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PII: S0223-5234(17)30038-7

DOI: 10.1016/j.ejmech.2017.01.028

Reference: EJMECH 9179

To appear in: *European Journal of Medicinal Chemistry*

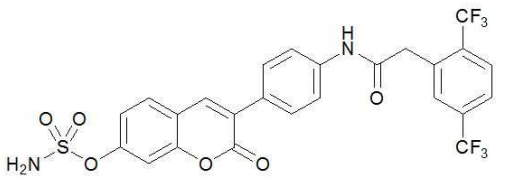
Received Date: 9 November 2016

Revised Date: 30 December 2016

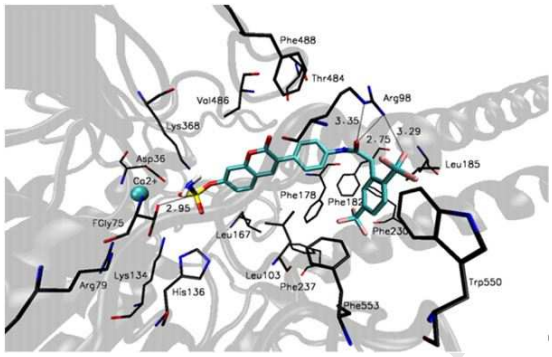
Accepted Date: 21 January 2017

Please cite this article as: M. Daśko, M. Przybyłowska, J. Rachon, M. Masłyk, K. Kubiński, M. Misiak, A. Składanowski, S. Demkowicz, Synthesis and biological evaluation of fluorinated *N*-benzoyl and *N*-phenylacetoyl derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate as steroid sulfatase inhibitors, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.01.028.

STS inhibitor



IC_{50} = 180 nM



Synthesis and biological evaluation of fluorinated *N*-benzoyl and *N*-phenylacetoyl derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate as steroid sulfatase inhibitors

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Keywords: steroid sulfatase, STS inhibitors, breast cancer, coumarin, sulfamates

Abstract

In the present work, we report convenient methods for the synthesis of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate derivatives *N*-acylated with fluorinated analogs of benzoic or phenylacetic acid as steroid sulfatase (STS) inhibitors. The design of these potential STS inhibitors was supported by molecular modeling techniques. Additionally, computational docking methods were used to determine the binding modes of the synthesized inhibitors and to identify potential interactions between inhibitors and amino acid residues located in the active site of STS. The inhibitory effects of the synthesized compounds were tested on STS isolated from human placenta and against estrogen receptor-(ER)-positive MCF-7 and T47D cells, as well as ER-negative MDA-MB-231 and SkBr3 cancer cell lines. In the course of our investigation, compounds **6c** and **6j** demonstrated the highest inhibitory effect in enzymatic STS assays, both with IC_{50} values of 0.18 µM (the IC_{50} value of coumarin-7-*O*-sulfamate is 1.38 µM, used as a reference). Compound **6j** exhibited the highest potency against the MCF-7 and T47D cell lines (15.9 µM and 8.7 µM, respectively). The GI_{50} values of tamoxifen (used as a reference) were 6.8; 10.6; 15.1; 12.5 µM against MCF-7, T47D, MDA-MB-231 and SkBr3 cancer cell lines, respectively. Despite the slightly lower activity of compounds **1** and **2** (both in enzymatic and cell-based experiments) compared to **6g** and **6j**, analogues **1** and **2** proved to selectively inhibit the growth of ER- and PR-positive cell lines.

1. Introduction

The World Health Organization (WHO) lists biologically active hormones, including estrogens, as one of the most important factors inducing the development of breast cancer. Novel treatment strategies for breast cancer involve inhibitors, which prevent the synthesis of estrogens in peripheral tissues [1]. Steroid sulfatase (STS) is one enzyme responsible for the formation of active estrogens in the breast tissue of postmenopausal women. STS hydrolyses estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) into estrone (E1) and dehydroepiandrosterone (DHEA), which can be converted into steroids that exhibit estrogenic properties (estradiol and androstenediol) [2].
The design and synthesis of compounds that regulate hormone levels in tissues through inhibition of STS activity is a major challenge for modern medicinal chemistry. Most known STS inhibitors act irreversibly. To date, various research groups have synthesized steroidal

and non-steroidal irreversible inhibitors of STS substituted with various functional groups including phosphates, thiophosphates or sulfamates [3-10]. Because the sulfamate moiety of STS inhibitors such as EMATE was designed to mimic sulfate moiety of natural substrates, it is assumed that the inhibition mechanism of these compounds involves an FGly75 residue located inside the active site of STS. The FGly75 residue coordinates to a calcium ion and plays a crucial role in the enzymatic hydrolysis of sulfated substrates. One proposed mechanism of inhibition assumes nucleophilic attack of the FGly75 hydroxyl group on the sulfur atom of the inhibitor containing the sulfamate moiety (Figure 1), which results in irreversible inactivation of the enzyme [11].

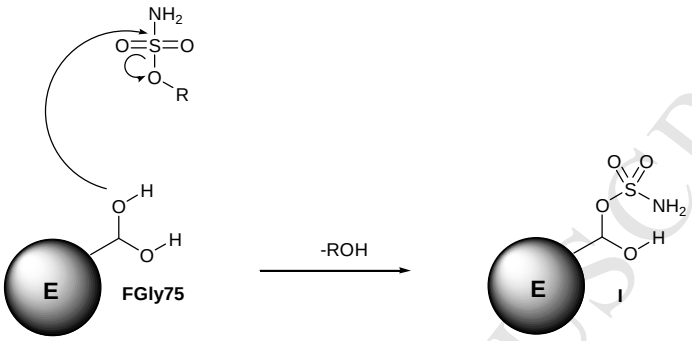


Fig. 1. Proposed mechanism of STS inhibition by compounds containing sulfamate moiety (E – enzyme; R – core of inhibitors).

Many STS inhibitors had been discontinued from clinical trials due to their estrogenic properties. For example, the steroid derivative EMATE (Figure 2) is an efficient inhibitor of STS activity (IC_{50} value of 65 pM in MCF-7 cell line assay) but was withdrawn from clinical trials due to its estrogenicity [12]. To avoid adversary side effects, several research groups have designed new compounds based on non-steroidal structures. An important class of potent inhibitors of STS includes coumarin derivatives. Coumarin-7-*O*-sulfamate and its derivatives, e.g., COUMATE (IC_{50} value of 380 nM in an assay with placental microsomes) [13] have been shown to lack significant estrogenicity.

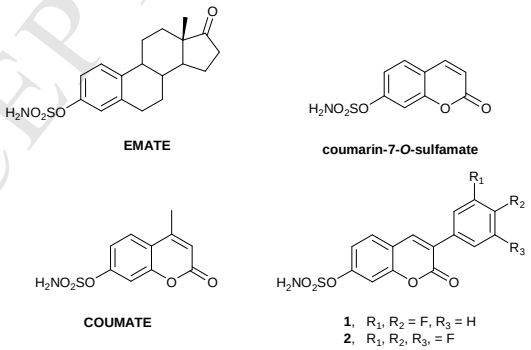


Fig. 2. Chemical structures of STS inhibitors.

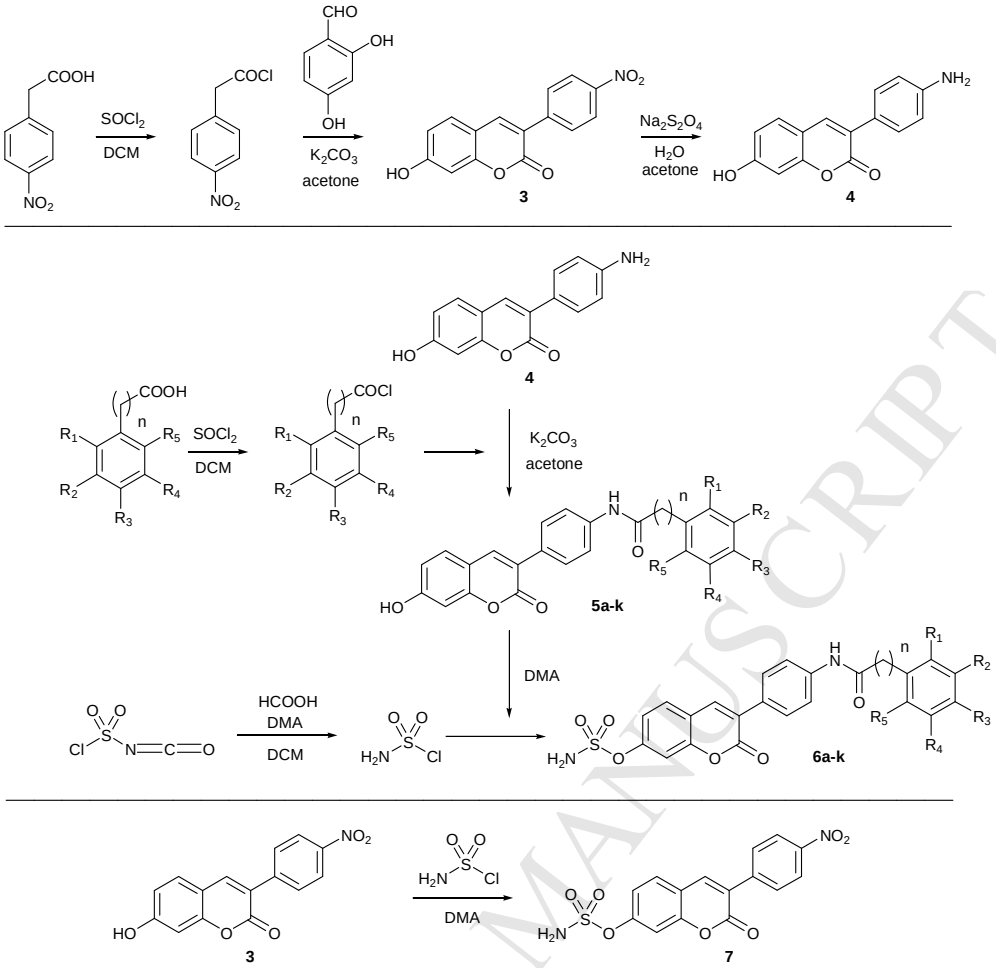
A key strategy in drug design is the introduction of fluorine atoms to their chemical structure. Since 1957, more than 150 fluorinated drugs have come to the market and now make up approximately 20% of all pharmaceuticals [14-16]. Organofluorine affects nearly all physical properties of the drug, as well as its absorption, distribution, metabolism, and excretion. In this paper we describe the synthesis of new fluorine-containing coumarins as potential inhibitors of STS. In the course of previous investigations with fluorinated 3-phenylcoumarin-

7-*O*-sulfamate derivatives [17], we found that introduction of fluorinated phenyl rings to coumarin scaffolds results in increased inhibitory activity. Two fluorinated compounds, **1** and **2**, demonstrated the highest activity in the STS enzyme assay, with IC_{50} values of 270 nM in both cases. In most cases, compounds containing C-F bonds showed slightly higher STS inhibition than analogues without fluorine atom in its structure.

2. Results and discussion

2.1. Chemistry

The synthesis of coumarin and their derivatives has been the subject of extensive research over many decades. Many convenient synthetic methods have been described including Pechmann [18], Perkin [19], Knoevenagel condensation [20], Reformatsky [21] and Wittig reactions [22]. Synthesis of newly designed STS inhibitors based on fluorinated *N*-benzoyl and *N*-phenylacetoyl derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate was accomplished using the pathway shown in Scheme 1. In the first step, 4-nitrophenyl acetyl chloride was obtained *in situ* by the treatment of 4-nitrophenyl acetic acids with thionyl chloride in dry dichloromethane. For the synthesis of 7-hydroxy-3-(4-nitrophenyl)-coumarin **3**, raw 4-nitrophenyl acetyl chloride was refluxed with 2,4-dihydroxybenzaldehyde in the presence of potassium carbonate. Next, 7-hydroxy-3-(4-nitrophenyl)-coumarin **3** was reduced to 7-hydroxy-3-(4-aminophenyl)-coumarin **4** using sodium hydrosulfite ($Na_2S_2O_4$). The progress of the reaction was monitored by TLC and, after full conversion of the starting material, product **4** was isolated from the reaction mixture in satisfactory yield (58%). In the next step, 7-hydroxy-3-(4-aminophenyl)-coumarin **4** was *N*-acylated with corresponding phenylacetoyl or benzoyl chlorides containing C-F bonds (obtained *in situ* from corresponding phenylacetic or benzoic acids reacted with thionyl chloride) in the presence of potassium carbonate. The reactions proceeded well and the desired products **5a-k** were obtained in good yield (62 - 75%). Finally, OH groups of the coumarin scaffolds **5a-k** were sulfamoylated. In brief, solutions of stable compounds **5a-k** in *N*,*N*-dimethylacetamide (DMA) were treated with H_2NSO_2Cl (previously generated by the reaction of chlorosulfonyl isocyanate and formic acid in the presence of a catalytic amount of *N*,*N*-dimethylacetamide). The yields of these reactions were high, reaching 90%. After standard isolation, we obtained the desired derivatives **6a-k**. The detailed synthesis is shown in Scheme 1. Additionally, we synthesized 3-(4-nitrophenyl)-coumarin-7-*O*-sulfamate **7** in the reaction of 7-hydroxy-3-(4-nitrophenyl)-coumarin **3** with H_2NSO_2Cl.



Scheme 1. Synthesis of 3-(4-benzoylamino-phenyl)- coumarin-7-*O*-sulfamate and 3-(4-phenylacetylamino-phenyl)-coumarin-7-*O*-sulfamate derivatives **6a-k** (n = 0, 1; R_1, R_2, R_3, R_4, R_5 = H, F, CF_3, OCF_3) and 3-(4-nitrophenyl)-coumarin-7-*O*-sulfamate **7**.

2.2. Molecular modeling

To examine the possible interactions of *N*-benzoyl and *N*-phenylacetoyl derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate with amino acid residues within the active site of STS, potential inhibitors were docked into the crystal structure of the human steroid sulfatase (Protein Data Bank accession code 1P49). The procedures for docking analyses as well as protein and inhibitor preparations are described in detail in the experimental section.

Our docking experiments revealed that fluorinated *N*-benzoyl and *N*-phenylacetoyl derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate could, at least theoretically, efficiently bind to the active site of STS. All candidates **6a-k** expressed satisfactory predicted free docking energies (in the range of -7.6 to -9.5 kcal/mol) and exhibited significantly lower AutoDock Vina scores compared to a reference inhibitor (the value of predicted free docking energy for coumarin-7-*O*-sulfamate, **1** and **2** were -2.9, -6.7 and -6.8 kcal/mol, respectively). The best docking result among the *N*-benzoyl derivatives was obtained for the **6c** analogue (predicted free docking energy was -8.9 kcal/mol), whereas the best docking result among the *N*-phenylacetoyl derivatives was obtained for the **6h** analogue (predicted free docking energy was -9.5 kcal/mol). Furthermore, we found that the AutoDock Vina score for compound **7** was -7.2 kcal/mol, lower than reference inhibitors (Table 1).

Figure 3 shows a putative enzyme-ligand complex before the presumed inactivation of STS and the superimposed best conformations of the three selected potential inhibitors **6c** (blue), **6h** (red), **6j** (CPK colored). The STS inhibitor candidates docked in a similar manner to the mode of the reported STS inhibitor coumarin-7-*O*-sulfamate (green). We found that sulfamate functional groups are directed to the catalytic amino acid FGly75 coordinated to the Ca^{2+} ion and are surrounded by several putative catalytic residues (Asp35, Asp36, Arg79, Lys134, His136 and Lys368). In each case, the distance between the sulfur atom of the designed compounds and a hydroxyl group of FGly75 are short (less than 3 Å) suggesting the possibility of an electrostatic interactions (*e.g.*, hydrogen bonds) between the sulfamate moiety and an OH group of FGly75 in its gem-diol form. Furthermore, the cores of the potential inhibitors are well accommodated by the cavity delimited by lipophilic amino acids residues located near the active site of the STS. Molecular modeling studies suggest that the hydrophobicity of coumarin cores could favor binding through the establishment of hydrophobic interactions with lipophilic amino acids in the enzyme active site (Leu103, Phe104, Leu167, Val177, Phe178, Phe182, Leu185, Phe230, Phe233, Phe237, Thr484, Val486, Phe488, Trp550 and Phe553). In addition, as shown in Figure 3 and exemplified by compound **6j**, amide moieties and fluorine atoms (*e.g.*, in CF_3 groups) of designed compounds are within a short distance of the backbone NH groups of Arg98, suggesting the presence of additional stabilizing interactions (*e.g.*, hydrogen bonds). These additional interactions may influence the binding of potential drug molecules to the enzyme active site.

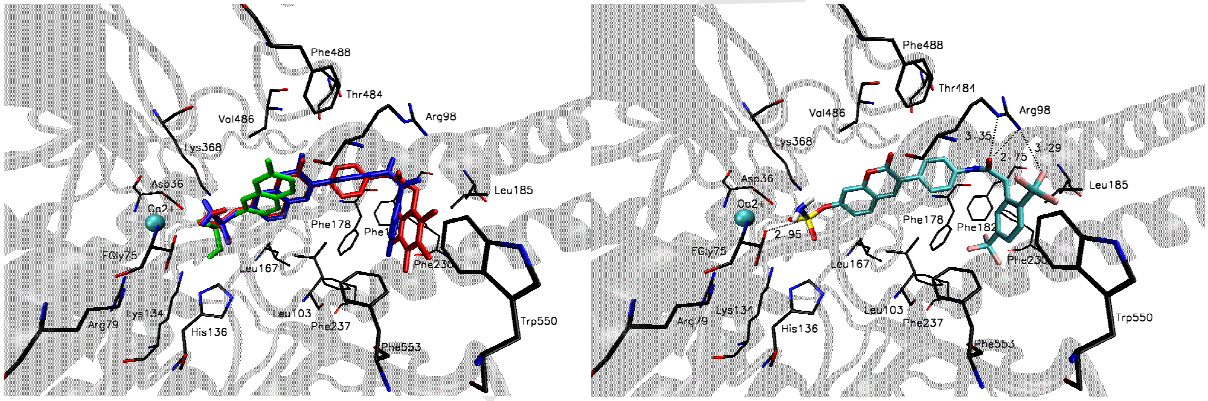


Fig. 3. Docked binding modes and distances to Arg98 for compounds **6c** (blue), **6h** (red), **6j** (CPK colored) and coumarin-7-*O*-sulfamate (green).

2.3. STS enzyme assays

The inhibitory effects of the synthesized compounds **6a-k** were tested using *in vitro* STS assays according to reported methods [23,24]. The screening assays were performed with STS enzyme extracted from human placenta and purified by three-step chromatography.

The results of the enzymatic assays are presented in Table 1. As observed in the data collected in the Table 1, the IC_{50} values are all fairly similar suggesting that the nature of R_1-R_5 substituents does not significantly affect the binding. Nevertheless, the compounds containing fluorine atoms in their structures, in most cases showed a higher activity against STS which was probably due to the stronger hydrophobic effect of the fluorine substituted aromatic ring. In the course of our investigation, we found that all the newly synthesized inhibitors possessed significantly greater inhibitory potency than reported for coumarin-7-*O*-sulfamate (IC_{50} value of 1.38 μM). The highest inhibitory activity among the *N*-benzoyl derivatives was exhibited by the **6c** analogue, with an IC_{50} value of 0.18 μM (consistent with molecular modeling data) whereas the highest inhibition among the *N*-phenylacetoyl derivatives was

observed for the **6j** analogue, with an IC_{50} value of 0.18 μM (the value of the predicted free docking energy calculated by AutoDock Vina software was -8.6 kcal/mol). Furthermore, the IC_{50} value for compound **7** was 0.29 μM. The best newly synthesized compounds **6c** and **6j** were slightly more potent than inhibitors **1** and **2**, whose design and evaluation we previously described [17].

Table 1.
Activities of the synthesized compounds **6a-k** and **7**, and a reference inhibitors (coumarin-7-*O*-sulfamate, **1** and **2**) in the STS enzyme assays.

No.	n	R_1	R_2	R_3	R_4	R_5	Free binding energy [kcal mol^{-1}]	IC_{50} [μM]
6a	0	H	H	H	H	H	-7.6	0.49 ± 0.04
6b		H	H	F	H	H	-8.6	0.21 ± 0.01
6c		H	F	F	H	H	-8.9	0.18 ± 0.01
6d		F	F	F	F	F	-8.8	0.23 ± 0.01
6e	1	H	H	H	H	H	-8.2	0.26 ± 0.02
6f		H	F	F	H	H	-8.7	0.36 ± 0.03
6 g		H	F	F	F	H	-7.8	0.30 ± 0.03
6h		F	F	F	F	F	-9.5	0.34 ± 0.03
6i		H	CF_3	F	H	H	-8.9	0.38 ± 0.02
6j		CF_3	H	H	CF_3	H	-8.6	0.18 ± 0.01
6k		H	H	OCF_3	H	H	-8.4	0.44 ± 0.02
7	-	-	-	-	-	-	-7.2	0.29 ± 0.03
1	-	-	-	-	-	-	-6.7	0.28 ± 0.02
2	-	-	-	-	-	-	-6.8	0.29 ± 0.02
coumarin-7-*O*-sulfamate	-	-	-	-	-	-	-2.9	1.38 ± 0.13

2.4. Cancer cell viability assay

According to the results of the enzymatic assay, we selected the six most active fluorinated derivatives from three different families (**6b** and **6c**, **6g** and **6j**, **1** and **2**) for cytotoxicity studies. In our panel of test cell lines, we included two estrogen- (ER-) and progesterone- (PR-) positive (MCF-7, T47D) and two ER- and PR-negative (SkBr3, MDA-MB-231) breast cancer cell lines. The results are presented in Table 2.
Despite the high activity of compounds **6b** and **6c** in *in vitro* experiments, their cytotoxicity against the tested cell lines ranged from moderate (mid-micromolar) to none. Analogue **6j** demonstrated the most potent antiproliferative activity (GI_{50} values for MCF-7 and T47D cell lines of 15.9 μM and 8.7 μM, respectively). However, it was not selective towards estrogen-dependent cells and effectively inhibited the growth of ER- and PR-negative cell lines (GI_{50} values in assays with SkBr3 and MDA-MB-231 cell lines of 18.8 μM and 8.1 μM, respectively). The activities of compounds **6g, 6j**, **1** and **2** were found to be comparable or approximately 2-3 times lower in comparison with tamoxifen used as a reference. The GI_{50} values of tamoxifen were 6.8; 10.6; 15.1; 12.5 μM against MCF-7, T47D, MDA-MB-231 and

SkBr3 cancer cell lines, respectively. In comparison, coumarin-7-*O*-sulfamate, used as a reference, was inactive against all of the aforementioned cells. No selectivity observed for compounds **6g** and **6j** towards estrogen-dependent cells could be a consequence of their low binding affinity to the human estrogen receptors ERα and ERβ (confirmed by independent experiments of molecular docking). In addition, other processes including a membrane transport and cellular metabolism could be crucial for the antiproliferative properties of these compounds. Despite the slightly lower activity of compounds **1** and **2** (both in enzymatic and cell-based experiments) in comparison with **6g** and **6j**, analogues **1** and **2** proved to selectively inhibit the growth of ER- and PR-positive cell lines (*e.g.*, for compound **2** the GI_{50} values were approximately 30 μM with MCF-7 and T47D cell lines and above 55 μM for SkBr3 and MDA-MB-231 cells). It is noteworthy that the compounds **1** and **2** showed selectivity for the estrogen-dependent cells, although molecular docking studies to the ERα and ERβ showed no affinity. These observations may suggest that the compounds **1** and **2** could be involved mainly in the inhibition of estrogen biosynthesis by interacting with the STS.

Table 2.
Antiproliferative activities of selected compounds and references (coumarin-7-*O*-sulfamate and tamoxifen).

No.	GI_{50} [μM]			
	MCF-7	T47D	SkBr3	MDA-MB-231
	ER^+; PR^+; $Her2^+$	ER^+; PR^+; $Her2^-$	ER^-; PR^-; $Her2^+$	ER^-; PR^-; $Her2^-$
6b	>100	>100	>100	>100
6c	>100	>100	>100	>100
6g	31.3 ± 6.9	13.7 ± 1.0	36.1 ± 0.3	11.7 ± 3.0
6j	15.9 ± 3.7	8.7 ± 0.1	18.8 ± 0.4	8.1 ± 1.7
1	30.0 ± 2.3	43.0 ± 1.7	>100	61.5 ± 3.9
2	32.6 ± 2.6	30.7 ± 3.9	61.3 ± 3.8	56.3 ± 0.6
coumarin-7-*O*-sulfamate	>100	>100	>100	>100
tamoxifen	6.8 ± 2.2	10.6 ± 0.6	12.5 ± 0.6	15.1 ± 0.1

[a] ER, estrogen receptor; PR, progesterone receptor; Her2 epithelial growth factor receptor 2.

3. Conclusion

We have efficiently synthesized and biologically evaluated a series of new STS inhibitors. Computational analyses supported the design of the structures of potential inhibitors. In the course of our docking studies, we found that fluorinated *N*-benzoyl and *N*-phenylacetoyl derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate are well accommodated to the active site of STS. Furthermore, the amide moieties and fluorine atoms (e.g., in CF_3 groups) of the designed compounds are within a short distance of the backbone NH groups of Arg98, suggesting the presence of additional stabilizing interactions (e.g., hydrogen bonds). The results of our molecular modeling studies were mostly confirmed in enzymatic experiments. An *in vitro* assay with isolated STS showed that all newly synthesized inhibitors possessed significantly greater inhibitory potency than reported for coumarin-7-*O*-sulfamate (IC_{50} value of 1.38 μM). The highest inhibitory activity among the *N*-benzoyl derivatives was exhibited by the **6c** analogue, whereas the highest inhibition among the *N*-phenylacetoyl derivatives was observed for the **6j** analogue, both with IC_{50} value of 0.18 μM. Six of the most active fluorinated derivatives in the enzymatic assay were grouped into three different families (**6b**

and **6c**, **6g** and **6j**, **1** and **2**) and were selected for cytotoxicity studies. In the course of our investigation, we found that compound **6j** exhibited the highest potency against the MCF-7 and T47D cell lines at 15.9 μM and 8.7 μM, respectively. However, it was not selective towards estrogen-dependent cells and effectively inhibited the growth of ER- and PR-negative cells. Despite the slightly lower activity of compounds **1** and **2** (both in enzymatic and cell-based experiments) compared to **6g** and **6j**, analogues **1** and **2** proved to selectively inhibit the growth of ER- and PR-positive cell lines.

4. Experimental

4.1. Materials and methods

Benzoic acid, 4-fluorobenzoic acid, 3,4-difluorobenzoic acid, pentafluorobenzoic acid, phenylacetic acid, 4-nitrophenylacetic acid, 3,4-difluorophenylacetic acid, 3,4,5-trifluorophenylacetic acid, 2,3,4,5,6-pentafluorophenylacetic acid, 2,5-Bis(trifluoromethyl)phenylacetic acid, [4-fluoro-3-(trifluoromethyl)phenyl]acetic acid, 4-(trifluoromethoxy)phenylacetic acid, thionyl chloride, potassium carbonate, 2,4-dihydroxybenzaldehyde, sodium hydrosulfite, chlorosulfonyl isocyanate, *N*,*N*-dimethylacetamide, formic acid, tamoxifen (TraceCERT®) are commercially available from Aldrich. Dichloromethane and acetone were dried and distilled using standard procedures. Melting points (uncorrected) were determined with a Stuart Scientific SMP30 apparatus. NMR spectra were recorded on a Varian Gemini 200 MHz and Varian Unity Plus 500 spectrometers. Chemical shifts are reported in ppm relative to the residual solvent peak (DMSO-d_6 2.49 ppm for ^{1}H, acetone-d_6 29.92 ppm for ^{13}C). Coupling constants are given in Hertz. IR spectra were measured on a Nicolet 8700 spectrometer. Elemental analysis was performed using CHNS-Carlo Erba EA-1108. Mass spectra were recorded on an Agilent 6540 Accurate Mass Q-TOF LC/MS System. Column chromatography was performed using silica gel 60 (230-400 mesh, Merck). Preparative thin-layer chromatography was performed with Polygram SIL G/UV_{254} silica gel (Macherey- Nagel GmbH & Co. KG, Düren, Germany).

*4.2. Preparation of 7-hydroxy-3-(4-nitrophenyl)-coumarin (**3**).*

$SOCl_2$ (33 mL) was added to a solution of 4-nitrophenylacetic acid (7.25 g, 40 mmol) in dry CH_2Cl_2 (33 mL), and the suspension was refluxed for 4 h. The resulting solution was evaporated, the residue was dissolved in dry acetone (200 mL) and 2,4-dihydroxybenzaldehyde (5.52 g, 40 mmol) was added. The mixture was refluxed with anhydrous K_2CO_3 (22.08 g, 160 mmol) for 4 h. Acetone was removed under reduced pressure and cold water (400 mL) was added; 3 N HCl was then added until the solution became acidic. The resulting precipitate was filtered, washed with water and recrystallized from ethanol to give the desired product **3**.

Yield 68%; mp 298-299 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3201, 1685, 1588, 1508, 1415, 1336, 1286, 1219, 1169, 1118, 993, 848; ^{1}H NMR δ_H (500 MHz, DMSO) 10.81 (1H, s, OH), 8.39 (1H, s, CH), 8.28 (2H, d, *J* 8.8, Ar-H), 8.00 (2H, d, *J* 8.3, Ar-H), 7.64 (1H, d, *J* 8.8, Ar-H), 6.85 (1H, d, *J* 8.8, Ar-H), 6.77 (1H, s, Ar-H); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 162.9, 160.3, 156.1, 147.3, 143.9, 142.5, 131.3, 129.9, 124.0, 120.4, 114.4, 112.4, 102.5. Anal. Calcd for: $C_{15}H_9NO_5$: C, 63.61; H, 3.20; N, 4.95. Found: C, 63.70; H, 3.27; N, 4.84%. HRMS (m/z) [M-H]$^-$ calcd 282.0402. Found 282.0444.

*4.3. Preparation of 3-(4-aminophenyl)-7-hydroxy-coumarin (**4**).*

First, 7-hydroxy-3-(4-nitrophenyl)-coumarin **3** (7.08 g, 25 mmol) was added to a mixture of acetone (1300 mL) and water (650 mL). The resulting solution was heated to 50 ˚C (until the solution became completely clear). Next, sodium hydrosulfite (43.53 g, 250 mmol) was added in three portions, and the reaction mixture was heated to reflux. After 2 h, the acetone was evaporated, and the resulting precipitate was filtered, washed with water and recrystallized from acetone to give the desired product **4**.

Yield 58%; mp 292-295 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3338, 3278, 1670, 1608, 1565, 1507, 1467, 1333, 1284, 1211, 1162, 1132, 994, 846; ^{1}H NMR δ_H (500 MHz, DMSO) 10.45 (1H, s, OH), 7.94 (1H, s, CH), 7.53 (1H, d, *J* 8.3, Ar-H), 7.41 (2H, d, *J* 8.8, Ar-H), 6.77 (1H, d, *J* 8.8, Ar-H), 6.71 (1H, s, Ar-H), 6.59 (2H, d, *J* 8.8, Ar-H), 5.43 (2H, br s, NH_2); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 137.6, 137.5, 131.4, 126.0, 114.8, 106.5, 106.2, 99.9, 99.4, 90.6, 90.4, 89.6, 78.8. Anal. Calcd for: $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.01; H, 4.41; N, 5.65%. HRMS (m/z) [M-H]$^-$ calcd 252.0661. Found 252.0705.

*4.4. General method for the synthesis of N-benzoyl and N-phenylacetoyl derivatives of 3-(4-aminophenyl)-7-hydroxy-coumarin (**5a-k**).*

$SOCl_2$ (1.65 mL) was added to a solution of benzoic or phenylacetic acid derivatives (2 mmol) in dry CH_2Cl_2 (1.65 mL), and the suspension was refluxed for 4 h. The resulting solution was evaporated, the residue was dissolved in dry acetone (10 mL) and 3-(4-aminophenyl)-7-hydroxy-coumarin **4** (0.51 g, 2 mmol) was added. The mixture was refluxed with anhydrous K_2CO_3 (1.104 g, 8 mmol) for 4 h. Acetone was removed under reduced pressure and cold water (20 mL) was added. 3 N HCl was added until the solution became acidic. The resulting precipitate was filtered, washed with water and recrystallized from methanol or ethanol to give the desired products **5a-k**.

3-(4-benzoylamino-phenyl)-7-hydroxy-coumarin **(5a).** Yield 72%; mp 281-284 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3363, 3198, 1696, 1664, 1601, 1516, 1410. 1325, 1283, 1202, 1158, 1129, 988, 837; ^{1}H NMR δ_H (500 MHz, DMSO) 10.59 (1H, s, OH), 10.37 (1H, s, NH), 8.16 (1H, s, CH), 7.97 (2H, d, *J* 7.1, Ar-H), 7.85 (2H, d, *J* = 8.8, Ar-H), 7.71 (2H, d, *J* 8.8, Ar-H), 7.62-7.50 (4H, m, Ar-H), 6.82 (1H, dd, *J* 8.5, 2.3, Ar-H), 6.75 (1H, d, *J* 2.2, Ar-H); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 166.3, 161.8, 160.8, 155.5, 141.0, 139.7, 135.5, 132.4, 130.9, 130.6, 129.2, 129.1, 128.4, 122.4, 120.5, 114.1, 112.8, 102.4. Anal. Calcd for: $C_{22}H_{15}NO_4$: C, 73.94; H, 4.23; N, 3.92. Found: C, 73.99; H, 4.16; N, 3.83%. HRMS (m/z) [M-H]$^-$ calcd 356.0923. Found 356.0960.

3-[4-(4-fluoro-benzoylamino)-phenyl]-7-hydroxy-coumarin **(5b).** Yield 66%; mp 321-324 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3544, 3337, 1705, 1665, 1599, 1501, 1412, 1321, 1282, 1213, 1153, 1118, 990, 822; ^{1}H NMR δ_H (500 MHz, DMSO) 10.59 (1H, s, OH), 10.38 (1H, s, NH), 8.16 (1H, s, CH), 8.08-8.02 (2H, m, Ar-H), 7.83 (2H, d, *J* = 8.8, Ar-H), 7.71 (2H, d, *J* 8.8, Ar-H), 7.59 (1H, d, *J* 8.3, Ar-H), 7.40-7.34 (2H, m, Ar-H), 6.82 (1H, dd, *J* 8.3, 2.4, Ar-H), 6.75 (1H, d, *J* 2.4, Ar-H); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 164.4, 164.1 (d, $^{1}J_{C\text{-}F}$ 249), 161.0, 160.1, 154.7, 140.2, 138.9, 131.2 (m). 130.5, 130.3, 129.8, 128.4, 121.7, 119.8, 115.3 (m), 113.3, 112.0, 101.7. Anal. Calcd for: $C_{22}H_{14}FNO_4$: C, 70.40; H, 3.76; N, 5.06. Found: C, 70.49; H, 3.83; N, 4.98%. HRMS (m/z) [M-H]$^-$ calcd 374.0829. Found 374.0883.

3-[4-(3,4-difluoro-benzoylamino)-phenyl]-7-hydroxy-coumarin **(5c).** Yield 65%; mp 334-337 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3577, 3337, 1703, 1666, 1600, 1503, 1411, 1322, 1288, 1214, 1160, 1122, 994, 824; ^{1}H NMR δ_H (200 MHz, DMSO) 10.61 (1H, s, OH), 10.44 (1H, s, NH), 8.18 (1H, s, CH), 8.16-8.00 (2H, m, Ar-H), 7.90-7.56 (6H, m, Ar-H), 6.88-6.76 (2H, m, Ar-H); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 163.2, 161.1, 159.7, 154.7, 150.0 (m), 147.8 (m), 140.3, 138.6, 132.1 (m), 130.5, 129.9, 128.5, 125.3 (m), 121.6, 119.9, 117.4 (m), 113.4, 112.0, 101.7. Anal. Calcd for: $C_{22}H_{13}F_2NO_4$: C, 67.18; H, 3.33; N, 3.56. Found: C, 67.14; H, 3.40; N, 3.63%. HRMS (m/z) [M-H]$^-$ calcd 392.0734. Found 392.0777.

7-hydroxy-3-(4-pentafluorobenzoylamino-phenyl)-coumarin **(5d).** Yield 62%; mp 243-245 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3307, 3201, 1695, 1666, 1608, 1502, 1412, 1335, 1282, 1214, 1158, 1126, 992, 844; ^{1}H NMR δ_H (500 MHz, DMSO) 11.11 (1H, s, OH), 10.61 (1H, s, NH), 8.17 (1H, s, CH), 7.78-7.68 (4H, m, Ar-H), 7.60 (1H, d, *J* = 8.8, Ar-H), 6.81 (1H, d, *J* 8.5, Ar-H), 6.75 (1H, s, Ar-H); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 161.2, 160.0, 154.8, 143.1 (d, $^{1}J_{C-F}$ 255), 140.6, 137.6, 137.0 (d, $^{1}J_{C-F}$ 252), 131.4, 129.9, 129.1, 128.9, 121.4, 119.2, 113.4, 112.7 (m), 112.0, 101.7. Anal. Calcd for: $C_{22}H_{10}F_5NO_4$: C, 59.07; H, 2.25; N, 3.13. Found: C, 59.11; H, 2.14; N, 3.21%. HRMS (m/z) [M-H]$^-$ calcd 446.0452. Found 446.0502.

7-hydroxy-3-(4-phenylacetylamino-phenyl)-coumarin **(5e).** Yield 75%; mp 237-239 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3351, 3190, 1705, 1655, 1609, 1517, 1410, 1321, 1285, 1211, 1165, 1129, 991, 840; ^{1}H NMR δ_H (500 MHz, DMSO) 10.59 (1H, s, OH), 10.30 (1H, s, NH), 8.12 (1H, s, CH), 7.68-7.54 (5H, m, Ar-H), 7.36-7.28 (4H, m, Ar-H), 7.26-7.22 (1H, m, Ar-H), 6.80 (1H, d, *J* 8.3, Ar-H), 6.73 (1H, s, Ar-H), 3.65 (2H, s, CH_2); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 169.9, 161.7, 160.8, 155.4, 140.8, 139.7, 136.6, 130.6, 130.5, 129.8, 129.3, 129.0, 127.3, 122.4, 122.4, 119.3, 114.1, 112.8, 102.4, 44.0. Anal. Calcd for: $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.52; H, 4.55; N, 3.86%. HRMS (m/z) [M-H]$^-$ calcd 370.1079. Found 370.1092.

3-{4-[2-(3,4-difluoro-phenyl)-acetylamino]-phenyl}-7-hydroxy-coumarin **(5f).** Yield 68%; mp 281-283 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3350, 3215, 1702, 1658, 1614, 1514, 1412, 1318, 1283, 1200, 1164, 1129, 994, 841; ^{1}H NMR δ_H (500 MHz, DMSO) 10.58 (1H, s, OH), 10.30 (1H, s, NH), 8.12 (1H, s, CH), 7.68-7.62 (4H, m, Ar-H), 7.58 (1H, d, *J* 8.3, Ar-H), 7.42-7.34 (2H, m, Ar-H), 7.20-7.14 (1H, m, Ar-H), 6.80 (1H, dd, *J* 8.8, 2.1, Ar-H), 6.73 (1H, d, *J* 2.1, Ar-H), 3.67 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.5, 161.0, 160.1, 154.7, 149.2 (d, $^{1}J_{C-F}$ 245), 148.5 (d, $^{1}J_{C-F}$ 244), 140.1, 138.9, 133.4 (m), 129.8, 128.6, 126.0 (m), 121.6, 118.7 (m), 118.1, 117.3, 117.0, 113.3, 112.0, 101.7, 42.0. Anal. Calcd for: $C_{23}H_{15}F_2NO_4$: C, 67.81; H, 3.71; N, 3.44. Found: C, 67.77; H, 3.58; N, 3.61%. HRMS (m/z) [M-H]$^-$ calcd 406.0891. Found 406.0929.

7-hydroxy-3-{4-[2-(3,4,5-trifluoro-phenyl)-acetylamino]-phenyl}-coumarin **(5 g).** Yield 67%; mp 280-282 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3342, 3243, 1691, 1662, 1614, 1525, 1413, 1322, 1282, 1201, 1161, 1130, 993, 842; ^{1}H NMR δ_H (500 MHz, DMSO) 10.58 (1H, s, OH), 10.31 (1H, s, NH), 8.12 (1H, s, CH), 7.68-7.60 (4H, m, Ar-H), 7.58 (1H, d, *J* 8.3, Ar-H), 7.32-7.26 (2H, m, Ar-H), 6.80 (1H, dd, *J* 8.8, 2.0, Ar-H), 6.74 (1H, d, *J* 2.0, Ar-H), 4.08 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.0, 161.0, 160.1, 154.7, 150.0 (d, $^{1}J_{C-F}$ 252), 140.2, 138.8, 135.2 (m), 133.0 (m), 129.8 (m), 128.6, 121.6, 118.7, 114.2, 113.8, 113.3, 112.0, 101.7, 41.8. Anal. Calcd for: $C_{23}H_{14}F_3NO_4$: C, 64.94; H, 3.32; N, 3.29. Found: C, 64.81; H, 3.35; N, 3.35%. HRMS (m/z) [M-H]$^-$ calcd 424.0797. Found 424.0868.

7-hydroxy-3-[4-(2-pentafluorophenyl-acetylamino)-phenyl]-coumarin **(5h).** Yield 73%; mp 291-293 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3322, 3245, 1685, 1655, 1606, 1503, 1416, 1327, 1291, 1194, 1159, 1132, 1001, 842; ^{1}H NMR δ_H (500 MHz, DMSO) 10.59 (1H, s, OH), 10.50 (1H, s, NH), 8.13 (1H, s, CH), 7.70-7.60 (4H, m, Ar-H), 7.58 (1H, d, *J* 8.8, Ar-H), 6.80 (1H, dd, *J* 8.8, 2.4, Ar-H), 6.74 (1H, d, *J* 2.4, Ar-H), 3.92 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 165.7, 161.0, 160.0, 154.7, 144.9 (d, $^{1}J_{C\text{-}F}$ 244), 140.2, 138.6 (d, $^{1}J_{C\text{-}F}$ 234), 138.5, 136.8 (d, $^{1}J_{C\text{-}F}$ 249), 130.1, 129.8, 128.7, 121.5, 118.7, 113.3, 112.0, 109.9 (m), 101.6, 29.8. Anal. Calcd for: $C_{23}H_{12}F_5NO_4$: C, 59.88; H, 2.62; N, 3.04. Found: C, 60.01; H, 2.53; N, 2.99%. HRMS (m/z) [M-H]$^-$ calcd 460.0608. Found 460.0670.

3-{4-[2-(4-fluoro-3-trifluoromethyl-phenyl)-acetylamino]-phenyl}-7-hydroxy-coumarin **(5i).** Yield 62%; mp 267-271 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3384, 3262, 1697, 1669, 1601, 1505, 1410, 1326, 1287, 1209, 1159, 1122, 990, 833; ^{1}H NMR δ_H (500 MHz, DMSO) 10.58 (1H, s, OH), 10.35 (1H, s, NH), 8.12 (1H, s, CH), 7.75 (1H, d, *J* 6.8, Ar-H), 7.74-7.62 (5H, m, Ar-H), 7.58 (1H, d, *J* 8.3, Ar-H), 7.52-7.40 (1H, m, Ar-H), 6.81 (1H, dd, *J* 8.3, 2.0, Ar-H), 6.74 (1H, d, *J* 2.0, Ar-H), 3.80 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.5, 161.0, 160.1, 157.7 (d, $^{1}J_{C\text{-}F}$ 252), 154.7, 140.1, 138.8, 136.1 (m), 132.9 (m), 129.8, 128.6, 127.9 (m), 122.7 (q, $^{1}J_{C\text{-}F}$ 272), 121.6, 120.0, 118.6, 117.1, 116.7, 113.3, 112.0, 101.6, 50.0. Anal. Calcd for: $C_{24}H_{15}F_4NO_4$: C, 63.02; H, 3.31; N, 3.06. Found: C, 62.97; H, 3.24; N, 3.11%. HRMS (m/z) [M-H]$^-$ calcd 456.0859. Found 456.0872.

3-{4-[2-(2,5-bis-trifluoromethyl-phenyl)-acetylamino]-phenyl}-7-hydroxy-coumarin **(5j).** Yield 68%; mp 271-272 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3308, 3262, 1669, 1611, 1516, 1412, 1318, 1290, 1187, 1168, 1114, 997, 838; ^{1}H NMR δ_H (500 MHz, DMSO) 10.58 (1H, s, OH), 10.41 (1H, s, NH), 8.12 (1H, s, CH), 7.98-7.94 (2H, m, Ar-H), 7.89 (1H, d, *J* 8.3, Ar-H), 7.70-7.54 (5H, m, Ar-H), 6.80 (1H, dd, *J* 8.3, 2.4, Ar-H), 6.74 (1H, d, *J* 2.4, Ar-H), 4.09 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 167.5, 161.0, 160.1, 154.7, 140.2, 138.8, 135.6, 132.5, 131.8, 131.6, 130.6 (m), 129.8, 128.7, 127.0 (m), 124.4 (m), 123.7 (q, $^{1}J_{C\text{-}F}$ 274), 123.5 (q, $^{1}J_{C\text{-}F}$ 274), 121.6, 118.6, 113.3, 112.0, 101.7. Anal. Calcd for: $C_{25}H_{15}F_6NO_4$: C, 59.18; H, 2.98; N, 2.76. Found: C, 59.26; H, 2.91; N, 2.81%. HRMS (m/z) [M-H]$^-$ calcd 506.0827. Found 506.0851.

7-hydroxy-3-{4-[2-(4-trifluoromethoxy-phenyl)-acetylamino]-phenyl}-coumarin **(5k).** Yield 70%; mp 261-262 ºC; ν_{max} (KBr)/cm^{-1} 3352, 3260, 1696, 1661, 1612, 1504, 1412, 1322, 1280, 1197, 1156, 1129, 991, 841; ^{1}H NMR δ_H (500 MHz, DMSO) 10.58 (1H, s, OH), 10.33 (1H, s, NH), 8.12 (1H, s, CH), 7.68-7.62 (4H, m, Ar-H), 7.58 (1H, d, *J* 8.8, Ar-H), 7.45 (2H, d, *J* 8.3, Ar-H), 7.32 (2H, d, *J* 8.3, Ar-H), 6.80 (1H, dd, *J* 8.3, 2.0, Ar-H), 6.74 (1H, d, *J* 2.0, Ar-H), 3.71 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.8, 161.0, 160.1, 154.7, 147.1, 140.1, 138.9, 135.4, 131.0, 129.8, 128.6, 121.6, 120.9, 120.1 (q, $^{1}J_{C\text{-}F}$ 256), 118.6, 113.3, 112.0, 101.6, 44.9. Anal. Calcd for: $C_{24}H_{16}F_3NO_5$: C, 63.30; H, 3.54; N, 3.08. Found: C, 63.19; H, 3.45; N, 3.15%. HRMS (m/z) [M-H]$^-$ calcd 454.0902. Found 454.0960.

*4.5. General method for the synthesis of 3-(4-benzoylamino-phenyl)-coumarin-7-O-sulfamate and 3-(4-phenylacetylamino-phenyl)-coumarin-7-O-sulfamate derivatives (**6a-k**).*

A mixture of formic acid (70.9 mg, 1.54 mmol) and *N*,*N*-dimethyl acetamide (1.4 mg, 0.016 mmol) was added to a stirred solution of chlorosulfonyl isocyanate (212.3 mg, 1.50 mmol) in dry dichloromethane (0.5 mL) at 40 ºC over 3.5 h. Then, a stirred solution of 3-(4-benzoylamino-phenyl)-7-hydroxy-coumarin and 7-hydroxy-3-(4-phenylacetylamino-phenyl)-coumarin derivatives **5a-k** (1.00 mmol) in *N*,*N*-dimethyl acetamide (3.4 mL) was added to the

mixture. The mixture was stirred at ambient temperature overnight and then poured into water (10 mL). Eventually, a white precipitate formed. The suspension was stirred at ambient temperature for an additional two hours. The resulting precipitate was filtered, washed with water and recrystallized from acetonitrile or acetone to give the desired products **6a-k**.

3-(4-benzoylamino-phenyl)-coumarin-7-O-sulfamate **(6a).** Yield 79%; mp 267-270 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3332, 3294, 1701, 1668, 1602, 1513, 1412, 1331, 1244, 1190, 1118, 979, 843; ^{1}H NMR δ_H (500 MHz, DMSO) 10.42 (1H, s, NH), 8.31 (1H, s, CH), 8.26 (2H, s, NH_2), 7.97 (2H, d, *J* 6.3, Ar-H), 7.92-7.84 (3H, m, Ar-H), 7.80-7.74 (2H, m, Ar-H), 7.66-7.52 (3H, m, Ar-H), 7.36 (1H, d, *J* 2.0, Ar-H), 7.29 (1H, dd, *J* 8.3, 2.0, Ar-H); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 166.4, 160.2, 153.9, 152.6, 140.4, 139.7, 135.5, 132.4, 130.4, 130.2, 129.5, 129.1, 128.4, 126.6, 120.5, 119.4, 118.7, 110.2. Anal. Calcd for: $C_{22}H_{16}N_2O_6S$: C, 60.54; H, 3.70; N, 6.42; S, 7.35. Found: C, 60.61; H, 3.74; N, 6.53; S, 7.31%. HRMS (m/z) [M-H]$^-$ calcd 435.0651. Found 435.0655.

3-[4-(4-fluoro-benzoylamino)-phenyl]-coumarin-7-O-sulfamate **(6b).** Yield 85%; mp 255-258 ºC; ν_{max} (KBr)/cm^{-1} 3399, 3345, 1701, 1660, 1610, 1499, 1412, 1327, 1253, 1181, 1112, 993, 834; ^{1}H NMR δ_H (200 MHz, DMSO) 10.44 (1H, s, NH), 8.33 (1H, s, CH), 8.27 (2H, s, NH_2), 8.14-8.02 (2H, m, Ar-H), 7.96-7.72 (5H, m, Ar-H), 7.48-7.26 (4H, m, Ar-H); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 164.4, 164.1 (d, $^{1}J_{C-F}$ 249), 161.0, 160.1, 159.4, 154.7, 153.2, 151.9, 140.2, 139.5, 139.0, 131.2, 130.4 (m), 129.6 (m), 128.6 (m), 125.8, 121.7, 119.8, 118.6, 117.9, 115.3 (m), 113.3, 112.0, 109.4, 101.7. Anal. Calcd for: $C_{22}H_{15}FN_2O_6S$: C, 58.15; H, 3.33; N, 6.16; S, 7.06. Found: C, 58.11; H, 3.41; N, 6.29; S, 6.99%. HRMS (m/z) [M-H]$^-$ calcd 453.0557. Found 453.0608.

3-[4-(3,4-difluoro-benzoylamino)-phenyl]-coumarin-7-O-sulfamate **(6c).** Yield 88%; mp 258-260 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3416, 3342, 1699, 1651, 1607, 1506, 1411, 1327, 1246, 1184, 1113, 991, 832; ^{1}H NMR δ_H (200 MHz, DMSO) 10.62 (1H, s, NH), 8.33 (1H, s, CH), 8.27 (2H, s, NH_2), 8.20-8.00 (1H, m, Ar-H), 7.92-7.56 (7H, m, Ar-H), 7.40-7.26 (2H, m, Ar-H); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 164.0, 160.1, 154.0, 152.6, 152.2 (d, $^{1}J_{C-F}$ 252), 149.8 (d, $^{1}J_{C-F}$ 247), 141.1, 139.8 (m), 132.8 (m), 130.5 (m), 129.6, 129.2, 126.5, 126.0, 120.6, 119.4, 118.3 (m), 114.1, 112.8, 110.1, 102.4. Anal. Calcd for: $C_{22}H_{14}F_2N_2O_6S$: C, 55.93; H, 2.99; N, 5.93; S, 6.79. Found: C, 56.01; H, 3.04; N, 5.81; S, 6.71%. HRMS (m/z) [M-H]$^-$ calcd 471.0462. Found 471.0486.

3-(4-pentafluorobenzoylamino-phenyl)-coumarin-7-O-sulfamate **(6d).** Yield 81%; mp 259-265 ºC (with decomposition); ν_{max} (KBr)/cm^{-1} 3257, 3194, 1711, 1666, 1601, 1501, 1417, 1336, 1253, 1191, 1118, 994, 846; ^{1}H NMR δ_H (500 MHz, DMSO) 11.16 (1H, s, NH), 8.31 (1H, s, CH), 8.25 (2H, s, NH_2), 7.87 (1H, d, *J* 8.3, Ar-H), 7.82-7.72 (4H, m, Ar-H), 7.36 (1H, d, *J* 2.4, Ar-H), 7.30 (1H, dd, *J* 8.3, 2.4, Ar-H); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 161.2, 1593, 154.8 (m), 153.3, 152.0, 143.1 (d, $^{1}J_{C-F}$ 252), 140.6, 139.4, 138.2, 137.6, 136.9 (d, $^{1}J_{C-F}$ 247), 131.4, 130.7, 129.9 (m), 129.2, 128.9, 125.6, 121.4, 119.2, 118.6, 117.8, 113.4, 112.0 (m), 109.4, 101.7. Anal. Calcd for: $C_{22}H_{11}F_5N_2O_6S$: C, 50.20; H, 2.11; N, 5.32; S, 6.09. Found: C, 50.32; H, 2.05; N, 5.29; S, 6.00%. HRMS (m/z) [M-H]$^-$ calcd 525.0180. Found 525.0177.

3-(4-phenylacetylamino-phenyl)-coumarin-7-O-sulfamate **(6e).** Yield 85%; mp 208-210 ºC; ν_{max} (KBr)/cm^{-1} 3289, 3185, 1712, 1665, 1601, 1499, 1415, 1347, 1248, 1182, 1112, 991, 843; ^{1}H NMR δ_H (500 MHz, DMSO) 10.30 (1H, s, NH), 8.26 (1H, s, CH), 8.24 (2H, s, NH_2), 7.84 (1H, d, *J* 8.8, Ar-H), 7.72-7.66 (4H, m, Ar-H), 7.36-7.22 (7H, m, Ar-H), 3.66 (2H, s,

CH_2); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 170.0, 160.1, 153.9, 152.6, 140.4, 139.6, 136.6, 130.4, 129.9, 129.8, 129.7, 129.6, 129.0, 127.3, 126.5, 119.4, 119.3, 118.7, 110.1, 44.0. Anal. Calcd for: $C_{23}H_{18}N_2O_6S$: C, 61.32; H, 4.03; N, 6.22; S, 7.12. Found: C, 61.26; H, 3.95; N, 6.11; S, 7.25%. HRMS (m/z) $[M-H]^-$ calcd 449.0807. Found 449.0813.

3-{4-[2-(3,4-difluoro-phenyl)-acetylamino]-phenyl}-coumarin-7-O-sulfamate **(6f).** Yield 87%; mp 212-214 ºC; ν_{max} (KBr)/cm^{-1} 3313, 3192, 1714, 1595, 1515, 1414, 1357, 1247, 1185, 1115, 992, 840; ^{1}H NMR δ_H (500 MHz, DMSO) 10.35 (1H, s, NH), 8.27 (1H, s, CH), 8.24 (2H, s, NH_2), 7.85 (1H, d, *J* 8.8, Ar-H), 7.74-7.64 (4H, m, Ar-H), 7.44-7.36 (2H, m, Ar-H), 7.35 (1H, d, *J* 2.0, Ar-H), 7.28 (1H, dd, *J* 8.3, 2.0, Ar-H), 7.20-7.16 (1H, m, Ar-H), 3.69 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.7, 161.0, 159.4, 153.2, 151.8, 149.2 (d, $^1J_{C-F}$ 244), 148.4 (d, $^1J_{C-F}$ 244), 140.1, 139.5, 138.9, 133.4 (m), 129.8, 129.6 (m), 126.1 (m), 118.7 (m), 117.0, 116.5, 113.3, 109.4, 101.7, 42.0. Anal. Calcd for: $C_{23}H_{16}F_2N_2O_6S$: C, 56.79; H, 3.32; N, 5.76; S, 6.59. Found: C, 56.91; H, 3.25; N, 5.79; S, 6.50%. HRMS (m/z) $[M-H]^-$ calcd 485.0619. Found 485.0621.

3-{4-[2-(3,4,5-trifluoro-phenyl)-acetylamino]-phenyl}-coumarin-7-O-sulfamate **(6 g).** Yield 90%; mp 216-219 ºC; ν_{max} (KBr)/cm^{-1} 3304, 3188, 1704, 1677, 1603, 1504, 1415, 1354, 1248, 1185, 1115, 991, 841; ^{1}H NMR δ_H (500 MHz, DMSO) 10.35 (1H, s, NH), 8.27 (1H, s, CH), 8.24 (2H, s, NH_2), 7.84 (1H, d, *J* 8.3, Ar-H), 7.74-7.64 (4H, m, Ar-H), 7.36-7.24 (4H, m, Ar-H), 3.72 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.1, 161.0, 159.4, 153.2, 151.8, 149.7 (d, $^1J_{C-F}$ 250), 139.4, 138.9, 135.3 (m), 132.9 (m), 129.7 (m), 128.9 (m), 125.7, 118.7, 117.9, 114.0 (m), 109.4, 101.7, 41.8. Anal. Calcd for: $C_{23}H_{15}F_3N_2O_6S$: C, 54.76; H, 3.00; N, 5.55; S, 6.36. Found: C, 54.71; H, 3.11; N, 5.48; S, 6.40%. HRMS (m/z) $[M-H]^-$ calcd 503.0525. Found 503.0586.

3-[4-(2-pentafluorophenyl-acetylamino)-phenyl]-coumarin-7-O-sulfamate **(6h).** Yield 82%; mp 248-250 ºC; ν_{max} (KBr)/cm^{-1} 3304, 3188, 1705, 1678, 1603, 1505, 1415, 1354, 1250, 1186, 1115, 991, 842; ^{1}H NMR δ_H (500 MHz, DMSO) 10.55 (1H, s, NH), 8.28 (1H, s, CH), 8.24 (2H, s, NH_2), 7.85 (1H, d, *J* 8.8, Ar-H), 7.73 (2H, d, *J* 8.8, Ar-H), 7.66 (2H, d, *J* 8.8, Ar-H), 7.35 (1H, d, *J* 2.0, Ar-H), 7.29 (1H, dd, *J* 8.3, 2.4, Ar-H), 3.93 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 165.8, 161.0, 159.4, 153.2, 151.9, 145.0 (d, $^1J_{C-F}$ 245), 139.0, 138.3 (d, $^1J_{C-F}$ 245), 132.2 (d, $^1J_{C-F}$ 245), 129.4 (m), 129.0 (m), 125.7, 118.7, 117.9, 113.3, 110.4 (m), 109.4, 101.6, 29.9. Anal. Calcd for: $C_{23}H_{13}F_5N_2O_6S$: C, 51.12; H, 2.42; N, 5.18; S, 5.93. Found: C, 51.23; H, 2.45; N, 5.21; S, 5.88%. HRMS (m/z) $[M-H]^-$ calcd 539.0336. Found 539.0396.

3-{4-[2-(4-fluoro-3-trifluoromethyl-phenyl)-acetylamino]-phenyl}-coumarin-7-O-sulfamate **(6i).** Yield 83%; mp 187-190 ºC; ν_{max} (KBr)/cm^{-1} 3299, 3188, 1711, 1667, 1611, 1508, 1413, 1356, 1244, 1187, 1115, 991, 842; ^{1}H NMR δ_H (500 MHz, DMSO) 10.39 (1H, s, NH), 8.26 (1H, s, CH), 8.24 (2H, s, NH_2), 7.84 (1H, d, *J* 8.8, Ar-H), 7.78-7.62 (6H, m, Ar-H), 7.52-7.44 (1H, m, Ar-H), 7.34 (1H, s, Ar-H), 7.28 (1H, d, *J* 9.3, Ar-H), 3.80 (2H, s, CH_2); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 169.4, 160.1, 156.2 (d, $^1J_{C-F}$ 245), 153.9, 152.6, 140.9, 140.2, 139.7, 136.9 (m), 133.6 (m), 130.4 (m), 129.7 (m), 128.7 (m), 126.5, 123.5 (q, $^1J_{C-F}$ 274), 119.4, 118.0, 117.6 (m), 114.1, 110.1, 102.4, 42.3. Anal. Calcd for: $C_{24}H_{16}F_4N_2O_6S$: C, 53.73; H, 3.01; N, 5.22; S, 5.98. Found: C, 53.69; H, 2.90; N, 5.14; S, 6.09%. HRMS (m/z) $[M-H]^-$ calcd 535.0587. Found 535.0593.

3-{4-[2-(2,5-bis-trifluoromethyl-phenyl)-acetylamino]-phenyl}-coumarin-7-O-sulfamate **(6j).** Yield 89%; mp 208-212 ºC; ν_{max} (KBr)/cm^{-1} 3305, 3211, 1710, 1671, 1598, 1517, 1414, 1342,

1249, 1185, 1115, 991, 843; ^{1}H NMR δ_H (500 MHz, DMSO) 10.46 (1H, s, NH), 8.27 (1H, s, CH), 8.24 (2H, s, NH_2), 8.00-7.94 (2H, m, Ar-H), 7.89 (1H, d, *J* 8.3, Ar-H), 7.85 (1H, d, *J* 8.8, Ar-H), 7.71 (2H, d, *J* 8.8, Ar-H), 7.66 (2H, d, *J* 8.8, Ar-H), 7.35 (1H, d, *J* 2.0, Ar-H), 7.29 (1H, d, *J* 8.3, Ar-H), 4.11 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 167.6, 161.0, 159.4, 153.2, 151.8, 140.2, 139.4, 138.9, 135.5, 130.5 (m), 129.6 (m), 129.0 (m), 127.0 (m), 125.7, 124.4 (m), 123.7 (q, $^{1}J_{C\text{-}F}$ 274), 123.5 (q, $^{1}J_{C\text{-}F}$ 274), 118.6 (m), 117.9, 113.3, 109.4, 101.7. Anal. Calcd for: $C_{25}H_{16}F_6N_2O_6S$: C, 51.20; H, 2.75; N, 4.78; S, 5.47. Found: C, 51.09; H, 2.68; N, 4.88; S, 5.52%. HRMS (m/z) $[M\text{-}H]^-$ calcd 585.0555. Found 585.0555.

3-{4-[2-(4-trifluoromethoxy-phenyl)-acetylamino]-phenyl}-coumarin-7-O-sulfamate **(6k).** Yield 88%; mp 252-254 ºC; ν_{max} (KBr)/cm^{-1} 3304, 3217, 1711, 1670, 1611, 1509, 1413, 1354, 1248, 1182, 1111, 991, 842; ^{1}H NMR δ_H (500 MHz, DMSO) 10.38 (1H, s, NH), 8.26 (1H, s, CH), 8.24 (2H, s, NH_2), 7.84 (1H, d, *J* 8.8, Ar-H), 7.74-7.64 (4H, m, Ar-H), 7.45 (2H, d, *J* 8.3, Ar-H), 7.36-7.30 (3H, m, Ar-H), 7.28 (1H, dd, *J* 8.3, 2.0, Ar-H), 3.72 (2H, s, CH_2); ^{13}C NMR (50 MHz, DMSO), δ (ppm): 168.9, 161.0, 154.7, 153.2, 151.8, 147.1, 140.1, 139.5, 138.9, 135.3, 131.0, 129.6 (m), 129.1 (m), 125.8, 121.6, 120.9, 120.1 (q, $^{1}J_{C\text{-}F}$ 255), 118.6, 117.5, 113.3, 112.0, 109.4, 101.6, 42.3. Anal. Calcd for: $C_{24}H_{17}F_3N_2O_7S$: C, 53.93; H, 3.21; N, 5.24; S, 6.00. Found: C, 60.02; H, 3.16; N, 5.15; S, 6.11%. HRMS (m/z) $[M\text{-}H]^-$ calcd 533.0630. Found 533.0681.

*4.6. Preparation of 3-(4-nitrophenyl)-coumarin-7-O-sulfamate (**7**).*

A mixture of formic acid (70.9 mg, 1.54 mmol) and *N*,*N*-dimethyl acetamide (1.4 mg, 0.016 mmol) was added to a stirred solution of chlorosulfonyl isocyanate (212.3 mg, 1.50 mmol) in dry dichloromethane (0.5 mL) at 40 ºC over 3.5 h. A stirred solution of 7-hydroxy-3-(4-nitrophenyl)-coumarin **3** (283.2 mg, 1.00 mmol) in *N*,*N*-dimethyl acetamide (3.4 mL) was added to the mixture. The mixture was stirred at ambient temperature overnight and then poured into water (10 mL). Eventually, a white precipitate formed. The suspension was stirred at ambient temperature for an additional two hours. The resulting precipitate was filtered, washed with water and recrystallized from acetonitrile or acetone to give the desired product **7**.

Yield 91%; mp 200-203 ºC; ν_{max} (KBr)/cm^{-1} 3387, 3217, 1697, 1595, 1507, 1341, 1247, 1191, 1111, 988, 853; ^{1}H NMR δ_H (500 MHz, DMSO) 8.5 (1H, s, CH), 8.33 (2H, d, *J* 9.3, Ar-H), 8.30 (2H, s, NH_2), 8.02 (2H, d, *J* 8.8, Ar-H), 7.90 (1H, d, *J* 8.3, Ar-H), 7.40 (1H, d, *J* 2.0, Ar-H), 7.33 (1H, d, *J* 8.8, 2.0, Ar-H); ^{13}C NMR (125 MHz, DMSO), δ (ppm): 159.7, 154.5, 153.4, 147.9, 142.7, 141.8, 131.1, 130.5, 125.1, 124.1, 119.5, 118.2, 110.2. Anal. Calcd for: $C_{15}H_{10}N_2O_7S$: C, 49.72; H, 2.78; N, 7.73; S, 8.85. Found: C, 49.65; H, 2.69; N, 7.80; S, 8.98%. HRMS (m/z) $[M+H]^+$ calcd 363.0287. Found 363.0277.

4.7. Molecular modeling

Prior to docking procedures, the potential inhibitors were constructed using Portable HyperChem 8.0.7 Release (Hypercube, Inc., Gainesville, FL, USA). Each ligand was optimized using a MM + force field and the Polak–Ribiere conjugate gradient algorithm (terminating at a gradient of 0.05 kcal mol^{-1} Å^{-1}). For docking the X-ray structure of human STS (obtained from Protein Data Bank – accession code 1P49) was prepared for docking using the standard procedure. First, the water molecules from crystallization were removed from the structure and the catalytic amino acid FGly75 were converted to the gem-diol form using the Protein Preparation Wizard module, delivered with Maestro (Schrödinger, LLC,

New York, NY, USA). Next, hydrogen atoms were built onto the structure and prepared model of enzyme will be optimized using the OPLS-AA force field. Docking of the optimized ligands to the prepared structure of human STS was carried out with Autodock Vina 1.1.2 software (The Molecular Graphic Laboratory, The Scripps Research Institute, La Jolla, CA, USA) [25]. For all of the docking studies, a grid box size of 30 Å x 30 Å x 30 Å, centered on the Cβ atom of amino acid 75, was used. The best poses for a particular ligand were inspected visually. Illustrations of the 3D model were generated using VMD 1.9 (University of Illinois at Urbana – Champaign, Urbana, IL, USA).

4.8. Biological assays

4.8.1. Enzyme purification

STS was extracted from human placenta and purified to homogeneity following a multi-step chromatography protocol as previously described [26].

4.8.2. In vitro activity assay

The reaction mixture, at a final volume of 100 μL, contained 20 mM Tris–HCl pH 7.4, 3 mM *p*-nitrophenyl sulfate (NPS), varied concentrations of an inhibitor (0.01–100 μM) and 5 U of purified enzyme (1 U is the amount of enzyme that hydrolyzes 100 μM of NPS in 1 h at 37 ˚C). The reaction was performed at 37 ˚C for 15 min and halted by the addition of 100 mL of 1 M NaOH. The absorbance of the released *p*-nitrophenol was measured at 405 nm using a Biotek ELx800 Microplate Reader (BioTek Instruments, Inc. Winooski, VT, USA). IC_{50} values were calculated using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). All measurements were performed in triplicate.

4.8.3. Cell culture and viability assay

MCF7 (ATCC® HTB-22™), T47D (ATCC ® HTB-133^{TM}), MDA-MB-231 (ATCC® HTB-26™) and SkBr3 (ATCC® HTB-30™) cells lines have been kindly provided by prof. Wesierska-Gadek (Medical University of Vienna, Vienna, Austria) in 2016. Cells were cultured in phenol red-free DMEM high glucose media supplemented with 10% FBS and antibiotics: penicillin (62.6 μg/mL) and streptomycin (40 μg/mL). All cells were maintained at 37 ˚C in a humidified atmosphere of 10% CO_2 and 90% air and routinely screened for *Mycoplasma* contamination. To determine cytotoxicity, exponentially grown cells were exposed to the indicated concentrations of the studied compounds for 120 hours, and viability was determined using the MTT assay. Dose–response curves were plotted using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) and represent data from two to three independent experiments performed in quadruplicate.

Acknowledgements

We gratefully acknowledge the National Science Centre (Poland) for financial support (grant no. 2015/19/N/NZ7/00938).

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Highlights:

1. A series of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate derivatives *N*-acylated with fluorinated analogs of benzoic or phenylacetic acid have been synthesized.
2. Computational docking methods were used to determine the binding modes of the synthesized inhibitors.
3. Compounds **6c** and **6j** demonstrated the highest inhibitory effect in enzymatic STS assays, both with IC_{50} values of 0.18 μM.
4. Compound **6j** exhibited the highest potency against the MCF-7 and T47D cell lines (15.9 μM and 8.7 μM, respectively).
5. Analogues **1** and **2** proved to selectively inhibit the growth of ER- and PR-positive cell lines.