Journal of Medicinal Chemistry

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Brief Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.7b00062 • Publication Date (Web): 13 Apr 2017

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

First Dual Inhibitors of Steroid Sulfatase (STS) and 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1): Designed Multiple Ligands as Novel Potential Therapeutics for Estrogen-Dependent Diseases

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KEYWORDS: steroidogenic enzyme inhibition; steroid sulfatase; 17β-hydroxysteroid dehydrogenase type 1; estrogen-dependent disease; designed multiple ligand.

ABSTRACT: STS and 17β-HSD1 are attractive targets for the treatment of estrogen-dependent diseases like endometriosis and breast cancer. The simultaneous inhibition of both enzymes appears more promising than blockage of either protein alone. We describe a designed multiple target approach resulting in highly potent dual inhibitors. The most interesting compound **9** showed nanomolar IC50s for both proteins, membrane permeability and no interference with estrogen receptors. It efficiently reversed E1S- and E1-induced T47D cell proliferation.

INTRODUCTION

Estrogens exert proliferative and antiapoptotic effects and are involved in the etiology of estrogen-dependent diseases (EDD) such as endometriosis and a high percentage of breast cancers. Therapeutic interventions comprise endocrine treatment with GnRH analogs, selective estrogen receptor modulators (SERMs) or aromatase inhibitors. These options, however, do not prevent relapses and often lead to severe side effects. Thus, there is considerable unmet medical need for novel treatments, and the exploration of novel biological targets is required.

Intriguingly, the progression of EDD is in many cases strongly coupled to the local estrogen biosynthesis, i.e. the formation of active estrogen within the diseased tissue itself. Normally, the activities of enzymes involved in local estrogen activation (steroid sulfatase (STS), aromatase, 17β-HSD1) and de-activation (17β-HSD2, sulfotransferase) are well balanced. In case of EDD, a mismatch between activation and de-activation results in elevated local estrogen levels, leading to increased cell proliferation and reduced apoptosis. This mismatch is caused by aberrant expression of the involved enzymes *in situ*.[1,2]

Therefore, the selective inhibition of local estrogen biosynthesis is a promising therapeutic approach, with the prospect of fewer side effects compared to existing therapies. In this context, STS and 17β-HSD1 play major roles as they catalyze the final steps in estrogen biosynthesis within the target cell (intracrinology): STS converts the inactive estrone-3-sulfate (E1S), the main transport and storage form of estrogens, to the weakly estrogenic estrone (E1).

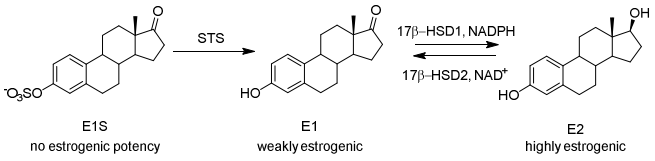


Figure 1. The sulfatase pathway of local estrogen biosynthesis

The latter is reduced to 17β-estradiol (E2), the most potent estrogen in humans, predominantly by the action of 17β-HSD1 (Figure 1).[3] This route of estrogen biosynthesis has been termed "sulfatase pathway".[4] It also includes the STS catalyzed transformation of androstenediol sulfate to the estrogenic androstenediol, whose proliferative effect on estrogen-sensitive cells is known.[5] 17β-HSD2 catalyzes the reverse reaction, i.e. the inactivation of E2 by oxidation to E1, and is the physiological adversary of the type 1 enzyme. Both STS and 17β-HSD1 are overexpressed in endometriotic lesions,[6,7] and there is strong evidence that elevated local E2 levels are mainly due to estrogen activation *via* the sulfatase pathway whereas local E2 formation from androgen precursors ("aromatase pathway") is of considerably less relevance.[8] The sulfatase pathway also plays a crucial role in estrogen-dependent breast cancer, and STS expression is an important prognostic factor in this disease.[9,10] Strikingly, the tumor tissue of breast cancer patients who were treated with aromatase inhibitors showed increased expression of both STS and 17β-HSD1.[11] Thus, STS and 17β-HSD1 are key enzymes for local estrogen activation in EDD. In conclusion, their inhibition is a promising approach for therapeutic intervention. The validity of this concept is supported by the observation that a 17β-HSD1 inhibitor led to a decrease of E2-levels in

endometriotic specimens.[12] In transgenic mice, 17β-HSD1 inhibitors reversed estrogen-induced endometrial hyperplasia.[13] 17β-HSD1 inhibitors were shown to reduce the E1-stimulated tumor cell growth *in vitro* and *in vivo*, suggesting the suitability of this target for the treatment of breast cancer.[14,15] In a mouse model, STS inhibition significantly reduced the growth of endometriotic lesions, while plasma E2 levels remained unchanged.[16]

Selective inhibition of either enzyme, however, bears intrinsic drawbacks: Selective 17β-HSD1 inhibition does not prevent the formation of the estrogenic agents E1 and androstenediol. Selective STS inhibition, on the other hand, does not block E2 formation from E1, which is produced from testosterone via the aromatase pathway. The latter should not be very distinct but could be of relevance for the progression of EDD.

Consequently, the idea of simultaneous inhibition of both STS and 17β-HSD1 arises as a novel and attractive treatment approach. This aim could be achieved by administration of two single inhibitors, each selective for one of the two targets (multi component therapy). Different patient-specific rates of biotransformation, however, may result in complex PK/PD-correlations, leading to difficult predictability of pharmacological effects. Another drawback is the risk of drug-drug-interactions.

An intriguing concept is the inhibition of both targets with a single drug whose structure is rationally derived for this dual mode of action (designed multiple ligand, DML).[17] Preferably, 17β-HSD2 should not be inhibited.

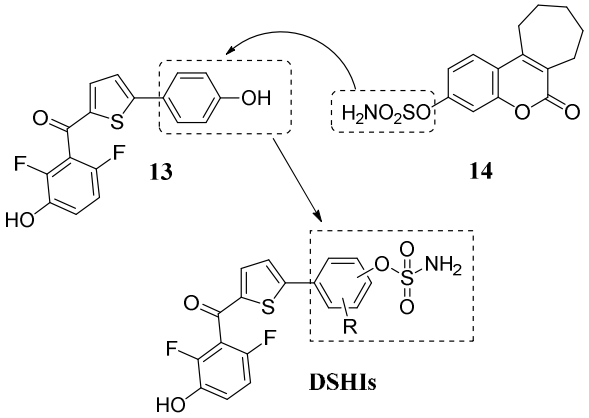


Figure 2. Structure of compounds **13, 14** and general structure of dual STS/17β-HSD1 inhibitors (DSHIs). R: e.g. Cl, F, CH_3.

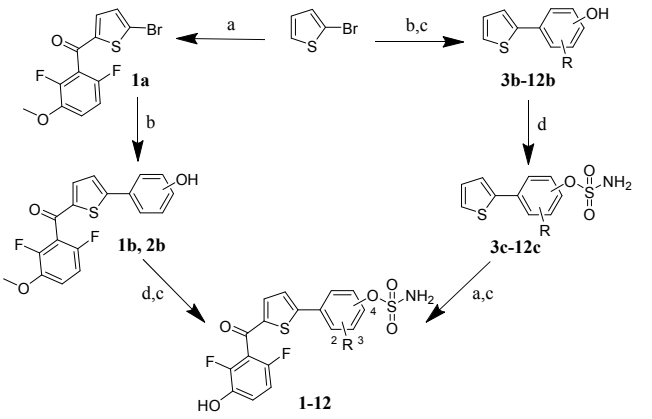
For 17β-HSD1 and STS, a number of steroidal and non-steroidal inhibitors have been described.[18–22] Examples are compounds **13** (17β-HSD1 inhibitor,)[23] and **14** (STX-64) which was the first STS inhibitor to enter clinical trials (figure 2).[24] Interestingly, DML approaches have successfully been applied for STS inhibitors, leading to the discovery of compounds combining STS inhibition with estrogen receptor modulation or inhibition of aromatase (dual aromatase sulfatase inhibitors, DASIs).[20] First described by Woo et al.,[25] the DASI concept has been thoroughly investigated and led to the discovery of many dual inhibitors from different compound classes, described in a series of publications and summarized in a recent review article.[20] Selected DASIs showed favourable properties *in vitro* and *in vivo*.[20,26] However, significant reduction of plasma E2 levels was reported indicating a not exclusively local mode of action.[25]

In this report we describe for the first time the rational design, synthesis and *in vitro* profilation of non-steroidal dual STS/17β-HSD1 inhibitors (DSHIs) as potential drugs for the treatment of EDD, with the prospect of local action.

RESULTS AND DISCUSSION

While STS inhibitors differ considerably regarding molecular structure, most of them bear an unsubstituted aryl sulfamate group (exemplified by cmpd **14**, figure 2) as a common feature which serves as the main pharmacophore for target inhibition. This structural motif was adopted for the design of dual inhibitors and transferred to an appropriate position of a highly potent inhibitor of 17β-HSD1.

Scheme 1. Synthesis of compounds 1-12



Cpd	R	Position $-OSO_2NH_2$	Cpd	R	Position $-OSO_2NH_2$
1	H	4	**7**	2-Me	4
2	H	2	**8**	3-F	4
3	H	3	**9**	3-Cl	4
4	2-F	3	**10**	3-Me	4
5	2-F	4	**11**	2,3-diF	4
6	2-Cl	4	**12**	2-F ,3-Cl	4

Reagents and conditions: **a)** 2,6-difluoro-3-methoxybenzoyl chloride, anhydrous $AlCl_3$, anhydrous CH_2Cl_2, 0 °C, 0.5h and then room temperature, 3h; **b)** corresponding phenyl boronic acid, Cs_2CO_3, $Pd(PPh_3)_4$, DME/water (1:1), 110 °C, 4h; **c)** BBr_3, CH_2Cl_2, -78°C to room temperature, overnight; **d)** DMA, sulfamoyl chloride, 0 °C, and then room temperature, overnight.

We reported on the discovery of bicyclic substituted hydroxyphenylmethanones (BSHs) such as compound **13** (figure 2) as highly active 17β-HSD1 inhibitors. [23] Their scaffold consists of a phenyl- and a benzoyl-moiety which

are linked by a thiophene ring. Extensive SAR revealed that their 17β-HSD1 inhibitory activity is maintained even if bulky substituents are attached to the phenyl moiety (Figure 2, compound **13**, dotted box). This characteristic triggered the implementation of the envisaged design strategy by attaching a sulfamate group essential for STS inhibition to the phenyl group of the BSHs. A general structure of the potential DSHIs based on the above mentioned strategy is shown in Figure 2. The potential dual inhibitors **1-12** were synthesized using standard methods according to scheme 1.

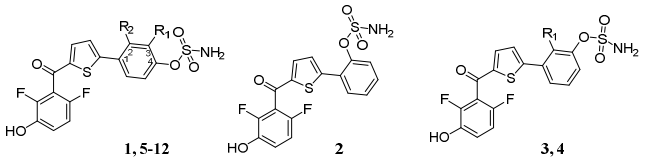
Inhibition of human STS, 17β-HSD1 and 17β-HSD2 in cell-free assays

STS inhibition was determined by incubation of human placental STS with E1S and inhibitor. E1 formation was quantified by ELISA (see Supporting Information). Inhibition of 17β-HSD1 and 17β-HSD2 was evaluated using the respective radiolabeled steroid (E1 or E2) and human placental 17β-HSD1 (cytosolic fraction) or 17β-HSD2 (microsomal fraction). The radiolabeled estrogens were separated and quantified using HPLC with scintillation detection (see Supporting Information). Inhibitory activities are expressed as IC_{50} values (Table 1). **13** and **14** were used as reference compounds.

Compounds **1-3** were highly active towards 17β-HSD1, but did not show inhibition of STS. Introduction of a fluorine atom to compound **3** led to the dual inhibitor **4**, whose inhibition of STS, however, was clearly less pronounced than that of 17β-HSD1. Shifting the sulfamate group from position 3 to position 4 resulted in the highly active compound **5** which equipotently inhibited STS and 17β-HSD1. The STS inhibitory potency of compound **5** matched that of the reference compound **14**, one of the most potent STS inhibitors. The fluorine atom of **5** could be replaced with chlorine, leading to compound **6** with practically identical inhibitory properties, whereas a methyl group slightly decreased potency (compound **7**). Compounds **5** and **6** only showed marginal selectivity over 17β-HSD2. Relocation of the substituents F, Cl and methyl from position 2 to position 3 (compounds **8-10**) on the one hand led to a decrease of STS inhibition. On the other hand, in case of the halogenated inhibitors **8** and **9** this modification further increased activity towards 17β-HSD1 and selectivity over 17β-HSD2. This finding prompted us to synthesize compounds **11** and **12**, bearing halogen atoms in both position 2 and 3, thus possibly combining strong STS inhibition (halogen in position 3) with strong 17β-HSD1 inhibition and selectivity over 17β-HSD2 (halogen in position 2).

In fact, a strong 17β-HSD1 inhibition was achieved, comparable to that of compounds **8** and **9**. In addition, **11** and **12** showed selectivity over 17β-HSD2. There was, however, no improvement concerning STS inhibition. Investigation of compound stability in buffer (20 mM Tris-HCl, pH = 7.2, 37 °C) revealed fast cleavage of the sulfamate moiety in case of **11** and **12**, under formation of the phenolic OH-group (28% and 22% of parent compound remaining after 30 min, resp.) whereas the other compounds proved to be stable under these conditions. The different stabilities can be correlated to the pKa values of the formed phenolic OH groups which are significantly lower in case of **11** and **12** compared to the other compounds (see Supporting Information, Table S2), making their phenolates better leaving groups in substitution reactions. The loss of the sulfamate "warhead" is in agreement with the comparably low STS inhibition by **11** and **12**. The fact that both compounds showed strong inhibition of 17β-HSD1 is not in conflict with their instability because the sulfamate group is not relevant for 17β-HSD1 inhibition.

Table 1: Inhibitory activities of compounds 1-14 towards *h*STS, *h*17β-HSD1 and 2 in cell-free assays



Cpd	R_1	R_2	IC_{50} [nM][a]			SF[e]
			*h*STS[b]	*h*17β-HSD1[c]	*h*17β-HSD2[d]	
1	H	H	ni	3.4	28.0	8.2
2	-	-	ni	22.5	4.1	0.2
3	H	-	ni	2.2	31.3	14.2
4	F	-	123.4	7.1	17.4	2.5
5	H	F	19.5	12.2	22.2	1.8
6	H	Cl	19.4	10.2	19.1	1.9
7	H	Me	35.0	43.5	40.0	0.9
8	F	H	81.8	2.5	13.7	5.5
9	Cl	H	143.1	1.1	36.1	33.0
10	Me	H	220.8	32.4	31.2	1.0
11	F	F	105.5	2.7	23.2	8.6
12	Cl	F	244.5	1.4	18.4	13.2
13	-	-	ni	3.1	71.3	23.1
14	-	-	15.1	ni	ni	NA

[a] Mean value of at least two independent experiments each conducted in duplicates , standard deviation less than 15%; [b] Human placenta, microsomal fraction, substrate E1S [300 nM]; [c] Human placenta, cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [0.5 mM]; [d] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1.5 mM]; [e] SF (selectivity factor): IC_{50}(17β-HSD2) / IC_{50}(17β-HSD1); ni: no inhibition (< 10% inhibition at 1 μM); NA: not applicable.

Intracellular inhibition of human STS and 17β-HSD1; Irreversible inhibition of human STS

The estrogen-dependent human breast cancer cell line T47D expresses both STS and 17β-HSD1.[27] Intact cells were incubated with compounds **5**, **6**, **8** and **9**, respectively, and the corresponding radiolabeled substrate, E1S or E1. After incubation, the steroids were separated and quantified using HPLC with radio-detection. Table 2 shows the IC_{50} values of the cellular inhibition assays.

All compounds displayed strong inhibition of the target enzymes with IC_{50} values in the nanomolar range, indicating good cell penetration. The fact that the compounds were able to strongly inhibit STS in spite of the long incubation time of 24h suggested an irreversible mode of STS inhibition, as described for other sulfamate-containing STS inhibitors, e.g. for **14**.[20]

For further support of this irreversible mode of action, T47D cells were pretreated with compounds **5**, **6**, **8**, **9** and the reference **14**, respectively. After removal of the compounds by extensive washing, STS activity was evaluated by incubation with E1S. In all cases, conversion to E1 was strongly inhibited with IC_{50} values very similar to those obtained in the "regular" assay for cellular STS inhibition (Table 2). In contrast, 17β-HSD1 activity was fully restored after incubation with **9** (Table S3), suggesting that the persisting STS inhibition is a result of irreversible inhibition rather than of compound retention.

Estrogen stimulation of T47D cell proliferation

The proliferation of T47D cells in response to estrogen treatment was evaluated by adding E1S, E1 or E2 to the culture medium at concentrations ranging from 0.1 nM to 500 nM and evaluation of cell viability after 7 days of incubation (Figure 3). Stimulation of proliferation was similar for E1S, E1 and E2.

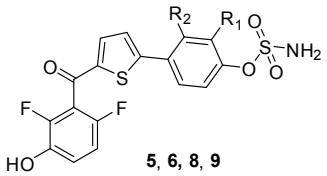
It was initially observed at an estrogen concentration of 10 nM and reached a maximum at 250 nM. In the following experiments, 100 nM of estrogen (either 50 nM E1S and 50 nM E1 –reflecting that both may be present *in vivo* - or 100 nM E2) was used.

Effect of DSHIs on estrogen stimulated cell proliferation

Compound **9** was applied to E1S/E1-stimulated T47D cells at concentrations of 100, 200 and 400 nM, approximately corresponding to 5, 10 and 20 times its IC_{50} value of cellular 17β-HSD1 inhibition. The reference compounds **13** and **14** were applied in concentrations of 50, 100 and 200 nM, approximately corresponding to the same multiples of their IC_{50} values, in case of **14** that of the cell-free assay. The dual inhibitor **9** was able to decrease the proliferative effect of E1S/E1 stimulation dose-dependently, reaching control levels when applied in a concentration of 400 nM (Figure 4). Similar results were obtained for compounds **5**, **6** and **8** (Figure S1). In contrast, the selective references **13** and **14** did not decrease the stimulatory estrogen effect below 150% and 200% of the control, respectively.

The stronger anti-proliferative effects of the dual inhibitors in comparison to the selective ones were assumed to be attributed to the differences in E2 and E1 levels in the

Table 2: Inhibitory activities of compounds 5, 6, 8, 9, 13 and 14 towards *h*STS and *h*17β-HSD1 in cellular assays



5, **6**, **8**, **9**

Cpd	R_1	R_2	IC_{50} [nM][a]		
			*h*STS[b]	*h*17β-HSD1[c]	*h*STS[b] irreversible
5	H	F	2.1	42.1	3.1
6	H	Cl	3.4	60.9	3.9
8	F	H	5.1	20.0	5.7
9	Cl	H	15.6	22.2	16.4
13	-	-	ni	7.9	ni
14	-	-	1.9	ni	2.1

[a] Mean value of at least two independent experiments each conducted in triplicates using intact T47D cells, standard deviation less than 15%; [b] Substrate [^{3}H]-E1S + E1S [5 nM]; [b] Substrate [^{3}H]-E1 + E1 [50 nM]; ni: no inhibition (< 5% inhibition at 1 μM).

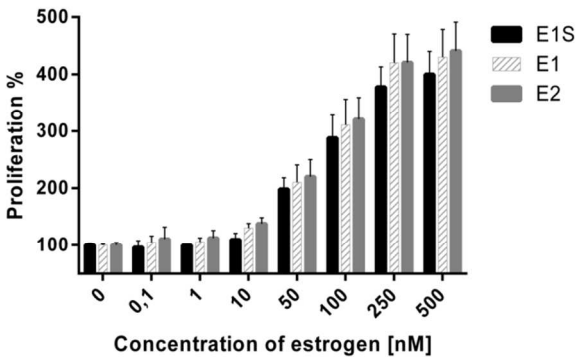


Figure 3. Concentration dependent stimulation of T47D cell proliferation. Black bars: E1S-stimulation, striped: E1-stimulation, gray: E2-stimulation. The control (no estrogen-stimulation) was arbitrarily set to 100%. Cells were incubated with the respective additives for 7 days without passage. Medium was changed every 2–3 days.

medium after feeding the cells with E1S and E1, depending on the presence of the different types of inhibitors. This point was investigated by incubating the cells with radiolabeled E1S and E1 in the presence or absence of inhibitors, and quantification of estrogen levels after 48 h using HPLC with radio-detection (Table 3). In the absence of inhibitor, almost complete conversion of both E1S and E1 to E2 was observed, whereas in the presence of the dual inhibitor **9** no significant conversion of E1S or E1 occurred. In the presence of **14** no conversion of E1S occurred but all

the E1 was transformed into E2. In case of **13**, E1S was almost completely consumed and 88.4% E1 were found, besides a minor amount of E2 (7.9%). These results are in agreement with the residual proliferation induction found in case of the selective inhibitors. In addition, they explain the more pronounced residual cell proliferation after application of **14** compared to **13** (200% vs. 150%), as in the first case the amount of the strongly estrogenic E2 was high (49.2%) whereas in the latter case the less estrogenic E1 was the predominant estrogen.

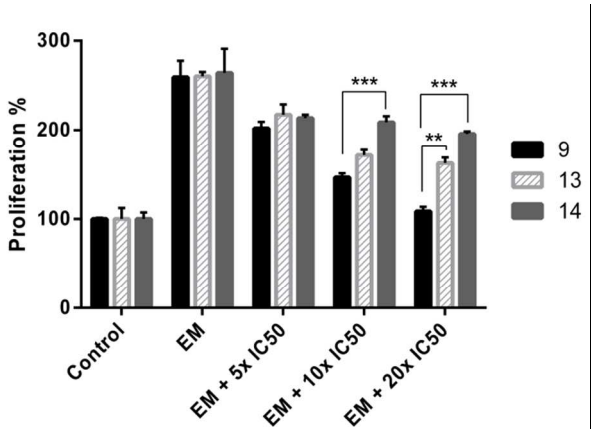


Figure 4. Concentration dependent inhibition of E1S- and E1-stimulated cell growth for compounds **9**, **13** and **14** on T47D cells. Cells were grown in phenol red-free RPMI 1640 medium supplemented with 5% stripped FCS. Control represents vehicle treated cells. EM (estrogenic medium) represents E1S [50 nM] and E1 [50 nM] treated cells. **9** was tested at 100, 200 and 400 nM, resp. **13** and **14** were tested at 50, 100 and 200 nM, resp. Cells were incubated with the respective additives for 7 days without passage. Medium was changed every 2–3 days. Vehicle: ethanol. ** $p < 0.01$. *** $p < 0.001$.

Table 3: Percentages of estrogens upon incubating T47D cells with E1S and E1 (50 nM each) for 48 hours, in the presence of vehicle, 9, 13 and 14

Cpd	% [a]		
	E1S	E1	E2
Vehicle [b]	3.9	0.9	95.2
9 [c]	50	48.9	1.1
13 [d]	3.5	88.4	7.9
14 [e]	50	0.8	49.2

[a] Mean value of at least two independent experiments each conducted in triplicates, standard deviation less than 10%; [b] vehicle: ethanol; [c] cmpd **9** [400 nM]; [d] cmpd **13** [200 nM]; [e] cmpd **14** [200 nM].

As depicted in Figure 5, compound **9** has no effect on non-stimulated cells at a concentration of 400 nM, which was the highest concentration in which **9** was applied in the anti-proliferation assay. Thus, the dual inhibitor **9** did not exert estrogenic or cytotoxic effects at this concentration. The lack of unspecific cytotoxicity was also confirmed using estrogen-independent cells: Compound **9** did not affect the viability of HEK293 cells, even in the highest applied concentration of 1 µM (see supporting information). Moreover, no influence of **9** on the proliferation of E2-stimulated cells could be detected, indicating that the compound did not deploy its anti-proliferative effect by estrogen receptor antagonism. These data clearly demonstrate that the effect of compound **9** on E1S/E1-stimulated cells is caused by inhibition of STS and 17β-HSD1, which results in the blockage of E2 formation.

CONCLUSIONS

A designed multiple target approach was successfully applied, leading to the discovery of potent dual inhibitors of STS and 17β-HSD1. Inhibitor design was facilitated by combining structural elements necessary for strong 17β-HSD1 blockage, identified using in-house SAR information, with a sulfamate function which is the major pharmacophore for STS inhibition. Twelve potential dual inhibitors were synthesized, all of which proved to be highly active against 17β-HSD1. Activity towards STS required an additional substituent at the aromatic moiety bearing the sulfamate group. An electron-withdrawing substituent is preferred; strong electron-withdrawing effects, however, impaired STS inhibition by reducing the chemical stability of the sulfamate function.Compound **9** turned out to be the most interesting dual inhibitor. In cellular assays it showed well-balanced activity against both target proteins, with IC_{50} values of about 20 nM and an irreversible mode of action towards STS. Moreover, it displayed the highest selectivity over 17β-HSD2. At 400 nM it efficiently reversed the E1S and E1 stimulated proliferation of T47D cells, showing neither cytotoxicity nor estrogen receptor interference.

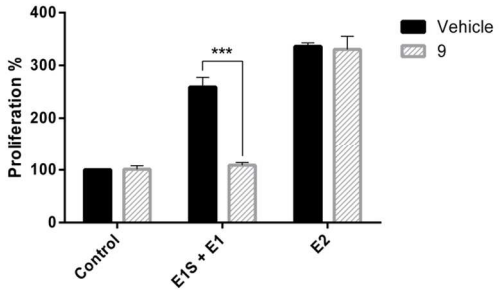


Figure 5. Effect of compound **9** on estrogen-stimulated cell proliferation of T47D. Cells were grown in phenol red-free RPMI 1640 medium supplemented with 5% stripped FCS. Proliferation was stimulated with E1S and E1 at a concentration of 50 nM each or E2 (100 nM). **9** (400 nM) was added in the presence or absence of estrogens. Cells were incubated with the respective additives for 7 days without passage. Medium was changed every 2–3 days. Vehicle: ethanol. *** $p < 0.001$.

In summary, compound **9** is the first rationally derived dual inhibitor of STS and 17β-HSD1. It may serve as a lead for the development of novel therapeutics for EDD.

EXPERIMENTAL SECTION

The purity of all tested compounds was ≥ 95%, as evaluated by LC/MS. Purchased chemicals were reagent grade and used without purification (supporting information).

Compound 9a was prepared according to method B by the reaction of 2-bromothiophene (0.82 g, 5 mmol, 1 equiv) and (3-chloro-4-methoxyphenyl)boronic acid (1.11 g, 6.00 mmol, 1.2 equiv) in the presence of caesium carbonate (6.50 g, 20.00 mmol, 4 equiv) and tetrakis-(triphenylphosphine) palladium (0.29 g, 0.25 mmol, 0.05 equiv) in DME/water 1:1 (50 ml). The product was purified by column chromatography (cyclohexane/dichloromethane 7:1) to give 1.01 g (4.50 mmol/ 90%) of the analytically pure compound. $C_{11}H_9ClOS$; MW 224; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 7.72 (d, *J* = 2.3 Hz, 1H), 7.57 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.50 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.47 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.11 (dd, *J* = 5.1, 3.6 Hz, 1H), 3.88 (s, 3H); MS (ESI): 225.07 $(M+H)^+$.

Compound 9b was prepared according to Method C by the reaction of **9a** (0.90 g, 4.00 mmol, 1 equiv) and boron tribromide (1 M) in dichloromethane (12.00 ml, 12.00 mmol, 3 equiv) in anhydrous dichloromethane (20 ml). The product was purified by column chromatography (cyclohexane/dichloromethane 4:1) to give 0.65 g (3.08 mmol/ 77%) of the analytically pure compound. $C_{10}H_7ClOS$; MW 210; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 10.36 (s, 1H), 7.62 (d, *J* = 2.3 Hz, 1H), 7.46 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.44 – 7.35 (m, 2H), 7.09 (dd, *J* = 5.1, 3.6 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H); MS (ESI): 211.03 $(M+H)^+$.

Compound 9c was prepared according to method D by the reaction of 9b (0.63 g, 3.00 mmol, 1 equiv) and sulfamoyl chloride (1.73 g, 15.00 mmol, 5 equiv) in DMA (20 ml). The product was purified by column chromatography (cyclohexane/ethylacetate 2:1) to give 0.45 g (1.56 mmol/ 52%) of the analytically pure compound. $C_{10}H_8ClNO_3S_2$; MW 289; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 8.29 (s, 2H), 7.88 (d, *J* = 2.2 Hz, 1H), 7.69 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.64 – 7.59 (m, 2H), 7.52 (d, *J* = 8.6 Hz, 1H), 7.16 (dd, *J* = 4.9, 3.9 Hz, 1H); MS (ESI): 290.01 $(M+H)^+$.

Compound 9d was prepared according to method A by the reaction of 2,6-difluoro-3-methoxybenzoyl chloride (0.41 g, 2.00 mmol, 1 equiv) and 9c (0.87 g, 3.00 mmol, 1.5 equiv) in the presence of anhydrous aluminum chloride (0.53 g, 4.00 mmol, 2 equiv) in anhydrous dichloromethane (10 ml). The product was purified by column chromatography (cyclohexane/ethylacetate 1:1) to give 0.59 g (1.30 mmol/ 65%) of the analytically pure compound. $C_{18}H_{12}ClF_2NO_5S_2$; MW 459; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 8.37 (s, 2H), 8.11 (d, *J* = 2.3 Hz, 1H), 7.88 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.79 (d, *J* = 4.1 Hz, 1H), 7.70 (d, *J* = 4.1 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.48 – 7.37 (m, 1H), 7.32 – 7.20 (m, 1H), 3.90 (s, 3H); MS (ESI): 459.99 $(M+H)^+$.

Compound 9 was prepared according to method C by the reaction of **9d** (0.23 g, 0.50 mmol, 1 equiv) and boron tribromide (1 M) in dichloromethane (1.50 ml, 1.50 mmol, 3 equiv) in anhydrous dichloromethane (10 ml). The product was purified by column chromatography (dichloromethane/methanol 98.5:1.5) to give 60.00 mg (0.13 mmol/ 27%) of the analytically pure compound. $C_{17}H_{10}ClF_2NO_5S_2$; MW 445; mp: 172-173; ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 9.00 (s, 1H), 7.97 (d, *J* = 2.3 Hz, 1H), 7.80 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.67 (d, *J* = 4.1 Hz, 1H), 7.64 – 7.62 (m, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.47 (s, 2H), 7.21 – 7.14 (m, 1H), 7.03 – 6.96 (m, 1H); ^{13}C NMR (126 MHz, $(CD_3)_2CO$) δ 180.76, 152.52 (dd, *J* = 240.6, 5.9 Hz), 152.31, 148.41 (dd, *J* = 246.0, 7.6 Hz), 148.06, 144.15, 142.67 (dd, *J* = 12.8, 3.2 Hz), 138.18, 133.45, 129.05, 129.03, 127.25, 127.04, 125.62, 120.44 (dd, *J* = 9.1, 3.9 Hz), 117.89 (dd, *J* = 23.9, 19.6 Hz), 112.45 (dd, *J* = 22.8, 3.9 Hz); MS (ESI): 446.12 $(M+H)^+$.

ASSOCIATED CONTENT

Supporting Information. Chemistry: Chemical methods; General synthetic methods (A, B, C, D, and E); Detailed synthesis procedures and compound characterization; Purity data; Molecular formula strings. Biology: Chemicals; Detailed biological assays; Statistical method. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

We are grateful to the Deutsche Forschungsgemeinschaft for financial support (FR 3002/1-1) of this work.

ACKNOWLEDGMENT

Thanks are due to C. Hoffmann and J. Ludwig for support in synthesis and biological evaluation.
Dedicated to Professor Rolf W. Hartmann on the occasion of his 65th birthday.

ABBREVIATIONS

BSHs, bicyclic substituted hydroxyphenylmethanones; DSHIs, dual STS/17β-HSD1 inhibitors; E2, 17β-estradiol; EDD, estrogen-dependent diseases; E1, estrone; E1S, estrone-3-sulfate; 17β-HSD1 or 2, 17β-hydroxysteroid dehydrogenase type1 or 2; STS, steroid sulfatase.

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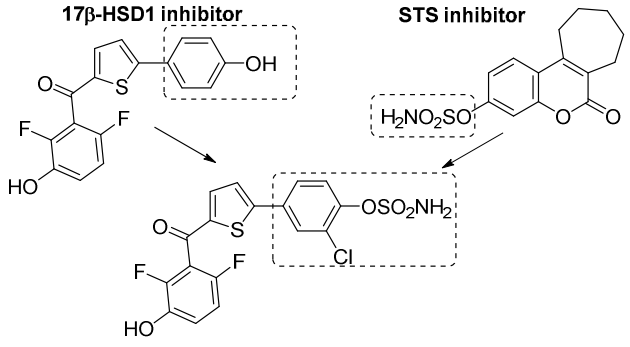
TOC/ Abstract Graphic



IC_{50}(whole cell assays) STS: 15.9 nM; 17β-HSD1: 22.2 nM
strongly reverses estrogen-induced T47D cell proliferation