# 2-(1-Adamantyl)-4-(thio)chromenone-6-carboxylic Acids: Potent Reversible Inhibitors of Human Steroid Sulfatase

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Steroid sulfatase (STS) is an attractive target for the potential therapy of a number of estrogen- and androgen-dependent disorders. Most potent STS inhibitors known so far act as irreversible enzyme blockers and feature an aryl sulfamate moiety; even minor modifications at the sulfamate group result in drastically decreased activity. On the basis of a recently reported subclass of highly potent STS inhibitors, i.e., chromenone sulfamates, we now extended the investigation of structure−activity relationships to hitherto unstudied sulfamate replacements. Thereby, we discovered 2-(1-adamantyl)-4-(thio)chromenone-6-carboxylic acids (**5d** and **5j**) as potent, reversible inhibitors of STS. In a cell-free system using purified human STS, both new inhibitors show similar $K_i$ values (0.50 $\mu$M and 0.53 $\mu$M, respectively). However, the thio analogue **5j** is superior to **5d** ($IC_{50}$ = 0.18 $\mu$M versus 9.4 $\mu$M) in a cellular assay system using CHO cells overexpressing STS. Compound **5j** is an example of a reversible STS inhibitor with potent activity toward the target enzyme in a cellular test system. Moreover, **5d**,**j** are stable and have no estrogenic potential.

## Introduction

Steroid sulfatase (STS), the enzyme catalyzing the hydrolysis of steroid-3-*O*-sulfates, has emerged as an attractive drug target, since it is crucial for the local production of active estrogens and androgens from their systemic circulating sulfated precursors, namely estrone sulfate and dehydroepiandrosterone (DHEA) sulfate, in diseased tissues.[1,2] Three areas where STS inhibitors could be used in therapy have been identified: (i) Breast cancer. There is evidence that the STS pathway is a major source of estrogens in breast tissue,[1−4] suggesting STS inhibitors as potential agents for the treatment of estrogen receptor-positive breast tumors. (ii) Androgen-dependent skin diseases. DHEAS is a precursor for active androgens in the skin; an important role of STS in the pathogenesis of androgenetic alopecia and acne has been proposed.[5,6] (iii) Cognitive dysfunction. In the brain STS may regulate the availability of DHEA as a neurohormone; treatment of rats with inhibitors of STS resulted in enhanced learning and spatial memory.[7−9] So far, development of STS inhibitors is mainly in preclinical phases, and the search for STS inhibitors which are suitable as therapeutics is actively ongoing (see ref 10 for a recent review).

To date, both reversible and irreversible STS inhibitors are known. As an example of steroidal compounds with a reversible mode of action, the 17α-4′-*tert*-butylbenzyl derivative of estradiol (**1**, Figure 1) with high inhibitory activity has been reported.[11] Recently, new classes of reversible inhibitors were discovered, namely nortropinyl-arylsulfonylureas[12] and substituted phenyl piperazines.[13] However, the majority of potent STS inhibitors known to date feature an aryl sulfamate moiety and act in an irreversible manner. Estrone sulfamate (EMATE, Figure 1),[14] the sulfamate analogue of the natural substrate estrone sulfate, has served as the lead for all sulfamate-type inhibitors.[10,15] One issue associated with this type of compounds is their chemical instability in solution (as reported for estrone sulfamate),[16−18] which might complicate or even impede development and clinical use. Hydrolytic lability appears to be an inherent problem for all aryl sulfamates, due to the reactivity of the aryl sulfamate functionality.

All attempts to replace the sulfamate moiety in EMATE by other functionalities reported in the literature[19] have so far resulted in compounds with substantially decreased or no STS inhibitory potency (examples are summarized under general structures **2** and **3** in Figure 1). We realized that in nearly all investigated structures the steroidal skeleton (or a mimic thereof) was connected to the headgroup functionality via a heteroatom (O in structures **2a**−**f**, S and N in structures **3a**−**k**). Therefore, we now explored sulfamate replacements by introducing carbon-linked functional groups (compounds **5a**−**j**; Figure 1). For this study, the 2-adamantyl-4-(thio)chromenone-6-yl scaffold, which we successfully applied in a series of highly potent sulfamate-type STS inhibitors (**4a**,**b**; Figure 1),[20] was used instead of the estrone skeleton. This led to the discovery of 4-(thio)chromenone carboxylic acids (**5d**,**j**) as potent nonsteroidal STS inhibitors with reversible mode of action. In this paper, we describe the discovery, synthesis and the biological activity of this novel class of inhibitors.

## Results and Discussion

**Chemistry.** For the synthesis of the chromenone-based test compounds, we started from the acyclic precursor **(6a**−**c)** and constructed the bicyclic core decorated with the 1-adamantyl residue at position 2 and three different substituents at position 6 (**9a** = Me, **9b** = $CH_2COOMe$, and **5c** = COOMe, Scheme 1). These

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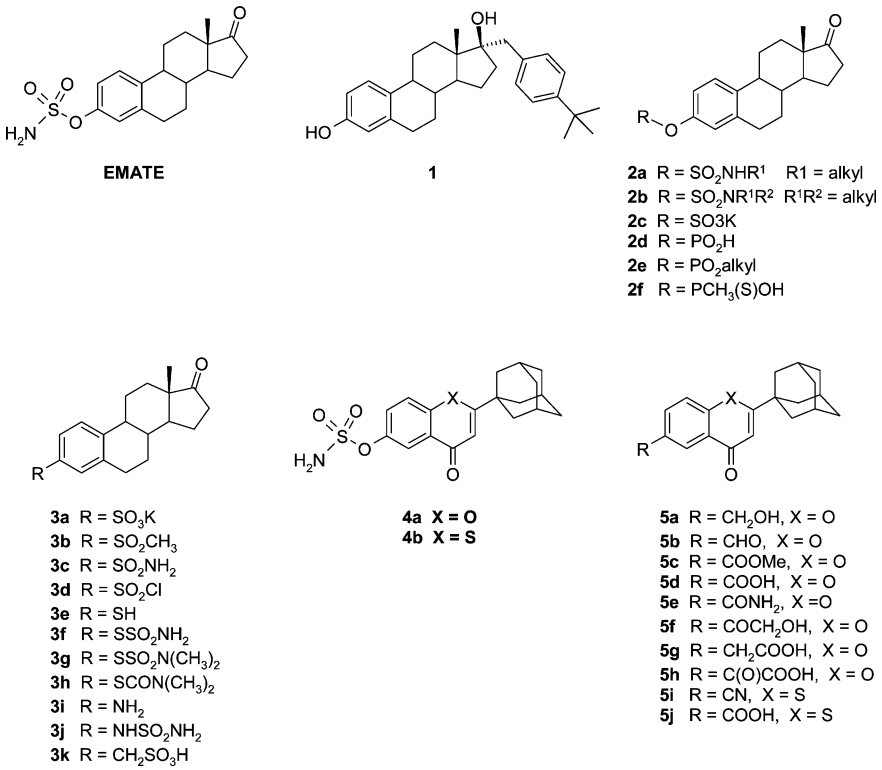
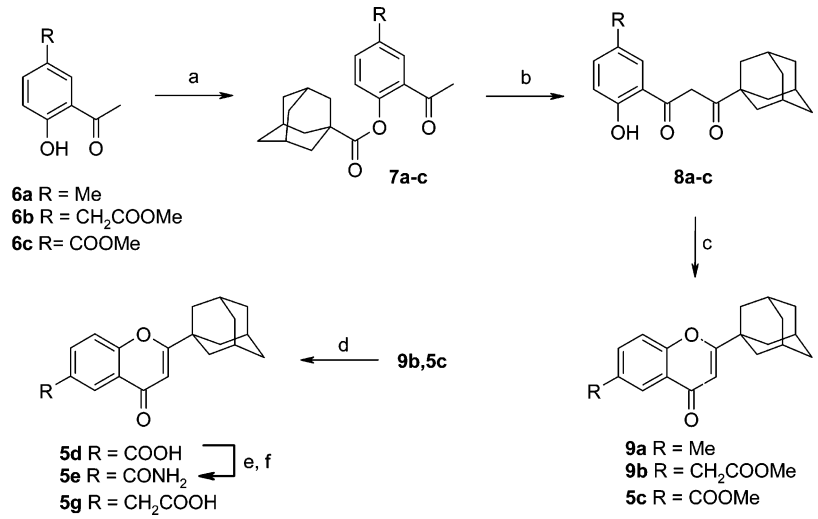



**Figure 1.** Structures of estrone sulfamate (EMATE), 17-α-(*tert*-butylbenzyl)estradiol (**1**), previously described (refs 16a−h) O-linked estrone derivatives **2a**−**f**, S-, N-, C-linked estrone derivatives **3a**−**k**, chromenone-based sulfamates **4a**,**b** (ref 17), and new test compounds **5a**−**j**.

**Scheme 1**[a]

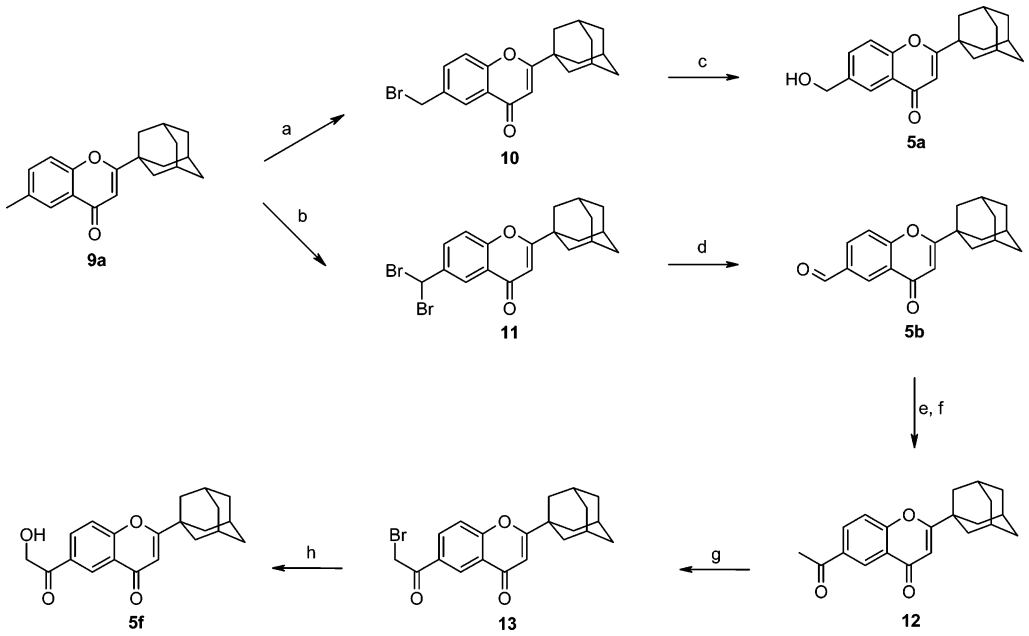



[a] Reagents and conditions: (a) adamantane-1-carbonyl chloride, DMAP, pyridine, rt, 18 h, 83−99%; (b) $K_2CO_3$, 2-propanol, reflux, 75 min; (c) HCOOH, 100 °C, 45 min, 52−84% over two steps; (d) 20% aqueous HCl, dioxane, 4 h, 100 °C, 74−90%; (e) $SOCl_2$, dichloroethane, cat. DMF, reflux, 4 h, quant.; (f) $NH_3$, EtOH, 0 °C, 30 min., 37%.

central intermediates were then transformed into test compounds **5a**,**b**,**d**−**h** by a variety of functional group manipulations (Schemes 1-3). Adamantyl esters **7a**−**c** were prepared by acylation of the appropriately substituted 4-hydroxy-3-acetophenones **6a**−**c** with adamantane-1-carbonyl chloride in the presence of (dimethylamino)pyridine in pyridine. Subsequent base-catalyzed Baker-Venkataraman rearrangement[21] provided the 1,3-diketones **8a**−**c**, which were used without purification in the next synthetic step. Cyclization with formic acid was performed analogously to published procedures[22] and yielded the chromenones **5c** and **9a**,**b**.

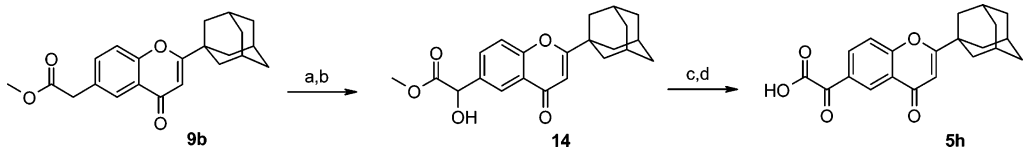
Treatment of 6-methyl chromenone **9a** with *N*-bromosuccinimide (NBS) and a catalytic amount of 2,2′-azobis(2-methylpropionitrile) (AIBN) in benzene afforded the monobromide **10**, which was converted into **5a** using Amberlite IRA 900 (carbonate form) exchange resin (Scheme 2).[23] Reaction of **9a** with an excess of NBS provided dibromide **11** in good yield, which was hydrolyzed with potassium acetate in acetic acid to the aldehyde **5b**. Compound **5b** was also an intermediate in the synthesis of the hydroxyacetyl derivative **5f**. Thus, methyl Grignard addition, followed by Swern oxidation provided ketone **12**, which after α-bromination

**Scheme 2**[a]



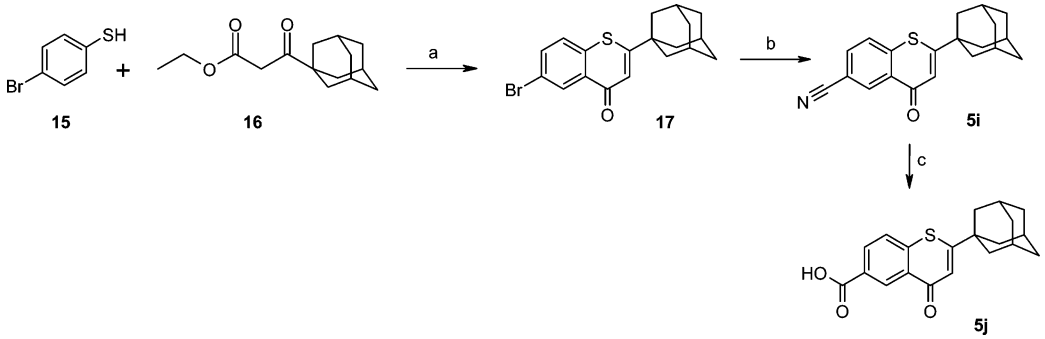

[a] Reagents and conditions: (a) 1.2 equiv of NBS, AIBN, benzene, reflux, 2 h, 72%; (b) 2.5 equiv of NBS, AIBN, benzene, reflux, 2 h, 84%; (c) Amberlite IRA 900, $HCO_3^-$ form, toluene, 2 h, reflux, 67%; (d) $CH_3COOK$, $CH_3COOH$, 120°C, 12 h, 56%; (e) $CH_3MgI$, THF, 0 °C, 1 h, 58%; (f) $(COCl)_2$, DMSO, $CH_2Cl_2$, −68 °C, 1 h, then $Et_3N$, 80%; (g) $Br_2$, $CH_3COOH$, 70 °C, 3 h, 68%; (h) $(CH_3)_3N^+CH_2COO^-$, DMF, $CH_2Cl_2$, EtOH; $Na_2CO_3$, $H_2O$, rt, 24 h, 61%.

**Scheme 3**[a]




[a] Reagents and conditions: (a) NBS, AIBN, benzene, 75%; (b) Amberlite IRA 900 $HCO_3^-$ form, toluene, reflux, 24 h, 59%; (c) DMSO, $(COCl)_2$, $CH_2Cl_2$, −68 °C, 1 h; then TEA, 59%; (d) 20% HCl, dioxane, 80 °C, 1.5 h, 91%.

**Scheme 4**[a]




[a] Reagents and conditions: (a) PPA, 90°C, 2 h, 34%; (b) $Pd(OAc)_2$, $Zn(CN)_2$, $Ca(OH)_2$, $PPh_3$, DMF, 100 °C, 1 h, 36%; (c) 20% HCl, dioxane, 120 °C, 16 h, 50%.

using bromine in acetic acid gave compound **13**. Treatment with betaine and subsequent ester hydrolysis yielded test compound **5f** (Scheme 2). The carboxylic acids **5d**,**g** were synthesized from the corresponding esters **5c** and **9b** by hydrolysis using 20% aqueous HCl in dioxane (Scheme 1). Conversion of the carboxylic acid **5c** into the acid chloride by treatment with thionyl chloride and subsequent reaction with ammonia in ethanol gave amide **5e** (Scheme 1). The oxo-acetic acid group in **5h** (Scheme 3) was introduced by first reacting acetic acid methyl ester **9b** with *N*-bromosuccinimide, followed by hydrolysis with Amberlite IRA 900 carbonate exchange resin to produce intermediate **14**. Compound **5h** was then obtained by Swern oxidation and subsequent acidic ester hydrolysis.

The thiochromenone-nitrile **5i** (Scheme 4) was prepared by condensation of commercially available 4-bromobenzenethiol (**15**) with 3-(adamantan-1-yl)-3-oxopropionic acid ethyl ester (**16**) in polyphosphoric acid to give the cyclic product **17**,[24] followed by palladium-mediated cyanation with zinc cyanide in the presence of calcium hydroxide. Acid-catalyzed hydrolysis of the nitrile **5i** gave the thiochromenone carboxylic acid **5j.**

**STS Inhibition Assays.** As described earlier,[20,25] we used highly purified human STS obtained from a recombinant cell line with 4-methylumbelliferyl sulfate

(4-MUS) as a synthetic substrate for a convenient colorimetric assay to determine $IC_{50}$ or $K_i$ values of inhibitors.

**Cellular STS Assay.** Cellular activities of both reversible and irreversible inhibitors were evaluated in CHO cells stably transfected with human STS (CHO−STS) using the fluorogenic substrate 4-MUS, as described.[26]

**Stability Studies in Aqueous Solution.** To assess potential degradation at physiological pH, test compounds were dissolved in 0.1 M Tris−HCl buffer, pH 7.5/acetonitrile 1:1 (acetonitrile being included to enhance solubility), and solutions (100 $\mu$M) were incubated at 37°C for up to 1 week. Aliquots were analyzed for degradation by HPLC.

**SAR.** Several studies exploring the SAR around the sulfamate moiety in prototype inhibitor EMATE and on surrogates of the natural substrate estrone sulfate have been published.[19] In Figure 1, compounds **2** are examples for estrone derivatives with O-linked headgroups and compounds **3a**−**j** for S- and N-linked variations. The common result of these studies was that any modification, even a minor one, such as alkylation at the nitrogen atom of the sulfamate moiety (**2a**),[27] led to a substantial decrease or complete loss of STS inhibitory activity. The most active compound coming out of these studies was analogue **2f** featuring the methylthiophosphonate moiety as sulfate surrogate; but its activity ($K_i$ = 14.6 $\mu$M) did not approach that of the potent sulfamate-type inhibitors. However, it was shown that **2f** and the corresponding 3-*O*-phosponate of estrone (**2d**) were able to inhibit STS activity in intact MCF-7 breast cancer cells.[19a] Interestingly, all active compounds with a modified headgroup were characterized as reversible inhibitors of STS. The only derivative of EMATE, where the modified headgroup is linked to the estrone skeleton via a carbon atom and STS inhibitory activity has been reported, is 3-desoxyestrone-3-methylenesulfonic acid **3k** (Figure 1) with a high $K_i$ value of 140 $\mu$M.[19d]

Searching for novel, potent, and stable STS inhibitors, we used the potent compounds **4a**,**b** (Figure 1) as starting points, based on our previous finding, that 4-(thio)chromenone 6-*O*-sulfamates with bulky alkyl groups at position 2 are more potent than EMATE. We prepared some analogues of the above-mentioned sulfate surrogates (e.g., sulfonic acid, phosphonic acid, phosphate) using the 2-(1-adamantyl)-4-(thio)chromenone-6-yl scaffold and basically confirmed the reported SAR findings on estrone-based derivatives: the compounds were inactive or only weakly active with $IC_{50}$ values ≥ 40 $\mu$M. The present report summarizes the results from extended SAR studies focusing on chromenone analogues with C-linked functionalities as headgroup (**5a**−**j**, Figure 1). Their ability to inhibit human STS was assessed in the enzyme assay using purified protein and in the cellular assay using CHO−STS cells. The results are summarized in Table 1 in comparison to prototype inhibitor EMATE and chromenone sulfamates **4a**,**b**.

We started our investigation with the primary alcohol **5a** and increased stepwise the oxidation level of the headgroup and the spacer length between the oxy function and the chromenone scaffold. Alcohol **5a** showed only very weak inhibitory activity (Table 1), and aldehyde **5b** was inactive at the highest test concentration.

**Table 1.** Effect of Inhibitors against Purified Human STS and against STS Overexpressed in CHO Cells

| compound | X | R | enzyme assay $K_i$ [$\mu$M] | cellular assay $IC_{50}$ [$\mu$M] |
|---|---|---|---|---|
| EMATE | − | − | 0.67[a] | 0.02[b] |
| **4a** | O | $OSO_2NH_2$ | 0.19 [a] | 0.026[c] |
| **4b** | S | $OSO_2NH_2$ | 0.21 [a] | 0.024[c] |
| **5a** | O | $CH_2OH$ | 32 | ∼30 |
| **5b** | O | CHO | >20 | >30 |
| **5c** | O | COOMe | >50 | >30 |
| **5d** | O | COOH | 0.50 | 9.4 |
| **5e** | O | $CONH_2$ | >50 | >30 |
| **5f** | O | $COCH_2OH$ | 3.2 | >30 |
| **5g** | O | $CH_2COOH$ | >50 | >30 |
| **5h** | O | C(O)COOH | 2.2 | 12.3 |
| **5i** | S | CN | >50 | >30 |
| **5j** | S | COOH | 0.53 | 0.18 |

[a] These values were determined by Purohit et al.[31] (for EMATE) and Nussbaumer et al.[20] (for **4a,b**) using the method of Kitz and Wilson for irreversible inhibitors. [b] This value was identified in the cellular assay setups for both irreversible and reversible inhibitors as described in Wolff et al.[26] [c] These $IC_{50}$ values were determined in the CHO−STS assay for irreversible inhibitors, using fixed cells.[26]

Also the carboxylic ester derivative **5c**, which was an intermediate in the synthesis of the corresponding acid **5d**, was devoid of inhibitory activity. In contrast, carboxylic acid **5d** showed marked inhibition in the enzyme assay and was also active in the cellular system, although the potency was not as pronounced as against the purified enzyme. This difference might be due to poor cell penetration properties of **5d**, which is quite frequently found for carboxylic acids. Amide derivative **5e** again did not exhibit inhibitory activity.

In the next series of test compounds the chain length was increased. Oxo alcohol **5f** was moderately active with a $K_i$ value of 3.2 $\mu$M in the enzyme assay. Surprisingly, carboxylic acid **5g**, the homologue of **5d**, was inactive, whereas the oxo derivative **5h** again showed inhibitory activity against the purified enzyme in the low $\mu$M range. Although analogue **5h** was inferior to **5d** in the enzyme assay roughly by a factor of 4, **5h** was almost as potent as **5d** in the cellular assay.

Because of the interesting potency obtained with **5d**, the corresponding thiochromenone analogue **5j** was also investigated. In the cell-free assay, **5j** was as potent as **5d**, but in the cellular test system compound **5j** was found to be substantially superior, displaying an $IC_{50}$ value of 180 nM (compared to 9.4 $\mu$M for **5d**). The determining factor for this remarkable difference has not been elucidated so far, but the increase in lipophilicity caused by the exchange of the oxygen to sulfur might result in enhanced cell penetration of the thio analogue.

The carboxylic acids **5d** and **5j** were analyzed in more detail. The compounds were found to be reversible inhibitors, as enzyme activity could be fully recovered upon incubation of the enzyme−inhibitor complexes with dextran-activated charcoal (data not shown). Kinetic measurements with STS and the substrate 4-MUS in the presence of the carboxylic derivative **5d** are shown in Figure 2A, where data are plotted in an S/V versus S diagram (Hanes plot). The linearity of the secondary plot of $K_{app}/V_{app}$ versus inhibitor concentration (I) (Figure 2B) indicates purely competitive inhibition, the $K_i$ value being 0.50 $\mu$M. In analogy, $K_i$= 0.53 $\mu$M was measured for **5j**. It is conceivable that the chromenone moiety of these inhibitors binds in a fashion

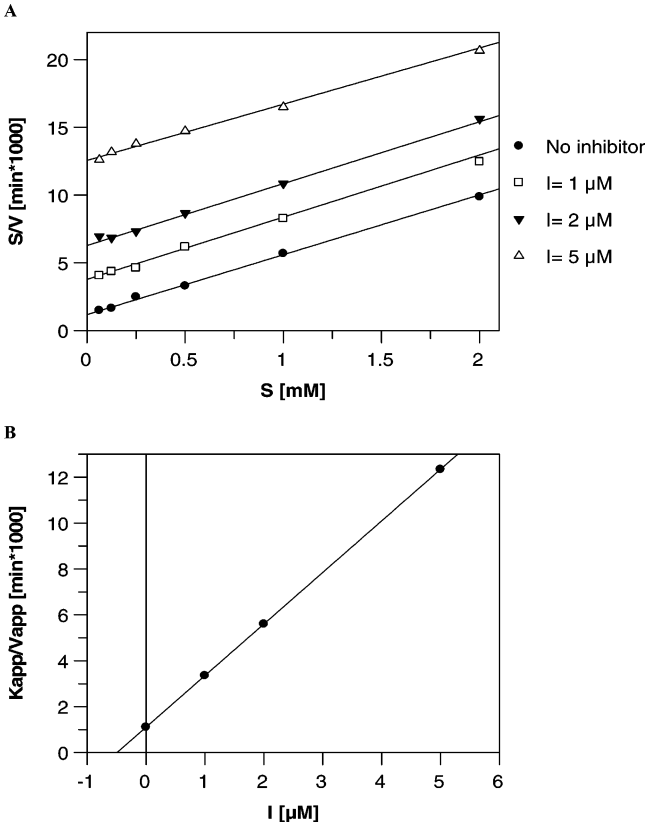



**Figure 2.** Mode of action and $K_i$ determination of inhibitor **5d**. A: rate of substrate turnover ($V$) at various substrate concentrations ($S$) in the presence of inhibitor **5d**, plotted in a Hanes diagram; B: secondary plot of $K_{app}/V_{app}$ ratios against inhibitor concentration (I).

similar to the active site of the enzyme as the A and B ring of the steroid sulfates, while the carboxylic acid moiety acts as a mimic of the sulfate group, both being negatively charged at physiologic pH. Although not formally proven, we assume that the weaker compounds **5a**, **5f**, and **5h** also act as reversible, competitive inhibitors.

When comparing the $K_i$ values in Table 1, it has to be taken into account that EMATE, **4a**, and **4b** are irreversible inhibitors; thus, their efficacy is not only determined by the binding to the active site (as indicated by the $K_i$ value), but also by the rate of covalent modification of the enzyme (as indicated by the $k_{inact}$ value).[20] Therefore, the $IC_{50}$ values for inhibition of STS in the cellular assays by the sulfamates are lower by at least 2 orders of magnitude than for the carboxylic acid derivatives **5d** and **5j**.

**Stability of Chromenone Carboxylic Acids and Corresponding Sulfamates.** One issue associated with sulfamate-type STS inhibitors is their limited stability in solution.[16−18] Therefore, the stability of carboxylic acid analogues **5d**,**j** and, as references, of their corresponding sulfamates **4a**,**b** in buffered aqueous solution at pH 7.5 was compared. Chromenone carboxylic acid derivatives **5d**,**j** did not show any measurable degradation during 1 week at 37 °C. In contrast, sulfamates **4a** and **4b** both decomposed to the corresponding phenols with half-lives of 36 and 20 h, respectively.

**Further Biological Evaluation of Chromenone Carboxylic Acids.** Since breast cancer is an important indication for STS inhibitors, we asked whether a chromenone carboxylic acid would also block the enzyme in MCF-7 cells, a commonly used breast cancer cell line. For compound **5d** an $IC_{50}$ value of 137 ± 15 nM was determined. Thus, STS inhibitors of the carboxylic acid type may also be useful to inhibit estrone sulfate-dependent growth of breast cancer cells.

Estrogenicity is an issue associated with many STS inhibitors which prevents their usefulness in estrogen-dependent diseases, although more recently potent nonestrogenic inhibitors have been identified.[10,17,28] Derivatives **5d**,**j** did not show any affinity to the human estrogen receptors α and $\beta$ up to the highest test concentration of 100 $\mu$M; for comparison, phenols **4a**,**b**, as the hydrolytic products of the corresponding sulfamates, showed binding to the receptors with $ED_{50}$ values in the range of 0.2 to 1.5 $\mu$M.[17]

Since the chromenone carboxylic acids represent a new class of STS inhibitors, we also asked whether they would exert any antiproliferative activity as a sign of cellular toxicity. We used the human keratinocyte cell line HaCaT as a sensitive assay system.[32] There was no effect of compounds **5d**,**g** on HaCaT cell proliferation up to the highest test concentration of 30 $\mu$M.

## Conclusions

By extending structure−activity relationship investigations to hitherto unstudied sulfamate replacements on STS inhibitors featuring the 2-adamantyl-4-(thio)-chromenone-6-yl scaffold, we discovered 2-(1-adamantyl)-4-(thio)chromenone-6-carboxylic acids (**5d** and **5j**) as potent, reversible inhibitors of STS. All other modifications, including variation of the oxidation state and chain length, gave inactive or poorly active compounds. The new inhibitors **5d** and **5j** show similar $K_i$ values (0.5 $\mu$M and 0.53 $\mu$M, respectively) against purified recombinant human STS. In a cellular assay system using CHO cells overexpressing STS, the thio analogue **5j** is substantially superior to **5d** ($IC_{50}$ value = 0.18 $\mu$M versus 9.4 $\mu$M). Compound **5j** is an example of a reversible STS inhibitor showing potent activity toward the target enzyme in a cellular test system.

## Experimental Section

**A. Chemical Synthesis. 1. General Methods.** 1-(2-Hydroxy-5-methylphenyl)ethanone (**6a**) and 4-bromobenzenethiol (**15**) were purchased from Aldrich. 3-Acetyl-4-hydroxybenzoic acid methyl ester (**6c**) and 3-acetyl-4-hydroxybenzeneacetic acid methyl ester (**6b**) were synthesized according to a published procedure.[22] Melting points were determined on a Reichert Thermovar microscope and are uncorrected. All reactions were monitored by thin-layer chromatography performed by use of silica gel F254 plates (Merck), detected by UV light or potassium permanganate. Column chromatography was carried out on silica gel 60 (40−63 $\mu$m, Merck) using pressures up to 5 bar with the indicated solvent system. The NMR spectra were recorded at 250 MHz (Bruker WM 250) and at 400 MHz (Bruker Avance 400 spectrometer) with tetramethylsilane as internal standard. Chemical shifts are given in $\delta$ units. All reactions were carried out under an atmosphere of argon unless otherwise noted. Tetrahydrofuran (THF) was obtained anhydrous by distillation over $LiAlH_4$; all other solvents were dried by storing over 3- or 4-Å molecular sieves. Elemental analyses were performed by Novartis Services AG and Solvias AG, respectively, both in Basle, Switzerland. High-resolution mass spectra (HMRS) were recorded on a Finnigan MAT900.

**2. General Procedure for the Synthesis of Test Compounds 5c−e and 5g. (a) Adamantane-1-carboxylic Acid 2-Acetyl-4-(methoxycarbonyl)phenyl Ester (7c).** To a solution of 3-acetyl-4-hydroxybenzoic acid methyl ester (**6c**, 1.86 g 10.22 mmol) and 4-(dimethylamino)pyridine (250 mg, 2.04 mmol) in anhydrous pyridine (18 mL) was added adamantane 1-carbonyl chloride (2.44 g, 12.26 mmol) at room temperature. After being stirred for 18 h, the mixture was diluted with toluene (10 mL) and evaporated in vacuo. The residue was partitioned between ethyl acetate (15 mL) and water (5 mL), the organic layer was washed with saturated aqueous sodium bicarbonate solution, water, and brine, and dried over $MgSO_4$. After evaporation of the solvent, the crude product was purified by column chromatography (toluene/ethyl acetate = 4/1) to afford **7c** (3.5 g, 99%) as colorless crystals; mp 97−101 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.36 (d, $J$ = 2.1 Hz, 1H), 8.10 (dd, $J$ = 2.1 + 8.5 Hz, 1H), 7.05 (d, $J$ = 8.5 Hz, 1H), 3.88 (s, 3H), 2.52 (s, 3H), 1.97−2.02 (m, 6H), 1.86−1.87 (m, 3H), 1.65−1.71 (m, 6H).

**(b) 2-Adamantan-1-yl-4-oxo-4*H*-chromene-6-carboxylic Acid Methyl Ester (5c).** A suspension of **7c** (10.67 g, 29.94 mmol) and potassium carbonate (12.41 g, 90 mmol) in 2-propanol (120 mL) was refluxed for 75 min. The reaction mixture was partitioned between ethyl acetate (400 mL) and water (100 mL), and the organic layer was separated, washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The crude product 3-[(3-adamantan-1-yl)-3-oxopropionyl]-4-hydroxybenzoic acid methyl ester **8c** (8.09 g, 76%) was used in the next step without further purification.

The crude product **8c** (8.09 g, 22.7 mmol) was heated in formic acid (60 mL) to 100 °C for 30 min. The solvent was removed under reduced pressure, and the product was recrystallized from acetone to afford pure **5c** (5.33 g, 69%) as colorless crystals; mp 211−212 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.88 (d, $J$ = 2.1 Hz, 1H), 8.29 (dd, $J$ = 2.1 + 8.8 Hz, 1H), 7.49 (d, $J$ = 8.8 Hz, 1H), 6.28 (s, 1H), 3.93 (s, 3H), 2.07−2.016 (m, 3H), 1.92−2.02 (m, 6H), 1.68−1.87 (m, 3H); HRMS $m/z$ 339.1600 [(M + 1) calcd for $C_{21}H_{22}O_4$ 339.1596]; Anal. ($C_{21}H_{22}O_4$) C, H.

According to the procedure described for **7c**, the following compounds were prepared.

**Adamantane-1-carboxylic acid 2-acetyl-4-methylphenyl Ester (7a).** Starting from 1-(2-hydroxy-5-methylphenyl)-ethanone (**6a**, 6 g, 39.95 mmol). Yield (11.17 g, 83.7%), colorless crystals; mp 94−96 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.55 (d, $J$ = 2 Hz, 1H), 7.29 (ddd, $J$ = 2 + 0.6 + 8.2 Hz, 1H), 6.89 (d, $J$ = 8.2 Hz, 1 H), 2.53 (s, 3H), 2.37 (s, 3H) 2.08 (s, br, 9H), 1.77 (s, br, 6H).

**Adamantane-1-carboxylic acid 2-acetyl-4-methoxycarbonylmethylphenyl Ester (7b).** Starting from (3-acetyl-4-hydroxyphenyl)acetic acid methyl ester (**6b**, 3 g, 14.4 mmol). Yield (5.28 g, 99%), colorless crystals; mp 103−107 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.66 (d, $J$ = 2.2 Hz, 1H), 7.43 (dd, $J$ = 2.3 + 8.3 Hz, 1H), 6.98 (d, $J$ = 8.3 Hz, 1H), 3.70 (s, 3H), 3.65 (s, 2H), 2.53 (s, 3H), 2.04−2.015 (m, 9H), 1.69−1.82 (m, 6H).

According to the procedure described for **5c**, the following compounds were prepared.

**2-Adamantan-1-yl-6-methylchromen-4-one (9a).** Starting from **7a** (5.2 g, 16.6 mmol). Yield (4.1 g, 84% over two steps), colorless crystals; mp 150 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.95 (d, $J$ = 2 Hz, 1H), 7.45 (dd, $J$ = 2 + 8.5 Hz, 1H), 7.34 (d, $J$ = 8.5 Hz, 1H), 6.18 (s, 1H), 2.43 (s, 3H), 2.06−2.18 (m, 3H), 1.91−2.03 (m, 6H), 1.70−1.86 (m, 6H); HRMS $m/z$ 295.1698 [(M + 1) calcd for $C_{20}H_{22}O_2$ 295.1694].

**(2-Adamantan-1-yl-4-oxo-4*H*-chromen-6-yl) acetic Acid Methyl Ester (9b).** Starting from **7b** (5.75 g, 14.2 mmol). Yield (3.55 g, 71%), colorless crystals; mp 93−97 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.05 (d, $J$ = 2.29 Hz, 1H), 7.59 (dd, $J$ = 2.3 + 8.6 Hz, 1H), 7.43 (d, $J$ = 8.6 Hz, 1H), 6.19 (s, 1H), 3.74 (s, 2H), 3.70 (s, 3H), 2.08−2.17 (m, 3H), 1.94−1.99 (m, 6H), 1.73−1.84 (m, 6H); HRMS $m/z$ 353.1754 [(M + 1) calcd for $C_{22}H_{24}O_4$ 353.1753].

**(c) Adamantan-1-yl-4-oxo-4*H*-chromene-6-carboxylic Acid (5d).** A suspension of **5c** (3 g, 8.86 mmol) was heated to 100 °C in a mixture of dioxane (100 mL) and 20% aqueous HCl for 4 h. The mixture was stored at 5 °C for 18 h, and the precipitate was collected and washed with a water/dioxane mixture (10:1) to give **5d** (74%) as colorless crystals; mp 250−251 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.83 (d, $J$ = 2.1 Hz, 1H), 8.27 (dd, $J$ = 2.1 + 8.8 Hz, 1H), 7.44 (d, $J$ = 8.8 HZ, 1H), 6.17 (s, 1H), 2.03−2.16 (m, 3H), 1.87−2.01 (m, 6H), 1.63−1.82 (m, 6H); HRMS $m/z$ 325.1444 [(M + 1) calcd for $C_{21}H_{20}O_4$ 325.1440]; Anal. ($C_{21}H_{20}O_4$) C, H.

**2-Adamantan-1-yl-4-oxo-4*H*-chromene-6-acetic acid (5g)** was prepared analogously, as described for **5d**. Yield 85%, colorless crystals; mp184−186 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.09 (d, $J$ = 2.2 Hz, 1H), 7.61 (dd, $J$ = 2.2 + 8.6 Hz, 1H), 7.43 (d, $J$ = Hz, 1H), 6.24 (s, 1H), 3.76 (s, 2H), 2.04−2.18 (m, 3H), 1.90−2.05 (m, 6H), 1.66−1.88 (m, 6H); HRMS $m/z$ 3339.1598 [(M + 1) calcd for $C_{21}H_{22}O_4$ 339.1596]; Anal. ($C_{21}H_{22}O_4$) C, H.

**2-Adamantan-1-yl-4-oxo-4*H*-chromene-6-carboxamide (5e).** A mixture of **5d** (1 g, 3.1 mmol) and thionyl chloride (0.47 g, 4 mmol) in dry 1,2- dichloroethane (4 mL) and DMF (0.2 mL) was heated to reflux for 4 h. The solvent was evaporated to dryness, and to the residue was added saturated ammonia solution in ethanol (15 mL) at 0 °C. After being stirred for 2 h at room temperature, the solvent was removed under reduced pressure and the residue was purified by column chromatography (toluene/ethyl acetate = 1/2) to afford **5e** (370 mg, 37%) as colorless crystals; mp 285−287 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.55 (d, $J$ = 2.2 Hz, 1H), 8.24 (dd, $J$ = 2.2 + 8.8 Hz, 1H), 7.71 (d, $J$ = 8.8 Hz, 1H), 7.51 (s, br, 2H), 6.18 (s, 1H), 2.02−2.14 (m, 3H), 1.82−2.01 (m, 6H), 1.67−1.82 (m, 6 H); HRMS $m/z$ 324.1603 [(M + 1) calcd for $C_{20}H_{21}NO_3$ 324.1600]; Anal. ($C_{20}H_{21}NO_3$) C, H, N.

**3. Synthesis of the Test Compound 5a. (a) 2-Adamantan-1-yl-6-bromomethylchromen-4-one (10).** A solution of **9a** (2 g, 6.79 mmol), *N*-bromosuccinimide (1.33 g, 7.47 mmol), and a catalytic amount of 2,2′-azobis(2-methylpropionitrile) in benzene (100 mL) was heated to reflux for 2 h. The cooled mixture was poured into saturated aqueous sodium bicarbonate solution (20 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed with water and brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Purification by column chromatography (toluene/ethyl acetate = 10/1) afforded **10** (1.87 g 72%) as colorless solid. $^1$H NMR ($CDCl_3$): $\delta$ 8.17 (d, $J$ = 2.3 Hz, 1H),7.69 (dd, $J$ = 2.3 + 8.7 Hz, 1H), 7.45 (d, $J$ = 8.7 Hz, 1H), 6.2 (s, 1H), 4.56 (s, 2H), 2.06−2.20 (m, 3H), 1.90−2.04 (m, 6H), 1.68−1.9 (m, 6H).

**(b) 2-Adamantan-1-yl-6-(hydroxymethyl)chromen-4-one (5a).** A mixture of **10** (270 mg, 0.72 mmol) and Amberlite IRA 900 carbonate form (820 mg, 2.87 mmol) in toluene (1.3 mL) was heated to 90 °C for 2 h. The reaction mixture was cooled to room temperature, filtered, and washed with toluene/methanol and evaporated to dryness. The residue was purified by column chromatography (cyclohexane/ethyl acetate = 1/1) to give **5a** as colorless crystals; mp 188−190 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.11 (d, $J$ = 2.2 Hz, 1H), 7.69 (dd, $J$ = 2.2 + 8.6 Hz, 1H), 7.42 (d, $J$ = 8.6 Hz), 6.17 (s, 1H), 4.77 (d, $J$ = 5.6 Hz, 2H), 2.66 (t, $J$ = 5.8 Hz, 1H), 2.08 − 2.23 (m, 3H), 1.89−2.08 (m, 6H), 1.65−2.08 (m, 6H); HRMS $m/z$ 311.1648 [(M + 1) calcd for $C_{20}H_{22}O_3$ 311.1647]; Anal. ($C_{20}H_{22}O_3$) C, H.

**4. Synthesis of the Test Compounds 5b and 5f. (a) 2-Adamantan-1-yl-6-(dibromomethyl)chromen-4-one (11).** According to the procedure described for **10**, prepared from **9a** with 2.5 equiv of *N*-bromosuccinimide to give colorless solid (28% yield). $^1$H NMR ($CDCl_3$): $\delta$ 8.21 (d, $J$ = 2.5, 1H), 8.0 (dd, $J$ = 2.5 + 8.8 Hz, 1H), 7.52 (m, $J$ = 8.8 Hz, 1H), 6.72 (s, 1H), 6.21 (s, 1H), 2.08−2.2 (m, 3H), 1.92−2.04 (m, 6H), 1.68−1.88 (m, 6H).

**(b) 2-Adamantan-1-yl-4-oxo-4*H*-chromene-6-carbaldehyde (5b).** A mixture of **11** (200 mg, 0.44 mmol) and potassium acetate (260 mg, 2.65 mmol) in acetic acid (10 mL) was heated to 120 °C for 12 h. To the hot reaction mixture was added 2 N HCl (1.6 mL), followed by further heating for 30 min. The cooled reaction mixture was evaporated to dryness, and the residue was partitioned between ethyl acetate (15 mL) and saturated aqueous sodium bicarbonate solution (5 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The crude product was purified by

chromatography on silica gel toluene/ethyl acetate = 6/4) to give **5b** (171 mg, 56%) as colorless crystals; mp 196−198 °C. $^1$H NMR ($CDCl_3$): $\delta$ 10.09 (s, 1H), 8.66 (d, $J$ = 2.1 Hz, 1H), 8.20 (dd, $J$ = 2.1 + 9 Hz, 1H), 7.58 (d, $J$ = 9 Hz, 1H), 6.25 (s, 1H), 2.10−2.26 (m, 3 H), 1.97−2.09 (m, 6H), 1.69−2.09 (m, 6H); Anal. ($C_{20}H_{20}O_3$) C, H.

**(c) Synthesis of 2-Adamantan-1-yl-6-(1-(hydroxyethyl))chromen-4-one.** To a stirred solution of **5b** (700 mg, 2.27 mmol) in THF (15 mL) was added methylmagnesium iodide (1 mL, 3 mmol of a 3 M solution in diethyl ether) at 0 °C. After the reaction mixture was stirred at room temperature for 1 h, the mixture was quenched with saturated ammonium chloride solution (2 mL) and ethyl acetate (15 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude product was purified by column chromatography (toluene /ethyl acetate = 4/1) to afford the title compound (431 mg, 59%) as colorless crystals; mp 142−147 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.08 (d, $J$ = 2.28 Hz, 1H), 7.29 (dd, $J$ = 2.2 + 8.7 Hz, 1H), 7.41 (d, $J$ = 8.8 Hz, 1H), 6.16 (s, 1H), 5.0 (qa. $J$ = 6.4 Hz, 1H), 2.02−2.17 (m, 3H), 1.90−2.04 (m, 6H), 1.75−1.87 (m, 6H), 1.52 and 1.49 (2s, 3H).

**(d) Synthesis of 6-Acetyl-2-adamantan-1-ylchromen-4-one (12).** To a stirred solution of oxalyl chloride (409 mg, 3.25 mmol) in anhydrous dichloromethane (15 mL) at −68 °C dimethyl sulfoxide (533 mg, 6.82 mmol) dissolved in dichloromethane (3 mL) was added over a period of 10 min. The reaction mixture was stirred for 15 min followed by addition of 2-adamantan-1-yl-6-(1-(hydroxyethyl))chromen-4-one (420 mg, 1.28 mmol) in 2 mL of dichloromethane within 15 min. After being stirred for 1 h at −68 °C the reaction was allowed to reach −30 °C, stirred for 15 min and then re-cooled to −68 °C. Triethylamine (471 mg, 4.6 mmol) was added and the mixture was allowed to reach room temperature. The clear yellow solution was partitioned between saturated aqueous sodium bicarbonate (5 mL) and ethyl acetate (20 mL), the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated. Purification by chromatography on silica gel (toluene/ethyl acetate = 20/1) afforded **12** (270 mg, 66%) as colorless crystals; mp 183−185 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.72 (d, $J$ = 2.2 Hz, 1H), 8.29 (dd, $J$ = 2.2 + 8.8 Hz, 1H), 7.52 (d, $J$ = 8.8 Hz, 1H), 6.23 (s, 1H), 2.69 (s, 3H), 2.12−2.26 (m, 3 H), 1.97−2.02 (m, 6H), 1.55−1.85 (m, 6H).

**(e) 2-Adamantan-1-yl-6-(2-bromoacetyl)chromen-4-one (13).** A suspension of ketone **12** (700 mg, 2.17 mmol) in acetic acid (15 mL) was heated to 70 °C followed by addition of bromine (347 mg, 2.17 mmol). After the mixture was stirred at 70 °C for 3 h, the solvent was evaporated and the residue was purified by column chromatography (toluene/ ethyl acetate = 10/1) to give **13** (591 mg, 68%). $^1$H NMR ($CDCl_3$): $\delta$ 8.73 (d, $J$ = 2.2 Hz, 1H), 8.31 (dd, $J$ = 2.2 + 8.8 Hz, 1H), 7.56 (d, $J$ = 8.7 Hz, 1H), 6.24 (s, 1H), 4.54 (s, 2H), 2.05−2.18 (m, 3 H), 1.96−2.04 (m, 6H), 1.61−1.88 (m, 6H).

**(f) 2-Adamantan-1-yl-6-(2-hydroxyacetyl)chromen-4-one (5f).** To a mixture of **13** (150 mg, 0.37 mmol) in anhydrous DMF (2 mL) and dichloromethane (2 mL) was added a solution of betaine (44 mg, 0.37 mmol) in anhydrous ethanol (1 mL). After being stirred for 22 h, the solvents were evaporated to dryness, the residue was taken up in dioxane (2 mL) and water (2.5 mL), and potassium carbonate (200 mg, 1.86 mmol) was added. The mixture was stirred for 30 min and partitioned between water and ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. Purification by column chromatography (toluene/ethyl acetate = 2/1) provided **5f** (77 mg, 61%) as colorless crystals; mp 208−212 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.51 (d, $J$ = 2 Hz, 1H), 8.27 (dd, $J$ = 2 + 8.4 Hz, 1H), 7.77 (d, $J$ = 8.5 Hz, 1H), 6.20 (s, 1H), 5.21 (s, br, 1H), 4.85 (s, 2H), 2.03−2.20 (m, 3H), 1.88−1.99 (m, 6 H), 1.71−1.77 (m, 6H); HRMS $m/z$ 339.1600 [(M + 1) calcd for $C_{21}H_{22}O_4$ 339.1596]; Anal. ($C_{21}H_{20}O_4$) C, H.

**5. Synthesis of the Test Compound 5h. (a) 2-(Adamantan-1-yl-4-oxo-4*H*-chromen-6-yl)bromoacetic acid methyl ester** was prepared analogously to the procedure as described for **10.** Yield 75%, light yellow crystals; mp 132−134 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.22 (d, $J$ = 2.4 Hz, 1H), 7.97 (dd, $J$ = 2.4 + 8.8 Hz, 1H), 7.5 (d, $J$ = 8 Hz, 1H), 6.20 (s, 1H), 5.45 (s, 1H), 3.80 (s, 3H), 2.06−2.23 (m, 3H), 1.93−2.00 (m, 6H), 1.70−1.88 (m, 6H).

**(b) (2-Adamantan-1-yl-4-oxo-4*H*-chromen-6-yl)hydroxyacetic Acid Methyl Ester (14).** Amberlite IRA 900, carbonate form (3.2 g, 11.2 mmol) was added to a solution of 2-adamantan-1-yl-4-oxo-4*H*-chromen-6-yl)bromoacetic acid methyl ester (1.36 g, 3.1 mmol) in dry toluene (60 mL), and the mixture was stirred for 48 h at room temperature. The ion-exchange resin was filtered off, the residue was washed with toluene, and the solvent was concentrated in vacuo. The pure compound **14** (726 mg, 59%) was obtained after column chromatography on silica gel (ethyl acetate) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 8.23 (d, $J$ = 2.26 Hz, 1H), 7.74 (dd, $J$ = 2.2 + 8.7 Hz, 1H), 7.47 (d, $J$ = 8.7 Hz, 1H), 6.20 (s, 1H), 5.29 (d, $J$ = 5.08 Hz, 1H), 3.75 (s, 3H), 3.66 (d, $J$ = 5.1 Hz, OH), 2.06−2.24 (m, 3H), 1.92−2.07 (m, 6H), 1.69−1.88 (m, 6H); HRMS $m/z$ 369.1704 [(M + 1) calcd for $C_{22}H_{24}O_5$ 369.1702].

**(c) (2-Adamantan-1-yl-4-oxo-4*H*-chromen-6-yl)oxoacetic Acid Methyl Ester.** According to the procedure described for **12**, prepared from **14.** Yield 72% yield, colorless crystals; mp 183−185 °C. $^1$H NMR ($CDCl_3$): $\delta$ 8.8 (d, $J$ = 2.2 Hz, 1H), 8.33 (dd, $J$ = 2.2 + 8.8 Hz, 1H), 7.57 (d, $J$ = 8.8 Hz, 1H), 6.24 (s, 1H), 4.02 (s, 3H), 2.04−2.09 (m, 3H), 2.03−1.94 (m, 6H), 1.7−1.8 (m, 6 H).

**(d) 2-Adamantan-1-yl-4-oxo-4*H*-chromene-6-yl)oxoacetic acid (5h).** Yield 90%, colorless crystals; mp 245−246 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.54 (d, $J$ = 2 Hz, 1H), 8.30 (dd, $J$ = 2 + 8.8 Hz, 1H), 7.85 (d, $J$ = 8.8 Hz, 1H), 6.23 (s, 1H), 2.02−2.14 (m, 3H), 1.87−2.02 (m, 6H), 1.65−1.82 (m, 6H); $^{13}$CNMR (DMSO-$d_6$): $\delta$ 187.1, 177.1, 176.3, 165.69, 159.7, 134.1, 129.1, 128.4, 123.2, 120.3, 107.1, 40.9, 40.5, 40.2, 39.9, 39.5, 39.2, 39.1, 38.9, 38.25, 36.2, 27.7; HRMS $m/z$ 339.1600 [(M + 1) calcd for $C_{21}H_{22}O_4$ 339.1596]; Anal. ($C_{21}H_{22}O_4$) C, H.

**6. Synthesis of the Test Compounds 5i and 5j. (a) 2-Adamantan-1-yl-6-bromothiochromen-4-one (17)** was prepared as described in the literature,[24] starting from 4-bromobenzenethiol (**15**, 1.89 g, 10 mmol) and ethyl 3-(1-adamantyl)-3-oxopropionate (**16**, 2.5 g, 10 mmol) in polyphosphoric acid (10 mL). Triturating of the crude product with hot EtOH gave **17** (1.25 g, 34%) as colorless crystals; mp 214−216 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.37 (dd, $J$ = 1.1 + 1,8 Hz, 1H), 7.91 (m, 3H), 6.94 (s, 1H), 2.05−2.14 (m, 3H), 1.96−2.03 (m, 6H), 1.69−1.78 (m, 6H).

**(b) 2-Adamantan-1-yl-4-oxo-4*H*-thiochromene-6-carbonitrile (5i).** A mixture of **17** (565 mg, 1.5 mmol), palladium(II) acetate (53 mg, 0.235 mmol), triphenylphosphine (123 mg, 0.47 mmol), and calcium hydroxide (115 mg, 1.55 mmol) in DMF (5 mL) was heated to 100 °C for 90 min. After being cooled to room temperature, the mixture was poured onto phosphate buffer (pH 7) and extracted with ethyl acetate (3×). The combined organic layers were dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by column chromatography (cyclohexane/ethyl acetate = 10/1) to give the title compound (176 mg, 36%) as colorless crystals; mp 261 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.59 (t, $J$ = 1.2 Hz, 1H), 8.12 (d, $J$ = 1.2 Hz, 3H), 6.96 (s, 1H), 2.04−2.15 (m, 3H), 1.93−2.04 (m, 6H), 1.67−1.80 (m, 6H). 8.91 (d, $J$ = 1.7 Hz, 1H); HRMS $m/z$ 322.1263 [(M + 1) calcd for $C_{20}H_{19}NOS$ 322.1266]; Anal. ($C_{20}H_{19}NOS$) C, H, N.

**(c) 2-Adamantan-1-yl-4-oxo-4*H*-thiochromene-6-carboxylic Acid (5j).** A suspension of **5i** (100 mg, 0. 31 mmol) in a mixture of dioxane (10 mL) and 20% aqueous HCl was heated to 100 °C for 16 h. After being cooled to room temperature, the reaction mixture was diluted with water (2 mL) and extracted with ethyl acetate (15 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were dried over $MgSO_4$ and evaporated. Purification by column chromatography (dichloromethane/methanol = 6/1) provided **5j** (53 mg, 50%) as colorless crystals; mp 275−280 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.9 (d, $J$ = 1.7 Hz, 1H), 8.28 (dd, $J$ = 1.2 + 8.2 Hz, 2H), 7.86 (d, $J$ = 8.3 Hz, 1H),6.96 (s, 1H), 2.03−2.16 (m, 3H), 2.04−

2.15 (m, 3H), 1.86−2.04 (m, 6H), 1.60−1.80 (m, 6H); HRMS *m*/*z* 341.1215 [(M + 1) calcd for $C_{20}H_{19}NOS$ 341.1211]; Anal. ($C_{20}H_{20}O_3S$) C, H.

**B. Biology. 1. Assay of Purified Human STS.** STS was produced from a clone of recombinant Chinese hamster ovary (CHO) cells that stably express the human enzyme as described previously.[26] The purity of the final enzyme preparation was estimated to > 95%. Sulfatase activity was assessed using the method by Eto et al.[29] with modifications as described.[20] Briefly, 0.5 mM 4-MUS (Sigma) was used as substrate at pH 7.5, 37 °C, and the formation of the reaction product 4-methylumbelliferone was measured fluorimetrically after alkalization.

For inhibition studies, compounds were included at graded concentrations from stock solutions in ethanol; final ethanol content did not exceed 2%. $IC_{50}$ values were calculated using nonlinear regression (GraFit, Erithacus Software Ltd.). Values reported are the mean of triplicate determinations, which typically lie in the range of ±20%.

In the case of compound **5d** and **5j**, $K_i$ values were determined by measuring substrate turnover (*V*) at various substrate concentrations (S) in the presence of various inhibitor concentrations (I), as detailed in Figure 2. Data were plotted in an S/V vs S diagram (Hanes plot), and replotted in a $K_m/V_{max}$ vs I diagram. $K_i$ values were calculated from the intercept of the linear fit with the abscissa using GraFit. For compounds **5a**, **5f**, and **5h**, the $K_i$ values were calculated from the $IC_{50}$ values, using the relation $IC_{50} = K_i(1 + S/K_m)$ for competitive inhibitors,[30] with $K_m$= 270 μM and S= 500 μM.

The reversibility of STS inhibition by **5d** and **5j** was determined using the enzyme activity in microsomes from human placenta as described.[20] The assay involves incubation of the microsomes with the inhibitor (for various times up to 6 h) followed by addition of dextran-coated charcoal to bind free and noncovalently bound inhibitor and determination of residual STS activity.

**2. CHO−STS Assay.** The cellular assay for both irreversible (**4a**,**b**) and reversible inhibitors (**5a**−**j**) was carried out as described,[26] using fixed and live cells, respectively. Briefly, cells were seeded into 96-well plates (Nunc, Roskilde, Denmark) at 30 000 cells/well in RPMI 1640 medium, supplemented with 10% FCS and 100 U/mL penicillin/100 μg/mL streptomycin, and allowed to adhere overnight. They were then treated with graded concentrations of inhibitors for 24 h or left untreated. For the assay using fixed cells, the cells were fixed with 4% paraformaldehyde for 10 min at room temperature and washed four times with PBS. They were then incubated with 100 μL/well 0.5 mM 4-MUS in 0.1 M Tris-HCl, pH 7.5 for 30 min. 50 μL/well of 1 M Tris-HCl, pH 10.4 (stop solution) were added to stop the reaction. The reaction solutions were transferred to white plates (Microfluor, Dynex, Chantilly, VA).

For the assay with live cells, cells were seeded into 96-well plates and incubated with test compounds as above, except that phenol red-free RPMI 1640 containing 10% FCS was used. After 24 h, the medium was replaced by fresh medium (200 μL/well) containing the same substance concentrations plus 0.5 mM 4-MUS. The cells were then incubated at 37°C for an additional 4 h. At this point, 100 μL supernatant were transferred to white plates containing 50 μL stop solution.

The plates were read in a Fluoroskan II fluorescence microtiter plate reader (Titertek, Salzburg, Austria) at excitation and emission wavelengths of 355 and 460 nm, respectively. Reagent blanks were subtracted from all values. The fluorescence units (FU) were divided by the optical density readings for cellular protein obtained after fixation and staining of cells with sulforhodamine B ($OD_{550}$), to correct for variations in cell number. $IC_{50}$ values were defined as the concentrations of test substances required for 50% inhibition of STS activity. All experiments were carried out in triplicate for each sample and concentration.

**3. Inhibition of STS Activity in MCF-7 Breast Cancer Cells.** Enzyme activity in confluent monolayers of MCF-7 cells was measured using tritiated estrone sulfate as substrate, as described.[25]

**4. HaCaT Keratinocyte Proliferation Assay**. HaCaT cells[32] were cultivated in DMEM (Gibco) containing 5% FCS. For the proliferation assay, cells were detached by trypsinization, suspended in fresh medium, and seeded into 96-well microtiter plates at 4000 cells/0.2 mL/ well. After 24 h, the medium was replaced with fresh medium containing graded concentrations of test compounds. After 4 days of incubation, the extent of cellular proliferation was measured by colorimetric assay using sulforhodamine.[33]

**5. Binding to Estrogen Receptors (ER) α and *β*.** Fluorescence polarization-based ligand displacement assays with ER-α and -*β* were performed as described,[17] using commercial assay kits obtained from PanVera (Madison, WI).

**6. Stability Studies in Solution.** Test compounds were dissolved at a concentration of 10 mM in acetonitrile and diluted 100-fold (final concentration: 100 μM) into 0.1 M Tris-HCl buffer, pH 7.5/acetonitrile 1:1. Samples contained in tightly closed vials were stored in the dark at 37 °C. Immediately after preparation of the solutions and at various time points up to 7 days, aliquots were analyzed by HPLC. The extent of degradation was determined and, in the case of sulfamates, first-order rate constants of hydrolysis and corresponding half-lives were calculated by linear regresssion using the software Grafit (Elsevier). HPLC analyses for **4a**,**b** were performed using a HP1090 chromatographic system equipped with a Kratos UV detector set at 230 nm; a Merck LiChrosorb column (RP-18, 7 μm, 250 × 4 mm) was eluted with acetonitrile/10 mM ammonium sulfate (55/45, v/v), pH 6.0, at a flow rate of 1.5 mL/min at ambient temperature. For **5d**,**j**, an Agilent 1100 system was used equipped with an XTerra Waters column (RP-8, 3.5 μm, 4.6 × 50 mm) using 95−5% phosphate buffer (pH 5.6) /acetonitrile/1%aqueous TFA (gradient) as eluent. Samples of the incubations were diluted with the corresponding eluent and then injected directly into the HPLC system.

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