2-Substituted 4-(Thio)chromenone 6-*O*-Sulfamates: Potent Inhibitors of Human Steroid Sulfatase

Peter Nussbaumer,* Philipp Lehr, and Andreas Billich

Novartis Research Institute Vienna, Brunnerstrasse 59, A-1235 Vienna, Austria

Received March 15, 2002

Steroid sulfatase (STS) has emerged as a highly attractive target for the therapy of a number of disorders. Starting with the known inhibitor estrone sulfamate (**1**) as lead compound and with the finding that steroid sulfamates containing a nonaromatic A-ring are inactive, chromen-4-one sulfamates were designed, prepared, and tested for their ability to block human STS. This new class of nonsteroidal inhibitors shows high potency when the sulfamate group and the side chain are situated in diagonally opposite positions (i.e., 2,6- and 3,7-substitution pattern). The highest activity is achieved with fully branched, bulky aliphatic side chains and with thiochromen-4-one as the core element. 2-(1-Adamantyl)-4*H*-thiochromen-4-on-6-*O*-sulfamate (**6c**) is the most potent STS inhibitor discovered so far, and it is about 170-fold superior to **1**. As with **1**, all chromenone sulfamates are irreversible inhibitors of STS with a biphasic time course of inactivation.

Introduction

The enzyme steroid sulfatase (E.C. 3.1.6.2., STS) catalyzes the hydrolysis of the sulfate esters of 3-hydroxy steroids, which are inactive transport or precursor forms of the active 3-hydroxy steroids. Major substrates are the 3-*O*-sulfates of estrone, dehydroepiandrosterone (DHEA), pregnelonone, and cholesterol.[1] STS is a 65 kDa membrane-bound protein, predominantly associated with the endoplasmatic reticulum,[2] and present in almost all mammalian tissues.[3] In recent years, this enzyme has received considerable attention due to its potential involvement in the pathogenesis of a number of diseases such as:

(i) Breast Cancer. Estrone sulfate appears to be a major source of active estrogens in mammary tumors, especially in women after menopause.[4,5] While estrone sulfate itself does not bind to the estrogen receptor, it is converted first by STS to unconjugated estrone and then by 17β-hydroxysteroid dehydrogenase to estradiol, which binds to the receptor with high affinity. STS levels are elevated in breast tumors,[6] and this is a predictor of tumor recurrence.[7] Inhibitors of STS block estrone sulfate-dependent breast cancer cell proliferation in vitro[8,9] and in vivo.[10,11] DHEA sulfate can also be converted in a metabolic pathway involving STS to an agonist of the estrogen receptor (androst-5-ene-3β,-17β-diol)[9,12] resulting in the stimulation of breast cancer cell growth. In addition to breast cancer, STS activity might also be involved in estrogen-dependent cancer of the endometrium and androgen-dependent prostate carcinomas.[5]

(ii) Androgen-Dependent Skin Diseases. The skin is capable of converting DHEA to active androgens, namely, testosterone and dihydrotestosterone.[13] DHEA is formed in the skin from the abundant systemic precursor DHEA sulfate. An involvement of STS in the development of androgenetic alopecia (male pattern hair loss)[14] and of acne[15] has been proposed.

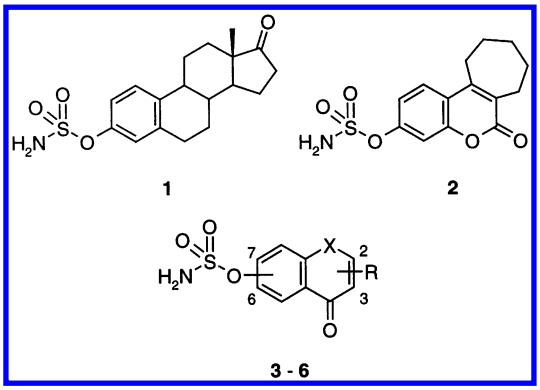


Figure 1. Structures of estrone sulfamate (**1**), the tricylic coumarin COUMATE 667 (**2**), and the chromenone-based sulfamates described here.

(iii) Cognitive Dysfunction. DHEA sulfate as a neurosteroid affects a number of neurotransmitter systems, including those involving acetylcholine, glutamate, and GABA, resulting in increased neuronal excitability.[16] STS inhibition was shown to enhance learning and spatial memory in the rat.[17]

(iv) Immune Functions. STS has been proposed to play a role in regulating T-helper cell functions by mobilizing DHEA in macrophages as an accessory signal for T-cells.[18] STS inhibition was shown to be protective in models of contact allergy and collagen-induced arthritis in rodents.[19]

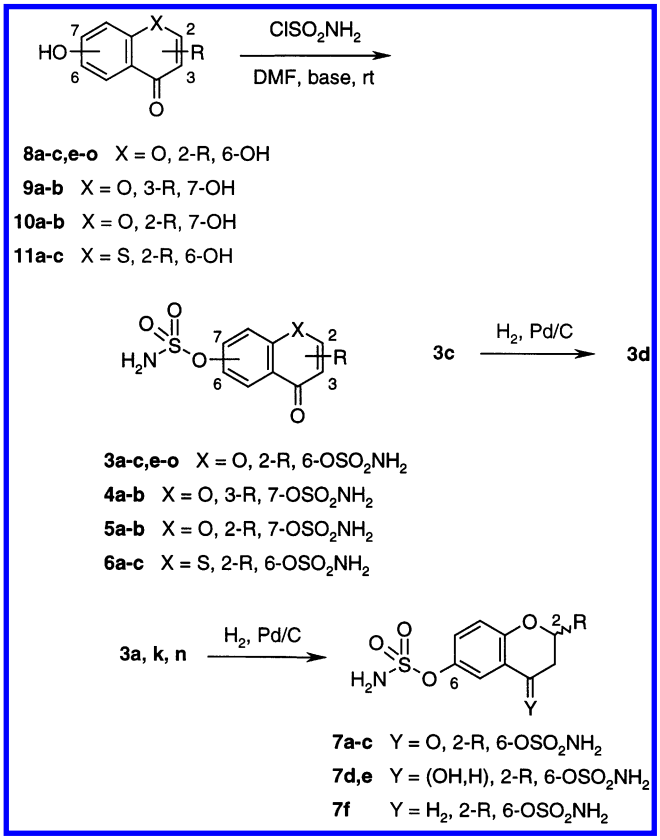
Thus, inhibitors of STS have been considered as potential new therapeutic agents for the treatment of a range of different diseases, with breast cancer having received the most attention so far (see ref 20 for review).

The most active STS inhibitors described so far are sulfamic acid esters, such as estrone sulfamate (**1**, Figure 1), which irreversibly inactivate the enzyme.[21] However, estrone sulfamate is not suitable for therapy due to its strong estrogenic activity.[22] Therefore, recent

* To whom correspondence should be addressed. Tel: ++43-1-86634-395. Fax. ++43-1-86634-354. E-mail: peter.nussbaumer@pharma.novartis.com.

10.1021/jm020878w CCC: $22.00
Published on Web 08/13/2002

Scheme 1. Synthesis of STS Inhibitors **3**−**7**[a]



[a] For a definition of substituent R, see Table 1.

efforts have focused on the synthesis of nonestrogenic sulfamate type STS inhibitors.[9,10,23−25] Among these, tricyclic coumarin sulfamates have been reported with good inhibitory activity and lacking estrogenic potential, e.g., COUMATE 667 (**2**, Figure 1).[11,24] In a complementary approach, we aimed at exploring aryl sulfamates with a bicyclic ring system, whereby the bicyclic core structure serves as an estrone A,B-ring mimic and the side chains fill the space of the steroidal C,D-rings. We designed and synthesized novel nonsteroidal aryl sulfamates featuring a 4-(thio)chromenone ring system (**3**−**6**, Figure 1) as the core element, which resulted in the discovery of highly potent STS inhibitors. Recently, we reported the synthesis and biological properties of two prototypes, i.e., **3f**,**h**.[9] In this paper, we describe the structure−activity relationships (SAR) obtained for this novel class of STS inhibitors.

Results and Discussion

Chemistry. Most of the test compounds **3**−**6** (for characteristic physicochemical properties, see Table 3) were prepared by introducing the sulfamate moiety in the final step (Scheme 1; for definition of substituent R, see Table 1). The sulfamoylation can be performed by reacting the corresponding phenolic precursors **8**−**11** with amidochlorosulfonic acid[26] (usually 2−3-fold excess) in dimethylformamide (DMF) or dimethylacetamide, either with or without addition of a base.[27] We routinely used sodium hydride (equimolar to excess amidochlorosulfonic acid) in DMF. After aqueous workup, the pure sulfamates were isolated directly either by crystallization (in most cases from 2-propanol) or by chromatography of the crude product mixture on silica gel followed by crystallization. The yield of isolated, analytically pure sulfamates depended on how quickly the pure product could be isolated (i.e., long purification and manipulation times reduced the yield). This may be due to reduced stability in solution as opposed to the solid state, where the compounds are stable.

Table 1. Inhibition of Purified Human STS by Chromenone-Based Sulfamates[a]

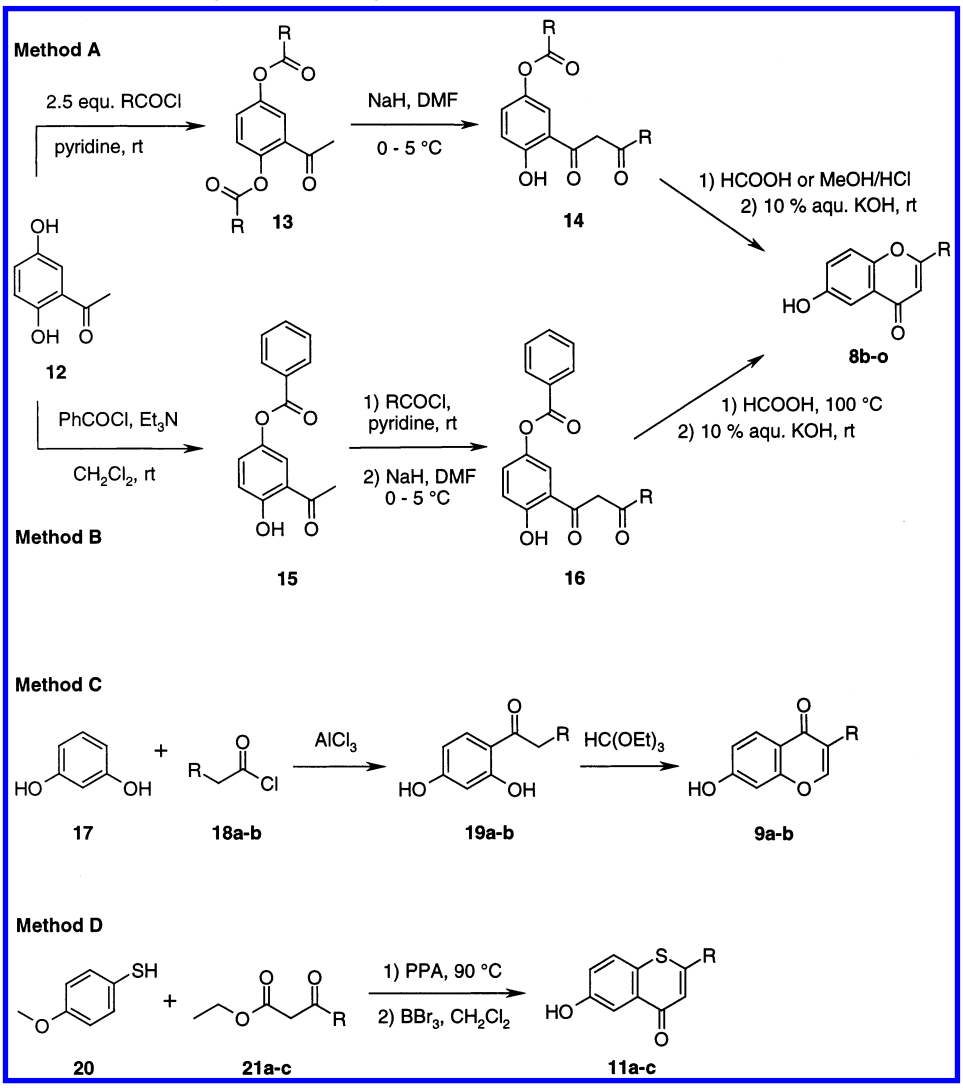
compd	X, Y	position of OSO_2NH_2	R	substituent (R)	IC_{50} (nM)
1					56
3a	O	6	2	phenyl	672
3b	O	6	2	benzyl	1620
3c	O	6	2	2-phenylethenyl	1620
3d	O	6	2	2-phenylethyl	190
3e	O	6	2	*n*-propyl	722
3f	O	6	2	*n*-nonyl	403
3g	O	6	2	1,1-dimethylnonyl	78
3h	O	6	2	*tert*-butyl	22
3i	O	6	2	2,2,3,3-tetramethyl-cyclopropyl	45
3j	O	6	2	cyclopentyl	134
3k	O	6	2	cyclohexyl	62
3l	O	6	2	cyclododecyl	45
3m	O	6	2	4-pentylbicyclo[2.2.2]-oct-1-yl	11
3n	O	6	2	1-adamantyl	5.6
3o	O	6	2	nor-adamantyl	11
4a	O	7	3	phenyl	437
4b	O	7	3	cyclohexyl	50
5a	O	7	2	*n*-propyl	>100 000
5b	O	7	2	cyclohexyl	6330
6a	S	6	2	phenyl	62
6b	S	6	2	*tert*-butyl	5.6
6c	S	6	2	1-adamantyl	0.34
7a	O	6	2	phenyl	2580
7b	O	6	2	cyclohexyl	280
7c	O	6	2	1-adamantyl	140
7d	OH, H	6	2	cyclohexyl	24 300
7e	OH, H	6	2	1-adamantyl	291
7f	H, H	6	2	cyclohexyl	11 200

[a] For generic structures, see Scheme 1.

Product **3d** was obtained by selective hydrogenation of the exocyclic double bond in **3c**. Catalytic hydrogenation was also used for the synthesis of chromane analogues **7** starting from chromenones **3a**,**k**,**n**, respectively. Depending on the reaction time, either the chroman-4-one derivatives **7a**−**c** or, after continued hydrogenation, the corresponding 4-hydroxychromanes (**7d**,**e**; stereochemistry not elucidated) and chromane (**7f**) could be obtained as the main products.

Scheme 2 summarizes the syntheses of the 6- and 7-hydroxy(thio)chromenone precursors **8**−**11** applying methods A−D. 2-Substituted 6-hydroxychromen-4-ones **8b**−**o** were prepared from a common starting material, i.e., 2,5-dihydroxyacetophenone (**12**), by following known methods A or B.[28] The hydroxy function at position 5 in **12** had to be protected for subsequent construction of the heterocyclic ring system. This could be achieved either by (i) using the same acyl residue as both building block and hydroxy protecting group generating intermediate **13** (method A) or (ii) by selective protection via benzoylation (intermediate **15**, method B), followed by acylation to introduce the desired R substituent. In both methods A and B, the double-acylated intermediates underwent O → C migration of the acyl residues within the hydroxyacetophenone moiety upon treatment with sodium hydride. The protecting groups in position 5 were not affected under these conditions. The resulting

Scheme 2. Methods A−D for the Synthesis of Key Intermediates **8**, **9**, and **11**[a]



[a] For a definition of substituent R, see Table 1.

crude (3-oxopropio)phenones **14** and **16** were then cyclyzed to the *O*-protected 2-alkyl chromen-4-ones without prior purification by treatment with formic acid or hydrochloric acid in methanol. Deprotection was achieved by basic hydrolysis (10% aqueous KOH in dioxane) yielding 6-hydroxychromen-4-ones **8**. For the 2-*tert*-butyl series, we developed a one pot reaction for the cyclization and deprotection steps. Thus, cleavage of the pivaloyl protecting group was accomplished by just adding aqueous hydrochloric acid to the reaction mixture.

2-Substituted 7-hydroxychromen-4-ones **10a**,**b** were synthesized analogously following method A but starting from 2,4-dihydroxyacetophenone. Method C (Scheme 2) was used to prepare 3-substituted 7-hydroxychromen-4-ones **9a**,**b**. Resorcinol (**17**) was acylated with acid chlorides **18a**,**b** to produce intermediates **19a**,**b**, which were cyclyzed to **9a**,**b** by treatment with triethyl orthoformate.[29]

4-Mercaptoanisol (**20**) was the starting material for the synthesis of thiochromen-4-ones **11a**−**c**. Condensation of **20** with appropriately substituted ethyl 3-oxopropionates **21a**−**c** in polyphosphoric acid, according to a published procedure[30] followed by ether cleavage using boron tribromide in dichloromethane, generated the substituted bicyclic heterocycles **11a**−**c**.

STS Inhibition Assay. Previous authors in the field have used either placental microsomes[21] or lysates of cells transfected with an STS expression vector[25] as the source of enzyme activity. In contrast, we used highly purified human STS obtained from a recombinant cell line for our inhibition assays.[9] Instead of radiolabeled estrone sulfate or DHEA sulfate as used by other authors, we used 4-methylumbelliferyl sulfate (4-MUS) as a synthetic substrate for a convenient colorimetric assay.[9] We demonstrated for a series of compounds that relative IC_{50} values measured with either estrone sulfate or 4-MUS were identical (data not shown).

SAR. Estrone sulfamate (**1**, Figure 1), the sulfamic acid analogue of the natural substrate estrone sulfate, was the most potent inhibitor of human STS known[21] when we started our search for novel inhibitors. In our assay system, the IC_{50} of estrone sulfamate is 56 ± 8 nM. STS accepts as substrates steroid sulfates not only with an aromatic A-ring (estrone sulfate) but also with a nonaromatic A-ring (e.g., DHEA sulfate and cholesterol sulfate).[1] When we synthesized and tested the corresponding sulfamate derivatives of DHEA and

cholesterol as potential inhibitors in analogy to estrone sulfamate, these compounds surprisingly turned out to be completely inactive up to the highest test concentrations of 30 μM. From these data, we concluded that an aromatic A-ring is essential for STS inhibition and thus concentrated our efforts on analogues with the sulfamate group attached to a phenolic group. All sulfamate type STS inhibitors described by other authors also fall into this structural category.[20]

In this study, we describe a series of novel nonsteroidal STS inhibitors featuring a 4-(thio)chromenone ring system, characterized by the general formulas **3**−**6** in Scheme 1. The inhibitory potencies of the compounds (IC_{50} values) against the pure enzyme were used for the establishment of SAR (Table 1).

Structural and biological similarities between steroidal estrogens and hydroxy-substituted flavones (= 2-phenylchromenones) are well-documented.[31] Therefore, it is not surprising that compound **3a** (flavone sulfamate) exhibits inhibitory activity against STS (Table 1), albeit with 12-fold less potency than the steroidal lead compound **1**. We were concerned about potential estrogenic activity of flavone derivatives and, therefore, proceeded with 2-alkyl-substituted 4-chromenone 6-*O*-sulfamates. When the phenyl substituent present in **3a** was shifted away from the bicyclic core by a C1 (**3b**) or a C=C spacer (**3c**), the inhibitory activity decreased roughly by a factor of 2.5. However, potency increased when the spacer in **3c** was hydrogenated to give an ethylene bridge between the phenyl ring and the bicyclic core. Compound **3d** was superior to all previous analogues with a phenyl-containing side chain (IC_{50} = 190 nM). The results obtained with **3a**−**d** indicated that there is considerable space available for side chain substituent R and that a substituent connected to the chromenone core by an sp^3 carbon might be preferred over those connected by an sp^2 carbon.

We then explored simple aliphatic chains, both unbranched and branched, for the substituent R. Within the unbranched series, a tendency for better activity by longer side chains (**3f** vs **3e**, Table 1) was observed. Moreover, bulkiness at the carbon atom attached to the heterocycle was identified as a key factor for high STS inhibitory potency of the compound class. This finding was deduced by comparing the results obtained with inhibitors **3f** (R = nonyl, IC_{50} = 403 nM) and **3g** (R = 1,1-dimethylnonyl, IC_{50} = 78 nM), as well as by the low IC_{50} value of 22 nM obtained with *tert*-butyl analogue **3h**. Thus, **3h** was the first chromenone-based inhibitor superior to the steroidal lead **1**. The importance of a branched, bulky substituent R for high activity was confirmed in a series of compounds with cycloalkyl side chains (**3i**−**o**). As observed for the linear alkyl substituents, we found that potency correlates with the size of the ring cycle (compare **3j**, R = cyclopentyl; **3k**, R = cyclohexyl; and **3l**, R = cyclododecyl; Table 1). The activity of the tetramethylcyclopropyl derivative **3i** is higher than expected for a small ring analogue, but it probably results from the bulkiness introduced by the four methyl substituents. Even higher activity was achieved by introduction of bi- and tricycloalkyl substituents, which are bulky and are fully branched at the attachment site to the chromenone core. With IC_{50} values of 11 nM, the inhibitors **3m**,**o** show potency significantly superior to estrone sulfamate (**1**). Furthermore, the 1-adamantyl analogue **3n** was the most potent inhibitor of the chromenone series (IC_{50} = 5.6 nM) with a 10-fold improved activity relative to **1**.

Then we asked whether the 3-R-7-OSO_2NH_2 substitution pattern on the chromenone core could also provide compounds with good inhibitory activities against human STS. This was confirmed by two derivatives, i.e., **4a** (R = phenyl) and **4b** (R = cyclohexyl). Comparable activity was obtained for both compounds relative to their 2,6-regioisomers **3a**,**k** (Table 1). Thus far, we only investigated chromenone compounds with the side chain R situated diagonally opposite to the sulfamate group. This gives the closest fit when overlaying the steroidal lead **1** with the substituted chromenone sulfamate inhibitors. Here, the bicyclic core and the side chain mimic the A,B- and the C,D-rings of estrone, respectively. To test the validity of this model, we synthesized and tested derivatives **5a**,**b** having a 2,7-substitution pattern on the chromenone core. As expected, both compounds were by at least 2 orders of magnitude less active than their 2,6-regioisomers (compare **5a** with **3f** and **5b** with **3k**, Table 1).

The inhibitory potency of chromenone sulfamates could be further increased by formally replacing the oxygen atom in the heterocycle by sulfur as demonstrated by compounds **6a**−**c** (Table 1). These thiochromenone analogues are 4- to 17-fold more potent than their respective oxo-analogues (**3a**,**h**,**n**). With an IC_{50} value of 0.34 nM, compound **6c** is the most potent STS inhibitor discovered so far and is about 170-fold more potent than the steroidal lead compound **1**.

Finally, we investigated the influence of reducing the keto function and the double bond in the chromenone sulfamates on inhibitory activity. The racemic chroman-4-one analogues (**7a**−**c**) were found to be slightly but consistently less potent than the corresponding chromen-4-one-based inhibitors (**3a**,**k**,**n**). Additional reduction of the keto function resulted in a further loss of activity (derivatives **7d**−**f**). Because the activities of compounds **7a**−**f** were substantially lower as compared to their parent chromenone derivatives, no efforts were made to explore the influence of stereochemistry on inhibitory potency.

Estrogenic activity of an STS inhibitor, as encountered in the case of **1**,[22] is prohibitive for its use in the treatment of estrogen-dependent cancer. We evaluated the potential estrogenicity of a selection of our chromenone-based STS inhibitors in two different systems and obtained a SAR that does not correlate with the SAR for STS inhibition. These results will be presented elsewhere.

Kinetics of STS Inactivation. We expected the chromenone-based sulfamates to be irreversible inhibitors of STS, similar to the lead compound estrone sulfamate (**1**).[21] We tested for enzyme inactivation by incubating human placental microsomes (which contain STS activity) with inhibitor, followed by separation of unbound inhibitor by binding to dextran-coated charcoal[21] (note that purified Triton-solubilized STS cannot be used in this assay since the detergent disturbs binding of the inhibitor to the charcoal). Using this method, we observed a time- and concentration-dependent inactivation of STS activity in placental microsomes

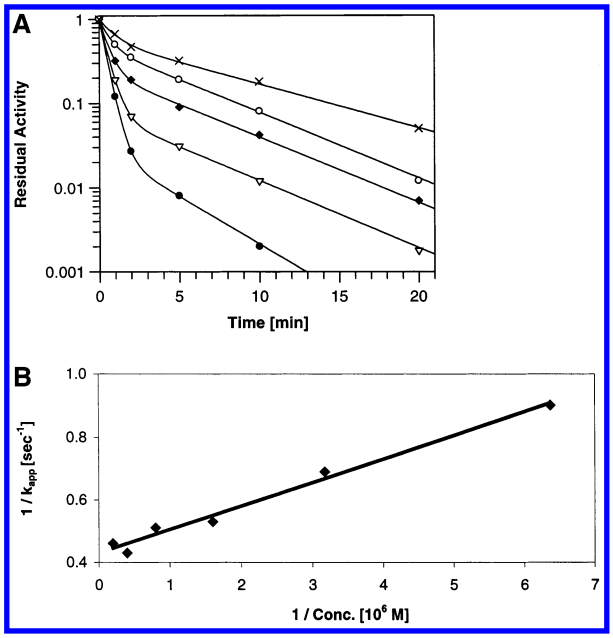


Figure 2. Time- and concentration-dependent inhibition of STS activity in placental microsomes by **3n**. (A) Time course of inactivation at different inhibitor concentrations (●, 5 μM; ▽, 2.5 μM; ◆, 1.25 μM; ○, 0.31 μM; ×, 0.16 μM). The initial enzyme activity was set to one; the curves are generated by fitting of the data to a double-exponential decay. (B) Double reciprocal plot of the apparent first-order rate constants k_{app} vs the inhibitor concentrations.

by our inhibitors, as exemplified in Figure 2A for compound **3n**. To explore whether inhibition of STS activity by our compounds was truly irreversible, we inactivated purified recombinant STS with inhibitors **3h**,**n** or **6c** and then extensively dialyzed the protein or passed it through a size exclusion column to separate it from unbound inhibitor. We did not observe any recovery of enzyme activity after these procedures, indicating that the test compounds act as irreversible inhibitors.

Similar to the case of estrone sulfamate,[21] a biphasic time course of inactivation was observed (Figure 2A). The data can be fitted to a double-exponential decay. Thus, the initial decrease in STS activity follows pseudo-first-order kinetics. From a double-reciprocal plot of the apparent first-order rates of inactivation (k_{app}) vs inhibitor concentration (see Figure 2B), the inhibition constant (K_I) and the rate constant of inactivation (k_{inact}) can be calculated.[32] Table 2 lists K_I and k_{inact} values for a selection of chromenone-based STS inhibitors. The ratio k_{inact}/K_I, as well as the IC_{50} value, are measures for the overall efficiency of an irreversible inhibitor; in fact, we observed a linear correlation ($r^2 = 0.962$) between these two parameters (Figure 3).

The K_I value, i.e., the dissociation constant of the noncovalent enzyme−inhibitor complex, is in the range of 0.2−2 μM for most compounds tested here. In contrast, compound **5b** (with substituents on the chromenone skeleton in 2,7-position) and compounds **7d**,**f** (which lack the keto function) have much higher K_I values (~24−54 μM), indicating that these configurations do not favor inhibitor binding.

The k_{inact} value describes the rate of covalent reaction of the inhibitor with the enzyme. The values determined here are in the range between 0.006 and 0.12 s^{-1}. Thus,

Table 2. Kinetic Parameters of Selected Chromenone-Based STS Inhibitors

compd	K_i (μM)	k_{inact} (sec^{-1})	k_{inact}/K_I (10^4/M/sec)	IC_{50} (nM)
3f	1.7	0.006	0.35	403
3h	0.9	0.070	7.8	22
3j	0.73	0.011	1.5	134
3k	1.0	0.035	3.5	62
3l	1.1	0.040	3.64	45
3m	0.5	0.074	14.8	11
3n	0.19	0.040	21.2	5.6
3o	0.22	0.042	19.1	11
4b	1.2	0.070	5.83	50
5b	24	0.020	0.083	6330
6c	0.21	0.115	54.8	0.34
7c	1.86	0.017	0.91	140
7d	53.7	0.018	0.034	24 300
7f	32	0.016	0.05	11 200

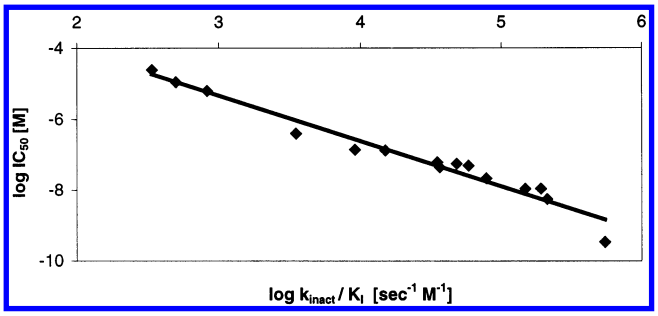


Figure 3. Correlation between k_{inact}/K_I and IC_{50} values for chromenone-based STS inhibitors.

the reactivity of the sulfamate group in the enzyme−inhibitor complex differs by factors of up to 20. This may be explained by a combination of (i) electronic effects of substituents on the electrophilic sulfur of the sulfamate group and (ii) steric effects influencing the orientation of the sulfamate moiety with respect to the nucleophilic reaction partner on the enzyme (see ref 24a for a postulated mechanism of STS inactivation by sulfamates). Analysis of the data set obtained suggests that steric effects may be the dominating factor. For instance, inhibitors **3k** (4-chromenone), **7c** (4-chromanone), **7d** (4-hydroxychromane), and **7f** (chromane) show very similar k_{inact} values, but the electronic properties of their heterocyclic core elements differ substantially. Similarly, we would have expected more than a factor of 2 in k_{inact} between the regioisomers **3k** (sulfamate functionality in meta position to the carbonyl group of the chromenone system) and **4b** (sulfamate in para position to the carbonyl), if electronic effects by the substituents play a major role. The most potent inhibitor found here (**6c**) features not only one of the lowest K_I values but also the fastest rate of inactivation.

Conclusions

We discovered novel nonsteroidal aryl sulfamates featuring a (thio)chromenone ring system as potent STS inhibitors and established the SAR of this compound class. The bicyclic ring system apparently serves as an estrone A,B-ring mimic, and the side chains, which have to be situated diagonally opposite relative to the sulfamate group (i.e., 2,6- or 3,7-substitution pattern), occupy the space of the steroidal C,D-rings. Aliphatic side chains yield higher activity than aromatic moieties, and fully branched, bulky groups are superior to linear chains. Most preferred are bicyclic and tricyclic aliphatic

Table 3. Physicochemical Properties of Chromenone-Based STS Inhibitors **3**−**7**

	mp (°C)	^{1}H NMR (DMSO-d_6) δ	method[a]/ yield[b] (%)
3b	157−160	8.08 (br.s, 2H), 7.85 (d, J = 2.8 Hz, 1H), 7.72 (d, J = 9 Hz, 1H), 7.63 (dd, J = 2.8 + 9 Hz, 1H), 7.26−7.40 (m, 5H), 6.31 (s, 1H), 4.06 (s, 2H)	B/58
3c	185−188	8.14 (br.s, 2H), 7.88 (d, J = 3 Hz, 1H), 7.86 (d, J = 9.2 Hz, 1H), 7.68−7.80 (m, 4H), 7.42−7.53 (m, 3H), 7.27 (d, J = 16.2 Hz, 1H), 6.54 (s, 1H)	A/44
3d	155−158	8.11 (br.s, 2H), 7.83 (d, J = 2.9 Hz, 1H), 7.74 (d, J = 9 Hz, 1H), 7.65 (dd, J = 2.9 + 9 Hz, 1H), 7.21−7.32 (m, 5H), 6.26 (s, 1H), 3.03 (s, 4H)	A/60
3e	148	8.10 (br.s, 2H), 7.86 (d, J = 2.8 Hz, 1H), 7.75 (d, J = 9 Hz, 1H), 7.65 (dd, J = 2.8 + 9 Hz, 1H), 6.30 (s, 1H), 2.66 (t, J = 7.4 Hz, 2H), 1.72 (sext, J = 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H)	A /61
3f	92	8.10 (br.s, 2H), 7.86 (d, J = 2.9 Hz, 1H), 7.76 (d, J = 9 Hz, 1H), 7.64 (dd, J = 2.9 +9 Hz, 1H), 6.30 (s, 1H), 2.68 (t, J = 7.5 Hz, 2H), 1.68 (qui, J = 7.5 Hz, 2H), 1.20−1.38 (m, 12H), 0.85 (t, J = 7.5 Hz, 3H)	B/75
3g	122	8.07 (br. s, 2H), 7.86 (d, J = 2.9 Hz, 1H), 7.78 (d, J = 9 Hz, 1H), 7.66 (dd, J = 2.9 +9 Hz, 1H), 6.25 (s, 1H), 1.62−1.71 (m, 2H), 1.29 (s, 6H), 1.13−1.23 (m, 12H), 0.80 (t, J = 7 Hz, 3H)	B/69
3h	180	8.10 (br s, 2H), 7.86 (d, J = 2.9 Hz, 1H), 7.79 (d, J = 9 Hz, 1H), 7.66 (dd, J = 2.9 + 9 Hz, 1H), 6.28 (s, 1H), 1.33 (s, 9H)	A/79
3i	148	8.08 (br s, 2H), 7.85 (d, J = 2.8 Hz, 1H), 7.76 (d, J = 9 Hz, 1H), 7.62 (dd, J = 2.8 + 9 Hz, 1H), 6.24 (s, 1H), 1.70 (s, 1H), 1.26 (s, 6H), 1.20 (s, 6H)	B/64
3j	158−160	8.10 (br s, 2H), 7.85 (d, J = 2.8 Hz, 1H), 7.75 (d, J = 9 Hz, 1H), 7.64 (dd, J = 2.8 + 9 Hz, 1H), 6.30 (s, 1H), 3.04−3.20 (m, 1H), 1.95−2.09 (m, 2H), 1.61−1.85 (m, 6H)	A/74
3k	170−171	8.09 (br s, 2H), 7.85 (d, J = 2.8 Hz, 1H), 7.76 (d, J = 9 Hz, 1H), 7.65 (dd, J = 2.8 + 9 Hz, 1H), 6.24 (s, 1H), 2.64 (tt, J = 3.2 + 11.3 Hz, 1H), 1.08−2.03 (m, 10H)	A/64
3l	168−170	8.09 (br s, 2H), 7.83 (d, J = 2.8 Hz, 1H), 7.76 (d, J = 9 Hz, 1H), 7.64 (dd, J = 2.8 + 9 Hz, 1H), 6.37 (s, 1H), 2.83 (qui, J = 6. 3 Hz, 1H), 1.23−1.85 (m, 22H)	B/64
3m	185	8.08 (br s, 2H), 7.84 (d, J = 2.8 Hz, 1H), 7.76 (d, J = 9 Hz, 1H), 7.65 (dd, J = 2.8 + 9 Hz, 1H), 6.17 (s, 1H), 1.78−1.88 (m, 6H), 1.40−1.49 (m, 6H), 1.08−1.34 (m, 8H), 0.86 (t, J = 7.4 Hz, 3H)	B/63
3n	166−168	8.08 (br s, 2H), 7.85 (d, J = 2.9 Hz, 1H), 7.78 (d, J = 9 Hz, 1H), 7.65 (dd, J = 2.9 + 9 Hz, 1H), 6.18 (s, 1H), 2.08 (br.s, 3H), 1.91−1.97 (m, 6H), 1.67−1.81 (m, 6H)	B/56
3o	165−167	8.09 (br s, 2H), 7.86 (d, J = 2.8 Hz, 1H), 7.74 (d, J = 9 Hz, 1H), 7.65 (dd, J = 2.8 + 9 Hz, 1H), 6.27 (s, 1H), 2.70 (t, J = 6.6 Hz, 1H), 2.36 (br.s, 2H), 2.05−2.13 (m, 2H), 1.61−1.99 (m, 8H)	B/74
4a	183	8.60 (s, 1H), 8.34 (br.s, 2H), 8.24 (d, J = 8.8 Hz, 1H), 7.55−7.63 (m, 3H), 7.38−7.49 (m, 4H)	C/34
4b	160−163	8.28 (br s, 2H), 8.20 (s, 1H), 8.14 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.37 (dd, J = 2.2 + 8.7 Hz, 1H), 2.62−2.75 (m, 1H), 1.65−1.83 (m, 5H), 1.24−1.40 (m, 5H)	C/27
5a	148−150	8.30 (br.s, 2H), 8.09 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.36 (dd, J = 2.2 + 8.7 Hz, 1H), 6.28 (s, 1H), 2.65 (t, J = 7.4 Hz, 2H), 1.72 (sext, J = 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H)	A/42
5b	185−187	8.29 (br.s, 2H), 8.08 (d, J = 8.7 Hz, 1H), 7.54 (d, J = 2.2 Hz, 1H), 7.35 (dd, J = 2.2 + 8.7 Hz, 1H), 6.22 (s, 1H), 2.64 (tt, J = 3.3 + 11.3 Hz, 1H), 1.18−2.03 (m, 10H)	A/54
6a	247−250	8.24 (d, J = 2.7 Hz, 1H), 8.20 (br.s, 2H), 8.12 (d, J = 8.8 Hz, 1H), 7.82−7.89 (m, 2H), 7.70 (dd, J = 2.7 + 8.8 Hz, 1H), 7.56−7.64 (m, 3H)	D/52
6b	150−153	8.17 (d, J = 2.7 Hz, 1H), 8.16 (br.s, 2H), 8.04 (d, J = 8.8 Hz, 1H), 7.64 (dd, J = 2.7 + 8.8 Hz, 1H), 6.98 (s, 1H), 1.39 (s, 9H)	D/85
6c	220	8.16 (d, J = 2.7 Hz, 1H), 8.14 (br.s, 2H), 8.02 (d, J = 8.8 Hz, 1H), 7.64 (dd, J = 2.7 + 8.8 Hz, 1H), 6.93 (s, 1H), 2.10 (br.s, 3H), 1.96−2.02 (m, 6H), 1.71−1.78 (m, 6H)	D/54
7a	178−180	$CDCl_3/CD_3OD$ = 5/1: 7.82 (d, J = 3 Hz, 1H), 7.37−7.55 (m, 6H), 7.12 (d, J = 9 Hz, 1H), 5.52 (dd, J = 3.2 + 13 Hz, 1H), 3.12 (dd, J = 13 + 17 Hz, 1H), 2.93 (dd, J = 3.2 + 17 Hz, 1H)	61
7b	136−138	$CDCl_3$: 7.73 (d, J = 3 Hz, 1H), 7.45 (dd, J = 3 + 9 Hz, 1H), 7.01 (d, J = 9 Hz, 1H), 5.49 (br.s, 2H), 4.10−4.21 (m, 1H), 2.58−2.70 (m, 2H), 1.63−2.00 (m, 7H), 1.03−1.35 (m, 6H)	20
7c	192−194	7.96 (br.s, 2H), 7.58 (d, J = 3 Hz, 1H), 7.43 (dd, J = 3 + 9 Hz, 1H), 7.15 (d, J = 9 Hz, 1H), 4.04 (dd, J = 2.5 + 14 Hz, 1H), 2.83 (dd, J = 14 + 16.7 Hz, 1H), 2.58 (dd, J = 2.5 + 16.7 Hz, 1H), 2.01 (br.s, 3H), 1.55−1.78 (m, 12H)	49
7d	147−149	7.82 (br.s, 2H), 7.30 (d, J = 2.9 Hz, 1H), 6.99 (dd, J = 2.9 + 8.8 Hz, 1H), 6.75 (d, J = 8.8 Hz, 1H), 5.56 (d, J = 6.3 Hz, 1H), 4.68−4.80 (m, 1H), 3.89−3. 98 (m, 1H), 2.06−2.17 (m, 1H), 1.49−1.92 (m, 7H), 1.00−1.34 (m, 5H)	73
7e	Foam	7.82 (br.s, 2H), 7.30 (d, J = 2.9 Hz, 1H), 6.99 (dd, J = 2.9 + 8.8 Hz, 1H), 6.76 (d, J = 8.8 Hz, 1H), 5.55 (d, J = 6.3 Hz, 1H), 4.69−4.80 (m, 1H), 3.65 (d, J = 11.1 Hz, 1H), 2.13 (dd, J = 5.6 + 12. 4 Hz, 1H), 1.99 (br.s, 3H), 1.49−1.77 (m, 12H)	31
7f	133	$CDCl_3$: 6.97−7.04 (m, 2H), 6.74−6.81 (m, 1H), 4.98 (br.s, 2H), 3.72 (ddd, J = 2.1 + 6.2 + 10 Hz, 1H), 2.68−2.89 (m, 2H), 1.02−2.05 (m, 13 H)	8

[a] Method used for the synthesis of the phenolic key intermediates **8**−**11**. [b] Yields (not optimized) of isolated, analytically pure products.

R groups such as the adamantyl residue in compounds **3n** and **6c**. Inhibitory potency is further increased by replacing the chromenone with the thiochromenone core element. 2-(1-Adamantyl)-4H-thiochromenone 6-*O*-sulfamate (**6c**) meets all of the defined criteria for high activity and is the most potent inhibitor discovered so far (about 170-fold superior to estrone sulfamate).

Like estrone sulfamate and related aryl sulfamates, these compounds are irreversible inhibitors of STS with a biphasic course of inactivation. When comparing highly and weakly potent analogues, the differences in K_I values are much more pronounced than those in k_{inact} values. This indicates that the binding to the enzyme, rather than the reactivity of the subsequent covalent modification, is the dominating factor for the overall efficiency of the chromenone-based inhibitors.

Experimental Section

A. Chemical Synthesis. 1. General Methods. 6-Hydroxy-2-phenyl-4H-1-benzopyran-4-one (**8a**) was purchased from Aldrich. 6-Hydroxy-2-phenyl-4H-1-thiobenzopyran-4-one (**11a**) was synthesized according to a published procedure,[30] and amidochlorosulfonic acid was prepared according to the method of Appel and Berger.[26]

Melting points were determined on a Reichert Thermovar microscope and are not corrected. The temperature is given in Celsius units. Thin-layer chromatography was performed using silica gel F254 plates (Merck) detection by UV light or potassium permanganate. Column chromatography was performed using silica gel 60 (0.040−0.063 mm; Merck), pressure 3−5 bar. NMR spectra were recorded at 250 and 400 MHz, respectively (Bruker WM 250 and Bruker Avance 400 spectrometer) with $(CH_3)_4Si$ as internal standard. Chemical shifts are given in δ units. Elemental analyses were performed by Novartis Services AG and Solvias AG, repectively, both in Basle, Switzerland.

2. General Procedure for the Synthesis of Test Compounds 3−6. Sulfamoylation Reaction. Synthesis of Sulfamic Acid 2-(1,1-Dimethylethyl)-4-oxo-4H-1-benzopyran-6-yl Ester (3h). Sodium hydride (95%, 0.6 g, 23.8 mmol) was carefully added to a solution of **8h** (4 g, 18.3 mmol) in dry DMF (40 mL). After it was stirred for 30 min at room temperature, the mixture was cooled (ice/water) and amidochlorosulfonic acid[26] (6.3 g, 55 mmol) was added in portions. The mixture was stirred for an additional 3 h at room temperature and subsequently concentrated in vacuo. The residue was partitioned between water and ethyl acetate, and after the layers were separated, the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried ($MgSO_4$) and evaporated to give the crude product, which was purified either by crystallization from toluene or by chromatography on silica gel (cyclohexane/ethyl acetate = 2/1) to yield the sulfamate **3h** (4.3 g, 79%) as colorless crystals; mp 180 °C. ^{13}C NMR (DMSO-d_6): δ 177.1, 176.6, 154.3, 147.2, 129.2, 123.9, 120.7, 117.8, 106.1, 36.8, 27.9.

3. General Procedure for the Synthesis of Test Compounds 7a−f. Hydrogenation. Synthesis of Sulfamic Acid 2-Cyclohexyl-3,4-dihydro-4-oxo-2H-1-benzopyran-6-yl Ester (7b), Sulfamic Acid 2-Cyclohexyl-3,4-dihydro-4-hydroxy-2H-1-benzopyran-6-yl Ester (7d), and Sulfamic Acid 2-Cyclohexyl-3,4-dihydro-2H-1-benzopyran-6-yl Ester (7f). A solution of **3k** (83 mg, 0.26 mmol) in ethyl acetate was hydrogenated over palladium (8 mg, 10% on charcoal) at atmospheric pressure and room temperature for 3 h. The mixture was filtered over Celite, and the filtrate was evaporated in vacuo. The residue was chromatographed on silica gel (cyclohexane/ethyl acetate = 2/1) to give **7b** (17 mg, 20%) as the first fraction, followed by **7d** (61 mg, 73%) as the second fraction. When the reaction time was extended to 14 h, a small amount of **7f** (8%) was isolated after chromatography as colorless crystals, mp 133 °C, in addition to **7d** as the main product. Analogously to the synthesis of **7b**,**d**, test compounds **7a**,**c**,**e** were prepared starting from **3a**,**n**, respectively. Compound **3d** was synthesized in 60% yield by the same procedure starting from **3c**, but the reaction time was limited to 40 min in order to achieve a selective reduction of the exocyclic double bond. Compound **3d** was purified by chromatography followed by crystallization from toluene; mp 155−158 °C.

4. Synthesis of Starting Materials. 4.1. Method A. Synthesis of 2-(1,1-Dimethylethyl)-6-hydroxy-4H-1-benzopyran-4-one (8h). 4.1.1. Synthesis of 2,2-Dimethylpropanoic Acid 2-Acetyl-1,4-phenylene Ester (13, R = *t*-Butyl). A solution of **12** (5 g, 32.8 mmol) in dry pyridine (35 mL) was treated with pivaloyl chloride (10 g, 83 mmol) under cooling with an ice-bath to keep the reaction temperature at about 20 °C. After it was stirred for 18 h at room temperature, the solvent was partially distilled off in vacuo. The residue was poured onto ice and hydrochloric acid (32%, 30 mL) and extracted with diethyl ether. The combined organic layers were washed with aqueous sodium carbonate solution and water, dried ($MgSO_4$), and evaporated to give the crude title compound (10.6 g, 100%), which was used in the next step without further purification, as yellow crystals. ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J = 2.8 Hz, 1H), 7.23 (dd, J = 2.8 + 8.7 Hz, 1H), 7.04 (d, J = 8.7 Hz, 1H), 2.54 (s, 3H), 1.38 (s, 9H), 1.36 (s, 9H).

4.1.2. Synthesis of 2,2-Dimethylpropanoic Acid 3-(4,4-Dimethyl-1,3-dioxopentyl)-4-hydroxyphenyl Ester (14, R = *t*-Butyl). Crude **13** (R = *tert*-butyl; 10.5 g, 32.8 mmol) was dissolved in dry DMF (30 mL) and added slowly at 0 °C under argon to a suspension of sodium hydride (95% pure, 870 mg, 34.4 mmol) in dry DMF (30 mL). After the mixture was stirred for an additional 2 h at 0−5 °C, acetic acid (2.5 mL) was added cautiously to the mixture, and then, it was poured into water (300 mL) and extracted with ethyl acetate (3 × 70 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution, dried ($MgSO_4$), and evaporated to yield the crude title compound (11 g, ∼100%) as an orange semicrystalline mass, which was used in the next step without further purification.

4.1.3. 2,2-Dimethylpropanoic Acid 2-(1,1-Dimethylethyl)-4-oxo-4H-1-benzopyran-6-yl Ester. A solution of crude **14** (R = *tert*-butyl; 4.33 g, 13.6 mmol) in methanol (30 mL) was treated with concentrated aqueous hydrochloric acid (15 mL). Then, dioxane was added until a clear solution was achieved. The mixture was stirred at ambient temperature for 3 h, poured into water, and extracted with ethyl acetate. The combined organic layers were washed cautiously with aqueous sodium carbonate solution, dried over magnesium sulfate, and evaporated in vacuo. The crude product was either directly used in the next step or purified by chromatography on silica gel to give the title compound as colorless crystals; mp 84−86 °C. ^{1}H NMR ($CDCl_3$): δ 7.82 (d, J = 2.8 Hz, 1H), 7.48 (d, J = 9 Hz, 1H), 7.36 (dd, J = 2.8 + 9 Hz, 1H), 6.28 (s, 1H), 1.37 (s, 9H), 1.36 (s, 9H).

4.1.4. Synthesis of 2-(1,1-Dimethylethyl)-6-hydroxy-4H-1-benzopyran-4-one (8h). 2,2-Dimethylpropanoic acid 2-(1,1-dimethylethyl)-4-oxo-4H-1-benzopyran-6-yl ester (2 g, 6.61 mmol) was dissolved in dioxane (60 mL) and treated with 2 N aqueous sodium hydroxide solution (12 mL, 24 mmol). The mixture was heated to 60 °C overnight, then poured into water, and washed with ethyl acetate. The aqueous layer was acidified with 15% aqueous hydrochloric acid followed by extraction with ethyl acetate. Subsequent drying over magnesium sulfate and evaporation yielded **8h** (1.34 g, 93%) as colorless crystals; mp 170 °C. ^{1}H NMR ($CDCl_3$): δ 8.10 (br s, 1H), 7.88 (d, J = 3 Hz, 1H), 7.39 (d, J = 9 Hz, 1H), 7.28 (dd, J = 3 + 9 Hz, 1H), 6.30 (s, 1H), 1.36 (s, 9H).

Alternatively, the ring closure of crude **14** to the corresponding *O*-acylated hydroxy-4H-1-benzopyran-4-ones can be achieved by heating in formic acid to 100 °C (see also method B), and further transformation of these intermediates into compounds **8** is also possible by acidic ester cleavage using concentrated aqueous hydrochloric acid in methanol and/or dioxane. For the synthesis of compound **8h** from **14h** (R = *tert*-butyl), a one pot procedure was also developed.

4.1.5. Alternative Synthesis of 2-(1,1-Dimethylethyl)-6-hydroxy-4H-1-benzopyran-4-one (8h). Crude **14** (R = *tert*-butyl; 10.4 g, 30.2 mmol) was heated in formic acid (60 mL) to 100 °C for 1 h. Then, hydrochloric acid (32%, 10 mL) and water (5 mL) were added and the mixture was stirred for an additional hour at 100 °C. The cooled mixture was poured onto ice/water (500 mL) and extracted with ethyl acetate. The combined organic layers were washed cautiously with saturated aqueous sodium bicarbonate solution (3 × 100 mL), dried over magnesium sulfate, and concentrated in vacuo. The dark green residue was chromatographed on silica gel (cyclohexane/ethyl acetate = 2/1) to give **8h** (4.9 g, 74%).

Analogously, the following compounds were prepared.

(*E*)-6-Hydroxy-2-(2-phenylethenyl)-4H-1-benzopyran-4-one (8c). Yield 93%, yellowish crystals, mp 219−222 °C. ^{1}H NMR ($CDCl_3$): δ 8.93 (br.s, 1H), 7.52−7.64 (m, 4H), 7.35−7.44 (m, 4H), 7.24 (dd, J = 3 + 9 Hz, 1H), 6.77 (d, J = 16 Hz, 1H), 6.28 (s, 1H).

Intermediate: (*E*,*E*)-3-Phenylpropenoic Acid 2-(2-Phenylethenyl)-4-oxo-4H-1-benzopyran-6-yl Ester. Yield 95%, yellow crystals, mp 179−181 °C. ^{1}H NMR ($CDCl_3$): δ 7.99 (d, J = 2.8 Hz, 1H), 7.92 (d, J = 16 Hz, 1H), 7.56−7.67 (m, 6H), 7.54 (dd, J = 2.8 + 9 Hz, 1H), 7.38−7.48 (m, 6H), 6.81 (d, J = 16 Hz, 1H), 6.66 (d, J = 16 Hz, 1H), 6.35 (s, 1H).

6-Hydroxy-2-propyl-4H-1-benzopyran-4-one (8e). Yield 86%, colorless crystals, mp 150 °C. ^{1}H NMR (DMSO-d_6): δ 9.97 (br.s, 1H), 7.47 (d, J = 9 Hz, 1H), 7.27 (d, J = 3 Hz, 1H), 7.19

(dd, $J = 3 + 9$ Hz, 1H), 6.15 (s, 1H), 2.60 (t, $J = 7.4$ Hz, 2H), 1.69 (sext, $J = 7.4$ Hz, 2H), 0.94 (t, $J = 7.4$ Hz, 3H).

2-Cyclopentyl-6-hydroxy-4H-1-benzopyran-4-one (8j). Yield 93%, colorless crystals, mp 174 °C. ^{1}H NMR (DMSO-d_6): δ 7.47 (d, $J = 9$ Hz, 1H), 7.27 (d, $J = 3$ Hz, 1H), 7.18 (dd, $J = 3 + 9$ Hz, 1H), 6.09 (s, 1H), 2.97−3.13 (m, 1H), 1.92−2.08 (m, 2H), 1.58−1.82 (m, 6H).

Intermediate: Cyclopentanecarboxylic Acid 2-Cyclopentyl-4-oxo-4H-1-benzopyran-6-yl Ester. Yield 56%, colorless crystals, mp 71 °C. ^{1}H NMR ($CDCl_3$): δ 7.83 (d, $J = 2.7$ Hz, 1H), 7.45 (d, $J = 9$ Hz, 1H), 7.36 (dd, $J = 2.7 + 9$ Hz, 1H), 6.21 (s, 1H), 2.93−3.07 (m, 2H), 1.60−2.13 (m, 16H).

2-Cyclohexyl-6-hydroxy-4H-1-benzopyran-4-one (8k). Yield 94%, colorless crystals, mp 171 °C. ^{1}H NMR ($CDCl_3$): δ 8.23 (br. s, 1H), 7.92 (d, $J = 2.9$ Hz, 1H), 7.37 (d, $J = 9$ Hz, 1H), 7.26 (dd, $J = 2.9 + 9$ Hz, 1H), 6.20 (s, 1H), 2.54 (tt, $J = 3.3 + 11.5$ Hz, 1H), 1.96−2.08 (m, 2H), 1.72−1.92 (m, 4H), 1.25−1.58 (m, 4H).

Intermediate: Cyclohexanecarboxylic Acid 2-Cyclohexyl-4-oxo-4H-1-benzopyran-6-yl Ester. Yield 51%, colorless crystals, mp 104−106 °C. ^{1}H NMR ($CDCl_3$): δ 7.83 (d, $J = 2.8$ Hz, 1H), 7.45 (d, $J = 9$ Hz, 1H), 7.35 (dd, $J = 2.8 + 9$ Hz, 1H), 6.16 (s, 1H), 2.47−2.66 (m, 2H), 2.02−2.14 (m, 4H), 1.23−1.95 (m, 16H).

7-Hydroxy-2-propyl-4H-1-benzopyran-4-one (10a). Yield 89%, colorless crystals, mp 140−143 °C. ^{1}H NMR (DMSO-d_6): δ 10.75 (br.s, 1H), 7.84 (d, $J = 8.7$ Hz, 1H), 6.88 (dd, $J = 2.2 + 8.7$ Hz, 1H), 6.80 (d, $J = 2.2$ Hz, 1H), 6.08 (s, 1H), 2.57 (t, $J = 7.5$ Hz, 2H), 1.68 (sext, $J = 7.5$ Hz, 2H), 0.94 (t, $J = 7.5$ Hz, 3H).

2-Cyclohexyl-7-hydroxy-4H-1-benzopyran-4-one (10b). Yield 84%, colorless crystals, mp 197 °C. ^{1}H NMR (DMSO-d_6): δ 10.78 (br.s, 1H), 7.83 (d, $J = 8.7$ Hz, 1H), 6.88 (dd, $J = 2.2 + 8.7$ Hz, 1H), 6.81 (d, $J = 2.2$ Hz, 1H), 6.02 (s, 1H), 2.47−2.60 (m, 1H), 1.20−1.98 (m, 10H).

Intermediate: Cyclohexanecarboxylic Acid 2-Cyclohexyl-4-oxo-4H-1-benzopyran-7-yl Ester. Yield 54%, colorless crystals, mp 110 °C. ^{1}H NMR ($CDCl_3$): δ 8.19 (d, $J = 8.7$ Hz, 1H), 7.24 (d, $J = 2.2$ Hz, 1H), 7.08 (dd, $J = 2.2 + 8.7$ Hz, 1H), 6.15 (s, 1H), 2.60 (tt, $J = 3.7 + 11$ Hz, 1H), 2.51 (tt, $J = 3.3 + 11$ Hz, 1H), 1.20−2.12 (m, 20H).

4.2. Method B. Synthesis of 6-Hydroxy-2-nonyl-4H-1-benzopyran-4-one (8f). 4.2.1. Synthesis of 5-Benzoyloxy-2-decanoyloxyacetophenone. Decanoyl chloride (3.8 g, 20 mmol) was added to a solution of **15**[9] (5.1 g, 20 mmol) in dry pyridine (50 mL). The solution was stirred for an additional 3 h at room temperature and then poured into 15% aqueous hydrochloric acid (300 mL). Extraction with ethyl acetate, followed by washing with aqueous sodium carbonate solution, drying over magnesium sulfate, and evaporation yielded the crude product (8.2 g, 100%), which was used in the next step without further purification, as yellowish crystals. ^{1}H NMR ($CDCl_3$): δ 8.18−8.23 (m, 2H), 7.62−7.72 (m, 1H), 7.67 (d, $J = 2.8$ Hz, 1H), 7.48−7.58 (m, 2H), 7.41 (dd, $J = 2.8 + 8.7$ Hz, 1H), 7.18 (d, $J = 8.7$ Hz, 1H), 2.64 (t, $J = 7.5$ Hz, 2H), 2.56 (s, 3H), 1.78 (qui, $J = 7.5$ Hz, 2H), 1.22−1.42 (m, 12H), 0.89 (t, $J = 7.5$ Hz, 3H).

4.2.2. 1-(5-Benzoyloxy-2-hydroxyphenyl)-1,3-dodecanedione (16, R = Nonyl). Following the procedure described for the synthesis of **14**, the title compound was obtained as crude product from 5-benzoyloxy-2-decanoyloxyacetophenone and used in the next step without further purification.

4.2.3. 6-Benzoyloxy-2-nonyl-4H-1-benzopyran-4-one. Crude **16** (R = nonyl, 8.2 g, 20 mmol) was heated in formic acid (50 mL) to 100 °C for 45 min. After it was cooled, the solvent was distilled off in vacuo and the residue was chromatographed on silica gel (cyclohexane/ethyl acetate = 8/1) to give the protected chromenone (3.95 g, 50%) as colorless crystals; mp 80 °C. ^{1}H NMR ($CDCl_3$): δ 8.18−8.23 (m, 2H), 8.00 (dd, $J = 1 + 2.3$ Hz, 1H), 7.62−7.72 (m, 1H), 7.48−7.58 (m, 4H), 6.20 (s, 1H), 2.64 (t, $J = 7.5$ Hz, 2H), 1.75 (qui, $J = 7.5$ Hz, 2H), 1.22−1.42 (m, 12H), 0.88 (t, $J = 7.5$ Hz, 3H).

4.2.4. 6-Hydroxy-2-nonyl-4H-1-benzopyran-4-one (8f). Treatment of 6-benzoyloxy-2-nonyl-4H-1-benzopyran-4-one with 10% aqueous potassium hydroxide solution in dioxane as described for the synthesis of **8h** (method A) gave the title compound in 93% yield as colorless crystals; mp 104 °C. ^{1}H NMR (DMSO-d_6): δ 9.96 (s, 1H), 7.47 (d, $J = 9$ Hz, 1H), 7.27 (d, $J = 3$ Hz, 1H), 7.18 (dd, $J = 3 + 9$ Hz, 1H), 6.15 (s, 1H), 2.61 (t, $J = 7.5$ Hz, 2H), 1.65 (qui, $J = 7.5$ Hz, 2H), 1.16−1.38 (m, 12H), 0.84 (t, $J = 7.5$ Hz, 3H).

Analogously, the following compounds were prepared.

2-Benzyl-6-hydroxy-4H-1-benzopyran-4-one (8b). Yield 79%. ^{1}H NMR ($CDCl_3$): δ 9.10 (br.s, 1H), 7.50 (d, $J = 2.9$ Hz, 1H), 7.20−7.35 (m, 6H), 7.18 (dd, $J = 2.9 + 9$ Hz, 1H), 6.03 (s, 1H), 3.91 (s, 2H).

2-(1,1-Dimethylnonyl)-6-hydroxy-4H-1-benzopyran-4-one (8g). Yield 83%. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, $J = 3$ Hz, 1H), 7.40 (d, $J = 9$ Hz, 1H), 7.29 (dd, $J = 3 + 9$ Hz, 1H), 6.31 (s, 1H), 1.62−1.72 (m, 2H), 1.32 (s, 6 H), 1.20−1.29 (m, 12H), 0.84 (t, $J = 7$ Hz, 3H).

6-Hydroxy-2-(2,2,3,3-tetramethylcyclopropyl)-4H-1-benzopyran-4-one (8i). Yield 69%, colorless crystals, 173 °C (from toluene). ^{1}H NMR (DMSO-d_6): δ 9.96 (s, 1H), 7.46 (d, $J = 9$ Hz, 1H), 7.26 (d, $J = 3$ Hz, 1H), 7.16 (dd, $J = 3 + 9$ Hz, 1H), 6.09 (s, 1H), 1.63 (s, 1H), 1.23 (s, 6H), 1.17 (s, 6H).

2-Cyclododecyl-6-hydroxy-4H-1-benzopyran-4-one (8l). Yield 78%, colorless crystals, mp 219 °C. ^{1}H NMR (DMSO-d_6): δ 10.02 (br.s, 1H), 7.47 (d, $J = 9$ Hz, 1H), 7.27 (d, $J = 3$ Hz, 1H), 7.18 (dd, $J = 3 + 9$ Hz, 1H), 6.20 (s, 1H), 2.76 (qui, $J = 6.2$ Hz, 1H), 1.22−1.86 (m, 22H).

Intermediate: 6-Benzoyloxy-2-cyclododecyl-4H-1-benzopyran-4-one. Yield 42%, colorless crystals, mp 105−107 °C. ^{1}H NMR ($CDCl_3$): δ 8.17−8.26 (m 2H), 7.97−8.02 (m, 1H), 7.62−7.72 (m, 1H), 7.47−7.58 (m, 5H), 6.20 (s, 1H), 2.78 (qui, $J = 6.5$ Hz, 1H), 1.32−1.90 (m, 22H).

6-Hydroxy-2-(4-pentylbicyclo[2.2.2]oct-1-yl)-4H-1-benzopyran-4-one (8m). Yield 88%, colorless crystals, 180−182 °C. ^{1}H NMR (DMSO-d_6): δ 9.96 (br.s, 1H), 7.47 (d, $J = 9$ Hz, 1H), 7.25 (d, $J = 3$ Hz, 1H), 7.18 (dd, $J = 3 + 9$ Hz, 1H), 6.03 (s, 1H), 1.75−1.86 (m, 6H), 1.37−1.50 (m, 6H), 1.04−1.35 (m, 8H), 0.86 (t, $J = 7$ Hz, 3H).

Intermediate: 6-Benzoyloxy-2-(4-pentylbicyclo[2.2.2]-oct-1-yl)-4H-1-benzopyran-4-one. Yield 49%, colorless crystals, mp 124−126 °C. ^{1}H NMR ($CDCl_3$): δ 8.17−8.25 (m 2H), 7.96−8.00 (m, 1H), 7.61−7.72 (m, 1H), 7.47−7.58 (m, 5H), 6.20 (s, 1H), 1.82−1.95 (m, 6H), 1.43−1.56 (m, 6H), 1.08−1.37 (m, 8H), 0.89 (t, $J = 7$ Hz, 3H).

6-Hydroxy-2-(tricyclo[3.3.1.13,7]dec-1-yl)-4H-1-benzopyran-4-one (8n). Yield 95%, colorless crystals, mp 232 °C (literature[33] 232−235 °C). ^{1}H NMR (DMSO-d_6): δ 9.98 (br.s, 1H), 7.49 (d, $J = 9$ Hz, 1H), 7.26 (d, $J = 3$ Hz, 1H), 7.19 (dd, $J = 3 + 9$ Hz, 1H), 6.04 (s, 1H), 2.02−2.12 (m, 3H), 1.88−1.96 (m, 6H), 1.66−1.78 (m, 6H).

Intermediate: 6-Benzoyloxy-2-(tricyclo[3.3.1.13,7]dec-1-yl)-4H-1-benzopyran-4-one. Yield 47%, colorless crystals, mp 185 °C. ^{1}H NMR (DMSO-d_6): δ 8.15−8.23 (m 2H), 7.84−7.88 (m, 1H), 7.72−7.83 (m, 3H), 7.58−7.68 (m, 2H), 6.18 (s, 1H), 2.04−2.13 (m, 3H), 1.95−2.01 (m, 6H), 1.70−1.80 (m, 6H).

2-[Hexahydro-2,5-methanopentalen-3a(1H)-yl]-6-hydroxy-4H-1-benzopyran-4-one (8o). Yield 95%, colorless crystals, mp 190 °C. ^{1}H NMR (DMSO-d_6): δ 9.98 (br.s, 1H), 7.46 (d, $J = 9$ Hz, 1H), 7.27 (d, $J = 3$ Hz, 1H), 7.18 (dd, $J = 3 + 9$ Hz, 1H), 6.13 (s, 1H), 2.66 (t, $J = 6.6$ Hz, 1H), 2.34 (br.s, 2H), 2.02−2.12 (m, 2H), 1.80−1.92 (m, 4H), 1.62−1.74 (m, 4H).

Intermediate: 6-Benzoyloxy-2-[Hexahydro-2,5-methanopentalen-3a(1H)-yl]-4H-1-benzopyran-4-one. Yield 71%, colorless crystals, mp 200 °C. ^{1}H NMR ($CDCl_3$): δ 8.18−8.26 (m 2H), 8.00−8.03 (m, 1H), 7.48−7.71 (m, 5H), 6.27 (s, 1H), 2.76 (t, $J = 6.6$ Hz, 1H), 2.42 (br.s, 2H), 2.10−2.18 (m, 2H), 1.84−1.98 (m, 4H), 1.64−1.78 (m, 4H).

4.3. Method C. Synthesis of 3-Cyclohexyl-7-hydroxy-4H-1-benzopyran-4-one (9b). 4.3.1. Synthesis of 2-Cyclohexyl-1-(2,4-dihydroxyphenyl)ethanone (19b). A suspension of **17** (1.54 g, 14.1 mmol) in dry dichloroethane was cooled to 0−5 °C and treated with aluminum chloride (1.87 g, 14.1 mmol). Cyclohexaneacetyl chloride (2.3 g, 14.1 mmol) was added slowly, and then, the mixture was stirred for 2 h at room

temperature and for 2 h at 70 °C. The cooled mixture was poured into 1 N aqueous hydrochloric acid and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate and evaporated. The residue was chromatographed on silica gel (cyclohexane/ethyl acetate = 4/1) to afford **19b** (1.31 g, 40%) as colorless crystals; mp 98−100 °C. ^{1}H NMR ($CDCl_3$): δ 12.8 (s, 1H), 7.63−7.69 (m, 1H), 6.35−6.40 (m, 2H), 2.74 (d, J = 6.9 Hz, 2H), 1.62−1.98 (m, 7H), 0.94−1.37 (m, 4H).

4.3.2. 3-Cyclohexyl-7-hydroxy-4H-1-benzopyran-4-one (9b). A mixture of **19b** (1.30 g, 5.59 mmol), triethyl orthoformate (1.24 g, 8.38 mmol), morpholine (2 g, 23 mmol), and DMF (8 mL) was heated to 150 °C for 2 h. Then, the solvent was distilled off and the residue was partitioned between water and ethyl acetate. The organic layer was separated, dried over magnesium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel (cyclohexane/ethyl acetate = 3/1) to yield **9b** (169 mg, 12%). ^{1}H NMR (DMSO-d_6): δ 10.70 (br.s, 1H), 7.99 (s, 1H), 7.88 (d, J = 8.8 Hz, 1H), 6.89 (dd, J = 2.3 + 8.8 Hz, 1H), 6.79 (d, J = 2.3 Hz, 1H), 2.52−2.73 (m, 1H), 1.60−1.80 (m, 5H), 1.05−1.45 (m, 5H).

Analogously, **19a** was prepared starting from **17** and phenylacetyl chloride (**18a**) in 70% yield and then converted into **9a**, which was obtained in 47% yield as colorless crystals; mp 202−205 °C (literature[34] 212−214 °C).

4.4. Method D. Synthesis of 6-Hydroxy-2-(tricyclo-[3.3.1.13,7]dec-1-yl)-4H-1-benzothiopyran-4-one (11c). 4.4.1. Synthesis of 6-Methoxy-2-(tricyclo[3.3.1.13,7]dec-1-yl)-4H-1-benzothiopyran-4-one. 4-Methoxybenzenethiol (**20**; 1.4 g, 10 mmol) was added to polyphosphoric acid (12 mL) preheated to 90 °C under mechanical stirring. At this temperature, ethyl 3-(1-adamantyl)-3-oxopropionate (**21c**; 2.5 g, 10 mmol) was added very slowly to the mixture and stirring was continued for 30 min after the addition. The cooled mixture was vigorously stirred with ice/water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated in vacuo. The crude product was purified by silica gel chromatography (cyclohexane/ethyl acetate = 6/1) to give the title compound as colorless crystals (2.09 g, 64%); mp 140 °C. ^{1}H NMR ($CDCl_3$): δ 7.93 (d, J = 2.9 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1H), 7.22 (dd, J = 2.9 + 8.8 Hz, 1H), 7.00 (s, 1H), 3.92 (s, 3H), 2.08−2.18 (m, 3H), 1.98−2.06 (m, 6H), 1.68−1.87 (m, 6H).

4.4.2. Synthesis of 6-Hydroxy-2-(tricyclo[3.3.1.13,7]dec-1-yl)-4H-1-benzothiopyran-4-one (11c). 6-Methoxy-2-(tricyclo-[3.3.1.13,7]dec-1-yl)-4H-1-benzothiopyran-4-one (1.1 g, 3.5 mmol) was dissolved in dry dichloromethane (30 mL) and treated with boron tribromide (14 mL of 1 M solution in dichloromethane, 14 mmol) under ice-cooling. The mixture was stirred for 1 h at room temperature and then poured onto ice/water. After vigorous stirring, the organic layer was separated, washed cautiously with aqueous sodium bicarbonate solution, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by chromatography on silica gel (cyclohexane/ethyl acetate = 4/1) to obtain **11c** (603 mg, 55%) as colorless crystals; mp 257 °C. ^{1}H NMR (DMSO-d_6): δ 10.20 (br.s, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.65 (d, J = 2.8 Hz, 1H), 7.21 (dd, J = 2.8 + 8.8 Hz, 1H), 6.83 (s, 1H), 2.03−2.14 (m, 3H), 1.90−2.00 (m, 6H), 1.64−1.80 (m, 6H).

Analogously, the following compounds was prepared.

2-(1,1-Dimethylethyl)-6-hydroxy-4H-1-benzothiopyran-4-one (11b). Yield 76%, colorless crystals, mp 188−190 °C from 2-propanol. ^{1}H NMR (DMSO-d_6): δ 10.20 (br.s, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.66 (d, J = 2.8 Hz, 1H), 7.21 (dd, J = 2.8 + 8.8 Hz, 1H), 6.87 (s, 1H), 1.37 (s, 9H).

Intermediate: 2-(1,1-Dimethylethyl)-6-methoxy-4H-1-benzothiopyran-4-one. Yield 20%, viscous oil. ^{1}H NMR ($CDCl_3$): δ 7.94 (d, J = 2.9 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1H), 7.22 (dd, J = 2.9 + 8.8 Hz, 1H), 7.03 (s, 1H), 3.93 (s, 3H), 1.43 (s, 9H).

B. Biology. 1. Expression and Purification of Human STS. STS was produced from a clone of recombinant Chinese hamster ovary cells that stably express the human enzyme.[9] Briefly, the enzyme contained in the microsomal fraction of the cells was solubilized using Triton X-100. The protein then was carried through a sequence of anion exchange and affinity chromatography (DEAE Sephacel, ConA Sepharose, Blue Sepharose); further purification was achieved on PBE94 chromatofocusing and Sephadex G-100 gel filtration columns. The purity of the final enzyme preparation was estimated to >95%. The identity of the protein was confirmed by its reaction with anti-STS polyclonal and monoclonal antibodies in enzyme-linked immunosorbent assay (ELISA) and on Western Blots (kindly provided by Dr. J.-I. Kawano, Miyazaki, Japan)[35] and by N-terminal sequencing.

2. Assay of Purified Human STS. Sulfatase activity was assessed using a method originally described by Eto et al.[36] with modifications. The assay was conducted in white 96 well plates (Packard). Enzyme (0.6 units in 50 μL buffer; one unit is defined as the amount of enzyme producing 1 nmol of 4-methylumbelliferone) was added to 100 μL of 0.75 mM 4-MUS (Sigma) solution in buffer (0.1 M Tris-HCl, pH 7.5, 0.1% Triton). After it was incubated for 60 min at 37 °C, 100 μL of 0.2 M NaOH was added and fluorescence was measured (λ_{ex} = 355 nm, λ_{em} = 448 nm) using a Titertek reader. For inhibition studies, compounds were included at graded concentrations from stock solutions in ethanol; the final ethanol content did not exceed 2%. IC_{50} values were calculated using nonlinear regression (GraFit, Erithacus Software Ltd.). Values reported here are the mean of triplicate determinations, which typically lie in the range of ±20%.

3. Microsomes from Human Placenta. Placenta obtained fresh after delivery was stripped of membranes and frozen at −70 °C for storage. After it was thawed, the tissue was cut into pieces and rinsed in 20 mM Tris-HCl, pH 7.4, 0.25 M sucrose, and 0.02% azide. The pieces were blotted on filter paper and then homogenized using an Ultraturax in 2 volumes of 50 mM Tris-HCl, pH 7.8. After the homogenate was centrifuged at 10 000g for 1 h, 4 °C, the supernatant was collected and the pellet was frozen again at −70 °C. The pellet was thawed and extracted again, and the whole procedure was repeated a third time. The supernatants were pooled and centrifuged at 100 000g, 4 °C, for 1 h. The microsomal pellet was suspended in 50 mM Tris-HCl, pH 7.8, and the protein concentration of the suspension was adjusted to about 10 mg/mL by dilution with the buffer.

4. Determination of K_I and k_{inact}. A 25 μL amount of microsomal suspension was added to 75 μL of inhibitor dilution in 0.1 M Tris-HCl, pH 7.5. The reaction was allowed to proceed for various periods (1−20 min) and was then stopped by adding a suspension of dextran-coated charcoal (5%/0.5%) in buffer. The samples were then centrifuged at 2000g for 1 min. A 100 μL amount of supernatant was transferred to white 96 well plates (Packard) followed by 75 μL of substrate solution (1.5 mM 4-MUS in 0.1 M Tris-HCl, pH 7.5). Plates were incubated at 37 °C for 20 min. Then, the assay was stopped by addition of 50 μL of 0.2 M NaOH. Fluorescence intensity was measured in a Titertek instrument with λ_{ex} = 355 nm and λ_{em} = 460 nm. The fluorescence data were normalized to the intensity of the uninhibited reaction and plotted against the reaction time. Data were fitted to a double-exponential decay using the software Grafit, and the apparent rate constant for the first phase (k_{app}) was obtained. As shown by Kitz and Wilson,[32] the relation $k_{app} = k_{inact}/(1 + K_I/[I])$ describes the kinetics of irreversible enzyme inhibition, where [I] is the inhibitor concentration, K_I is the inhibition constant, and k_{inact} is the rate constant of inactivation. K_I and k_{inact} were obtained from a double-reciprocal plot of k_{app} vs [I]. Finally, the ratio k_{inact}/K_I was calculated as a measure for the overall efficiency of inactivation.

Acknowledgment. We thank Dr. Anthony Winiski for a critical reading of the manuscript.

References

(1) Dibbelt, L.; Kuss, E. Human placental sterylsulfatase. *Biol. Chem. Hoppe-Seyler* **1991**, *372*, 173−185.

(2) Stein, Ch.; Hille, A.; Seidel, J.; Rijnbout, S.; Waheed, A.; Schmidt, B.; Geuze, H.; von Figura, K. Cloning and expression of human steroid-sulfatase. *J. Biol. Chem.* **1989**, *264*, 13865−13872.

(3) Martel, C.; Melner, M. H.; Gagné, D.; Simard, J.; Labrie, F. Widespread tissue distribution of steroid sulfatase, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD), 17β-HSD, 5α-reductase and aromatase activities in the rhesus monkey. *Mol. Cell. Endocrinol.* **1994**, *104*, 103−111.

(4) Pasqualini, J. R.; Gelly, C.; Nguyen, B. L.; Vella, C. Importance of estrogen sulfates in breast cancer. *J. Steroid Biochem.* **1989**, *34*, 155−163.

(5) Reed, M. J.; Purohit, A. Sulphatase inhibitors: The rationale for the development of a new endocrine therapy. *Rev. Endocr.-Relat. Cancer* **1993**, *45*, 51−62.

(6) Utsumi, T.; Yoshimura, N.; Takeuchi, S.; Maruta, M.; Maeda, K.; Harada, N. Elevated steroid sulfatase expression in breast cancers. *J. Steroid Biochem. Mol. Biol.* **2000**, *73*, 141−145.

(7) Utsumi, T.; Yoshimura, N.; Takeuchi, S.; Ando, J.; Maruta, M.; Maeda, K.; Harada, N. Steroid sulfatase expression is an independent predictor of recurrence in human breast cancer. *Cancer Res.* **1999**, *59*, 377−381.

(8) (a) Selcer, K. W.; Jagannathan, S.; Rhodes, M. E.; Li, P. K. Inhibition of placental estrone sulfatase activity and MCF-7 breast cancer cell proliferation by estrone-3-amino derivatives. *J. Steroid Biochem. Mol. Biol.* **1996**, *59*, 83−91. (b) Selcer, K. W.; Hedge, P. V.; Li, P.-K. Inhibition of estrone sulfatase and proliferation of human breast cancer cells by nonsteroidal (p-*O*-sulfamoyl)-*N*-alkanoyl tyramines. *Cancer Res.* **1997**, *57*, 702−707.

(9) Billich, A.; Nussbaumer, P.; Lehr, P. Stimulation of MCF-7 breast cancer cell proliferation by estrone sulfate and dehydroepiandrosterone sulfate: inhibition by novel nonsteroidal steroid sulfatase inhibitors. *J. Steroid. Biochem. Mol. Biol.* **2000**, *73*, 225−235.

(10) Purohit, A.; Woo, L. W. L.; Singh, A.; Winterborn, C. J.; Potter, B. V. L.; Reed, M. J. In vivo actvity of 4-methylcoumarin-7-*O*-sulfamate, a nonsteroidal nonestrogenic steroid sulfatase inhibitor. *Cancer Res.* **1996**, *56*, 4950−4955.

(11) Purohit, A.; Woo, L. W.; Potter, B. V.; Reed, M. J. In vivo inhibition of estrone sulfatase activity and growth of nitrosomethylurea-induced mammary tumors by 667 COUMATE. *Cancer Res.* **2000**, *60*, 3394−3396.

(12) Le Bail, J. C.; Allen, K.; Nicolas, J. C.; Habrioux, G. Dehydroepiandrosterone sulfate estrogenic action at its physiological plasma concentration in human breast cancer cell lines. *Anticancer Res.* **1998**, *18*, 1683−1688.

(13) (a) Faredin, I.; Kokai, K.; Toth, I.; Fazekas, A. G.; Julesz, M. The metabolism of [^{14}C]dehydroepiandrosterone by human skin in vitro. *Acta Med. Acad. Sci. Hung.* **1970**, *27*, 95−102. (b) Voigt, W.; Sawaya, M. E.; Hsia, S. L.; Amthor, C. Cutaneous production of potent androgens from dehydroepiandrosterone in adult women. *IRSC Med. Sci.* **1982**, *10*, 529−530. (c) Sawaya, M. E. Steroid chemistry and hormone controls during the hair follicle cycle. *Ann. N. Y. Acad. Sci.* **1991**, *642*, 376−384.

(14) (a) Hoffmann, R.; Happle, R. Current understanding of androgenetic alopecia. Part I: etiopathogenesis. *Eur. J. Dermatol.* **2000**, *10*, 319−327. (b) Hoffmann, R.; Rot, A.; Niiyama, S.; Billich, A. Steroid sulfatase in the human hair follicle concentrates in the dermal papilla. *J. Invest. Dermatol.* **2001**, *117*, 1342−1348.

(15) Billich, A.; Nussbaumer, P.; Schreiner, E.; Schuster, I. Chromanone and thiochromanone derivatives. **1999**, WO 9952890.

(16) Wolf, O. T.; Kirschbaum, C. Actions of dehydroepiandrosterone and its sulfate in central nervous system: effects on cognition and emotion in animals and humans. *Brain Res. Rev.* **1999**, *30*, 264−288.

(17) (a) Li, P. K.; Rhodes, M. E:, Jagannathan, S.; Johnson, D. A. Reversal of scopolamine induced amnesia in rats by the steroids sulfatase inhbitor estrone-3-*O*-sulfamate. *Cognit. Brain Res.* **1995**, *2*, 251−254. (b) Rhodes, M. E.; Li, P. K.; Burke, A. M.; Johnson, D. A. Enhanced plasma DHEAS, brain acetylcholine and memory mediated by steroid sulfatase inhibition. *Brain Res.* **1997**, *773*, 28−32. (c) Johnson, D. A.; Wu, T.; Li, P.; Maher, T. J. The effect of steroid sulfatase inhibition on learning and spatial memory. *Brain Res.* **2000**, *865*, 286−290.

(18) Daynes, R. A:, Araneo, B. A:, Dowell, T. A.; Huang, K.; Dudley, D. Regulation of murine lymphokine production in vivo. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell function. *J. Exp. Med.* **1990**, *171*, 979−996.

(19) (a) Suitters, A. J.; Shaw, S.; Wales, M. R.; Porter, J. P.; Leonard, J.; Woodger, R.; Brand, H.; Bodmer, M.; Foulkes, R. Immune enhancing effects of dehydroepiandrosterone and dehydroepiandrosterone sulphate and the role of steroid sulphatase. *Immunology* **1997**, *91*, 314−21. (b) Foulkes, R.; Shaw, S.; Suitters, A. Immunological consequences of inhibiting dehydroepiandrosterone (DHEA) sulfatase in vivo. In *Steroid Hormones and the T-Cell Cytokine Profile*; Rook, G. A. W., Lightman, S., Eds.; Springer: New York, 1998.

(20) Poirier, D.; Ciobanu, L. C.; Maltais, R. Steroid sulfatase inhibitors. *Exp. Opin. Ther. Pat.* **1999**, *9*, 1083−1099.

(21) Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. Inactivation of steroid sulfatase by an active site directed inhibitor, estrone 3-*O*-sulfamate. *Biochemistry* **1995**, *34*, 11508−11514.

(22) Elger, W.; Schwarz, S.; Hedden, A.; Reddersen, G.; Schneider, B. Sulfamates of various estrogens − prodrugs with increased systemic and reduced hepatic estrogenicity at oral application. *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 395−403.

(23) (a) Woo, L. W. L.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Active site directed inhibition of estrone sulfatase by nonsteroidal coumarin sulfamates. *J. Med. Chem.* **1996**, *39*, 1349−1351. (b) Li, P. K.; Milano, S.; Kluth, L.; Rhodes, M. E. Synthesis and sulfatase inhibitory activities of nonsteroidal estrone sulfatase inhibitors. *J. Steroid Biochem. Mol. Biol.* **1996**, *59*, 41−48. (c) Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Jejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. Steroidal and nonsteroidal sulfamates as potent inhibitors of steroid sulfatase. *J. Med. Chem.* **1998**, *41*, 1068−1083. (d) Li, P. K.; Chu, G. H.; Guo, J. P.; Peters, A.; Selcer, K. W. Development of potent nonestrogenic estrone sulfatase inhbitors. *Steroids* **1998**, *63*, 425−432. (e) Poirier, D.; Boivin, R. P. 17α-Alkyl- or 17α-substituted-17β-estradiols: a new family of estrone-sulfatase inhibitors. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1891−1896. (f) Purohit, A.; Vernon, K. A.; Hummelinck, A. E.; Woo, L. W.; Hejaz, H. A.; Potter, B. V.; Reed, M. J. The development of A-ring modified analogues of oestrone-3-O-sulphamate as potent steroid sulphatase inhibitors with reduced oestrogenicity. *J. Steroid Biochem. Mol. Biol.* **1998**, *64*, 269−275. (g) Kolli, A.; Chu, H.-H.; Rhodes, M. E.; Inoue, K.; Selcer, K. W.; Li, P.-K. Development of (*p*-*O*-sulfamoyl)-*N*-alkanoylphenylalkylamines as nonsteroidal estrone sulfatase inhibitors. *J. Steroid Biochem. Mol. Biol.* **1999**, *68*, 31−40. (h) Ciobanu, L. C.; Boivin, R. P.; Luu-The, V.; Labrie, F.; Poirier, D. Potent inhibition of steroid sulfatase activity by 3-*O*-sulfamate 17α-benzyl (or 4-*tert*butylbenzyl)estra-1,3,5(10)-trienes: combination of two substituents at positions C3 and 17α of estradiol. *J. Med. Chem.* **1999**, *42*, 2280−2286. (i) Boivin, R. P.; Labrie, F.; Poirier, D. 17Alpha-alkan (or alkyn) amide derivatives of estradiol as inhibitors of steroid-sulfatase activity. *Steroids* **1999**, *64*, 825−833. (j) Boivin, R. P.; Luu-The, V.; Lachance, R.; Labrie, F.; Poirier, D. Structure−activity relationships of 17alpha-derivatives of estradiol as inhibitors of steroid sulfatase. *J. Med. Chem.* **2000**, *43*, 4465−4478.

(24) (a) Woo, L. L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates. *Chem. Biol.* **2000**, *7*, 773−791. (b) Malini, B.; Purohit, A.; Ganeshapillai, D.; Woo, L. W.; Potter, B. V.; Reed, M. J. Inhibition of steroid sulphatase activity by tricyclic coumarin sulphamates. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 253−258.

(25) Ciobanu, L. C.; Boivin, R. P.; Luu-The, V.; Poirier, D. Synthesis and steroid sulphatase inhibitory activity of C19- and C21-steroidal derivatives bearing a benzyl-inhibiting group. *Eur. J. Med. Chem.* **2001**, *36*, 659−671.

(26) Appel R.; Berger G. Über das Hydrazidosulfamid. (On hydrazidosulfamide.) *Chem. Ber.* **1958**, *91*, 1339−1340.

(27) Schwarz, S.; Elger, W. Estrogen sulfamates, a novel approach to oral contraception and hormone replacement therapy. *Drugs Future* **1996**, *21* (1), 49−61.

(28) Erickson, R. H.; Natalie, K. J., Jr.; Bock, W.; Lu, Z.; Farzin, F.; Sherrill, R. G.; Meloni, D. J.; Tach, R. J.; Rzesotarski, W. J.; Clifton, J.; Pontecorvo, M. J.; Bailey, M. A.; Naper, K.; Karbon, W. (Aminoalkoxy)chromones. Selective σ receptor ligands. *J. Med. Chem.* **1992**, *35*, 1526−1535.

(29) Ferrari, M. Multistep preparation of high-purity ipriflavone. EP 941992 A (15.9.1999).

(30) Wang, H.-K.; Bastow, K. F.; Cosentino, L. M.; Lee, K.-H. Antitumor agents. 166. Synthesis and biological evaluation of 5,6,7,8-substituted-2-phenylthiochromen-4-ones. *J. Med. Chem.* **1996**, *39*, 1975−1980.

(31) (a) Miksicek, R. J. Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol.* **1993**, *44* (1), 37−43. (b) Miksicek, R. J. Estrogenic flavonoids: structural requirements for biological activity. *Proc. Soc. Exp. Biol. Med.* **1995**, *208*, 44−50. (c) Davis, S. R.; Dalais, F. S.; Simpson, E. R.; Murkies, A. L. Phytoestrogens in health and disease. *Recent Prog. Horm. Res.* **1999**, *54*, 185−211.

(32) Kitz, R.; Wilson, I. B. Esters of methanesulfonic acid as irreversible inhibitors of acetylcholin-esterase. *J. Biol. Chem.* **1962**, *237*, 3245−3249.

(33) Le Floc'h, Y.; Lefeuvre, M. Synthesis of (dihydroxyphenacylidene)triphenylphosphoranes, new flavonoid precursors. Synthesis of 6- and 7-hydroxychromones. *Tetrahedron Lett.* **1986**, *27* (24), 2751−2752.

(34) Baker, W.; Chadderton, J.; Harborne, J. B.; Ollis, W. D. A new synthesis of isoflavones. Part I. *J. Chem. Soc.* **1953**, 1852−1860.
(35) Kawano, J.-I.; Kotani, T.; Umeki, K.; Oinuma, T.; Ohtaki, S.; Aikawa, E. A monoclonal antibody to rat liver arylsulfatase C and its application in immunohistochemistry. *J. Histochem. Cytochem.* **1989**, *37*, 683−690.
(36) Eto, Y.; Rampini, S.; Wiesmann, U.; Herschkowitz, N. N. Enzymic studies of sulphatases in tissues of the normal human and in metachromatic leukodystrophy with multiple sulphatase deficiencies: arylsulphatases A, B and C, cerebroside sulphatase, psychosine sulphatase and steroid sulphatases. *J. Neurochem.* **1974**, *23*, 1161−1170.
(37) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

JM020878W