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Nortropinyl-Arylsulfonylureas as Novel, Reversible Inhibitors of Human Steroid Sulfatase

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Abstract—Steroid sulfatase (STS) has emerged as an attractive target for a range of estrogen- and androgen-dependent diseases. Searching for novel chemotypes as STS inhibitors, we identified nortropinyl-arylsulfonylurea **3** as a hit from high-throughput screening. A series of analogues was prepared in order to explore the essential structural elements for STS inhibition, and first structure–activity relationships were established. Mechanistic investigations revealed that the compounds are reversible, competitive inhibitors of STS.

Steroid sulfatase (STS) acts as the first enzyme in metabolic pathways converting sulfated steroid hormone precursors (e.g., estrone sulfate and dehydroepiandrosterone sulfate) to the active hormones which bind to estrogen or androgen receptors. Therefore, STS has been recognized as a potential drug target for hormone-dependent diseases.[1–4] STS inhibitors have been suggested for the therapy of breast, endometrial and prostate cancer,[1,2] and for androgen-dependent skin diseases, such as acne.[5] To date, the therapeutic concept of STS inhibition is still at a preclinical stage, with only one compound (667COUMATE[6]) being announced to enter clinical trials in the near future.

The most potent STS inhibitors known to date feature an aryl sulfamate moiety and act in an irreversible manner. Estrone sulfamate (**1**, Fig. 1), discovered by Potter and co-workers in 1994,[7] has served as the lead for all sulfamate-type inhibitors.[3,4] In general, there are two issues associated with this compound class: potential estrogenicity and chemical instability. Several recent studies have addressed the estrogenicity issue, resulting in the identification of potent, non-estrogenic inhibitors (for review, see ref 4). However, the chemical instability in solution (as described for estrone sulfamate[8]) appears to be an inherent problem for all aryl sulfamates, due to the reactivity of the sulfamate functionality, and might complicate or even impede development and clinical use. While one class of steroidal reversible STS inhibitors lacking the sulfamate group (see compound **2** in Fig. 1 as an example) has been described by Poirier and co-workers,[9,10] its activity does not approach that of the most potent sulfamate-type inhibitors.

In this situation, discovery of new, preferentially non-steroidal inhibitor classes would be welcome. As a result of a high-throughput screening for STS inhibitors, we obtained compound **3** (Fig. 1), initially synthesized by Fulvio Gadient in 1967 as potential hypoglycemic agent (for closely related analogues, see ref 11), as a hit. In this paper we describe the inhibitory profile of **3** and some initial structure–activity relationships (SARs) of a novel class of STS inhibitors.

Chemistry

The synthesis of the nortropinyl-arylsulfonylureas **6** was accomplished by reacting the central intermediate **4**[12] with various arylsulfonyl isocyanates **5** (Scheme 1) in toluene or pyridine. **5a** (R = 4-Cl), **5b** (R = 4-F), **5d** (R = H), and **5e** (R = 4-Me) were commercially available, while **5c** (R = 4-Br), **5f** (R = 4-CF_3), and **5g** (R = 3,5-diCF_3) were prepared in situ from the corresponding sulfonyl chlorides by treatment with NaOCN and pyridine in acetonitrile according to a published

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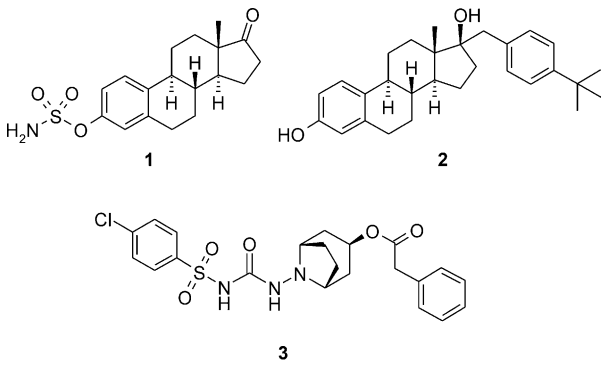
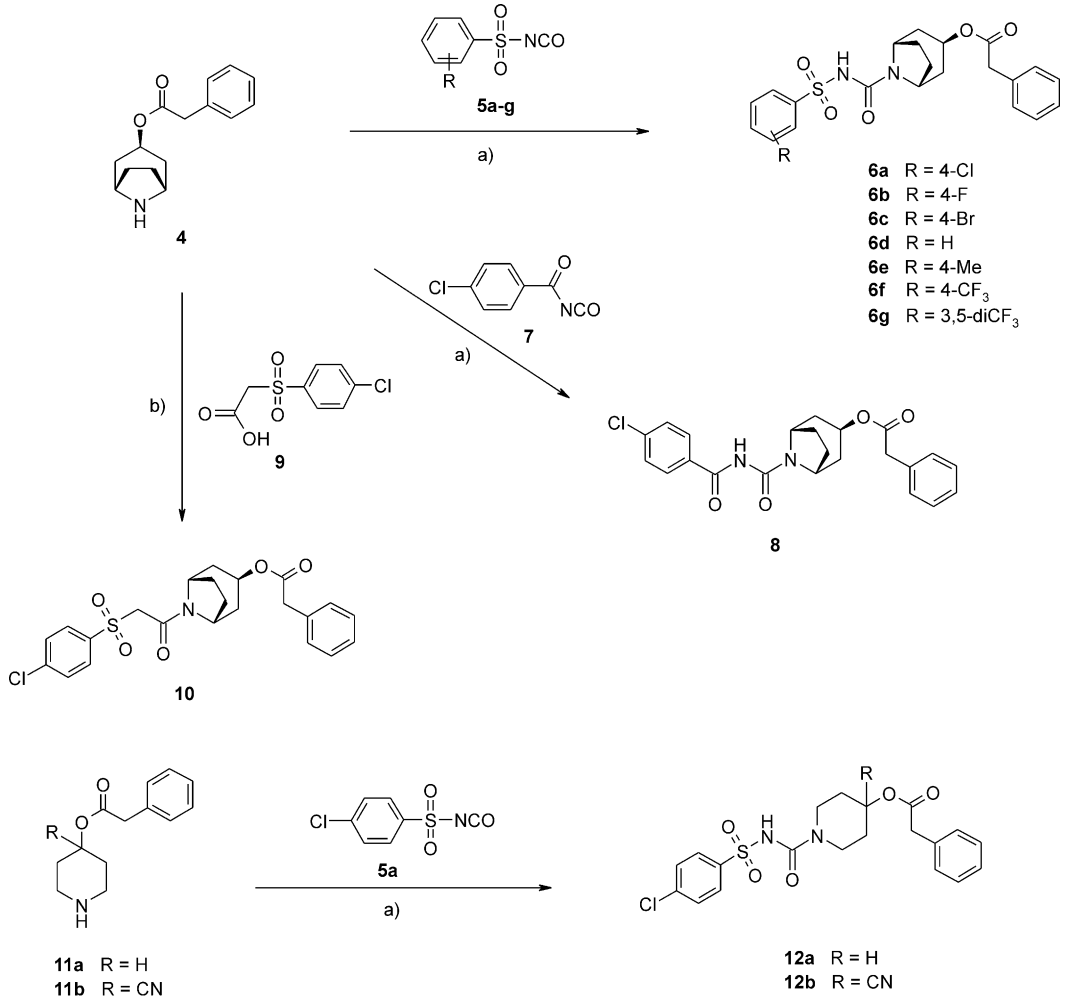


Figure 1. Structural formulae of STS inhibitors.

method.[13] In this case, the isolation and purification of the products was more laborious (repeated chromatography), resulting in low to moderate yields (8–50%), but no efforts have been invested so far to optimise the procedure. In contrast, usually good yields (60–85%) were obtained when the purchased starting materials **5** were used. Benzoylurea analogue **8** was synthesised by reacting amine **4** with benzoyl isocyanate **7**, which was prepared from 4-chlorobenzamide and oxalyl chloride in analogy to a literature procedure and used without purification.[14] Coupling of the commercially available acid **9** with the secondary amine **4** using *n*-propylphosphonic acid anhydride (PPA) provided the carba-analogue **10**, and the unbridged piperidine derivatives **12** were prepared by treatment of amines **11a**,**b** with sulfonyl isocyanate **5a**. Intermediate **11b** was obtained by acylation of the cyanohydrin of *N*-benzyl-4-piperidone followed by debenzylation using catalytic hydrogenation. All test compounds were characterized by ^{1}H NMR, MS, HRMS, TLC, and HPLC, and the structures fully complied with the analytical data.

Steroid sulfatase assays

Compounds were tested in an enzymatic assay using human STS (purified to homogeneity from recombinant CHO cells) as described.[15] In brief, the STS-catalysed cleavage of 4-methylumbelliferyl sulfate (4-MUS) was monitored in a discontinuous fluorimetric assay in the absence or presence of inhibitor; initial velocity v was calculated from the linear phase of the reaction as FU (fluorescence units) per min. Inhibition constants (K_i values) were calculated from kinetic data obtained at different inhibitor concentrations using [S]/v versus [S] diagrams



Scheme 1. Synthesis of nortropinyl-arylsulfonylureas. Reaction conditions: (a) toluene or pyridine, rt, 4–16 h, 6–85% yield; (b) Et_3N/PPA/cat. DMAP, DMF, rt, 16 h, 30% yield.

(Hanes plots). In some instances, IC_{50} values were measured using the method described previously.[16]

To assess the effect of test compounds on the activity of STS in intact cells, an assay using recombinant CHO cells and 4-MUS as substrate was used as previously described,[17] using the assay variant for reversible inhibitors.

Characterisation of the hit compound (3)

High-throughput screening (HTS) of our substance collections for STS inhibitors using the assay with purified enzyme yielded compound **3** (Fig. 1) as a hit. Kinetic analysis (Fig. 2) revealed that the compound is a pure competitive inhibitor of STS with an inhibition constant (K_i) of 890 nM. Pre-incubation of STS with **3** did not enhance inhibition, indicating that it does not act as a time-dependently inactivating inhibitor. Furthermore, enzyme activity was fully recovered when the enzyme-inhibitor complex was treated with dextran-activated charcoal (data not shown), demonstrating that **3** acts as a reversible inhibitor of STS.

The K_i for estrone sulfamate (**1**) has been determined to be 670 nM.[18] Thus, the binding affinities of **1** and **3** towards STS are of the same order of magnitude. However, since **1** irreversibly inactivates STS, its IC_{50} was found to be approximately 10-fold lower than the K_i value of **1** and **3** after a 1 h incubation period under our assay conditions.

Cellular activity of **3** was measured in CHO cells overexpressing STS.[17] In this assay, **3** was about 128 times less potent than the irreversible standard compound **1**.

Some STS inhibitors, in particular **1**,[19] have been shown to be estrogenic, which might limit their potential clinical usefulness. Compound **3** did not stimulate the growth of the estrogen-dependent breast cancer cell line MCF-7 (assayed as described in ref 15) up to the highest test concentration of 10 μM, and thus is devoid of estrogenicity, at least in this in vitro cell culture system.

Structure–activity relationships

After having established the mode of action of **3** we explored which structural features were essential for the inhibitory activity. When the tractability and synthetic accessibility of the hit compound were analyzed, the hydrazine moiety was recognized as a hurdle for rapid SAR exploration. To reduce the synthetic complexity, analogue **6a** lacking the exocyclic nitrogen atom was prepared and tested. Since **6a** was only 2.7-fold less active than **3** (based on the comparison of the K_i values against purified STS, Table 1), we decided to prepare a collection of further derivatives without the original hydrazine structural element while keeping the phenylacetate residue constant. This led to **6a–g** as the first series of derivatives; their STS inhibitory activities are listed in Table 1. Among the halogen substituents investigated, chlorine (**6a**) and bromine (**6c**) gave comparable results, whereas the fluorine derivative **6b** was

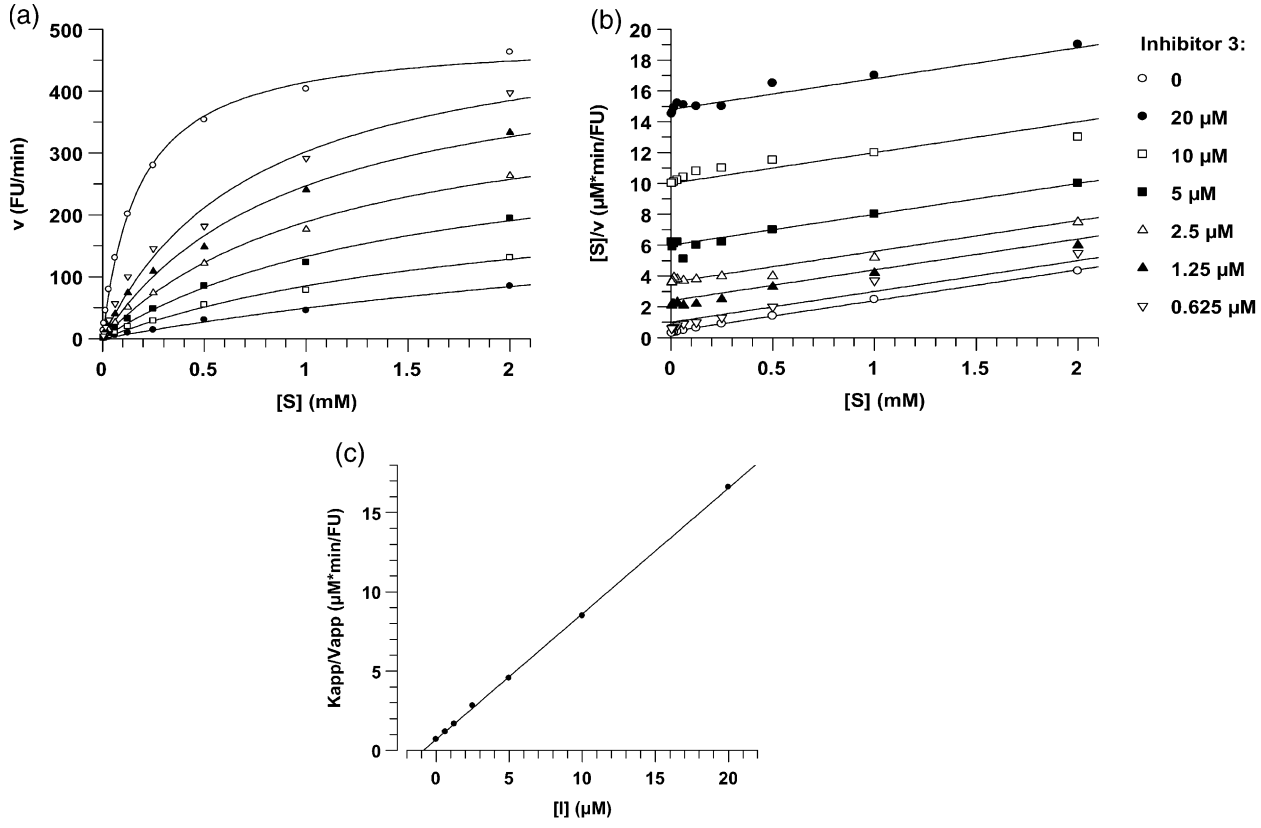


Figure 2. Mode of action and K_i determination of inhibitor **3**: (a) rate of substrate turnover (v) at various concentrations of **3**; (b) same data plotted in a Hanes diagram; (c) plot of K_{app}/V_{app} ratios against inhibitor concentration [I]; the linear correlation indicates pure competitive inhibition of the enzyme, the K_i value is obtained from the intercept with the abscissa.

Table 1. Inhibitory activities of test compounds against purified human STS and against STS over-expressed in CHO cells

Compd	Purified STS		CHO-STS cells
	K_i (μM)	IC_{50} (μM)	IC_{50} (μM)
1	0.67[a]	0.056[b]	0.03
3	0.89	—[c]	3.84
6a	2.4	—	6.72
6b	—	50	39.2
6c	1.85	—	6.15
6d	—	>100	>30
6e	—	16.6	37.1
6f	0.53	—	7.47
6g	0.076	0.084	1.89
8	5.8	—	10.4
10	—	6.2	4.32
12a	—	>100	>30
12b	—	13	24.5

[a]Taken from ref 17 (there calculated by the method of Kitz and Wilson for an irreversible inhibitor).
[b]Note that the IC_{50} value for **1**, an irreversible inhibitor, depends on incubation time; the value given is for the 1 h time point of our standard assay.[15]
[c]Not done.

substantially less effective. Analogue **6d**, lacking an additional substituent at the sulfonylated phenyl ring, did not show any inhibition of STS up to the highest test concentrations (30 or 100 μM). The formal introduction of a *para*-methyl group (**6e**) restored some inhibitory activity, but **6e** was about 10-fold less potent than the chloro and bromo analogues **6a** and **6c**. This indicated that not only the size but also electronic effects of the substituents were important for potent inhibitory activity of this compound class. This finding was further supported by the trifluoromethyl derivatives **6f** and **6g**. While **6f** was already more active than **6a** against purified STS, compound **6g** with two trifluoromethyl groups at positions 3 and 5 of the sulfonylated phenyl ring was the best inhibitor out of this series. Based on the K_i values as a measure for binding of the compound to the enzyme, **6g** (76 nM) was found to be superior to the irreversible inhibitor **1** (670 nM) by almost one order of magnitude. Using purified STS, the IC_{50} values of both compounds were determined to be in a similar range (84 nM for **6g** and 56 nM for **1**, respectively).

Next, we investigated whether the sulfonylurea and the nortropine moieties were essential elements for STS blocking. Compounds **8**, the benzoylurea analogue of **6a**, and **10**, a carba-analogue of **6a**, still showed inhibitory potency in the low micromolar range, but were less active than the corresponding sulfonylurea analogue **6a**. When the nortropine core was replaced by 4-hydroxypiperidine (**12a**), activity was completely lost. Some potency could be regained by introducing a cyano group at position 4 of the piperidine residue (**12b**), forcing the phenylacetate side chain into a slightly different orientation compared to **12a**. This result indicates that in the nortropine compounds the bridge itself might not be required for high potency, but that it controls the orientation of the side chain via the stereochemistry of the hydroxy functionality. More detailed investigations are needed to further clarify this aspect.

The compounds of this study were also tested for STS inhibition in intact CHO cells over-expressing the enzyme (Table 1). The cellular potencies of the new compounds were relatively weak when compared to the irreversibly acting standard **1**. In general, the cellular results correlated with the cell-free data with regard to the ranking of the compounds. However, the improved cell-free potency of derivatives **6f** and, particularly, **6g** did not translate into significantly increased cellular activity relative to the screening hit **3**, indicating a cell penetration problem for **6f**,**g**. As the cell-free and cellular data for compounds **3** and its 'simplified' analogue **6a** correlate very nicely, it can be ruled out that the discrepant results obtained with **3** in comparison to **6f**,**g** are due to the structural modification in the core region, that is elimination of the exocyclic nitrogen atom. Both analogues **6f**,**g** feature the trifluoromethyl group as substituent. One can speculate that physicochemical properties of this moiety, that is high lipophilicity, render the compounds less cell permeable. In any case, insufficient cellular potency was recognized as weakness of these first representatives of the novel inhibitor class.

In summary, we have discovered a novel class of reversible, competitive inhibitors of STS and established first insight into its SAR. Work is in progress to improve cellular activity of the compounds.

References and Notes

1. Pasqualini, J. R.; Gelly, C.; Nguyen, B. L.; Vella, C. *J. Steroid Biochem.* **1989**, *34*, 155.
2. Reed, M. J.; Purohit, A. *Rev. Endocrine Relat. Cancer* **1993**, *45*, 51.
3. Poirier, D.; Ciobanu, L. C.; Maltais, R. *Exp. Opin. Ther. Pat.* **1999**, *9*, 1083.
4. Nussbaumer, P.; Billich, A. *Exp. Opin. Ther. Pat.* **2003**, *13*, 605.
5. Billich, A.; Rot, A.; Lam, C.; Schmidt, J. B.; Schuster, I. *Horm. Res.* **2000**, *532*, 92.
6. Woo, L. L.; Purohit, A.; Malini, B.; Reed, M. J.; Potter, B. V. *Chem. Biol.* **2000**, *7*, 773.
7. Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 219.
8. Sahm, U. G.; Williams, G. J.; Purohit, A.; Hidalgo Aragones, M. I.; Parish, D.; Reed, M. J.; Potter, B. V. L.; Pouton, C. W. *Pharm. Sci.* **1996**, *2*, 17.
9. Poirier, D.; Boivin, R. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1891.
10. Ciobanu, L. C.; Boivin, R. P.; Luu-The, V.; Labrie, F.; Poirier, D. *J. Med. Chem.* **1999**, *42*, 2280.
11. Jucker, E.; Lindenmann, A.; Schenker, E.; Gadient, F. FR 6273, 1968. *Chem. Abstr* **1971**, *74*, 125454.
12. Bertholdt, H.; Pfleger, R.; Schulz, W. *Drug Res.* **1967**, *17*, 719.
13. Chiang, G.C.; Temeng, K.O.; Davis, R.F. EP0759431, 1997.
14. Weikert, R. J.; Bingham, S., Jr.; Emanuel, M. A.; Fraser-Smith, E. B.; Loughhead, D. G.; Nelson, P. H.; Poulton, A. L. *J. Med. Chem.* **1991**, *34*, 1630.
15. Billich, A.; Nussbaumer, P.; Lehr, P. *J. Steroid Biochem. Mol. Biol.* **2000**, *73*, 225.
16. Nussbaumer, P.; Lehr, P.; Billich, A. *J. Med. Chem.* **2002**, *45*, 4310.

17. Wolff, B.; Billich, A.; Brunowsky, W.; Herzig, G.; Lindley, I.; Nussbaumer, P.; Pursch, E.; Rabeck, C.; Winkler, G. *Anal. Biochem* **2003**, *318*, 276.
18. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508.
19. Elger, W.; Schwarz, S.; Hedden, A.; Reddersen, G.; Schneider, B. *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 395.