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N-Acyl arylsulfonamides as novel, reversible inhibitors of human steroid sulfatase

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Abstract—Steroid sulfatase (STS) is an attractive target for a range of oestrogen- and androgen-dependent diseases. In search of novel chemotypes of STS inhibitors, we had previously identified nortropinyl–arylsulfonylureas **1**; however, while these compounds were good inhibitors of purified STS (lowest K_i = 76 nM), they showed only weak inhibition of STS activity in cells (lowest IC_{50} around 2 μM). Extended structure–activity relationship studies involving modification of the phenylacetyl side chain and replacement of the nortropine element by simpler scaffolds led to the discovery of *N*-acyl arylsulfonamides, more specifically *N*-(Boc-piperidine-4-carbonyl)-benzenesulfonamides, as STS inhibitors, some of which exhibit improved cellular potency (best IC_{50} = 270 nM).

1. Introduction

Steroid sulfatase (STS) catalyses the desulfation of steroid hormone precursors, such as oestrone and dehydroepiandrosterone sulfate. The steroid sulfates are delivered from the circulation to the target tissues, where they are cleaved by STS as the first step in the local production of oestrogens and androgens. Inhibition of STS should result in reduced local levels of the active hormones, and thus presents a potential new therapeutic option in the treatment of oestrogen- and androgen-dependent diseases, such as breast, endometrial and prostate cancers, acne and androgenic alopecia.[1–6] Clinical validation of the concept is still missing, which might reflect that the appropriate compound for clinical testing has not been identified yet, although numerous potent STS inhibitors have been described in the literature (for recent review see Ref. 6).

Recently, we reported on the discovery and preliminary structure–activity relationship (SAR) of nortropinyl–arylsulfonylureas **1** as reversible inhibitors of steroid sulfatase.[7] Starting from **1a**, compounds featuring high inhibitory activity towards purified STS were identified, with analogue **1b** as the most active derivative (Table 1); however, the compounds were only poor inhibitors of STS in cells (best IC_{50} = 1.89 μM) (**1b**, Table 1). In this paper, we present extended SAR studies leading to the discovery of *N*-acyl arylsulfonamide as novel scaffold for STS inhibitors (**4a**–**i**). Among these compounds,

Table 1. Inhibitory activity of test compounds against purified human STS and against STS over-expressed in CHO cells (for structures see Fig. 1)

Compound	Purified STS		CHO–STS cells
	K_i (μM)	IC_{50} (μM)	IC_{50} (μM)
EMATE	0.67[a]	0.056[b]	0.03
1a	2.4	0.91	6.72
1b	0.076	0.084	1.89
2	—[c]	>100	—
3a	—	>100	—
3b	14.5	3.6	12.0
4a	16.1	8.6	9.8
4b	—	>100	—
4c	—	71	—
4d	—	6.1	9.90
4e	—	0.45	1.26
4f	0.012	0.026	0.27
4g	—	2.8	—
4h	—	1.90	—
4i	0.22	0.21	0.78
4j	—	1.79	—
4k	—	10.2	—

[a] Taken from Ref. 11.
[b] Note that the IC_{50} value for the irreversible inhibitor EMATE depends on incubation time; the value given is for the 1 h time point of our standard assay (Ref. 9).
[c] Not done.

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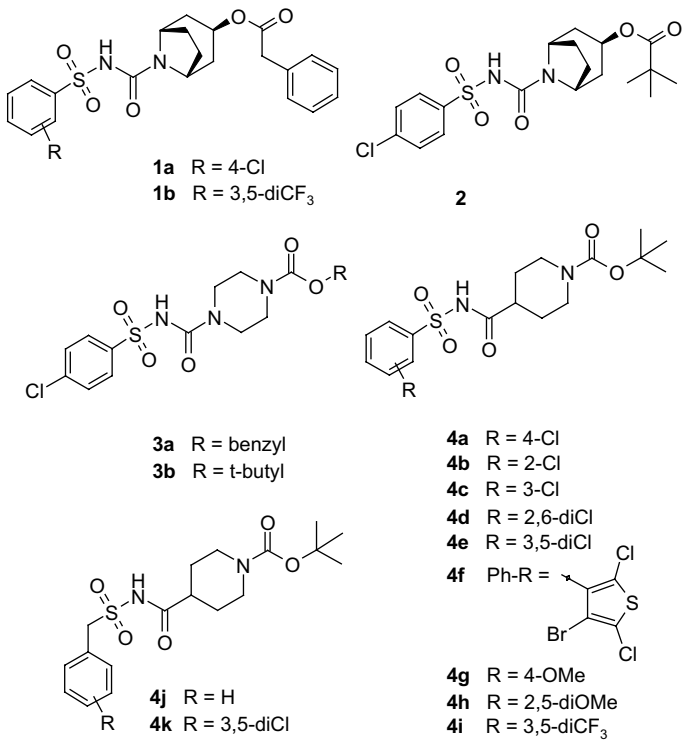


Figure 1. Structural formulae of STS inhibitors.

derivatives with improved cellular activity were identified (Fig. 1).

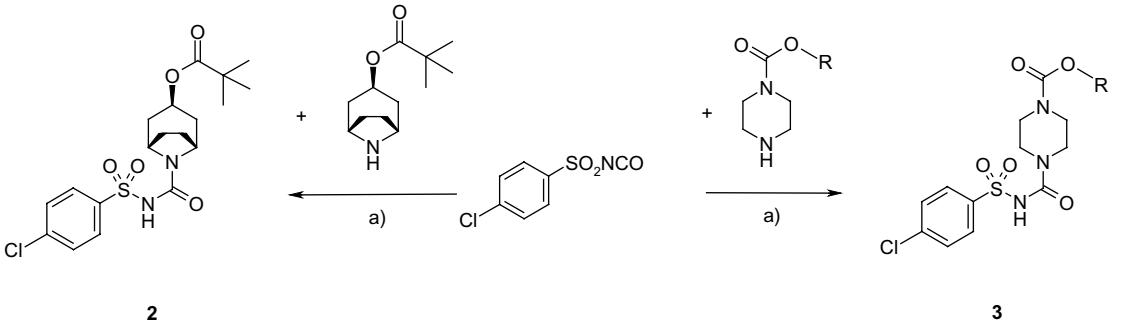
2. Chemistry

Sulfonylurea-type inhibitors **2** and **3** were synthesized by reacting 4-chlorophenylsulfonyl isocyanate with the corresponding secondary amines (Scheme 1). The required nortropine building block was prepared by *O*-acylation with pivaloyl chloride using an *N*-Boc-protection/deprotection protocol. Synthesis of acylsulfonamides **4a**–**i** (Scheme 2) started from substituted arylsulfonyl chlorides, which were readily converted into the corresponding sulfonamides using ion exchange resin Dowex 50W X4 to sequester excess of ammonia. Without further purification, crude sulfonamides were *N*-acylated in high yields with *N*-Boc-piperidinecarboxylic acid applying propylphosphonic anhydride (PPA) as coupling reagent. Benzyl analogues **4j**,**k** were prepared analogously.

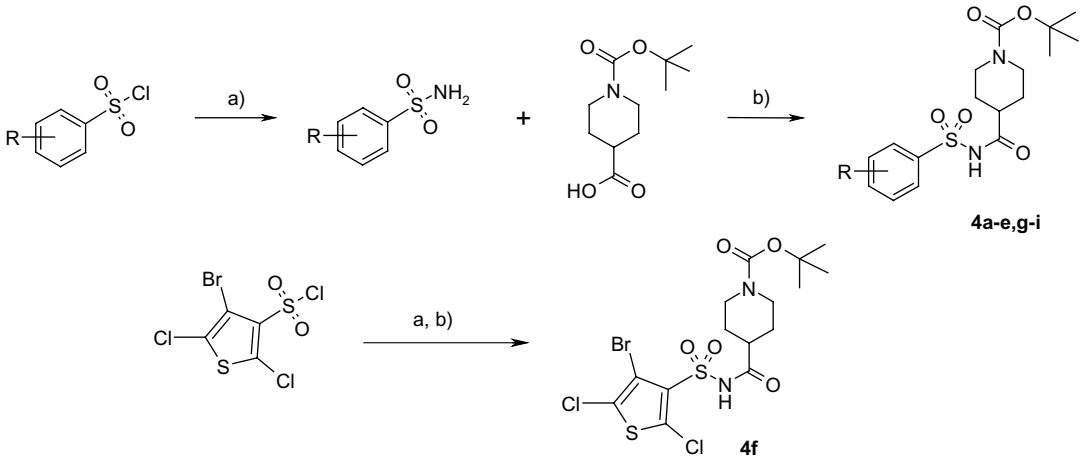
All test compounds were characterized by ^{1}H NMR, (HR)MS, TLC and HPLC, and the structures fully complied with the analytical data.

3. Steroid sulfatase assays

Compounds were tested in an enzymatic assay using human STS (purified to homogeneity from recombinant CHO cells) as described.[8] In brief, the STS-catalyzed cleavage of 4-methylumbelliferyl sulfate (4-MUS) was monitored at 37 °C in a fluorimetric assay (1 h incubations) 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 (Hanes plots). IC_{50} values were measured using the method described previously.[9] The concentrations of 4-MUS were varied between 0.05



Scheme 1. Synthesis of arylsulfonylureas. Reaction conditions: (a) toluene or pyridine, rt, 4–16 h, 60–85% yield.



Scheme 2. Synthesis of acylsulfonamides **4a**–**i**. Reaction conditions: (a) NH_4OH/ethyl acetate, rt, 16 h, then Dowex 50WX4; (b) PPA/DIEA, cat. DMAP, DMF, rt, 16 h, 85–96% overall yield.

and 2 mM in the case of measurement of steady-state kinetics, and were fixed at 0.75 mM in the case of IC_{50} determinations.

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 (0.5 mM) was used as previously described,[10] using the assay variant for reversible inhibitors (using live cells and 4 h incubations).

4. Discussion of biological results

Starting from lead compound **1a**, we had been able to enhance the potency of this new STS inhibitor class by appropriate aryl substitution at the arylsulfonylurea moiety.[7] The present study deals with modification of the bridged nortropine and the attached phenylacetyl side chain, particularly motivated by the search for simpler central scaffolds.

Initial attempts in this direction, partly disclosed in the previous publication,[7] had been unsuccessful. Thus, cutting out the bridge in the nortropine element to generate the corresponding piperidine analogue resulted in loss of activity. Furthermore, the new derivative **2** featuring the pivaloyl instead of the phenylacetyl ester in **1a** was found to be inactive (Table 1). When investigating additional analogues, we discovered that moderate inhibitory activity can be achieved by a concomitant change to a simpler template (i.e., piperazine in **3b**) and to the *t*-butoxycarbonyl residue as side chain. Surprisingly, the corresponding benzyloxycarbonyl piperazine **3a** with a side chain similar to that of **1a** was inactive. Further variation of the central scaffold yielded compound **4a** in which formally one nitrogen atom of the piperazine scaffold in **3b** was replaced by carbon. Although this compound shows 10-fold less inhibitory potency relative to the lead **1a** against purified STS, the cellular activities of both compounds are in the same range (Table 1). This result encouraged us to further investigate the potential of acylsulfonamides of type **4a** as STS inhibitors.

Based on the SAR finding that the potency of sulfonylurea-based STS inhibitors **1** was strongly dependent on the aryl substitution pattern, we synthesized and tested a collection of derivatives of **4a** with different aryl substituents. The results with selected analogues **4b**–**i** are shown in Table 1. When the *para*-chloro substituent in **4a** is moved to the *ortho* and *meta* position (**4b**,**c**) potency is substantially reduced. Considering that the *ortho*-chloro analogue **4b** does not show inhibitory activity up to 100 μM, it is surprising that the 2,6-dichloro derivative **4d** with an IC_{50} value of 6.1 μM is as potent as **4a**. Substantial improvement in potency was achieved with the 3,5-dichloro compound **4e**, which shows an IC_{50} value against purified STS in the submicromolar range. In the cellular assay system **4e** displays an IC_{50} value of 1.26 μM, which is only about 3-fold higher than the inhibitory potency in the cell-free STS assay. Good agreement between the CHO and purified enzyme data were also observed for the other chlorophenyl acylsulfonamides tested, that is, **4a** and **4d**. The highest potency in this series was obtained with the fully halogenated analogue **4f**, which features a 4-bromo-2,5-dichloro-3-thienyl moiety as the aryl element. The IC_{50} and K_i values of **4f** against purified STS are 26 and 12 nM, respectively. Thus, compound **4f** binds much better to the target and reaches a comparable IC_{50} relative to the standard irreversible inhibitor EMATE. This is remarkable because acylsulfonamides **4** were proven to be reversible inhibitors of STS (data not shown). In STS-overexpressing CHO cells, **4f** is also the most potent inhibitor out of this series with an IC_{50} value of 270 nM, exceeding the cellular activity of all representatives of the original sulfonylurea lead class **1**.[7]

The results obtained with additional analogues **4g**–**i** (Table 1) demonstrate that STS inhibition can also be achieved with other aryl substituents in acylsulfonamides **4**. As seen in the previous sulfonylurea series with **1b**,[7] the 3,5-diCF_3 substitution pattern yields a compound with high inhibitory potency. Acylsulfonamide analogue **4i** is not as active as **1b** against purified STS but is superior to **1b** with regard to cellular activity. In

general, acylsulfonamide-based STS inhibitors **4** show improved cellular activity over sulfonylureas **1** when comparing activity towards purified and cellular STS. So far, we have not identified a rationale for the obtained SAR with regard to the aryl substitution, as both electron-withdrawing and electron-donating groups can yield potent inhibitors and a clear correlation with *o*-, *m*-, and *p*-substitution is missing. However, in general, double (or multiple) halogen substitution at positions 3 and 5 is preferred and consistently provides high potency. We also investigated the potential of benzylsulfonamides (**4j**,**k**) as STS inhibitors. Surprisingly, the (aryl)-unsubstituted compound **4j** was found to be superior to its dichloro derivative **4k**. As this subclass did not show any advantage over the arylsulfonamide analogues it was not followed further.

For selected compounds (**3b**, **4a**,**f**,**i**), measurement of steady-state kinetics was performed and inhibition constants (K_i) were determined. In all cases, the compounds showed pure competitive inhibition, similar to our previous finding for the nortropinyl–arylsulfonylureas **1a** and **1b**.[7] Selected test compounds were investigated for chemical stability and oestrogenicity, issues associated with some STS inhibitor classes, in particular the irreversibly acting arylsulfamates.[6] As expected, the results indicate that acylsulfonamides **4** are chemically stable in bulk and do not exhibit oestrogenic activity in vitro (data not shown).

In summary, by modification of the nortropine scaffold and the phenylacetyl side chain we obtained *N*-acyl arylsulfonamides **4** as STS inhibitors with improved cellular potency. These compounds are currently further profiled in order to assess their potential use for a clinical proof-of-concept trial.

References and notes

1. Poirier, D.; Ciobanu, L. C.; Maltais, R. *Expert Opin. Ther. Pat.* **1999**, *9*, 1083.
2. Nussbaumer, P.; Billich, A. *Expert Opin. Ther. Pat.* **2003**, *13*, 605.
3. Winum, J. Y.; Scozzafava, A.; Montero, J. L.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2004**, *14*, 1273.
4. Purohit, A.; Woo, L. W.; Chander, S. K.; Newman, S. P.; Ireson, C.; Ho, Y.; Grasso, A.; Leese, M. P.; Potter, B. V.; Reed, M. J. *J. Steroid Biochem. Mol. Biol.* **2003**, *86*, 423.
5. Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Med. Chem.* **2003**, *10*, 925.
6. Nussbaumer, P.; Billich, A. *Med. Res. Rev.* **2004**, *24*, 529.
7. Nussbaumer, P.; Geyl, D.; Horvath, A.; Lehr, P.; Wolff, B.; Billich, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3673.
8. Billich, A.; Bilban, M.; Meisner, N. C.; Nussbaumer, P.; Neubauer, A.; Jäger, S.; Auer, M. *Assay Drug Dev. Technol.* **2004**, *2*, 21.
9. Nussbaumer, P.; Lehr, P.; Billich, A. *J. Med. Chem.* **2002**, *45*, 4310.
10. Wolff, B.; Billich, A.; Brunowsky, W.; Herzig, G.; Lindley, I.; Nussbaumer, P.; Pursch, E.; Rabeck, C.; Winkler, G. *Anal. Biochem.* **2003**, *318*, 276.
11. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508.