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6-(2-Adamantan-2-ylidene-hydroxybenzoxazole)-*O*-sulfamate: A Potent Non-steroidal Irreversible Inhibitor of Human Steroid Sulfatase

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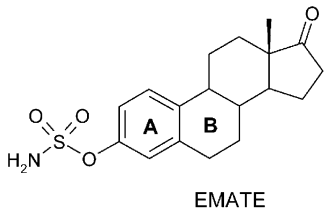
Abstract—We report the synthesis and results from the in vitro evaluation of 6-(adamantan-2-ylidene-hydroxybenzoxazole)-*O*-sulfamate **1** as an irreversible inhibitor of human steroid sulfatase (STS). Highly straightforward, condensation of 2-methyl-6-hydroxybenzoxazole with 2-adamantanone, subsequent elimination of water and sulfamoylation provide the title compound in 45% overall yield from the inexpensive 2,4-dihydroxyacetophenone. **1** was found to be a potent irreversible inhibitor of purified human steroid sulfatase (STS) and specific for this enzyme relative to human arylsulfatases A and B. In cellular assays with human keratinocytes, sebocytes and fibroblasts, **1** blocked STS activity with IC_{50} values in the range of 0.15–0.8 nM, and in MCF-7 breast cancer cells with $IC_{50} = 2.3$ nM, while it did not bind to estrogen receptors α and β. Thus, **1** is a candidate for further investigation of its potential as a drug to be used in androgen- and estrogen-dependent diseases.

Steroid sulfatase (E.C. 3.1.6.2., STS) catalyses the hydrolysis of steroid-3-*O*-sulfates to yield the corresponding steroids. The enzyme has emerged as an attractive drug target, because it is crucial for the local production of active estrogens and androgens from their systemic circulating sulfated precursors, such as estrone sulfate and dehydroepiandrosterone sulfate (DHEAS), in diseased tissues.[1] In particular, there is evidence that the STS pathway is a major source of estrogens in breast cancer tissue,[1,2] suggesting inhibitors of STS as potential therapeutic agents for the treatment of breast cancer.

In the skin, DHEAS is cleaved to DHEA, which is further converted into the androgens testosterone and dihydrotestosterone.[3] An important role of STS in the pathogenesis of androgen-dependent skin diseases, such as androgenetic alopecia and acne, has been proposed.[4]

Finally, STS may regulate the availability of DHEA as a neurohormone in the brain: treatment of rats with inhibitors of STS resulted in enhanced learning and spatial memory.[5]

The most potent STS inhibitors described so far are aryl sulfamates which act as irreversible inhibitors. These include steroidal compounds such as estrone sulfamate (EMATE, Fig. 1), the first potent inhibitor of STS,[6] and non-steroidal inhibitors featuring partial replacements of the steroid skeleton (see refs. 7 and 8 for reviews). While EMATE as the prototype compound is highly estrogenic,[9] a variety of compounds devoid of estrogenicity have been described which may be better suited for therapeutic use.[10]



EMATE

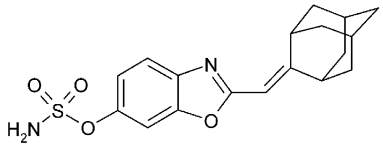


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Figure 1.

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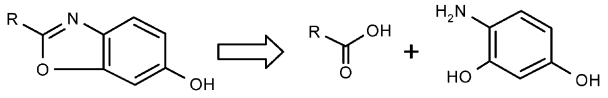
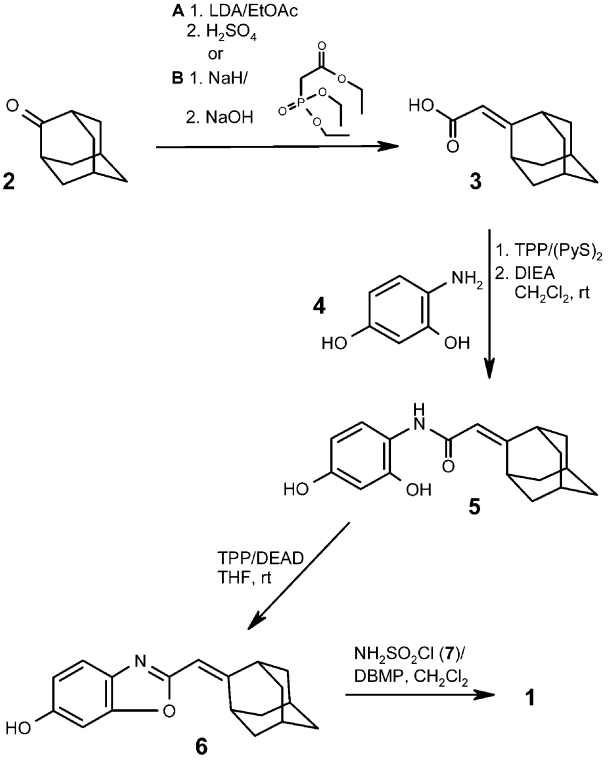
We aimed at identifying non-estrogenic irreversible inhibitors of STS as a new therapeutic approach for the treatment of acne. In addition to our efforts to identify inhibitors by replacement of the steroid skeleton with 6,6-bicyclic ring systems,[11] we also investigated benzoxazoles as a mimicry for the steroidal A- and B-rings, as exemplified by **1** (Fig. 1).[12]

We devised a general synthetic approach for substituted 6-hydroxy-benzoxazoles featuring a hydrophobic residue in the 2-position, starting from the appropriate 2-aminophenol and the corresponding acids:



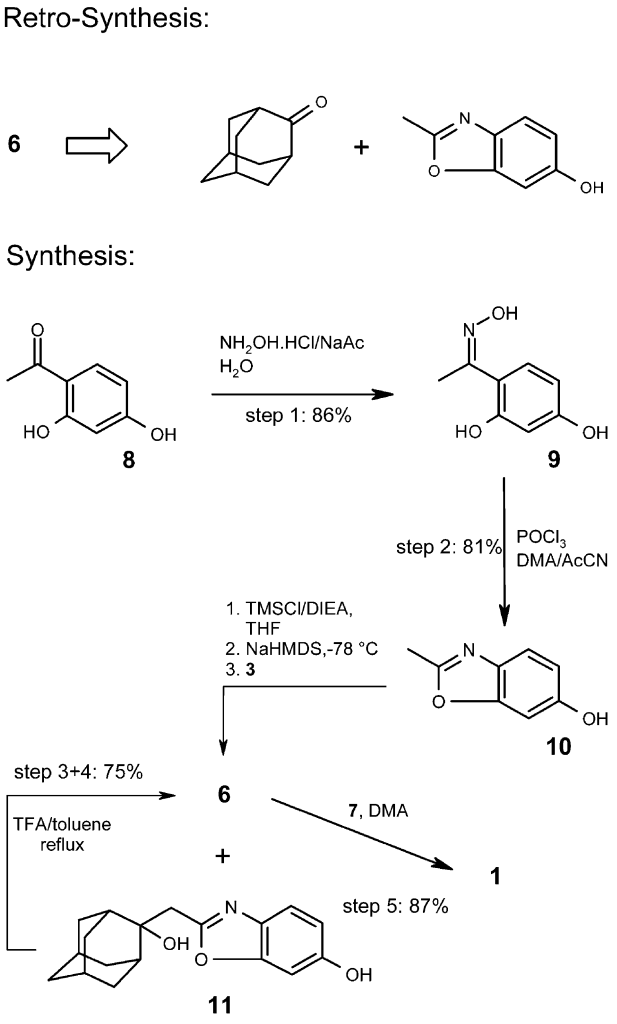
For the preparation of **1**, 2-adamantanone (**2**) was transformed into adamantylidene acetic acid (**3**) either by addition of the sodium enolate of EtOAc and subsequent acid-induced elimination of water (A)[13] or by Horner–Emmons olefination (B)[14] in 72 and 46% yield, respectively. Coupling of **3** with 2,4-dihydroxyaniline (**4**) was achieved via the 2-thiopyridyl ester obtained from treatment with triphenylphosphane (TPP) and 2,2′-dipyridyl-disulfide in a one-pot reaction producing the anilide **5** in 91% yield. Cyclisation of **5** performed with TPP/diethyl azodicarboxylate (DEAD) gave the corresponding benzoxazole **6** in 67% yield. Finally, reaction of **6** with amidochlorosulfonic acid[15] (**7**) in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) produced the sulfamate **1** in 88% yield (Scheme 1).[16]



Scheme 1.

While this sequence provided **1** in an overall yield of 39%, however, the building blocks **2** and **4** are quite expensive and used early in the synthesis. Furthermore, three chromatographic purification steps are involved and the potential need for a replacement of the reagents TPP/2,2′-dipyridyldisulfide and TPP/DEAD during large-scale synthesis made this approach unattractive for up-scaling. Therefore, we developed an improved synthetic route towards **1**, devoid of these disadvantages (Scheme 2).

Starting from the inexpensive 2,4-dihydroxyacetophenone (**8**), treatment with an aqueous solution of hydroxylammonium chloride/sodium acetate gave the crystalline oxime **9** in 86% yield.[17] Beckmann rearrangement of **9**, induced by means of $POCl_3$ in a mixture of MeCN/dimethylacetamide (DMA), gave crude 6-hydroxy-2-methylbenzoxazole (**10**)[18] (81%), which was used in the next step without further purification. In situ protection of the phenolic hydroxyl group as trimethylsilyl ether, subsequent deprotonation with sodium bis(trimethylsilyl)amide and condensation with 2-adamantanone at −78 °C produced a mixture (1:2.5) of the desired olefin **6** and the primarily formed addition product **11** in almost quantitative yield. To achieve complete conversion of **11** into **6**, the crude reaction



Scheme 2.

mixture was refluxed with TFA in toluene for 7 h. At this stage purification was achieved by crystallisation of the trifluoroacetic acid salt of **6** from ethanol to give pure **7** (75%).[19] Finally, heating of **6** with **7** in DMA at 50 °C for 1 h and crystallization after aqueous workup cleanly provided **1** in 87% yield. Within this sequence the most expensive synthone **2** is introduced late in the synthesis and no chromatographic purification is needed. Overall yield amounted to 45%.

The target compound **1** inactivated purified human STS irreversibly. From the kinetics of inactivation, followed by measuring residual enzyme activity after incubation with various concentrations of the inhibitor,[11,20] an inhibition constant (K_i) of 6 μM and a rate constant of inactivation (k_{inact}) of 0.097 μs^{-1} was obtained. These values may be compared with $K_i = 0.47$ μM and $k_{inact} = 0.009$ s^{-1} reported for EMATE as a reference compound.[20] While **1** shows weaker binding to STS than EMATE, the efficiency of inactivation, as expressed by the ratio of k_{inact}/K_i^{-1}, is about equal (1.6 vs $1.9 \cdot 10^4$ s^{-1} M^{-1}) for the two compounds.

To assess the selectivity of **1** for STS over human arylsulfatases A (ASA) and B (ASB), both enzymes were isolated from human placenta,[21] and assayed in presence of **1**.[22] Whereas ASA was not inhibited by **1** up to the highest test concentration of 10 μM, μASB was weakly inhibited (by 25% at 10 μM) in a time-independent fashion, suggesting reversible binding to the active site of this enzyme.

Measurement of enzyme inhibition in cellular systems is one step closer to the in vivo situation. First, we assessed the ability of **1** to inhibit human STS in CHO cells over-expressing STS:[23] an IC_{50} of ~37.5 nM was determined (Table 1). Furthermore, we tested for inhibition of STS-catalysed hydrolysis of DHEAS in different cell types of the skin, namely keratinocytes, fibroblasts, and sebocytes (Table 1).[24] IC_{50} values in the range of 0.15–0.8 nM were obtained. Finally, the IC_{50} value for inhibition of STS-dependent cleavage of estrone sulfate by the human breast cancer cell line MCF-7 was 2.3 nM.[25]

These IC_{50} values roughly follow the amount of enzyme activity in the various cell types (data not shown), which is in line with the irreversible action of **1** and hence a dependence on enzyme concentration.

The title compound **1** did not show any measurable affinity to the human estrogen receptors α and β ($EC_{50} > 100$ μM) whereas for EMATE EC_{50} values of 2.6 and 3.7 μM were determined.[26]

Table 1. Inhibition of human STS in different cell types

Cell type	IC_{50} (nM)
STS-expressing CHO cells	31.8±3.6
Keratinocytes	0.77±0.32
Fibroblasts	0.75±0.15
Sebocytes	0.15±0.01
MCF-7 breast cancer cells	2.3±0.8

In conclusion, we identified **1** as the first potent non-estrogenic irreversible inhibitor of human steroid sulfatase featuring a 5,6-bicyclic ring system as a mimicry for the steroidal A- and B-ring. **1** is a candidate for further evaluation of its potential as a drug in the treatment of estrogen- and androgen-dependent diseases.

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16. For **1**: ^1H NMR (250 MHz, DMSO-d_6) δ 8.02 (br.s, 2H), 7.76 (d, $J = 8.6$ Hz, 1H), 7.62 (d, $J = 2.2$ Hz, 1H), 7.26 (dd, $J = 8.6$ and 2.2 Hz, 1H), 4.46 (s, 2H), 6.24 (s, 1H), 4.22 (s, 1H), 2.68 (s, 1H), 1.79–2.05 (m, 12H). ^{13}C NMR (250 MHz, DMSO-d_6) δ (ppm): 2167.76, 163.44, 148.82, 147.28, 140.02, 119.44, 105.43, 103.60, 40.97, 40.03, 39.01, 36.62, 33.50, 27.45, IR (neat) ν_{max} 1192, 1388 cm^{-1}, MS (APCI) m/z 361.1 $[MH]^+$.
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19. Procedure: 60 g (0.4 mol) of 6-hydroxy-2-methyl-benzoxazole (**10**) were dissolved in 1 L of dry THF, cooled to 0 °C, and 75 mL (0.44 mol) of DIEA and 52 mL (0.42 mol) trimethylsilyl chloride were added. Then the ice bath was removed and stirring continued for 2 h. For the deprotonation the reaction mixture was cooled to −78 °C

and 680 mL of a 2 M NaHMDS solution in THF were added within 2 h. After one additional hour solid 66 g (0.44 mol) 2-adamantanone was added and kept at −78 °C for another hour. Then, the reaction mixture was allowed to warm up to rt, poured into 3 L of an 1M aqueous $NaHSO_4$ solution and 5 L of ethyl acetate were added. The layers are separated, and the organic phase was washed once with 1 L of a 1M aqueous $NaHSO_4$ solution and with 1 L of brine, and was dried over sodium sulfate. The solvent is evaporated and the residue obtained is re-dissolved in 2.4 L of toluene and 32 mL of TFA by heating at 115 °C for 7 h. Filtration of the precipitate formed in the course of cooling to rt afforded 125 g of the trifluoroacetic acid salt of **6**. A second-crystallisation from ethanol gave 66 g (59%) 6-hydroxy-2-adamantan-2-ylidenemethyl-benzoxazole (**6**). Concentration of the mother liquor yielded a second crop of 18 g (16%). MP 262 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (br.s, 1H), 7.44 (d, J = 8.5 Hz, 1H), 6.96 (d, J = 2.2 Hz, 1H), 6.78 (dd, J = 8.5 and 2.2 Hz, 1H), 6.11 (s, 1H), 4.18 (s, 1H), 2.60 (s, 1H), 1.75–1.99 (m, 12H). ^{1}H NMR (125 MHz, DMSO-d_6) δ 164.53, 160.61, 155.77, 149.99, 134.10, 119.17, 113.03, 103.081, 96.89, 40.56, 38.64, 36.41, 32.94, 27.45.

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25. STS activity in MCF-7 cells was measured using estrone sulfate as substrate, as described in: Billich, A.; Nussbaumer, P.; Lehr, P. *J. Steroid Biochem. Mol. Biol.* **2000**, *73*, 225.

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