Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4999–5002
Bioorganic & Medicinal Chemistry Letters

Estrone formate: a novel type of irreversible inhibitor of human steroid sulfatase

Erwin P. Schreiner* and Andreas Billich

Novartis Institutes for BioMedical Research, GDC, Brunner Strasse 59, A-1235 Vienna, Austria

Received 10 June 2004; revised 2 July 2004; accepted 6 July 2004
Available online 29 July 2004

Abstract—A series of estrone conjugates of the type estrone-3-O-C(O,S)-X have been prepared and evaluated for inhibition of human steroid sulfatase (STS). Among the carbamate (**6**), thiocarbamate (**8**), cyanate (**7**), formate (**9**), and acetate (**10**) analogs of estrone, only **9** was found to inhibit STS in a time- and concentration-dependent manner. With an IC_{50} of 0.42 μM **9** is the first potent inactivator of STS which does not feature the sulfamate group. Furthermore a formate-type inhibitor featuring a benzoxazole moiety in place of the steroid skeleton (**14**) was prepared, suggesting a general principle of inactivation by the formate group. As the mode of action we propose an immediate transfer of the formyl moiety to a nucleophilic residue in the active site of STS.

1. Introduction

Steroid sulfatase (E.C. 3.1.6.2., STS) has received considerable interest as a drug target, due to its role in the local production of androgens and estrogens within target tissues.[1] Indications envisaged for STS inhibitors comprise steroid hormone-dependent cancers (of breast, endometrium, and prostate), androgen-dependent skin diseases (acne, androgenetic alopecia), and cognitive dysfunction.

The first steroidal inhibitors of STS were phosphonate, thiophosphonate[2], and sulfonate[3] conjugates of steroids such as compounds **1**–**3** (Fig. 1) designed as analogs of the substrate estrone sulfate. Whereas **1**–**3** act as reversible inhibitors, the first highly potent STS blocker estrone sulfamate (**4**, EMATE) inhibits STS in an irreversible manner, presumably by transferring the sulfamoyl moiety to the active site of the enzyme.[4] In addition to studies[5,6] addressing the replacement of the steroid moiety by bicyclic scaffolds we searched for novel types of STS inhibitors by replacing the sulfamoyl residue of **4**. For this purpose we synthesized a series of estrone conjugates of the type estrone-3-O–C(O,S)–X– and tested them for inhibition of STS.

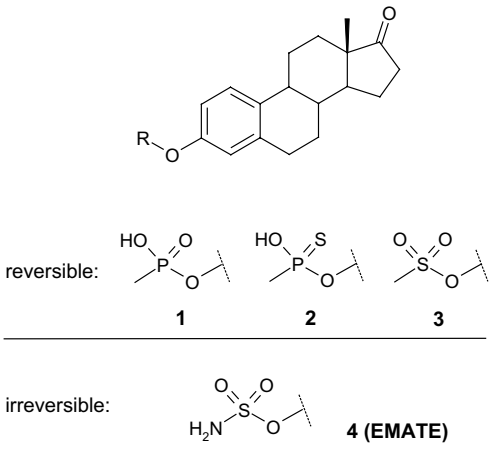


Figure 1.

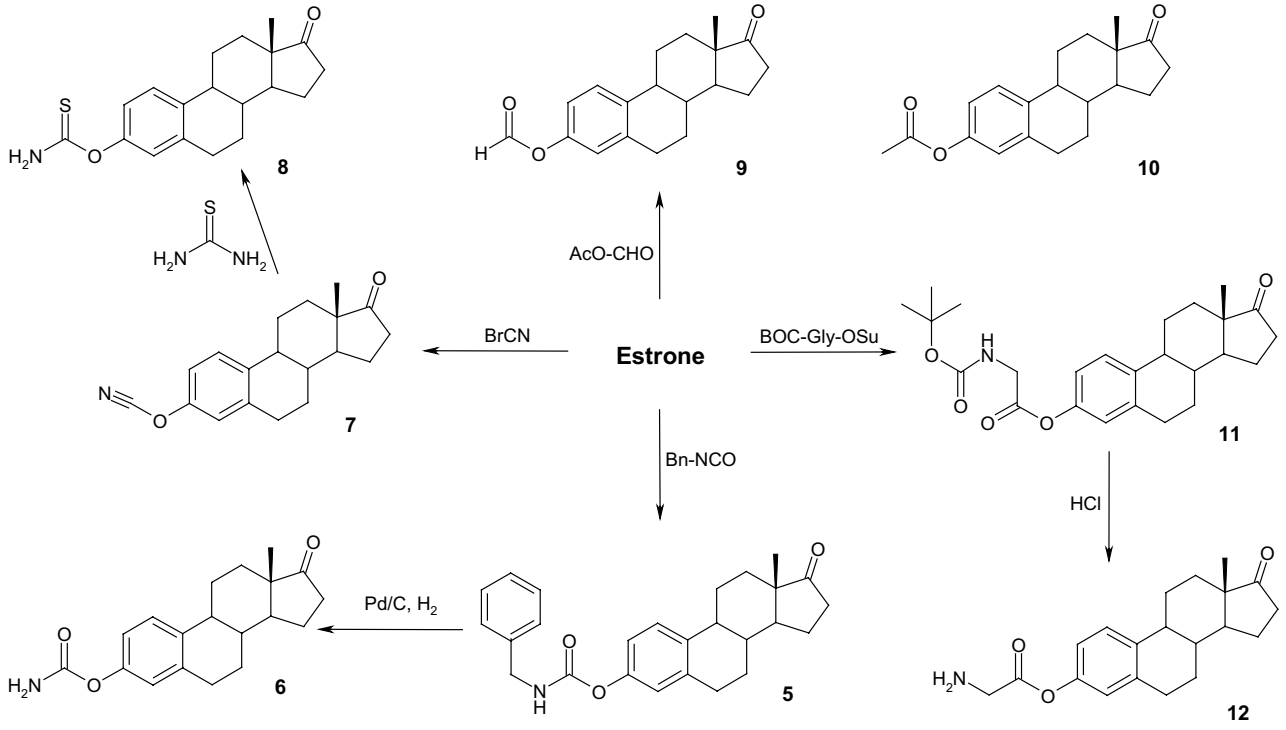
2. Results

To obtain the carba analog of EMATE (**4**) we treated estrone with benzyl isocyanate providing the *N*-benzylcarbamate of estrone **5**[7] in 75% yield (Scheme 1). Subsequent removal of the benzyl group by hydrogenolysis (Pd/C, H_2, *i*PrOH/EtOAc, rt, 52%) gave the carbamate **6**.[8]

Due to the incompatibility of the thiocarbonyl with a Pd-catalyzed hydrogenation reaction we chose the

Keywords: Steroid sulfatase; Inactivator; Estrone formate.
* Corresponding author. Tel.: +43-1-866349004; fax: +43-1-8663-4354; e-mail: erwin.schreiner@pharma.novartis.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.07.013



Scheme 1. Preparation of estrone conjugates **5**–**12**.

cyanate **7**, which can be accessed by treatment of estrone with BrCN,[9] as starting material for the preparation of the thiocarbamate **8**. Treatment of **7** with thiourea[10] in acetone then produced **8** in 60% yield.[11] Estrone formate (**9**)[12] was prepared by treatment of estrone with formyl acetate. Estrone acetate (**10**) was obtained from a commercial source (Sigma).

Furthermore, we synthesized the glycine ester of estrone as a homolog of **6**. Coupling of estrone with the succinimide ester of *N*-Boc-protected glycine in presence of DMAP afforded **11** in 92% yield. Subsequent acidic cleavage of the *N*-Boc group gave the glycine ester of estrone as its stable hydrochloride salt **12**.[13]

Compounds were tested in an enzymatic assay using human STS (purified to homogeneity from recombinant CHO cells) as described.[14] In brief, the STS-catalyzed cleavage of 4-methylumbelliferyl sulfate (4-MUS) was monitored in a discontinuous fluorimetric assay in the absence or presence of inhibitor. IC_{50} values were calculated using the method described previously.[5]

Only one compound out of our first series, that is, the formate derivative **9**, showed inhibitory activity against STS (Table 1). Its IC_{50} of 0.42 μM is only 7.5-fold above the value for EMATE (**4**).

We asked, whether **9** would act as a reversible or irreversible inhibitor of the enzyme, and measured residual activity of STS after incubation with **9** or the reference compound **4**, following the protocol described in Ref. 5. In fact, both compounds time dependently inactivated the enzyme (Fig. 2) demonstrating the irreversible mode of action of **9**.

Table 1. Inhibition of purified human STS by **4** (EMATE) and **5**–**12**

Compound	IC_{50} [μM]
4	0.056
5	>50
6	>50
7	>50
8	>50
9	0.42
10	>50[a]
11	>50
12	>50
14	1.5
15	>30
17	>10

[a] In line with data in Ref. 20.

To obtain a first idea whether the SAR for the formate esters is similar to that of the sulfamates we chose a clearly distinct phenol, 6-adamantan-2-ylidene-hydroxybenz-oxazole **13**, as an alternative parent compound.[6] The corresponding nonestrogenic sulfamate of **13** showed an IC_{50} of 0.26 μM in our assay system. Using the same procedure as described for **9**, we obtained the corresponding formate **14** in 32% yield and determined an IC_{50} value of 1.5 μM for STS inhibition. The IC_{50} value for this structurally quite different aryl formate is about 5.8-fold above the IC_{50} for the sulfamate. This may indicate that aryl formates are a novel general class of inactivators of STS with a SAR quite similar to that observed for the sulfamates. In fact, simple phenyl

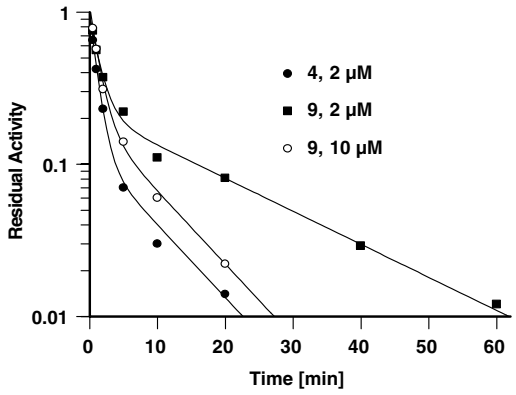
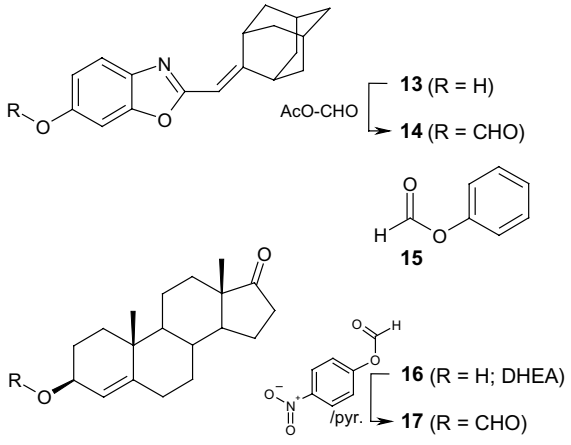


Figure 2. Time-dependent inhibition of human STS by **4** and **9**; data are fitted to a bis-exponential decay, as described for **4**.[4b]

formate **15** was inactive up to a concentration of 30 µM, in line with an IC_{50} value of >10 mM reported for the corresponding sulfamate.[15]

Although, DHEAS is a natural substrate of human STS, the corresponding sulfamate does not inactivate STS.[16] To investigate whether the formate derived from DHEA (**16**) is an inhibitor, we transformed **16** into **17** by treatment with 4-nitrophenyl formate in pyridine (48 h, rt; 76%) (Scheme 2).[17] Similar to the DHEA-derived sulfamate, DHEA formate **17** was found to be inactive up



Scheme 2. Preparation of formates **14** and **17**.

to the highest test concentration of 10 µM (which was limited by the poor aqueous solubility of the compound).

3. Discussion

For the transfer of the sulfamate residue of EMATE to the active site of STS that causes irreversible inhibition the following mechanisms have been proposed: (a) formation of an *N*-sulfonylated imine,[18,19] (b) generation of a reactive aminosulfene intermediate, which subsequently reacts with a nucleophilic amino acid side chain in the active site;[20] and (c) nucleophilic attack by an amino acid side chain at the sulfur atom subsequent to a proton transfer process.[4] For formates, a mechanism equivalent to (a) cannot be formulated. A mechanism in analogy to (b) for formates may be supported by ab initio calculations in the gas phase,[21] as well as experimental observation of carbon monoxide formation upon treatment of formates with strong bases in aprotic solvents[22]. However, considering these artificial conditions we think this unlikely to occur in the active site of STS.

Finally, several reports[23–25] on reaction of aryl formates with aliphatic and aromatic amines suggest attack by a nucleophile at the electrophilic formate carbonyl atom as the most likely mechanism (outlined by the mechanism depicted in Fig. 3). Since the formyl conjugates of a hydrated oxo-serine or histidine are rather instable and, therefore, not appropriate to cause long-lasting irreversible inhibition, we suggest one of two lysines in the active site, Lys134 or Lys368,[26] as the final formyl acceptor. Details about this proposed mechanism remain to be elucidated.

In summary, we identified aryl formates, such as **9** and **14**, as a novel type of STS inhibitors, causing irreversible inactivation of the enzyme in a concentration-dependent manner. As a potential mode of action we suggest an immediate transfer of the formyl group to a nucleophile in the active site of STS.

Acknowledgements

We thank Peter Nussbaumer and Dieter Scholz for critical reading of the manuscript.

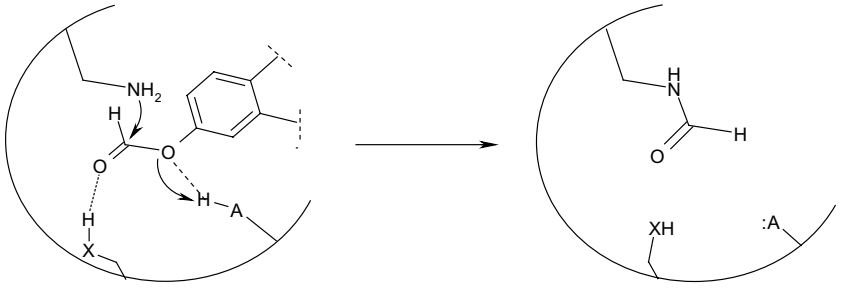


Figure 3. Potential mechanism for the inactivation of STS by transfer of the formyl moiety from **9**.

References and notes

1. For reviews see: (a) Pasqualini, J. R.; Gelly, C.; Nguyen, B. L.; Vella, C. *J. Steroid Biochem.* **1989**, *34*, 155; (b) Reed, M. J.; Purohit, A. *Rev. Endocrine Related Cancer* **1993**, *45*, 51; (c) Poirier, D.; Ciobanu, L. C.; Maltais, R. *Exp. Opin. Ther. Patents* **1999**, *9*, 1083; (d) Nussbaumer, P.; Billich, A. *Exp. Opin. Ther. Patents* **2003**, *13*, 605.
2. Howarth, N. M.; Cooper, G.; Purohit, A.; Duncan, L.; Reed, M. J.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 313.
3. Li, P.-K.; Pillai, R.; Dibbelt, L. *Steroids* **1995**, *60*, 299.
4. (a) Howarth, N. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 219; (b) Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. L. V.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508.
5. Nussbaumer, P.; Lehr, P.; Billich, A. *J. Med. Chem.* **2002**, *73*, 4310.
6. Billich, A.; Schreiner, E. P.; Wolff-Winiski, B. **2001**, WO 0136398 A1.
7. Procedure: 270 mg (1 mmol) of estrone and 122 μL (1 mmol) of benzylisocyanate in toluene (4 mL) were stirred at 100 °C. Then the solvent was evaporated and the residue obtained purified by filtration over silica gel (Et_2O/*c*-Hex = 2/1) followed by crystallization affording 302 mg of **5** (75%). mp = 152–154 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.36–7.25 (m, 6H), 6.93–6.88 (m, 2H), 5.38 (t, J = 5.5 Hz, 1H), 4.45 (d, J = 6.0 Hz, 2H), 2.91 (dd, J = 8.5, 4.0 Hz, 2H), 2.57–1.39 (series of multiplets, 13H), 0.91 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 220.5, 154.5, 137.5, 136.0, 128.7, 127.6, 126.2, 121.6, 118.8, 50.3, 47.9, 45.2, 44.0, 37.9, 35.8, 31.5, 29.3, 26.3, 25.7, 21.5, 13.7.
8. Onken, D.; Kutz, K.; Martin, D. *Zeitschr. f. Chem.* **1971**, *11*, 65.
9. Onken, D.; Martin, D.; Rackow, S. *Zeitschr. f. Chem.* **1969**, *9*, 228.
10. Grigat, E.; Puetter, R. *Chem. Ber.* **1965**, *98*, 1186.
11. Procedure: 300 mg (0.97 mmol) of **7** and 150 mg (2 mmol) of thiourea in acetone (2 mL) were stirred at room temperature overnight. The reaction mixture was diluted with water (10 mL), the precipitate was separated by filtration and purified by chromatography over silica gel (EtOAc/*c*-Hex = 2/1) providing 113 mg of **8** (34%). mp = 198–202 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.31 (d, J = 8.5 Hz, 1H), 6.89 (dd, J = 8.5, 2.6 Hz, 1H), 6.83 (d, J = 2.6 Hz 1H), 6.70 (br s, 1H), 6.48 (br s, 1H), 2.95 (dd, J = 8.5, 4.0 Hz, 2H), 2.57–1.42 (series of multiplets, 13H), 0.91 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 220.8, 192.1, 151.3, 137.9, 126.2, 122.2, 119.5, 50.4, 47.9, 44.2, 37.9, 35.8, 31.5, 29.4, 26.3, 25.7, 21.6, 13.8.
12. Edwards, R. W. H. *J. Chromatogr.* **1963**, *12*, 212.
13. **11**: To a solution of 270 mg (1 mmol) of estrone and 548 mg (2 mmol) of Boc-Gly-OSuc in dichloromethane (10 mL) 300 mg (2.5 mmol) of DMAP was added and stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAC/*c*-Hex (1/1; 30 mL), and extracted with aq NaH_2PO_4 solution (1 M) and brine. Evaporation of the solvent and purification by chromatography over silica gel (EtOAc/*c*-Hex = 1/2) gave 405 mg of **11** (92%). mp = 60–62 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.28 (d, J = 8.5 Hz, 1H), 6.87 (dd, J = 8.5, 2.6 Hz, 1H), 6.83 (d, J = 2.6 Hz 1H), 5.10 (br s, 1H), 4.15 (d, J = 5.7 Hz, 2H), 2.95 (dd, J = 8.5, 4.0 Hz, 2H), 2.56–1.43 (series of multiplets, 13H), 0.91 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 220.7, 169.3, 148.1, 138.1, 137.7, 126.4, 121.3, 118.4, 80.2, 50.4, 47.9, 44.1, 42.6, 37.9, 35.8, 31.5, 29.3, 28.3, 26.3, 25.7, 21.5, 13.8 **12**: To a solution of 220 mg (0.5 mmol) of **11** in Et_2O (4 mL) etheral hydrochloric acid (3 M; 4 mL) was added dropwisely and stirred at room temperature for 4 h. The precipitate was filtered off, washed with dry ether and dried in vacuo delivering 185 mg of **12** (98%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.48 (br s, 1H), 7.14 (d, J = 8.5 Hz, 3H), 6.92 (dd, J = 8.5, 2.6 Hz, 1H), 6.82 (d, J = 2.6 Hz 1H), 4.02 (br s, 2H), 4.15 (d, J = 5.7 Hz, 2H), 2.88 (m, 3H), 2.47 (dd, J = 17.8, 8.0 Hz, 2H), 2.22–1.23 (series of multiplets, 11H), 0.79 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 220.3, 167.0, 147.8, 138.1, 138.0, 126.3, 121.2, 118.5, 50.4, 47.7, 44.0, 42.5, 37.8, 35.7, 31.5, 29.3, 26.3, 21.5, 13.7.
14. Billich, A.; Nussbaumer, P.; Lehr, P. *J. Steroid Biochem. Mol. Biol.* **2000**, *73*, 225.
15. Ahmed, S.; Owen, C. P.; James, K.; Patel, C. K.; Sampson, L. *J. Steroid Biochem. Mol. Biol.* **2002**, *80*, 429.
16. Ciobanu, L. C.; Boivin, R. P.; Luu-The, V.; Poirier, D. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 15.
17. Procedure: To a solution of 288 mg (1.0 mmol) of DHEA in 5 mL of dry dichloromethane 200 μL (1.2 mmol) of DIEA and 167 mg (1.0 mmol) of 4-nitrophenylformate were added and the reaction mixture was stirred at rt overnight. Since the starting material was not consumed, the same amount of the reagent and base was added again and stirring was continued for additional 24 h. The solvent was evaporated and the residue was dissolved in EtOAc and washed with 1 M aq $NaHSO_4$ solution and brine. The solvent was evaporated and the residue was purified by chromatography over silica gel with EtOAc/*c*-hexane (1/2) providing 240 mg (76%) of **17** as a white crystalline solid.
18. Pliego, J. R.; Riveros, J. M. *Chem. Eur. J.* **2001**, *7*, 169.
19. Ahmed, S.; James, K.; Owen, C. P.; Patel, K. C.; Sampson, L. *J. Steroid Biochem. Mol. Biol.* **2002**, *80*, 429.
20. Woo, L. W. L.; Lightowler, M.; Purohit, A.; Reed, M. J.; Potter, B. L. V. *J. Steroid Biochem. Mol. Biol.* **1996**, *57*, 79.
21. Powers, J. C.; Seidner, R.; Parsons, T. G.; Berwin, H. J. *J. Org. Chem.* **1966**, *31*, 2623.
22. Lithiated amines react with carbon monoxide even in the absence of a transition metal to form formamides, see: Nudelman, N. S.; Perez, D. *J. Org. Chem.* **1983**, *48*, 133.
23. Yale, H. L. *J. Org. Chem.* **1971**, *36*, 3238.
24. Laplante, D. G.; Manku, S.; Nagendran, J. *J. Org. Chem.* **1999**, *64*, 698.
25. Martinez, J.; Laur, J. *Synthesis*, **1982**, 979.
26. Howarth, N. M.; Purohit, T.; Robinson, J. J.; Vicker, N.; Reed, M. J.; Potter, B. L. V. *Biochemistry* **2002**, *41*, 14801.