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Estrone sulfonates as inhibitors of estrone sulfatase

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*In our continuing quest to design efficient inhibitors of estrone sulfatase activity and to assess the recognition of estrone sulfate surrogates by estrone sulfatase, we synthesized and evaluated several sulfonate derivatives of 5,6,7,8-tetrahydronaphth-2-ol and estrone. 5,6,7,8-Tetrahydronaphth-2-methanesulfonate (**11**), and 5,6,7,8-tetrahydronaphth-2-(*p*-toluene)sulfonate (**12**) were found not to inhibit estrone sulfatase activity; estrone-3-methanesulfonate (**5**), estrone-3-ethanesulfonate (**6**), estrone-3-butanesulfonate (**7**), and estrone-3-[(+)10-camphor]sulfonate (**8**) all weakly inhibited estrone sulfatase, and the best inhibitor, from this class of compounds, was estrone-3-(*p*-toluene)sulfonate (**9**). At 10 μM, it inhibited estrone sulfatase activity by 91%. These results emphasize some of the requirements needed for high-affinity binding to the enzyme.* (*Steroids* **62**:346–350, 1997) © *1997 by Elsevier Science Inc.*

Keywords: sulfonate; analog; estrone sulfatase; inhibitor

Introduction

Breast cancer is the most prevalent cancer in Western countries, and approximately one-third of breast tumors are hormone dependent.[1] In recent years, considerable evidence has accumulated that implicates estrogens as playing a central role in supporting growth of these tumors.[2] In postmenopausal women, in whom breast cancer most commonly occurs, breast tumor concentrations of estrogens are much higher than the corresponding estrogen concentrations in the plasma.[3–5] Although one possible explanation for this finding might be attributable to intratumoral aromatization of androgen to estrogen, recent studies have shown that ten times more estrone (**1**) (Scheme 1) originates from its sulfate conjugate, estrone sulfate (**2**) (Scheme 1), in breast cancer tissue than from androstenedione.[6] In addition to this, it has been found that aromatase activity in breast tumors is one million-fold lower than estrone sulfatase activity[7] and that plasma and breast tissue concentrations of estrone sulfate are much higher than those of unconjugated estrone.[8,9] All these facts imply that estrone sulfate, as a result of its ability to become converted readily to estrone by estrone sulfatase, acts as an abundant pool of potentially available estrogens and that estrone sulfatase, therefore, has a pivotal role in regulating estrogen production in postmenopausal women.

The development of specific inhibitors of estrogen synthesis could be an important advance in the treatment of hormone-dependent breast cancer. Until now, considerable effort has gone into producing efficient inhibitors of aromatase activity, and such compounds as aminoglutethimide and 4-hydroxyandrostenedione have been developed. However, although these compounds greatly reduce peripheral aromatase activity, plasma estrone and estrone sulfate concentrations are reduced by only some 50%.[10,11] With the recognition of the importance of estrone sulfatase in regulating estrogen production, it is possible that inhibitors of estrone sulfatase activity, used alone or in conjunction with an aromatase inhibitor, might enhance the response to this type of endocrine therapy.

In contrast to the development of aromatase inhibitors, this area of research is still in its early stages. However, in the past few years, with more awareness of the significance of the sulfatase, studies on a number of estrone sulfatase inhibitors have been published. Recently, Li et al. have reported on the synthesis and biochemical studies undertaken on a series of sulfonate analogues of 3-desoxyestrone,[12,13] estrone-3-methanesulfonate, estrone-3-phosphate, 3-desoxyestradiol-3-methylenesulfonate and 3-desoxyestrone-3-methylenesulfonate.[14] We have also

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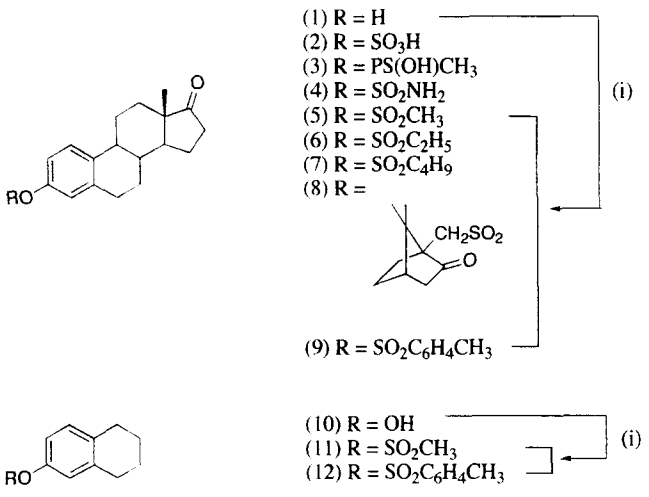

Scheme 1 Structure of compounds **1–12** and synthesis of compounds **5–9, 11** and **12.** Reagents and conditions: (i) RSO_2Cl/anhydrous pyridine/0°C-R.T./24 h.

developed a wide range of estrone sulfatase inhibitors, including estrone-3-methylthiophosphonate (E1-3-MTP) **(3)** (Scheme 1),[15,16] and estrone-3-*O*-sulfamate (EMATE) **(4)** (Scheme 1), our most potent inhibitor to date.[17] In a placental microsomal preparation, EMATE was found to inhibit estrone sulfatase activity by >99% at 10 μM, and it had an IC_{50} value of 80 nM.[18] Subsequent enzyme kinetic studies confirmed that EMATE inhibits estrone sulfatase in a time- and concentration-dependent manner, which indicates that it acts as an active site-directed inactivator.[17,18]

In our continuing quest to design efficient inhibitors of estrone sulfatase activity and to assess the recognition of estrone sulfate surrogates by estrone sulfatase, we synthesized and evaluated several sulfonate derivatives of estrone; namely, estrone-3-methanesulfonate **(5),** estrone-3-ethanesulfonate **(6),** estrone-3-butanesulfonate **(7),** estrone-3-[(+)10-camphor]sulfonate **(8),** and estrone-3-(*p*-toluene)sulfonate **(9)** (Scheme 1). We also prepared the methanesulfonate and *p*-toluenesulfonate analogs of 5,6,7,8-tetrahydronaphth-2-ol **(11, 12)** (Scheme 1) in order to determine the effect on inhibition of removal of part of the steroidal skeleton and, hence, to establish whether or not the entire estrone nucleus is required for enzyme recognition. We present here synthetic and structure-activity data on these compounds.

Experimental

All organic solvents were of AR grade and were supplied by Fisons plc (Loughborough, UK). These were dried according to the procedures described by Perrin and Armarego.[19] Estrone and 5,6,7,8-tetrahydronaphth-2-ol were purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany), respectively, and were dried under vacuum prior to use.

Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica $60F_{254}$, Art. No. 5554). Products were visualized either by ultraviolet light or by spraying the plate with a 7% solution of phosphomolybdic acid in methanol followed by heating. Flash chromatography refers to the method of Still et al.[20] and was carried out using Sorbsil C60 silica gel.

^1H and ^{13}C NMR spectra were run on Jeol FX90Q and GX270 NMR spectrometers. ^1H and ^{13}C NMR chemical shifts were measured in ppm relative to tetramethyl silane (TMS). J values are given in Hz. Melting points (uncorrected) were determined using a Reichert–Jung Thermo Galen Kofler block. Microanalysis was performed by the University of Bath Microanalysis Service. Mass spectra were recorded at the University of Bath Mass Spectrometry Service Centre. Synthetic compounds were tested for activity in MCF-7 cells, as described previously.[15,17] Potential time-dependent inactivation of estrone sulfatase by compounds **5** and **9** was performed, as previously described.[21]

Estrone-3-methanesulfonate (5)

Methanesulphonyl chloride (172 μl; 2.22 mmol; 2 eq) was added dropwise to a stirred solution of estrone (300 mg; 1.11 mmol; 1 eq) in anhydrous pyridine (5 mL) at 0°C. Subsequently, the reaction mixture was allowed to warm to room temperature, and stirring was continued overnight. The reaction mixture was then poured onto ice (20 mL), and the aqueous solution was extracted with ethyl acetate (6 × 15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo. Final traces of pyridine were removed by repeated co-evaporation with toluene (3 × 20 mL).

The crude product was purified by precipitation from dichloromethane by addition of pentane, and **5** was obtained as a pale yellow solid (390 mg; 100%): mp 152–154°C [Lit. mp 152–155°C (22)]; ^1H NMR ($CDCl_3$) δ 0.92 (s, 3H, C18-CH_3), 1.49–1.64(m, 6H), 1.96–2.57 (series of m, 7H), 2.94(m, 2H), 3.14(s, 3H, SO_2CH_3), 7.04(d, 2H, J = 8.43Hz, C2-*CH* and C4-*CH*), 7.32(d, 1H, J = 8.24Hz, C1-*CH*); ^{13}C NMR ($CDCl_3$)δ 13.74(q, C18-CH_3), 21.50(t), 25.66(t), 26.11(t), 29.32(t), 31.43(t), 35.76(t), 37.23(q, SO_2CH_3), 37.80(d), 44.05(d), 47.83(s, *C*13), 50.31(d), 118.94(d), 121.94(d), 126.87(d), 138.81(s), 139.11(s), 147.09(s), 220.53(s, *CO*); M.S. (E.I., 70eV) *m/z*(%) 348(100)[M^+], 304(13), 291(21), 251(14), 213(27), 159(17), 145(16), 133(17), 115(15), 97(24), 81(17), 65(46), 57(50), 41(53), 29(21); Analysis calculated for $C_{19}H_{24}O_4S$: C 65.49, H 6.94. Found C 65.20, H 6.98.

Estrone-3-ethanesulfonate (6)

This was prepared in a manner identical to **5.** Ethanesulphonyl chloride (210 μL; 2.22 mmol; 2 eq), estrone (300 mg; 1.11 mmol; 1 eq) and pyridine (5 mL) were used. Purification of the crude product was effected by flash chromatography (chloroform) to yield **6** as a white solid. Ultimate purification was achieved by precipitation from chloroform on addition of pentane to afford **6** as a white crystalline material (98 mg; 24%): mp 149–151°C; R_f 0.43 (chloroform); ^1H NMR ($CDCl_3$) δ 0.91(s, 3H, C18-CH_3), 1.49–1.64(m, 6H), 1.54(t, 3H, J = 6.75Hz, $SO_2CH_2CH_3$), 1.96–2.57(series of m, 7H), 2.94(m, 2H), 3.27(q, 2H, J = 6.75Hz, $SO_2CH_2CH_3$), 7.03(d, 2H, J = 8.61Hz, C2-*CH* and C4-*CH*), 7.31(d, 1H, J = 8.24Hz, C1-*CH*); ^{13}C NMR ($CDCl_3$) δ 8.21(q, $SO_2CH_2CH_3$), 13.72(q, C18-CH_3), 21.50(t), 25.66(t), 26.11(t), 29.32(t), 31.43(t), 35.78(t), 37.79(d), 44.05(d), 44.82(t, $SO_2CH_2CH_3$), 47.81(s, *C*13), 50.31(d), 118.94(d), 121.92(d), 126.82(d), 138.69(s), 138.85(s), 146.93(s), 220.59(s,CO); M.S. (E.I., 70eV) *m/z*(%) 362(35)[M^+], 270(17), 213(16), 172(16), 159(16), 149(36), 133(16), 120(17), 97(20), 85(27), 71(46), 57(100), 43(77), 29(40); Analysis calculated for $C_{20}H_{26}O_4S$: C 66.27, H 7.23. Found C 66.00, H 7.24.

Estrone-3-butanesulfonate (7)

This was synthesised according to the method used to prepare **5.** Butanesulphonyl chloride (290 μl; 2.22 mmol; 2 eq), estrone (300 mg; 1.11 mmol; 1 eq) and pyridine (5 mL) were used. The crude product was purified by flash chromatography [chloroform/

methanol (98:2)] to afford **7** as a pale yellow viscous oil (200 mg; 45%): R_f 0.86 [chloroform/methanol (98:2)]; ^{1}H NMR ($CDCl_3$) δ 0.78(s, 3H, C18-CH_3), 0.83(m, 5H, $SO_2(CH_2)_2CH_2CH_3$), 1.12–1.59(m, 8H, 6 steroidal *H* and $SO_2CH_2CH_2CH_2CH_3$), 1.76–2.42(series of m, 9H, 7 steroidal *H* and $SO_2CH_2(CH_2)_2CH_3$), 2.79(m, 2H), 6.88(d, 2H, J = 8.25Hz, C2-C*H* and C4-C*H*), 7.16(d, 1H, J = 8.25Hz, C1-C*H*); ^{13}C NMR ($CDCl_3$) δ 12.73(q, $SO_2(CH_2)_3CH_3$), 13.18(q, C18-CH_3), 21.14(t), 21.36(t), 24.77(t), 25.23(t), 26.14(t), 28.86(t), 30.90(t), 35.45(t), 37.50(d), 43.64(d), 47.27(s, *C*13), 50.00(t), 50.23(d), 118.86(d), 121.82(d), 126.82(d), 139.09(s), 139.18(s), 146.59(s), 220.91(s, *C*O); M.S. (C.I., isobutane) *m/z*(%) 391(4)[(M + H)$^+$], 211(100), 177(13), 149(6), 123(8), 105(7), 91(27), 81(9), 73(33); Empirical formula: $C_{22}H_{30}SO_4$.

Estrone-3-[(+)10-camphor]sulfonate (8)

This was prepared using the same method used to form **5.** (+)10-Camphorsulphonyl chloride (560 mg; 2.22 mmol; 2 eq), estrone (300 mg; 1.11 mmol; 1 eq) and pyridine (5 mL) were used. The crude product was purified by flash chromatography (chloroform) to yield **8** as a colorless glassy solid (570 mg; 100%): R_f 0.25 (chloroform); ^{1}H NMR ($CDCl_3$) δ 0.91(s, 6H, C18-CH_3 and 1 camphor C-7 CH_3), 1.16(s, 3H, 1 camphor C-7 CH_3), 1.40–1.73(m, 8H), 1.93–2.55(series of m, 12H), 2.93(m.2H), 3.06(d, 1H, J = 15.21Hz) and 3.80(d, 1H, J = 15.21Hz) [SO_2CH_2], 7.06(d, 2H, J = 8.79Hz, C2-C*H* and C4-C*H*), 7.31(d, 1H, J = 9.34Hz, C1-C*H*); ^{13}C NMR ($CDCl_3$) δ 13.49(q, C18-CH_3), 19.40(q, 1 camphor C-7 CH_3), 19.62(q, 1 camphor C-7 CH_3), 21.24(t), 24.81(t), 25.40(t), 25.88(t), 26.53(t), 29.06(t), 31.20(t), 35.48(t), 37.53(d), 42.13(t), 42.49(d), 43.75(d), 47.06(d), 47.52(t), 47.61(s, *C*13), 50.01(d), 57.80(s, *C*SO_2CH_2), 118.78(d), 121.70(d), 126.53(d), 138.40(s), 138.56(s), 146.83(s), 213.71(s, *C*O), 220.14(s, *C*O); M.S. (C.I., isobutane) *m/z*(%) 485(25)[(M + H)$^+$], 270(44), 215(31), 151(26), 123(25), 109(30), 81(50), 69(100); Empirical formula: $C_{28}H_{36}SO_5$.

Estrone-3-(p-toluene)sulfonate (9)

This was prepared in an identical manner to **5.** *p*-Toluenesulphonyl chloride (350 mg; 1.85 mmol; 2 eq), estrone (250 mg; 0.92 mmol; 1 eq) and pyridine (5 mL) were used. The crude product was purified by precipitation from ethyl acetate upon addition of hexane, and **9** was afforded as a white crystalline solid (310 mg; 78%): mp 125-126°C; ^{1}H NMR ($CDCl_3$) δ 0.91(s, 3H, C18-CH_3), 1.34–1.67(m, 6H), 1.93–2.56(series of m, 7H), 2.46(s, 3H, $SO_2C_6H_4CH_3$), 2.84(m, 2H), 6.65(d, 2H, J = 8.43Hz, C2-C*H* and C4-C*H*), 7.16(d, 1H, J = 8.61Hz, C1-C*H*), 7.32(d, 2H, J = 8.06Hz, $SO_2C_6H_4CH_3$), 7.74(d, 2H, J = 8.24Hz, $SO_2C_6H_4CH_3$); ^{13}C NMR ($CDCl_3$) δ 13.78(q, C18-CH_3), 21.54(t), 21.70(q, $SO_2C_6H_4CH_3$), 25.62(t), 26.17(t), 29.26(t), 31.46(t), 35.81(t), 37.79(t), 44.05(d), 47.87(s, *C*13), 50.37(d), 112.81(s), 119.20(d), 122.44(d), 126.40(d), 128.44(d), 129.68(d), 132.72(s), 138.51(s), 145.15(s), 147.45(s), 220.72(s, *C*O); M.S. (E.I., 70eV) *m/z*(%) 424(3)[M$^+$], 270(11), 147(9), 137(13), 123(10), 109(11), 95(21), 81(53), 69(100), 57(61), 43(53), 29(19); Analysis calculated for $C_{25}H_{28}O_4S$: C 70.72, H 6.65. Found C 70.80, H 6.77.

5,6,7,8-Tetrahydronaphth-2-methanesulfonate (11)

The same procedure was used to prepare this compound as that used for the preparation of **5.** Methanesulphonyl chloride (320 μl; 4.05 mmol; 2 eq), 5,6,7,8-tetrahydronaphth-2-ol (310 mg; 2.07 mmol; 1 eq) and pyridine (5 mL) were used. The crude product was purified by flash chromatography (chloroform) to afford **11** as a pale yellow oil (450 mg; 99%): R_f 0.45 (chloroform); ^{1}H NMR ($CDCl_3$) δ 1.79 (quintet, 4H, J = 3.30Hz, C6-CH_2 and C7-CH_2), 2.77(m, 4H, C5-CH_2 and C8-CH_2), 3.11(s, 3H, SO_2CH_3), 6.97–7.07(m, 3H, C1-C*H*, C3-C*H*, and C4-C*H*); ^{13}C NMR ($CDCl_3$) δ 22.61(t), 22.83(t), 28.80(t), 29.35(t), 37.11(q, SO_2CH_3), 118.81(d), 121.99(d), 130.39(d), 136.49(s); M.S. (E.I., 70eV) *m/z*(%) 226(33)[M$^+$], 147(100), 119(17), 91(35), 41(16), 28(19); Empirical formula: $C_{11}H_{14}SO_3$.

5,6,7,8-Tetrahydronaphth-2-(p-toluene)sulfonate (12)

This compound was prepared in a manner identical to **5.** *p*-Toluenesulphonyl chloride (770 mg; 4.05 mmol; 2 eq), 5,6,7,8-tetrahydronaphth-2-ol (310 mg; 2.07 mmol; 1 eq) and pyridine (5 mL) were used. The crude product was purified by flash chromatography (chloroform) to yield **12** as a waxy, white solid (510 mg; 83%): mp 55–57°C; R_f 0.60 (chloroform); ^{1}H NMR ($CDCl_3$) δ 1.74(t, 4H, J = 3.21Hz, C6-CH_2 and C7-CH_2), 2.45(s, 3H, $SO_2C_6H_4CH_3$), 2.68(br d, 4H, J = 6.23Hz, C5-CH_2 and C8-CH_2), 6.60–6.94(m, 3H, C1-C*H*, C3-C*H* and C4-C*H*), 7.31(d, 2H, J = 8.43Hz, $SO_2C_6H_4CH_3$), 7.72(d, 2H, J = 8.25Hz, $SO_2C_6H_4CH_3$); ^{13}C NMR ($CDCl_3$) δ 21.63(q, $SO_2C_6H_4CH_3$), 22.61(t), 22.83(t), 28.77(t), 29.26(t), 119.04(d), 122.47(d), 128.44(d), 129.61(d), 129.90(d), 136.03(s), 138.66(s), 145.05(s), 147.13(s); M.S. (E.I., 70eV) *m/z*(%) 302(40)[M$^+$], 155(29), 147(100), 119(16), 91(100), 77(13), 65(29), 57(20), 41(35), 28(75); Analysis calculated for $C_{17}H_{18}O_3S$: C 67.55, H 5.96. Found C 67.77, H 5.96.

Results and discussion

All of the sulfonates reported in this paper were prepared from either estrone (**1**) or 5,6,7,8-tetrahydronaphth-2-ol (**10**) by addition of the appropriate sulphonyl chloride to a solution of the alcohol in anhydrous pyridine at 0°C, as shown in Scheme 1. After work-up and purification, these compounds were afforded in yields ranging from 24 to 100%, with the lowest yield obtained in the synthesis of estrone-3-ethanesulfonate (**6**). The ability of these compounds to inhibit estrone sulfatase activity in intact MCF-7 breast cancer cells was then examined.

As shown in Table 1, both the methanesulfonate and the *p*-toluenesulfonate derivatives of 5,6,7,8-tetrahydronaphth-2-ol (**11** and **12**, respectively) did not exhibit any inhibition of estrone sulfatase activity at 10 μM. The lack of inhibition observed for these nonsteroidal compounds can be explained simply by the fact that they do not retain sufficient

Table 1 Percentage inhibition of estrone sulfatase activity in intact MCF-7 breast cancer cells by E1-3-MTP **(3)**, EMATE **(4)**, compounds **5–9**, **11** and **12**.

	% Inhibition		
Compound	10 μM	1 μM	0.1 μM
E1-3-MTP **(3)**	95	74	52
EMATE **(4)**	99	99	99
(5)	28	ND	ND
(6)	27	0	ND
(7)	17	0	ND
(8)	22	0	ND
(9)	91	ND	30
(11)	0	ND	ND
(12)	0	0	ND

(ND = not determined). Mean results of duplicate incubations at each concentration are shown. The coefficient of variation for the assay was less than 10%.

recognition elements of the steroidal nucleus to enable them to bind with high affinity to the enzyme. It has recently been postulated that high-affinity binding to estrone sulfatase is facilitated by the presence of the following groups, which are then able to form important interactions with amino acid residues at the active site of the enzyme: (1) an oxygen anion or an uncharged but highly electronegative substituent at the central ester atom, which is available for ionic interactions; (2) an oxygen atom or a sterically/or electronically similar link between the ring and the sulfonate or analogous ester moiety, which is able to be involved in hydrogen-bonding interactions; (3) a large carbon skeleton, such as the steroidal structure, which can provide hydrophobic interactions; and (4) C-17 carbonyl group for hydrogen bonding.[14,21] Thus, because compounds **11** and **12** have very few of these motifs, it is not surprising that they showed no inhibitory activity. These findings are also in accord with our observation that the 2-ring sulphamate, 5,6,7,8-tetrahydronaphth-2-sulphamate, is a much weaker inhibitor of estrone sulfatase activity than its corresponding steroidal analog, EMATE (i.e., at 10 μM, it was found to inhibit estrone sulfatase activity by 97% and at 1 μM by 47%).[17]

The alkysulfonate derivatives of estrone; i.e., the methanesulfonate **(5),** the ethanesulfonate **(6),** and the butanesulfonate **(7),** all showed some inhibition of estrone sulfatase activity at 10 μM, although they were much weaker inhibitors than either E1-3-MTP or EMATE (Table 1). As previously mentioned, estrone-3-methanesulfonate **(5)** has also been prepared and examined for estrone sulfatase inhibitory activity by Li et al.[14] They found that at 30 μM, it caused roughly 50% inhibition and that it had a K_i value approximately 30-fold greater than the substrate, estrone sulfate. The low-inhibitory potency observed for all these compounds, as compared to E1-3-MTP and EMATE, can be attributed to their lack of an electronegative substituent at the central ester atom. Although the effects are modest, the degree of inhibition can also be seen to decrease with increasing size of the alkyl chain; i.e., **5 > 6 > 7.** Presumably, this can be explained by the fact that, as the alkyl group increases in size and becomes more mobile, steric hindrance becomes more important, causing disruption of the already weak binding of the molecule to the active site.

The camphorsulfonate derivative of estrone **(8)** was found to be a slightly better inhibitor of estrone sulfatase activity than the butanesulfonate derivative **7** (i.e., at 10 μM, **8** inhibited estrone sulfatase activity by 22%, as compared to 17% for **7**) (Table 1). This could be because, although camphor is a bulky moiety, it is constrained in a bicyclic ring and, therefore, is not as mobile as the butyl group. It also possesses more motifs for hydrophobic interaction and a hydrophilic carbonyl group. However, this compound, too, shows only a weak inhibitory activity as compared to our lead compounds of E1-3-MTP and EMATE, again underlying the importance of an electronegative substituent at the central ester atom.

The best inhibitor of estrone sulfatase activity from this class of compounds was found to be that of the *p*-toluenesulfonate derivative of estrone **(9).** Surprisingly, at 10 μM, it exhibited an inhibitory activity close to that of E1-3-MTP, although at 0.1 μM, the difference in percentage inhibition between the two compounds markedly increases, with **9** being the poorer inhibitor (Table 1). Nevertheless, this compound definitely exhibits a much higher affinity for estrone sulfatase than do all the other sulfonates examined here. The reason for this is unclear, because it also does not have an electronegative substituent at the central ester atom. One possible explanation, however, is that the aromatic ring of the *p*-toluene moiety becomes involved in pi-stacking or hydrophobic interaction with some amino acid residues at the active site of the enzyme.

The time-dependent inactivation of placental microsomal estrone sulfatase activity by estrone-3-methanesulfonate **(5)** and estrone-3-(*p*-toluene)sulfonate **(9)** compared to E1-3-MTP and EMATE was also explored. As can be seen from Figure 1, after 30 minutes, **5** and **9,** as did E1-3-MTP, failed to show the irreversible time-dependent inactivation so clearly demonstrated by EMATE. This implies that neither **5** nor **9** acts by an active site-directed mode of inhibition and, hence, unlike EMATE, they are not irreversible inhibitors.

In conclusion, we have prepared a range of sulfonate derivatives of estrone and 5,6,7,8-tetrahydronaphth-2-ol and examined their abilities to inhibit estrone sulfatase activity. Neither the methanesulfonate nor *p*-toluenesulfonate derivatives of 5,6,7,8-tetrahydronaphth-2-ol (**11** or **12,** respectively) showed any inhibition of estrone sulfatase activity at 10 μM. This is presumably because of their lack of sufficient recognition elements of the steroid nucleus to enable high-affinity binding to the enzyme. The estrone sulfonates **5, 6, 7** and **8** all showed modest inhibition of estrone sulfatase at 10 μM. This is because they do not have an electronegative substituent at the central ester atom; a requirement that seems to be of increasing importance as a result of these studies. The best inhibitor of estrone sulfatase activity from this class of compounds was estrone-3-(*p*-toluene)sul-

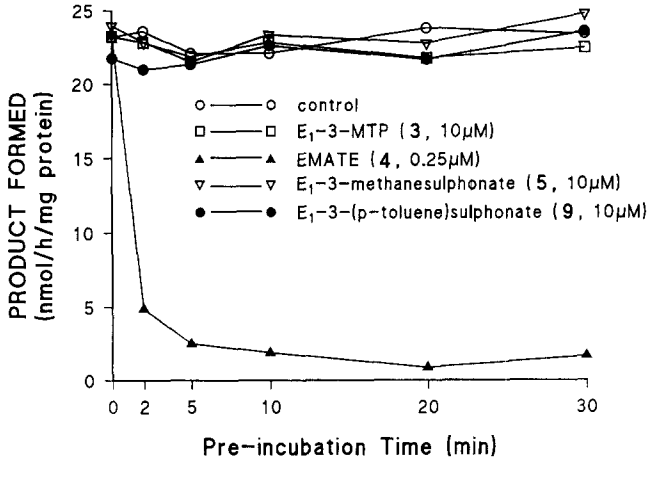

Figure 1 Time-dependent inactivation of estrone sulfatase by E1-3-MTP **(3)** (□—□), EMATE **(4)** (▲—▲), **5** (▽—▽) and **9** (●—●). Placental microsomes (200 μg of protein) were preincubated with **3–5** and **9** for 0–30 min at 37°C followed by incubation with dextran-charcoal for 10 min at 4°C. Dextran-charcoal was sedimented by centrifugation, and portions of the supernatant were then incubated with [^{3}H]estrone sulfate (20 μM) for 1 h at 37°C to assess remaining sulfatase activity. Duplicate experiments were performed, but assays for residual activity were taken at different times for each experiment.

fonate (**9**). At 10 μM, it showed comparable inhibition to E1-3-MTP. Its high binding affinity might be attributed to pi-stacking or hydrophobic interaction of the aromatic ring in the *p*-toluene group and some amino acid residues at the active site. Compounds **5** and **9** did not exhibit any time-dependent inactivation of estrone sulfatase, thus they do not act by an active site-directed mode of inhibition. Nevertheless, the tosylate **9** should be a reasonable lead compound for further developmental work relating to the use of sulfonates as sulfate surrogates for inhibition of steroid sulfatase.

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References

1. James VHT, Reed MJ (1980). Steroid hormones and human cancer. *Prog Cancer Res Ther* **14**:471–487.
2. Lippman ME, Dickson RB, Gelmann EP, Rosen N, Knabbe C, Bates S, Bronzert D, Huff K, Kasid A (1988). Growth regulatory and peptide production by human breast carcinoma cells. *J Steroid Biochem* **30**:53–61.
3. Bonney RC, Reed MJ, Davidson K, Beranek PA, James VHT (1983). The relationship between 17β-hydroxysteroid dehydrogenase activity and estrogen concentrations in human breast tumors and in normal breast tissue. *Clin Endocrinol* **19**:727–739.
4. Van Landeghem AAJ, Poortman J, Nabuurs M, Thijssen JHH (1985). Endogenous concentration and sub-cellular distribution of estrogens in normal and malignant breast tissue. *Cancer Res* **45**: 2900–2906.
5. Vermeulen A, Deslypere JP, Pavidaens R, Leclercq G, Roy F, Henson JC (1986). Aromatase, 17β-hydroxysteroid dehydrogenase and intra-tissular sex hormone concentrations in cancerous and normal breast tissue in postmenopausal women. *Eur J Cancer Clin Oncol* **26**:515–525.
6. Santner SJ, Feil PD, Santen RJ (1984). In situ estrogen production via the estrone sulfatase pathway in breast tumors: Relative importance versus the aromatase pathway. *J Clin Endocrinol Metab* **59**: 29–33.
7. James VHT, McNeill JM, Lai LC, Newton CJ, Ghilchik MW, Reed MJ (1987). Aromatase activity in normal breast and breast tumor tissues: In vivo and in vitro studies. *Steroids* **50**:269–279.
8. Noel CT, Reed MJ, Jacobs HS, James VHT (1981). The plasma concentration of estrone sulfate in postmenopausal women: Lack of diurnal variation, effect of ovariectomy, age, and weight. *J Steroid Biochem* **14**:1101–1105.
9. Pasqualini J, Getty C, Nguyen B-L, Vella C (1989). Importance of oestrogen sulphates in breast cancer. *J Steroid Biochem* **34**:155–163.
10. Santen RJ, Santner SJ, Davis B, Veldhius J, Samojlik E, Ruby E (1978). Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. *J Clin Endocrinol Metab* **47**:1257–1265.
11. Dowsett M, Goss PE, Powles TJ, Hutchinson G, Brodie AM, Jeffcoate SL, Coombes RC (1987). Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: Optimization of therapeutic dose and route. *Cancer Res* **47**:1957–1961.
12. Li P-K, Pillai R, Young BL, Bender WH, Martino DM, Lin F-T (1993). Synthesis and biochemical studies of estrone sulfatase inhibitors. *Steroids* **58**:106–111.
13. Dibbelt L, Li P-K, Pillai R, Knuppen R (1994). Inhibition of human placental sterylsulfatase by synthetic analogs of estrone sulfate. *J Steroid Biochem Mol Biol* **50**:261–266.
14. Li P-K, Pillai R, Dibbelt L (1995). Estrone sulfate analogs as estrone sulfatase inhibitors. *Steroids* **60**:299–306.
15. Duncan LJ, Purohit A, Howarth NM, Potter BVL, Reed MJ (1993). Inhibition of estrone sulfatase activity by estrone-3-methylthiophosphonate: A potential therapeutic agent in breast cancer. *Cancer Res* **53**:298–303.
16. Howarth NM, Cooper G, Purohit A, Duncan LJ, Reed MJ, Potter BVL (1993). Phosphonates and thiophosphonates as sulfate surrogates: Synthesis of estrone 3-methylthiophosphonate, a potent inhibitor of estrone sulfatase. *Bioorg Med Chem Lett* **3**:313–318.
17. Howarth NM, Purohit A, Reed MJ, Potter BVL (1994). Estrone sulfamates: Potent inhibitors of estrone sulfatase with therapeutic potential. *J Med Chem* **37**:219–221.
18. Purohit A, Williams GJ, Howarth NM, Potter BVL (1995). Inactivation of steroid sulfatase by an active site-directed inhibitor, estrone-sulfamate. *Biochemistry* **34**:11,508–11,514.
19. Perrin DD, Armarego WLF (1988). *Purification of Laboratory Chemicals*. Pergamon, Oxford.
20. Still WC, Kahn M, Mitra A (1978). Rapid chromatographic technique for preparative separations with moderate resolution. *J Org Chem* **43**:2923–2925.
21. Woo LWL, Lightowler M, Purohit A, Reed MJ, Potter BVL (1996). Heteroatom-substituted analogues of the active-site directed inhibitor estra-1,3,5(10)-triene-17-one-3-sulfamate inhibit estrone sulfatase by a different mechanism. *J Steroid Biochem Mol Biol* **57**:79–88.
22. Baldwin JE, Barton DHR, Dainis I, Pereira JLC (1968). Photochemical transformations. Part XXIV. The synthesis of 18-hydroxyestrone. *J Chem Soc (C)*:2283–2289.