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Structure-activity relationship studies of the amide functionality in (*p-O*-sulfamoyl)-*N*-alkanoyl tyramines as estrone sulfatase inhibitors

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Recently, we reported the synthesis and biochemical studies of a series of (p-O-*sulfamoyl*)-N-*alkanoyl tyramines as nonsteroidal estrone sulfatase inhibitors. One of the most potent inhibitors in this series is* (p-O-*sulfamoyl*)-N-*tridecanoyl tyramine* **1** *with an* IC_{50} *value of 61.3 nM. In this study, we synthesized four analogs of* **1** *(compounds* **2–5***) to investigate the structure-activity relationships of the amide functionality in* (p-O-*sulfamoyl*)-N-*tridecanoyl tyramine. Replacement of the amide functionality in* **1** *with an ethylene moiety to form the alkyl analog* **5** *resulted in complete loss of sulfatase inhibitory activity (*IC_{50} *of 61.3 nM vs. >20 μM). The keto, hydroxy, and ester analogs (inhibitors* **2–4***) are 8–15 times less in affinity to the sulfatase than inhibitor* **1***. However, their inhibitory activities are significantly higher than the alkyl analog* **5***. The results suggest that the amide functionality is favorable for sulfatase inhibitory activity and that there may be a hydrogen bonding component to the enzyme interaction in this region.* (*Steroids* **62**:530–535, 1997) © *1997 by Elsevier Science Inc.*

Keywords: estrone sulfatase; mammary carcinoma; sulfatase inhibitors; hormone dependence

Introduction

Estrogen levels in breast tumors of postmenopausal women are at least 10 times higher than estrogen levels in plasma.[1,2] The high levels of estrogen in these tumors are due to in situ formation of estrogen, possibly through conversion of estrone sulfate to estrone by the enzyme estrone sulfatase.[3,4] Therefore, inhibitors of estrone sulfatase are potential agents for the treatment of estrogen-dependent breast cancers. Of all the estrone sulfatase inhibitors reported in the literature, estrone 3-*O*-sulfamate (Figure 1) is the most potent estrone sulfatase inhibitor ever reported. It inhibited over 99% of estrone sulfatase activity in intact MCF-7 cells at 0.1 μM. In addition, it exhibited time- and concentration-dependent inactivation of estrone sulfatase and is classified as an active-site directed irreversible inhibitor.[5–7] However, a recent report by Elger et al. demonstrated that estrone-3-*O*-sulfamate is a potent estrogen.[8] Therefore, there remains a real and substantial need for a potent nonsteroidal sulfatase inhibitor that is metabolically stable, more selective, and devoid of estrogenic activity. Reed and coworkers[5,9] have reported that the C and D rings of estrone 3-*O*-sulfamate are not required for binding to the enzyme. Tetrahydronaphth-2-ol sulfamate and coumarin sulfamate (Figure 1), which mimic the A/B ring of estrone 3-*O*-sulfamate, inhibited estrone sulfatase activity in intact MCF-7 breast cancer cells.[5,9] Recently, we reported the design, synthesis, and enzyme studies of a series of (*p-O*-sulfamoyl)-*N*-alkanoyl tyramines as nonsteroidal estrone sulfatase inhibitors (Figure 1).[10] The phenyl sulfamoyl group of the inhibitors mimic the A ring of 3-*O*-sulfamate, and the long alkanoyl chain is proposed to provide additional binding through insertion into the membrane because estrone sulfatase is a membrane-bound enzyme. Detail enzyme studies showed that (*p-O*-sulfamoyl)-*N*-alkanoyl tyramines inhibited estrone sulfatase from human placental microsomes and act as active-site directed irreversible inhibitors.[10] One of the most potent inhibitors in this series is (*p-O*-sulfamoyl)-*N*-tridecanoyl tyramine **1** with an IC_{50} value of 61.3 nM[10] (Figure 2). In our continuous effort to design nonsteroidal estrone sulfatase inhibitors, four analogs of **1** (compounds **2–5**) were synthesized to investigate the structure-activity relationships of the amide functionality in (*p-O*-sulfamoyl)-*N*-tridecanoyl tyramine (Figure 2).

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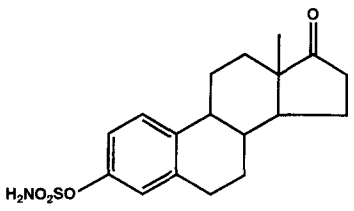
Received November 6, 1996; accepted March 13, 1997.

Steroids 62:530–535, 1997

655 Avenue of the Americas, New York, NY 10010

0039-128X/97/$17.00
PII S0039-128X(97)00038-X



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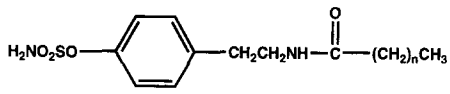
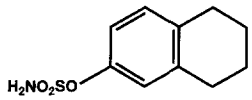


(p-O-sulfamoyl)-N-alkanoyl tyramines
n = 5 - 13



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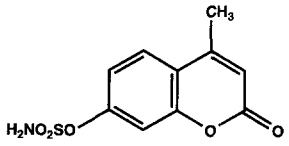


4-methylcoumarin 7-O-sulfamate

Figure 1 Structures of steroidal and nonsteroidal estrone sulfatase inhibitors.

Experimental

Chemicals and reagents

Chemicals and silica gel were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). The chemicals were checked for purity by thin layer chromatography and NMR. Biochemicals and estrone sulfate were obtained from Sigma Chemical Company (St. Louis, Missouri, USA). [6,7-^{3}H]estrone sulfate and [C4,^{14}C]estrone were purchased from DuPont NEN (Boston, Massachusetts, USA). Melting points were determined on a Thomas Hoover capillary melting point apparatus and were uncorrected. Proton NMR spectra were obtained with a Bruker WH-300 (300 MHz) spectrophotometer. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, Georgia, USA). Centrifugation was performed on a Damon CRU-5000 Centrifuge and ultracentrifugation on a Beckman Type B Ultracentrifuge. Radioactive samples were analyzed with a Packard Tri-Carb 4530 Liquid scintillation Counter. The liquid scintillation cocktail was Ecolume (ICN, Costa Mesa, California, USA). Mass spectra (MS) were obtained by Hewlett Packard 5972 Mass Spectrometer (Palo Alto, California, USA).

Chemistry

Synthesis of (±)-1-(4-methoxyphenyl)-4-hexadecanol (8). To a suspension of pyridinium chlorochromate (PCC; 13 g, 60.3 mmol) and Celite (5 g) in 200 mL of anhydrous dichloromethane (CH_2Cl_2) was added to a solution of 4-(methoxyphenyl)-1-butanol

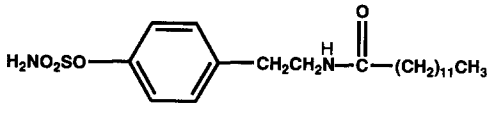
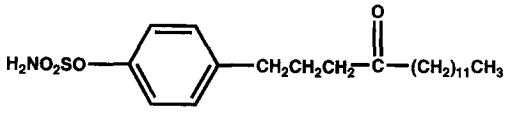
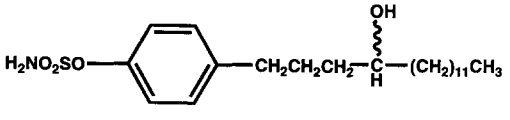
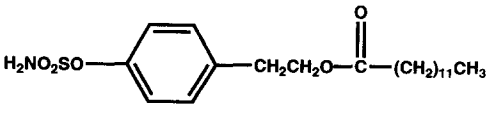
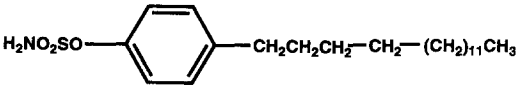
Compound #		IC_{50} (nM)
1		61.3 ± 3.4
2		652 ± 35
3		524 ± 32
4		960 ± 47
5		> 20000

Figure 2 Structures and inhibitory effects of compounds **1–5** to estrone sulfatase from human placental microsome, utilizing estrone sulfate (20 μM) as the substrate. Each value represents the mean of two determinations in triplicate.

6 (3.64 g, 20.22 mmol) in 20 mL of anhydrous CH_2Cl_2. After the reaction mixture was stirred at room temperature (r.t.) for 45 min, 100 mL of anhydrous ether was added. The mixture was passed through a pad of florisil, and the florisil pad was washed with ether. The filtrate was concentrated to give the crude 4-(methoxyphenyl)-1-butanal **7** (3.6 g, ~100%), which was taken on directly to the next step without purification. To a suspension of magnesium turnings (622 mg, 25.6 mmol) in anhydrous tetrahydrofuran (THF; 50 mL) was added 1-bromododecane (6.35 g, 6.12 mL, 25.6 mmol). The initiation of the reaction was achieved by mild heating. After the complete formation of the Grignard reagent 1-dodecanyl magnesium bromide (1 h), a solution of the above crude aldehyde **7** (3.6 g, 20.22 mmol) in 15 mL of anhydrous THF was added to the Grignard reagent at 0°C. The reaction mixture was stirred at r.t. for 2.5 h and then quenched with saturated ammonium chloride (NH_4Cl) solution and extracted with ethyl acetate (EtOAc, 2 × 80 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography and eluted with petroleum ether/ethyl acetate (3:1), yielding 1-(4-methoxyphenyl)-4-hexadecanol **8** (6.37 g, 90.5% for two steps). m.p. 60–62°C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.6 Hz, CH_3), 1.2–1.7 (m, 27H, 13 × CH_2 and OH), 2.57 (m, 2H, benzyl CH_2), 3.60 (m, 1H, −CH–OH) 3.77 (s, 3H, OCH_3), 6.83 (d, 2H, J = 8.2 Hz, arom H), 7.09 (d, 2H, J = 8.2 Hz, arom H). MS (*m/z*) 348 (M^+).

Synthesis of 1-(4-methoxyphenyl)-4-hexadecanone (9). To a suspension of PCC (10.1 g, 46.9 mmol) and Celite (4.0 g) in 150 mL of CH_2Cl_2 was added a solution of **8** (4.4 g, 12.64 mmol) in 30 mL of CH_2Cl_2 in one portion. The solution was stirred at r.t. for 24 h, and the reaction mixture was diluted with ether (100 mL) and passed through a florisil column. The filtrate was concentrated and purified by silica gel chromatography and eluted with petroleum ether/ethyl acetate (4:1), yielding the pure ketone **9** (4.25 g, 97.1%). m.p. 47–49°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 6.6 Hz, CH_3), 1.25–2.56 (m, 28H, 14 × CH_2), 3.78 (s, 3H, OCH_3), 6.82 (d, 2H, J = 8.2 Hz, arom H), 7.08 (d, 2H, J = 8.2 Hz, arom H). MS (*m/z*) 346 (M^+).

Synthesis of 1-(*p*-hydroxyphenyl)-4-hexadecanone (10). A 1 M solution of boron tribromide (BBr_3) in CH_2Cl_2 (10 mL, 10 mmol) was added dropwise to a solution of ketone **9** (1.30 g, 3.76 mmol) in 40 mL of CH_2Cl_2 at −78°C. The solution was stirred at −78°C for 1.5 h and was allowed to warm to r.t. in 45 min and then quenched by adding H_2O and 10% HCl. The reaction mixture was extracted with CH_2Cl_2. The organic extracts were washed with brine, separated, and dried under reduced pressure. After concentration, the residue was purified by silica gel chromatography and eluted with petroleum ether/methylene chloride/ethyl acetate (4:1:1), giving the phenol **10** (874 mg, 70%). m.p. 65.5–66.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.29–2.53 (m, 28H, 14 × CH_2), 5.02 (br s, 1H, OH), 6.75 (d, 2H, J = 8.2 Hz, arom H), 7.00 (d, 2 H, J = 8.2 Hz, arom H). MS (*m/z*) 332 (M^+).

Synthesis of 1-(*p*-*O*-sulfamoylphenyl)-4-hexadecanone (2). Sodium hydride (122 mg, 4.8 mmol) was added in a stirred solution of 1-(*p*-hydroxyphenyl)-4-hexadecanone **10** (800 mg, 2.4 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF) (30 mL) at 0°C under nitrogen. The solution was stirred for 30 min and chlorosulfonamide[5] (0.56 g, 9.6 mmol) was added in one portion. The solution was then stirred at r.t. for 24 h. The mixture was poured into a cold saturated sodium bicarbonate solution, and the resulting solution was extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure to give a white solid (556 mg, 56.2% yield). The product was purified by chromatography on a silica gel column and eluted with methylene chloride/ethyl acetate (20:1). m.p. 87.5–89°C; ^{1}H NMR (300 MHz, DMSO-d_6) 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.24–2.55 (m, 28H, 14 × CH_2), 7.18 (d, 2 H, J = 8.2 Hz, arom H), 7.26 (d, 2 H, J = 8.2 Hz, arom H), 7.93 (br s, 2H, NH_2). Analysis calculated for $C_{22}H_{37}NO_4S$: C, 64.20; H, 9.06; N, 3.40. Found: C, 64.08; H, 9.09; N, 3.45.

Synthesis of 1-(4-*p*-*O*-sulfamoylphenyl)-4-hexadecanol (3). To a solution of sulfamate **2** (165 mg, 0.4 mmol) in 5 mL of anhydrous THF at −78°C was added dropwise to 0.72 mL of diisobutylaluminum hydride DIBAL (1 M solution in CH_2Cl_2). The reaction mixture was stirred for 45 min at −78°C and then quenched with saturated aqueous NH_4Cl (50 mL), and the aqueous solution was extracted with CH_2Cl_2 (2 × 50 mL). The organic layer was dried and concentrated. The residue was purified by silica gel chromatography and eluted with petroleum ether/methylene chloride/ethyl acetate (3:2:2) to afford the pure alcohol **3** (150 mg, 90.5%). m.p. 93.5–95°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.24–1.65 (m, 26H, 13 × CH_2), 2.57 (m, 2H, benzyl H), 3.39 (m, 1H, CHOH), 4.28 (br s, 1H, OH), 7.17 (d, 2H, J = 8.2 Hz, arom H), 7.26 (d, 2H, J = 8.2 Hz, arom H), 7.93 (br s, 2H, NH_2). Analysis calculated for $C_{22}H_{39}NO_4S$: C, 63.88; H, 9.50; N, 3.39. Found: C, 63.99; H, 9.41; N, 3.27.

Synthesis of 2-(*p*-benzyloxyphenyl)-1-ethanol (12). 2-(*p*-Hydroxyphenyl)-1-ethanol **11** (2.76 g, 20 mmol) was dissolved in 100 mL of acetone. Potassium carbonate (4.15 g, 30 mmol) was added to the acetone solution, followed by benzyl bromide (2.86 mL, 24 mmol). The reaction mixture was refluxed for 18 h. The mixture was then filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography and eluted with petroleum ether/ethyl acetate (1:1), giving pure **12** (4.53 g, 99.3%). m.p. 85–86°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47 (br s, 1H, OH), 2.66 (t, 2H, J = 6.6 Hz, CH_2), 3.66 (t, 2H, J = 6.6 Hz, CH_2), 4.90 (s, 2H, CH_2Ph), 6.78 (d, 2H, J = 8.2 Hz, arom H), 6.99 (d, 2H, J = 8.2 Hz, arom H), 7.20–7.30 (m, 5H, arom H). MS (*m/z*) 228 (M^+).

Synthesis of 2-(*p*-benzyloxyphenyl)-1-ethyl tridecanoate (13). 1-Tridecanoic acid (3.3 g, 15.5 mmol) was treated with excess oxalyl chloride (78 mmol, 5 eq) in dry CH_2Cl_2 (50 mL) at r.t. for 3 h. Evaporation of the solvent gave the crude 1-tridecanoyl chloride. To a solution of the alcohol **12** (2.2 g, 9.65 mmol), triethylamine (2.8 mL, 20 mmol) and 4-dimethylaminopyridine (122 mg, 1 mmol) in dry CH_2Cl_2 (60 mL) was added dropwise the above 1-tridecanoyl chloride in 8 mL of CH_2Cl_2 at 0°C. The reaction mixture was stirred at r.t. overnight and then washed with saturated $NaHCO_3$ solution, water, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel chromatography and eluted with petroleum ether/ethyl acetate (8:1), yielding ester **13** (3.74 g, 91.4%). m.p. 68.5–70°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.24–1.54 (m, 20H, 10 × CH_2), 2.26 (t, 2H, J = 7.2 Hz, CH_2), 2.85 (t, 2H, J = 7.2 Hz, CH_2), 4.22 (t, 2H, J = 7.2 Hz, CH_2), 5.03 (s, 2H, CH_2Ph), 6.90 (d, 2H, J = 8.2 Hz, arom H), 7.11 (d, 2H, J = 8.2 Hz, arom H), 7.30–7.40 (m, 5H, arom H). MS (*m/z*) 228 (M-$CH_3(CH_2)_{11}CO)^+$.

Synthesis of 2-(*p*-hydroxyphenyl)-1-ethyl tridecanoate (14). Ester **14** (2.3 g, 6.88 mmol) was debenzylated by hydrogenation over 5% Pd-C in methanol at r.t. overnight. The catalyst was removed by filtration, and the solvent was evaporated to give the phenol **15** (1.8 g, 100%). m.p. 43–45°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.23–1.59 (m, 20H, 10 × CH_2), 2.26 (t, 2H, J = 7.2 Hz, CH_2), 2.84 (t, 2H, J = 7.2 Hz, CH_2), 4.21 (t, 2H,

J = 7.2 Hz, CH_2), 6.73–7.23 (m, 4H, arom H). MS (*m/z*) 197 $[CH_3(CH_2)_{11}CO)^+]$.

Synthesis of 2-(*p*-sulfamoylphenyl)-1-ethyl tridecanoate (4). The inhibitor was synthesized in a similar manner as compound **2.** m.p. 74.5–76°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.24–1.50 (m, 20H, 10 × CH_2), 2.27 (t, 2H, J = 7.2 Hz, CH_2), 2.83 (t, 2H, J = 7.2 Hz, CH_2), 4.20 (t, 2H, J = 7.2 Hz, CH_2), 7.13–7.25 (m, 4H, arom H), 7.91 (s, 2H, SO_2NH_2). Analysis calculated for $C_{21}H_{35}NO_5S$: C, 60.99; H, 8.53; N, 3.39. Found: C, 60.76; H, 8.59; N, 3.42.

Synthesis of 1-(4-benzyloxyphenyl)-1-hexadecanol (16). 1-Bromopentadecane (6.64 g, 6.58 mL, 22.8 mmol) was added to a suspension of magnesium turnings (555 mg, 22.8 mmol) in 60 mL of anhydrous THF. Initiation of the reaction was achieved by mild heating. After the complete formation of the Grignard reagent 1-pentadecanyl magnesium bromide (1.5 h), a solution of 4-benzyloxybenzaldehyde **15** (3.82 g, 18 mmol) in 15 mL of anhydrous THF was added dropwise, and the reaction mixture was stirred for 3 h at r.t. The reaction mixture was quenched with saturated NH_4Cl and extracted with ethyl acetate (3 × 60 mL). The organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel chromatography and eluted with petroleum ether/ethyl acetate (5:1), giving the alcohol **16** (6.6 g, 86.5%). m.p. 70–72°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 7.0 Hz, CH_3), 1.20–1.83 (m, 29H, 14 × CH_2 and OH), 4.62 (t, 1H, J = 6.6 Hz, CH–OH), 5.08 (s, 2H, $PhCH_2O$), 6.97 (d, 2H, J = 8.4 Hz, arom H), 7.43 (m, 5H, arom H). MS (*m/z*) 406 $(M\text{-}H_2O)^+$.

Synthesis of 1-(4-benzyloxyphenyl)-1-acetyl-hexadecane (17). To a solution of the alcohol **16** (2.12 g, 5 mmol) in 50 mL of CH_2Cl_2 at 0°C was added 4-dimethylaminopyridine (62 mg, 0.5 mmol), triethylamine (Et_3N; 1.4 mL, 10 mmol), and finally acetic anhydride (Ac_2O; 0.71 ml, 7.5 mmol). The reaction mixture was stirred at r.t. for 24 h and then washed with saturated $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluted with petroleum ether/methylene chloride (1:3), affording pure **17** (2.32 g, ~100%). m.p. 62–63°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 6.6 Hz, CH_3), 1.25–1.90 (m, 28H, 14 × CH_2), 2.03 (s, 3H, OAc), 5.04 (s, 2H, $PhCH_2O$), 5.67 (t, 1H, J = 6.6 Hz, CH-OAc), 6.93 (d, 2H, J = 8.2 Hz, arom H), 7.24–7.40 (m, 6H, arom H). MS (*m/z*) 318 $(M\text{-}CH_3COOH)^+$.

Synthesis of 4-hexadecanylphenol (18). Compound **17** (1.6 g, 3.43 mmol) in 100 mL of methanol was hydrogenated over 5% Pd on activated carbon at r.t. for 18 h. The reaction mixture was filtered through Celite. The filtrate was concentrated and purified by silica gel chromatography and eluted with petroleum ether/methylene chloride/ethyl acetate (4:1:1) to obtain pure phenol **18** (1.1 g, 85.3%). m.p. 78–79.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 6.9 Hz, CH_3), 1.25–1.56 (m, 28H, 14 × CH_2), 2.52 (t, 2H, J = 7.5 Hz, $PhCH_2$), 4.63 (br s, 1H, OH), 6.74 (d, 2H, J = 8.2 Hz, arom H), 7.03 (d, 2H, J = 8.2 Hz, arom H). MS (*m/z*) 318 (M^+).

Synthesis 4-(*p-O*-sulfamoylphenyl)-1-hexadecane (5). The inhibitor was synthesized in a similar manner as compound **2.** m.p. 92.5–94°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (t, 3H, CH_3), 1.10–1.53 (m, 28H, 14 × CH_2), 2.51 (t, 2H, $PhCH_2$), 7.12–7.23 (m, 4H, arom H), 7.88 (s, 2H, SO_2NH_2). Analysis calculated for $C_{22}H_{39}O_3NS$: C, 66.46; H, 9.89; N, 3.52. Found: C, 66.72; H, 9.77; N, 3.47.

Biochemical evaluation of inhibitors

Estrone sulfatase assay. The final volume of the enzyme assay was 1 mL. An inhibitor at various concentrations in ethanol was added to a 5 mL test tube. The ethanol was removed with a stream of nitrogen. Estrone sulfate (20 μM per tube; 300,000 dpm per tube) and Tris-Cl buffer (0.02 M, pH 7.2, 0.2 mL) were then added to each tube. The assay tubes containing the estrone sulfate, Tris-HCl buffer, and inhibitor were preincubated for 5 min at 37°C in a water bath shaker. The assay began by the addition of placental microsomes (150 μg) diluted with 0.02 M Tris-Cl buffer, pH 7.2 (0.8 mL). After 20 min of incubation at 37°C, 4 mL of toluene was added to quench the assay. [^{14}C]Estrone (10,000 dpm per tube) was added concurrently with the toluene as the internal standard for the determination of extraction efficiency. Control samples with no inhibitor were incubated simultaneously. Blank samples were obtained by incubating boiled microsomes. The quenched samples were vortexed for 45 s and centrifuged (2000

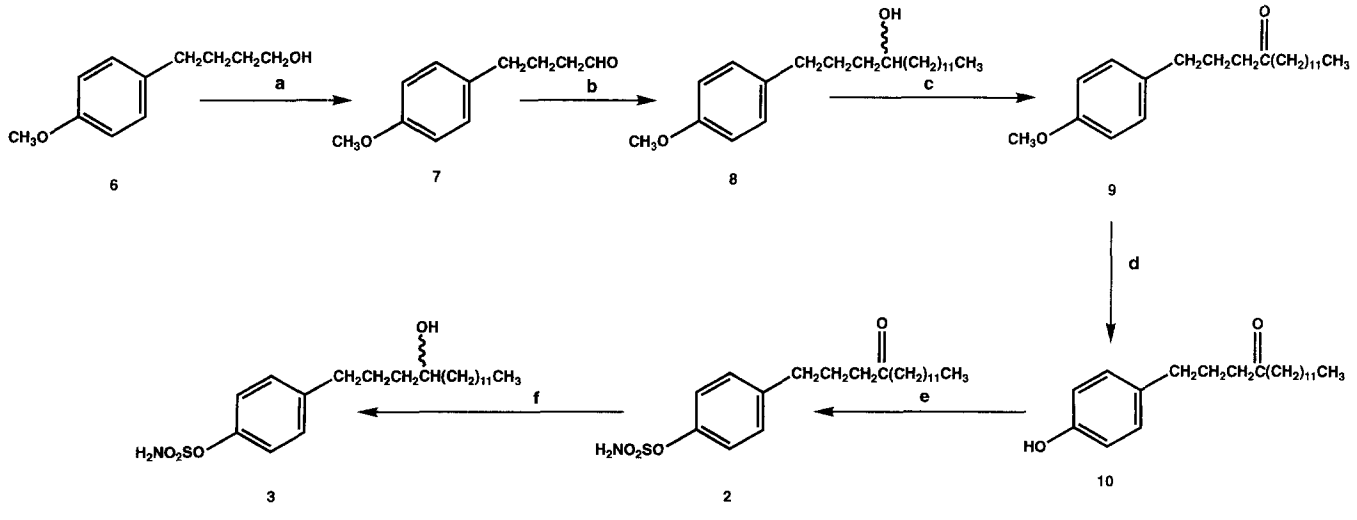


Figure 3 (a) PCC/Celite, CH_2Cl_2, r.t., 45 min; (b) $CH_3(CH_2)_{11}MgBr$, THF, r.t., 2 h; (c) PCC/Celite, r.t., 45 min; (d) BBr_3, CH_2Cl_2, −78°C to r.t. in 45 min; (e) $ClSO_2NH_2$, NaH, DMF, 0°C, 24 h; (f) DIBAL/THF, −78°C, 45 min.

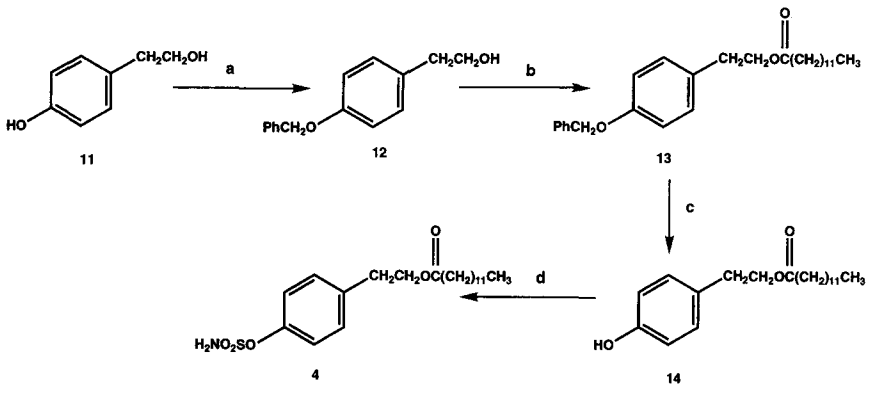


Figure 4 (a) $PhCH_2Br$, K_2CO_3, acetone, reflux 18 h; (b) $CH_3(CH_2)_{11}COCl$, Et_3N, CH_2Cl_2, r.t., 24 h; (c) 5% Pd-C/H_2, MeOH, r.t., 18 h; (d) $ClSO_2NH_2$, NaH, DMF, 0°C, 24 h.

rpm for 10 min). One milliliter of toluene was obtained from each quenched sample to determine the amount of product formation. All the samples were run twice in triplicate with variation of less than 7%. The IC_{50} value, in nanomolar (nM) or micromolar (μM) concentration, represents the concentration of the inhibitor needed to achieve 50% inhibition of estrone sulfatase activity in the microsome when compared to the control with no inhibitor.

Results and discussion

Numerous reports have suggested the importance of estrone sulfate and estrone sulfatase in regulating the supply of estrogens to estrogen-dependent breast cancers in the last 10 years.[3,4] Estrone sulfatase inhibitors may prove to be useful for the treatment of breast cancers. In our continuous effort to design and synthesize potent estrone sulfatase inhibitors lacking estrogenic activity, we recently reported the design, synthesis, and enzyme studies of a series of (*p*-*O*-sulfamoyl)-*N*-alkanoyl tyramines as nonsteroidal estrone sulfatase inhibitors (Figure 1).[10] One of the most potent inhibitors in this series is (*p*-*O*-sulfamoyl)-*N*-tridecanoyl tyramine **1**, with an IC_{50} value of 61.3 nM (Figure 2). The goal of this study is to investigate the structure-activity relationships of the amide functionality in (*p*-*O*-sulfamoyl)-*N*-tridecanoyl tyramine. Four analogs of **1** were synthesized: the keto analog **2**, the hydroxy analog **3**, the ester analog **4**, and the alkyl analog **5** (Figure 2). The syntheses of inhibitors **2** and **3** are shown in Figure 3. Their syntheses began with the PCC oxidation of alcohol **6** to yield the aldehyde **7**. Aldehyde **7** was reacted with 1-dodecyl magnesium bromide to obtain the alcohol **8**. Compound **8** was then oxidized to ketone **9** with PCC. Demethylation of ketone **9** with BBr_3 in CH_2Cl_2 afforded the phenol **10**. Inhibitor **2** was obtained by sulfamoylation of the phenolic group of **10** with sodium hydride and chlorosulfonamide. Reduction of the keto group of **2** with DIBAL in THF yielded **3**.

The synthesis of inhibitor **4** (Figure 4) is similar in procedure for the synthesis of **1**.[10] The synthesis began with the protection of the phenolic group of 2-(*p*-hydroxyphenyl)-1-ethanol **11**. Refluxing **11** in acetone with benzyl bromide and potassium carbonate yielded **12**. Esterification of **12** with 1-tridecanoyl chloride afforded the compound **13**. Hydrogenation of **13** with 5% Pd-C as catalyst formed the debenzylated product **14**. Inhibitor **4** was obtained by sulfamoylation of **14** with sodium hydride and chlorosulfonamide.

The synthesis of **5** (Figure 5) was accomplished in four steps. The synthesis started with reacting the aldehyde **15** with 1-pentadecanyl magnesium bromide to form the alcohol **16**. The alcohol **16** was then converted to acetate **17** with

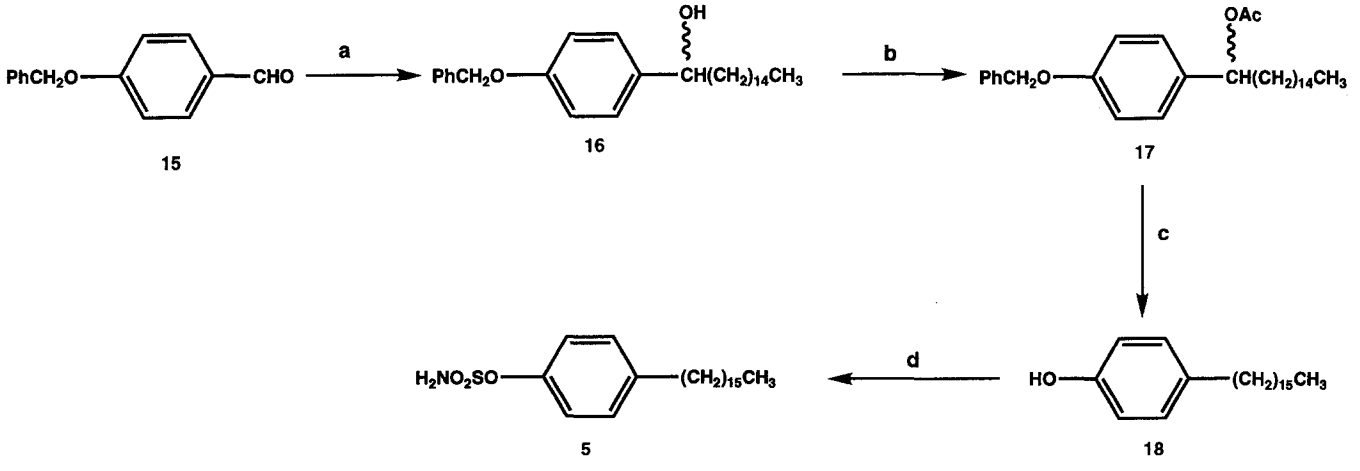


Figure 5 (a) $CH_3(CH_2)_{14}MgBr$, THF, r.t., 3 h; (b) AC_2O, 4-dimethylaminopyridine, Et_3N/CH_2Cl_2, r.t., 24 h; (c) 5% Pd-C/H_2, MeOH; (d) $ClSO_2NH_2$, NaH, DMF, 0°C, 24 h.

acetic anhydride. Hydrogenation of **17** with 5% Pd-C as catalyst not only removed the benzyl protecting group but also removed the acetate group to afford compound **18**. Inhibitor **5** was obtained by sulfamoylation of **18** with sodium hydride and chlorosulfonamide.

The inhibitors were evaluated according to the reported procedure.[10] The sulfatase activities in the presence of increasing amounts of inhibitor concentration were determined to evaluate the relative potency of the inhibitors. The IC_{50} values of compounds **1–5** are shown in Figure 2. The IC_{50} values ranged from 61.3 nM for compound **1**[9] to >20 μM for compound **5**. Replacement of the amide functionality in **1** with an ethylene moiety to form the alkyl analog **5** resulted in complete loss of sulfatase inhibitory activity (IC_{50} of 61.3 nM vs. >20 μM). The keto, hydroxy, and ester analogs (inhibitors **2–4**) are 8–15 times less in affinity to the sulfatase than inhibitor **1**. However, their inhibitory activities are significantly higher than the alkyl analog **5**. The results suggest that the amide functionality is favorable for sulfatase inhibitory activity and that there may be a hydrogen bonding component to the enzyme interaction in this region.

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