Synthesis of a steroidal A ring aromatic sulfonic acid as an inhibitor of steroid 5α-reductase

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The synthesis of 17β-(N,N-diisopropylcarbamoyl)estra-1,3,5(10)-triene-3-sulfonic acid ***(3)*** *has been accomplished. Sulfonate* ***3*** *was designed as a novel inhibitor of human steroid 5α-reductase based on considerations of enzyme mechanisms, and exhibits an inhibition constant in the low nanomolar range.* (*Steroids* **56:**4–7, 1991)

Keywords: steroids; steroid 5α-reductase; enzyme inhibition; benign prostatic hyperplasia; sulfonic acid

Introduction

Our continuing search for potent and selective inhibitors of human steroid 5α-reductase, the enzyme responsible for the conversion of testosterone (T) to 5α-dihydrotestosterone (DHT), has been driven by the potential utility of such compounds in the treatment of DHT-related disorders, such as benign prostatic hyperplasia, male pattern baldness, and acne.[1] The chemical mechanism of this enzyme is proposed to involve a direct, stereospecific hydride transfer from NADPH to C-5 of T, leading to an enolate intermediate that is ultimately protonated at C-4 to yield DHT (Figure 1).

We recently described two new classes of carboxylic acid inhibitors of steroid 5α-reductase, exemplified by **1** and **2,** which were designed as mimics of the putative enzyme-bound enolate intermediate.[2,3] Each compound incorporates sp^2-hybridized centers at C-3 and C-4 and, most significantly, anionic carboxylic acids at C-3 as charged replacements for the enolate oxyanion. Each class of compounds displays apparent inhibition constants with human prostatic steroid 5α-reductase in the tens of nanomolar range (30 nM and 20 nM for **1** and **2,** respectively). In contrast, related primary alcohols, aldehydes, esters, and primary amides are devoid of substantial activity, emphasizing the importance of the negatively charged A ring functionality in these series. Furthermore, both classes of compounds display inhibition kinetics consistent with the formation of ternary complexes with enzyme and $NADP^+$, rather than NADPH, owing presumably to a charge complementarity between the carboxylates and positive nicotinamide.[4,5]

Extending this inhibitor design strategy, we have explored the suitability of other charged functional groups as surrogates for the oxyanion of the putative intermediate state. This report describes the synthesis and in vitro activity of aryl sulfonic acid **3.** The structures of compounds **1, 2,** and **3** are illustrated in Figure 2.

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Experimental

General methods

Melting points (mp) are uncorrected. ^1H nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ solutions (unless otherwise noted) with Bruker AM-250 or Varian EM390 spectrometers and are reported (in part) as parts per million downfield from tetramethylsilane. Infrared (IR) spectra were obtained with a Perkin-Elmer model 783 spectrometer. Mass spectra (MS) were obtained with a Finnigan-MAT quadrupole instrument with desorptive chemical ionization. Chromatography refers to flash chromatography using Kieselgel 60 (230 to 400 mesh) silica gel.

17β-(N,N-diisopropylcarbamoyl)-3-[(N,N-dimethylthiocarbamoyl)-oxy]estra-1,3,5(10)-triene **(5).**

A suspension of compound **4** (2.0 g, 5.2 mmol) in DMF (30 ml) was added under argon to NaH (0.45 g, 11

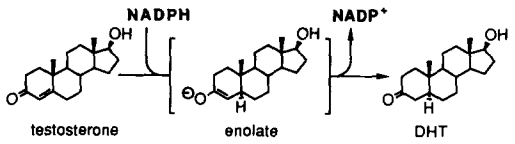


Figure 1 Chemical mechanism of steroid 5α-reductase.

mmol). After the evolution of hydrogen had ceased, a solution of dimethylthiocarbamoyl chloride (0.7 g, 5.66 mmol) in DMF (10 ml) was added, and the resulting reaction mixture was heated at 80 C for 1 hour. The reaction mixture was then cooled to room temperature, diluted with water, and thoroughly extracted with ethyl acetate. The organic extract was subsequently washed with water and brine, dried (Na_2SO_4), and concentrated. Trituration of the resulting solid with acetone provided 2.0 g (82%) of product **5.** An analytic sample was obtained by recrystallization from methanol/acetone; mp, 225 to 228 C. The following values were obtained: ^{1}H NMR (δ): 0.8 (s, 3H), 1.16 (d, J = 6.5 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H), 1.41 (d, J = 6.5 Hz, 3H), 1.43 (d, J = 6.5 Hz, 3H), 3.33 (s, 3H), 3.42 (m, 1H), 3.46 (s, 3H), 4.25 (m, 1H), 6.8 (bs, 1H), 6.85 (d, 1H), 7.3 (d, 1H); IR (Nujol): 1,630 cm^{-1}. Analysis calculated for $C_{28}H_{42}N_2O_2S$: C, 71.45; H, 8.99; N, 5.95. Found: C, 71.69; H, 8.81; N, 5.95.

17β-(N,N-diisopropylcarbamoyl)3-[(N,N-dimethylcarbamoyl)thio]estra-1,3,5(10)-triene **(6)**

A mixture of compound **5** (1.4 g, 3.0 mmol) and sulfolane (15 ml) was heated at reflux for 30 minutes after which the reaction mixture was cooled, diluted with water, and extracted with ethyl acetate. The organic extract was washed with water, dried (Na_2SO_4), and concentrated. Chromatography of the residue (1 : 3 ethyl acetate/hexanes) afforded **6** as a white solid (1.0 g; 71%; mp, 202 to 204 C). The following values were obtained: ^{1}H NMR (δ): 0.8 (s, 3H), 1.16 (d, J = 6.5 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H), 1.41 (d, J = 6.5 Hz, 3H), 1.43 (d, J = 6.5 Hz, 3H), 3.05 (s, 6H), 3.42 (m, 1H), 4.25 (m, 1H), 7.2 to 7.4 (M, 3H); IR (Nujol): 1,630 and 1,670 cm^{-1}. Analysis calculated for $C_{28}H_{42}N_2O_2S$: C, 71.45; H, 8.99; N, 5.95. Found: C, 71.58; H, 8.98; N, 5.78.

17β-(N,N-diisopropylcarbamoyl)-3-mercaptoestra-1,3,5(10)-triene **(7)**

A mixture of compound **6** (1.5 g, 3.2 mmol), 10% aqueous NaOH (15 ml), and ethanol (150 ml) was heated at reflux for 1.5 hours. The resulting reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated to remove ethanol and then diluted with ice water, acidified with dilute HCl, and extracted with ethyl acetate. The organic extract was washed with water and brine, dried (Na_2SO_4), and concentrated to a yellow foam. Chromatography (3 : 17 ethyl acetate/hexanes) provided 0.74 g (58%) of thiophenol **7** as a white solid (mp, 105 to 106 C). The following values were obtained: ^{1}H NMR (δ): 0.8 (s, 3H), 1.16 (d, J = 6.5 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H), 1.41 (d, J = 6.5 Hz, 3H), 1.43 (d, J = 6.5 Hz, 3H), 3.42 (m, 1H), 4.25 (m, 1H), 7.01 (s, 1H), 7.05 (d, 1H), 7.14 (d, 1H); IR (Nujol): 1,625 and 2,520 cm^{-1}; MS (m/z): 400 (M + H$^+$). Analysis calculated for $C_{25}H_{37}NOS$: C, 75.14; H, 9.33; N, 3.51. Found: C, 74.92; H, 9.51; N, 3.75.

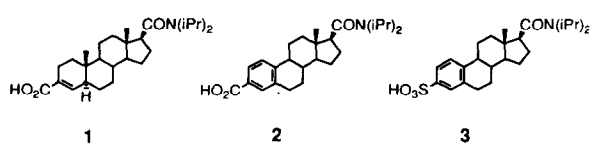


Figure 2 Structure of compounds **1, 2,** and **3.**

17β-(N,N-diisopropylcarbamoyl)-3-(benzylthio)estra-1,3,5(10)-triene **(8)**

Sodium hydride (0.14 g of a 60% suspension in mineral oil, 3 mmol) was added to a solution of product **7** (0.6 g, 1.5 mmol) in 10 ml DMF. After the evolution of hydrogen had ceased, benzyl bromide (0.3 g, 1.75 mmol) was added, and the resulting mixture was stirred at 25 C for 2 hours. The reaction mixture was then poured into ice water and extracted twice with ethyl acetate. The combined organic extracts were washed with water, dried (Na_2SO_4), and concentrated. Trituration of the residue with petroleum ether afforded **8** (0.57 g, 78%) as a white solid. Recrystallization from methanol/acetone afforded an analytic sample; mp, 166 to 168 C. The following values were obtained: ^{1}H NMR (δ): 0.8 (s, 3H), 1.16 (d, J = 6.5 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H), 1.41 (d, J = 6.5 Hz, 3H), 1.43 (d, J = 6.5 Hz, 3H), 3.42 (m, 1H), 4.1 (s, 2H), 4.25 (m, 1H), 7.0 to 7.4 (m, 8H). Analysis calculated for $C_{32}H_{43}NOS$: C, 78.48; H, 8.85; N, 2.86. Found: C, 78.66; H, 8.97; N, 2.80.

17β-(N,N-diisopropylcarbamoyl)estra-1,3,5(10)-triene-3-sulfonic, potassium salt **(3)**

Chlorine gas was bubbled for 3 minutes through a cooled (0 C) suspension of compound **8** (0.5 g, 1.02 mmol) in acetic acid (25 ml glacial) and water (3.3 ml). The resulting reaction mixture was diluted with water and extracted with ethyl acetate. The organic extract was washed with water, 5% aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated to provide crude sulfonyl chloride **9,** which was not further purified. The sufonyl chloride residue was mixed with dioxane (10 ml) and aqueous potassium carbonate (1.5 g in 20 ml), then heated at reflux for 3 hours. The dioxane was then removed in vacuo and the residue was diluted with ice water. The resulting precipitate was collected by filtration, washed with water, and finally triturated with acetone to provide **3** as the potassium salt (0.264 g, 54%). An analytic sample was obtained by precipitation from an aqueous methanol solution with acetone (mp, 325 C). The following values were obtained: ^{1}H NMR (CD_3OD) (δ): 0.78 (s, 3H), 1.18 (d, J = 6.5 Hz, 3H),

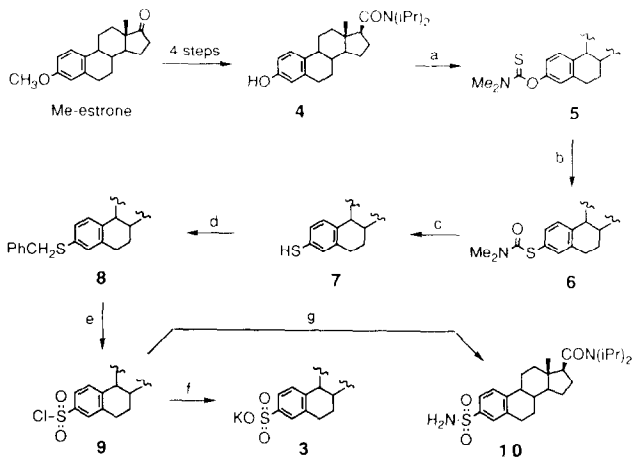
1.28 (d, J = 6.5 Hz, 3H), 1.40 (d, J = 6.5 Hz, 6H), 3.50 (m, 1H), 4.35 (m, 1H), 7.32 (d, 1H), 7.53 (s, 1H), 7.56 (d, 1H); IR (Nujol): 1,640 cm^{-1}; MS (m/z): 448 ($M + H^+$). Analysis calculated for $C_{25}H_{36}NO_4SK \cdot 1/2\ H_2O$: C, 60.69; H, 7.54; N, 2.83. Found: C, 60.42; H, 7.42; N, 2.76.

17β-(N,N-diisopropylcarbamoyl)estra-1,3,5(10)-triene-3-sulfonamide (**10**)

To a solution of sulfonyl chloride **9** (crude, 0.1 g, 0.2 mmol) in acetone (5 ml) was added cold concentrated ammonium hydroxide (5 ml). The resulting mixture was stirred at room temperature for 10 minutes, after which the solid product was collected by filtration. The crude product was dissolved in dichloromethane, washed with water and brine, dried, and concentrated to give 50 mg (52%) of sulfonamide **10** as a white solid. An analytic sample was obtained by recrystallization from acetone (mp, 204 to 207 C). The following values were obtained: ^1H NMR (δ): 0.8 (s, 3H), 1.16 (d, J = 6.5 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H), 1.41 (d, J = 6.5 Hz, 3H), 1.43 (d, J = 6.5 Hz, 3H), 3.42 (m, 1H), 4.25 (m, 1H), 4.75 (s, 2H), 7.41 (d, 1H), 7.65 (s, 1H), 7.68 (d, 1H); IR (Nujol): 1,620 cm^{-1}; MS (m/z): 447 ($M + H^+$). Analysis calculated for $C_{25}H_{38}N_2O_3S$: C, 67.23; H, 8.58; N, 6.27. Found: C, 67.14; H, 8.53; N, 6.25.

Inhibitor evaluation

Assays for steroid 5α-reductase activity were performed with microsomal-associated enzyme from surgically derived benign hyperplastic human prostatic tissue and whole rat ventral prostates. Prostatic microsomes were prepared as previously described for the rat[6] and human[7] tissues. Enzyme activity was determined by measuring the conversion of T to total 5α-reduced metabolites, represented by the sum of DHT and 5α-androstanediol (ADIOL).[8] Briefly, [^{14}C]T (55 mCi/mmol, Amersham) and inhibitors in ethanol were deposited in test tubes and the solvent was removed to dryness. Following the addition of incubation buffer to the tubes, the solutions were equilibrated to 37 C. A 20-μl aliquot of freshly prepared 10 mM NADPH solution was added to each tube immediately before initiation of the reaction with enzyme. The final concentration of cofactor in the 0.5-ml incubation was 400 mM. The rat enzyme incubation buffer consisted of 20 mM sodium phosphate, pH 6.6; that for human microsomes was 50 mM sodium citrate, pH 5.0. Following 20- to 30-minute incubations, the reactions were quenched with 4 ml ethyl acetate containing 0.15 mmol each of T, DHT, androstenedione, and ADIOL. The mixture was vortexed, centrifuged to separate the solvent layers, and the organic layer removed. On evaporation of solvent in vacuo, the residue was dissolved in 40 μl 1 : 1 methanol/chloroform. Substrate and products were separated by thin-layer chromatography on silica gel plates (Baker, Si250F-PA), developed twice with 1 : 9 acetone/chloroform, and evaluated with a Bioscan imaging scanner (Washington, DC, USA). The relative amounts of radiolabel in substrate and products were used to calculate enzyme activity. Assays were conducted such that no more than 20% of initial T concentration was consumed in the reaction. Typically, the Michaelis constants for T with the rat and human prostatic enzymes were determined to be 0.9 mM and 4.5 mM, respectively. Experiments to determine the potency of potential inhibitors were conducted at 400 mM NADPH, 1.2 mM T, and 0 to 10 mM of test compound. Apparent inhibition constants ($K_{i,app}$) were determined for compounds that followed a linear response by Dixon analysis.[9]



Scheme 1 (a) NaH, Me_2NCSCl; (b) 285 C; (c) NaOH, $EtOH/H_2O$; (d) NaH, $PhCH_2Br$; (e) Cl_2, $HOAc/H_2O$; (f) K_2CO_3, dioxane/H_2O; (g) NH_4OH.

Results and discussion

The synthesis of sulfonic acid **3** was accomplished in five steps from phenol **4** (prepared as previously described in four steps from *O*-methyl estrone[3]), as shown in Scheme 1. Sequential treatment of phenol **4** with sodium hydride and dimethylthiocarbonyl chloride provided an 82% yield of thioncarbamate **5.** Newman-Kwart rearrangement[10,11] was effected in 30 minutes in refluxing tetramethylene sulfone (285 C) to provide **6** (71%). Hydrolysis (sodium hydroxide in ethanol-water) then afforded thiophenol **7** (76%). Although direct oxidation of the thiol to the sulfonate was accomplished with oxygen and potassium hydroxide in DMF, a more efficient protocol proved to be benzylation of **7** (sodium hydride, benzyl bromide) to give **8** (78%) followed by oxidation of the benzyl thioether with chlorine in acetic acid-water.[12] The resulting sulfonyl chloride **9** was not purified or fully characterized, but was hydrolyzed immediately (aqueous potassium carbonate, dioxane, reflux) to the sulfonic acid **3** (isolated and characterized as the potassium salt hemihydrate; mp, 325 C; 54% from **8**). Treatment of intermediate **9** with concentrated ammonium hydroxide afforded sulfonamide **10** in 56% yield.

Sulfonate **3** proved to be a potent in vitro inhibitor of human prostatic steroid 5α-reductase, exhibiting an apparent inhibition constant of 20 to 40 nM (comparable

to the analogous carboxylic acid **2**[3]). When tested against the enzyme isolated from rat prostate, however, compound **3** displayed much weaker activity ($K_{i,app}$ = 1,700 nM), further illustrating the previously described species differences in enzymes. In keeping with our proposal that the negatively charged acidic moiety plays a critical role in the enzyme inhibitory activity, the neutral isosteric sulfonamide derivative **10** lacked significant activity on the human enzyme ($K_{i,app}$ > 5 μM). By analogy to the carboxylic acid inhibitors, inhibition of steroid 5α-reductase by **3** is proposed to involve a ternary complex with enzyme and $NADP^+$. Studies examining the in vivo activity of aryl sulfonic acids are in progress and will be reported in due course.

Acknowledgments

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