Nonsteroidal Inhibitors of Human Type I Steroid 5-α-Reductase

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Elevated dihydrotestosterone (DHT), arising from the reduction of testosterone through the action of steroid 5α-reductase, has been implicated in a number of conditions including benign prostatic hyperplasia (BPH),[1] acne,[2] hirsutism,[3] and androgenic alopecia (male-pattern baldness).[4] A number of compounds have been identified as inhibitors of 5α-reductase, including both steroidal inhibitors, MK-906[5] (1, Proscar, finasteride) and SKF 105687[6] (2), and a nonsteroidal inhibitor, ONO-3805[7] (3) (Chart I). Recently, Russell and co-workers have demonstrated the existence of different genes encoding for two 5α-reductase enzymes, types I and II.[8] The physiological role of the type I enzyme versus the type II enzyme is under active investigation. We would like to report a novel class of agents, the benzoquinolinones, which are potent and selective inhibitors of the human type I 5α-reductase enzyme.[9]

Benzoquinolinones shown in Table I were synthesized in a fashion analogous to that employed by Cannon[10] as shown in Scheme I.[11] The 2-tetralones 4 were converted to the pyrrolidine enamines 5 and condensed with acrylamide to afford the hexahydrobenzoquinolinones 6. Methylation under standard conditions provided 7. Ionic reduction[12] of 7 with triethylsilane provided mixtures of cis (8) and trans (9) isomers in a ratio of approximately 1 to 3, respectively. Assignment of stereochemistry for the 4a and 10b hydrogens in 8 and 9 was based upon the magnitude of the coupling constants between them (5.6 Hz for cis, 11.4 Hz for trans) and the presence of a NOE (4a–10b) for the cis isomer. Alternatively, 6 could be reduced with triethylsilane, affording 10 (along with the corresponding cis isomer), and subsequently methylated to obtain 9. Formation of the angular methyl compounds, 11 and 12, followed similar lines. The initial cyclization of tetralone 13 with acrylamide afforded the $\Delta_{4a,5}$ hexahydro derivative 14, which was methylated to provide 15. Ionic reduction of 14 as before led to the trans isomer 11, whereas reduction of 15 afforded 12.

Compounds were evaluated for their ability to inhibit the type I 5α-reductase enzyme in cultured Hs68 human foreskin fibroblast cells.[9] Results are reported as the micromolar concentration required for 50% inhibition (IC_{50}) of enzyme activity (Table I). The parent hexahydrobenzoquinolinone, **6a**,[10] had an IC_{50} of 6 μM. In general, potency was enhanced by fluorine substitution on the aromatic ring, with the greatest increase seen in the 8-fluoro derivative **6b** (IC_{50} of 0.6 μM). The 7-fluoro (**6c**) and 9-fluoro (**6d**) substituents also increased potency, while the 10-fluoro (**6e**) derivative showed diminished activity. The 8-chloro substituent (**6f**) provided an increase in potency similar to its fluoro counterpart and was held constant during evaluation of the effect of inhibitory activity of modifications to the ring juncture and of N-alkylation.

The *N*-methyl analogs showed substantial increases in potency over their nonalkylated counterparts. The octahydro derivatives were typically more potent inhibitors than the corresponding hexahydro compounds, with the trans isomers demonstrating significantly greater activity than the cis isomers. Inclusion of an angular methyl substituent led to a modest decrease in inhibitory activity, contrary to our expectations of increased potency based on structural analogy of the benzoquinolinone nucleus to

Chart I

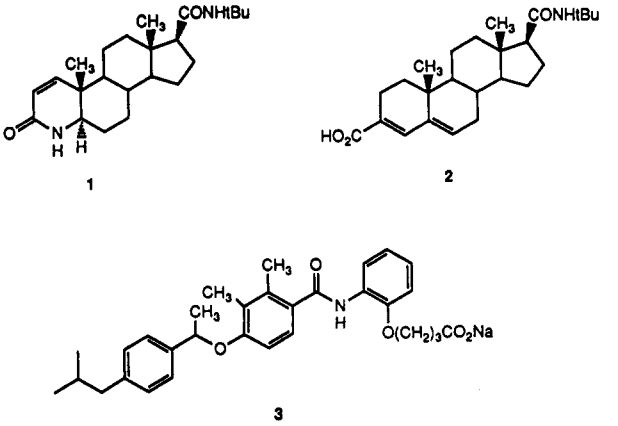

Table I. Inhibition of Type I 5α-Reductase in Hs68 Foreskin Fibroblast Cells[a] by Benzoquinolinone Series

no.	R	Y	X	IC_{50}[b] (μM)
6a	H		H	6.5
6b	H		8-F	0.60
6c	H		7-F	3.2
6d	H		9-F	2.3
6e	H		10-F	10.0
6f	H		8-Cl	0.46
7	CH_3		8-Cl	0.030
8a	CH_3	H	8-Cl	0.041
8b	CH_3	H	8-F	0.32
9a	CH_3	H	8-Cl	0.008
10	H	H	8-Cl	0.060
11	H	CH_3	8-Cl	1.8
12	CH_3	CH_3	8-Cl	0.017
14	H	CH_3	8-Cl	3.6
15	CH_3	CH_3	8-Cl	0.12
9b	CH_3	H	H	0.56
9c	CH_3	H	8-CH_3	0.011
9d	CH_3	H	8-F	0.035
9e	CH_3	H	8-OCH_3	0.12

[a] Hs68 (CRL 1635) human genital skin fibroblasts were plated in Falcon 6-well (35 mm) plates at a density of 50 000 per well and allowed to grow for 4–5 days or until they reached 80% confluence. Test compounds were dissolved in absolute ethanol and diluted with Dulbecco's Modified Eagles Media (DMEM) plus 10% stripped fetal bovine serum. Typical concentration ranges for test compounds were initially 0.032–10.0 μM, and adjusted to 0.001–1.0 μM in subsequent experiments to allow determination of IC_{50} for the highly active analogs. The compounds and 12 μM of [^{14}C]testosterone (50 mCi/mmol) were added to the sample wells in a final volume of 2.0 mL of medium. Following a 4-h incubation in 5% CO_2–95% air at 37 °C, the media was extracted, the individual steroids were separated by TLC, and their radioactivity was counted. Results were expressed as the amount of DHT produced as a percentage of control values. [b] $N = 2$ with average variability less than 15%. For detailed methodology, see the supplementary materials section.

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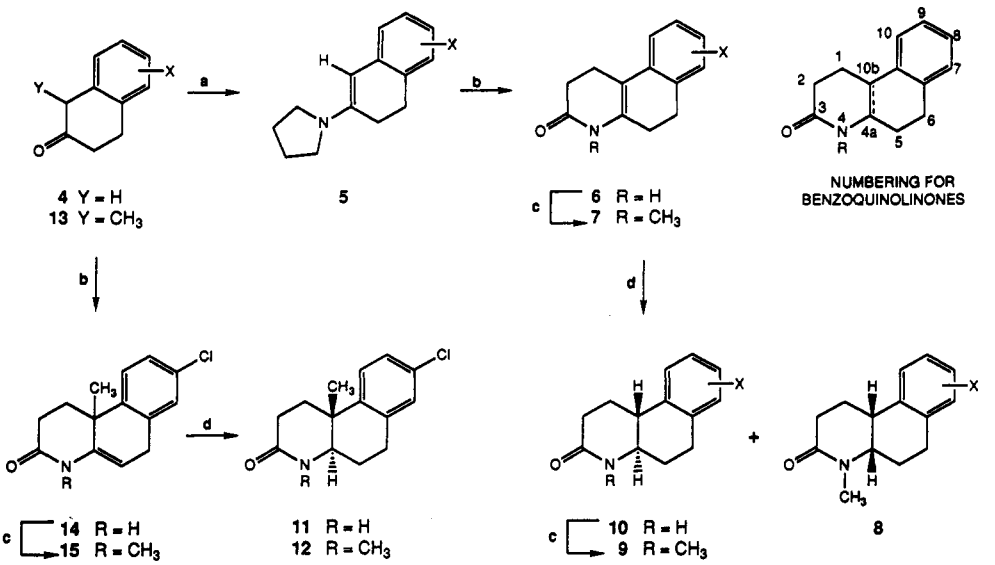
Scheme I[a]



[a] (a) Pyrrolidine, *p*-TSA; (b) methyl acrylamide, Δ; (c) NaH, CH_3I; (d) Et_3SiH, TFA.

Table II. Inhibition of Type I 5α-Reductase in Hs68 Foreskin Fibroblast Cells[a] and Type II 5α-Reductase in Human Prostate Homogenates (HPH)[b] by **9a**, 1, and 2

	IC_{50} (nm)	
compd	Hs68 type I	HPH type II[c]
9a	8	10000
1	62	10
2	7500	8

[a] For methodology, see the legend of Table I. [b] The human prostate enzyme was prepared and assayed as described in ref 13 with the following exceptions. The assay was done using a 40 mM sodium acetate buffer, pH 5.5. The compounds were added in 20 μL of 50% methanol. The incubation was for 30 min at 25 °C. The samples were applied to solid matrix extraction columns (C-18 reverse phase Bond Elut, Analytichem International), washed with acetone/water (1:4), and eluted with methanol. The products were analyzed by HPLC using a Beckman 171 in-line flow radioisotope detector. [c] $N = 2$ with the average variability less than 6%.

the natural steroids. Compound **9a** (LY191704), which possesses the optimal *N*-methyl, trans, angular hydrogen arrangement, is an extremely potent inhibitor of human type I steroid 5α-reductase with an IC_{50} of 0.008 μM.

The structure–activity relationship between substituents on the 8-position and activity was explored briefly. It is noteworthy that both electron-withdrawing and electron-donating substituents demonstrate increased potency over the parent structure, **9b**. Among the series studied, the greatest increases were seen with the chloro (**9a**) and methyl (**9c**) substituents, but significant increases were also noted with fluoro (**9d**) and methoxy (**9e**) groups. Although the potency equivalence for the chloro and methyl substituents is suggestive that lipophilicity is an important determinant of activity in the benzoquinolinone series, a QSAR analysis is not possible with this small of a dataset.

In contrast to their potent inhibition of the human type I 5α-reductase enzyme, the benzoquinolinones as a class are weak inhibitors of the human type II enzyme. The relative potency of a representative benzoquinolinone, **9a**, aza steroid 1, and diene acid 2 versus type I and II enzyme preparations[8] is shown in Table II. Benzoquinolinone **9a** is a very weak inhibitor in human prostate homogenates (type II) with an IC_{50} of greater than 10 000 nM; 1 and 2 both exhibited IC_{50}s of approximately 10 nM against the type II enzyme. Thus, aza steroid 1 is relative nonselective, favoring the type II enzyme, while diene acid 2 is a selective type II inhibitor. In addition, the benzoquinolinones were found to inhibit only weakly the rat 5α-reductase enzyme, possibly due to the low sequence homology exhibited between the human and rat enzymes.[8a,13]

In conclusion, the benzoquinolinones, as a class, are potent and selective inhibitors of the human type I 5α-reductase enzyme with IC_{50} values in the low nanomolar range. They represent a novel opportunity to intervene clinically in conditions due to local overproduction of DHT via the type I enzyme. Furthermore, this series demonstrates that potent inhibition of the type I 5α-reductase enzyme can be realized by a compact, rigid, tricyclic nucleus which does not require the full steroid ring system.

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Supplementary Material Available: General experimental, spectral data for representative compounds, procedures for measurement of 5α-reductase activity in the Hs68 assay, and tables listing physical data for final products (10 pages). Ordering information is given on any current masthead page.

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