Communications to the Editor

6-Azasteroids: Potent Dual Inhibitors of Human Type 1 and 2 Steroid 5α-Reductase

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Received October 1, 1993

In humans, two isozymes of 5α-reductase (5AR) catalyze the conversion of testosterone (T) to the more potent androgen dihydrotestosterone (DHT).[1] Male pseudohermaphrodites with 5AR deficiency have mutations in type 2 5AR, vestigial prostates, decreased acne, and facial and body hair and do not develop male pattern baldness.[2] Type 1 5AR is normal in these individuals and is the likely source of much of their residual plasma DHT (ca. 30% of normal).[2d] Interestingly, these pseudohermaphrodites virilize to different extents at puberty, and this coincides with expression of type 1 5AR in the skin (present in the liver from birth).[3] The relative roles of human type 1 and 2 5AR in developmental physiology and in the pathophysiology of benign prostatic hyperplasia (BPH), and other androgen related disorders, is the subject of intense research. A number of 5AR inhibitors have been identified, including finasteride (MK-906, 1)[4] and epristeride (SK&F 105657, 2),[5] which have been assessed clinically (Chart I).[6] We report here on novel 6-azaandrost-4-en-3-ones that are potent inhibitors of both human 5ARs with exceptional—*picomolar*—potency versus the type 2 enzyme.

The stimulus for preparing 6-azaandrost-4-en-3-ones as potential 5AR inhibitors was based on the transition state inhibitor paradigm.[4,5] The synthesis of the 6-azaandrost-4-en-3-one nucleus, outlined in Scheme I, followed the general strategy which Lettré employed to introduce nitrogen at the 6-position of cholesterol.[7] Accordingly, 3β-hydroxyetienic acid methyl ester[4a] (3) was protected as the triisopropylsilyl ether and treated with ozone followed by reductive workup (Zn, acetic acid), and the intermediate aldehyde was oxidized to keto-acid 4 with Jones reagent. Conversion of 4 to an acyl azide followed by heating and stirring with silica gel effected the Curtius rearrangement and subsequent cyclization to give 17β-carbomethoxy-3β-[(triisopropylsilyl)oxy]-6-azaandrost-5-ene, 5. The discovery that the silica gel induced cyclization of the intermediate isocyanate proceeded in excellent yield avoided the major pitfall of similar approaches to the 6-azasteroid nucleus where basic conditions employed for cyclization led to β-elimination of the 3-oxy substituent.[7,8] Compound 5 was then treated with di-*tert*-butyl dicarbonate, which acylated the 6-nitrogen with migration of the double bond, the silyl group was removed with TBAF, and the resulting alcohol was oxidized with PDC to give 6. Lithium hydroxide hydrolysis of 6 yielded the C-17

Table I. Inhibition of Recombinant Type 1 and 2 Human 5α-Reductase[a] by 6-Azaandrost-4-en-3-ones

no.	R	type 1 K_i (nM)[b]	type 2 IC_{50} (nM)[c]
7	OCH_3	150	3.2
8	O-2-adamantyl	6.9	0.04
9	NEt_2	750	1.5
10	NMeOMe	2300	2.4
11	NH-1-adamantyl	11	0.07
12	NH-*t*-Bu	820	0.88
13	N-*i*-Pr_2	190	0.36
14	NH-*i*-Pr	6000	6.7
15	NMeEt	720	2.8
16	$NHCH_2Ph$	360	0.58
17	$N(CH_2Ph)_2$	75	0.13
18	$NHCHPh_2$	30	0.09
19	$NHCPh_3$	8.2	0.09
20	NHCH(4-fluorophenyl)$_2$	20	0.16
21	NHCH(4-chlorophenyl)$_2$	20	0.12
22	NHCH(cyclohexyl)$_2$	20	0.40
23	$NHNPh_2$	14	0.23
24	piperazine	8600	33
25	morpholine	2200	7.1
26	thiomorpholine	570	1.3
27	*i*-Bu	9	0.08
2	epristeride	>5000	30
1	finasteride	150[d]	0.18

[a] Frozen stocks of microsomes, prepared from baculovirus infected SF-9 cells expressing either human type 1 or 2 5AR, were diluted immediately before use to final enzyme concentrations ranging from 0.05 to 5 nM. With the exception of the inhibitors, all dilutions and enzyme assays were performed at 37 °C in the following standard assay buffer: 17.6 mM diethylamine, 17.6 mM imidazole, 14.2 mM succinic acid, 0.26 M KCl, and 1 mM dithiothreitol. The pH of the buffer was adjusted to either pH 7 or 6 for type 1 and 2 5AR, respectively. A regenerating system containing 1 mM glucose 6-phosphate and 20 units/mL of glucose 6-phosphate dehydrogenase was included in the assay buffer for type 2 5AR since reaction times exceeded 20 min. [b] The type 1 assay was carried out in polystyrene 96-well plates, and the total assay volume was 300 μL. On a separate plate, inhibitor concentrations were serially diluted 2-fold in 100% DMSO followed by 2-fold further dilution with buffer. A 5-μL aliquot of this inhibitor solution was preincubated for 10 min in standard buffer containing 1 mM NADPH and 1–5 nM type 1 5AR. The assay was initiated by addition of 45 μL of 0.55 μM [1,2,6,7-^{3}H]testosterone (100 nM final concentration). After 20 min a 60-μL aliquot from the assay was added to 150 μL of ethanol, and the percent T and DHT were quantitated by radiochemical analysis following separation by HPLC. The error in the K_i determinations estimated at the 95% confidence limit was between 10 and 75% of the reported value. [c] The type 2 assay was carried out in polystyrene 96-well plates as above. On a separate plate, inhibitor concentrations were serially diluted 2–3-fold in 100% DMSO followed by 4-fold further dilution with buffer. A 7.2-μL aliquot of this inhibitor solution was preincubated for 20 min (see ref 10) in standard buffer containing an NADPH regenerating system and 0.1 nM type 2 5AR. The assay was initiated by addition of 20 μL of 80 nM [1,2,6,7-^{3}H]testosterone (8 nM final concentration). After 20–40 min the assay was quenched with ethanol, and the percent T and DHT were quantitated by radiochemical analysis following separation by HPLC. The error in the IC_{50} determinations estimated at the 95% confidence limit was between 10 and 75% of the reported value. [d] This is the IC_{50} determined under these assay conditions. Finasteride is a slow, time-dependent inhibitor of type 1 and 2 5AR (see refs 10 and 11).

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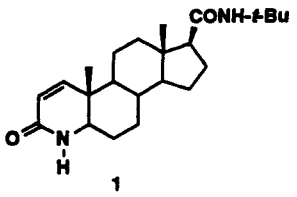
0022-2623/93/1836-4313$04.00/0

Chart I



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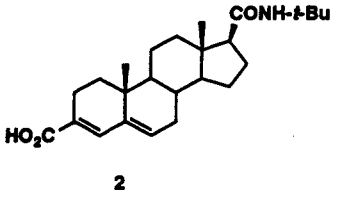

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acid which was converted to the corresponding acid chloride, coupled with the appropriate amine or alcohol and deprotected to give compounds **8–26**. Alternatively the acid chloride could be treated with cuprous iodide followed by isobutylmagnesium chloride and deprotected to give ketone **27**.[9] Deprotection of **6** produced C-17 methyl ester **7** directly.

Compounds were assayed using microsomes prepared from recombinant baculovirus infected SF9 cells expressing either type 1 or 2 human 5AR.[1,10] Conversion of [1,2,6,7-^{3}H]T to DHT was monitored by radiochemical detection of peaks separated by HPLC and K_i's versus type 1 5AR were determined by titration of enzyme with inhibitor while IC_{50}'s versus type 2 5AR were determined after a 30-min preincubation of inhibitor with enzyme.[10] Table I summarizes the results for compounds **7-27** as well as finasteride[4,10,11] and epristeride.[5] Within this limited series, potency versus type 1 and 2 5AR appears to be dominated by the lipophilicity of the C-17 substituent.[12] For example with type 1 5AR, compare **7** to **8** and **12** to **11** where replacement of methyl and *tert*-butyl by adamantyl results in a 20–50-fold greater type 1 potency. The series resulting from replacement of benzylic hydrogens by phenyl in **16** to give **18** and **19**, as well as the heteroatom series in **24**, **25**, and **26** (NH, O, S), are also consistent with this trend.

The results of in vivo evaluation of promising dual inhibitors dosed orally in a chronic castrated rat model[4c,13] are summarized in Table II. In vivo activity varies substantially with the C-17 substituent in this series, and the most active compounds are equivalent to finasteride in their ability to inhibit T-stimulated prostate growth in this model.[14,15] With this indication of oral activity in the rat, the half-lives of compounds **18**, **20**, and **21** were determined to be 4.8, 5.2, and 12 h, respectively, in the dog, and **18** was shown to be 80% orally bioavailable.[16]

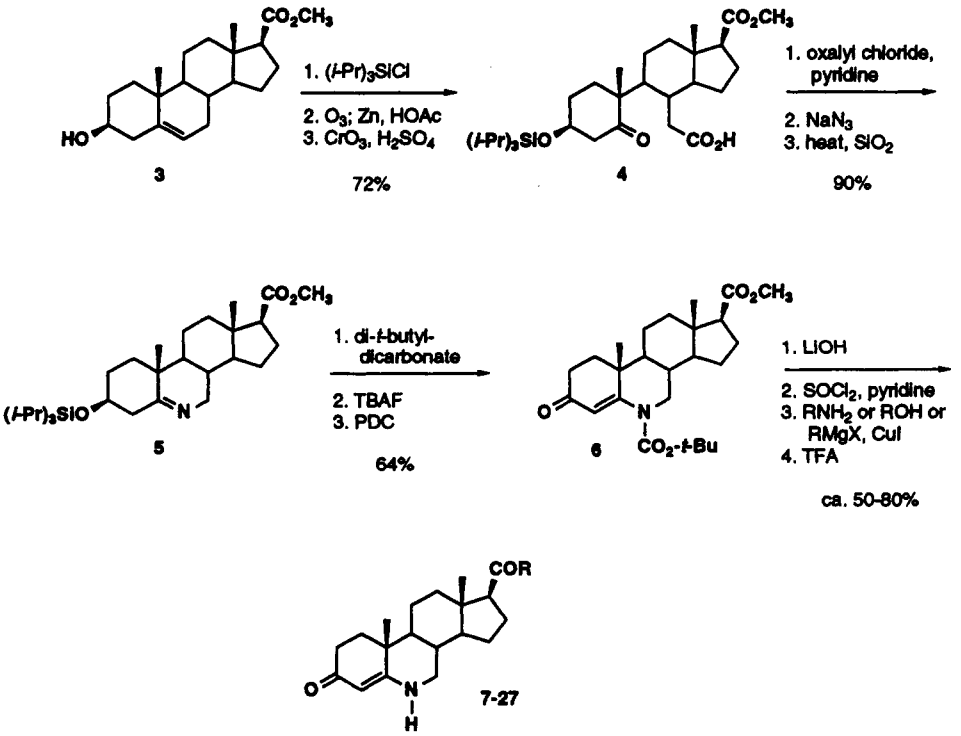
Table II. In Vivo Evaluation of 6-Azaandrost-4-en-3-ones in the Castrated Rat[a]

no.	% redn in prostate wt vs T-treated controls	no.	% redn in prostate wt vs T-treated controls
8	26	21	31
11	18	22	14
18	43	23	38
19	4	27	18
20	43	1	41

[a] In vivo efficacy testing was performed by a modification of the methods of Dorfman.[13] Juvenile male Sprague–Dawley rats (40–50 g) were castrated under halothane anesthesia. Seven days after castration, the rats were dosed orally with the test compound at 10 mg/kg/day, finasteride (1) 10 mg/kg–day or vehicle (n = 8 rats/group, 7 days). Four hours after oral dosing with the test compound, the animals were dosed with testosterone (40 μg/kg, sc). On the eighth day after the start of dosing, the rats were sacrificed and the ventral prostates were removed, cleaned of adherent tissue, and weighed. Data are expressed as percent reduction in prostate weight which was calculated as follows: 100–100[(prostate weight of test compound + T group)/(prostate weight of vehicle + T group)]. Mean prostate weights for the vehicle + T groups were 17–28 mg. Standard errors for prostate weights were generally 10% of the mean or less.

On the basis of the clinical efficacy of finasteride,[4d] its in vitro profile, and the residual circulating DHT in patients treated with the drug,[17] a more effective dual inhibitor of type 1 and 2 human 5AR may show advantages in the treatment of disease states which depend upon DHT.[1b] The 17β-[*N*-(diphenylmethyl)carbamoyl]-6-azaandrost-4-en-3-ones (compounds **18**, **20**, and **21**) are potent inhibitors of type 1 and 2 human 5AR with good in vivo efficacy in the rat and adequate half-life in the dog. Clinical evaluation of such potent dual 5AR inhibitors may help define the relative roles of human type 1 and 2 5AR in the pathophysiology of benign prostatic hyperplasia and other androgen dependent diseases.

Scheme I



Acknowledgment. We gratefully acknowledge Professor David W. Russell for providing cDNA of the human type 1 and 2 5AR and Ms. Sue H. Kadwell, Dr. Thomas A. Kost, Ms. Laurie Overton, Dr. Indravadan Patel, and George B. Wisely (Department of Molecular Biology, Glaxo Inc. Research Institute) for expression cloning of human type 1 and 2 5AR. We also thank Ms. Catherine J. Mader, Dr. Alan Millar (Department of Synthetic Organic Chemistry, Glaxo Inc. Research Institute), and Dr. Michael R. Peel (Department of Medicinal Chemistry, Glaxo Inc. Research Institute) for supplying some intermediates.

Supplementary Material Available: General experimental and spectral data for representative compounds and physical data for final products (18 pages). Ordering information is given on any current masthead page.

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(12) Log *P*'s calculated for MeCO-R (available for all but **10**, **15**, and **23**) plotted versus pK_i gave an $R^2 = 0.74$ for type 1 and an $R^2 = 0.65$ for type 2.

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(15) Compounds **7**–**27** showed no significant binding to the rat androgen receptor (<10% at 5μM).

(16) The determination of half-lives of compound **18**, **20**, and **21** in the dog was based on the disappearance rate of parent drug in the terminal phase from the semilog blood concentration versus time plot. The half-life of finasteride in the dog is 4 h.

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