6-Azasteroids: Structure–Activity Relationships for Inhibition of Type 1 and 2 Human 5α-Reductase and Human Adrenal 3β-Hydroxy-Δ⁵-steroid Dehydrogenase/3-Keto-Δ⁵-steroid Isomerase

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6-Azaandrost-4-en-3-ones were synthesized and tested versus human type 1 and 2 steroid 5α-reductase (5AR) and human adrenal 3β-hydroxy-Δ⁵-steroid dehydrogenase/3-keto-Δ⁵-steroid isomerase (3BHSD) to explore the structure–activity relationship of this novel series in order to optimize potency versus both isozymes of 5AR and selectivity versus 3BHSD. Compounds with picomolar IC_{50}'s versus human type 2 5AR and low nanomolar K_i's versus human type 1 5AR with 100-fold selectivity versus 3BHSD were identified (**70**). Preliminary in vivo evaluation of some optimal compounds from this series in a chronic castrated rat model of 5AR inhibitor-induced prostate involution and dog pharmacokinetic measurements identified a series of 17β-[*N*-(diphenylmethyl)carbamoyl]-6-azaandrost-4-en-3-ones (compounds **54**, **66**, and **67**) with good in vivo efficacy and half-life in the dog. Inhibitors with, at the minimum, low nanomolar potency toward both human 5AR's and selectivity versus 3BHSD may show advantages over previously known 5AR inhibitors in the treatment of disease states which depend upon dihydrotestosterone, such as benign prostatic hyperplasia.

Introduction

Benign prostatic hyperplasia (BPH) is the most common neoplastic disease of mankind and is virtually a universal condition in the aging male.[1] The incidence of BPH detected at autopsy increases from approximately 30% at age 50 to >80% at age 80. A substantial portion of men with BPH develop urinary symptoms, collectively known as prostatism, and undergo transurethral resection of the prostate (TURP) to alleviate these symptoms.[2] Although the etiology of BPH is unclear, the permissive role of dihydrotestosterone (DHT) in the hyperplastic growth of the prostate is well established.[1,3] 5α-Reductase (5AR) is the enzyme which catalyzes the conversion of testosterone (T) to the more potent androgen DHT, and two isozymes are known in humans.[3] 5AR-deficient male pseudohemaphrodites have mutations in type 2 5AR, vestigal prostates, and decreased acne, facial, and body hair and do not develop male pattern baldness.[4] Type 1 5AR is normal in these individuals and is the likely source of much of their residual plasma DHT (ca. 30% of normal).[4d] These pseudohemaphrodites experience varying degrees of virilization of their external genitalia at puberty, and this coincides with expression of type 1 5AR in the skin (present in the liver from birth).[5] With the discovery of two isozymes of 5AR, the relative roles of these enzymes in developmental physiology and in the pathophysiology of benign prostatic hyperplasia, and other androgen related disorders, are the subject of much current research.[3-8] A number of 5AR inhibitors have been identified including finasteride (MK-906, **1**)[6] and epristeride (SK&F 105657, **2**)[7] which have been assessed clinically (Chart 1).[8] Finasteride has proven somewhat less effective in treating BPH than originally expected.[9] Among the likely causes of the clinical shortcomings of finasteride, certain obstacles (the heterogeneity of the disease,[10] the large placebo effect,[6d,e] and the possible role of T[7b,11]) will be faced by any 5AR inhibitor developed for BPH. However, the residual circulating DHT in patients treated with the drug (20–40% of base line)[6e,12] is a clear target for improvement, and a more effective dual inhibitor of type 1 and 2 human 5AR may lower circulating DHT to a greater extent than finasteride and show advantages in the treatment of BPH and other disease states which depend upon DHT.[4a,b]

In addition to potent dual inhibition of type 1 and 2 5AR, the issue of selectivity in a 5AR inhibitor for the treatment of BPH has been emphasized by reports of toxicity, specifically hepatotoxicity in dogs, in the 4-azasteroid series[6] exemplified by an early Merck lead, 4-MA (**3**).[13] While selectivity in the 4-azasteroid series versus the rat androgen receptor has been discussed,[6b,14] it is unlikely that hepatotoxicity was related to this activity. Although not obviously related to hepatotoxicity either, the potent inhibition of bovine adrenal and porcine granulosa cell 3β-hydroxy-Δ⁵-steroid dehydrogenase/3-keto-Δ⁵-steroid isomerase (3BHSD) by 4-MA and not finasteride (Table 1)[15] and the critical role of this enzyme in steroid biosynthesis[16] led us to incorporate selectivity versus human adrenal 3BHSD as an in vitro criteria for evaluating compounds. We report here on the synthesis and structure–activity relationship (SAR) for a novel series of 6-azaandrost-4-en-3-ones optimized for potency versus human type 1 and 2 5AR with

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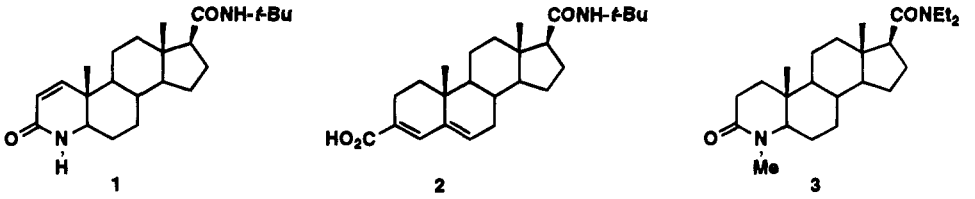
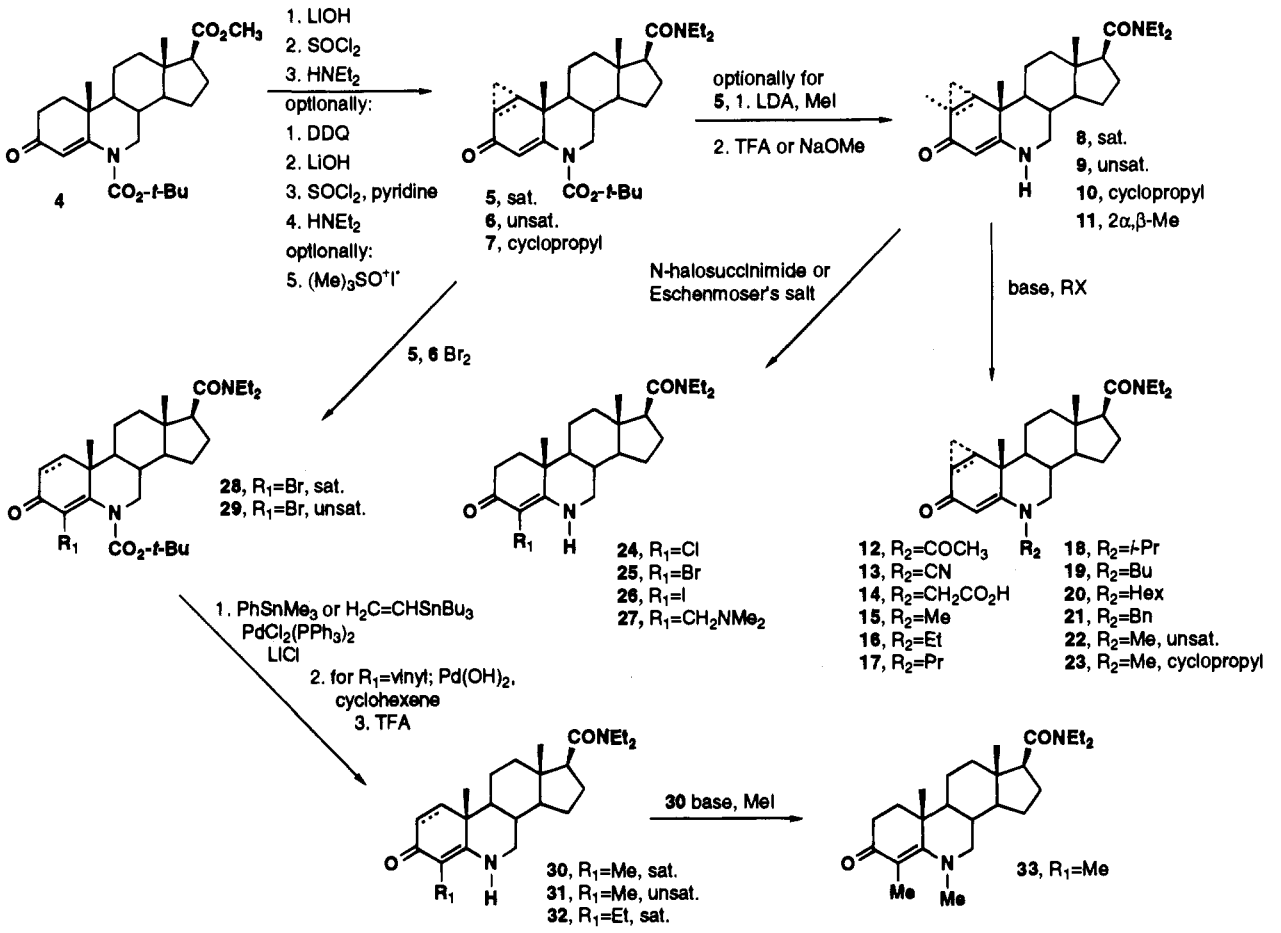
Chart 1



Scheme 1

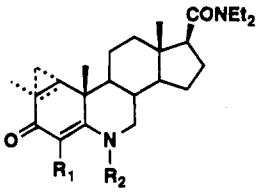


selectivity versus human adrenal 3BHSD.[17] Rat androgen receptor binding, in vivo evaluation in a chronic castrated rat model of 5AR inhibitor-induced prostate involution,[6c] and dog pharmacokinetic measurements are also presented for some optimal compounds from this series.

Chemistry

Starting from 3β-hydroxyetienic acid methyl ester,[6a] compound **4** was prepared in nine steps and 50% overall yield on a 100 g scale as previously described.[17] The observation that the reactivity of the 6-aza Δ^4-3-one system can be very enone-like, when the 6-nitrogen is protected with an electron-withdrawing substituent, or ketoenamine-like, when the 6-nitrogen is unprotected, forms the conceptual basis for functionalization of the A-ring in this system. The chemistry employed on **4** to explore the SAR of N-6, C-1, C-2, and C-4 is summarized in Scheme 1. Hydrolysis of **4**, acid chloride formation, and treatment with diethylamine gives **5**. Optionally, Δ^1-unsaturation was introduced by oxidation of **4** with DDQ followed by hydrolysis of the methyl ester and standard acid chloride coupling to diethylamine to give **6**. Compound **6** was cyclopropanated with trimethylsulfoxonium iodide to give **7**. A methyl group was introduced as a 1.4:1 mixture of α- and β-epimers at C-2 by treatment of **5** with LDA followed by MeI. The 6-nitrogen was then deprotected with either TFA or sodium hydroxide in methanol to give **8**–**11**, and these compounds (and **30**) could be alkylated on nitrogen by treatment with sodium hydride followed by an alkyl iodide or acetyl chloride to give **12**, **14**–**23**, and **33**. Compound **13** was prepared by treating **8** with potassium *tert*-butoxide followed by 4-chlorophenyl cyanate. Compound **8** reacted with *N*-halosuccinimides or *N*,*N*-dimethylmethyleneammonium iodide (Eschenmoser's salt) on C-4 to give compounds **24**–**27**. Bromine was also introduced at C-4 with the *N*-6-*tert*-butoxycarbonyl group in place by treatment of **5** and **6** with bromine in the presence of potassium carbonate to yield **28** and **29**. The bromination of **6** appeared completely selective for the Δ^4-double bond. The C-4 bromides, **28** and **29**, were converted to the C-4 methyl derivatives, **30** and **31**, by treatment with phenyltrimethyltin and palladium chloride. An ethyl group at C-4 was similarily provided by reaction of **28** with vinyltributyltin/palladium chloride

Table 1. Inhibition of Recombinant Type 1 and 2 Human 5α-Reductase and Human Adrenal 3β-Hydroxy-Δ^5-steroid Dehydrogenase/3-Keto-Δ^5-steroid Isomerase by 6-Azaandrost-4-en-3-ones **8**–**27** and **30**–**34**



no.[a]	R_1	R_2	other	type 1 5AR K_i (nM)	type 2 5AR IC_{50} (nM)[b]	3BHSD K_i (nM)
8	H	H	–	750	1.5	60
9	H	H	Δ^1	5800	3.5	80
10	H	H	1,2-α-methano	14 000	1.8	95
11[c]	H	H	2α,β-Me	3500	3.4	510
12	H	$COCH_3$		40 000	3000	34 000
13	H	CN		8300	42	1500
14	H	CH_2CO_2H		10 000	1500	180
15	H	Me		180	2.3	3.6
16	H	Et		1300	3.5	49
17	H	Pr		24 000	4.2	520
18	H	*i*-Pr		3400	4.9	1100
19	H	Bu		7800	29	3600
20	H	Hex		5900	12	11 000
21	H	Bn		10 000	40	16 000
22	H	Me	Δ^1	1300	5.7	22
23	H	Me	1,2-α-methano	10 000	5.0	10
24	Cl	H		51	1.9	27
25	Br	H		97	2.1	13
26	I	H		690	6.0	15
27	CH_2NMe_2	H		10 000	170	1200
30	Me	H		40	3.9	18
31[d]	Me	H	Δ^1	280	8.1	91
32	Et	H		4100	180	47
33	Me	Me		82	4.5	11
34[e]	H	H	homo-B-ring	1000	3.8	1500
1			finasteride	150[f]	0.18	11 000
2			epristeride	1600[g]	0.18[g]	160
3			4-MA	5.0	0.23	20

[a] Satisfactory elemental analyses were obtained (C, H, N; ±0.4% of calculated values) unless otherwise noted. [b] Time-dependent inhibition of type 2 5AR was observed for many of these compounds (see ref 21 and text). [c] 1.4:1 ratio of α- to β-isomers by reverse-phase HPLC. [d] An elemental analysis was not obtained for this compound; ^1H NMR and MS were consistent with the structure and purity of ≥90%. [e] See ref 18 for the synthesis of this compound. [f] This is an IC_{50}. Finasteride is a time-dependent inhibitor of type 1 5AR; see ref 21(b). [g] These are values determined using current experimental procedures reported herein which compare favorably to the values reported in ref 20 (830 and 0.7 nM, type 1 and prostatic homogenate IC_{50}'s, respectively). Reference 17, Table 1, reported literature values for this compound and inadvertently omitted a note to that effect [ref 7(a) and 8].

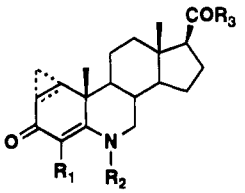
followed by transfer hydrogenation with palladium hydroxide and cyclohexene to give **32**. The other compounds of Scheme 1 and Tables 1–3 were prepared by the methods described here or previously.[17,18]

Results and Discussion

In Vitro SAR. The initial impetus for preparing 6-azaandrost-4-en-3-ones as potential 5AR inhibitors was based on the transition state inhibitor paradigm[19] whereby the ketoenamine functionality would mimic structural and charge-polarization features of the transition state for the enzyme-catalyzed transfer of hydride from NADPH to testosterone. Synthesis and 5AR inhibition studies of **8** confirmed that this series merited further investigation, and the compounds of Tables 1–3 were prepared to explore the SAR of the 6-azaandrost-4-en-3-ones versus type 1 and 2 5AR and human adrenal 3BHSD. The 5AR assay methods employed were as previously described (Table 1).[17,21b] 3BHSD enzyme activity in microsomes derived from human adrenal tissues was measured by monitoring (HPLC) the conversion of dehydroepiandrosterone to androstenedione in the presence of test compounds compared to the corresponding conversion in the control study.

Initially, a set of N-6-, C-1-, C-2-, and C-4-substituted derivatives of **8** were prepared to explore the SAR of the A- and B-rings. The results of this study are presented in Table 1, with epristeride, finasteride, and 4MA included for comparison.[20] Type 1 5AR activity was decreased by Δ^1-unsaturation (**9**), 1,2-cyclopropanation (**10**), C-2 methylation (**11**), and electron-withdrawing groups on the 6-nitrogen (**12** and **13**). Although methylation of N-6 increased type 1 5AR potency 4-fold (**15**) and ethylation was tolerated (**16**), larger alkyl groups (**17**–**21**) or carboxymethylation (**14**) significantly reduced activity. The largest gains in type 1 potency resulted from substitution of C-4 with small lipophilic groups such as chlorine (**24**), bromine (**25**), and methyl (**30**). Larger lipophilic or polar functionality at C-4 resulted in decreased type 1 potency (**27** and **32**). The increased potency upon C-4 methylation mirrors the effect of N-4 methylation in the 4-azasteroids.[20] The SAR for 3BHSD inhibition appears to be a somewhat attenuated version of the SAR versus type 1 5AR with subtle differences. Accordingly, Δ^1-unsaturation and 1,2-cyclopropanation were better tolerated, N-6 methylation increased 3BHSD potency the most (**15**, 15-fold), ethylation was neutral, and larger alkyl groups or, to a

Table 2. Inhibition of Recombinant Type 1 and 2 Human 5α-Reductase and Human Adrenal 3β-Hydroxy-Δ^5-steroid Dehydrogenase/3-Keto-Δ^5-steroid Isomerase by 6-Azaandrost-4-en-3-ones **35**–**58**



no.[a]	R_1	R_2	R_3	other	type 1 5AR (K_i(nM)	type 2 5AR IC_{50} (nM)[b]	3BHSD K_i (nM)
35	H	H	NH-*t*-Bu		820	0.88	150
36	H	H	NH-*t*-Bu	Δ^1	2400	1.8	2200
37	H	H	NH-*t*-Bu	1,2-α-methano	49 000	4.4	300
38	H	Me	NH-*t*-Bu		87	1.7	6.6
39	H	Me	NH-*t*-Bu	Δ^1	1400	6.7	62
40	Me	H	NH-*t*-Bu		12	1.4	9.2
41	Me	Me	NH-*t*-Bu		60	8.6	23
42	H	H	*i*-Bu		9	<0.10[c]	10
43	H	Me	*i*-Bu		3.1	0.10	1.1
44	Br	H	*i*-Bu		3.2	0.40	2.3
45	Me	H	*i*-Bu		0.40	<0.10	1.2
46	H	H	NH-1-Ad[d]		11	<0.10	78
47	H	H	NH-1-Ad	Δ^1	74	<0.10	1000
48	H	Me	NH-1-Ad		8.5	<0.10	2.7
49	H	Me	NH-1-Ad	Δ^1	280	0.30	62
50	Br	H	NH-1-Ad		4.5	<0.10	13
51	Me	H	NH-1-Ad		1.1	<0.10	9.0
52[e]	Br	Me	NH-1-Ad		8.6	1.7	8.3
53	Me	Me	NH-1-Ad		6.2	0.4	18
54	H	H	$NHCHPh_2$		30	<0.10	150
55	H	H	$NHCHPh_2$	Δ^1	48	<0.10	990
56	H	Me	$NHCHPh_2$		6.4	0.20	11
57	H	Pr	$NHCHPh_2$	Δ^1	340	1.3	180
58	Me	H	$NHCHPh_2$		3.6	<0.10	18

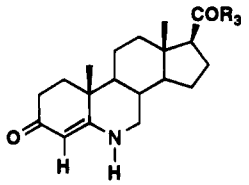
[a] Satisfactory elemental analyses were obtained (C, H, N; ±0.4% of calculated values) unless otherwise noted. [b] Time-dependent inhibition of type 2 5AR was observed for many of these compounds (see ref 21 and text). [c] The enzyme concentration of ca. 100 pM establishes a lower limit for measurable IC_{50}'s. [d] 1-Adamantylamine. [e] A satisfactory elemental analysis was not obtained for this compound. C: calcd, 66.29; found, 66.98.

lesser extent, carboxymethylation reduced activity. Again, as with type 1 5AR, electron-withdrawing groups on the 6-nitrogen greatly diminished inhibitory activity. Substitution of C-4 with lipophilic substituents increased potency versus 3BHSD, and this enzyme was less sensitive to steric bulk at this position than type 1 5AR. For example, substitution of C-4 with iodine and ethyl increased the 10-fold 3BHSD to type 1 5AR selectivity in **8** to 50- and 100-fold, respectively, in **26** and **32**. Interestingly, substitution of C-4 with chlorine transformed the 10-fold 3BHSD-selective **8** to a roughly equipotent (30–50 nM) inhibitor of these two enzymes (**24**).

Many of the compounds of Tables 1–3 demonstrate time-dependent inhibition of type 2 5AR,[21] and IC_{50}'s determined after 20 min prequilibrations versus this enzyme are reported. In general, potency versus type 2 5AR is relatively insensitive to many of the changes that significantly influenced type 1 5AR and 3BHSD activity. It is possible, even likely, that true K_i's would reveal a greater range of SAR. Acetylation and carboxymethylation of N-6 (**12** and **14**) are the only changes which result in 1000-fold loss of activity. Substitution of N-6 with cyano or benzyl (**13** and **21**) and C-4 with (dimethylamino)methylene or ethyl (**27** and **32**) results in >10-fold loss in activity. Alkyl substituents at N-6 are especially ineffectual at changing the IC_{50} versus type 2 5AR (**15**–**20**).

When the data of Table 1 are viewed with the overall objective of optimizing potency versus both isozymes of 5AR and selectivity versus 3BHSD in mind, it is obvious that achieving type 1 5AR potency while maintaining selectivity versus 3BHSD is the major challenge in this series. Within the A- and B-ring chemistry explored, C-4 chlorination and methylation increase type 1 5AR selectivity versus 3BHSD. 6-Azasteroids are such potent inhibitors of type 2 5AR that the SAR versus this isozyme is of lesser importance. Table 2 presents the results of combining some of the A- and B-ring substitutions with different C-17 groups. The most important initial result from these data is the ability of the C-17 group to change the 3BHSD versus type 1 5AR selectivity from 12- and 5-fold 3BHSD selective in **8** and **35** to equivalent potency in **42** and to 5–7-fold type 1 5AR selective in **54** and **46**. When the effect of A- and B-ring substitutions is superimposed upon these intial selectivities, the trends discussed above for **8** (Table 1) hold to a large extent [although the magnitude of the A- and B-ring substituent effect is clearly C-17 substituent dependent; compare the effect of Δ^1-unsaturation on 3BHSD for **8** to **46** (1.3-fold versus 90-fold decrease in potency)], and these general conclusions may be reached concerning the differential effect on type 1 5AR versus 3BHSD inhibition: Δ^1-unsaturation diminishes potency against both enzymes although 3BHSD is more sensitive; 1,2-cyclopropanation greatly diminishes type 1 5AR potency and that of 3BHSD less so; and C-4 or N-6 methylation enhances potency for both type 1 5AR and 3BHSD with type 1 preferring C-4 and 3BHSD preferring N-6.

Table 3. Inhibition of Recombinant Type 1 and 2 Human 5α-Reductase and Human Adrenal 3β-Hydroxy-Δ^5-steroid Dehydrogenase/3-Keto-Δ^5-steroid Isomerase by 6-Azaandrost-4-en-3-ones **59**–**77**



no.[a]	R_3	type 1 5AR K_i (nM)	type 2 5AR IC_{50} (nM)[b]	3BHSD K_i (nM)
59[c]	OCH_3	150	3.2	12
60	O-2-Ad[d]	6.9	<0.10[e]	180
61	NMeOMe	2300	2.4	50
62	piperazine	8600	33	220
63	morpholine	2200	7.1	190
64	thiomorpholine	570	1.3	31
65	piperidine	85	0.50	110
66	NHCH(4-fluorophenyl)$_2$	20	0.16	320
67	NHCH(4-chlorophenyl)$_2$	20	0.12	510
68	$NHNPh_2$	14	0.23	160
69	NOH-*t*-Bu	38	0.60	62
70	NHCH(cyclohexyl)$_2$	20	0.40	4000
71	$NHCPh_3$	8.2	<0.10	100
72	*n*-Pr	12	0.30	11
73	*n*-octyl	1.0	<0.10	7.5
74	CH_2(cyclohexyl)	4.0	<0.10	16
75	2,6-difluorophenyl	39	<0.10	11
76	1-naphthyl	15	<0.10	7.8
77	2,4,6-triisopropylphenyl	280	0.50	1900

[a] Satisfactory elemental analyses were obtained (C, H, N; ±0.4% of calculated values) unless otherwise noted. [b] Time-dependent inhibition type of 2 5AR was observed for many of these compounds (see ref 21 and text). [c] A satisfactory elemental analysis was not obtained for this compound. H: calcd, 9.10; found, 8.10. [d] 2-Adamantyl alcohol. [e] The enzyme concentration of ca. 100 pM establishes a lower limit for measurable IC_{50}'s.

Given the favorable effect of changing C-17 upon the overall in vitro profile of 6-azasteroids (Table 2), a series of compounds were prepared which differed only in the C-17 group (Table 3).[17] A comparison of compounds **59** and **60**, where swapping a methyl ester for an adamantyl ester transforms a 12-fold selective 3BHSD inhibitor into a 26-fold selective type 1 5AR inhibitor, dramatically confirms the differential sensitivity of these enzymes to the C-17 substituent present in the inhibitor. For esters and amides, type 1 5AR appears to prefer large, highly lipophilic groups at C-17 (compare **61**–**65**) while 3BHSD better tolerates small groups and polar functionality (**59** and **61**–**65**). If these generalities hold, the 20-fold increase in type 1 5AR potency in going from **35** to **69** may be consistent with a preferred *cis*-amide conformation for interaction with type **1** 5AR since hydroxamic acids favor such a conformation.[22] Although ketones at C-17 proved extremely potent inhibitors of type 1 5AR (**42** and **72**–**77**), selectivity toward 3BHSD was not obtained in this series. Preparation of analogues of **54** resulted in **66**, **67**, and **70** which are 16–200-fold selective for type 1 5AR over 3BHSD.

SAR for binding of 4-azasteroids to the rat androgen receptor has been presented,[6b] and this is an activity to be minimized in a 5AR inhibitor if it is to be free of antiandrogen activity.[14b] Most of the compounds in Tables 1–3 were screened for their ability to compete with either [^{3}H]mibolerone or [^{3}H]methyltrienolone for specific binding to the rat androgen receptor.[23] Only compounds **15** and **22** displayed competition at 10 μM, and they were roughly equivalent to 4-MA (**3**) in their potency.

Table 4. In Vivo Evaluation of 6-Azaandrost-4-en-3-ones

no.	castrated rat: % of finasteride (1) reduction in prostate weight vs T-treated controls, 10 mg/kg/day po (8 rats/group)[a]	blood half-life (h) following iv administration to male beagles, n = no. of animals (dose, % bioavailability)[b]
15	52	nd[c]
30	0	nd
38	54	1.4, n = 4 (10 mg/kg, 80%)
40	0	nd
43	5	2.2, n = 1 (5 mg/kg, nd)
46	38	1.8, n = 1 (10 mg/kg, nd)
47	14	nd
48	12	nd
51	24	nd
54	95	4.8, n = 2 (5 mg/kg, 79%)
55	0	nd
56	13	nd
58	38	nd
66	90	5.2, n = 1 (5 mg/kg, nd)
67	74	12, n = 1 (5 mg/kg, nd)
1	100	4.2, n = 5 (10 mg/kg, 69%)

[a] Standard errors for prostate weights were generally 10% of the mean or less. [b] Standard errors for half-lives, when repeated, were 12% of the mean or less. [c] Not done.

In Vivo Results. Several 6-azasteroids were dosed orally in a chronic castrated rat model,[17] and the results are summarized in Table 4. Since finasteride was one of the more active 4-azasteroids reported in this assay,[6c] it served as an internal standard in each experiment, and the resulting inhibition of T-stimulated prostate growth for each compound is reported relative to finasteride. In vivo activity varies substantially within this series, and the most active compounds are equivalent to finasteride in their ability to inhibit T-stimulated prostate growth.[24] Some SAR for this assay is apparent within this set of compounds. For example, Δ^1-unsaturation and C-4 or N-6 methylation diminish in vivo activity relative to the parent compounds (compare **46** to **47**, **48**, and **51**; and **54** to **55**, **56**, and **58**). Activity also varies considerably with C-17 as previously reported.[17] Although human and rat 5AR differ in their inhibitor profile[25] and we initially determined the potency of some 6-azasteroids toward rat prostatic 5AR,[17] the activity of compounds in this assay appears to be dominated by factors other than in vitro potency (see below).

With this indication of oral activity in the rat, the half-lives of selected compounds were determined following iv administration in the dog (Table 4).[26] The 6-azasteroids with good activity in the rat tended to have a longer half-life in the dog relative to compounds with poor activity in the rat. This suggests that metabolic stability may be a major component of the variation in in vivo activity in the rat, although no metabolism experiments have been done in the rat to confirm this speculation. The activity of finasteride in the rat and human pharmacodynamics[6c-e,9,26] are consistent with an apparently irreversible inhibition[21] mechanism, as opposed to a metabolic clearance rate, dominating its in vivo potency.[27] The C-17 diphenylmethyl amides **54**, **66**, and **67** all have good half-lives in the dog, and **67** combines potent dual inhibition of type 1 and 2 5AR and

selectivity versus 3BHSD with good in vivo activity and pharmacokinetics.

Conclusions

On the basis of the clinical efficacy of finasteride,[6d,e] its in vitro profile,[28] and the residual circulating DHT in patients treated with the drug,[9] 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. 17β-*N*-Bis[[(4-chlorophenyl)methyl]-carbamoyl]-6-azaandrost-4-en-3-one (compound **67**) is a potent dual inhibitor of type 1 and 2 human 5AR with selectivity versus 3BHSD and good oral in vivo efficacy in the rat and half-life in the dog. Further in vivo evaluation of **67** will be necessary to establish an in vivo therapeutic index for 5AR- versus 3BHSD-mediated effects,[16] and an increase in in vitro selectivity may be desirable. On the basis of the SAR presented above, further investigation of C-17 substituents appears promising.

Experimental Section

Starting materials were obtained from commercial suppliers and used without further purification. Finasteride (1) and epristeride (**2**) were prepared as previously reported.[6,7] The amines used in the preparation of compounds **66** and **67** were prepared as described by Cram.[29] ^{1}H NMR spectra were acquired on a Varian 300 MHz spectrometer with TMS as an internal reference. Chromatography was performed on silica gel with ethyl acetate/hexanes as eluant unless otherwise noted. All compounds gave satisfactory MH^+ on low-resolution FAB-MS. *Caution*: Inhibitors of 5α-reductase are likely to be teratogenic to the male fetus.[1-5]

General Procedure for Preparation of C-17 Acid Chloride, Conversion to Amides, Esters, and Ketones, and Deprotection (8, 35, 42, 46, 54, and 59–77). A sample of 17β-carboxy-6-(*tert*-butylcarboxy)-6-azaandrost-4-en-3-one prepared by LiOH hydrolysis[17] of **4** (502 mg, 1.20 mmol) was suspended in toluene (15 mL), treated with pyridine (0.15 mL, 1.5 equiv) and 1 drop of DMF, and cooled to 0 °C and thionyl chloride added (0.13 mL, 1.5 equiv). After 1 h at 0 °C, the reaction mixture was filtered and concentrated to a yellow solid, and this crude acid chloride was dissolved in methylene chloride (15 mL) and treated with the appropriate amine or alcohol to give the 6-*tert*-butylcarboxy precursors (i.e., for diethylamine compound **5**) to final compounds **8**, **35**, **46**, **54**, and **59–71**. These products were dissolved in methylene chloride (15 mL) and treated with trifluoroacetic acid (2 mL) at room temperature. After 3 h, the reaction mixture was concentrated, methylene chloride (50 mL) and saturated aqueous bicarbonate (50 mL) were added, and the layers were separated, methylene chloride washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated to give **8**, **35**, **46**, **54**, and **59–71**.[30] Direct TFA treatment of **4** under these conditions yielded **59**. Alternatively, the crude acid chloride (prepared from 260 mg, 0.62 mmol, of acid) was dissolved in THF (6 mL), and CuI was added (120 mg, 0.62 mmol). The mixture was cooled to −78 °C and treated with an excess (1.4 equiv) of the appropriate Grignard reagent. The reaction mixture was allowed to warm to room temperature, stirred for 30 min, and worked up as above to give the 6-*tert*-butylcarboxy precursors to final compounds **42** and **72–77**. These products were then deprotected as described above to give **42** and **72–77**.[30] Satisfactory analyses (C, H, N) were obtained on these compounds unless otherwise indicated in Tables 1–3 (compound **59**). Diagnostic ^{1}H NMR resonances[30] for **54**: ($CDCl_3$) δ 7.36–7.21 (m, 10H), 6.28 (d, 1H, J = 7.8 Hz, NHC*H*Ph_2), 5.94 (d, 1H, J = 7.8 Hz, N*H*CHPh_2), 5.01 (s, 1H, C4-*H*), 4.81 (b s, 1H, N6-*H*), 3.31 (ddd, 1H, J = 2.5, 5.6, 11.3 Hz, C7-H_{eq}), 2.83 (t, 1H, J = 11.3 Hz, C7-H_{ax}).

General Procedure for Introduction of Δ^1-Unsaturation: 17β-(*N*,*N*-Diethylcarbamoyl)-6-azaandrosta-1,4-dien-3-one (9). A solution of **4** (2.00 g, 4.63 mmol) in dioxane (50 mL) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1.37 g, 6.02 mmol) and *p*-nitrophenol (10 mg). The reaction mixture was heated to reflux for 2 h, poured into ice water (150 mL), and extracted with ethyl acetate (3 × 100 mL); the extracts were washed with saturated aqueous $NaHSO_3$, 2 N NaOH, and saturated aqueous NaCl, dried over $MgSO_4$, concentrated, and chromatographed on silica gel to give crude 17β-carbomethoxy-6-(*tert*-butylcarboxy)-6-azaandrosta-1,4-dien-3-one as a tan solid of sufficient purity to carry on to the following step: yield 1.53 g (76%). The crude 17β-carbomethoxy-6-(*tert*-butylcarboxy)-6-azaandrosta-1,4-dien-3-one (1.50 g, 3.50 mmol) was hydrolyzed with LiOH[17] and then coupled with diethylamine as described above to give, after flash chromatography, **6** as a white foam: yield 1.07 g (65%). Anal. ($C_{28}H_{42}N_2O_4$) C, H, N. A sample of **6** (188 mg, 0.400 mmol), prepared above, was treated with trifluoroacetic acid as described to give, after trituration with diethyl ether/hexanes, **9** as a pale yellow solid: yield 98 mg (66%); mp >250 °C; diagnostic ^{1}H NMR resonances[30] ($CDCl_3$) δ 6.74 (d, 1H, J = 6.5 Hz, C1-*H*), 6.12 (d, 1H, J = 6.5 Hz, C2-*H*), 5.50 (b s, 2H, C4-*H*, N6-*H*). Anal. ($C_{23}H_{34}N_2O_2 \cdot {}^1/_2H_2O$) C, H, N. Using this procedure on the appropriate starting material, compounds **36**, **47**, and **55** were prepared. Satisfactory analyses (C, H, N) were obtained on these compounds.

General Procedure for Introduction of 1α,2α-Cyclopropyl: 17β-(*N*,*N*-Diethylcarbamoyl)-1α,2α-methano-6-azaandrost-4-en-3-one (10). A solution of **6** (500 mg, 1.06 mmol), in dimethyl sulfoxide (DMSO, 4 mL) was added to a solution of trimethylsulfoxonium iodide (1.31 g, 5.93 mmol) which had stirred with sodium hydride (237 mg, 5.93 mmol) in DMSO (4 mL) for 1 h. After stirring overnight at room temperature, the reaction mixture was poured into ice water and extracted with diethyl ether (2 × 50 mL); the extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, and concentrated to give **7** as a white foam: yield 411 mg (80%). This material was treated with trifluoroacetic acid as described above to give, after crystallization from diethyl ether/hexanes, **10** as a pale yellow solid: yield 186 mg (57%); mp >250 °C; diagnostic ^{1}H NMR resonances[30] ($CDCl_3$) δ 5.95 (b s, 1H, N6-*H*), 4.98 (s, 1H, C4-*H*), 0.58 (m, 1H, C$H_{cyclopropyl}$). Anal. ($C_{24}H_{36}N_2O_2 \cdot {}^1/_4H_2O$) C, H, N. Using this procedure on the appropriate starting material, compound **37** was also prepared. Satisfactory analyses (C, H, N) were obtained on this compound.

17β-(*N*,*N*-Diethylcarbamoyl)-2α,β-methyl-6-azaandrost-4-en-3-one (11). A solution of **5** (1.00 g, 2.12 mmol; see above) in tetrahydrofuran (10 mL) was treated at −78 °C with lithium diisopropylamide (2 equiv in tetrahydrofuran), warmed slightly by removal of the dry ice–acetone bath for 10 min, recooled to −78 °C, and treated with excess methyl iodide. The solution was then warmed to room temperature, poured into saturated aqueous $NaHSO_4$, and extracted with ethyl acetate (2 × 50 mL); the extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, concentrated, and chromatographed to give 17β-(*N*,*N*-diethylcarbamoyl)-6-(*tert*-butylcarboxy)-2α,β-methyl-6-azaandrost-4-en-3-one: yield 582 mg (58%). This material was treated with trifluoroacetic acid as described above to give, after recrystallization from methylene chloride/hexane, 11 as a pale yellow crystalline solid, as a 1.4:1 mixture of epimers at C-2 (RP-HPLC, BDS Hypersil C8, 290 nm, gradient elution 40–70% CH_3CN:0.1% TFA/H_2O): yield 390 mg (85%); mp 246–252 °C dec; diagnostic ^{1}H NMR resonances[30] ($CDCl_3$) δ 5.21 (b s, 1H, N6-*H*), 5.12 (s, 1H, C4-*H*). Anal. ($C_{24}H_{38}N_2O_4$) C, H, N.

General Procedure for Alkylation of N-6: 17β-(*N*,*N*-Diethylcarbamoyl)-6-methyl-6-azaandrost-4-en-3-one (15). To a solution of **8** (24 mg, 0.065 mmol) in DMF (2 mL) at room temperature were added NaH (16 mg, 80% oil dispersion, 0.5 mmol) and, after 30 min, methyl iodide (50 μL, excess). The reaction mixture was allowed to stir for 30 min further, ethyl acetate (30 mL) was added, and the solution was washed with water and saturated aqueous NaCl, dried over $MgSO_4$, concentrated, and flash chromatographed to give **15** as a white solid which was recrystallized from hexanes/methylene chloride: yield 14 mg (56%); mp 158–159 °C; diagnostic ^{1}H NMR

resonances[30] ($CDCl_3$) δ 5.03 (s, 1H, C4-*H*), 2.79 (s, 3H, NC*H*$_3$). Anal. ($C_{24}H_{38}N_2O_2$) C, H, N. Using this procedure and the appropriate starting materials, compounds **12**, **14**, **16**–**23**, **38**, **39**, **41**, **43**, **48**, **49**, **52**, **53**, **56**, and **57** were also prepared. Satisfactory analyses (C, H, N) were obtained on these compounds unless otherwise indicated in Tables 1–3 (compound **52**).

17β-(*N*,*N*-Diethylcarbamoyl)-6-cyano-6-azaandrost-4-en-3-one (13). To a suspension of **8** (150 mg, 0.40 mmol) in THF (3 mL) and DMPU (0.5 mL) at 0 °C was added potassium *tert*-butoxide (135 mg, 1.2 mmol); the yellow mixture was stirred for 2 min and 4-chlorophenyl cyanate (155 mg, 1.0 mmol) added. After 5 min, the reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was then diluted with saturated aqueous ammonium chloride and extracted with ethyl acetate (2 × 40 mL); the ethyl acetate was washed with water and saturated aqueous NaCl, dried over $MgSO_4$, concentrated, and flash chromatographed to give **13** as a tan solid (108 mg, 67%) which was recrystallized from 10:1 hexane/ethyl acetate to give a yellow solid: yield 61 mg (38%); mp 196–197 °C; diagnostic ^{1}H NMR resonance[30] ($CDCl_3$) δ 5.92 (s, 1H, C4-*H*). Anal. ($C_{24}H_{35}N_3O_2 \cdot {}^1/_4H_2O$) C, H, N.

17β-(*N*,*N*-Diethylcarbamoyl)-4-chloro-6-azaandrost-4-en-3-one (24). A solution of **8** (152 mg, 0.408 mmol) in THF (3 mL) was treated with *N*-chlorosuccinimide (110 mg, 0.82 mmol). After 4 h at room temperature, the reaction was quenched with saturated aqueous Na_2SO_3. The mixture was diluted with water, extracted with methylene chloride (2 × 30 mL), dried over $MgSO_4$, concentrated, and flash chromatographed to give **24** as a yellow solid: yield 81 mg (49%); mp 134–136 °C; diagnostic ^{1}H NMR resonance[30] ($CDCl_3$) δ 5.72 (s, 1H, N6-*H*). Anal. ($C_{23}H_{35}N_2O_2Cl \cdot {}^3/_4H_2O$) C, H, N. Using this procedure and the appropriate succinimide, compounds **25**, **26**, **44**,0 and **50** were also prepared. Satisfactory analyses (C, H, N) were obtained on these compounds.

17β-(*N*,*N*-Diethylcarbamoyl)-4-[(dimethylamino)methylene]-6-azaandrost-4-en-3-one (27). A solution of **8** (160 mg, 0.430 mmol) in acetonitrile (2 mL) was treated with *N*,*N*-dimethylmethylene ammonium iodide (Eschenmoser's salt, 160 mg, 0.87 mmol) at room temperature for 4 h. The reaction mixture was then diluted with saturated aqueous sodium bicarbonate and extracted with ethyl acetate (2 × 30 mL); the extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, concentrated, and flash chromatographed (30% isopropyl alcohol in 70% ethyl acetate/hexanes followed by 10% ammonium hydroxide in 20% acetonitrile/chloroform) to give **27** as a white solid: yield 110 mg (60%); mp 84–88 °C; diagnostic ^{1}H NMR resonances[30] ($CDCl_3$) δ 8.38 (s, 1H, N6-*H*), 3.34 (d, 1H, J = 14.2 Hz, Me_2N-C*H*$_a$H$_b$), 3.25 (d, 1H, J = 14.2 Hz, Me_2N-CH$_a$*H*$_b$), 2.10 (s, 6H, N(C*H*$_3$)$_2$). Anal. ($C_{26}H_{43}N_3O_2 \cdot {}^1/_4H_2O$) C, H, N.

17β-(*N*,*N*-Diethylcarbamoyl)-6-(*tert*-butylcarboxy)-4-bromo-6-azaandrost-4-en-3-one (28). A solution of **5** (5.20 g, 11.0 mmol) in methylene chloride (100 mL) containing anhydrous K_2CO_3 (10 g, 74 mmol) at 0 °C was treated with bromine (1.7 mL, 33 mmol) dissolved in methylene chloride (10 mL). After 2 h, the mixture was diluted with water and extracted with methylene chloride (3 × 50 mL); the extracts were washed with saturated aqueous Na_2SO_3, dried over $MgSO_4$, concentrated, and flash chromatographed to give **28** as a white solid: yield 5.18 g (85%). Recrystallization from ethyl acetate gave an analytical sample: mp 215–216 °C dec; diagnostic ^{1}H NMR resonance[30] ($CDCl_3$) δ 4.38 (dd, 1H, J = 4.2, 12.5 Hz, C7-*H*$_{eq}$). Anal. ($C_{28}H_{43}N_2O_4Br$) C, H, N. Using this procedure on **6**, compound **29** was also prepared. Similar treatment of the appropriate substrate produced the 6-*tert*-butylcarboxy 4-bromo precursors to **40**, **45**, **51**, and **58**.

17β-(*N*,*N*-Diethylcarbamoyl)-4-methyl-6-azaandrost-4-en-3-one (30). A mixture of **28** (250 mg, 0.453 mmol), phenyltrimethyltin (320 mg, 1.33 mmol), $PdCl_2(PPh_3)_2$ (45 mg), and lithium chloride (20 mg) in DMF (1.5 mL) was heated at 140 °C for 8 h. The reaction mixture was allowed to cool to room temperature and the reaction quenched with 5 M aqueous potassium fluoride (5 mL). The mixture was extracted with ethyl acetate (3 × 30 mL), and the extracts were washed with 5 M aqueous potassium fluoride, dried over $MgSO_4$, concentrated, and flash chromatographed to give 17β-(*N*,*N*-diethylcarbamoyl)-6-(*tert*-butylcarboxy)-4-methyl-6-azaandrost-4-en-3-one as a clear oil: yield 204 mg (93%). A portion of this material (181 mg, 0.372 mmol) was treated with trifluoroacetic acid as described above to give, after chromatography (0–30% isopropyl alcohol in 70% ethyl acetate/hexanes), **30** as a white powder: yield 75 mg (52%); mp 115–118 °C; diagnostic ^{1}H NMR resonances[30] ($CDCl_3$) δ 4.57 (s, 1H, N6-*H*), 1.67 (s, 3H, C4-C*H*$_3$). Anal. ($C_{24}H_{38}N_2O_2 \cdot {}^1/_2$isopropyl alcohol) C, H, N. Using this procedure on **29**, compound **31** was also prepared. Identical treatment of the requisite 6-*tert*-butylcarboxy 4-bromo precursors produced **40**, **45**, **51**, and **58**. Satisfactory analyses (C, H, N) were obtained on these compounds unless otherwise indicated in Tables 1–3 (compound **31**).

17β-(*N*,*N*-Diethylcarbamoyl)-4-ethyl-6-azaandrost-4-en-3-one (32). A mixture of **28** (510 mg, 0.925 mmol), vinyltributyltin (800 mg, 2.52 mmol), $PdCl_2(PPh_3)_2$ (70 mg), and lithium chloride (50 mg) in DMF (1.5 mL) was heated at 140 °C for 4 h. The reaction mixture was allowed to cool to room temperature and the reaction quenched with 5 M aqueous potassium fluoride (3 mL). The mixture was extracted with ethyl acetate (2 × 30 mL), and the extracts were washed with 5 M aqueous potassium fluoride, dried over $MgSO_4$, concentrated, and flash chromatographed to give 17β-(*N*,*N*-diethylcarbamoyl)-6-(*tert*-butylcarboxy)-4-vinyl-6-azaandrost-4-en-3-one as a white foam: yield 395 mg (84%). A portion of this material (180 mg, 0.353 mmol) was dissolved in isopropyl alcohol and treated with 20% palladium hydroxide on carbon (100 mg) and cyclohexene (2 mL) at reflux for 8 h. The reaction mixture was then filtered, concentrated, and chromatographed to give 17β-(*N*,*N*-diethylcarbamoyl)-6-(*tert*-butylcarboxy)-4-ethyl-6-azaandrost-4-en-3-one as an amorphous solid: yield 173 mg (96%); mp 163–165 °C. A portion of this material (157 mg, 0.306 mmol) was treated with trifluoroacetic acid as described above to give, after chromatography (0–50% isopropyl alcohol in 70% ethyl acetate/hexanes), **32** as a white powder: yield 85 mg (67%); mp 112–116 °C; diagnostic ^{1}H NMR resonances[30] ($CDCl_3$) δ 4.64 (s, 1H, N6-*H*), 0.89 (t, 3H, J = 7.4 Hz, N6-CH_2C*H*$_3$). Anal. ($C_{25}H_{40}N_2O_2 \cdot {}^3/_8i$-propanol) C, H, N.

Bioassays. Type 1 and 2 Recombinant Human 5α-Reductase Assays. The cDNA of type 1 and 2 5α-reductases were provided by David W. Russell. The subsequent subcloning, transfection, expression, and microsome preparation was carried out as previously described.[21b] 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. The 5AR assays were carried out in poly(styrene) 96 well plates in a total assay volume of 250 μL. In a separate plate, inhibitor concentrations were serially diluted 2-fold in 100% DMSO followed by a 2-fold dilution of each concentration into assay buffer. A 5 or 4 μL aliquot (for type 1 and 2 5AR, respectively) of inhibitor in 50% DMSO or 50% DMSO alone was preincubated[21] for 10 min (type 1) or 20 min (type 2) in 150 μL of standard assay buffer containing an NADPH-regenerating system (1 mM glucose-6-phosphate and 13 units/mL glucose-6-phosphate dehydrogenase, final assay concentrations) and 50 μL of the appropriate 5AR isozyme. Solutions of [1,2,6,7-^{3}H]testosterone (New England Nuclear) were prepared by evaporating ethanol from a stock solution under a stream of nitrogen and resuspending in standard buffer containing either 1% DMSO or 1% DMSO and 1% ethanol (for type 1 and 2 5AR, respectively). Each assay was initiated by addition of testosterone solution (100 and 8 nM final assay concentration for type 1 and 2, respectively). After either 120 or 40 min (for type 1 and 2, respectively), reaction aliquots were quenched by addition to equal volumes of ethanol. The percent T and DHT was

quantitated by HPLC (Beckman, Spectraphysics or LDC pumps, Regis C18 column, 15 cm × 4.6 mm) using an in-line radioisotope detector (Radiomatic or Beckman) and 50:50 CH_3CN:water as eluant. The percent inhibition at each concentration was determined by calculating the ratio of enzyme activity with inhibitor to enzyme activity without inhibitor. Nonlinear regression analysis according to the equation $y = (\%\ \text{inhibition max} \cdot x)/(IC_{50} \cdot x)$ of the plot of percent inhibition versus inhibitor concentration allowed the IC_{50} (or K_i) to be determined. The error in the IC_{50} or K_i determinations estimated at the 95% confidence limit was 10–75% of the reported values.

Human Adrenal 3β-Hydroxy-Δ⁵-steroid Dehydrogenase/3-Keto-Δ⁵-steroid Isomerase Assay. Enzyme activities were measured using microsomes derived from human adrenal tissues. Microsomes were prepared by homogenization of the tissue followed by differential centrifugation of the homogenate. The 3BHSD assay was carried out in poly(styrene) 96-well plates in a total assay volume of 250 μL. In a separate plate, inhibitor concentrations were serially diluted 2-fold in 100% DMSO followed by a 2-fold dilution in standard assay buffer (100 mM potassium phosphate, pH = 7.5). A 5 μL aliquot of inhibitor in 50% DMSO or 50% DMSO alone was preincubated for 10 min at 37 °C in 150 μL of standard assay buffer, 50 μL of NAD^+ (1 mM final concentration), and 50 μL microsomes. The assay was initiated by addition of [1,2,6,7-^{3}H]dehydroepiandosterone (DHEA, 100 nM final assay concentration). After 15 min, an 80 μL reaction aliquot was quenched by addition to 120 μL of ethanol. The percent DHEA and androstenedione was quantitated by HPLC (Beckman, Spectraphysics or LDC pumps, Regis C18 column, 15 cm × 4.6 mm) using an in-line radioisotope detector (Radiomatic or Beckman) and 44:35:11:10 water:methanol:CH_3CN:tetrahydrofuran as eluant. The percent inhibition at each concentration was determined by calculating the ratio of enzyme activity with inhibitor to enzyme activity without inhibitor. Nonlinear regression analysis according to the equation $y = (\%\ \text{inhibition max} \cdot x)/(IC_{50} \cdot x)$ of the plot of percent inhibition versus inhibitor concentration allowed the IC_{50} (or K_i) to be determined. The error in the IC_{50} or K_i determinations estimated at the 95% confidence limit was 10–75% of the reported values.

Chronic Rat Model. In vivo efficacy testing was performed by a modification of the methods of Dorfman.[31] 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 as a solution (vehicle, 1 part cremophor:2 parts saline:0.5% Tween 80) at 10 mg/kg/day, finasteride (1) as a solution at 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 in corn oil). 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 of the finasteride reduction in prostate weight. 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.

Dog Pharmacokinetics. Adult male beagle dogs were dosed intravenously with compound **38** ($n = 2$, 10 mg/kg), **43** ($n = 1$, 5 mg/kg), **46** ($n = 1$, 10 mg/kg), **54** ($n = 1$, 5 mg/kg), **66** ($n = 1$, 5 mg/kg), or **67** ($n = 1$, 5 mg/kg). Only compounds **38** and **54** were given orally to dogs to determine the oral bioavailability ($n = 2$ and 1, respectively). Compound **1** was given as an iv dose to three dogs and as an oral dose to two dogs at 10 mg/kg. The dosing solutions (5 mg/mL) were prepared by dissolving the test compound in aqueous 40% (w/v) Molecusol (Pharmos Corp., Alachua, FL). Heparinized blood samples were collected at 0, 5, 15, 30, and 45 min and 1, 1.5, 2.5, 4, 6, 8, and 24 h following dose administration. An aliquot of 400 μL of CH_3CN was mixed with 200 μL of a blood sample to precipitate protein. The supernatant was removed and evaporated to dryness under a gentle stream of nitrogen at 45 °C. The residue was redissolved in 100 μL of the HPLC mobile phase and injected onto HPLC. HPLC analysis was performed using a Hypersil BDS C8 (25 × 0.46 cm^2) HPLC column. All the compounds except compound 1 were eluted with CH_3CN and 50 mM ammonium acetate buffer (pH = 4.2) over a 10 min linear gradient from 50% to 80% CH_3CN at a flow rate of 1 mL/min and detected by ultraviolet spectroscopy at a wavelength of 304 nm. Compound **1** was eluted isocratically with CH_3CN:50 mM ammonium acetate buffer (pH = 6.8) (55:45, v/v) and detected by thermospray mass spectrometry. The determination of half-lives of compounds 1, **38**, **43**, **46**, **54**, **66**, and **67** in the dog was based on the disappearance rate of parent drug in the terminal phase from the semilog blood concentration versus time plot using the RSTRIP computer program (MicroMath Scientific Software, Salt Lake City, UT). The area under the blood concentration versus time curve to infinity (AUC_∞) was determined using the trapezoidal rule with the RSTRIP computer program. The oral bioavailabilities were calculated using the formula [(AUC_∞/dose)oral/(AUC_∞/dose)iv] × 100%.

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