Structure–Activity Relationships for Inhibition of 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: Optimization of the C17 Substituent

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A variety of C17 amide-substituted 6-azaandrost-4-en-3-ones were prepared and tested versus human type 1 and 2 steroid 5α-reductase (5AR) and human adrenal 3β-hydroxy-Δ^5-steroid dehydrogenase/3-keto-Δ^5-steroid isomerase (3BHSD) in order to optimize potency versus both isozymes of 5AR and selectivity versus 3BHSD. Two series of potent and selective C17 amides were discovered, 2,5-disubstituted anilides and (arylcycloalkyl)amides. Compounds from each series with picomolar IC_{50}'s versus human type 2 5AR and low nanomolar to picomolar IC_{50}'s versus human type 1 5AR possessing 100–500-fold selectivity versus 3BHSD were identified. A conformational model to predict 3BHSD potency was developed which could rationalize 3BHSD potency within three different series of compounds. Evaluation of some optimal compounds from this series in a chronic castrated rat model of 5AR inhibitor induced prostate involution, and pharmacokinetic measurements identified compounds (**9**, **12**, **16**, and **29**) with good in vivo efficacy and half-life in the dog. An intact rat model of in vivo selectivity for 5AR versus 3BHSD inhibition was also developed. Dual inhibitors of both human 5AR's may show advantages over type 2 selective 5AR inhibitors, such as finasteride (**1**), in the treatment of disease states which depend upon dihydrotestosterone.

Introduction

Benign prostatic hyperplasia (BPH) is a ubiquitous condition in the aging male in so much as the incidence of BPH detected at autopsy increases from approximately 30% at age 50 to >80% at age 80.[1] Unfortunately, the urinary symptoms attributed to BPH lead to significant erosion in the quality of life for affected men, and many undergo surgery as a result.[2] Although the etiology of BPH is unclear, the permissive role of dihydrotestosterone (DHT) in the hyperplastic growth of the prostate is well established.[1,2] 5α-Reductase (5AR) is the enzyme which catalyzes the conversion of testosterone (T) to the more potent androgen DHT. With the discovery of two isozymes of 5AR, the relative roles of these enzymes in developmental physiology and the pathophysiology of androgen-related disorders are the subject of much current research.[3] Given the limited efficacy of selective type 2 5AR inhibitors, such as finasteride (**1**), in the treatment of BPH, and the residual circulating DHT present in treated patients (20–40% of base line),[4] we have previously made the case that dual inhibitors of type 1 and 2 5AR may be advantageous.[5] Combination of finasteride with a potent type 1 5AR inhibitor has also been suggested as an alternative strategy to achieve complete DHT reduction.[6]

We report here on the optimization of the 6-azaandrost-4-en-3-ones for potent dual inhibition of type 1 and 2 5AR and selectivity versus 3β-hydroxy-Δ^5-steroid dehydrogenase/3-keto-Δ^5-steroid isomerase (3BHSD),[5a] a crucial enzyme in steroid biosynthesis.[7] Substitution at C17 was found to influence 3BHSD structure–activity relationships (SAR) most strongly, and a conformational model was developed which accounts for the observed potency trends. Optimized compounds were evaluated for efficacy and selectivity in the rat as well as for pharmacokinetics in the dog.

Results and Discussion

In Vitro SAR. On the basis of our initial studies, variation of the C17 substituent of the 6-azaandrost-4-en-3-ones appeared to be especially effective in terms of the ability to modulate both potency and selectivity. After sampling a variety of C17 groups,[8] two series of amides emerged, 2,5-disubstituted anilides and (arylcycloalkyl)amides, which were the focus of more extensive optimization. Other than straightforward preparation of several novel amines (see the Experimental Section), compounds were prepared by the general methods which have been described.[5] The results of this study are presented in Tables 1 and 2 with finasteride included for comparison. The assays employed are as previously described.[5b,c]

Table 1 summarizes the results of substitution effects for C17 anilides with unsubstituted anilide **2** as the base line for comparison. The compounds of Table 1 demonstrate the powerful influence of the C17 substituent on the potency of these compounds—especially as inhibitors of 3BHSD. For example, introduction of a 2-*tert*-

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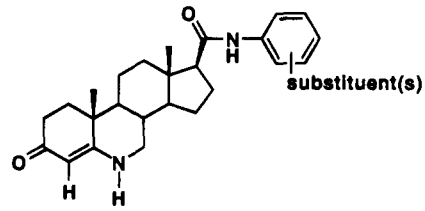
Table 1. Inhibition of Recombinant Type 1 and 2 Human 5α-Reductase and Human Adrenal 3β-Hydroxy-Δ^5-steroid Dehydrogenase/3-Keto-Δ^5-steroid Isomerase by 17β-(*N*-Phenylcarbamoyl)-6-azaandrost-4-en-3-ones **2**–**15**



no.[a]	substituent(s)	type 1 5AR K_i (nM)	type 2 5AR IC_{50} (nM)[b]	3BHSD K_i (nM)
2		240	1.4	10
3	2-*tert*-butyl	27	0.2	85
4	5-chloro, 2-*tert*-butyl	7.5	<0.1[c]	760
5	5-bromo, 2-*tert*-butyl	4.2	<0.1	820
6	4-bromo, 2-*tert*-butyl	5.3	<0.1	390
7	2,5-di-*tert*-butyl	5.0	<0.1	500
8	2-*tert*-butyl, 5-phenyl	4.6	<0.1	830
9	2-*tert*-butyl, 5-trifluoromethyl	8.8	<0.1	1600
10	2-*tert*-butyl, 5-(4-chlorophenyl)	3.4	<0.1	1200
11	2-*tert*-butyl, 5-(4-*tert*-butylphenyl)	1.3	<0.1	8900
12	2,5-bis(trifluoromethyl)	4.0	<0.1	300
13	2-(4-*tert*-butylphenyl), 5-trifluoromethyl	1100	0.5	19
14	3,5-bis(trifluoromethyl)	26	0.2	58
15	3,5-di-*tert*-butyl	8.0	<0.1	7.8
1	finasteride	150[d]	0.18[d]	11000

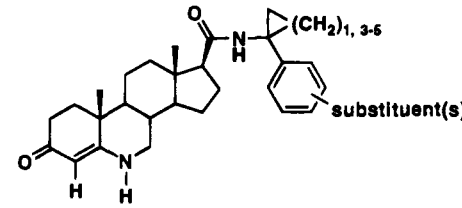
[a] Satisfactory elemental analyses were obtained (C, H, N; ±0.4% of calculated values). [b] Time-dependent inhibition of type 2 5AR was observed for many of these compounds; see ref 5. [c] The enzyme concentration of *ca.* 100 pM establishes a lower limit for measurable IC_{50}'s. [d] This is an IC_{50}. Finasteride is a time-dependent inhibitor of type 1 and 2 5AR; see ref 13.

butyl substituent decreases 3BHSD potency by 8-fold (**2** versus **3**), and further substitution with chlorine, bromine, *tert*-butyl, or phenyl in the 5-position decreases potency an additional 10-fold (**3** versus **4**, **5**, **7**, and **8**), while 4-substituents have a somewhat diminished effect (**6** versus **5**). Simultaneous interest in decreasing the metabolic lability of the C17 substituent led to introduction of trifluoromethyl in the 5-position which results in a further decrease in 3BHSD potency (**9**). The overall effect of these changes is to convert the 24-fold selective (versus type 1 5AR) 3BHSD inhibitor, **2**, to the 180-fold selective type 1 5AR inhibitor, **9**. Although the major effect is on 3BHSD potency, the effect on type 1 5AR is also beneficial and consistent with more lipophilic C17 substituents being preferred. The introduction of greater steric bulk in the 5-position was explored in the biphenyl derivatives **10** and **11**, and this resulted in 300–6000-fold type 1 5AR and 3BHSD selectivity, respectively.

Although a number of other 2-substituents were examined,[8] the 2-*tert*-butyl group appeared to be optimal in terms of overall in vitro and in vivo properties. For example, replacement of the 2-*tert*-butyl group of **9** with trifluoromethyl caused a 5-fold increase in 3BHSD potency; however, this resulted in a compound with quite interesting in vivo properties (**12**, see below). Surprisingly, when the 2-*tert*-butyl substituent of **9** was replaced with the 4-*tert*-butylphenyl substituent used to advantage in **11**, a reversal in selectivity occurred such that **13** became a 60-fold selective 3BHSD inhibitor. The importance of the 2,5-disubstitution pattern is exemplified in compounds **14** and **15** which lose their selectivity upon shifting to 3,5-disubstitution as a result of an increase in 3BHSD potency.

On the basis of the results with C17 anilides outlined above, and the notion that rigidity and steric bulk were important factors controlling the SAR, a series of (arylcycloalkyl)amides were prepared. Table 2 presents

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 17β-[*N*-(1-Phenylcycloalkyl)carbamoyl]-6-azaandrost-4-en-3-ones **16**–**21**



no.[a]	ring size, substituent(s)	type 1 5AR K_i (nM)	type 2 5AR IC_{50} (nM)[b]	3BHSD K_i (nM)
16	5, 4-chloro	6.8	<0.1[c]	1600
17	6, 4-chloro	3.1	<0.1	1800
18	3, 2,4-dichloro	6.7	<0.1	130
19	5, 4-*tert*-butyl	3.0	<0.1	1500
20	6, 4-*tert*-butyl	1.5	<0.1	2200
21	7, 4-*tert*-butyl	3.6	<0.1	1500

[a] Satisfactory elemental analyses were obtained (C, H, N; ±0.4% of calculated values). [b] Time-dependent inhibition of type 2 5AR was observed for many of these compounds; see ref 5. [c] The enzyme concentration of *ca.* 100 pM establishes a lower limit for measurable IC_{50}'s.

selected compounds from this series. With the possible exception of the cyclopropyl compound **18**, all of these compounds proved to be highly potent and selective dual 5AR inhibitors.

Conformational Model of 3BHSD Potency. As this work was proceeding, it became clear that the ability of the C17 substituent to influence 3BHSD potency was quite sensitive to the size and position of the substituents in the aniline series. Additionally, prior work with simple alkylamides had also pointed to subtle effects upon 3BHSD potency.[8] For example, the 6-azaandrost-4-en-3-one amides prepared from $H_2NCH_2CH_2CMe_3$ (**22**) and $H_2NCMe_2CH_2CMe_3$ (**23**) are

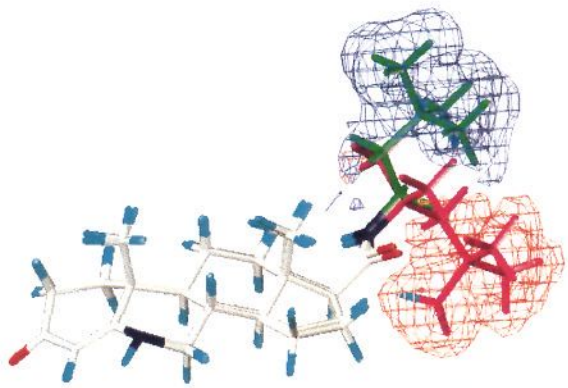


Figure 1. Overlap of low-energy conformers of **22** (green, C17 = -$CH_2CH_2CMe_3$ amide) and **23** (red, C17 = -$CMe_2CH_2CMe_3$ amide) including the excluded volumes (shaded blue for **22** and orange for **23**).

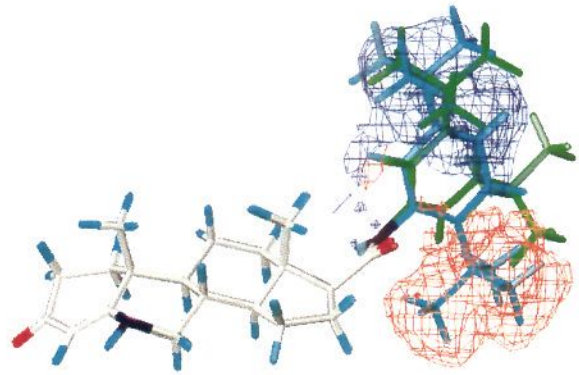


Figure 2. Overlap of low-energy conformers of **7** (blue, C17 = 2,5-di-*tert*-butylphenyl anilide) and **15** (green, C17 = 3,5-di-*tert*-butylphenyl anilide) including the excluded volumes of **22** and **23** (shaded blue for **22** and orange for **23**).

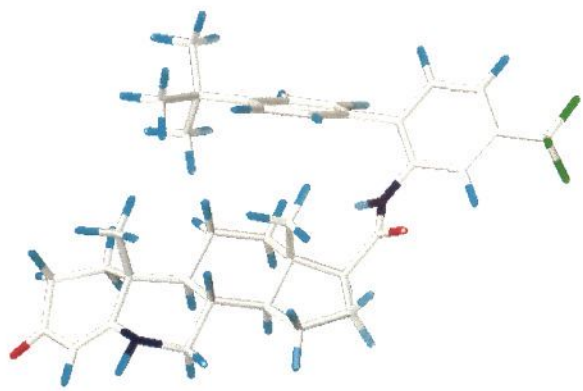


Figure 3. Low-energy conformer of **13**.

8.3 and 150 nM inhibitors of 3BHSD, respectively. Therefore, a molecular modeling study was conducted on a set of selected compounds to determine if the differential 3BHSD activity might be related to common conformational attributes. For each compound, a full search of the conformational hyperspace about all rotatable bonds in the C17 substituent was performed using Monte Carlo methods within MacroModel. A comparison of the lowest energy conformers of **22** and **23** reveals that the terminal -CMe_3 groups occupy quite different regions of space. This can be attributed to the different steric interaction between the substituent and the C19 methyl group of the steroid. This interaction of the substituents on the quaternary carbon α to the amide nitrogen in **23** steers the terminal -CMe_3 group to a unique region relative to the -CMe_3 group of **22**. Figure 1 illustrates the overlap of the lowest energy conformers and the excluded volume due to the difference in conformation at C17, based on a rigid fit of their common steroid framework. The alkyl group of **22** is shaded green while that of **23** is shaded red, and the corresponding excluded volume is shaded blue and orange, respectively. This substantial difference in *shape of the lowest energy conformers of* **22** *and* **23** *was* somewhat surprising as was their 18-fold difference in potency versus 3BHSD. When the more constrained anilides **7** and **15** were likewise overlaid in their low-energy conformers and compared to **23**, it was striking that the excluded volume defined in Figure 1 by the orange shading was well occupied by the 2-*tert*-butyl group of **7** (blue, 500 nM versus 3BHSD) and not by the 3-*tert*-butyl group of **15** (green, 7.8 nM versus 3BHSD) (Figure 2). Similarly the aryl group of the compounds of Table 2 projects into this same region in their low-energy conformations (data not shown; see, however, Figure 4). The leap from such a correlation to assumptions about the bound conformation of these compounds and implications for specific interactions with the protein involved is necessarily tenuous; however, it may suggest that the orange-shaded regions of Figures 1 and 2 are occupied by a portion of the 3BHSD protein itself in the enzyme−inhibitor complex.

Compound **13** represents an interesting and dramatic change in the simple SAR of the 2,5-disubstituted anilides discussed above (Table 1). When the 2-*tert*-butyl substituent of **9** was replaced with a 2-(4-*tert*-butylphenyl) to give **13**, the type 1 5AR potency decreased 125-fold while the 3BHSD potency increased 80-fold. When this compound was subjected to the same modeling discussed above, the low-energy conformer displayed a unique orientation of the C17 substituent as seen in Figure 3. Due to the ability of the 2-phenyl substituent to turn face on to the C19 methyl group, the 2-(4-*tert*-butylphenyl) group is oriented over the C/D-ring of the steroid instead of occupying the orange region defined by the 2-substituent of the less potent 3BHSD inhibitors described above (Figures 1 and 2). Compound **13** also represents the only significant departure from the trend that more lipophilic substituents generally increase type 1 5AR potency (compared to **11**), and this may also be due to the distinct conformation adopted by the C17 group.

Effect of 4-Substitution. In a previous study of A- and B-ring SAR for the 6-azaandrost-4-en-3-ones,[5a] substitution of the 4-position with either chlorine or methyl was found to increase type 1 5AR potency and selectivity versus 3BHSD. In order to further optimize the aniline and (arylcycloalkyl)amide series, compounds **9**, **12**, and **16** from Tables 1 and 2 were substituted with methyl and chlorine, and the resulting compounds are depicted in Table 3. As expected, substitution of C4 increased potency against both type 1 5AR and 3BHSD, but the increase in type 1 5AR averaged 20-fold for 4-methyl substitution and 15-fold for 4-chloro substitution, while the increase in potency for 3BHSD averaged 11-fold for 4-methyl substitution and only 3-fold for 4-chloro substitution. Overall 4-chloro substitution produced the best results providing picomolar dual 5AR inhibitors with 600−900-fold selectivity versus 3BHSD. Compound **29** was selected for in vivo assessment (see

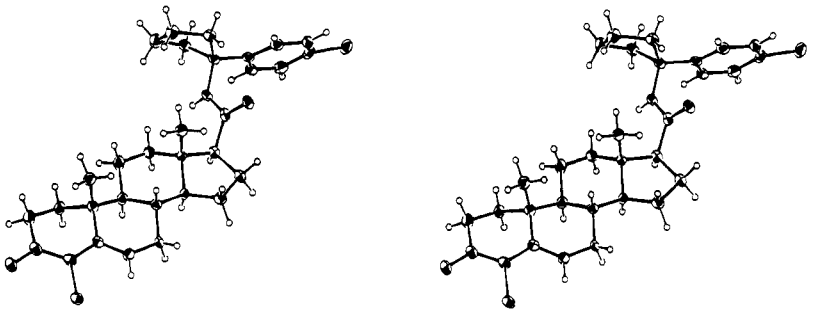
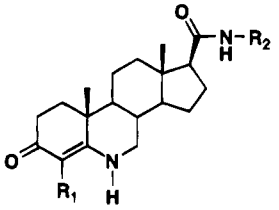


Figure 4. Stereoplot of the crystal structure of **29**.

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 17β-[*N*-(1-Phenylcycloalkyl)carbamoyl]-4-substituted-6-aza-androst-4-en-3-ones **24**–**29**



no.[a]	R_1	R_2	type 1 5AR K_i (nM)	type 2 5AR IC_{50} (nM)[b]	3BHSD K_i (nM)
24	Me	2-*tert*-butyl-5-(trifluoromethyl)-phenyl	0.5	<0.1[c]	200
25	Cl		0.6	<0.1	370
26	Me	2,5-bis(trifluoromethyl)phenyl	0.2	<0.1	19
27	Cl		0.2	<0.1	190
28[d]	Me	*N*-[1-(4-chlorophenyl)cyclopentyl]	0.3	<0.1	160
29	Cl		0.6	<0.1	490

[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 5. [c] The enzyme concentration of *ca.* 100 pM establishes a lower limit for measurable IC_{50}'s. [d] An elemental analysis was not obtained for this compound: HRMS: calcd for $C_{31}H_{41}N_2O_2Cl$ (MH^+), 509.2935; found, 509.2941.

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]
9	100	8.8, n = 4 (4 mg/kg, 67%)
12	130	31, n = 4 (4 mg/kg, 100%)
16	130	12, n = 5 (5 mg/kg, 79%)
29	49	19, n = 2 (5 mg/kg, 74%)
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 were 10% of the mean or less.

below), and upon scaleup and crystallization from acetonitrile, crystals suitable for X-ray diffraction were obtained. The X-ray crystal structure of compound **29** is depicted stereoscopically in Figure 4, and the conformation at C17 is consistent with the analysis of 3BHSD selectivity based upon molecular modeling (see above).

In Vivo Results. The results for several 6-azasteroids dosed orally in a castrated rat model and evaluated for pharmacokinetics in the dog are summarized in Table 4. Since finasteride was one of the more active 4-azasteroids reported in this assay,[9] it served as an internal standard in each experiment. With the exception of **29**, the compounds tested were as potent as or more potent than finasteride in the chronic rat assay, and all were longer lived in the dog. Although the general correlation between rat efficacy and dog half-life noted previously also holds for these compounds,[5] **29** was a sufficiently interesting anomaly to determine its pharmacokinetic parameters in the rat. As it turns out, the limited efficacy of **29** in the rat is consistent with its half-life of 0.7 h and 12% bioavailability in that animal. Upon subsequent administration subcutaneously in corn oil at 5 mg/kg, to avoid poor absorption and first-pass clearance, **29** was fully effective in the castrated rat assay.

In addition to the assessment of efficacy and pharmacokinetics in vivo, some indication of in vivo selectivity which would substantiate the in vitro selectivity versus 3BHSD was desired. On the basis of reports of significant adrenal hyperplasia in rats on dosing with the 3BHSD inhibitor trilostane,[10] 14-day multiple-dose studies were carried out in intact rats in order to simultaneously determine the effect of compounds on prostate and adrenal weight. Compounds **9**, **12**, **16**, and **30** (17β-[*N*-[2-chloro-5-(trifluoromethyl)phenyl]carbamoyl]-6-azaandrost-4-en-3-one) were assayed in this model at 10 and 100 mg/kg/day, and finasteride was included at equimolar doses of 7.2 and 72 mg/kg/day. It was anticipated that compound **30** would serve as a positive control in this assay since it had been shown to be a 90 nM inhibitor of human adrenal 3BHSD. Figure 5 depicts the effect of these compounds on prostate and adrenal weight as a percent of the vehicle control values. While all the compounds were selective for prostate shrinkage at the low dose, compounds **12** and **30** caused significant adrenal hyperplasia at the high dose.

In order to interpret these in vivo results in the rat with greater confidence, in vitro assays versus the rat enzymes of interest (rat type 1 and 2 5AR and rat adrenal 3BHSD) were carried out, and the data from those assays are presented in Table 5. In the rat in vitro assays, all of the 6-azasteroids proved to be potent, selective inhibitors of both rat 5AR's. However, the absolute potency versus rat type 1 5AR and rat 3BHSD were roughly 10-fold lower than their potency versus the human enzymes. When the in vivo selectivity is compared to these in vitro results, an excellent correlation is seen between the compounds which caused adrenal hyperplasia, **12** and **30**, and potency versus rat 3BHSD. It is interesting to note that efficacy in the rat may track more strongly with the rat type 2 5AR enzyme than rat type 1 given finasteride's (1) activity in this model versus its rat type 1 potency. This is unexpected given the similar expression levels of the rat 5AR isozymes in the rat prostate.[3]

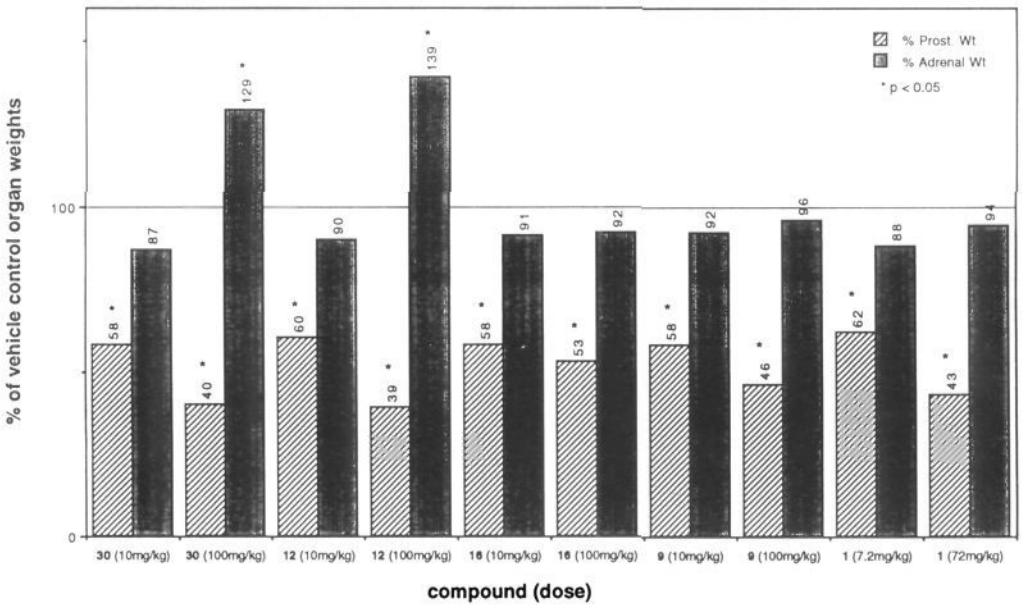


Figure 5. 14-Day intact rat experiment with compounds **30**, **12**, **16**, **9**, and **1**: in vivo selectivity for inhibition of prostate growth versus adrenal hyperplasia.

Table 5. Inhibition of Recombinant Type 1 and 2 Rat 5α-Reductase and Rat Adrenal 3β-Hydroxy-Δ^5-steroid Dehydrogenase/3-Keto-Δ^5-steroid Isomerase by 6-Azaandrost-4-en-3-ones **9**, **12**, **16**, and **30**

	K_i (nM)		
no.	rat type 1 5AR	rat type 2 5AR	rat 3BHSD
9	0.10	0.17	140
12	0.27	1.10	50
16	0.07	0.12	97
30[a,b]	0.13	0.27	13
1	6.8	0.28[c]	150

[a] 17β-[*N*-[2-Chloro-5-(trifluoromethyl)phenyl]carbamoyl]-6-aza-androst-4-en-3-one. [b] A satisfactory elemental analysis was obtained for this compound (C, H, N; ±0.4% of calculated values). [c] This is an IC_{50}; see ref 13.

Effect of Optimized C17 Substituents on Other Steroidal 5AR Inhibitors. Having discovered two novel series of C17 amides which significantly improve the potency, selectivity, and in vivo properties of 6-aza-steroids, it seemed worthwhile to determine the effect of these substituents on the 4-azasteroid[11] and 3-carboxysteroid[12] framework. To that end, compounds **31** and **33**, which bear the same C17 [(4-chlorophenyl)-cyclopentyl]amide as **16**, **28**, and **29**, were prepared and assayed versus the 5AR isozymes and 3BHSD, and the results appear in Table 6. When compared to finasteride (**1**) and epristeride (**32**),[11b,c] **31** and **33** are 30–150-fold more potent inhibitors of type 1 5AR. In contrast to the 6-azasteroids, where the change from *tert*-butylamide to [(4-chlorophenyl)cyclopentyl]amide resulted in a 10-fold diminution in 3BHSD potency, this modification of C17 resulted in a relatively small effect on 3BHSD potency for the 4-aza- and 3-carboxysteroids. This differential effect of C17 substituents on 3BHSD potency within each series may result from subtle differences in the structure of the enzyme–inhibitor complex for each series. If instead the general trends between series are examined (**16** versus **31** versus **33**), the changes in 3BHSD potency can be rationalized by reference to the proposed transition state for the reduction/isomerization reaction catalyzed by 3BHSD which proceeds through a $\Delta^{3,5}$-dienolate-like structure.[7b] Apparently, the 3-carboxysteroid mimics this structure best followed by the 6-azasteroid and the 4-azasteroid—possibly the least good mimic because of the more limited planarity across the C3–C6 carbons.

Overall, the 4-azasteroid **31** is a remarkably potent dual inhibitor of 5AR with 10 000-fold selectivity versus 3BHSD. Considering the apparently irreversible nature of inhibition of type 1 and 2 5AR by Δ^1-4-azasteroids,[13] and the exceptional selectivity versus 3BHSD of compounds such as **31**, further studies in this series appear judicious.[14]

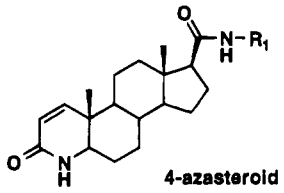
Conclusions

Optimization of the C17 substituent of 6-azasteroids for dual 5AR inhibition, selectivity versus 3BHSD, in vivo efficacy, and pharmacokinetics has yielded potent and selective compounds which also demonstrate selectivity in vivo. During this work, two series of novel C17 amides were discovered which endow these properties: 2,5-disubstituted anilides and (arylcycloalkyl)amides. On the basis of these compounds, a conformational model for potency versus human adrenal 3BHSD was developed which consistently correlated with activity and which could prove useful in the design of C17 groups with greater conformational bias. Interestingly, substitution of either finasteride or epristeride with [(4-chlorophenyl)cyclopentyl]amide results in significant improvement of type 1 5AR potency yielding potentially more effective dual 5AR inhibitors within the 4-aza- and 3-carboxysteroid framework as well. On the basis of the residual circulating DHT in patients treated with epristeride and finasteride,[4,12c] 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.

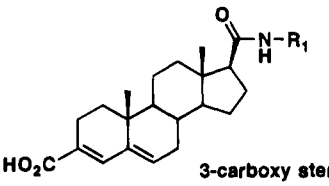
Experimental Section

Starting materials were obtained from commercial suppliers and used without further purification. Finasteride (**1**) and epristeride (**2**) were prepared as reported.[11,12] Amines required

Table 6. 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 3-Carboxy- and 4-Azasteroids



4-azasteroid



3-carboxy steroid

no.[a]	steroidal framework	R_1	IC_{50} (nM)[b] type 1 5AR	IC_{50} (nM)[b] type 2 5AR	3BHSD K_i (nM)
1	4-aza	NH-*tert*-butyl (finasteride)	150	0.18	11000
31	4-aza	N-[1-(4-chlorophenyl)cyclopentyl]	0.9	<0.1[c]	9000
32	3-carboxy	NH-*tert*-butyl (epristeride)	1600	0.18	160
33	3-carboxy	N-[1-(4-chlorophenyl)cyclopentyl]	60	<0.1	280

[a] Satisfactory elemental analyses were obtained (C, H, N; ±0.4% of calculated values). [b] Time-dependent inhibition of type 1 and 2 5AR was observed for the 4-azasteroids; see ref 13. [c] The enzyme concentration of *ca.* 100 pM establishes a lower limit for measurable IC_{50}'s.

for the preparation of amides of Tables 1 and 2 which were not commercially available were prepared by methods described in the literature as reported previously.[8] *Caution:* Inhibitors of 5α-reductase are likely to be teratogenic to the male fetus.

Molecular Modeling. Molecular modeling calculations were performed on a Silicon Graphics Indigo workstation. All molecules were built in MacroModel V3.5[15] and energy minimized to a gradient of <0.05 A2 with the MM2 force field. This resulted in the A-ring of each molecule adopting the normal (1α,2β) conformation. Using the minimized structure and keeping the steroid skeleton rigid, a search of conformational hyperspace about all rotatable bonds within the C17 side chain was conducted via the Monte Carlo Search within MacroModel. The search was run for 1000 steps, and those conformations with an energy window of 50 kJ/mol (12.5 kcal/mol) were stored. These structures were further minimized using the MULT option within MacroModel to arrive at a final set of low-energy conformers. This set of structures was then imported into SYBYL 5.5 (Tripos Assoc., 1699 S. Hanley Rd., Suite 303, St. Louis, MO 63144) for further analysis. The lowest energy conformations of **22** and **23** were superimposed through a rigid fit of the steroid backbone atoms, and volume calculations were carried out via the mvolume command within SYBYL. The excluded volumes were obtained by applying the difference operator to the arithmetic sums of the volumes for **22** and **23**.

Bioassays. Rat Adrenal 3β-Hydroxy-Δ^5-steroid Dehydrogenase/3-Keto-Δ^5-steroid Isomerase Assay. Enzyme activities were measured using microsomes derived from rat adrenal tissue by homogenization of the tissue followed by differential centrifugation. The assay was carried out in poly(styrene) 96-well plates in a total assay volume of 250 μL. A solution of the substrate, [1,2,6,7-^{3}H]dehydroepiandosterone (DHEA; New England Nuclear), was prepared by evaporating ethanol stock solution in a Savant SpeedVac and resuspending in standard assay buffer (100 mM potassium phosphate, pH = 7.5, 0.1 mM EDTA, 20% glycerol, 0.1 mM DTT) containing 1% DMSO. In a separate plate, inhibitor concentrations were serially diluted 2-fold in 100% DMSO followed by a 2-fold dilution in standard assay buffer. A 4-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 and 50 μL of 5 mM NAD^+ solution (dissolved in standard assay buffer) containing adrenal microsomes. The assay was initiated by addition of 46 μL of DHEA solution (8 nM final concentration) which had been preheated to 37 °C. After 15 min, a 100-μL aliquot from the reaction mixture was quenched by addition to an equal volume of ethanol. The substrate and product (androstenedione) were quantitated by HPLC (Spectra-Physics pump, BioRad autosampler, Regis C18 column, 15 cm × 4.6 mm, Radiometric in-line radioisotope detector) with water:methanol:acetonitrile:tetrahydrofuran (44:35:11:10) as mobile phase at a flow rate of 1.5 mL/min. The percent inhibition at each inhibitor concentration was determined by calculating the ratio of enzyme activity in the presence and absence of inhibitor. The apparent K_i (K_{iapp}) of each inhibitor was determined by nonlinear regression analysis according to the equation $y = (\%\ \text{inhibition max}\,x)/(K_{iapp} x)$. K_i's were then calculated from the relationship $K_i = K_{iapp}/(1 + [\text{substrate}]/K_m)$, where the substrate concentration was 8 nM and the K_m (Michaelis constant) was previously determined to be 21 nM. The error in K_i determinations estimated at the 95% confidence limit was 4–7% of the reported values.

Type 1 and 2 Recombinant Rat 5α-Reductase Assays. The rat isozymes of 5AR were assayed as previously described for the human type 2 assay[5] with the following exceptions:

Rat Type 1. The assay was carried out at pH 7 in the presence of 94 nM testosterone. After a 10-min preincubation of enzyme with test compound, the reaction was initiated by addition of substrate and allowed to proceed for 60 min. Following quenching as previously described, product formation was determined by HPLC analysis of 55-μL aliquots. The error in K_i determinations estimated at the 95% confidence limit was 5–20% of the reported values.

Rat Type 2. Due to its high K_m for this isozyme of 5AR, corticosterone (9 nM) was substituted for testosterone in order to allow reactions to be carried out under pseudo-first-order conditions. Substrate and product were resolved for quantitation using a Vydac C18 HPLC column (4.6 × 150 mm, 5 μm) and a mobile phase consisting of H_2O:acetonitrile (65:35) at a flow rate of 2 mL/min. The error in K_i determinations estimated at the 95% confidence limit was 5–40% of the reported values.

Intact Rat Model. Male Sprague–Dawley rats, 200–225 g, were grouped by body weight (n = 8/group). Test compound or vehicle was adminstered daily, by oral gavage, for 14 days. On the 15th day, animals were sacrificed by asphyxiation. Body weight was recorded. The ventral prostate, seminal vesicles, adrenal glands, liver, and right testicle were removed, cleared of adherent tissue, weighed, and preserved in 20% formalin. Standard errors for organ weights were generally 10% of the mean or less.

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Supporting Information Available: Further information concerning the X-ray analysis of **29**—fractional atomic coordinates, anisotropic temperature factors, bond angles and distances, and atomic coordinates (15 pages). Ordering information is given on any current masthead page.

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