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Communications to the Editor

4-Aza-3-oxo-5α-androst-1-ene-17β-*N*-aryl-carboxamides as Dual Inhibitors of Human Type 1 and Type 2 Steroid 5α-Reductases. Dramatic Effect of *N*-Aryl Substituents on Type 1 and Type 2 5α-Reductase Inhibitory Potency

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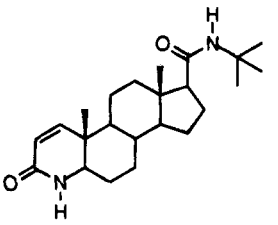
Steroid 5α-reductase type 1 and type 2 are expressed tissue specifically in humans[1] and are responsible for local production of the more potent androgen dihydrotestosterone (DHT) from testosterone (T). DHT is believed to play an important role in the pathology of benign prostatic hyperplasia (BPH),[2] acne,[3] hirsutism,[4] and male pattern baldness.[5] Inhibitors of human type 1 and type 2 5α-reductase are the subject of intense research for therapeutic intervention in the above conditions. Finasteride (1),[2b] which is a selective inhibitor of the human type 2 isozyme, has been approved for the treatment of BPH. At the therapeutic dose of 5 mg/day, 1 lowers the serum DHT levels[6] in men by 65–80% compared to baseline values. The residual DHT in the above patients may be due to its production by type 1 5α-reductase, which is not effectively inhibited by **1**. Epristeride (**2**),[7] another type 2 5α-reductase selective inhibitor, is less effective in lowering DHT in humans compared to finasteride. MK-386 (**3**)[8] and LY 191704 (**4**)[9] are the only reported human type 1 selective 5α-reductase inhibitors. However, the physiological role of type 1 5α-reductase in humans is not yet clear.[10]



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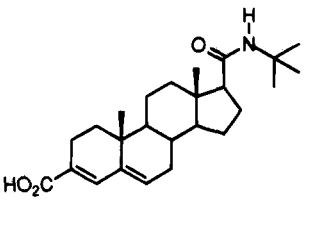

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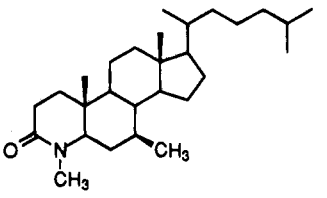

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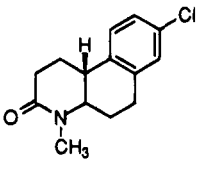

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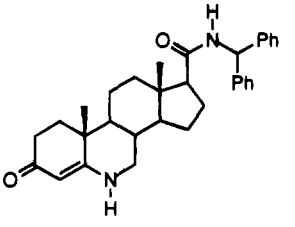

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Inhibition of both enzymes (type 1 and 2) by a dual inhibitor or combination of selective type 1 and type 2 inhibitors should lower DHT more effectively compared to use of a type 1 or type 2 inhibitor separately and thus may be more efficacious for clinical treatment of BPH, acne, hirsutism, or male pattern baldness, if circulating DHT levels are important in these androgenic disorders. 6-Azasteroid **5** has been reported[11] as a dual inhibitor of both human 5α-reductases. In continuation of our interest in the inhibition of 5α-reductase,[8,14] we report herein the synthesis and *in vitro* 5α-reductase activities of 4-aza-3-oxo-5α-androst-1-ene-17β-*N*-arylcarboxamides.[12] Some of these azasteroids are potent inhibitors of both human type 1 and type 2 5α-reductase isozymes. In a dog model of prostate shrinkage by systemic

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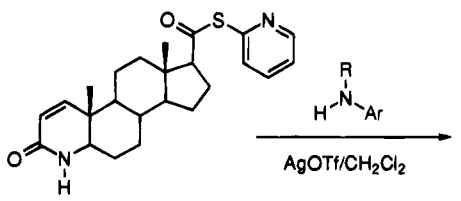
treatment with 5α-reductase inhibitors, finasteride 1 has been shown to be effective in reducing prostate volume which correlated with a decrease in prostatic DHT.[13a] 4-Aza-3-oxo-5α-androst-1-ene-17β-*N*-phenylcarboxamides (**7**, L-697,818), potent dual inhibitors described herein for the first time, have similarly been shown to be very effective in reducing the same parameters in this animal model.[13b]

The synthesis of the azasteroids starts with thiopyridyl ester (**6**), which was prepared by following previ-



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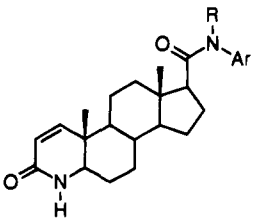


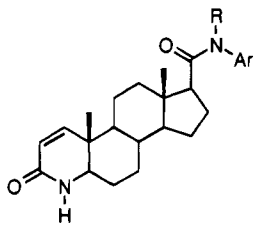
(7)

ously described chemistry from these laboratories.[14] Reaction of the thiopyridyl ester **6** with anilines in presence of AgOTf resulted in formation of anilides **7**. The above coupling reaction is very slow and in some cases does not occur if AgOTf is not used as catalyst. Coordination of Ag^+ with the thiophilic *S*-pyridyl group presumably activates the thiopyrdyl ester **6** toward the weak nucleophiles. The use of the AgOTf in activating thiopyridyl esters for coupling reaction with weakly nucleophilic species has not been previously reported.

The azasteroids were assayed[1a,8a] using recombinant human type 1 and type 2 isozymes.[15] The results are reported as the nanomolar (nM) concentration required for 50% inhibition (IC_{50}) of the conversion of T to DHT. Some Δ^1- azasteroids have been reported[16] to be time dependent inhibitors of human type 1 and type 2 5α-reductase. For this reason an altered protocol with preincubation of cofactor and inhibitor with enzyme prior to substrate addition was employed in the assay to allow equilibration of the system. The results are summarized in Table 1. Additionally, five of the compounds (**7**, **9a**, **9d**, **13b**, and **14a**) described in this paper were selected for further evaluation regarding the time dependent nature of the inhibition. Each of the compounds were found to be slow binding inhibitors of both isozymes of 5α-reductase. The K_i for the preliminary enzyme–inhibitor complex and the second-order rate constants for formation of the high-affinity complex are presented in Table 2.

Compound **7** was found to be a potent inhibitor of human type 2 enzyme (IC_{50} 0.2 nM) with a reasonable potency against type 1 isozyme (IC_{50} 20 nM). Introduction of an alkyl group at the anilide amide nitrogen (Table 1, **7a**, **7b**) results in a drastic loss of both type 1 and type 2 inhibitory activity. Modeling studies with these compounds indicate that addition of the alkyl

Table 1. Inhibition of Recombinant Human Type 1 and Type 2 5α-Reductase by 4-Aza-3-oxo-5α-androst-1-ene-17β-*N*-arylcarboxamides



			IC_{50} (Nm)[a]	
no.	R	Ar	Type 2	Type 1
1		finasteride	<0.1	52.0
2		epristeride	0.6	>1000.0
3		MK-386	154.0	0.9
4		LY191704	1750.0	8.6
5		6-azasteroid	<0.1	186.0
7	H	C_6H_5	0.2	20.0
7a	Me	C_6H_5	24.6	350.0
7b	Et	C_6H_5	7.9	614.0
7c	Ph	C_6H_5	25.2	>1000.0
7d		1-indolinyl	0.4	120.2
7e		1-(1,2,3,4-tetrahydroquinolinyl)	1.4	154.0
7f		1-(2,3,4,5-tetrahydro-1*H*-benzazepinyl)	10.2	595.0
8a	H	$2\text{-}FC_6H_4$	<0.1	7.9
8b	H	$3\text{-}FC_6H_4$	0.2	23.0
8c	H	$4\text{-}FC_6H_4$	0.2	22.5
9a	H	$2\text{-}CF_3C_6H_4$	<0.1	5.6
9b	H	$3\text{-}CF_3C_6H_4$	0.1	12.5
9c	H	$4\text{-}CF_3C_6H_4$	0.4	20.9
9d	H	$2,5\text{-}(CF_3)_2C_6H_3$	<0.1	5.8
10a	H	$2\text{-}ClC_6H_4$	<0.1	14.5
10b	H	$3\text{-}ClC_6H_4$	0.1	18.2
10c	H	$4\text{-}ClC_6H_4$	0.2	6.8
10d	H	$3\text{-}BrC_6H_4$	0.2	20.5
11a	H	$2\text{-}MeC_6H_4$	<0.1	30.3
11b	H	$3\text{-}MeC_6H_4$	0.3	24.6
11c	H	$4\text{-}MeC_6H_4$	0.1	17.2
11d	H	$2,6\text{-}Me_2C_6H_3$	<1.0	14.0
12a	H	$2\text{-}MeOC_6H_4$	<1.0	11.0
12b	H	$3\text{-}MeOC_6H_4$	<1.0	19.0
12c	H	$4\text{-}OMeC_6H_4$	<1.0	26.0
13a	H	$2\text{-}C_6H_5C_6H_4$	<0.1	38.3
13b	H	$3\text{-}C_6H_5C_6H_4$	<0.1	14.0
13c	H	$4\text{-}C_6H_5C_6H_4$	2.2	34.9
14a	H	1-naphthyl	0.2	8.1
14b	H	2-naphthyl	0.2	17.2
15a	H	$2\text{-}OHC_6H_4$	<1.0	12.9
15b	H	$3\text{-}OHC_6H_4$	<1.0	52.0
15c	H	$4\text{-}OHC_6H_4$	<1.0	74.0
16a	H	$2\text{-}NH_2C_6H_4$	<1.0	130.0
16b	H	$3\text{-}NH_2C_6H_4$	<1.0	94.0
16c	H	$4\text{-}NH_2C_6H_4$	<1.0	120.0
17a	H	$2\text{-}C_6H_4COC_6H_5$	<1.0	28.7
17b	H	$3\text{-}C_6H_4COC_6H_5$	<1.0	12.0
17c	H	$4\text{-}C_6H_4COC_6H_5$	1.6	19.0

[a] The IC_{50} values were determined by following product formation in the presence and absence of inhibitor using the standard assay conditions described previously.[8a]

substituent changes the conformational preference of the amide bond and reduces the rotational energy barrier between rotamers, which may explain the loss of activity for these compounds. Conformational energies calculated by the Optimol program[17] for 360° rotation about the amide bond in 10° increments show a 2 kcal/mol preference for the *Z* rotamer in **7a** and **7b** as compared to the 5 kcal/mol preference for the *E* isomer in **7** (Figure 1). This implies that the preferred binding conformation of the anilide amide is *trans* in both type 1 and type 2 5α-reductase. In the *cis* amide conformation (**7a**,**b**), either the alkyl group which occupies the *trans* position is not big enough to effectively

Table 2. Kinetic Parameters for Inhibition of 5α-Reductase by Selected Azasteroids[a]

	type 1		type 2	
no.	K_i (nM)	k_3/K_i (M^{-1} s^{-1})	K_i (nM)	k_3/K_i (M^{-1} s^{-1})
13b	24	5.1×10^4	1.4	5.0×10^6
14a	70	1.7×10^4	0.5	2.1×10^6
7	230	3.9×10^3	1.7	1.7×10^6
9a	12	3.5×10^4	1.3	9.7×10^6
9d	2.4	2.7×10^5	0.5	7.0×10^6

[a] The second order rate constants were determined by following product formation in the presence and absence of inhibitor using the standard assay conditions described previously.[8a] The data were fit to the integrated first-order rate equation reviewed by Morrison and Walsh.[18]

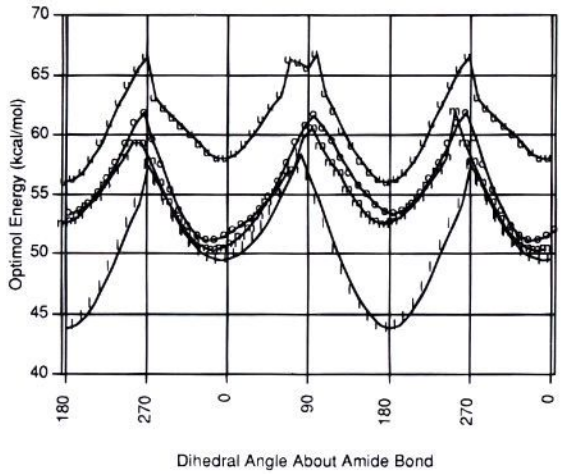


Figure 1. Conformational energies calculated by the Optimol program[17] for 540° rotation about the torsion CC—NC in 10° increments. Energies were calculated for an additional 180° over 360° referred to in the text to show that the conformational energy profile is repetitive. Each conformer was completely energy minimized after rotation to the desired dihedral angle from the starting structure. Legend (|) compound **7**, (m) compound **7a**, (o) compound **7b**, and (u) compound **7d**.

fill up the binding pocket and/or the *cis* phenyl group has unfavorable interactions (in both the type1 and 2 enzyme) which are reflected in the ~150-fold increase in the IC_{50} values. The diphenyl amide **7c**, which contains the phenyl group in both *cis* and *trans* positions, has 5α-reductase (type 1 and 2) inhibitory activity similar to **7a** and **7b**, which clearly suggests that a *cis*-phenyl group has unfavorable interactions with binding pockets in the type 1 and type 2 enzymes. Interesting results are obtained when an *N*-alkyl group is attached to the aryl ring as a means of controlling its conformation [Figure 2, **7d** (energy minimized conformation)]. Indolinylamide **7d** in which the *trans*-amide is slightly more stable than the *cis* (Figure 1), regains only the type 2 inhibitory activity (IC_{50} 0.4 nM). This indicates that the requirements for binding of the phenyl ring of *trans*-anilide amide for type 1 inhibition are more stringent than for those of type 2 reductase. The other cyclic anilide amides **7e** and **7f** behave similarly to the anilides **7a** and **7b** because of close *cis*/*trans*-amide rotational energies.

Further efforts were focused on the optimization of substitutents on the aryl ring in the NH series because of the preference for the *trans*-amide conformation. Introduction of a lipophilic or electron-donating substituent like Me or OMe (**11a-d**, **12a-c**) at the *ortho*, *meta*, or *para* position have mild effects on type 1 and

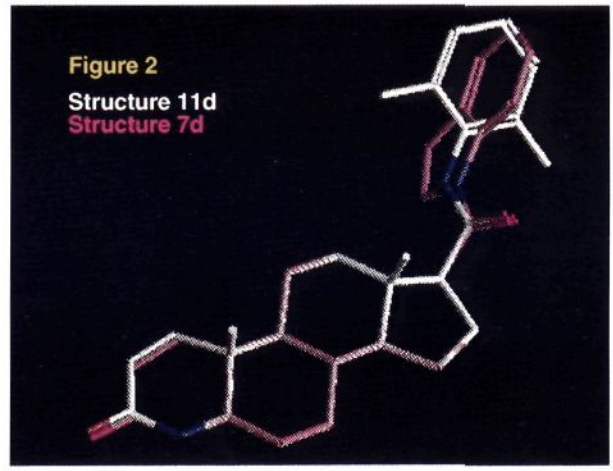


Figure 2.

type 2 inhibition. However, polar groups (OH, NH_2) on the aryl ring (**15a-c**, **16a-c**) generally lead to a drop of 2−6-fold for type 1 5α-reductase activity, except in the case of **15a**. The increase of 2-fold for the type 1 inhibitory potency of **15a** may be due to hydrogen bonding of the oxygen of *o*-hydroxyl group with the enzyme. Hydrogen bond acceptor groups like F or CF_3 at *ortho* position (**8a**, **9a**, **9d**, **10a**) lead to a ~2−3-fold increase in type 1 inhibitory potency. These results corroborate our earlier assumption that the *ortho* substituent is involved in the hydrogen bonding with the type 1 enzyme. The above substituents at the *meta* and *para* position do not seem to influence type 1 or type 2 5α-reductase inhibitory potency.

The binding pocket of the type 1 and type 2 5α-reductase seems to be quite accommodating for flat groups. In the biphenyl (**13a-c**) and benzophenone (**17a-c**) series *meta* substituents have slightly more type 1 inhibitory activity compared to the *ortho* and *para* analogs. This suggests the presence of a lipophilic binding pocket at the *meta* position. The α-naphthylamide (**14a**) is 2 times more potent for type 1 inhibition than the β-naphthylamide (**14b**). All the anilide azasteroids with the Δ^1-NH A-ring are very potent inhibitors for type 2 enzyme with IC_{50} values of < 1 nM.

In conclusion, we have shown that (a) anilides bind most favorably to both type 1 and type 2 isozymes in a *trans* conformation; (b) introduction of a F or CF_3 group at the *ortho* position leads to an increase in the type 1 inhibitory potency; (c) good type 1 inhibitory potency is seen with the α-naphthylamide (**14a**) and *meta*-biphenylamide (**13b**); (d) these azasteroids are time-dependent inhibitors of human type 1 and type 2 enzyme (Table 2) and are far more potent than the fixed-time assay results in Table 1 would imply. Furthermore, we have not only shown important differences in the binding pocket of the type 1 and type 2 enzyme around C-17 but have also demonstrated that compounds could be optimized to potent dual inhibitors of both human type 1 and type 2 5α-reductase. Additionally, azasteroid **7** has shown *in vivo* efficacy in reduction of prostate size in systemically treated dogs.

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Supporting Information Available: Experimental procedures, analytical data, conformational energies graphs for

compounds **7**, **7a**, **7b**, **7d**, **7e**, and **7f**, and enzymological method (13 pages). Ordering information is given on any current masthead page.

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(18) The values presented represent an average of at least three determinations. The kinetic model used

$$E + I \overset{K_i}{\rightleftharpoons} EI \overset{k_3}{\underset{k_4}{\rightleftharpoons}} EI^*$$

was described by Morrison and Walsh: Morrison, J. F.; Walsh, C. T. The behavior and significance of slow-binding enzyme inhibitors. *Adv. Enzymol.* **1988** *61*, 201–301. The data were fit to $y = v_s t + (v_o - v_s)(1 - e^{-kt})/k$ where y = product, v_o is the initial velocity, v_s is the velocity at infinity time, and k is the pseudo-first-order rate constant. The second order rate constants (k_3/K_i) were estimated using the equation $k = k_4 + k_3 I/\{I + K_i(1 + S/K_m)\}$ where k_4 was taken to be zero and K_i was determined using the equation $v_o = V_{max}S/(K_m(1 + I/K_i)) + S)$.

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