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5α-reductase inhibitors, chemical and clinical models

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An active site model of 5α-reductase type 2 isoenzyme on an "active-analog approach" and based on 4-azasteroidal inhibitors has been constructed to evaluate the effects on the inhibitory potency of substituents on the steroid A ring. This model has proven able to predict the potential inhibitory activity of 19-nor-10-azasteroid and 6-azasteroid compounds. A model for the evaluation of clinical efficacy of an inhibitor, based on in vitro data, has also been developed and applied to finasteride. This inhibitory potency evaluation of finasteride in human scalp homogenates, plus pharmacokinetic data, allows the calculation of a theoretical in situ inhibition value for human scalp. From the IC_{50} curve of finasteride in scalp homogenates, it is possible to calculate that for an inhibition level similar to that obtained in prostate with 5 mg of finasteride, the necessary plasma concentration of the drug is 1 μM, a level obtained after the acute administration of 50 mg of finasteride. (*Steroids* **63**:355–361, 1998) *© 1998 by Elsevier Science Inc.*

Keywords: 19-nor-10-azasteroids; 5α-reductase; inhibition; finasteride; clinical efficacy

Introduction

Steroid 5α-reductase (EC 1.3.99.5) is a membrane-bound NADPH-dependent enzyme that catalyzes the reduction of testosterone (T) to the more potent androgen dihydrotestosterone (DHT).[1,2] Two isozymes of 5α-reductase have been cloned, expressed, and characterized (5αR-1 and 5αR-2). They have different chromosomal localization, tissue expression patterns, and enzyme kinetic parameters. The Type 2 isozyme is found predominantly in the prostate, genital skin, seminal vesicles, epididymis, and liver. The Type 1 isozyme occurs predominantly in hair follicle and sebaceous glands of the skin (including the scalp) and liver.[1,2] The Type 2 isozyme is essential for differentiation of male external genitalia during foetal life, as assessed by studies on male pseudohermaphroditism in which total or partial deficiency of 5αR-2 has been found.[3–6] In addition to the obvious abnormalities in male phenotype, in such patients there are clinical signs suggesting a therapeutic potential for 5αR inhibition in normal sexually mature individuals in that the prostate remains undeveloped, facial and body hair growth patterns are more feminine in character, temporal regression of the hair line is significantly reduced, and there is a decrease in the incidence of acne.

As a logical consequence of these clinical observations, 5αR inhibitors have been synthesized as potential drugs for treatment of human BPH (benign prostatic hyperplasia), male pattern hair loss, acne, and hirsutism.[7–10] The recent discovery of the two isozymes and the determination of their tissue localization has enhanced prospects for the selective intervention in one or more of these disorders that exhibit isozyme dependence, and studies directed toward the synthesis of selective inhibitors of Type 1 or Type 2 isozyme and their clinical evaluation have been performed. However, since X-ray structures of 5αR-1 and 5αR-2 are to be yet determined, computer-assisted modeling of the active site of the two isozymes, based on the known structure–activity relationship data, might be useful to predict the potential inhibitory activity of new compounds. In this paper, we report the application of an active site model for 5αR-2 for the evaluation of new steroidal compounds as potential inhibitors of this isozyme.

A different kind of problem is the correct evaluation of in vitro data to extend them to clinical practice. Therefore, we discuss a possible approach to predicting the clinical efficacy of an inhibitor (finasteride) for the treatment of the male pattern baldness based on in vitro and in vivo data.

Design of new inhibitors: A 5αR-2 active site model

Inhibitors based on the steroidal structure, such as 4-azasteroids, 6-azasteroids, and carboxylic acid derivatives, have been studied extensively.[11] Among these inhib-

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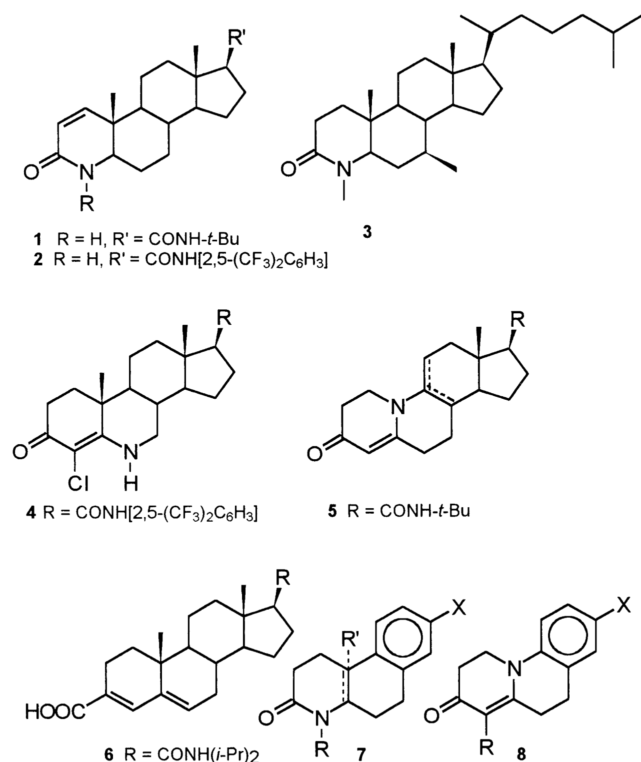


Figure 1 Some 5α-reductase inhibitors.

itors, finasteride (**1**; Figure 1) has been assessed clinically and is now on the market for treatment of BPH.[12,13] Other inhibitors in clinical trials for BPH are GG745 (**2**), one of the most potent dual inhibitors to date (it is reported to reduce circulating DHT levels by 90%),[14] and epristeride (**6**), a potent selective 5αR-2 inhibitor (which clinically reduces DHT to a lesser extent than finasteride).[15] Among 6-azasteroids, compound **4** is a potent dual inhibitor of both isoenzymes.[16] Compound **3** (MK386) is a potent 5αR-1 inhibitor and is now undergoing human clinical trials for acne, alopecia, and hirsutism.[17,18]

Nonsteroidal tricyclic inhibitors, such as compound **7** and others, conceptually derived from 4-azasteroids, 6-azasteroids, and steroidal carboxylic acids (Figure 1) have been described recently. They are very potent inhibitors of human 5α-reductases with, in most cases, higher activity against the Type 1 isozyme.[11]

We have described recently a novel class of 5α-reductase azasteroidal inhibitors with a N atom at the position 10 of the steroidal skeleton (19-nor-10-azasteroids).[19,20] In particular, 17β-*N*-(*t*-butyl)carbamoyl substituted compound **5** (Figure 1) displayed an inhibitory potency against 5αR-2 comparable with that of finasteride and was more active against 5αR-1.

The design of 19-nor-10-azasteroids as 5α-reductase inhibitors is based on the transition state inhibitor paradigm,[21] a concept that states that the affinity for the enzyme should be greater for molecules that mimic the transition state of the enzymatic process. The proposed mechanism (Figure 2) of T reduction to DHT by 5αR catalysis[8–10] involves as a key step the activation of the 4-en-3-one moiety of T by the interaction of the carbonyl group with an electrophilic residue E^+ of the active site, followed by hydride transfer from NADPH to the position 5. Thus, it is possible to postulate two different transition states (TS): the "substrate like" TS in which the C-5 has not yet changed its sp^2 hybridisation, and the "product like" TS, in which the C-5 has assumed the final sp^3 hybridisation.

Accordingly, 4-azasteroids, similar to the DHT enol, are mimics of the "product like" TS, whereas 6-azasteroids and 10-azasteroids, which have an enone structure in the A ring,

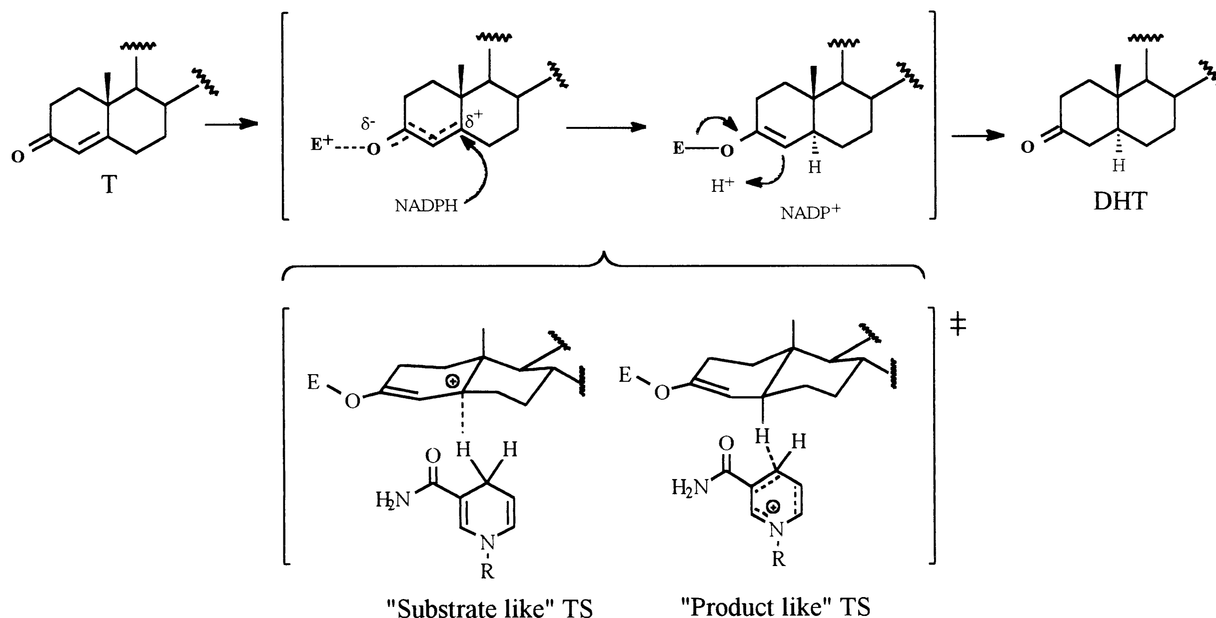


Figure 2 Mechanism of inhibition of 5α-reductase.

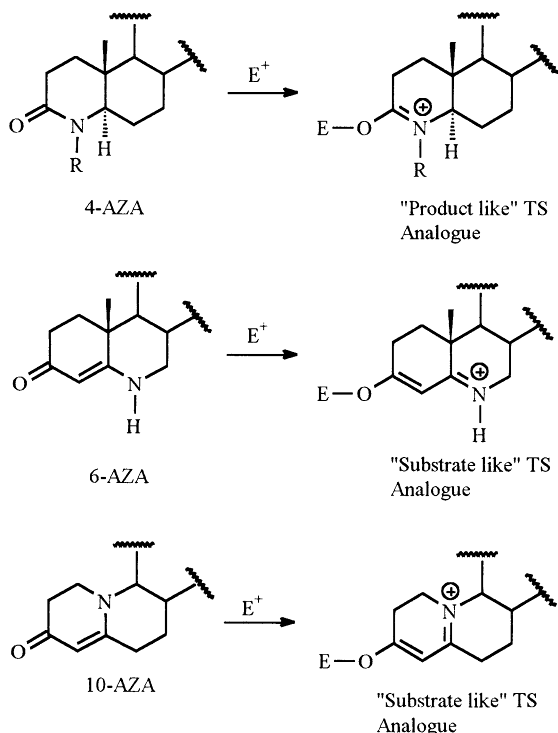


Figure 3 4-, 6-, and 10-azasteroids as "product- or substrate-like" 5α-reductase inhibitors.

are mimics of the "substrate like" TS (Figure 3). In these compounds, the presence of the nitrogen atom increases the nucleophilicity of the carbonyl group and thus favors the interaction with an electrophilic residue in the active site. In general, azasteroids interact with the enzyme-NADPH complex and are competitive with respect to testosterone. It has been demonstrated recently that finasteride **1** and other $\Delta^{1(2)}$ unsaturated 4-azasteroids are slow offset, essentially irreversible inhibitors. This is due to the formation of a covalently bound complex between finasteride and the cofactor, after 1,4 reduction of the enone moiety and subsequent alkylation of the enolate.[22] Preliminary studies performed on 10-azasteroids have excluded any time dependence in inhibition activity of the tested compounds.[19] The androstenecarboxylic acids such as compound **6** display noncompetitive kinetics and are believed to interact preferentially with the enzyme-NADP$^+$ complex.[23]

In a conformational study performed on 19-nor-10-azasteroids and on 4- and 6-azasteroids aimed at determining the number and energy of the possible conformers in addition to the molecular flexibility of the azasteroidal skeletons, it emerged that 10-azasteroids are very flexible molecules with transitional barriers between four thermally accessible conformers (each conformer having an energy in the 0–3 kcal/mol range with respect to the global minimum conformer) lower than 4 kcal/mol.[20] 4- and 6-azasteroids were generally more rigid structures than 10-azasteroids, and thus may be more suitable than 10-azasteroids for developing models of the 5αR active site. With this in mind, we have recently proposed a model for 5αR-2 on the "active-analog approach" based on 4-azasteroids, and have applied it successfully to predict the activity of different inhibitors.[24]

This model was obtained by determining the differences between the combined van der Waals volumes of a set of inactive 4-azasteroids and those of a set of active 4-azasteroids. The resulting three-dimensional area represents part of the space occupied by the enzyme ("excluded volume"). The set of active molecules included inhibitors with a potency of up to 10-fold lower than the most active inhibitor (4-MA,[25] IC_{50} 8.5 nM). The second group, defined as inactive molecules, included 4-azasteroids having a lower inhibitory potency (IC_{50} > 100-fold 4-MA). Thus, the compatibility of the enzyme toward a series of inhibitors can be established by evaluating the intersection volume (V_i) between the molecules studied and the excluded volume of the active site. The higher the V_i, the lower the expected affinity of the molecule for the enzyme.

All 4-azasteroids used to construct the model had the diethylamide group at position 17 to maintain constant the effect of the C-17 substituent on the inhibitory potency, as well as the same substitution on B and C rings; thus, the model was designed to predict the effect on inhibitory activity of A ring substituents. Compatibility of the A ring with the region of the active site responsible for the enzymatic reduction process is of fundamental importance for recognition of the inhibitor.

Since "product-like" inhibitors such as 4-azasteroids have been used for the construction of the model, to extend

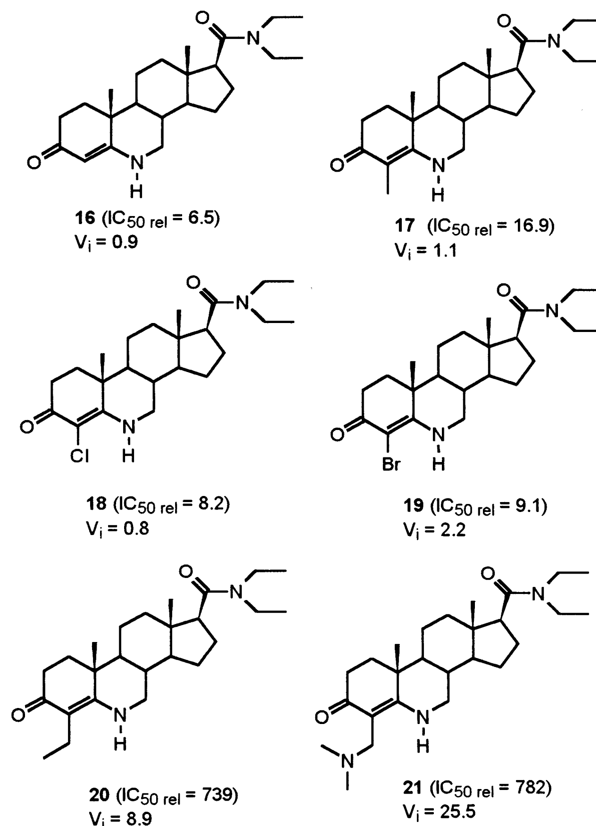
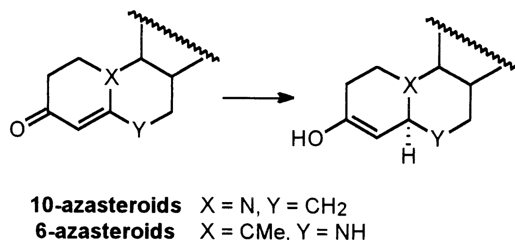


Figure 4 Intersection volumes V_i (Å^3) of some 6-azasteroids.

it to compounds such as 6- and 10-azasteroids or other steroids possessing a 4-en-3-one moiety requires conversion of these compounds to the corresponding 5α-reduced enol form, assuming the latter is the active form (Scheme 1).



Scheme 1

After complete conformational analysis, performed as reported for the corresponding 4-en-3-one compounds,[20] two thermally accessible conformers were found in the 0–3 kcal/mol range for 10-azasteroids, while 6-azasteroids were represented by only one conformer in that range.

The V_i (Å^3) were thus calculated for the series of active and inactive 6-azasteroids **16-21** and 10-azasteroids **5a**, **5b**, **12**, and **15** (Figures 4 and 5). For active 6-azasteroids **16-19** V_i values were in the 0.8–2.2 range, and for inactive compounds **20-21**, values were in the 8.9–25.5 range. For 10-azasteroid **5a** ($\Delta^{9(11)}$ unsaturated), V_i was 1.1 (and 1.3 for the other thermally accessible conformer); for its regioisomer **5b**, V_i was 1.7 (and 1.0 for the other conformer). For the inactive compound **12**, a greater intersection volume was found ($V_i = 2.6$).

As is evident from these results, when the model is applied to inhibitors of the same class, it indicates a trend of potential inhibitory activity. For example, 6-azasteroids with $V_i < 2.2$ are active compounds, while those having $V_i > 2.2$ are inactive. However, comparison of the V_i of inhibitors of different classes may be inappropriate. For example, in the 6-azasteroid series, an active compound such as **19** has a V_i value comparable to that of the inactive 10-azasteroid **12**; in the 10-azasteroid series active compounds have $V_i < 1.7$.

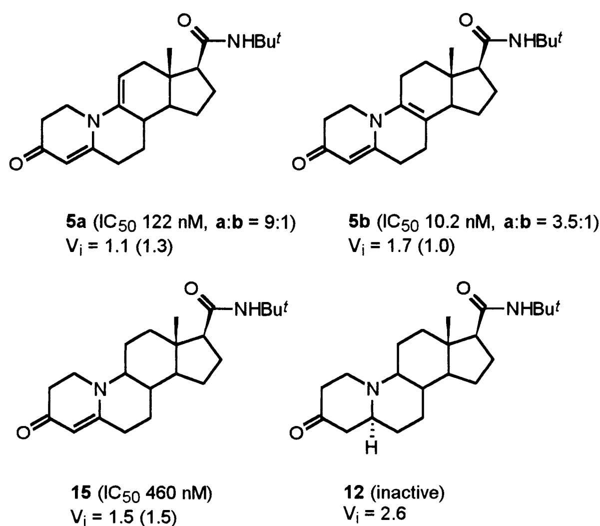


Figure 5 Intersection volumes V_i (Å^3) of some 10-azasteroids.

The model is also not sufficiently accurate to predict slight differences of inhibitory potency. For example in the 6-azasteroids series, while the model correctly gives a greater V_i for compound **17** than for **18**, which is twice as active, it also predicts a greater V_i for compound **19**, which is also twice as active. Similarly, in the 10-azasteroid series the increase of potency of compound **5b** with respect to **5a** does not correspond to a reduction of the V_i. However, in this case the intersection volume of the other conformer ($V_i = 1.0$) of **5b** is lower than that of both conformers of **5a**. Thus, the increase of potency associated with the shift of the double bond in correspondence of the A/B ring junction can be explained by assuming that the enzyme recognizes the higher energy conformer instead of the global minimum with a very low energy cost, reflecting the high flexibility of 10-azasteroids.

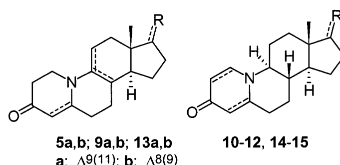
The model has been developed to evaluate the compatibility of the steroidal A ring of an inhibitor with the enzyme active site. Since it has been established that C-17 substitution strongly affects the inhibitory activity of a compound, suitable substitutions at this position on a steroidal skeleton with a low intersection volume could produce a substantial enhancement of inhibitory potency.

For example, azasteroid **9** (Table 1, entries 1 and 2), which has low 5αR-2 inhibition activity (IC_{50} 4.6 μM) and a low V_i (1.0–1.1), could be a potent inhibitor if the appropriate C-17 substituent was present. A 17β-OH group does not seem appropriate for this purpose, since compound **13** is only slightly more potent than **9** (Table 1, entry 6). Instead, with the 17β-*N*-(*t*-butyl)carbamoyl substitution, the same as in finasteride **1**, a potent inhibitor such as **5** is obtained (IC_{50} 122 nM; Table 1, entry 8). Similarly, compound **10** has low V_i values (1.5) for both its two conformers but displays only weak activity (IC_{50} 4.4 μM; Table 1, entry 3). However, introducing the above mentioned 17β substituent provides more effective 5αR-2 inhibition (see compound **15**, IC_{50} 460 nM; Table 1, entry 10). In the case of **15**, the model is able to predict the relative potency with respect to compound **5a**, since to a lower potency of **15** for 5αR-2 corresponds a greater intersection volume (Figure 5).

The model proposed is general and can be applied to all steroidal structures. With this model, for example, we predicted that 4-hydroxyandrostenedione, having $V_i = 0.4$ in its reduced enol form, would be a potent 5αR-2 inhibitor if suitably substituted at C-17, whereas 4-methoxy- and 4-*O*-acetyl-androstenediones (with $V_i = 6.9$ and 25, respectively) would not. This was later demonstrated by Labrie et al.[26] who found that 17β-*N*-(t-butyl)carbamoyl substituted 4-hydroxyandrostenedione was a potent ($IC_{50} = 172$ nM) 5αR-2 inhibitor.

In the evaluation of activity by this model, only repulsive steric interactions between inhibitor and enzyme are taken into account, with other contributions to the binding affinity not considered, which might explain the inability of the model to establish slight differences of inhibitory potency. A particular case is represented by the completely inactive compound **12**, in which other than steric effects may deter-

Table 1 IC_{50} Values of 19-nor-10-azasteroids toward 5αR-1 and -2 Isoenzymes



Entry	Compd	A ring unsatn	R	5αR-2 IC_{50} (nM)	5αR-2 IC_{50} rel	5αR-1 IC_{50} (nM)	5αR-1 IC_{50} rel
1	**9a**	$\Delta^{4(5)}$	=O	4600	1389		
2	**9a**:**9b** = 5:1	$\Delta^{4(5)}$	=O	4600	1123	263	6.7
3	**10**	$\Delta^{4(5)}$	=O	4400	1265	299	6.4
4	**11**	$\Delta^{1(2)}$	=O	46000	18843		
5	**12**	—	=O	≫100000		≫100000	
6	**13a**:**13b** = 22:1	$\Delta^{4(5)}$	β-OH	2900 860[a]	981 614[b]		
7	**14**	$\Delta^{4(5)}$	β-OH	4200	1581	409	10.4
8	**5a:5b = 9:1**	$\Delta^{4(5)}$	β-CONHBu^t	122	21.5	127	2.8
9	**5a:5b = 3.5:1**[c]	$\Delta^{4(5)}$		10.2 7.3	2.9 2.4		
10	**15**	$\Delta^{4(5)}$	β-CONHBu^t	460	83	1134	24.4

[a]This is a K_i value.
[b]This value is a ratio between K_is.
[c]Freshly prepared solution.

mine its inactivity, for instance the lack of conjugation between the N atom and the 3-oxo group.

On the basis of the results reported in Table 1, the presence of a C=C bond on the A ring allowing conjugation between the carbonyl group and the nitrogen appears an essential feature for inhibitory activity against both isoenzymes. The presence of a nitrogen at position 10 increases the nucleophilic character of the carbonyl, which causes a stronger interaction with the active site of 5α-reductase.[20]

If the partial negative charge on the oxygen is taken as a measure of the nucleophilic character of the C-3 carbonyl in the above compounds, the increase of this partial charge could be related to a greater interaction with an electrophile in the cavity. Thus, the presence of the nitrogen at position 10, conjugated with the carbonyl group, confers on the oxygen in 10-azasteroids the same increase in negative charge character (−0.63) as in 6-azasteroids, whereas the reduction of the C-4 C-5 double bond decreases the partial charge to a value corresponding to that of testosterone (−0.59).[19] These calculations suggest that the observed inactivity of compound **12** may derive from the electronic effect mentioned above, as well as from steric effects associated with the high intersection volume found. In terms of the C ring unsaturated compound **5**, the variation of 5αR-2 inhibition found by changing the ratio between $\Delta^{9(11)}$ to $\Delta^{8(9)}$ isomers does not seem related to an electronic effect because no changes in the partial charge on the oxygen were observed. Thus, it is possible that a steric effect is involved and the comparison of **5a** and **5b** taking into account only steric effects correctly predicts the relative potency of the two compounds.

The presence of a double bond in the C ring affects the potency of compound **5** against 5αR-1, which is worthy of discussion. It has been recently pointed out that in tricyclic inhibitors such as the series represented by **7** (Figure 1), the extended planarity of the structure may be an important factor in determining the increased potency against this isoenzyme.[27] The same feature appears to be the basis of the high inhibition potency of benzo[c]quinolizin-3-ones **8**, which we have recently synthesized (WO 97/29107), and which are selective 5αR-1 inhibitors. Thus, the 10-fold greater activity of **5** than **15** may reflect the presence of a C=C bond in the C ring of **5**, which to some extent makes the molecule more planar.

Evaluation of the clinical efficacy

While substantial progress has been made toward the synthesis of more potent and isozyme specific inhibitors, clinical experimental models remain less well established. In the case of DHT-dependent disorders, animal models appear unsuitable for human pathology (for instance hirsutism and alopecia). Moreover, substantial differences among 5αR isozymes from different animal species are reported.[28] On the other hand, based on appropriate in vitro inhibitory potency data, an attempt can be made to extrapolate such results to clinical practice.

The pharmacological properties of 5αR inhibitors are generally studied in various cells transfected with the cDNA of human 5αR-1 and 2, respectively. To extend in vitro data to the human situation, prostate homogenates and human scalp homogenates are optimal sources of enzyme, since data from tissue homogenates are sometime different from those in transfected cells,[29] and probably more representative of the human situation in vivo.

Pharmacokinetic data for 5αR inhibitors in humans are

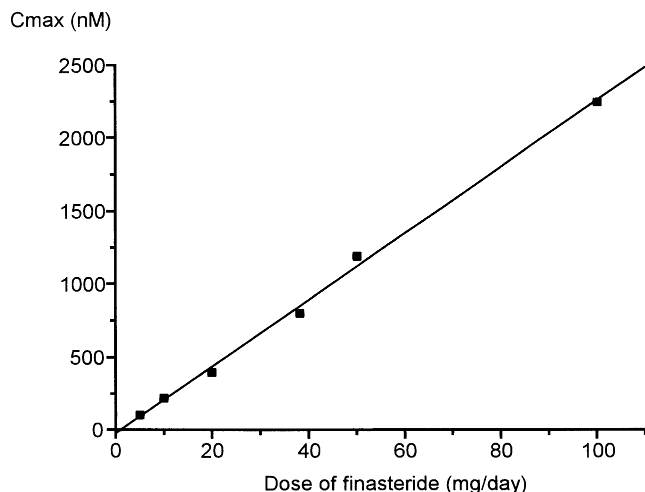


Figure 6 Correlation between C_{max} values of finasteride in plasma and administerd dose (single administration).[36]

scarce and generally reflect results of bolus administration of the drug with parameters measured only in plasma and not tissue (it has been established that the tissular concentration of some steroidal enzyme inhibitors may be much higher than the plasma concentration; Reference 30). Evaluation of inhibitory potency of 5αR inhibitors using human homogenates, accompanied by appropriate pharmacokinetic data, would allow calculation of in situ inhibition that in turn could be useful in clinical practice. This is the case for finasteride, the only 5αR inhibitor used extensively in clinical practice for human BPH, and to a lesser extent for hirsutism.[31] In both situations, the drug has marked limitations; in BPH only 25% of patients show reduction of prostatic size on ultrasound,[32] and in hirsutism a major limitation is the possible feminilizing effect on a male fetus during pregnancy.

For finasteride, which is already used for the treatment of BPH and was recently proposed for the treatment of alopecia, IC_{50} in prostate and scalp homogenates have been measured as 5.9 ± 0.3 nM and 310 ± 33 nM,[33] respectively. We have also studied the pharmacokinetics of finasteride after acute administration of a 5-mg dose using GC/MS,[34] and found a C_{max} of 35 ± 9 ng/mL (94 nM). This value and other pharmacokinetic parameters are in good agreement with those obtained by other authors using HPLC.[35] Othawa et al. [36] studied the pharmacokinetics of finasteride after single and multiple administration of the drug over an increasing dose range (5–100 mg). They demonstrated a linear correlation of C_{max} with dose after bolus administration (Figure 6) and no significant differences in this parameter after multiple administration over 7 days, indicating that no appreciable accumulation of finasteride occurs.

With these experimental data, a concentration of 94 nM causes a calculated 85% inhibition of prostate homogenate 5αR. McConnell et al.[37] measured the DHT reduction in prostatic tissue after 7 days administration of finasteride at 5 mg/day in BPH patients and found ≈85% DHT reduction versus placebo. The same concentration causes only 25% inhibition of scalp homogenate 5αR. Dallob et al.[18] measured the DHT reduction in scalp skin after 28 days administration of 5 mg/day of finasteride to balding men and found a 35% reduction from baseline.

It is possible to extrapolate from the IC_{50} curve of finasteride in scalp homogenates that to obtain a level of inhibition similar to that with 5 mg of finasteride in the prostate the concentration in plasma needs to be 1 μM, a value obtained after bolus administration of 50 mg of finasteride.[36] On the basis of these calculations, the proposed 1 mg/day dose of finasteride appears insufficient for effective treatment of male pattern baldness.

In the case of 5αR-1 inhibitors, only two compounds, LY191704 and MK386, have been tested for potency in human scalp homogenates (IC_{50} 9.7 and 20 nM, respectively),[29,33] and pharmacokinetic data are not available. Unfortunately MK386, which according to the reported results seems to be specific for the scalp, appears to have hepatotoxic effects. These are probably due to the presence of a lipophilic chain at position 17, and occur despite of the short administration period (2 weeks).[38] Short-term treatment would in any case be unsuitable for treatment of chronic conditions such as male baldness, acne, and hirsutism.

In conclusion, for a rational approach to the use of 5αR inhibitors in clinical practice, it is necessary to have data on in vitro activity obtained in human tissue homogenates together with pharmacokinetic data in blood and preferably in human tissue. In such conditions, calculation of the theoretical effective dose for clinical treatment can be attempted with the validity of this calculation tested by the reduction of DHT in the prostate and skin of treated patients.

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