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STRUCTURE ACTIVITY RELATIONSHIP STUDY OF KNOWN INHIBITORS OF THE ENZYME 5α-REDUCTASE (5AR)

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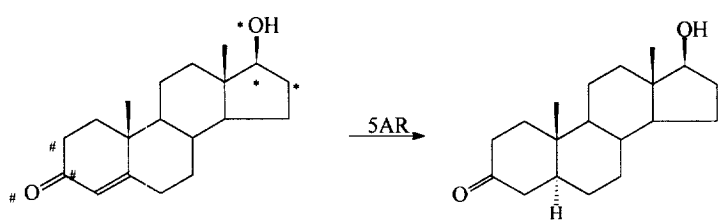
ABSTRACT

Preliminary results of a modelling study of both steroidal and non-steroidal inhibitors of 5α-reductase (5AR) are described in order to elucidate the essential structural requirements needed for the design of novel non-steroidal inhibitors. The study suggests that : (i) there is a requirement for groups to mimic the C(3)=O of the steroid substrate A-ring; (ii) the area of the active site about the C(17)-OH position of the substrate does not appear to possess hydrogen bonding groups and is unrestricted.

The disease state benign prostate hyperplasia (bph) is a benign enlargement of the prostate gland caused by augmented levels of the androgen dihydrotestosterone (DHT). In the treatment of bph, extensive research has been undertaken to produce compounds which are both potent and selective inhibitors of 5α-reductase (5AR), the enzyme responsible for the conversion of testosterone (T) to the more potent androgen DHT (Figure 1). Interest in the design and synthesis of 5AR inhibitors for prostate cancer has recently increased. This stems from the discovery that prostatic growth is regulated through a series of events which are initiated with the binding of DHT to the androgen receptor in the cytoplasm of the prostatic cell.



Testosterone Dihydrotestosterone

Figure 1. Reaction catalysed by 5AR.

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To date, derivatives of 4- and 6-azasteroids have proved to be potent inhibitors of 5AR[1], one example being 17β-N,N-diethylcarbamoyl-4-methyl-4aza-5α-androstan-3-one (4-MA), which is believed to behave as a transition-state inhibitor[2]. Although extensive structure-activity studies into steroidal inhibitors have been undertaken[3], research into novel non-steroidal inhibitors is still in its early stages, but may benefit from a clearer understanding of the active sites of 5AR. Here, we report the initial results of a structure activity relationship study, involving molecular modelling, undertaken to determine the essential structural requirements of inhibitors through the superimpositioning of a number of steroidal and non-steroidal inhibitors onto T (a full report of the study, together with the inhibitory activity data for novel non-steroidal inhibitors, will be submitted elsewhere). It should be noted that within this report, we have not attempted to clarify differences between Types I and II isozymes of 5AR (studies are underway in an attempt to use molecular modelling techniques to determine the existence of any differences).

The structures of T, 4-MA, sodium 4-methyl-3-oxo-4-aza-5α-pregnane-20(*S*)-carboxylate (4-MAPC), N-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (Finasteride, MK-906), 4-[2-(4-pentyl-βmethyl cinnamoylamino)-phenoxy] butanoic acid, 4-[2'-[4"-(4'"-isobutyl phenyl methoxy) benzoyl amino] phenoxy] butanoic acid, 4-[2'-(4"-pentyl-βmethyl cinnamoylamino)-phenoxy] butanoic acid, 6-decyl-5,6-dihydro-2H-pyran-2-one and some benzoquinolinone and indole acid based compounds (Figure 2) were all constructed within the Alchemy III[4] molecular modelling software on a P100 Intel microprocessor based IBM PC compatible microcomputer. The completed structures were then subjected to an initial minimisation using the conjugate-gradient algorithm within Alchemy III until the gradient fell below 10^{-6} resulting, in general, in 500 or more iterations per structure. Conformational analysis was performed (using the systematic search method with energy windows of 20-40 kcal mol^{-1} and bond rotation between 20-50°) on flexible parts of the inhibitors using Powersearch[4] in order to determine the low energy conformers. On the assumption that the shape of T would reflect the nature of the binding site of 5AR, the lowest energy conformer of the inhibitors were superimposed by specification of three or more points on both the assumed binding ring of the inhibitor and ring A or ring D of T, using the polar groups in the fitting process (each pair of three or more points is highlighted using * or # on T, Figure 1).

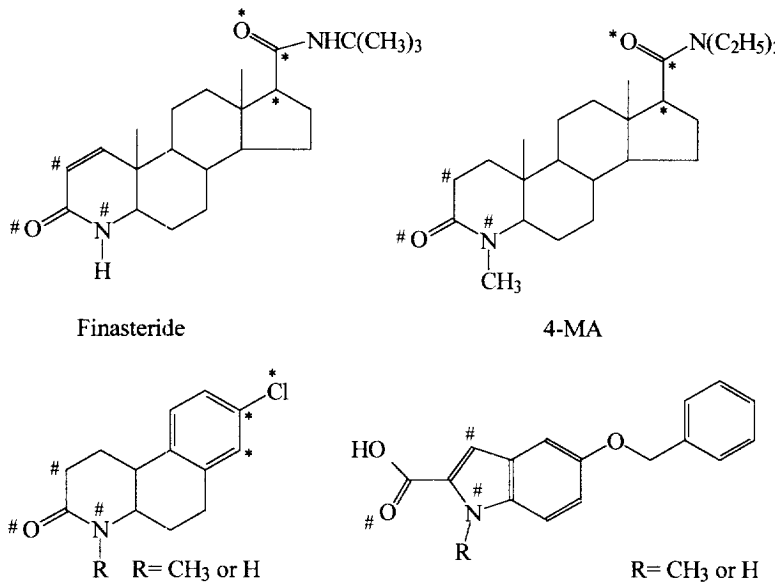

Figure 2. Some of the inhibitors of 5AR considered within the present study.

When the steroidal inhibitors were superimposed onto T using the C(17)-OH group of T (shown by '*' in Figure 1) and the carbonyl group-containing side chain of the inhibitor (Figure 2), it was discovered that the positions of the C(5) moiety of the different inhibitors did not correspond well [for example, Finasteride (IC_{50}=4.2nm for type I) in Figure 3]. Using the C(3) carbonyl of the inhibitors and the substrate however (shown by '#' in Figures 1 and 2), the C(5) position of all the inhibitors were discovered to closely approach the C(4), C(5), C(6) area of T, all within 0.5Å (for example, Finasteride in Figure 4). We therefore conclude that any steroid-derived structure, possessing a C(3) polar group, should possess 5AR inhibitory activity, other factors then determining the extent of inhibition. The validity of this hypothesis is supported by the recently reported 17α-hydroxylase/17,20-lyase inhibitor, 17-(3'-pyridyl) androsta-4,16-dien-3-one, which has been shown to be a good inhibitor of 5AR[5] (IC_{50}=10μM) [we postulate that this compound, lacking the 4-aza, is not a transition state inhibitor but acts as a competitive inhibitor possibly resulting in the reduction of the Δ5 moiety to the 5α-dihydro product; this is also presumed to be true for steroidal inhibitors which lack the 4 or 6 aza-grouping but contain Δ4 or Δ5 C=C bonds].

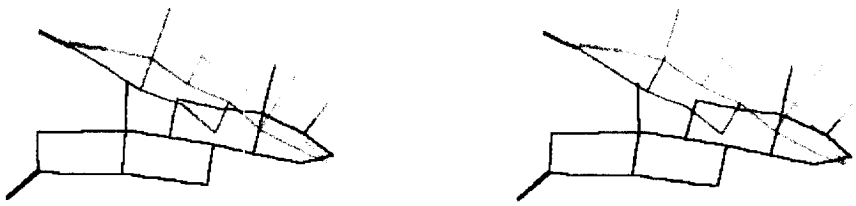

Figure 3. Finasteride superimposed onto substrate using C(17)-OH of T.

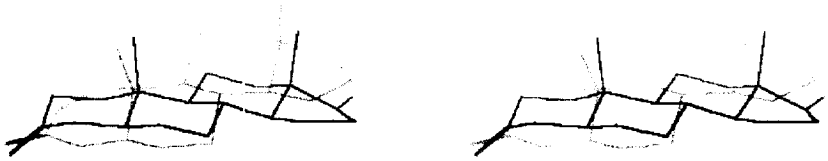

Figure 4. Finasteride superimposed onto substrate using C(3)=O of T.

From the consideration of the numerous steroidal inhibitors of this enzyme and in particular the observed activity of 17-(3'-pyridyl) androsta-4,16-dien-3-one, we hypothesise that the C(17) hydroxyl group is not essential for inhibition. This is further supported by potent steroidal inhibitors where the D-ring has been altered to a six-membered lactam[6]. Furthermore, it can be observed that when the C(3)=O group is used to fit the steroidal inhibitors onto T, the volume of space occupied by the low energy conformers of the C(17) side group of the inhibitors is extensive (for example, Figure 5 shows the volume of space occupied by the low energy conformers of 4-MA). We therefore postulate from these observations that hydrogen bonding groups, expected to bind to the C(17)-OH of T, may be absent from this area of the active site and that due to the extensive volume of space available to the C(17) side arm of the steroidal inhibitor, the entry/exit to the 5AR active site exists about this position. Furthermore, it is believed that the increased inhibitory activity associated with increasing side chains[1] is related to logP, as opposed to binding to the active site via hydrogen bond formation. This hypothesis is supported by the inhibitory data[3] in Table 1.

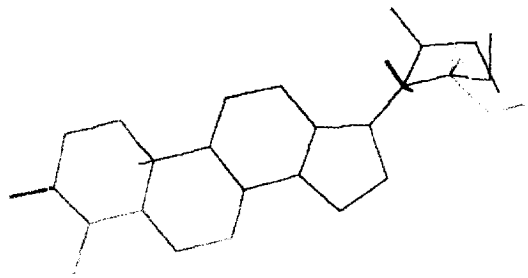

Figure 5. Low energy conformers of the C(17) side chain of 4-MA.

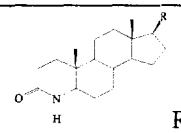 R =	rat 5AR ($IC_{50}x10^{-8}M$)	Calculated LogP
$CON(C_2H_5)_2$	4.1	3.91
$CONHC(CH_3)_3$	0.63	4.55
$CONH(CH_2)_7CH_3$	0.013	5.64

Table 1. Reported inhibitory data for some derivatives of 4-MA.

Consideration of the non-steroidal inhibitors, such as those based on the benzoquinolinones[7] (Figure 6) or the indole acid[8] (Figure 7) show that these types of compound also appear to utilise the steroid substrate C(3)=O binding region (Figure 7) and show a poor fit with respect to the C(17) hydroxy group, further supporting the previous hypothesis regarding the lack of hydrogen bonding groups at the active site corresponding to the steroid C(17) position [it is to be noted that groups such as Cl of, for example benzoquinolinone inhibitors, do not undergo hydrogen bonding but undergo a polar-polar interaction]. Also, compounds such as 4-[2'-(4"-pentyl-βmethyl cinnamoylamino)-phenoxy] butanoic acid and, in particular, their conformers, support the earlier hypothesis that the volume about the C(17) area of T appears to be unlimited.

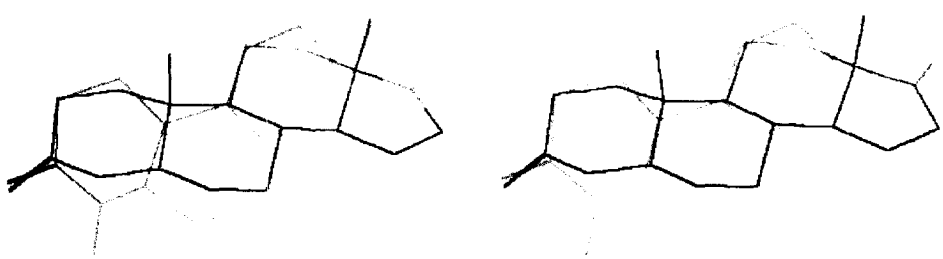

Figure 6. Benzoquinolinone based inhibitor superimposed onto T using C(3)=O.

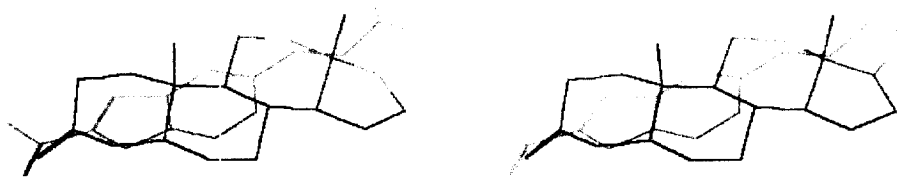

Figure 7. Indole acid based inhibitor superimposed onto T using C(3)=O.

In conclusion, this preliminary study of the inhibitors of 5AR has allowed us to hypothesise that : (i) there is a requirement for groups to mimic the steroid substrate A-ring, in particular the C(3)=O; (ii) the area of the active site about the C(17)-OH of the substrate does not appear to possess hydrogen bonding groups, and; (iii) the volume of space available to groups about the steroid C(17) position is not restricted, suggesting that the exit/entry into the active site may exist about this area.

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