Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2615–2620

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

MECHANISM BASED REPRESENTATION OF THE ACTIVE SITE OF 5α-REDUCTASE (5AR)

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Received 25 September 1997; accepted 13 August 1998

ABSTRACT

In the present study, we have attempted to determine a detailed representation of the 5α-Reductase (5AR) active site involving the elucidation of the transition state for the steroid Δ4 reduction reaction (the 'NADPH-substrate' complex), onto which steroidal and non-steroidal inhibitors were superimposed. We conclude that : (i) there is a requirement for groups to mimic the steroid substrate A-ring; (ii) the area about C(3), C(4), C(5) and C(6) of T appears to be sterically hindered, and; (iii) the area of the active site about the C(17) of the steroid substrate does not possess hydrogen bonding groups and is not restricted.

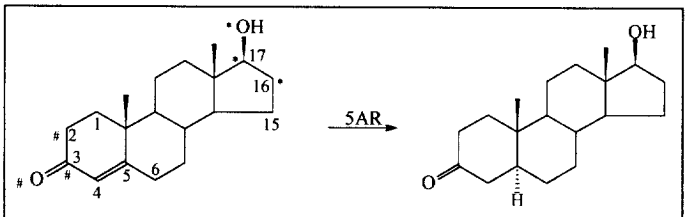

Figure 1. Reaction catalyzed by 5AR.

The disease state benign prostate hyperplasia (bph) is a benign enlargement of the prostate gland caused by augmented levels of dihydrotestosterone (DHT), the more potent androgen. In the treatment of bph and other androgen related conditions (e.g. acne), 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 DHT (Figure 1).

To date, derivatives of 4- and 6-azasteroids have proved to be potent inhibitors of 5AR, for example, 17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA) and N-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (Finasteride, MK-906), both of which are believed to behave as 'transition-state inhibitors'[1] and have provided useful information regarding the probable mechanism of 5AR[2]. Research into novel non-steroidal inhibitors is, however, still in its early stages, but may benefit from a clearer understanding of the active sites of this membrane-bound and non-isolable enzyme. Molecular modelling of both steroidal and non-steroidal inhibitors, and more specifically the determination

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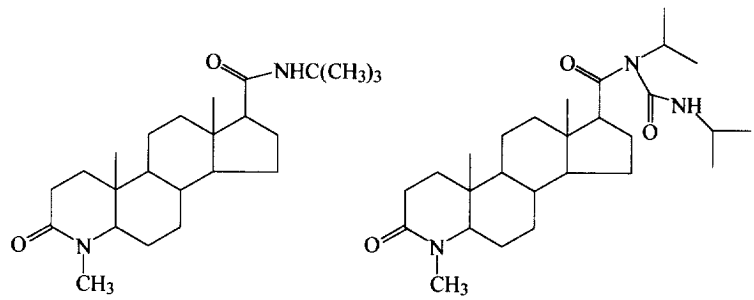
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00463-6

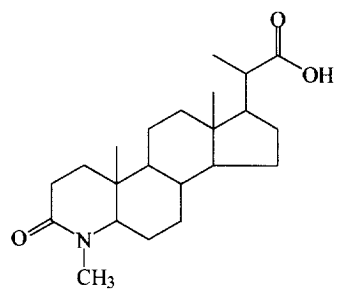
of the transition-state, offers a route by which further information may be deduced. Here, we report the elucidation of a representation of the active site from a consideration of the proposed reaction mechanism[2]. The structures of T, partial NADPH structure, 4-MA, sodium 4-methyl-3-oxo-4-aza-5α-pregnane-20(*S*)-carboxylate(4-MAPC), N-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (MK-906), 17-(3'-pyridyl)androsta-4,16-dien-3-one, 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 CACHE[3] molecular modelling software suite on a P100 Intel microprocessor based IBM PC compatible microcomputer. The completed structures were then refined performing a pre-optimisation calculation in mechanics using augmented MM2, followed by a geometry optimisation in Mopac using PM3 parameters. In order to determine the transition state, the hydrogen of the pyridine ring of NADPH was attached to the C(5) carbon of T, via a weak bond. The remainder of the groups were also attached: hydrogen bonding carboxyl group to the C(3) carbonyl group, and a hydrogen donor to the β position of C(4). The initial structure was minimised using the MM2 and Mopac/PM3 routines as previously described (reactant file) - it should be noted that partial deletion of the NADPH molecule was required so as to allow calculations within Mopac atom limits. A 'product file' was also created (involving the conversion of the NADPH moiety to NAD^+) and the structures minimised as previously described. Using routines within CACHE, the saddle point for the reaction was computed. The resulting transition-state structure was then refined by performing a minimise gradient calculation using Mopac and PM3 parameters[4] following which the molecule's vibrational transitions were calculated (once again with Mopac using PM3 parameters) in order to 'verify' the TS. A single negative vibration was indeed observed upon viewing the molecule file (Figure 3) - the figure obtained -239.51cm^{-1} (six other small frequencies were also obtained : -18.36, -8.48, -1.05, 6.43, 11.04, 17.62 cm^{-1}). Conformational analysis of the inhibitors was performed (with a sequence of conformers generated by sequentially searching a number of geometry labels, calculations being performed in mechanics using augmented MM2 parameters) on flexible parts of the inhibitors in order to determine probable low energy conformers (Figure 4 shows the low energy conformers obtained for 4-MA superimposed onto the transition-state). In order to obtain a measure of the fit, the inhibitors and the transition-state were read into Alchemy III[5] molecular modelling software for the superimpositioning study involving the 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 are highlighted using * or # on T, Figure 1).

Transition-state (TS) of 5AR : Consideration of the transition-state shows that the NADPH moiety is positioned close to the C(4) position of the steroidal backbone such that the C(4) is hindered - the nearest NADPH atom to steroid C(4) being 3.1Å (Figure 3). This observation is consistent with experimental data[6] which shows that aza-steroid derivatives containing groups larger than methyl result in poorer inhibition. We therefore propose that these larger groups are involved in steric interaction with the NADPH molecule leading to destabilisation of the enzyme-inhibitor complex - Figure 5 shows one of the low energy conformers of the propyl derivative of 4-MA superimposed onto the transition-state and the resulting interaction between the NADPH moiety of the transition-state and the inhibitor.

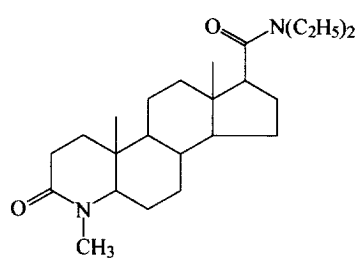


Finasteride

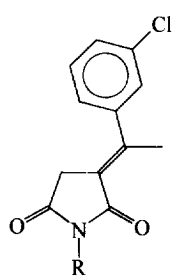
Turosteride (FCE 26073)



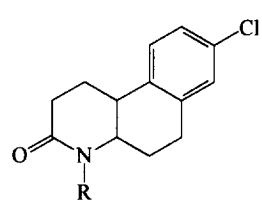
4-MAPC



4-MA



Pyrrolidine-2,5-dione based inhibitors
R = H or 2,4-dichlorophenyl.



Benzoquinolinones based inhibitors

Figure 2. Some steroidal and non-steroidal inhibitors of 5AR.

Superimposition of Inhibitors onto the transition-state : The data and observations of the present study support our previous report. For example, when the steroidal inhibitors (such as, 4-MA) were superimposed onto T utilising the C(17)-OH group of T and the carbonyl group-containing side chain of the inhibitor, it was found that the positions of the C(5) of the different inhibitors did not correspond well. Using the C(3) carbonyl of the inhibitors and the substrate, however, the C(5) positions of all the inhibitors were found to closely approach the C(4), C(5), C(6) area of T, all within 0.5Å (for example, 4MA in Figure 4).

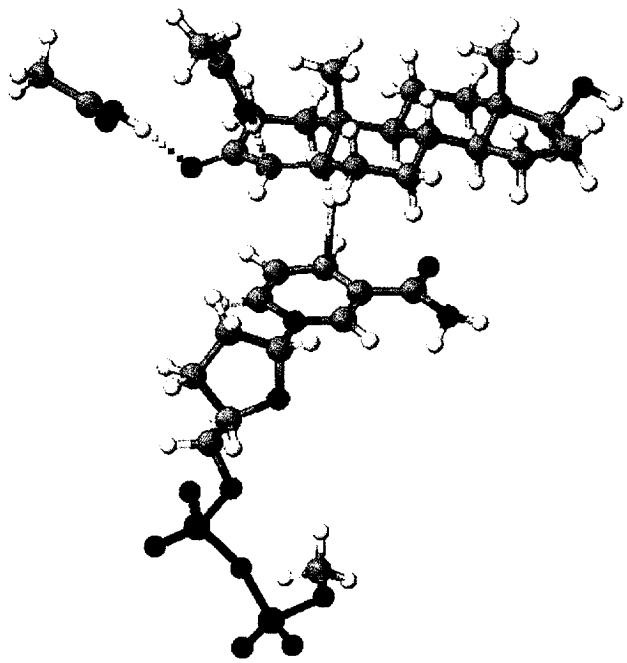


Figure 3. Derived transition-state for the reductase reaction undertaken by 5AR.

In our earlier report[7], we also hypothesised the lack of hydrogen bonding groups which could interact with the C(17)-β-OH group of the substrate. From consideration of the results of the superimpositioning of the low energy conformers of, for example FCE26073, we observe that the conformers closely approach the C(17)-β-OH hydrogen bonding group (resulting in nearest NADPH atom to steroid atom distances of 1.3Å). With compounds possessing larger groups, we find that these groups take up conformations which would, in effect, interact with any hydrogen atoms of the NADPH moiety. As such, we constructed an alternative transition-state with a hydrogen bonding group attached to the substrate's C(17) hydroxy group. We discovered that the side chain of the FCE26073 inhibitor is involved in steric interaction with

the hypothesised hydrogen bonding group at the active site. Consideration of alternative inhibitors possessing bulky side chains show similar results. This observation is consistent with results from the other steroidal inhibitors with large flexible groups about this area of the inhibitor. This observation would therefore appear to support our previous hypothesis[7] that the area of the active site corresponding to the C(17) of the substrate is extensive and that there is a lack of hydrogen bonding groups which could undergo interaction with the C(17)-β-OH group of the substrate.

Consideration of the non-steroidal inhibitors, such as the benzoquinolinones[8] (Figure 5) or the recently reported novel pyrrolidine-2,5-dione based compounds[8], show that these also appear to utilise the steroid substrate C(3) carbonyl binding region (Figure 7). Indeed, the results with the non-steroidal inhibitors are consistent with those obtained for the steroidal inhibitors and those previously reported[7].

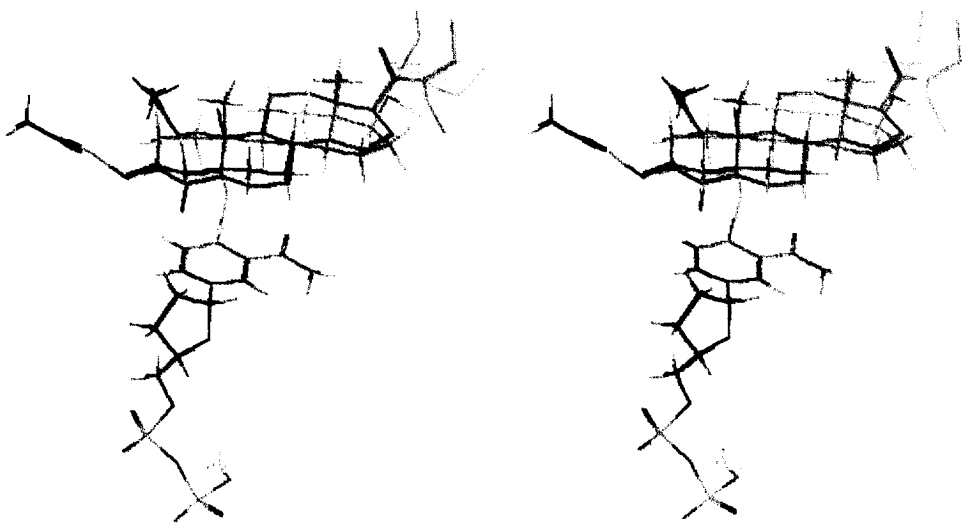


Figure 4. Low energy conformers of 4-MA (in different colour) superimposed onto the transition-state.

In conclusion, the representation of the 5AR active site derived from the consideration of the proposed reaction mechanism has allowed us to successfully rationalise the inhibitory data of a number of compounds.

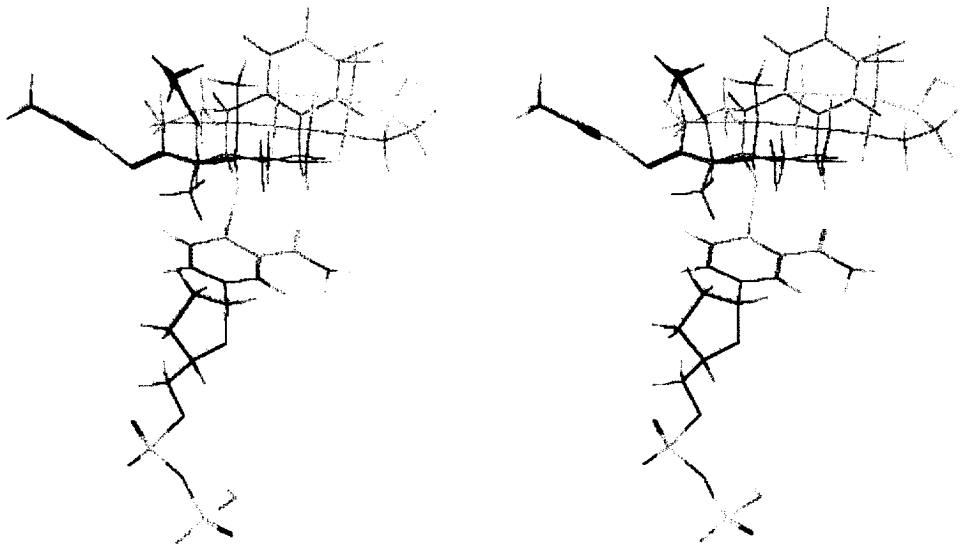


Figure 5. Benzoquinolinone based inhibitor superimposed onto the transition-state.

ACKNOWLEDGEMENTS : The authors would like to thank Kingston University for funding SD.

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