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ACTIVE-SITE CONFORMATION OF 17-(3-PYRIDYL) ANDROSTA-5,16-DIEN-3β-OL, A POTENT INHIBITOR OF THE P450 ENZYME C17α-HYDROXYLASE/C17-20 LYASE

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Abstract:
17-(3-pyridyl)androsta-5,16-dien-3β-ol, a nanomolar inhibitor of the P450 enzyme C17α-hydroxylase/C17-20 lyase, is a target for prostate cancer chemotherapy. A model is presented for the inhibitor docked into the structure of the enzyme.

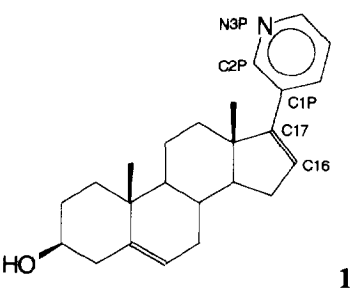
Prostate cancer is the second most prevalent cancer in men in the UK. Some 80% of patients respond to androgen deprivation, reflecting a requirement for circulating testosterone for their growth. Considerable attention has been given to the development of drugs active against prostate cancer that intervene in the processes of tumour growth especially in the pathways of steroid metabolism leading to testosterone[1].

The biosynthesis of the androgenic steroids such as testosterone involves two key reactions, which occur sequentially. The first is 17α-hydroxylation of pregnenolone and progesterone, the second is the cleavage of the C17-C20 bond resulting in formation of the C17 carbonyl function. Both reactions are catalysed by a single enzyme, the cytochrome P450 monooxygenase C17α-hydroxylase/C17-20 lyase[2-4]. There is currently no structural information on this enzyme (or indeed any eukaryotic P450), although we have developed a structural model for it based on the X-ray structure of $P450_{cam}$ from *Pseudomonas putida* by means of homology modelling

techniques[5]. The active site of the enzyme contains a hydrophobic region and a haem group, in common with that of all P450 enzymes.

A number of inhibitors of C17α-hydroxylase/C17-20 lyase have been developed[6-8]. We have used molecular modelling methods to examine structure-activity relationships in one such series, those based on pyridyl esters[9]. It has been shown that an extended conformation of the active esters is required, for the compounds to resemble the conformation of the natural steroid substrate. It was assumed in this work that the pyridyl ring nitrogen atom directly coordinated to the haem group.

A second inhibitor containing a pyridyl residue has also been developed[10] in which the steroidal skeleton is substituted at the 17-position by a 3-pyridyl group (whose nitrogen atom is presumed to coordinate to the haem Fe atom in the active site of the enzyme).



1

This compound, **1**, has exceptionally high activity against the human 17α-hydroxylase/17-20 lyase enzyme, with an IC_{50} value of ~1nM. It has recently been approved for Phase I clinical trials in the UK. The conformational features of **1** are important for understanding its modes of interaction with the enzyme; they have been examined here by a combined crystallographic and molecular modelling approach.

Methods

Colourless prismatic crystals of **1** were obtained from ethanolic solution. Intensity data were collected on the diffractometer, using a rotating-anode source. The structure was solved by direct methods and refined by full-matrix least-squares[11,12] to a final R factor of 0.037. Coordinates and derived geometric parameters have been deposited at the Cambridge Crystallographic Data Centre.

All molecular mechanics calculations were performed with the MM2P(85) computer program[13], running on an Alliant FX40/3 computer. Quantum-mechanical calculations

were run on the same machine using the AMPAC package (QCPE No. 506). All molecular dynamics calculations were performed using the AMBER suite of programs[14]. Computerised model-building and visualisations were carried out with locally-written programs and the MIDAS package[15], running on Silicon Graphics workstations.

A three-dimensional structure for **1** was built by computerised modelling. This was subjected to full minimisation using the force-field parameters within MM2P(85). Atom-centred point charges for the molecule in the minimised conformation, were then calculated with AMPAC. Rotations about the bond linking the pyridyl ring and the steroid (C17-C1P) were performed with the MM2P(85) program, between 0 and 360° in increments of 10°.

A procedure involving initial hand-docking, molecular mechanics minimisation and simulated annealing was then used to dock **1** into the active site of a model for the hydroxylase enzyme (this model, which was originally derived[5] from the known crystal structure of the bacterial $P450_{cam}$ enzyme, has been subsequently been refined by us taking into account the more recently-determined[16] crystal structure of $P450_{BM-3}$, which is functionally closer to eukaryotic P450s than is $P450_{cam}$). All atoms of **1** were allowed to move throughout the simulation, keeping the active site residues fixed. The inhibitor/protein complex was allowed to equilibrate at 3000°K for 10ps and then cooled to 0°K over a further 20ps[17]. Full minimisation was then performed with all residues within 9Å of the active site being allowed to move.

Results and Discussion

The crystal structure of **1** is shown in Fig 1, with its absolute configuration as defined by the chemical route to **1**. Rings A, B and C of the steroid all adopt chair-like conformations, and ring D has an envelope shape. The methyl substituents at C10 and C13 are both on the β face of the steroid, as is the ring nitrogen atom N3P of the pyridine ring. The torsion angle around the C17-C1P bond (defined by the atoms C16-C17-C1P-C2P) connecting the pyridine ring to the D ring, is 208.3(3)°.

The molecular mechanics-minimised structure has a rms deviation of 0.15Å compared to the crystal structure (with hydrogen atoms included); the C17-C1P torsion angle in the minimised structure is 207.2°. The energy profile for rotation

about the C17-C1P bond is shown in Fig 2, with the inclusion of electrostatic contributions to the total force-field energy.

The plot has two equi-energetic local minima, at 27° and 207° for the C17-C1P

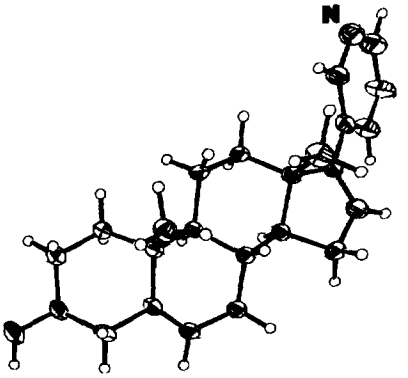


Fig 1. ORTEP plot of the molecular structure of **1**.

torsion angle, with an energy barrier of ~2.5 kcal mol^{-1} between them. The fact that these two minima are separated by 180° and are equi-energetic, show that the potential asymmetry introduced by the presence of the pyridine nitrogen atom has no effect on them; the nitrogen atom is beyond the influence of, in particular, the angular methyl group at C13. The crystal packing of **1** involves a hydrogen bond between the pyridine ring nitrogen atom N3P and the hydroxyl group (O3), of length 2.963(3)Å. Presumably it is this hydrogen bond which stabilises one conformation over the other in the crystal lattice.

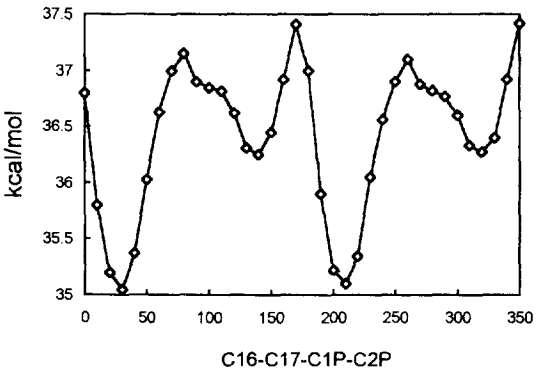


Fig 2. Energy profile for **1** with respect to rotation about the C17-C1P bond, in °

The minimised structure of **1**, docked into the active site model of the enzyme is shown in Fig 3. The torsion angle around the C17-C1P bond is 223.3°. This is close to one of the two minimum energy conformations, and is thus rotated by ~180° from its conformation in the crystal. The rest of the steroid is kept fairly tightly in place in the binding site due to numerous non-bonded interactions with the active site residues. The most important interactions are with residues Arg65, Pro66, Gln67, Met68, Thr70, Phe83, Thr275, Met 338, Leu339, Ile340 and Val451. This orientation for the steroid **1** is in principle an appropriate position for C17α hydroxylation to occur.

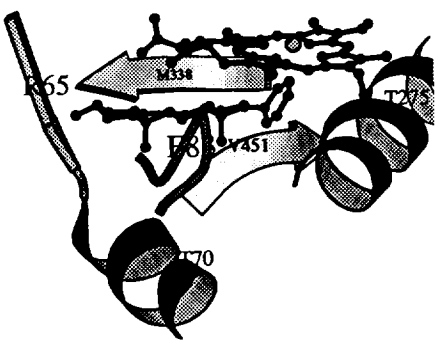


Fig 3. Plot of **1** bound within the active site of the enzyme, drawn with the MOLSCRIPT program[18]. The pyridine ring of **1** is seen close to the lower face of the haem group.

It was found that **1** would not readily dock into the "lyase" lobe of the enzyme, where rings A to D of the steroid would have to be perpendicular to the heme. This is due to unfavourable interactions with residues at the distal end of this lobe. This seems to suggest only one mode of binding, but it could reflect inaccuracies in the model itself or, less likely in the docking procedure; since the active site residues were held rigid during the annealing stage, alternative sidechain conformations were not exhaustedly sampled.

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