# Structure-based design and analysis of cytochrome P450 inhibitors for the treatment of prostate cancer

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**Abstract** - *Structure-based drug design approach is used to design and analyze cytochrome P450 inhibitors for the treatment of prostate cancer. The structural efficiency of inhibitors is measured by the ability of the designed inhibitors to form interactions with atoms in the active site of cytochrome P450 that includes a heme group. Docking studies are conducted to analyze the structural effectiveness of inhibitors available in the literature. We also present novel inhibitors and analyze their structural characteristics. The drug compounds in two different groups, steroidal and steroidomimetic inhibitors. It is shown with in-silico tests that steroidomimetic compounds show stronger binding interactions compared to steroidal compounds.*

**Keywords:** cytochrome P450, prostate cancer, drug design

## 1 Introduction

Prostate cancer is the most common malignancy and age-related cause of cancer deaths among male worldwide.[1] Androgen is a major growth factor in the normal prostate and determines the overall number of prostate cells. A number of studies indicate a correlation between serum testosterone levels and increased risk of prostate cancer.[2] Furthermore, about 90% of patients respond to androgen deprivation, reflecting a requirement of circulating testosterone for their growth.[3] The two most important androgens in this regard are testosterone and dihydrotestosterone. The testes synthesize about 90% of testosterone, and the rest (10%) is synthesized by the adrenal glands. Testosterone is further converted to the more potent androgen dihydrotestosterone by the enzyme steroid 5R-reductase that is localized primarily in the prostate. In the testes and adrenal glands, the last step in the biosynthesis of testosterone involves two key reactions, which act sequentially, and they are both catalyzed by a single enzyme, the cytochrome P450 monooxygenase 17αhydroxylase/17,20-lyase (CYP17). It catalyzes the hydroxylation of progesterone and pregnenolone into the corresponding 17 α-products, as well as the cleavage of the $C_{17}$-$C_{20}$ bond to yield androstenedione and dehydro-epiandrosterone (DHEA). These steroids are weak androgens which subsequently are converted by other enzymes (17β-HSD, 3β-HSD, 5α-reductase) to the most potent androgens testosterone and dehydro-testosterone. A promising alternative to treatment with antiandrogens and GnRH (gonadropin releasing hormone) analogues might be the use of inhibitors of CYP 17[1-5] as androgens have been implicated in the development and progression of prostatic cancer. For that reason CYP 17 attracted attention as a therapeutic for the development of nonsteroidal inhibitors an iron complexing group.

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CYP17 is a microsomal enzyme and encoded by a single gene[4] that is located at 10q24.3. This locus has not been previously reported in hereditary prostate cancer families.[5] CYP17 is found in the mammalian adrenal[6, 7], the testes[6, 8-10], and ovarian theca tissue[11-14]. Enzyme functions in the biosynthesis of testosterone through two catalytic activities[15]: Steroid 17α-hydroxylation in which an oxygen is inserted into $C_{17}$-H bond; and 17,20-lyase activity, in which 21-carbon 17 α-hydroxysteroids are cleaved to 19-carbon, 17-ketosteroids.[16–19] $\Delta^5$-pregnenolone and $\Delta^4$-progesterone are good substrates for the 17α-hydroxylase reaction, but $\Delta^5$ 17-OH-pregnenolone is preferred over $\Delta^4$-17-OH-progesterone for the 17,20-lyase reaction.[20]

Enzyme structure is crucial for understanding the catalytic activities, substrate and reaction selectivity. Therefore, knowing the structure of CYP17 is mandatory for designing specific drugs to inhibit the catalytic activities of the enzyme. Although a crystal structure of CYP17 has not been reported in the literature or databases, a computer generated model exists as shown in Fig. 1 with PDB ID code 2c17.[21]

There are also other models for cytochrome P450 available in the literature. Laughton *et al.*[22] also build a model for CYP17, and Lin *et al.*[23] modeled the active site of the protein. Both of these models were based on the crystal structure of P450cam, a class I P450. Lin *et al.* defines a bi-lobed substrate binding pocket.[23] These studies were based on the crystal structures of class I P450s, those that use ferrodoxin intermediate as electron donor. However; class II (microsomal) P450s, like CYP17, modeled using class I P450s as templates have been suboptimal.[23] Therefore, the more recent model based on a class II P450 crystal structure, P450BMP, will be used in this study.

The mono-lobed substrate binding pocket is defined with heme group, as the floor of the pocket; residues I288 to H321 which makes the I-helix and stands on one side of heme group; residues P368 to K374 and K91 to G95 forming the 4th and 5th strands of β-sheet 1, standing opposite the I-helix; I112; residues 365 to 367 that makes a loop after

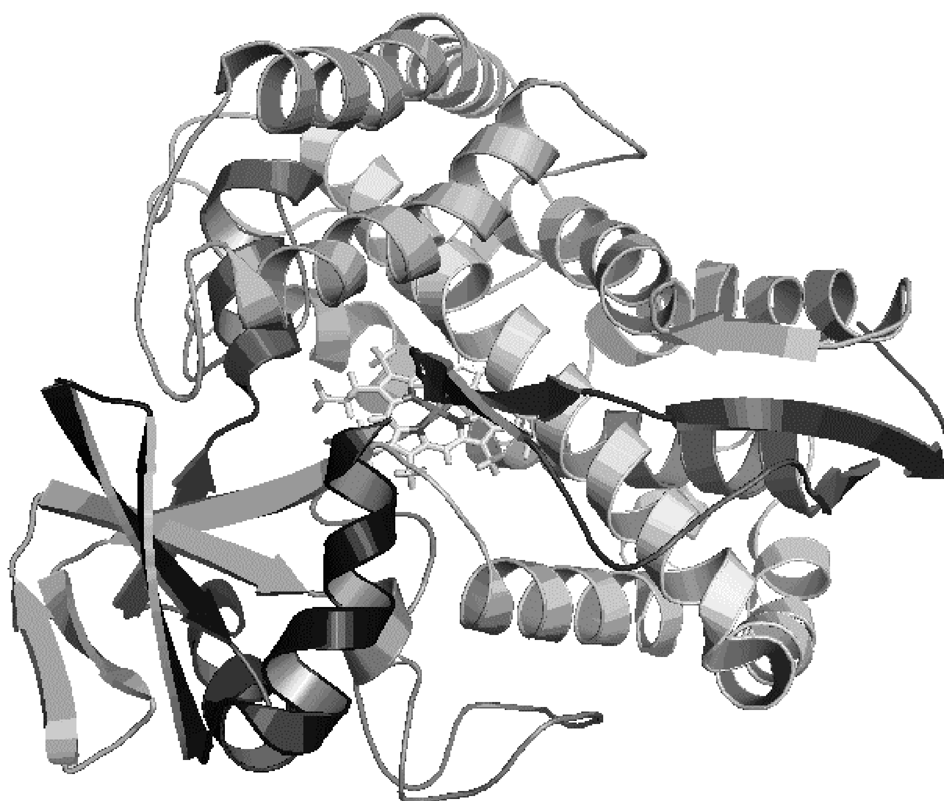

*Fig. 1 Computer generated model for cytochrome P450 with PDB access code, 2c17.*

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K-Helix; and finally V482 and V483 making up the top portion of the pocket. In this model, $C_{17}$ of substrate steroid molecule would be above heme iron. Residues P368, V482, V483 and A302 would have hydrophobic interactions with $C_{18}$ and $C_{19}$. Oxygen on $C_3$ of steroids would be making hydrogen bonds with residue G95. More specifically, $\Delta^4$ (3 keto) steroids forms hydrogen bond with amide hydrogen of G95, and $\Delta^5$ (3β-hydroxy) steroids form a hydrogen bond with the carbonyl oxygen, on MD simulations. All these residues form a smaller substrate binding pocket than the previous models, letting only the planar substrates like steroids to accommodate. Catalytic activity of the enzyme relies on a protein donor, residue T306. The hydroxyl group of this residue is placed across the heme ring from the D ring of steroid substrate. The place of T306 is homologous to other P450 crystal structures, and the hydroxyl proton at this location enables 17α-hydroxylase reaction by taking role in dioxygen protonation and O-O bond cleavage.[21] The active site is shown in Fig.2.

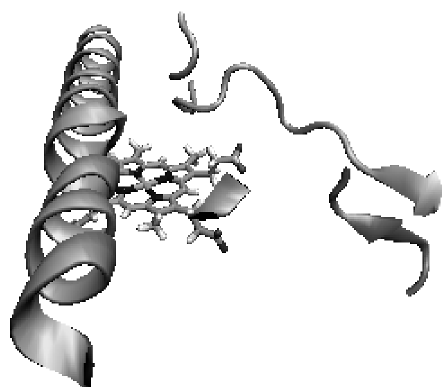

*Fig. 2 Graphical representation of the substrate- binding pocket of 2c17.*

## 2 Materials and Methods

The drug design problem has been addressed by experimental and computational methods. The experimental methods concentrate on high throughput screening methods; it is possible to test about 1 million chemical substances for interaction with a protein active site using robotics integrated systems and combinatorial libraries. The main difficulties with the experimental methods include: small number of available chemical substances for testing, high experiment costs, and the possibility that the chemical substance may be interacting with another active site of the target protein. Computational simulation methods test chemical structures stored in databases for binding to the active site of the protein. These methods are based on testing for activity on the target protein. The drawbacks of this method include: availability of only a very small number of chemical structures in databases for evaluation (databases contain only a very small fraction of vast number of possible molecular structures), databases often do not contain suitable molecules for the target protein, and the availability of the same database to every database user. Therefore, it is necessary to develop new strategies for drug discovery and design that will overcome the drawbacks of the traditional methods.

This paper is based on the idea that compounds providing a number of specific interactions with the backbone atoms and side chain atoms at the active site of the protein can be designed using structure-based drug design techniques. Structure-based drug design has been accepted as an established approach in pharmaceutical industry and academia.[24] One of the important requirements in the structure-based drug design is the structural analysis of the active site of the protein that is responsible from the onset and progress of the disease of interest.[25] The main steps of the design method used in this paper is schematically represented in Fig.3.

### 2.1 Target Selection and Protein Structure

Selection of a target for the treatment of a disease is one of the important steps in designing drugs to prevent or control the progress of a disease. Androgen is a major growth factor in the normal prostate and determines the overall number of prostate cells. As discussed in introduction, CYP 17 has a key role in androgen synthesis. Therefore, this enzyme is selected as the target protein to inhibit androgen synthesis.

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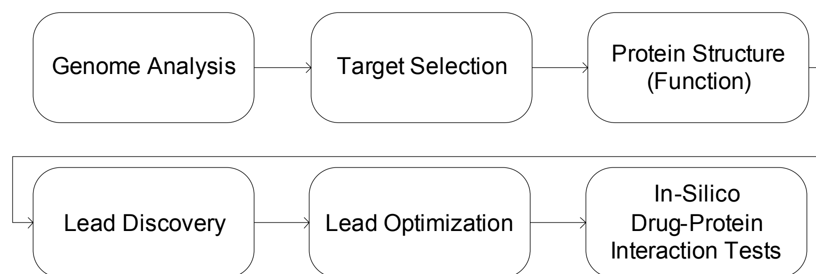


*Fig. 3 Schematic representation of the steps in drug design and analysis method used.*

The three dimensional structure of the cytochrome P450 was determined computationally and is available in PDB with ID code 2c17.[21] The binding site of the cytochrome P450 is defined with heme group, as the floor of the pocket; and surrounding residues. The three dimensional structure obtained from PDB is neutralized with the addition of a $Cl^-$ ion and solvated with water. Then, the complete system is minimized to eliminate as many poor contacts as possible. The MD simulations are carried out with CHARMM[26] force field parameters. In the MD simulations, NVT ensemble and periodic boundary conditions with a rectangular box are applied. The temperature of the simulations was kept constant at 300 K by using Langevin Dynamics. The time step is 2 fs and trajectories are sampled at 40-ps intervals. Initial minimization is done for 10,000 steps, followed by ~2-ns simulation runs. The results of MD simulations indicate that a stable configuration of the system at physiological conditions is obtained after 30 time steps as shown in Fig.4. RMSD, root mean square deviation in the coordinates of all atoms in the protein, is used to decide whether protein has a stable configuration or not.

## 2.2 Lead Discovery and Optimization

The next step in the drug discovery process is to identify drug candidates that will interact with the binding site of the target protein. In general, a large number of chemical substances that may be suitable drugs are tested on the target protein to observe the effect for interaction. The chemical substances that show any level of interaction are categorized as a hit. The drug candidates interact with the active site to have therapeutic effect by blocking, accelerating, decelerating, reversing or initiating reactions depending on

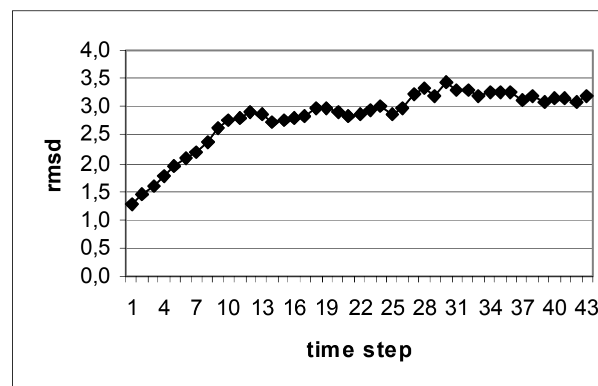


*Fig.4 RMSD results for MD simulations.*

the way to cure the disease. This phase ends up with a list of drug candidates interacting successfully with the target in laboratory conditions.

In this paper, we consider drug compounds available in literature.[27-29] Steroidal inhibitors are widely used in inhibiting proteins with heme group. It was also suggested using steroidomimetics instead of steroidal drugs. Steroidal drugs are likely to interact with some other proteins due to strong affinity of steroidal compounds with one or more steroid receptors including estrogen and gestagen receptor resulting in side effects.[29, 30] We also designed novel inhibitors of cytochrome P450 using the procedure given in Turkay.[31] The compounds that we considered in this study are shown in Fig.5. The Lead Compounds A and B are reported in Hartmann et al.[29] The Lead Compound B is modified by replacing the OH group of the aromatic ring with $NH_2$ to illustrate the change in the binding and docking energies by a structural difference in the drug molecule. MHE001 is designed using the method presented in Turkay.[31] The rest of the compounds shown in Fig. 5 are reported in Clement et al.[27]

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### 2.3 In-Silico Drug-Protein Interaction Tests

The strength of interaction between the drug compounds and the target protein can be analyzed using docking studies. We studied docking of compounds given in Fig. 5 with the binding site of the cytochrome P450 using AutoDock 3.05.[32] AutoDock allows flexible docking of ligand molecules using a grid based approach and incorporates an empirical free energy function that is used to measure the strength of interactions created by inhibitors based on binding free energy. In the preliminary screening of the virtual library, all rotatable bonds of the molecules except for the amide linkages were allowed to rotate and each molecule was evaluated using the Lamarckian Genetic Algorithm (LGA) implementation for 100 runs of maximum 5 million energy evaluations.

## 3 Results and Discussion

The results of the docking calculations are given in Table 1. The drug molecules belonging to set of steroidal and steroidomimetic compounds considered in this paper are ranked according to their binding free energy.

As shown in Table 1, Lead Compound B creates strong interaction with the atoms at the active site of cytochrome P450. Lead Compound interacts with the O atoms of the heme group and atoms in the side chains Ser-94, Gly-95, Ala-115 and Arg-440 as shown in Fig. 6. These interactions give docking energy of -10.55 kcal/mol and a binding energy of -9.93 kcal/mol. These values are the lowest among the compounds studied in this paper.

An interesting result that we are reporting in this paper is the comparison of

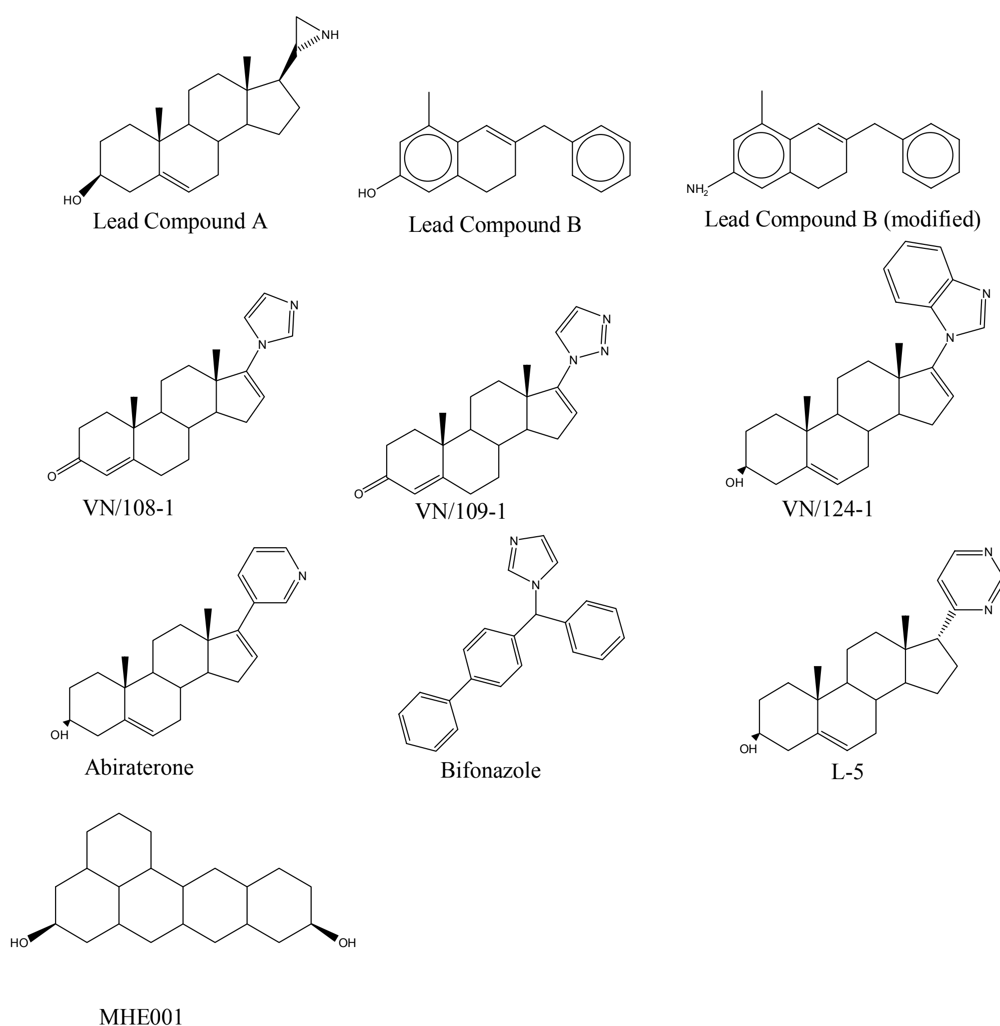


*Fig.5 Drug compounds considered in this paper.*

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*Table 1* Predicted binding free energy values for different drugs.

| | Energies | (kcal/mol) | |
|---|---|---|---|
| **Inhibitors** | **Docking** | **Binding** | **Chemistry** |
| Lead Compound B[29] | -10,55 | -9,93 | Steroidomimetic |
| Lead Compound B (modified) | -9,98 | -9,32 | Steroidomimetic |
| Bifonazole[27] | -8,59 | -8,89 | Steroidomimetic |
| MHE001 | -7,3 | -7,26 | Steroidomimetic |
| Abiraterone[27] | -5,23 | -4,74 | Steroidal |
| Lead Compound A[29] | -4,73 | -4,44 | Steroidal |
| VN/108-1[27] | -3,67 | -3,17 | Steroidal |
| VN/109-1[27] | -3,35 | -2,77 | Steroidal |
| VN/124-1[27] | -1,46 | -1,05 | Steroidal |
| L-5[27] | -0,92 | -1,25 | Steroidal |

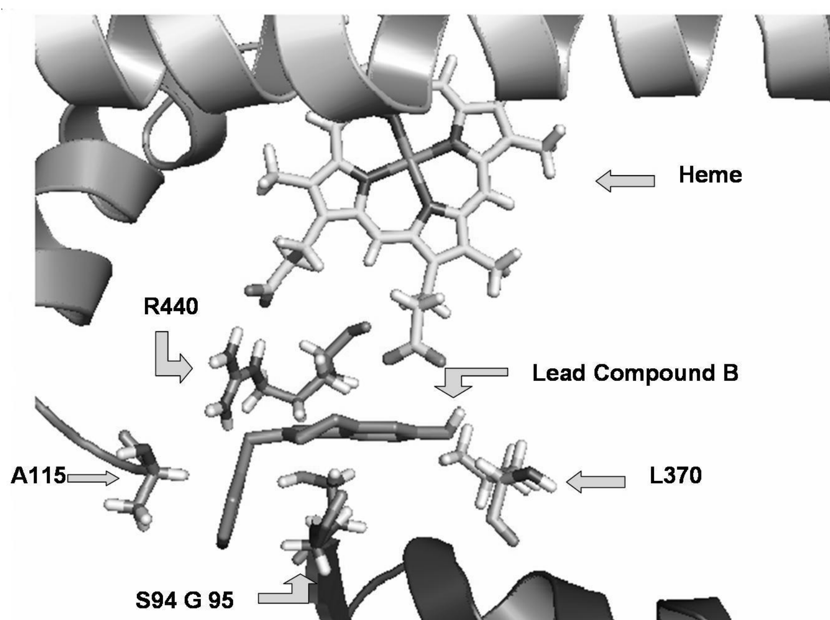


*Fig.6 Minimum energy state for candidate lead compound B obtained using AutoDock 3.05.*[32] *4 amino acids surrounding the candidate molecule (backbone shown in yellow) and heme ring are also shown.*

docking and binding energies for two distinct groups of compounds studied: steroidal and steroidomimetic compounds. It is argued in the literature that steroidomimetics may offer better results compared to steroidal drugs due to interaction of steroidal drugs with some other steroid receptors including estrogen and gestagen receptor resulting in side effects.[29, 30] However, no structural justification of these arguments were provided. It is shown in this paper that all of the steroidomimetic compound we studied categorically have better docking and binding energies compared to steroidal drugs. Our results show that steroidomimetic compounds can be more effective inhibitors of CYP 17 compared to steroidal compounds.

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