Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK–001

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Cytochrome P450 17A1 (also known as CYP17A1 and cytochrome P450c17) catalyses the biosynthesis of androgens in humans. As prostate cancer cells proliferate in response to androgen steroids, CYP17A1 inhibition is a new strategy to prevent androgen synthesis and treat lethal metastatic castration-resistant prostate cancer, but drug development has been hampered by lack of information regarding the structure of CYP17A1. Here we report X-ray crystal structures of CYP17A1, which were obtained in the presence of either abiraterone, a first-in-class steroidal inhibitor recently approved by the US Food and Drug Administration for late-stage prostate cancer, or TOK–001, an inhibitor that is currently undergoing clinical trials. Both of these inhibitors bind the haem iron, forming a 60° angle above the haem plane and packing against the central I helix with the 3β-OH interacting with asparagine 202 in the F helix. Notably, this binding mode differs substantially from those that are predicted by homology models and from steroids in other cytochrome P450 enzymes with known structures, and some features of this binding mode are more similar to steroid receptors. Whereas the overall structure of CYP17A1 provides a rationale for understanding many mutations that are found in patients with steroidogenic diseases, the active site reveals multiple steric and hydrogen bonding features that will facilitate a better understanding of the enzyme’s dual hydroxylase and lyase catalytic capabilities and assist in rational drug design. Specifically, structure-based design is expected to aid development of inhibitors that bind only CYP17A1 and solely inhibit its androgen-generating lyase activity to improve treatment of prostate and other hormone-responsive cancers.

CYP17A1 is a membrane-bound dual-function monooxygenase with a critical role in the synthesis of many human steroid hormones. The 17α-hydroxylase activity of CYP17A1 is required for the generation of glucocorticoids such as cortisol, but both the hydroxylase and 17,20-lyase activities of CYP17A1 are required for the production of androgenic and oestrogenic sex steroids (Supplementary Fig. 1). CYP17A1 is an important target for the treatment of breast and prostate cancers that proliferate in response to oestrogens and androgens. In the absence of structural information, CYP17A1 inhibitors have been designed that are thought to bind the cytochrome P450 haem iron, but it has been difficult to rationalize or predict other structural features that are critical for effective and selective CYP17A1 inhibition. In addition, structural information is important for understanding 17-hydroxylase deficiencies and may also improve our understanding of polycystic ovary disease. We determined the structures of human CYP17A1 when bound to two clinically relevant CYP17A1 inhibitors (Supplementary Fig. 2). Abiraterone is the active form of a prodrug that was recently approved by the US Food and Drug Administration for metastatic prostate cancer and is under investigation for breast cancer. TOK–001 is currently undergoing clinical trials for prostate cancer.

A truncated, His-tagged version of the human CYP17A1 protein was generated from a synthetic complementary DNA that was engineered to remove the single amino-terminal transmembrane helix, and this CYP17A1 was expressed in Escherichia coli. The resulting CYP17A1 was membrane bound and so it was solubilized with detergent before purification. This CYP17A1 binds abiraterone (17-(3-pyridyl)androst-5,16-dien-3β-ol) (Fig. 1a) and TOK–001 (17-(1H-Benzoimidazol-1-yl)androst-5,16-dien-3β-ol) with decreases in absorbance at 402 nm and increases in absorbance at 424 nm, consistent with nitrogen binding to the haem iron (type II interaction) with dissociation constant (Kd) values of <100 nM (Fig. 1a, inset). Similar titrations with the substrates progesterone (Fig. 1b) and pregnenolone revealed decreases in absorbance at 419 nm and increases in absorbance at 385 nm, which is indicative of the ligand displacing water from the haem (type I interaction). CYP17A1 binds pregnenolone (Kd < 100 nM) more tightly than progesterone (Kd 229 ± 14 nM; Fig. 1b, inset). We found that full-length enzyme had a similar kcatal and three-fold higher Michaelis constant (Km 1.31 ± 0.03 min−1, Km 11.4 ± 0.7 μM) compared to the truncated form (Kcatal 1.31 ± 0.03 min−1, Km 3.7 ± 0.3 μM). The half-maximum inhibitory concentration (IC50) values for abiraterone (201 ± 1 nM) were lower than for TOK–001 (503 ± 1 nM) (Fig. 1c). Thus, truncated human CYP17A1 is a functional enzyme in terms of ligand binding, catalytic function and inhibition.

Both structures with abiraterone (2.6 Å) and TOK–001 (2.4 Å) demonstrate the characteristic cytochrome P450 fold (Fig. 2a) and have four very similar protein copies in each asymmetric unit.

Figure 1 | Function of CYP17A1 and inhibition by clinical compounds.

a, CYP17A1 titration with abiraterone (10–274 nM) yields progressive shifts in the ultraviolet–visible difference spectrum that indicate nitrogen binding to haem iron. b, Similar titration with progesterone (10–1535 nM) indicates water displacement from the haem iron. c, IC50 of abiraterone (circles) and TOK–001 (squares) for progesterone 17α-hydroxylase.

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(Supplementary Table 1). Consistent with spectral binding data, abiraterone and TOK-001 bind with the nitrogen of the C17 pyridine or benzimidazole, respectively, forming a coordinate covalent bond with the haem iron (Fig. 2b, d). The steroidal cores of these inhibitors rise at an angle of 60° above the haem plane, directed between the F and G helices (Fig. 2b, d), and essentially overlap with each other (Fig. 2f). The unsubstituted α-face packs against the I helix where G301, A302 and adjacent residues form a highly complementary hydrophobic planar surface (Fig. 2b). The 3β-OH groups of abiraterone (Fig. 2b) and TOK-001 (Fig. 2d) hydrogen bond with N202 in the F helix (∼2.6 Å and ∼2.4 Å, respectively).

Although inhibitors occupy the majority of the enclosed active site, the void extends beyond these ligands in several directions. First, the active site wall nearest the inhibitor β-face is not as complementary to the steroid core as to the α-face. The C18 and C19 methyl groups project towards a crevice between the B′ helix, the β4 loop and the loop following the F helix (Fig. 2b). Only three side chains of the cavity wall come within 4 Å of C18 or C19. The cavity wall facing the haem is occupied by benzimidazole in the TOK-001 structure (Fig. 2e). Last, the most substantial active site cavity extension is from the 3β-OH of the inhibitors over the top of helix I and along the underside of helices F and G. This cavity is mostly lined by hydrophobic residues (1198, L243 and F300), but its ‘roof’ is bordered by several polar F and G helix residues (Y201, N202 and R239; Fig. 2c) that interact with, or are located near, water molecules in this region. The overall cavity containing TOK-001 is similar to that observed when abiraterone is bound, except that the available void volume over helix I is slightly smaller (Fig. 2c).

The single direct hydrogen bond between inhibitors and the protein is part of a larger hydrogen bonding network. In the abiraterone complex this network involves N202, E305, several conserved water molecules, R239, the backbone carbonyl of G297 and, in some molecules, Y201 (Fig. 3). Although Y201 is not within hydrogen bonding distance to these waters for molecules A and B, the side chain rotates slightly towards abiraterone in molecules C and D to interact with one or both of the water molecules. TOK-001 has a very similar hydrogen bonding network (Supplementary Fig. 3). These interactions are strongly reminiscent of the interactions that are conserved in the androgen, oestrogen, glucocorticoid, mineralocorticoid and progesterone receptors11 (Fig. 4a, b). In each receptor, the 3β-OH or 3-keto group of steroids binds within a deep receptor pocket and forms hydrogen bonds with a glutamine or glutamate, an arginine and often a conserved water ring bordered by V366, A367, I371 and V483 (Fig. 2c), and this volume is occupied by benzimidazole in the TOK-001 structure (Fig. 2e).

![Figure 2](https://example.com/figure2.png) **Figure 2** | CYP17A1 ligand binding. In the stick and sphere representations, non-carbon atoms are indicated in blue (N), red (O), black (haem), grey (abiraterone), cyan (TOK-001) and by a dark red sphere (Fe). a, A coloured representation of the CYP17A1–abiraterone structure, from the N terminus (blue) to the C terminus (red). b, Abiraterone binds [2 F, |F| density at 1σ; blue mesh] at an angle of approximately 60° from haem against helix I (yellow). c, Abiraterone cavity (grey mesh), rotated approximately 180° in relation to d, TOK-001 binding [2 F, |F| density at 1σ, blue mesh]. e, TOK-001 cavity (grey mesh). f, Abiraterone structure (yellow) superimposed on TOK-001 structure (purple) with respective voids (mesh). The B‘ helix has been removed from panels c-f so that it is possible to view the ligands.
Hormone receptors and may also contribute to CYP17A1 selectivity molecule. These interactions are critical for ligand recognition by different networks, with the main difference being the involvement of Y201. Water molecules are indicated by small spheres. Hydrogen bonds are represented by dashed lines and the distances between them are indicated.

Notably, TOK-001 is both a CYP17A1 inhibitor and androgen receptor for pregnenolone, progesterone and their 17α-hydroxy derivatives. Notably, TOK-001 is both a CYP17A1 inhibitor and androgen receptor antagonist, and the similarity of these binding modes is probably the reason for this dual mechanism of action.

Figure 3 | Hydrogen bond network with abiraterone. CYP17A1 has a hydrogen bonding network at the top of the active site that interacts with abiraterone. Molecules A and B (yellow), and C and D (green) have slightly different networks, with the main difference being the involvement of Y201. Water molecules are indicated by small spheres. Hydrogen bonds are represented by dashed lines and the distances between them are indicated.

Figure 4 | CYP17A1 compared to the androgen receptor and CYP11A1. A. The hydrogen bonding network near the abiraterone 3β-OH involves N202, R239 and conserved waters. B. The androgen receptor (PDB 3L3X) has a similar hydrogen bond network with R752, Q711 and several waters that interact with the dihydrotestosterone ketone. C. The structure of CYP17A1 with abiraterone (yellow) superimposed on the structure of CYP11A1 with 20,22-dihydroxycholesterol (PDB 3NA0, pink). This shows that there are markedly different steroid orientations between the two structures.

Methods Summary
A synthetic cDNA for human CYP17A1 was modified to delete residues 2–19, substitute the hydrophilic sequence Arg20-Arg21-Cys22-Pro23 with Ala20-Lys22-Thr23 and add a carboxy-terminal four-histidine tag (Supplementary Fig. 6) before cloning into the pCWori plasmid and overexpression in Escherichia coli.
coli JM109 cells. Protein was purified by nickel affinity, cation exchange and size exclusion chromatography. Abiraterone was synthesized (see Methods). Binding affinities were determined using an ultraviolet–visible spectral shift assay. Progesterone 17α-hydroxylation was evaluated using HPLC separation and ultraviolet detection. For crystallography, inhibitors were included throughout purification. Crystals were grown from CYP17A1 (30 mg ml⁻¹) complexed with inhibitor using hanging-drop vapour diffusion to equilibrate against 30% PEG 3350, 0.175 M Tris, pH 8.5, 0.30 M ammonium sulphate and 3% glycerol. Diffraction data were collected and phased by molecular replacement. Iterative model building and refinement generated the final model. Substrates were docked using Surflex-Dock.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Miller, W. L. & Auchus, R. J. The molecular biology, biochemistry, and physiology of human aromatogenesis and its disorders. Endocr. Rev. 32, 81–151 (2011).
2. Attard, G., Reid, A. H., Olims, D. & de Bono, J. S. Antitumor activity with CYP17 blockade indicates that castration-resistant prostate cancer frequency remains hormone driven. Cancer Res. 69, 4937–4940 (2009).
3. Yap, T. A., Carden, C. P., Attard, G. & de Bono, J. S. Targeting CYP17: Established and novel approaches in prostate cancer. Curr. Opin. Pharmacol. 8, 449–457 (2008).
4. Vasaitis, T. S., Bruno, R. D. & Njar, V. C. CYP17 inhibitors for prostate cancer therapy. J. Steroid Biochem. Mol. Biol. 125, 23–31 (2011).
5. de Bono, J., S. et al. Abiraterone and increased survival in metastatic prostate cancer. N. Engl. J. Med. 369, 1995–2005 (2011).
6. Molina, A. & Belledgrun, A. Novel therapeutic strategies for castration resistant prostate cancer: inhibition of persistent androgen production and androgen receptor mediated signaling. J. Urol. 185, 787–794 (2011).
7. Auchus, R. J., Geller, D. H., Lee, T. C. & Miller, W. L. The regulation of human P450sc17 activity: relationship to premature androgenesis, insulin resistance and the polycystic ovary syndrome. Trends Endocrinol. Metab. 9, 47–50 (1998).
8. Attard, G. et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. J. Clin. Oncol. 26, 4563–4571 (2008).
9. Brodie, A., Njar, V., Macedo, L. F., Vasiti, T. S. & Sabnis, G. The Coffey Lecture: steroidogenic enzyme inhibitors and hormone dependent cancer. Urol. Oncol. 27, 53–63 (2009).
10. Imai, T. et al. Expression and purification of functional human 17α-hydroxylase/17,20-lyase (P450c17) in Escherichia coli. Use of this system for study of a novel form of combined 17α-hydroxylation/17,20-lyase deficiency. J. Biol. Chem. 268, 19681–19689 (1993).
11. Huang, P., Chandra, V. & Rastinejad, F. Structural overview of the nuclear receptor superfamily: insights into physiology and therapeutics. Annu. Rev. Physiol. 72, 247–277 (2010).
12. Pereira de Jesús-Tran, K. et al. Comparison of crystal structures of human androgen receptor ligand-binding domain complexes with various agonists reveals molecular determinants responsible for binding affinity. Protein Sci. 15, 987–999 (2006).
13. Vasaitis, T. et al. Androgen receptor inactivation contributes to antitumor efficacy of 17α-hydroxylation/17,20-lyase inhibitor 3β-hydroxy-17(11H-benzimidazole-1-yl)androst-5,16-diene in prostate cancer. Mol. Cancer Ther. 7, 2348–2357 (2008).
14. Ghosh, D., Griswold, J., Erman, M. & Pangborn, W. Structural basis for androgen specificity and oestrogen synthesis in human aromatase. Nature 457, 219–223 (2009).
15. Mast, N. et al. Structural basis for three-step sequential catalysis by the cholesterol side chain cleavage enzyme CYP11A1. J. Biol. Chem. 286, 5607–5613 (2011).
16. Mast, N. et al. Crystal structures of substrate-bound and substrate-free cytochrome P450 46A1, the principal cholesterol hydroxylase in the brain. Proc. Natl Acad. Sci. USA 105, 9546–9551 (2008).
17. Dhir, V. et al. Steroid 17α-hydroxylase deficiency: Functional characterization of four mutations (A174E, V187D, R440C, L465P) in the CYP17A1 gene. J. Clin. Endocrinol. Metab. 94, 3058–3064 (2009).
18. Rosa, S. et al. Clinical, genetic and functional characteristics of three novel CYP17A1 mutations causing combined 17α-hydroxylase/17,20-lyase deficiency. Horm. Res. Paediatr. 73, 198–204 (2010).
19. Katsumata, N., Ogawa, E., Fujiwara, I. & Fujikura, K. Novel CYP17A1 mutation in a Japanese patient with combined 17α-hydroxylation/17,20-lyase deficiency. Metabolism 59, 275–278 (2010).
20. Ergun-Longmire, B. et al. Two novel mutations found in a patient with 17α-hydroxylase enzyme deficiency. J. Clin. Endocrinol. Metab. 91, 4179–4182 (2006).
21. Sahakianruangta, T., Tee, M. K., Speiser, P. W. & Miller, W. L. Novel P450sc17 mutation H373D causing combined 17α-hydroxylase/17,20-lyase deficiency. J. Clin. Endocrinol. Metab. 94, 3089–3092 (2009).
22. Biaison-Laufer, A. et al. 17α-hydroxylase/17,20-lyase deficiency as a model to study enzymatic activity regulation: role of phosphorylation. J. Clin. Endocrinol. Metab. 85, 1226–1321 (2000).
23. Lee-Robichaud, P. et al. The cationic charges on Arg347, Arg358 and Arg449 of human cytochrome P450sc17 (CYP17) are essential for the enzyme’s cytochrome b5-dependent acyl-carbon cleavage activities. J. Steroid Biochem. Mol. Biol. 92, 119–130 (2004).
24. Gupta, M. K., Geller, D. H. & Auchus, R. J. Pitfalls in characterizing P450sc17 mutations associated with isolated 17,20-lyase deficiency. J. Clin. Endocrinol. Metab. 86, 4416–4423 (2001).
25. Tosano, D. et al. Metabolic evidence for impaired 17α-hydroxylase activity in a kindred bearing the E305G mutation for isolate 17,20-lyase activity. Eur. J. Endocrinol. 158, 385–392 (2008).
26. Auchus, R. J., Lee, T. C. & Miller, W. L. Cytochrome b5 augments the 17α,20-lyase activity of human P450sc17 without direct electron transfer. J. Biol. Chem. 273, 3158–3165 (1998).
27. Swart, A. C., Storbeck, K. H. & Swart, P. A single amino acid residue, Ala 105, confers 16α-hydroxylase activity to human cytochrome P450 17α-hydroxylation/17,20-lyase. J. Steroid Biochem. Mol. Biol. 119, 112–120 (2010).
28. Haider, S. M., Patel, J. S., Poojari, C. S. & Neidle, S. Molecular modeling on inhibitor complexes and active-site dynamics of cytochrome P450 C17, a target for prostate cancer therapy. J. Mol. Biol. 400, 1078–1098 (2010).
29. Jagusch, C. et al. Synthesis, biological evaluation and molecular modelling studies of methyleneimidazole substituted biaryls as inhibitors of human 17α-hydroxylase-17,20-lyase (CYP17). Part I: heterocyclic modifications of the core structure. Bioorg. Med. Chem. 16, 1992–2010 (2008).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions N.M.D. performed the docking studies of CYP17A1. X-ray diffraction experiments, solved and refined the structures, and wrote the manuscript. N.M.D. performed the docking studies of CYP17A1.

Author Information Atomic coordinates and structure factors for the reported crystal structures have been deposited with the Protein Data Bank under the accession codes 3RUK for CYP17A1 with abiraterone and 3SWZ for CYP17A1 with TOK-D01. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to E.E.S. (eescott@kiu.edu).


**METHODS**

**Synthesis and characterization of abiraterone.** A stirred solution of 17-
iodoandrosta-5,16-dien-3β-d (600 mg, 1.5 mmol) in tetrahydrofuran (20 ml) in a 100-ml round-bottomed flask was stirred with argon. Bis(triphenylphosphine) palladium (II) dichloride catalyst (11 mg, 0.016 mmol) and then diethyl(3-pyridyl) borane (265 mg, 1.8 mmol) were added. To the resultant orange solution, an aqueous solution of sodium carbonate (2.0 M, 5 ml) was added. The flask was fitted with a reflux condenser and the apparatus was purged again with argon. The mixture was then heated under reflux (at ∼80 °C) and stirred for 4 days then allowed to cool. The mixture was poured into water and extracted with hot toluene (3 × 30 ml). The toluene extracts were dried (using Na2CO3) and concentrated.

**Column chromatography** was performed with EtO and toluene (with a ratio of 1:2) as the eluent to give abiraterone (350 mg, 66%) as a white crystalline solid: melting point 228–230 °C; IR O-H stretching frequency νmax 3307 cm−1; H NMR peak shifts in ppm (δ) 1.07 (s, 3, H-19), 1.09 (s, 3, H-18), 3.54 (m, 1, H-3z), 5.41 (dm, 1, J = 5.2 Hz, H-6), 6.01 (m, 1, H-16), 7.24 (dd, 1, pyridyl H-5), 7.66 (dd, 1, pyridyl H-4), 8.47 (dd, 1, pyridyl H-6), 8.63 (d, 1, pyridyl H-2); 13CN M Rδ 151.69, 147.92, 147.84, 141.19, 133.68, 132.98, 129.98, 129.24, 120.98, 121.31, 71.55, 57.56, 50.36, 47.34, 42.32, 37.19, 36.71, 35.26, 31.81, 31.64, 30.45, 20.88, 19.35, 16.59. The high resolution mass spectrum calculated the mass to charge ratio (m/z) C24H32NO+ [M+H]+ to be 350.2484. The experimental value was 350.2491. Abiraterone was 99% pure by liquid chromatography mass spectrometry.

**CYP17A1 design, expression and purification.** The human CYP17A1 cDNA was synthesized with codon optimization for CYP17A1 design, expression and purification. 

**WHATCHECK36 and PROCHECK37.** Ramachandran plot analysis reveals per cent favourable/additional allowed/generously allowed/disallowed residues are 86.3/13.2/0.5/0.0 (abiraterone structure) and 86.2/13.3/0.5/0.0 (TOK-001 structure). X-ray statistics are provided (Supplementary Table 1). Organ-purposed voids were calculated using VOIDOO38 (probe radius = 1.4 Å; grid mesh = 0.4 Å). All figures were prepared using MacPyMOL.

**Docking.** The CYP17A1 active site was defined as described for other cytochromes P450 (ref. 40) with the addition of an oxygen molecule directly coordinated to the haem to mimic compound I of the cytochrome P450 catalytic cycle. Substrate coordinates were prepared and energy was minimized with SYBYL (Tripos International). Charges were assigned using the Gasteiger and Marsili method. Surflex-Dock41 (Tripos International) was used to dock ligands as previously described39. The active site was a 10 Å sphere around the haem and pregnenolone. Movement of pregnenolone within the active site was not substantial, with the distances from C17 to O Fe(IV) as 4.5 Å, from C16 to O Fe(IV) as 4.5 Å, and from C21 to O Fe(IV) as 3.0 Å for the lowest energy pose.

**Enzyme activity and IC50 determinations.** Progesterone 17α-hydroxylase was evaluated using a modified HPLC method with ultraviolet detection42. CYP17A1 (30 pmol) and rat NADPH-cytochrome P450 reductase43 were mixed with a ratio of 1:4, incubated on ice (20 min) and added to buffer (50 mM Tris, pH 7.4 and 5 mM MgCl2) containing progesterone (0–50 μM) to a total volume of 500 μl. Phosphatidylcholine (25 μg) was included for side-by-side kinetic comparisons with the full-length enzyme44. For IC50 determinations, inhibitor concentrations were 0–1.5 μM for abiraterone and 0–3.0 μM for TOK-001. After warming (37 °C, 3 min), reactions were initiated by the addition of NADPH (20 μl 25 mM), incubated for 10 min (37 °C), quenched with 20% trichloroacetic acid (300 μl) and placed on ice. The 17α-hydroxypregnenolone metabolite was identified by ultraviolet detection at 248 nm after HPLC separation and co-eluted with authentic standards. The HPLC mobile phase was 40% acetonitrile, 60% water with 1% acetic acid and run at 1 ml min−1 (Luna (Phenomenex), 5 μm, C18, 50 × 4.6 mm).

**Ligand binding assays.** Ligand binding assays were based on spectral differences that were detected after ligand titration and were performed as described44, except that the CYP17A1 concentration was 0.1 μM, the path length was 5 cm and the tight binding equation was used.

Functional data were analysed using Prism (GraphPad Software) and presented as mean ± standard error.

30. Pechurskaya, T. A., Lukashevich, O. P., Gilep, A. A. & Usanov, S. A. Engineering, expression, and purification of “soluble” human cytochrome P450 17αx and its functional characterization. *Biochemistry* 73, 806–811 (2008).

31. Leslie, A. G. W. MOSFILM 6.0 (Cambridge, 1998).

32. Collaborative Computational Project, Number 4, The CCP4 suite: programs for protein crystallography. *Acta Crystallogr. D* 50, 760–763 (1994).

33. Long, F., Vagin, A. A., Young, P. & Murshudov, G. N. BALBES: a molecular replacement pipeline. *Acta Crystallogr. D* 64, 125–132 (2008).

34. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr. D* 60, 2126–2132 (2004).

35. Murshudov, G. N., Vagin, A. A. & Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr. D* 53, 240–255 (1997).

36. DeLano, W. L. The PyMol Molecular Graphics System (DeLano Scientific, 2002).

37. Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* 26, 283–291 (1993).

38. DeVore, N. M. *Biochemistry* 17, 1127–1135 (1978).

39. Key residues controlling binding of diverse ligands to human cytochrome P450 2A1 enzymes. *Drug Metab. Dispos.* 37, 1319–1327 (2009).

40. DeLano, W. L. The PyMol Molecular Graphics System (DeLano Scientific, 2002).

41. Kleywegt, G. J. & Jones, T. A. Detection, delineation, measurement and display of cavities in macromolecular structures. *Acta Crystallogr. D* 50, 178–185 (1994).

42. Jain, A. N. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* 46, 499–511 (2002).

43. Hutschenreuter, T. U., Eherm, P. B. & Hartmann, R. W. Synthesis of hydroxysteroidal non-steroidal CYP 17 inhibitors as potential metabolites and evaluation of their activity by a non cellular assay using recombinant human enzyme. *J. Enzyme Inhib.* Med. Chem. 19, 17–32 (2004).

44. Shen, A. L., Porter, T. D., Wilson, T. E. & Kasper, C. B. Structural analysis of the FMN binding domain of NADPH-cytochrome P450 oxidoreductase by site-directed mutagenesis. *J. Biol. Chem.* 264, 7584–7589 (1989).