Three Dimensional Pharmacophore Modeling of Human CYP17 Inhibitors. Potential Agents for Prostate Cancer Therapy

Omoshile O. Clement,*,† Clive M. Freeman,† Rolf W. Hartmann,‡ Venkatesh D. Handratta,§ Tadas S. Vasaitis,§ Angela M. H. Brodie,*,§ and Vincent C. O. Njar*,§

Accelrys, 9685 Scranton Road, San Diego, California 92121, Department of Pharmaceutical and Medicinal Chemistry, University of Saarlandes, P.O. Box 151150, D-66041, Saarbrucken, Germany, and Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201

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We report here a molecular modeling investigation of steroidal and nonsteroidal inhibitors of human cytochrome P450 17α-hydroxylase-17,20-lyase (CYP17). Using the pharmacophore perception technique, we have generated common-feature pharmacophore model(s) to explain the putative binding requirements for two classes of human CYP17 inhibitors. Common chemical features in the steroid and nonsteroid human CYP17 enzyme inhibitors, as deduced by the Catalyst/HipHop program, are one to two hydrogen bond acceptors (HBAs) and three hydrophobic groups. For azole-steroidal ligands, the 3*β*-OH group of ring A and the N-3 of the azole ring attached to ring D at C-17 act as hydrogen bond acceptors. A model that permits hydrogen bond interaction between the azole functionality on ring D and the enzyme is consistent with experimental deductions for type II CYP17 inhibitors where a sixth ligating atom interacts with Fe(II) of heme. In general, pharmacophore models derived for steroid and nonsteroidal compounds bear striking similarities to all azole sites mapping the HBA functionality and to three hydrophobic features describing the hydrophobic interactions between the ligands and the enzyme. Using the pharmacophore model derived for azole-steroidal inhibitors as a 3D search query against several 3D multiconformational Catalyst formatted databases, we identified several steroidal compounds with potential inhibition of this enzyme. Biological testing of some of these compounds show low to high inhibitory potency against the human CYP17 enzyme. This shows the potential of our pharmacophore model in identifying new and potent CYP17 inhibitors. Further refinement of the model is in progress with a view to identifying and optimizing new leads.

Introduction

Prostate cancer (PC) is the second leading cause of cancer deaths in men in the U.S.[1] Androgens play an important role in the development, growth, and progression of PC.[2,3] A number of studies indicate a correlation between serum testosterone levels and increased risk of PC.[4] Furthermore, about 90% of patients respond to androgen deprivation, reflecting a requirement of circulating testosterone for their growth.

The cytochrome P450 enzymes involved in steroid hormone biosynthesis represent an important target in the chemotherapeutic route to the treatment of hormone-dependent cancers.[5] Perhaps most extensively developed are inhibitors of the P450 enzyme aromatase, which catalyzes the conversion of androgens to estrogens and is a target in the treatment of breast cancer.[6] Indeed, research efforts in this area have proved to be very successful, resulting in four aromatase inhibitors that are currently used clinically for the treatment of breast cancer. While only about 50% of breast tumors are hormone-sensitive, approximately 90% of prostate tumors are androgen-dependent. The last step in the biosynthesis of androgens (for example, testosterone) involves two key reactions that occur sequentially and are both catalyzed by a single enzyme, the cytochrome P450 monooxygenase 17α-hydroxylase/17,20-lyase (CYP17 or 17-lyase).[7] Ketoconazole, an antifungal agent that is also a modest CYP17 inhibitor, has been used clinically for the treatment of PC.[8] However, ketoconazole has now been withdrawn from use because of liver toxicity and other side effects due mainly to its inhibition of other CYP enzymes. This highlights the need for discovery and development of *more potent* and *specific* CYP17 inhibitors.

Several categories of highly potent and specific steroidal and nonsteroidal CYP17 inhibitors have been reported (reviewed by Njar and Brodie, 1999).[9] The design strategy of these new inhibitors is based on the knowledge that the active site of the enzyme contains a hydrophobic region and a heme group, which is common with all P450 enzymes. Some of these compounds are under development and are expected to show improved selectivity and reduced side effects. The rational design of CYP17 inhibitors would be greatly helped by knowledge of the three-dimensional structure of the enzyme. However, there is currently no crystal structural information on CYP17. Indeed, this enzyme

* To whom correspondence should be addressed. For O.O.C.: phone, (858) 799-5336; fax, (858) 799-5100; e-mail, oclement@accelrys.com. For A.M.H.B.: phone, (410) 706-3137; fax, (410) 706-0032; e-mail, abrodie@umaryland.edu. For V.C.O.N.: phone, (410) 706-5885; fax, (410) 706-0032; e-mail, vnjar001@umaryland.edu.
† Accelrys.
‡ University of Saarlandes.
§ University of Maryland School of Medicine.

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and all related mammalian P450 enzymes are membrane-bound and practical problems have so far prevented their crystallization and structural elucidation.

To date, only two molecular modeling studies of CYP17 based on the crystal structure of the related class II P450 enzyme, P450BM-3[10] (a fatty acid monooxygenase from *Bacillus megaterium*) have been reported.[11,12] However, whereas the predicted structure of CYP17 by Bruke et al.[11] has a *bilobal* active-site cavity, that by Auchus and Miller[12] is reported to possess a *monolobal* active-site cavity. These studies were conducted with the hope that knowledge of the three-dimensional structure of CYP17 will facilitate the design of new and specific CYP17 inhibitors useful in chemotherapy of androgen-dependent cancers. Unfortunately, new CYP17 inhibitors based on these molecular modeling studies are yet to be reported.

A molecular modeling (2D) study for the binding of nonsteroidal antimycotic inhibitors of CYP17 to the enzyme's active site has been reported.[13] However, the inhibitors used in the study were not specific for CYP17 because they also inhibit other P450 enzymes. Recently, our group[9,14] and others[15,16] have developed highly potent and specific inhibitors of CYP17. We therefore considered it a worthy research endeavor to analyze the CYP17 inhibitory activity data determined in vitro for these compounds using the three-dimensional quantitative structure−activity relationship (3D-QSAR) software program Catalyst.[17] The Catalyst program can be used to probe how ligands interact with a receptor by evaluating chemical features common to a set of active ligands (HipHop)[18] or by elucidating the correlation between activity and chemical binding features (Hypogen).[19]

One application of the Catalyst program is the generation of hypotheses that attempt to correlate the biological activity observed for a series of compounds to their chemical structures. The hypotheses generated are three-dimensional descriptions of a pharmacophore model proposed for the series of compounds examined. The hypotheses are represented by chemical features that describe a series of compounds (e.g., hydrogen bonding acceptors (HBAs), positive and negative ionizable groups, etc). The hypotheses generated may be used to estimate the biological activity of proposed targets, allowing a rank ordering of synthetic priorities. In addition, the hypotheses generated may be used as three-dimensional queries to search databases of proprietary and/or commercially available compounds. These three-dimensional searches could identify novel compounds that might exhibit potent inhibition of the target enzyme.

In this study, we used the Catalyst program to establish a hypothetical CYP17 active site by analyzing a variety of steroidal CYP17 inhibitors that *were evaluated under the same assay conditions*. Following validation of the hypotheses that were generated, we used the hypotheses to query three databases. These studies resulted in the discovery of new and potent CYP17 inhibitors and are the subject of this paper.

Experimental Section

Chemistry. Most of the CYP17 inhibitors used in this study were synthesized in our laboratories, and the methodology for their preparation has been previously published.[14,16] 17-(3-Pyridyl)androsta-5,16-diene-3β-ol was synthesized as reported,[15b] and spectral and analytical data were as previously described.[15] Ketoconazole was purchased from Sigma Chemical Co., St. Louis, MO. The compounds identified by searching the Maybridge[20] database were obtained from Ryan Scientific, Inc., Mt. Pleasant, SC.

In Vitro Assay of CYP17. The in vitro CYP17 inhibitory activities of the compounds were evaluated using our rapid acetic acid releasing assay (AARA), utilizing intact P450c17-expressing *E. coli* or P450c17-LNCaP cells as the enzyme source.[21] It involves the use of [21-^{3}H]-17α-hydroxypregnenolone as the substrate, and CYP17 activity was measured by the amount of tritiated acetic acid formed during the cleavage of the C-21 side chain of the substrate. We have firmly established that the method is comparable in terms of accuracy and reliability to the HPLC analysis procedure used by researchers in the field.[21] IC_{50} values were obtained directly from plots relating the percentage inhibition versus inhibitor concentration over appropriate ranges. Each compound was tested at a minimum of five different concentrations. The assays were performed in triplicate, and the IC_{50} values reported are the mean of triplicate experiments. The standard deviations were ±5% of the mean values.

Computational Methods. The study was performed using the Catalyst suite of programs available in Catalyst, release 4.7,[17] with graphic display on a 195 MHz MIPS R10K Octane (IRIX 6.5.8) and a compute server on a dual processor, 1.5 GHz Intel based PC running the LINUX RedHat 7.1 (Kernel 2.4) operating system.

All compounds were constructed using the 2D/3D sketcher of the Catalyst software. By use of conformational Poling,[22] a family of diverse conformational models for each compound were generated within a 10 kcal/mol range of the computed minimum for each compound, as found in the BEST search method in Catalyst. The average numbers of conformations range from about 5 to 20 for all rigid steroidal compounds.

The pharmacophore-based investigation of CYP17 inhibitors involved using the Catalyst/HipHop program to generate feature-based 3D pharmacophore alignments.[23,24] This was performed in a three-step procedure:[25] (a) a conformational model for each molecule in the training set was generated; (b) each conformer was examined for the presence of chemical features; (c) a three-dimensional configuration of chemical features common to the input molecules was determined. Catalyst provides a dictionary of chemical features found to be important in drug−enzyme/receptor interactions. These are hydrogen bond donors, hydrogen bond acceptors, hydrophobic groups, and positive and negative ionizable groups. For the pharmacophore modeling runs, common features selected for the run were ring aromatic (R), hydrogen bond donor (D), hydrogen bond acceptor (A), and hydrophobic groups (H).

Results and Discussion

Common Feature-Based Pharmacophore Models. Steroidal CYP17 Inhibitors. Figure 1 shows the structures of 23 compounds and their IC_{50} values for CYP17 inhibition obtained in our laboratory under the same assay conditions. The range of CYP17 inhibition activity exhibited by these compounds was 50−12000 nM. This range of inhibition activity (<2.5 log units) was not large enough to allow us to generate meaningful activity-based (predictive) pharmacophone models using the Catalyst/Hypogen technology. However, on the assumption that the most active compounds bind in a similar fashion at the enzyme's active site, we employed the Catalyst/HipHop approach to evaluate the common features required for binding and the hypothetical geometries adopted by these ligands in their most active forms. Thus, a training set consisting of seven of the most active steroidal CYP17 inhibitors (VN/85-1, VN/

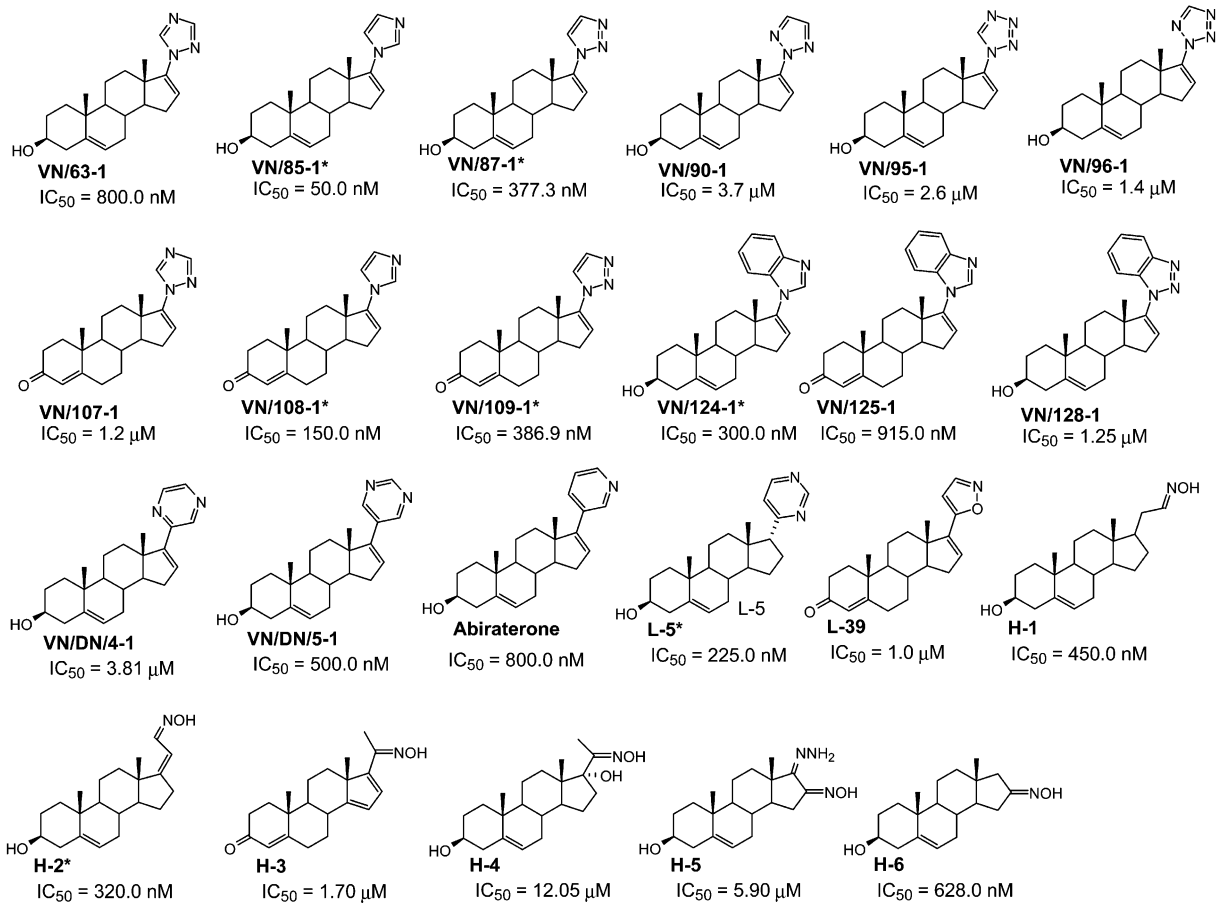

Figure 1. CYP17 inhibitors and their IC_{50} values. The asterisk (∗) represents the training set of steroidal CYP17 inhibitors (seven) used to generate the common-feature (HipHop) pharmacophore model.

87-1, VN/108-1, VN/109-1, VN/124-1, H-2, and L-5; see Figure 1) was submitted for pharmacophore model generation based on common chemical features.

In the model generation methodology, the highest weighting was given to the most "active" compound in the training set (VN/85-1; IC_{50} = 50.0 nM). The 10 hypotheses (Hypos) generated had scores ranging from 58 to 92. Of these 10 hypotheses, three (Hypos 1, 3, and 4) were retained for further analysis as determined by the ranking of the HipHop models and by elimination of redundancies based on hierachical clustering of all 10 hypotheses. Further pruning of the remaining three hypotheses was performed using the Güner−Henry (GH) scoring method.[26] This methodology has been successfully applied to quantification of model selectivity and coverage of activity space from database mining[27] and to the evaluation of the effectiveness of similarity searches in databases containing both structural and biological activity data.[28] The method consists of computing the following: the percent yield (% *Y*), which is a measure of the selectivity of the model, the percent active (% *A*), which represents the coverage of activity space by the model, the enrichment factor *E*, and the Güner−Henry (GH) score. These variables are determined using information derived from the total number of compounds in the drug database (*D*), the number of actives in the database (*A*), the number of actives retrieved by the model (H_a), and the total number of hits retrieved by the model (H_t). These variables are defined in Chart 1.

Chart 1. Metrics for Analyzing Hit Lists in Pharmacophore-Based Modeling[26,a]

$$\%A = \frac{H_a}{A} \times 100$$

$$\%Y = \frac{H_a}{H_t} \times 100$$

$$E = \frac{H_a/H_t}{A/D}$$

$$\mathrm{GH} = \frac{H_a(3A + H_t)}{4(H_tA)}\left(1 - \frac{H_t - H_a}{D - A}\right)$$

[a] Ht = no. of hits retrieved; Ha = no. of actives in hit list; % *A* = ratio of actives retrieved in hit list; % *Y* = fraction of hits relative to size of database (hit rate or selectivity); *E* = enrichment of active bin by model relative to random screening; GH = Güner−Henry score.

A text-based (1D) query using mechanism of action (MA) in the search field was used to search for *all lyase inhibitors* in the Catalyst formatted version of the Derwent World Drug Index (1999 release)[29] (Catalyst/WDI99: 53 964 compounds). This version of the drug database contained 15 compounds (6 steroids, 9 nonsteroids) described as lyase- or 5α-reductase inhibitors. Five of the six steroidal compounds have previously been reported by our group as potent CYP17 inhibitors.[14a] The nonsteroidal compounds were mainly either antimycin analogues or imidazolecarbozoles that are potent (low nanomolar IC_{50}) inhibitors of CYP17.[30] Using Hypos 1, 3, and 4 as search queries against the same

Table 1. Pharmacophore Model Evaluation Based on the Güner−Henry Scoring Method[23,a]

hypothesis	features	H_t	H_a	% *A*	% *Y*	*E*	GH
Hypo 1	HHHHAA	3043	6	40	0.2	7.1	0.096
Hypo 3	HHHHA	6100	9	60	0.15	5.4	0.134
Hypo 4	HHHAA	2380	9	60	0.38	13.6	0.146

[a] Total number of compounds in Catalyst/WDI99 (*D*) is 53 964, and the total number of lyase inhibitors in this database (*A*) is 15. Ht = no. of hits retrieved; H_a = no. of actives in hit list; % *A* = ratio of actives retrieved in hit list; % *Y* = fraction of hits relative to size of database (hit rate or selectivity); *E* = enrichment of active bin by model relative to random screening; GH = Güner−Henry score.

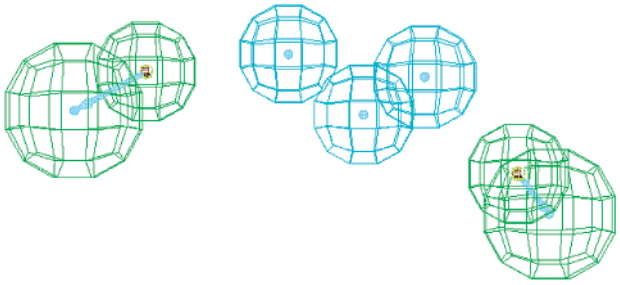

Figure 2. Common feature-based (Catalyst/HipHop) pharmacophore model of azole steroid-based human CYP17 inhibitors. The model contains five features: three hydrophobes (cyan) and two hydrogen bond acceptors (green).

database, we retrieved $^6/_{15}$, $^9/_{15}$, and $^9/_{15}$, respectively, of the lyase inhibitors in the WDI database. The search results were then subjected to the GH scoring analysis. The results are presented in Table 1. On the basis of the data in Table 1, Hypo 4 with the highest enrichment factor (13.5), highest coverage of activity space (*A* = 60%), highest selectivity (*Y* = 0.38%), and highest GH score (0.146) was chosen as the overall best pharmacophore model for this class of inhibitors.

This pharmacophore model contained five chemical features: three hydrophobes (cyan) and two hydrogen bond acceptors (green) (see Figure 2). The HBAs map the oxo/hydroxo group on ring A and the N-3 atom of the azole moiety attached to ring D. Out of the nine known lyase inhibitors identified by the model, it included all six steroid-based inhibitors in the Catalyst/Derwent WDI99 database.

We note that the distance between the two HBAs in the pharmacophore model is ca. 13 Å. This is similar to the distance between the 3β-OH group on ring A and the heteroatom N-3 atom of the azole attached to ring D (ca. 13.2 Å), as determined from the low-energy conformer with the best mapping to the pharmacophore model. It is also noteworthy that type II CYP17 inhibitors such as the steroidal compounds investigated here are known to act as a sixth ligating atom with Fe(II) of heme as part of their bioactive functionality.[9] Such interaction is accounted for by the model with the mapping of an HBA site described by the N-3 atom of the azole moiety on ring D.

Hypo 4 has five features, and hence, the maximum fit value of any ligand alignment with this model will be 5.0. Alignment of Hypo 4 with all training compounds were performed (see Figure 3) and found to give fit scores[23] ranging from 3.8 to 5.0.

To identify potential new CYP17 inhibitors, Hypo 4 was used as a search query against three commercial

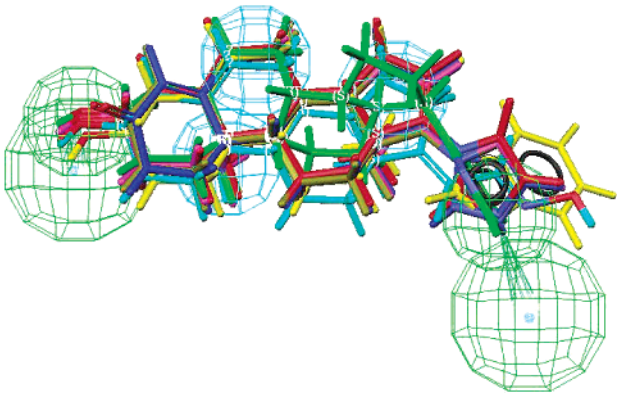

Figure 3. Alignment of common-feature pharmacophore model with training set CYP17 inhibitors.

Table 2. Results of 3D Search of Three Databases (Maybrdige, ACD, and BioByteMasterFile) Using the Pharmacophore Model Derived for Steroid-Based Human CYP17 Inhibitors (Hypo 4) as Search Query

database	DB size	no. of hits	% of database	hits with fit score >3.5
Maybridge, 2001	55 273	1.951	3.5	21
ACD, 2001	266 812	12.536	4.7	567
BioByteMasterFile, 2001	39 389	2643	6.7	170

databases: Catalyst/Maybridge2001 (55 273 compounds), Catalyst/ACD2001 (238 000 compounds), and Catalyst/BioByteMasterFile2001 (39 389 compounds). The search results are provided in Table 2. The hits retained for further evaluation were those with calculated fit scores (of model alignment and ligand) greater than or equal to 3.5 (this is based on the lowest fit score from the alignment of the HipHop model with all seven training set compounds (fit scores range from 3.8 to 5.0)). For example, the pruned results of hits retrieved from the Catalyst/Maybridge2001 database yielded 21 compounds with fit scores greater than or equal to 3.5, 15 of which were steroids of interest to this study (see Figure 4). Six compounds, BTB 13785, CD 10709, NRB 03689, NRB 03731, NRB 03742, and NRB 03849, were selected from the 21 compounds identified because of availability for the CYP17 inhibition assay. Except for CD 10709, the structures of the other five compounds were similar (possessing the steroid scaffold with C17 polar groups) to those of the training set. The in vitro IC_{50} values for inhibition of CYP17 by these compounds are presented in Table 3. Again, except for CD 10709, the other five compounds exhibited potent inhibition of the enzyme, with IC_{50} values in the top 10% of the training set compounds (see Figure 1). The high (83%) hit ratio obtained with these compounds retrieved from the Maybridge database is significant, suggesting that our modeling strategy is reliable and that it may be a useful procedure to identify novel and potent CYP17 inhibitors. However, except for NRB 03731 with a relatively stable 17-hydrazine-1-carboxamide group, the other compounds are metabolically unstable (will be easily hydrolyzed in vivo) because of the presence of ester moieties and may not be useful in vivo as CYP17 inhibitors. Nevertheless, it is conceivable that potentially stable (unhydrolyzable) analogues of these compounds may be designed to yield useful CYP17 inhibitors with in vivo activity.

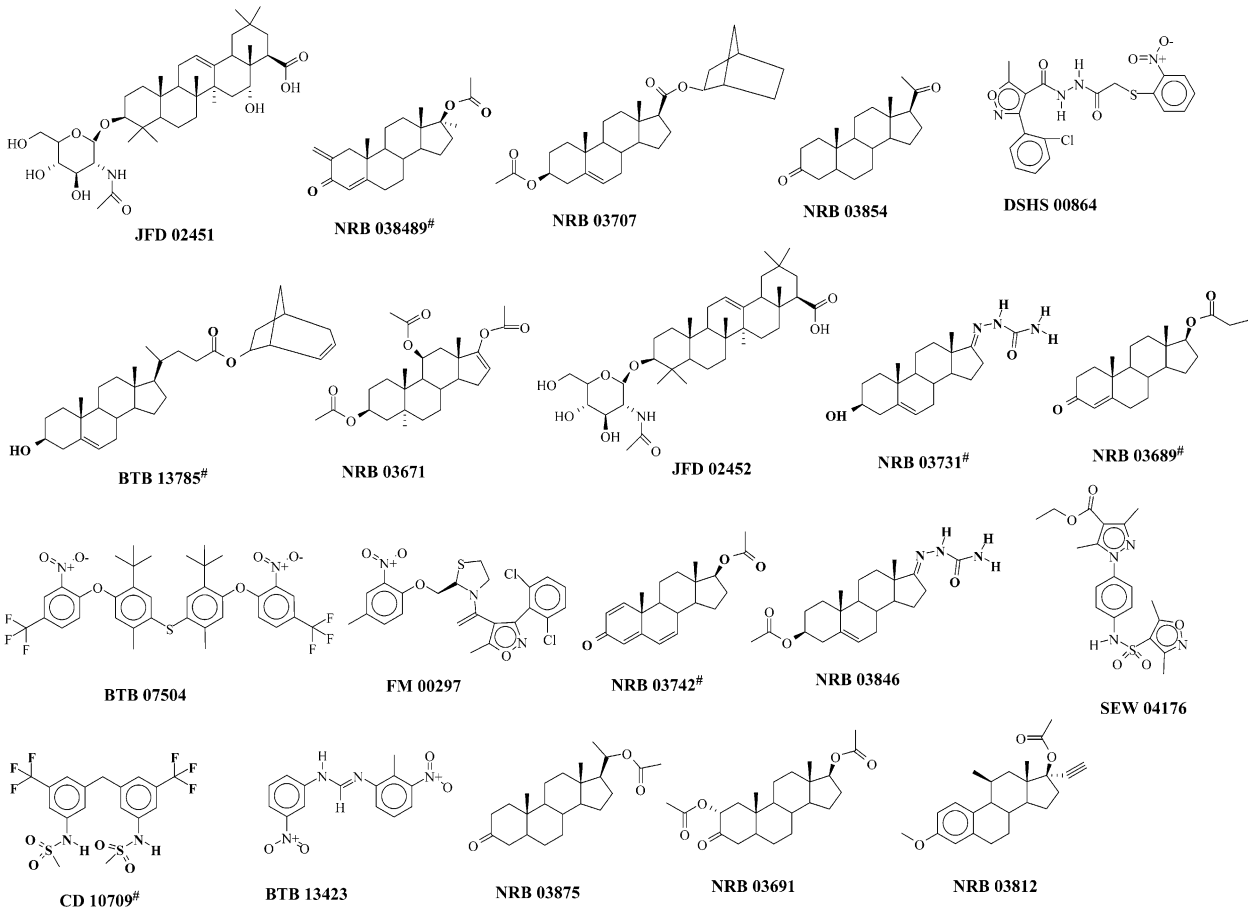

Figure 4. Structures of the compounds retrieved as "hits" from a 3D search of Catalyst formatted Maybridge Database (Maybridge2001) using the HipHop model derived for azole steroid-based human CYP17 inhibitors. The pound sign (#) indicates compounds (six) that were assayed for CYP17 inhibition.

Table 3. Inhibition of CYP17[a] by Compounds Retrieved from the Maybridge Database Using the HipHop Model (Hypo 4) as Search Query

compd	IC_{50} (nM)
BTB 13785	616.50
CD 10709	*b*
NRB 03689	56.0
NRB 03731	178.20
NRB 03742	660.25
NRB 03849	562.0
VN/85-1 (for comparison)	28.0

[a] CYP17-LNCaP enzyme system was used in these assays. [b] Approximately 25% inhibition at 1 mM concentration.

Common Feature-Based Pharmacophore Models. Nonsteroidal CYP17 Inhibitors. In a previous study on the molecular modeling of azole-based antimycotic agents (Figure 5a) with inhibitory potency against human CYP17 enzyme,[13] the higher activity of bifonazole against the CYP17 enzyme was rationalized on the ability of this ligand to undergo hydrophobic binding similar to rings A, B, and C of the steroidal progesterone, despite the lack of a ligating heteroatomic site on the compound.[13] The study, though in a 2D evaluation of the binding/alignment of these ligands relative to the natural substrates (progesterone and pregnenolone), provided an interesting finding nonetheless. Results of the 3D pharmacophore-based analysis of the heterocyle-based human CYP17 inhibitors are described here.

In the present study, we investigated 3D pharmacophore elucidation of the binding requirements for inhibiting human CYP17 by this class of compounds as part of our overall investigation of type II inhibitors of this enzyme. Our training set consisted of six of these ligands (Figure 5a). As in the steroid-based ligands, diverse conformations within a 10 kcal/mol energy range were generated and submitted to the Catalyst/HipHop program. The chemical feature-based pharmacophore model identified in the study is shown in Figure 5b, and alignment of the model with the most potent members of this class of compounds (ketoconazole, bifonazole, and clotrimazole) is shown in Figure 5c. The pharmacophore model for inhibition of the human CYP17 enzyme by these ligands consisted of three hydrophobic groups (HYD) and one hydrogen bond acceptor (HBA).

The common binding features in these compounds as identified by the Catalyst/HipHop program suggest the requirement of a hydrogen bond acceptor site mapped by the imidazole N-3 site and three hydrophobic sites mapped by three aromatic rings (see Figure 5c). It can be seen that the imidazole N-3 atom maps to the HBA feature of our model in all the compounds. Since the model derived in this study did not include activity values, it is difficult to speculate if the lack of mapping of all three aromatic rings by ketoconazole and clotrimazole accounts for their slightly lower inhibitory potency against human CYP17. We scored the alignment of the model against low-energy conformers of the

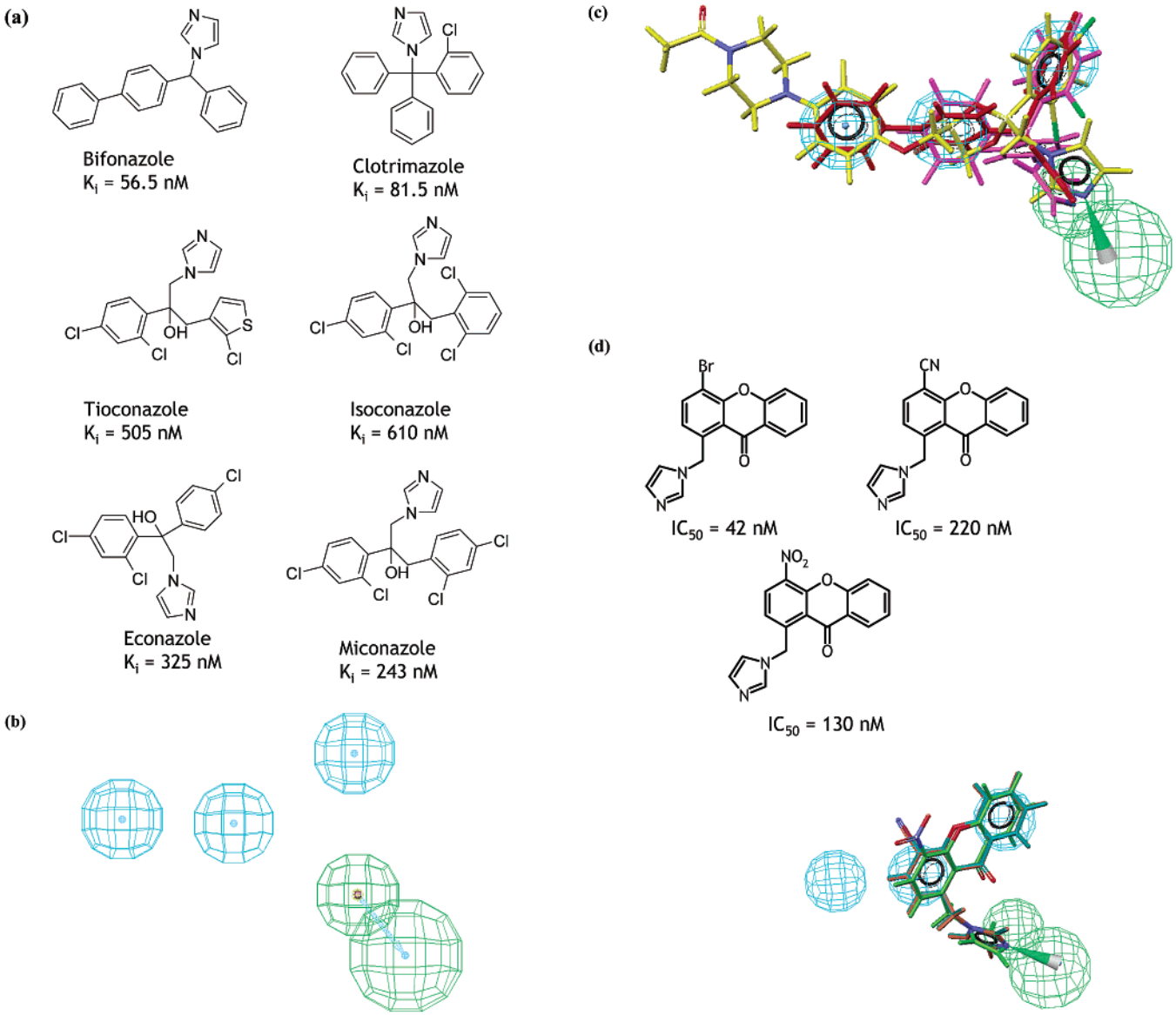

Figure 5. (a) Structures of nonsteroidal human CYP17 inhibitors[13] used in pharmacophore model generation; (b) common chemical-feature-based pharmacophore model generated for nonsteroidal inhibitors (contains four features of three hydrophobes (cyan) and one hydrogen bond acceptor (green)); (c) alignment of ketoconazole (yellow), clotrimazole (magenta), and bifonazole (red) to the pharmacophore model for nonsteroid CYP17 inhibitors; (d) alignment of xanthene-based human CYP17 inhibitors[16b] with pharmacophore model for nonsteroidal inhibitors.

non-steroid-based CYP17 inhibitors, and the fit scores ranged between 2.2 and 4.0.

Recently, three xanthene-based CYP17 inhibitors (Figure 5d) were reported by Recanatini and co-workers,[16b] with activity ranging between 42 and 200 nM. We wanted to evaluate if there were similarities in the binding motifs for heterocyclic CYP17 inhibitors and the xanthene-based ligands by aligning our non-steroid-based pharmacophore model against the Recanatini compounds. This alignment is shown in Figure 5d. When compared with the features mapped by the reference ligand bifonazole, the xanthene-derived compounds map three of four features in the model, with average fit scores of ca. 2.2 out of a maximum score of 4.0. These results suggest that the binding requirements in the heterocyclic and xanthene-based nonsteroidal CYP17 inhibitors are quite similar, and these are largely hydrophobic interactions in accord with the inference of a largely hydrophobic pocket in the active site of this enzyme based on homology models.[12]

Conclusions

In the absence of detailed structural information on the CYP17 binding site, we have employed a ligand-based computational approach to identify ligand requirements for inhibiting this enzyme. This study has provided the first insight into hypothetical ligand binding requirements for steroidal and nonsteroidal inhibition of the CYP17 enzyme. The pharmacophore model proposes a largely hydrophobic interaction between ligand and enzyme, with important hydrogen bond acceptor functionality by a one or two donor atoms on a heteroatomic group. In all cases, the N-3 atom of the imidazole moiety in the two classes of compounds examined here acts as a hydrogen bond acceptor and can be classified as mimicking the proposed interaction

with heme for all type II CYP17 inhibitors.[9] Using these models as search queries, we have identified potent steroidal compounds with nanomolar inhibition of human CYP17. Further work is ongoing to develop unhydrolyzable analogues of these new lead CYP17 inhibitors that may be used in living organisms.

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References

(1) Greenlee, R. T.; Murray, T.; Hill-Harmon, M. B.; Thun, M. J. Cancer Statistics, 2001. *CA—Cancer J. Clin.* **2001**, *51*, 15−36.
(2) Kozlowski, J. M.; Grayhack, J. T. Carcinoma of the prostate. In *Adult and Pediatric Urology*; Gillen-water, J. J., Grayhack, J. T., Howards, S. S., Duckett, J. W., Eds.; Mobsby Year Book: St. Louis, MO, 1991; Chapter 34.
(3) McConnell, J. D. Physiologic basis of endocrine therapy for prostatic cancer. *Urol. Clin. North Am.* **1991**, *18*, 1−13.
(4) Carter, B. S.; Carter, H. B.; Isaacs, J. T. Epidemiologic evidence regarding predisposing factors to prostate cancer. *Prostate* **1990**, *16*, 187−197.
(5) Van Wauwe, J. P.; Janssen, P. A. J. Is there a case for P-450 inhibitors in cancer treatment? *J. Med. Chem.* **1989**, *32*, 2231−2239.
(6) Brodie, A. M. H.; Njar, V. C. O. Aromatase inhibitors and their application in breast cancer treatment. *Steroids* **2000**, *65*, 171−179.
(7) Hall, P. F. Cytochrome P-450 C_{21scc}: one enzyme with two actions: Hydroxylase and lyase. *J. Steroid Biochem. Mol. Biol.* **1991**, *40*, 527−532.
(8) Trachtenberg, J.; Halpern, N.; Pont, A. Ketoconazole: A novel and rapid treatment for advanced prostatic cancer. *J. Urol.* **1983**, *130*, 152−153.
(9) Njar, V. C. O.; Brodie, A. M. H. Inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase (CYP17): Potential agents for the treatment of prostate cancer. *Curr. Pharm. Des.* **1999**, *5*, 163−180.
(10) Ravichandran, K. G.; Boddupalli, S. S.; Haseman, C. A.; Peterson, J. A.; Deisenhofer, J. Crystal structure of hemoprotein domain of P450bm3, a prototype for microsomal P450's. *Science* **1993**, *261*, 731−736.
(11) Bruke, D. F.; Laughton, C. A.; Neidle, S. Homology modelling of the enzyme P450 17α-hydroxylase/17,20-lyase—a target for prostate cancer chemotherapy—from the crystal structure of P450BM-3. *Anti-Cancer Drug Des.* **1997**, *12*, 113−123.
(12) Auchus, R. J.; Miller, W. L. Molecular modeling of human P450c17 (17α-hydroxylase/17,20-lyase): Insights into reaction mechanisms and effects of mutations. *Mol. Endocrinol.* **1999**, *13*, 1169−1182.
(13) Ahmed, S. Molecular modelling of known non-steroidal inhibitors of 17α-hydroxylase/17,20-lyase using antimycotic compound. *Drug Des. Discovery* **1994**, *12*, 77−88.
(14) (a) Ling, Y.; Li, J.; Liu, Y.; Kato, K.; Klus, G. T.; Brodie, A. 17-Imidazolyl, pyrozolyl, isoxaloly androstatene derivatives. Novel steroidal inhibitors of human cytochrome $C_{17,20}$-lyase ($P450_{17\alpha}$). *J. Med Chem.* **1997**, *40*, 3297−3304. (b) Njar, V. C. O.; Kato, K.; Nnane, I. P.; Grigoryev, D. N.; Long, B. J.; Brodie, A. M. H. Novel 17-azolyl steroids; potent inhibitors of cytochrome P450 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$): Potential agents for the treatment of prostate cancer. *J. Med. Chem.* **1998**, *41*, 902−912.
(15) (a) Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. Novel steroidal inhibitors of human cytochrome P450(17α) (17α-hydroxylase-C17,20-lyase): potential agents for the treatment of prostate cancer. *J. Med. Chem.* **1995**, *38*, 2463−2471. (b) Potter, G. A.; Hardcastle, I. R.; Jarman, M. A convenient, large-scale synthesis of abiraterone acetate [3*β*-acetoxy-17-(3-pyridyl)-androsta-5,16-diene], a potential new drug for the treatment of prostate cancer. *Org. Prep. Proced. Int.* **1997**, *29*, 123−134.
(16) (a) Hartmann, R. W.; Hector, M.; Haidar, S.; Ehmer, P. B.; Reichert, W.; Jose, J. Synthesis and evaluation of novel steroidal oxime inhibitors of P450 17 (17α-hydroxylase/17,20-lyase) and 5α-reductase types 1 and 2. *J. Med Chem.* **2000**, *43*, 4266−4277. (b) Recantini, M.; Bisi, A.; Cavalli, A.; Belluti, F.; Gobbi, S.; Rampa, A.; Valenti, P.; Palzer, M.; Palusczak, A.; Hartmann, R. W. A new class of nonsteroidal aromatase inhibitors: Design and synthesis of chromone and xanthone derivatives and inhibition of the P450 enzymes aromatase and 17α-hydroxylase/C17,20-lyase. *J. Med. Chem.* **2001**, *44*, 672−680.
(17) *Catalyst*, release version 4.7; Accelrys, 9685 Scranton Road, San Diego, CA 92121.
(18) Li, H.; Sutter, J.; Hoffmann, R. HypoGen: An automated system for generating 3D predictive pharmacophore models. In *Pharmacophore Perception, Development, and Use in Drug Design*; Güner, O. F., Ed.; International University Line: La Jolla, CA, 2000; pp 171−189.
(19) Barnum, D.; Greene, J.; Smellie, A.; Sprague, P. Identification of Common Functional Configurations among Molecules. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 563−571.
(20) Maybridge Database is available from Maybridge Plc, Trevillett, Tintagel, Cornwall PL34 OHW, England.
(21) Grigoryev, D. N.; Kato, K.; Njar, V. C. O.; Long, B. J.; Ling, Y.-Z.; Wan, X.; Mohler, J.; Brodie, A. M. H. Cytochrome P450c 17-expressing *Escherichia coli* as a first-step screening system for 17α-hydroxylase-$C_{17,20}$-lyase inhibitors. *Anal. Biochem.* **1999**, *267*, 319−330.
(22) Smellie, A.; Teig, S. L.; Towbin, P. Poling: Promoting Conformational Coverage. *J. Comput. Chem.* **1995**, *16*, 171−187.
(23) Sprague, P. W. Automated Chemical Hypothesis Generation and Database Searching with Catalyst. In *Perspectives in Drug Discovery and Design*; Müller, K., Ed.; ESCOM Science Publishers B. V.: Leiden, The Netherlands, 1995; Vol. 3, pp 1−20.
(24) Greene, J.; Kahn, S.; Savoj, H.; Sprague, P.; Teig, S. Chemical Function Queries for 3D Database Search, *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 1297−1308.
(25) Clement, O. O.; Trope-Mehl, A. HipHop: Pharmacophores based on multiple common-feature alignments. In *Pharmacophore Perception, Development, and Use in Drug Design*; Güner, O. F., Ed.; International University Line: La Jolla, CA, 2000; pp 69−83.
(26) Güner, O. F.; Henry, D. R. Metric for analyzing hit lists and pharmacophores. In *Pharmacophore Perception, Development, and Use in Drug Design*; Güner, O. F., Ed., International University Line: La Jolla, CA, 2000; pp 191−211.
(27) Güner, O. F.; Waldman, M.; Hoffmann, R.; Kim, J.-H. Strategies for database mining and pharmacophore development. In *Pharmacophore Perception, Development, and Use in Drug Design*; Güner, O. F., Ed.; International University Line: La Jolla, CA, 2000; pp 213−236.
(28) Edgar, S. J.; Holliday, J. D.; Willett, P. Effectiveness of retrieval in similarity searches of chemical databases: A review of performance measures. *J. Mol. Graphics Modell.* **2000**, *18*, 343−375.
(29) Derwent WDI database is available from Derwent Information Americas, 1475 Duke Street, Suite 250, Alexandria, VA 22314.
(30) Web site: http://www.derwent.com/worlddrugindex/index.html.
(31) (a) Ideyama, Y.; Kudoh, M.; Tanimoto, K.; Susaki, Y.; Nanya, T.; Nakahara, T.; Isikawa, H.; Yoden, T.; Okada, M.; Fujikura, T.; Akaza, H.; Shikama, H. Novel nonsteroidal inhibitor of cytochrome $P450_{17\alpha}$ (17α-Hydroxylase/C17-20 lyase), YM116, decreased prostatic weights by reducing serum concentrations of testosterone and adrenal androgens in rats. *Prostate* **1998**, *37*, 10−18. (b) Yoden, T.; Okada, M.; Kinoyama, I.; Isihara, T.; Sakuda, S.; Ideyama, Y.; Kudoh, M. World Patent, WO 96/26927, 1996.

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