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Letters

Looking for Selectivity among Cytochrome P450s Inhibitors

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Abstract: Cytochrome P450s 19 and 17 are very important pharmacological targets in two different fields of cancer chemotherapy. We present here a theoretical study aimed at explaining the molecular basis of inhibitor affinity and selectivity for either P450 19 or P450 17. Docking simulations of two compounds pointed out the major physicochemical features associated with inhibitory activity. Our results, in agreement with site-directed mutagenesis experiments, could be of relevant utility when designing new P450 19 and P450 17 inhibitors.

Introduction. Cytochrome P450 19, known as aromatase, is an important target of pharmacological interest for the treatment of breast cancer.[1,2] At the same time, cytochrome P450 17 has been recently identified as a very promising target for prostatic androgen dependent diseases, such as benign hypertrophy and carcinoma.[3,4] To design molecules able to specifically inhibit one of the two enzymes is a very important step for obtaining new drugs against these kinds of cancer. In this letter, we present a theoretical study carried out in order to explain the molecular basis of the affinity and selectivity toward these different pharmacological targets. By taking advantage of our previous study,[5] in which molecules able to specifically bind either P450 19 or P450 17 were obtained, we have carried out docking simulations of the most active inhibitors. The molecular criteria at the basis of affinity and selectivity have thus been identified. Therefore, what we finally describe are the physicochemical requirements for the inhibitory activity toward both P450 19 and P450 17. We also discuss the fundamental issue of the selectivity.

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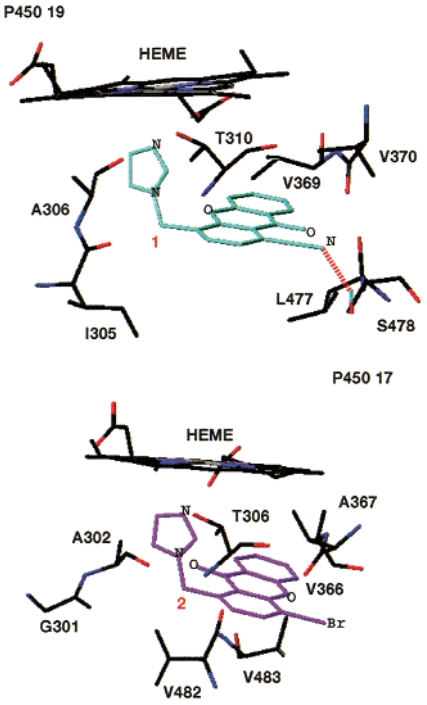

Figure 1. Docking of **1** (cyan) and **2** (magenta) in the active site of P450 19 and P450 17, respectively. Aliphatic residues predominantly make the two active sites. Therefore, either for P450 19 or P450 17 inhibition, hydrophobic ligands are required. The H-bond between the CN group of **1** and S478 of P450 19 is drawn in red. Theoretical models of **1** and **2** were first built and then docked into the active sites of P450 19 and P450 17 by following the procedure already described in a previous study.[6]

In this context, we hopefully provide a good pharmacophoric starting point for the design of new molecules able to specifically act toward one of these two cytochrome P450s.

Results and Discussion. The three-dimensional models of the two cytochrome P450s recently appeared in the literature.[6,7] This allowed us to perform a theoretical investigation of ligands in the active site of their biological counterparts. In particular, two highly active inhibitors of our previous series of compounds[5]

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          30        40        50        60        70        80
P450 19   LLLTGLFLLVWNYEGTSSIPGPGYCMGIGPLISHGRFLWMGIGSACNYYN--RVYGEFMR
          ::: : : : . . : ... ::.. :: :... . :: ..
P450 17   LLLLTLAYLFWPKRRCPGAKYPKSLLSL-PLVGSLPFLPRHGHMHNNFFKLQKKYGPIYS
            10        20        30        40        50        60

            90       100       110       120       130       140
P450 19   VWIS*G*EETLIISKSSSMFHIM--KHNHYSSRFGSKLGLQCIGMHEKGIIFNNNPELW*K*TT
          : .. . :.:... . ... : . .:.: . :. . ..::: : .. :.
P450 17   VRM*G*TKTTVIVGHHQLAKEVLIKKGKDFSGR-PQMATLDIASNNRKGIAFADSGAHW*Q*LH
            70        80        90       100       110       120

            150       160       170       180       190       200
P450 19   RPFFMK--ALSGPGLVRMVTVCAESLKTHLDRLEEVTNESGYVDVLTLLRRVMLDTSNTL
          : . : :: : .. . . ..: : : .:... .:. . .. .. . .
P450 17   RRLAMATFALFKDGDQKLEKIICQEISTLCDML--ATHNGQSIDISFPVFVAVTNVISLI
            130       140       150       160       170       180

              210       220       230       240       250
P450 19   FLRIPLDESAIVVK-IQGYFDAWQALLIKPDIFFKISWLYKKYEKSVKDLKDAIEVL---
          . .. .. ::.: .. : : .. . :: .:... ::. ...
P450 17   CFNTSYKNGDPELNVIQNYNEGIIDNLSKDSLVDLVPWLKIFPNKTLEKLKSHVKIRNDL
              190       200       210       220       230       240

            260       270       280       290               300
P450 19   ---IAEKRRRISTEEKLEECMDFATELILAEKRGD---------LTRENVNQCIL*E*ML*IA*
            : :. .. ... . .: . . :. :. ... : ... :
P450 17   LNKILENYKEKFRSDSITNMLDTLMQAKMNSDNGNAGPDQDSELLSDNHILTTIG*D*IF*GA*
              250       260       270       280       290       300**

           310       320       330       340       350       360
P450 19   APD*T*MSVSLFFMLFLIAKHPNVEEAIIKEIQTVIG-ERDIKIDDIQKLKVMENFIYESM*R*
          . .: . . . : .. ..:.:.. . .:::. .: : :.: ..: ..: : : .:
P450 17   GVE*T*TTSVVKWTLAFLLHNPQVKKKLYEEIDQNVGFSRTPTISDRNRLLLLEATIREVL*R*
            * 310       320       330       340       350       360

            370       380       390       400       410       420
P450 19   YQPVVDLVM-*R*KALEDDVIDGYPVKKGTNIILNIGRMHRLE-FFPKPNEFTLENFAKNV-
          .::. ... .:: :. : . : :::..:.:. .:. : . .:..: : : . .
P450 17   LRPVAPMLIP*H*KANVDSSIGEFAVDKGTEVIINLWALHHNEKEWHQPDQFMPERFLNPAG
            **370       380       390       400       410       420

                  430       440       450       460       470
P450 19   -----PYRYFQP*F*GF*G*PRG*C*A*G*KYI*A*MVMMKAILVTL*L*RRFHVKTLQGQCVESIQKIHDL
                : . ::: :::.: :. .: . :.. ::.:: ... . . :.. : .
P450 17   TQLISPSVSYLP*F*GA*G*PRS*C*I*G*EIL*A*RQELFLIMAWL*L*QRFDLEVPDDGQLPSLEGIPKV
              430       440       450       460       470       480 *

          480
P450 19   SLHPDETK
          . : :
P450 17   VFLIDSFK
          *    490
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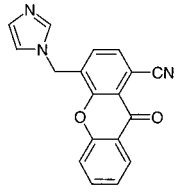
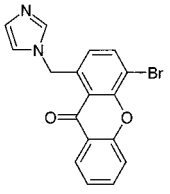
Figure 2. Sequence alignment between P450 19 and P450 17. The identical and conserved amino acids are labeled by two and one point(s), respectively. In ***italic-bold*** are indicated the residues invariant or highly conserved in the cytochrome P450s family.[6,15] The amino acids that have been identified as involved in interactions with **1** and **2** are labeled with asterisks. All of these except S478 and V483 are conserved hydrophobic amino acids in both enzyme active sites. The pairwise alignment of the two sequences was carried out by means of LALIGN program[16] of the FASTA package.[17] The opening gap penalty and the extending gap penalty were set to −14 and −4, respectively. PAM250 scoring matrix was used.

(**1** and **2**; Chart 1) were docked into the active site of P450 19 and P450 17, respectively. Minimum energy conformations of the two docking complexes are shown in Figure 1. The inhibitors bind to the two enzymes in a similar fashion. The most important interaction is indeed the coordination bond between the lone pair carrying the N atom of the imidazole ring and the heme iron of the two enzymes. As already discussed, the imidazole ring showed to be a very good sixth coordinating moiety of the heme because of its ability to carry the highest occupied molecular orbital (HOMO) involved in the bond with the iron atom.[5] This can be considered

Chart 1



1



2

Compound	*P450 19 inhibition (nM)*[5]	*P450 17 inhibition (nM)*[5]
1	43	Inactive
2	940	42

the primary interaction between enzyme and inhibitors. The other moieties of the ligands are surrounded by highly hydrophobic aliphatic amino acids. Namely, they are I305, A306, T310, V369, V370, and L477 of P450 19, and G301, A302, T306, V366, A367, and V482 of P450 17. As shown in Figure 2, in which the alignment between the two protein sequences is reported, the hydrophobic residues above indicated are very well conserved between the two enzymes. This is reflected by the strong chemical similarity between the two inhibitors' structure. On the contrary, S478 of P450 19, which is directly involved in a H-bond interaction with the inhibitor **1** (see Figure 1), is not conserved between the two enzymes. Actually, in P450 17 there is a Val (V483, see Figure 2) instead of a H-bond donor amino acid. This makes the active site of P450 17 more hydrophobic than the P450 19 one and unable to H-bond ligands. Therefore, the most striking difference between the two enzyme active sites seems to be the capability to establish a H-bond interaction with inhibitors. It is now clear why **1** is very active toward P450 19, while **2** is the most potent inhibitor we obtained toward P450 17. In fact, as shown in Figure 1, **1** is able to H-bond S478 by means of the CN group and maybe even through the carbonyl group of the xanthone moiety. On the other side, **1** is a very weak inhibitor toward P450 17, because it would bring hydrophilic groups (i.e., CN and xanthone carbonyl group) in a very hydrophobic environment of the enzyme active site. Concerning **2**, its weak ability to inhibit P450 19 can be explained by the lacking of a strong H-bond acceptor group. In fact, the bromine atom present on the xanthone ring has electrons delocalized on the aromatic moiety, and therefore, its ability to accept proton in H-bond interaction is rather low. On the contrary, the bromine atom greatly increases the overall hydrophobicity of the molecule (log $P = 2.57$[5]), and consequently the interaction of **2** with V483 is indeed better than that established by **1**. Moreover, in **2** the xanthone ring is "inverted" with respect to **1**. This further reduces the hydrophilicity of the molecule in that specific active site region and therefore ameliorates the interaction with P450 17.

Summarizing, the physicochemical features required for strong inhibitor binding to either P450 19 or P450 17 can be indicated as follows.

(i) First, molecule candidates to become cytochrome P450s inhibitors should be able to strongly interact with the iron atom of the heme group, always present in this enzyme family. To this aim, the imidazole ring has shown to be the best-suited heterocycle. However, other electron rich heterocycles might be accepted in this position as well.[8]

(ii) Second, the inhibitors should have a high hydrophobic character because of the predominant presence of aliphatic amino acid residues in both enzyme active sites. As previously mentioned,[5] positive log *P* values are associated with a good biological activity against aromatase, and moreover, higher log *P* values might be actually required for the inhibition of P450 17. The fact that P450 17 has one more hydrophobic residue in the active site pocket with respect to P450 19 makes log *P* a fundamental parameter to consider when P450 17 inhibitors are looked for.

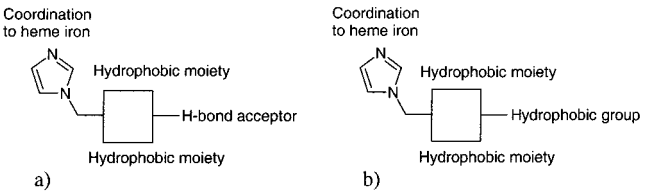


Figure 3. Physicochemical features required for good P450 19 (a) and P450 17 (b) inhibitory activity. As reported in the text, S478 of P450 19, which corresponds to V483 of P450 17, might be the critical point to consider for the selectivity. This is reflected in the requirement of a H-bond acceptor group for P450 19 inhibition (a). On the contrary, such a group should not be present when P450 17 inhibition is pursued (b). The overall hydrophobicity should be in both cases sufficiently high.

Then, one has to consider the issue of the selectivity. Actually, the physicochemical features of points i and ii depict quite well inhibition of *both* enzymes. This is in agreement with the high sequence homology between the two active sites (Figure 2)—probably due to the fact that these enzymes catalyze very similar reactions (a C−C bond cleavage[9])—and also it may well explain the striking structural similarity between the inhibitors **1** and **2**. However, S478 of P450 19 and V483 of P450 17 are key residues to consider when designing selective ligands. While P450 19 inhibitors need a chemical group able to accept a H-bond from S478, for those active toward P450 17 such requirement is unnecessary. Due to the lack of a H-bond donor residue in the position correspondent to S478, in the case of P450 17 inhibitors, it may be better to increase the overall hydrophobicity to ameliorate the affinity toward this enzyme. These concepts are schematically depicted in Figure 3, where the most important pharmacophoric requirements characterizing a good inhibitor toward either P450 19 or P450 17, are shown.

Remarkably, the role of a H-bond acceptor group in the binding of nonsteroidal P450 19 inhibitors was previously pointed out in a molecular modeling study performed by Furet et al.[10] Furthermore, in a docking model developed by Koymans et al.,[11] such a pharmacophoric group was suggested to bind in an active site region where residues such as D309, S478, and H483 are present. Our results are in-line with these earlier studies.

In addition, the above-presented hypothesis is in very good agreement with site-directed mutagenesis experiments recently carried out on both P450 19[12] and P450 17.[13,14] In particular, concerning P450 19, it was shown that S478 is located in the active site of the enzyme and, moreover, that it might play a crucial role in the first and second hydroxylation reactions occurring in the reactive process catalyzed by aromatase.[12] At the same time, V483 of P450 17 is certainly an amino acid of the enzyme active site,[7] which can be argued also by studies concerning the molecular basis of carboxyl-terminal mutations. In particular, either a frame shift after P480 or premature stop codon at Q461 completely eliminated all P450 17 enzymatic activities.[13,14]

Finally, we wish to remark that this work might be considered as a first step in determining the molecular basis of selectivity among cytochrome P450s inhibitors. Actually, the enzymes considered in this study were two

out of the great number of proteins belonging to this family. We can expect that further research will increase the number of resolved mammalian cytochrome P450 structures and, therefore, bring us the opportunity to carry out similar work on a widespread set of protein targets. Work in progress aimed to obtain new molecules able to fit the pharmacophoric scheme of Figure 3 will hopefully support such a hypothesis.

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