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Cataloging the Repertoire of Nature's Blowtorch, P450

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Veith et al. (2009) analyzed over 17,000 compounds for interaction with the five major human cytochrome P450 drug-metabolizing enzymes. Analysis of the results provides some structural insight and framework for expansion.

Cytochrome P450 (P450 or CYP) enzymes are involved in approximately three fourths of the reactions of drug metabolism in humans, and five liver P450s (1A2, 2C9, 2C19, 2D6, 3A4) account for greater than 90% of these reactions (Guengerich, 2005). Many drug-drug interactions and drug side effects can be attributed to variations in these P450s and to the interaction of drugs with the P450s (e.g., inhibiting metabolism of each other via competitive or irreversible interactions). In silico prediction of P450 liganding would be desirable, but even with P450 crystal structures available, the malleability of many of the P450s has confounded docking predictions. Collectively these five P450s probably have millions of substrates and inhibitors, if one considers all of the potential natural and synthetic substrates. Oxidation of drugs by the high-valent iron chemistry of P450 (the ''blowtorch'') seems to be largely a function of getting a particular atom of a molecule into the right place in the active site (Ortiz de Montellano and De Voss, 2005). Many drug candidates are discarded because metabolism is too fast, and some drugs are almost completely resistant to oxidation for unknown reasons (e.g., rosuvastatin, varenicline).

Veith et al. (2009) screened libraries containing a total of 17,193 compounds for the inhibition of activity of these five P450s using high-throughput luminescence assays developed by Promega. Although the term ''activity'' was used here, the authors really mean inhibition, although (as pointed out by the authors) the results do not discriminate between a test compound being a substrate or an inhibitor. With regard to the title, the individual P450 enzymes are not ''isozymes,'' in the sense that they do not necessarily catalyze the same reaction. The results, obtained by varying concentrations of each test compound, were compiled and used to propose some structure-activity relationships.

Although information of this type has been accumulated in some pharmaceutical companies (Afzelius et al., 2007) this effort of Veith et al. (2009) appears to be the largest public effort of this type to date. Several interesting features have come out of the analysis. The authors found that the U.S. Food and Drug Administration (FDA)-approved drugs within the set showed less P450 inhibition than did the rest of the library, which is logical because modern screening strategies should discriminate against strong P450 inhibitors. An interesting point is that a substantial fraction of the compounds tested (5%–10%) had IC_{50} values $< 1\ \mu M$, which has been a rough guide for potential concern in some companies. Another interesting result is that 350 of the 17,143 compounds ($\sim$2%) inhibited all five P450s. The authors state that amines were overrepresented in such ''pan-activity.'' In Figure 4 of Veith et al. (2009), the analysis shows an overrepresentation of thiophenes and methylenedioxyphenyl moieties in the inhibition of all P450s, which may not be surprising in light of the known tendencies of these two classes of compounds to be oxidized to reactive products (Nelson, 1982; Guengerich and MacDonald, 2007). Indoles, in this class, are often substrates for P450s (Gillam et al., 2000), as are naphthalenes. The inclusion of pyrimidines as pan-active is a surprise, in light of general knowledge of drug metabolism. What is missing from the analysis (and may not be feasible) is a definition of patterns of inhibition, regarding where in a large molecule a particular group is positioned. One group of compounds that was not reported for pan-activity was azoles, which have been historically recognized as P450 inhibitors due to heme liganding (and have even been used as drugs to block some P450s, such as yeast P450 51A1).

One of the intriguing areas of interest in the P450 field is the stimulation of some reactions by ligands, which still defies mechanistic explanation. Such stimulation was reported for $\sim$3% of the compounds with P450s 2C9, 2C19, and 3A4. The in vivo relevance of this phenomenon in humans is still a matter of speculation.

One limitation of this work is that the results were collected using only the luminescence-generating substrates, which the authors acknowledge. These (and fluorescence assays) have lost some favor in the pharmaceutical industry in screening assays in recent years. However, the authors do a reasonable job of correlating their results with standardized P450 assays, in subsets of the library.

Veith et al. (2009) do not distinguish between P450 substrates and inhibitors, as the authors acknowledge. That is, a P450 substrate would also be an inhibitor in this assay (Figure 1). It is also possible that a substrate might be oxidized to attenuate inhibition in the assay (Shimada et al., 2008). Another limitation of the work, acknowledged by the authors, is that this screen is not designed to identify mechanism-based inhibitors (also called time-dependent or suicide inhibitors), which are fairly common (particularly with P450 3A4) and more problematic than simple competitive inhibitors. In principle, these should show up in the primary screen because they are also necessarily competitive inhibitors and

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could then be screened in pre-incubation settings (Silverman, 1995).

The results of Veith et al. (2009) can be used to predict inhibition by drug candidates, but a number of other factors are involved (Food and Drug Administration, 2006) and ultimately the true test for inhibition is an in vivo human experiment. The results can also be used to predict if compounds are substrates, and the methods for following these up are relatively straightforward. Finally, the information, if available, could be coupled with similar screens of other libraries—perhaps even if run on different platforms—to expand the results. The database used in this analysis is available in the online supplemental information.

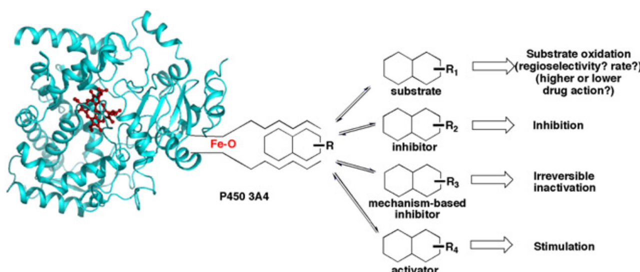

Figure 1. Outcomes of Interaction of a Ligand with a P450
The interaction of a compound with a P450 (P450 3A4 structure is shown, pdb code 1TQN) can be related to the chemical being a substrate, inhibitor, irreversible inhibitor, or stimulator—or combinations thereof. Also note that many P450 ligands can interact via multiple binding modes, leading to multiple products in the case of substrates (Guengerich, 2005).

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Targeting Multiple Biofilm Pathways

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Cegelski et al. (2009) demonstrate the importance of amyloid production for biofilm formation and host colonization using several mutant strains of pathogenic *E. coli* and small molecule inhibitors. This work reveals a path forward for studying the role of bacterial amyloids in vivo and suggests the potential for small molecules to target multiple biofilm formation pathways.

The increased prevalence of antibiotic resistant bacteria heralds a need for new drugs and novel strategies for identifying better drug targets. One such strategy is to target microbial virulence factors, which are important for causing pathology but are not required for the microbe to survive in vitro. This strategy avoids targeting essential gene functions, which may result in strong evolutionary selection for resistant strains. While this idea remains theoretical, efforts have been increased to develop new antibiotics based on this principle. One virulence process of particular interest to target is biofilm formation because of the associated antibiotic insensitivity of bacteria surviving within biofilms. Cegelski et al. (2009) have recently generated tools to allow researchers to address the relative importance of different bacterial attachment strategies during biofilm formation in vivo in a model for urinary tract infection.

Many pathogenic bacteria elaborate virulence factors in order to cause disease. Examples of factors include secretion systems to inject effector molecules into host cells, secreted toxins that manipulate host cell processes or outright kill host cells, quorum-sensing systems that

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