High throughput P450 inhibition screens in early drug discovery

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This review of high throughput (HT) P450 inhibition technologies and their impact on early drug discovery finds the field at a mature stage. The relationship between P450 inhibition and drug–drug interactions is well understood. A wide variety of P450 inhibition detection technologies are readily available off-the-shelf, but what seems still to be missing is a general agreement on how much weight one should give to the various types of early discovery HT P450 inhibition data. Method-dependent potency differences are a cause of concern, and to resolve this issue the authors advocate calibration of the HT methods with a large set of marketed drugs.

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Rationale for early compound profiling

Drug discovery is a multi-parameter optimization process in which compounds are optimized for interaction with the desired target and minimal off-target activities, while imparting drug-like properties on the candidate compounds. Project teams often pursue a parallel optimization approach in which multiple scaffolds are explored along multiple axes, including potency, selectivity, physicochemical properties and absorption, distribution, metabolism, excretion and toxicity (ADME–Tox) parameters [1–6]. This is to minimize the risk of making potent and target-class selective compounds in a chemical space that might be entirely incompatible with modifications needed to address other liabilities. Hence, all these molecular activities and properties need to be determined early on many compounds, to progress the overall best leads or scaffolds to the pre-clinical candidate stage. Such an approach requires a high throughput (HT) assay infrastructure, providing all relevant parameters for many, preferably all, members of privileged scaffolds at a reasonable cost and speed. Optimally, all data should be available concurrently with primary-target affinity information.

Multi-parameter optimizations are complex and project teams need to decide early upon which parameters, in addition to potency and selectivity, to focus on and to devise strategies to tackle the optimization challenges. Ideally, some liabilities can be addressed with targeted scaffold modifications that are tolerated with regard to target potency and class selectivity. Examples relating to CYP3A4 inhibition will be presented below.

Case study

As part of ADME–Tox profiling, compounds are routinely tested *in vitro* for the potential to cause drug–drug interactions [7]. These are adverse events in which a drug influences the levels of a co-administered drug, either increasing or decreasing its levels. The most prominent cases involve the inhibition of the drug's metabolism, which can raise drug levels to toxic concentrations [8]. Prominent contributors to drug metabolism are a family of oxidizing enzymes

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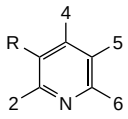
TABLE 1

CYP3A4 inhibition within a series of pyridine-containing analogues



	IC_{50} (μM) with substituent at position:			
Substituent	**2**	**4**	**5**	**6**
H	3	3	3	3
F	71	–	5	>200
OCH_3	>200	7	1	>200
CH_{2OH}	74	–	–	–
NMe_2	13	5	1	–
OH	–	–	10	–
Cl	–	15	–	–
CH_3	–	5	–	–
OCH_2Ph	–	2	–	–

Key: R, side chain; –, not tested.
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called cytochromes P450 (P450s). These monooxygenases are involved in the metabolism of >60% of all marketed drugs. Cimetidine (Tagamet®, GlaxoSmithKline), a drug for the treatment of heartburn and acid reflux, quickly lost sales to competing products as its drug–drug interaction liability emerged in clinical and post-market approval studies [9]. Mibefradil (Posicor®, Roche) was withdrawn from the market in 1998 because it potently inhibits CYP3A4, the predominant drug metabolizing P450, after having led to adverse events in patients concurrently taking a variety of other commonly prescribed medicines [10]. Alerted to the dire consequences of bringing P450 inhibitors to market, the pharmaceutical industry began testing candidate drugs sooner in discovery to be able to act early and move away from unwanted interactions.

Examples of the use of P450 inhibition data in drug discovery are CYP3A4 inhibitions by two pyridine-attached scaffolds. In the first example, which relies on a small dataset generated by traditional low throughput HPLC-based methods [11], the authors investigated CYP3A4 inhibition by changing the steric hindrance of the pyridine nitrogen via 2- or 6-substitution relative to the pyridine nitrogen, as shown in Table 1. Steric bulk next to the pyridine nitrogen weakens the coordination of the nitrogen to the P450 prosthetic heme iron. This approach is very efficient in changing CYP3A4 activity and several small substituents reduced CYP3A4 inhibition by more than one order of magnitude.

The other example is from an in-house project that employed HT screens to monitor the progress of an optimization for target potency while minimizing CYP3A4 inhibition, shown in Figure 1. The medicinal chemistry effort focused on a 4-substituted pyridine series. The initial 2,6-unsubstituted 4R-pyridines showed a wide spread of target as well as CYP3A4 activities (Figure 1, gray diamonds), with the majority of compounds being sub-micromolar inhibitors of CYP3A4. Although one could argue that a few potent inhibitors of the target had a lower CYP3A4 liability, it was realized that further optimization of these series for other parameters ran the danger of creating compounds with activities closer to the center of the distribution and towards sub-micromolar inhibition of CYP3A4 (Figure 1, region indicated by the arrow). It seemed safer to substitute positions 2 or 6 of the pyridine and indeed, for most compounds, various 2-substituents (Figure 1, blue triangles, yellow circles and green square) reduced CYP3A4 inhibition potency to >1 μM, whereas, for instance, 3-fluoro substitution (Figure 1, red stars) was ineffective in dealing with the CYP3A4 liability. In both examples, the early optimization of the CYP3A4 inhibition parameter in scaffold evolution by targeted substitution removed this factor from the multi-parameter optimization matrix, allowing teams to focus on other issues, probably without the need to revisit CYP3A4 inhibition at a later stage.

In addition to pyridine moieties, terminal imidazoles are notorious for inhibiting multiple P450 isozymes, and azole antifungals as well as the antihistamine cimetidine [9] have been reported to display drug–drug interactions as a result of this activity. To ameliorate the P450–imidazole interactions, substitution patterns on imidazole derivatives have been explored. In an SAR study on imidazoles, 2- and/or 4-substitution led to compounds that are essentially inactive in inhibiting aldrin epoxidation in rat liver microsomes [12]. In humans, however, 2-methylimidazoles are known precursors to reactive metabolites, and potent P450 inhibition has been observed upon metabolism of this moiety [13]. There are several other chemical features that lead to inhibition of P450 enzymes, such as terminal olefins and acetylenes, quinolines, amines, hydrazines, hydrazones and methylenedioxy phenyls, to mention a few [14].

Prediction of potential drug–drug interactions involving lesser-studied P450 isoforms

In a recent publication, Walsky *et al.* [15] demonstrated the use of large P450 inhibition datasets in uncovering the potential of a drug to cause drug–drug interactions as a result of the inhibition of the P450 isoform CYP2C8. They tested the inhibition of CYP2C8 by 209 commonly prescribed drugs, to identify medicines with the potential to interact with other drugs that involve in their metabolism this P450 isozyme, such as paclitaxel, repaglinide, rosiglitazone and cervistatin. Although most tested drugs were not expected to cause clinically relevant drug interactions, montelukast was identified as a potent inhibitor of CYP2C8, with the potential of causing clinically relevant interactions with CYP2C8 drug substrates. Armed with such knowledge, companies should gauge the risk of inhibiting a minor P450 isoform with a new candidate drug. To establish *in vivo* relevance, companies should