



Codexis Metabolite Platform

Background and New Approaches

Drugs are metabolized in the liver primarily via oxidative and conjugative transformations. The new compounds generated can have different toxicity and activity profiles than the parent. It has been estimated that most drug candidates fail due to problems resulting from their metabolites rather than the parent compound.¹ Recently, there has been increased emphasis in drug discovery in the area of safety testing of drug metabolites.² This has led to proposals that provide a standard framework for metabolite characterization.³ Human Cytochrome P450 enzyme systems are involved in the metabolism of approximately 90% of drugs currently on the market.⁴ The question is: how best to produce these often low abundance compounds?

Codexis has developed a line of products and services to make the identification and generation of metabolites easier. The recombinant Codexis Human Cytochrome Biocatalysts screening kit and Codexis MicroCyp screening plate allow for the efficient screening of drug metabolites and hydroxylated lead compounds related to a compound of interest. The Human Cytochrome and MicroCyp lyophilized enzyme powders enable the preparation of microgram to gram quantities of the resulting metabolites or lead compounds. The metabolite synthesis services utilize Codexis' expertise in making and purifying metabolites. Tools used in this service include the Human Cytochrome Biocatalysts, the Codexis MicroCyp enzymes, an in-house diverse library of microbial strains, and third party microsomes. Codexis has experience in the preparation of oxidized, de-alkylated and conjugated metabolites like glucuronides, glutathione and *N*-acetyl-cysteine derivatives.

Codexis Human Cytochrome Biocatalysts (HCB)

Codexis has developed recombinant Codexis Human Cytochrome Biocatalysts (HCB) to facilitate the production of metabolites. The HCBs are human cytochrome P450 enzymes recombinantly expressed in *E. coli* at high levels along with their human reductase domain. The HCB set is available as a screening kit for the rapid identification of metabolites related to a compound of interest. The screening kits are formulated with all the components necessary for a fully functional catalytic system to generate metabolites, including cofactor and associated recycling system. Each HCB is also available as a stable, lyophilized powder with everything necessary for metabolite synthesis. The user only needs to add water and the compound to be metabolized. Because of the high enzyme expression levels, the cost of the system is up to 75% less than with other expressed CYPs, making preparation of metabolites with this system practical.

Codexis MicroCyp Platform

For the past twenty years, synthetic organic chemists have asked the biotechnology community for enzymatic tools to generate hydroxylated compounds for drug discovery applications such as lead diversification and library expansion. In addition, while the HCB products are useful for generating material for structure

ID and limited testing applications, these enzymes are often not practical for use in synthesizing larger amounts of metabolites or lead compounds. Now, there is a solution to both these needs in the Codexis MicroCyp Platform.

The Codexis MicroCyps are a collection of bacterial enzymes (variants of *Bacillus megaterium* cytochrome P450s, or BM3s) that have been evolved to produce mammalian-type metabolites.⁵ The BM3 variants show broad substrate specificity and are expressed at high levels in their bacterial host. MicroCyps have shown up to 100-fold increases in productivity when compared with human P450 activity. Combined with their lower cost of manufacture, MicroCyp variants can produce even gram quantities of metabolites cost effectively (Figure 2). In addition, the MicroCyps are capable of other types of transformations that can be useful in lead diversification programs (Figure 3). Codexis MicroCyps are available in two formats. A screening plate contains 86 unique BM3 variants and is provided with all the necessary reagents and protocols to enable rapid identification of the BM3 enzyme(s) producing the compound of interest. The plate is designed to be run in a typical synthetic organic chemistry laboratory or a standard screening laboratory. In addition, each individual BM3 variant is available as a lyophilized enzyme powder for scale up synthesis of the hydroxylated product.

Codexis MicroCyp Platform (CONTINUED)

Figure 1 illustrates the results of an experiment screening the Codexis MicroCyp plate with two generic drugs and one proprietary compound. The MicroCyp enzymes are significantly active for all of the compounds. Additionally, these enzymes can accommodate higher starting substrate concentrations allowing for more metabolite to be made per unit volume. Thus the generation of milligram to gram quantities of metabolite can be obtained using the MicroCyp enzymes.

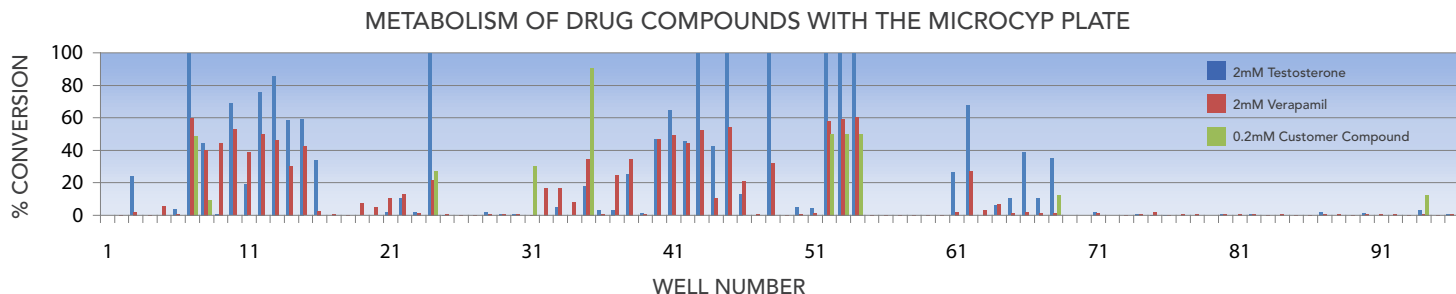


Figure 1 – Results of a MicroCyp screening plate used to prepare metabolites of testosterone, verapamil and a proprietary compound. Percent conversion is on the y-axis and well number is on the x-axis.

Codexis Metabolite Screening and Synthesis Service

If resources within your company are not available, Codexis offers metabolite screening and synthesis services—through Codexis' Metabolite Synthesis Group (CMSG).

With state of the art LCMS systems and a team of dedicated scientists at our facility, CMSG can quickly screen the appropriate biotransformation system and then prepare your metabolites or lead compounds for you. Any aspect of this work, or all of it can be contracted out to CMSG. Oxidative and conjugative metabolites have been prepared for over 30 drug candidate compounds at a scale from micrograms to multi-milligrams.

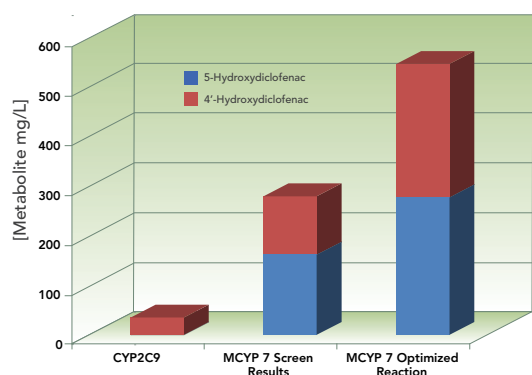


Figure 2 – Gram scale production of 2 diclofenac metabolites using a MicroCyp enzyme (2.5L optimized reaction yields ~1g metabolites).

Summary

Making metabolites or expanding lead compound libraries has never been easier. Codexis can provide recombinant human cytochrome P450s in easy-to-use formulations and MicroCyps demonstrating very high productivity, enabling metabolite synthesis to be more efficient and cost-effective than ever before. Using these novel products, Codexis provides the tools and services you need to support your drug development efforts.

For sales and services, contact pharmaservices@codexis.com.

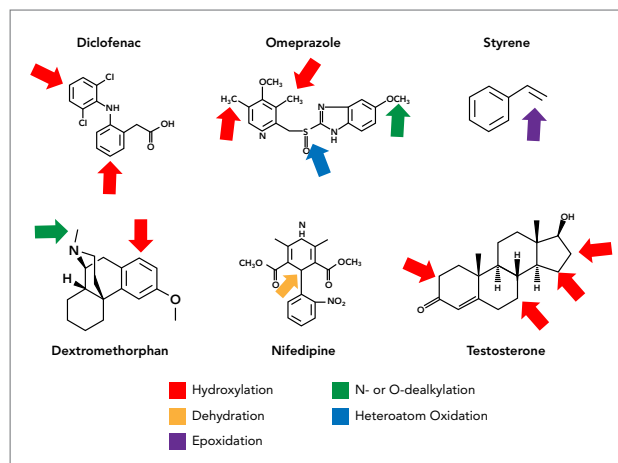


Figure 3 – Many known p450 chemistries are also observed using MicroCyp enzymes.

References

- 1 C.K. Svensson. 2006. Commentary: Drug Hypersensitivity — Where Do We Stand? *AAPS J.* 8: E236-8.
- 2 US-FDA. 2008. Safety Testing of Drug Metabolites. www.fda.gov/cder/guidance/#Pharmacology/Toxicology
- 3 L. Leclercq et al. 2009. Which human metabolites have we MIST? Retrospective analysis, practical aspects, and perspectives for metabolite identification and quantification in pharmaceutical development. *Chem Res Toxicol* 22: 280-93.
- 4 T. Lynch et al. 2007. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician* 76: 391-6.
- 5 F.H. Arnold et al. 2006. Preparation of human metabolites of propranolol using laboratory-evolved bacterial cytochromes P450. *Biotech Bioeng* 93: 494-9.