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Emerging technologies for metabolite generation and structural diversification

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ABSTRACT

Multiple technologies have emerged for structural diversification and efficient production of metabolites of drug molecules. These include expanded use of enzymatic and bioorganic transformations that mimic biological systems, biomimetic catalysis and electrochemical techniques. As this field continues to mature the breadth of transformations is growing beyond simple oxidative processes due in part to parallel development of more efficient catalytic methods for functionalization of unactivated scaffolds. These technologies allow for efficient structural diversification of both aromatic and aliphatic substrates in many cases via single step reactions without the use of protecting groups.

The generation of metabolites of active drug molecules is an important part of drug discovery as we attempt to understand the fate of pharmaceutical agents in the body. Typically, a circulating metabolite is sought out and isolated from an in vivo study as the result of poor PK or unexpected pharmacology. Confounding this analysis can be the transient or reactive nature of some metabolites.[1] Subsequent to tedious mass spectral analysis, a structure is proposed and a first attempt to prepare sufficient material might be carried out using liver slices or microsomes, for example.[2] When larger quantities are required, the task of producing a particular metabolite is assigned to the medicinal chemist via a dedicated synthetic route that may or may not intersect common building blocks. In addition this process occasionally requires redesign when the proposed molecule fails to match the isolated metabolite. Out of this need, various more efficient biomimetic technologies have been developed to aid medicinal chemistry teams in the production and study of metabolites.[2,3] The field of biomimetic chemistry has grown beyond simple oxidative transformations and now includes many other related disciplines that allow for structural diversification.[4] The purpose of this review is to highlight technologies related to structural diversification and generation of metabolites including enzymatic, catalytic and electrochemical methods. Together, these and other technologies are making single point structural modifications more efficient.[5]

The desire to selectively functionalize lead molecules is not new. For example, during the golden age of steroid research, teams utilized enzymatic transformations to create a diversity of biologically active steroids from plant based starting materials.[6] In the decades since, the understanding of the mechanisms underlying biological oxidation processes has increased[7] and research teams have been inspired to prepare novel metalloporphyrin catalysts that mimic these reactions. Research in this area continues to expand beyond metabolite production into the novel functionalization of common building blocks. However as Groves, et al. have pointed out, even with the recent advances in bioinorganic chemistry, the structure and reactivity of all of the metalloenzymes is not fully understood.[8] With the recent growth in the use of electrochemistry, the collection of efficient technologies available for structural diversification is beginning to grow. The ability to manipulate unfunctionalized molecules in ways that were previously inaccessible via traditional methods is particularly relevant

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to drug discovery for example.[9,10] Structural diversification allows the designer to navigate through safety and toxicity challenges while the ability to selectively oxidize molecules can be advantageous to physicochemical properties via lowering of the log *P*.

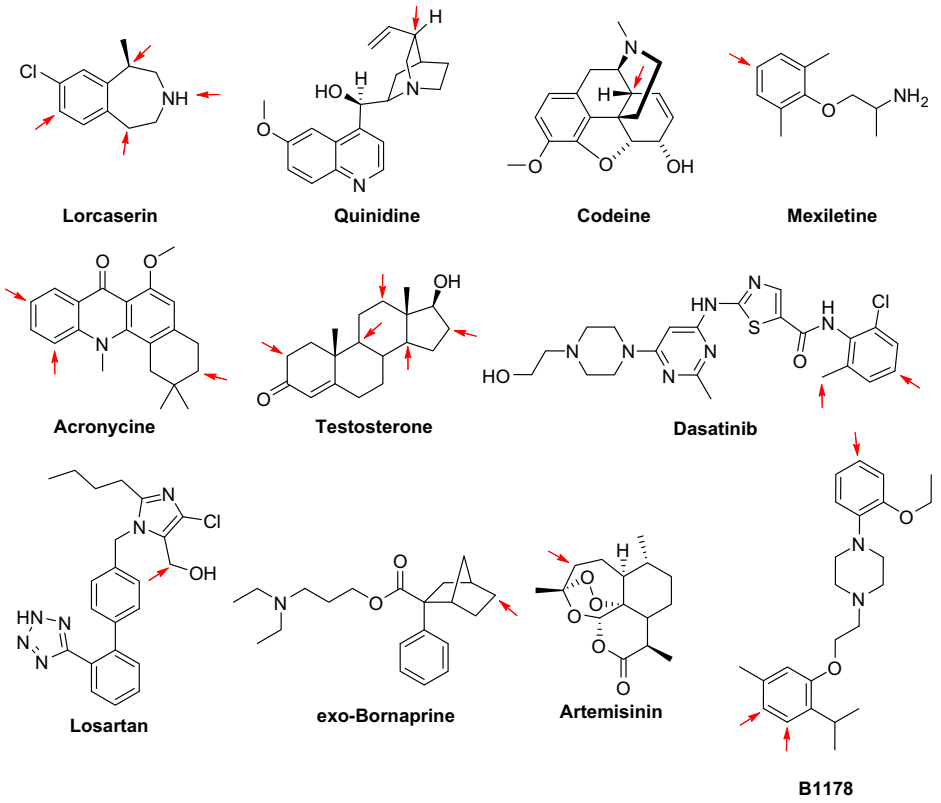
Biocatalysis: Access to specific drug metabolites is often key for the success of drug discovery programs and enzymatic transformations can contribute to the synthesis of metabolites and pseudometabolites (metabolites not observed in mammals). This section focuses on oxidative phase I metabolic transformations[11] involving cytochrome P_{450}s (CYPs), flavin-dependent monooxygenases (FMOs), monoamineoxidases (MAOs) and dehydrogenases.[12] Phase II transformations, while important, are beyond the scope of this digest.[13] Metabolic phase I transformations include a wide variety of reactions like dealkylations, epoxidations and isomerizations, but site-selective hydroxylations by direct C–H functionalization are among the most intriguing (Scheme 1).[14] The vast majority of hydroxylations in mammalian metabolism of xenobiotics results from the action of cytochrome P450s, in particular CYP1A2, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4.[15]

Besides microsomal preparations, pure recombinant CYP (rCYP) and FMO lyophilisates are standard tools with high value in metabolism-related R&D assays. However, compared to the use of recombinant enzymes, the expression of a certain enzyme in a bacterial host represents a modification of this technique being suitable for small scale preparative metabolite generation. Furthermore, the high potential of microorganisms in whole cells has been used for the creation of molecular diversity far beyond the observed mammalian metabolic transformations. One example being the construction of a CYP expression library based on *Escherichia coli* expression for P450 monooxygenases, which after careful screening and optimization revealed a rapid biotransformation-system on multi-well plates (Fig. 1).[16]

Microbial cultures provide higher enzyme activities, long-term stability and easier scale-up to prepare purified metabolites. These parameters support the use of whole cell enzymatic preparations as they have proven to be more efficient in terms of scalability of metabolite production, enzyme activity and costs. In addition, no regeneration system is required when using whole cell systems, offering another advantage.[17] Current practices within the pharmaceutical industry are trending towards the establishment of in-house screening technologies of representative diversity using both microbial biocatalysts as well as microsomal preparations and recombinant systems and the types of biocatalyst applications are summarized in Table 1.[18]

The direct correlation of bacterial or fungal P450s with specific human CYP isoforms or certain mammalian metabolite patterns still represents a future challenge but would tremendously facilitate strain selection. Remarkable progress has been made to optimize the correlation of microbial and mammalian oxidative drug metabolism during the last years by directed biocatalyst engineering.[19] Within the area of bacterial mutants, BM-3 variants of P450 derived from *Bacillus megaterium* (CYP 102A1) are of particular interest, as they accept a broader substrate range and offer greater potential for use at larger scale than human CYPs.[20] In this context, a drug library screening for metabolic activity towards a structurally diverse set of 43 drug-like compounds has been reported using BM3 mutants in cytosolic fractions.[21,22]

The application and combination of different methods such as human liver (HLM) and renal (HRM) microsomes, recombinant P450s and FMOs is illustrated by a recent report of Usmani et al. (Scheme 2).[23] The enzymes involved in the primary metabolism of Lorcaserin, a 5-HT_{2C} agonist, are described along with CYP inhibition experiments revealing the contribution of CYPs to the metabolic pathway.



Scheme 1. Diversity of enzymatic hydroxylation sites in various pharmacologically active compounds.[14]