Access to Drug Metabolites using Biomimetic Oxidation Systems
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ABSTRACT
The MIST guidance issued by FDA requires the safety evaluation of drug metabolites for regulatory approval. However, the lack of reliable methods to produce metabolites makes their study difficult, time consuming and expensive. We have developed a catalytic platform to produce drug candidate metabolites rapidly and efficiently, thus addressing a significant bottleneck in modern drug development. This innovative technology consists of the exploitation of a panel of chemical reaction conditions in a multi-well parallel format, which by its diversity mimics the panel of enzymes (cytochrome P450) present in human hepatocytes. In this poster we describe the process we use to generate and authenticate metabolites and demonstrate that this chemical catalysis platform combines the advantages of biocatalysis and organic chemistry.

INTRODUCTION
Biomimetic systems have been intensively studied in the last decade. Metalloporphyrins have been described to be the very efficient catalysts to perform biomimetic oxidation and produce metabolites. Previous works have shown their ability to mimic the main biological oxidation such as aliphatic hydroxylation, N and O-dealkylation, and aromatic hydroxylation. However, due to the complexity of these systems, they were not often used in the pharmaceutical industry. We developed a process allowing main biological oxidations occurring in Phase I metabolism. This process allows for rapid and efficient production of mg quantities of metabolites.

CURRENT TECHNOLOGIES AND THEIR LIMITATIONS IN GENERATING METABOLITES
• Conventional Organic Synthesis
  - Difficult routes
  - Multiple-step synthesis with low yield
• Biological Models (microsomes, recombinant cytochrome P450 and isolated cells, perfused organs, animal experiments)
  - Prohibitive cost
  - Variable efficiency of biological systems
  - Problematic isolation of hydrophilic metabolites
  - Animal use

ADVANTAGES OF THE BIOMIMETIC OXIDATION APPROACH
• No prior structure elucidation necessary
• One step synthesis from the parent
• Authentication performed by direct comparison with an actual in-vitro or in-vivo reference sample using LC/MS/MS
• High success rate (>70%)
• Production of major and minor metabolites
• Large scale synthesis (mg to gram)

REFERENCES
(2) T. Higuchi, M.Hirobe; J. Mol. Cat. A, 1996, 173, 403-422

Selectivity of Biomimetic Systems

Oxo-Metalphorphyrin Intermediate
The reactive intermediate that oxidizes an oxo-metalphorphyrin intermediate. According to several publications, the nature of this reactive intermediate directly affect the chemoselectivity of the reaction. Depending on the nature of metalloporphyrin, ligand solvent and oxidant, it’s possible to stabilize one reactive intermediate and consequently to favor one reaction.

Biomimetic Oxidation of Dimethylaminopyrine
Biomimetic systems show remarkable chemo-selectivity. The same porphyrin can promote different oxidation reactions depending on the condition (oxidant, solvent, ligand, etc.). For example, the system using Fe(TDCIPP) and PHIO allows N-demethylation while Fe(TDCIPP) and H2O2 in presence of imidazole allows main formylation.

Tolbutamide is a known substrate of CYP2C9. Two major metabolites have been isolated from human, rat and dog (compounds 11 and 7). Compound 8 is resulting from aliphatic hydroxylation and has been synthesized by Makaya et al. using unidirect method. Mn(TDCIPP) and H2O2 system was able to hydroxylate a less reactive position on the alkyl chain (compounds 8 and 9) as well as to produce the two known metabolites H1 and H7.

Metabolite Production

Tolbutamide 4 is a known substrate of CYP2C9. Two major metabolites have been isolated from human, rat and dog (compounds 11 and 7). Compound 8 is resulting from aliphatic hydroxylation and has been synthesized by Makaya et al. using unidirect method. Mn(TDCIPP) and H2O2 system was able to hydroxylate a less reactive position on the alkyl chain (compounds 8 and 9) as well as to produce the two known metabolites H1 and H7.

Synthetic metalloporphyrins to mimic Cytochrome P450

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