Access to Drug Metabolites using Biomimetic Oxidation Systems

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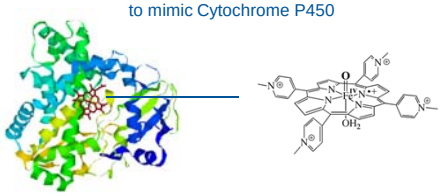
ABSTRACT

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.

Synthetic metalloporphyrins to mimic Cytochrome P450



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.[1,2] 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 metabolite.

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)

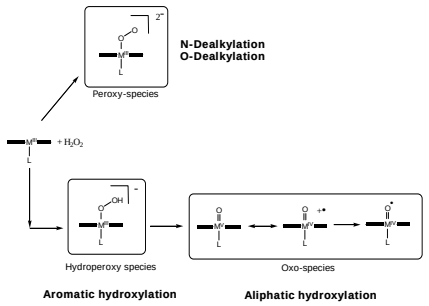
REFERENCES

(1) J. Bernadou, B. Meunier; Adv.Synth. Catal. **2004**, *346*, 171-184
(2) T. Higuchi, M.Hirobe; J. Mol. Cat. A **1996**, *113*, 403-422
(3) J. T. Groves, et al. PNAS 2003; "Cytochrome P450" Chapter 1, 3rd Ed. 2005; Angew. Chem. 2006; Chem. Biol. 2007, J. Am. Chem. Soc. 2009.
(4) S. L. H. Rebelo, J. A. S. Cavaleiro; J.Catal., **2005**, *234*, 76-87
(5) J. T. Groves; Journal of Porphyrins and Phthalocyanines, **2000**, 4, 350-352.
(6) W. Nam ,et al..; J. Am. Chem. Soc., **2000**, *122*, 6641-6647.
(7) O. Makaya, H. Irie, J. Shibasaki; Chem. Pharm. Bull., **1983**, *31*, 2518-2519
(8) M. A. Brodney et al.: J. Med. Chem., **2012**, *55* (21), 9240–9254

Selectivity of Biomimetic Systems

OXO-METALLOPORPHYRIN INTERMEDIATE

The reactive intermediate that oxidizes is an oxo-metalloporphyrin intermediate. According to several publications,[4,5,6] 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.

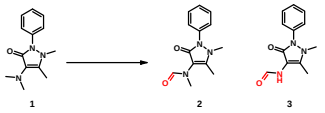


BIOMIMETIC OXIDATION OF DIMETHYLAMINOPYRINE

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(TDClPP) and PhIO allows N-demethylation while Fe(TDClPP) and H_2O_2 in presence of imidazole allows mainly formylation.

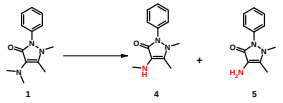
Examples

Fe(TDClPP) and H_2O_2: Formylation



MeOH/CH_3CN: 57% of compound **2** and 8% of compound **3**

Fe(TDClPP) and PhIO : N-Demethylation



MeOH/CH_3CN: 49 % of compiund **5** and 15 % of compound **4**
In $PhCF_3$: 5% of compound **5** and 41 % of compound **4**

Systematic Process to Access Metabolite

Step 1: Screening

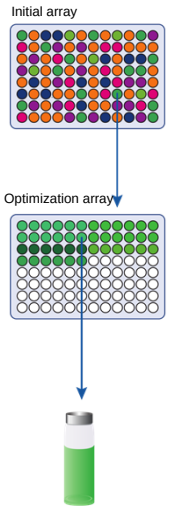
- Evaluation of chemistry using a predefined array
- Selection of the well containing the desired metabolite.

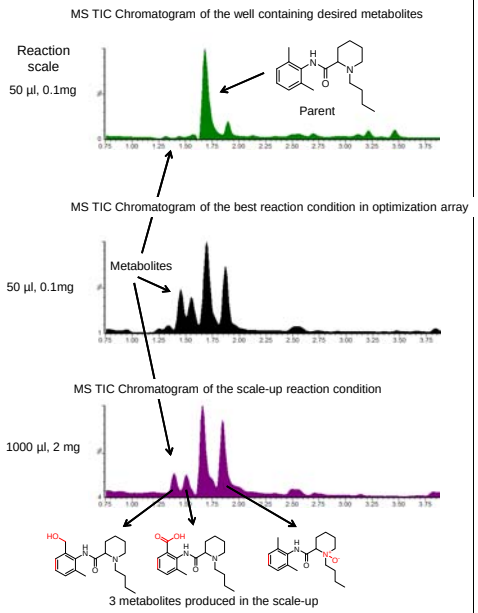
Step 2: Optimization

- Optimization of initial reaction condition using a predefined array.
- Selection of the well containing the most desired metabolite.

Step 3: Production

- Scale-up reaction condition and isolation of desired metabolite.
- Sufficient material for NMR and in-vitro testing.



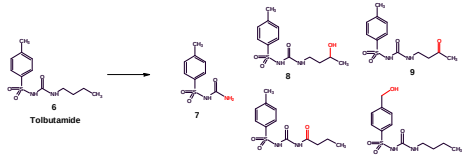


Metabolite Production

TOLBUTAMIDE METABOLITES

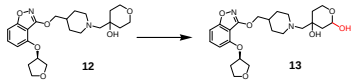
Tolbutamide **6** is a known substrate of CYP2C9. Two major metabolites have been isolated from human, rat and dog (compounds **11** and **7**). Compound **8** is resulting from aliphatic hydroxylation and has been synthesized by Makaya[7] using undirect method., Mn(TDClPP) and H_2O_2 system was able to hydroxylate less reactive position on the alkyl chain (compounds **8** and **9**) as well as to produce the two known metabolites **11** and **7**.

Mn(TDClPP) and H_2O_2 with imidazole: Aliphatic Hydroxylation of Tolbutamide



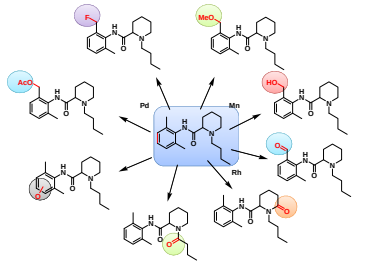
TETRAHYDROPYRAN COMPOUND HYDROXYLATION

The compound **12** is a compound submitted by a sponsor. We authenticated the metabolite **13** produced by our biomimetic system using LC/MS/MS. Then we scaled up the reaction to produce 20 mg of the pure metabolite. The structure of the metabolite was determined by NMR.[8] This metabolite was not accessible using any other method.



BEYOND BIOTRANSFORMATION

We expanded our catalyst platform with other catalysts than metalloporphyrins using metals such as rhodium or palladium. This allows to perform a wider range of transformations including methoxylation, acetoxylation or even fluorination. On a test compound like bupivacaine we observed the formation a variety of products with modification happening at different positions.



CONCLUSION

- ✓ Biomimetic systems using metalloporphyrin can produce metabolites efficiently.
- ✓ These systems are selective and their reactivity can be tuned using different reagents.
- ✓ We designed a fast process to screen, optimize and scale-up biomimetic chemistry.
- ✓ The process can be applied to generate analogue sof drug candidate.