Washington University in St. Louis
Office of Technology Management

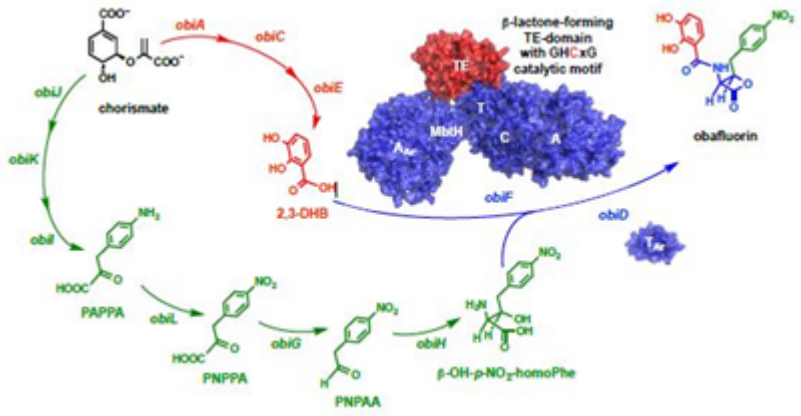
LOW COST, VERSATILE ENZYMATIC SYNTHESIS OF BETA-LACTONE PEPTIDES FOR ANTIBIOTIC OR SERINE HYDROLASE INHIBITOR DRUG DISCOVERY

Reck, Margaret, Schaffer, Jason, Wencewicz, Timothy

Hardin, Clyde "Frank"

T-016508

Chemists from Washington University have discovered and characterized an enzyme pathway that could be harnessed as an efficient platform to produce peptide beta-lactone libraries for drug discovery. Beta-lactones are a family of molecules with a structure similar to penicillin and therefore could potentially be used as antibiotics. In addition, beta-lactones are known to inhibit serine hydrolases and lipases, families of proteins that have been targeted with drug candidates to treat cancer, obesity, Type II diabetes, thrombosis and respiratory disease.



The inventors synthesized the β-lactone antibiotic obafluorin in vitro by reconstituting five enzymes (ObiL, G, H, F, and D) discovered in Prof. Tim Wencewicz' laboratory. ObiF performs the critical step of cyclizing the highly strained β-lactone ring.

Problem

Natural product molecules in the beta-lactone family have the potential to be used as novel therapeutic agents with applications as antibiotics, serine hydrolase inhibitors or lipase inhibitors. However, these molecules are highly unstable and difficult to synthesize due their complex chemical structure that includes a highly strained 4-membered beta-lactone ring. Conventional chemical synthesis of beta-lactones requires a complicated multi-step process performed at very low temperatures, with variable yield and lack of stereochemical control. An alternative synthetic approach that is simple, cost-effective, high-yield and versatile could generate a variety of new drug candidates for a wide range of indications.

Solution

This invention solves that problem with a five-enzyme bio-catalytic pathway that can reliably synthesize beta-lactones at room temperature *in vitro* from simple aldehyde precursors. This technology is based on the discovery and characterization of a non-ribosomal peptide synthetase (NRPS) that is responsible for producing the obaflourin, a broad-spectrum antibiotic made in soil bacteria. The unusual chemistry

of “Obi” enzymes in this system allows it to overcome the energy barrier that otherwise prevents the formation of a strained ring, thereby offering an alternative to complicated chemical synthesis of these valuable molecules. Further protein engineering of the pathway could produce compounds targeted to specific downstream applications.

This flexible biosynthetic machinery could be used to produce drug discovery libraries. Furthermore, the biochemical blueprint for forming beta-lactone rings can be employed to identify additional enzymes for producing a wider variety of molecules with unique peptide backbones. There are hundreds of known serine hydrolases and they are implicated in many human diseases. This beta-lactone synthesis technology could unlock the ability to design therapies directed to those targets with the potential for developing new antimicrobial, anticancer, antiviral and anti-obesity agents.

Key Advantages

- **Simple, cost-effective synthesis –** compared with chemical synthesis that requires unusual reagents and very low reaction temperature, this enzymatic synthesis uses:
  - simple starting materials (aldehyde precursors)
  - room temperature reactions
  - enzymes that are inexpensive, easy to obtain and long lasting
- **Versatile, modular system:**
  - enzymes and pathways can be adapted to generate a range of peptides
  - any aldehyde could potentially be used as the starting substrate
  - potential to design beta-lactone for specific molecular target
- **High yield synthesis:**
  - very high yield as measured by mass spectroscopy
  - highly stereoselective products
  - robust performance in batch and flow applictions

Applications

- **Drug discovery libraries –** flexible enzymatic platform for producing libraries of peptide beta-lactone molecules with the potential for identifying novel drug candidates such as:
  - antibiotics
  - serine hydrolase inhibitors – a family of molecules being developed for indications such as Alzheimer’s disease, Type II diabetes, obesity, thrombosis and respiratory disease
  - lipase inhibitors – molecules that could be used to treat obesity
- **Bio-catalysis of beta-lactone natural products** for industrial and other applications

Stage of Research

- **Characterization** - The inventors have characterized the complete biosynthetic machinery used by *P. fluorescens* (a soil bacteria) to synthesize the beta-lactone ring in obafluorin – a 5-enzyme pathway comprised of ObiL, G, H, F and D.
- **Synthetic demonstration** – The inventors have harnessed the Obi enzymes to synthesize a peptide beta-lactone from simple starting materials and demonstrated that a structural analog can be produced by the same process. They have also shown that the ObiH enzyme can successfully catalyze a reaction on a wide range of beta-lactone precursor molecules.
- **Continued discovery** – The inventors are using the genetic signature of the Obi enzyme gene cluster to perform genome mining studies to discover additional enzymes that a capable of

synthesizing beta-lactones.

Publications

- Schaffer, J. E., Reck, M. R., Prasad, N. K., & Wencewicz, T. A. (2017). β-Lactone formation during product release from a nonribosomal peptide synthetase. *Nature chemical biology*, *13*(7), 737.
- The next enchanted ring? *The Record* (Washington University in St. Louis), May 30, 2017.

Patents

- Chemoenzymatic synthesis of peptide beta-lactones and beta-hydroxy acids (U.S. Patent Application, Publication No. 20180265905)

Website

- Wencewicz Lab