example, it is contemplated that the mixed disulfide conjugates of thienopyridine compounds of the present disclosure improve dosing consistency because production of the active metabolite from the conjugates is predictable. In addition, it is contemplated that use of the mixed disulfide conjugates of thienopyridine compounds of the present disclosure as antiplatelet agents reduce the toxicity as toxic reactive metabolites will not be produced by the thiol-exchange reaction. In addition, the therapeutic onset time for the mixed disulfide conjugates of thienopyridine compounds of the present disclosure is shortened, which greatly benefits patients who experience acute cardiovascular events. For example, the standard regimen for clopidogrel requires continuously dosing patients for 3-5 days as only a small percentage of the ingested drug are converted to the active metabolite. In contrast, it is contemplated that the mixed disulfide conjugates of thienopyridine compounds of the present disclosure will release the active metabolites with high yields in less than 30 min. In addition, it is contemplated that the mixed disulfide conjugates of thienopyridine compounds of the present disclosure will have superior stability over the active metabolites and therefore can be used to quantitatively generate the active metabolites for basic and clinical research in vitro.

Accordingly, the present disclosure relates to a process for efficiently synthesizing highly optically active mixed disulfide conjugates of thienopyridine compounds with genetically engineered biocatalysts, i.e., a one-step process for preparing highly optically active mixed disulfide conjugates of thienopyridine compounds by mixing 2-oxo thienopyridine and heterocyclic thiols in the presence of reducing reagent NADPH or NADH and a genetically engineered variant of cytochrome P450 BM3 or CYP102A1 as a catalyst. The operation of the process is simple, and the raw materials and reagents are readily available. The methods selectively produce the cis stereoisomer of the conjugates at a higher yield than prior art methods.

In one aspect, the present disclosure provides a mutant CYP102A1 enzyme capable of catalyzing conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent, wherein an amino acid sequence of the enzyme has a specific set of the following muations within SEQ ID NO: 2: A82F/L188Q/R47L/F87V; A82F/F87V/L188Q; R47L/F87V/L188Q; and A82F/F87V/L188Q/W1046L.

In one example, the present disclosure provides a mutant CYP102A1 enzyme capable of catalyzing conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent. In some examples, the catalyzing of conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent results in the generation of mixed disulfide conjugates of thienopyridine compounds. In some examples, the catalyzing of conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent selectively generates cis stereoisomers of the mixed disulfide conjugates of thienopyridine compounds. In some examples, the reducing agent is NADPH or NADH.

What Is Claimed Is:

1. A mutant CYP102A1 enzyme capable of catalyzing conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent, wherein an amino acid sequence of the enzyme has a specific set of following muations within SEQ ID NO: 2:.

A82F/L188Q/R47L/F87V;

A82F/F87V/L188Q;

R47L/F87V/L188Q; and

A82F/F87V/L188Q/W1046L.

2. The mutant CYP102A1 enzyme of claim 1, wherein the catalyzing of conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent results in the generation of mixed disulfide conjugates of thienopyridine compounds.

3. The mutant CYP102A1 enzyme of claim 1, wherein the catalyzing of conjugation between 2-oxo thienopyridine and heterocyclic thiols in the presence of a reducing reagent selectively generates cis stereoisomers of the mixed disulfide conjugates of thienopyridine compounds.

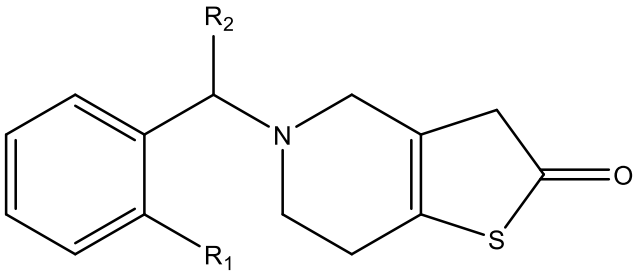
4. The mutant CYP102A1 enzyme of claim 1, wherein the enzyme comprises nucleic acid having at least 99% homology with SEQ ID NO: 1.

5. The mutant CYP102A1 enzyme of claim 1, wherein the enzyme comprises nucleic acid having at least 100% homology with SEQ ID NO: 3.

6. A method for synthesizing cis stereoisomers of mixed disulfide conjugates of thienopyridine compounds, comprising mixing a 2-oxo thienopyridine moiety, a heterocyclic thiol moiety, and the mutant CYP102A1 enzyme of claim 1 in the presence of a reducing reagent.

7. The method of claim 6, wherein the reducing agent is NADPH or NADH.

8. The method of claim 6, wherein the 2-oxo thienopyridine moiety is represented by



; wherein R1 is either Chlorine or Fluorine; wherein R2 is H, COOCH3, or COCHCH2CH2.

9. The method of claim 6, wherein the heterocyclic thiol moiety is represented by R3-SH; wherein R3 is selected from 3-nitropyridine-2-thiol, 2-mercaptopyridine, 2-mercapto-6-methylpyridine, 5-chloropyridine-2-thiol, 2-mercapto-5-trifluoromethyl-pyridine, 3-(trifluoromethyl)pyridine-2-thiol, 2-mercaptopyridine-3-carbonitrile, 4,6-dimethyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile, 2-quinolinethiol, 1-amino-3-mercaptoisoquinoline, 6-chloropyridazine-3-thiol, and 2,5-dimethylfuran-3-thiol.

10. The method of claim 6, wherein the mixing occurs at ambient temperature.

11. The method of claim 6, wherein the mixing occurs for a time period between twenty and sixty minutes.

12. The method of claim 6, wherein the mutant CYP102A1 enzyme is comprised within a bacterial cytosolic fraction.

13. The method of claim 6, wherein the amount of mutant CYP102A1 enzyme is between approximately 0.1 and 1 μM.

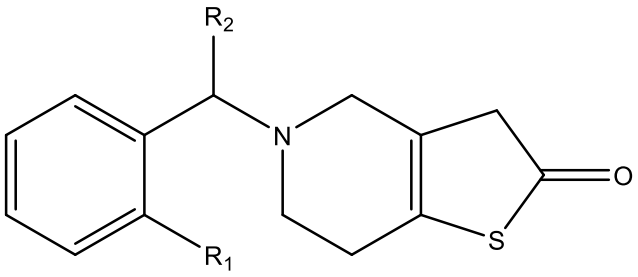
14. The method of claim 6, wherein the mixing results in the generation of approximately 100 mg of cis stereoisomers of mixed disulfide conjugates of thienopyridine compounds per liter of the 2-oxo thienopyridine moiety, the heterocyclic thiol moiety, the mutant CYP102A1 enzyme, and the reducing agent.

15. A kit comprising a 2-oxo thienopyridine moiety, a heterocyclic thiol moeity, and the mutant CYP102A1 enzyme of claim 1.

16. The kit of claim 15, further comprising a reducing reagent.

17. The kit of claim 16, wherein the reducing agent is NADPH or NADH.

18. The kit of claim 15, wherein the 2-oxo thienopyridine moiety is represented by



; wherein R1 is either Chlorine or Fluorine; wherein R2 is H, COOCH3, or COCHCH2CH2.

19. The kit of claim 15, wherein the heterocyclic thiol moiety is represented by R3-SH; wherein R3 is selected from 3-nitropyridine-2-thiol, 2-mercaptopyridine, 2-mercapto-6-methylpyridine, 5-chloropyridine-2-thiol, 2-mercapto-5-trifluoromethyl-pyridine, 3-(trifluoromethyl)pyridine-2-thiol, 2-mercaptopyridine-3-carbonitrile, 4,6-dimethyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile, 2-quinolinethiol, 1-amino-3-mercaptoisoquinoline, 6-chloropyridazine-3-thiol, and 2,5-dimethylfuran-3-thiol.