CLAIMS

What is claimed is:

1. A modified galactose oxidase comprising a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6,

wherein SEQ ID NO: 6 is a polypeptide sequence of galactose oxidase variant M3-5, wherein the galactose oxidase variant M3-5 comprises mutations of S33P, M93V, G218E, W313F, R353M, Q429T, V517A, and N558D relative to the wildtype galactose oxidase from *Fusarium graminearum* (SEQ ID NO: 4),

and wherein the polypeptide sequence of the modified galactose oxidase further comprises at least one mutation at one or more positions selected from the group consisting of 217, 268, and 616, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6,

wherein the mutation is selected from the group consisting of:

- T616V; and
- a combination of F217A, N268W and T616V.

2. The modified galactose oxidase of claim 1, wherein the modified galactose oxidase comprises the mutations of F217A, N268W, and T616V.

3. The modified galactose oxidase of claim 2, wherein the polypeptide sequence of the modified galactose oxidase has at least 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:300.

4. The modified galactose oxidase of any one of claims 1-3, wherein the modified galactose oxidase comprises the property of:

increased enzymatic activity by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase with the polypeptide sequence set forth in SEQ ID NO: 68, wherein the enzymatic activity comprises converting a secondary alcohol to a ketone, wherein optionally the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol.

5. A polynucleotide sequence encoding the modified galactose oxidase of any one of claims 1-4.

6. The polynucleotide sequence of claim 5, wherein the polynucleotide sequence comprises at least 95%, 96%, 97%, 98%, or 99% or more sequence identity to SEQ ID NO: 5, wherein SEQ ID NO: 5 is a polynucleotide sequence of the galactose oxidase variant M3-5, wherein the polynucleotide sequence of claim 5 comprises at least one mutation at one or more positions which leads to at least one mutation in the polypeptide sequence of the modified galactose oxidase in any one of claims 1-4.

7. The polynucleotide sequence of claim 5 or 6, wherein the polynucleotide is codon optimized, wherein optionally the polynucleotide is codon optimized for protein expression in *Escherichia coli*.

8. The polynucleotide sequence of any one of claims 5-7, wherein the polynucleotide sequence is further linked to a control sequence, wherein optionally the control sequence is a promoter sequence or a termination sequence, wherein optionally the promoter sequence is a lac promoter sequence.

9. The polynucleotide sequence of any one of claims 5-8, wherein the polynucleotide comprises set forth in SEQ ID NO: 299.

10. An expression vector comprising the polynucleotide sequence of any one of claims 5-9.

11. The expression vector of claim 10, wherein the expression vector is selected from the group consisting of a plasmid and a viral vector, wherein optionally the expression vector is a pET28a plasmid vector.

12. A host cell comprising the polynucleotide sequence of any one of claims 5-9 or the expression vector of claim 10 or 11.

13. The host cell of claim 12, wherein the host cell is a prokaryotic cell or eukaryotic cell, wherein optionally the host cell is a prokaryotic cell, and wherein optionally the prokaryotic cell is *Escherichia coli*.

14. A method of producing a modified galactose oxidase, comprising culturing the host cell of claim 12 or 13 under suitable culture conditions such that the modified galactose oxidase is produced.

15. The method of claim 14, further comprises recovering the modified galactose oxidase from the culture and/or the host cell.

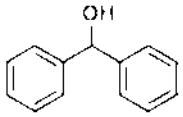
16. The method of claim 14 or 15, further comprises purifying the modified galactose oxidase.

17. A method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase of any one of claims 1-4 under suitable conditions for an oxidation reaction.

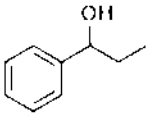
18. The method of claim 17, wherein the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol, wherein the bulky secondary alcohol comprises one or more bulky substituents, and wherein the unactivated secondary alcohol is an aliphatic secondary alcohol without a benzene ring in the alpha position adjacent to the alcohol group.

19. The method of claim 17 or 18, wherein the secondary alcohol comprises the formula R^1R^2CHOH, wherein R^1 and R^2 are independently selected from the group consisting of cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl, wherein the cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl is optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, alkyl halide, alkylene, alkynyl, alkoxy, cyano, oxo, nitro, amino, thiol, carboxyl, ester and hydroxyl.

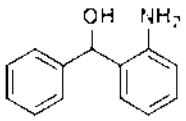
20. The method of any one of claims 17-19, wherein the secondary alcohol is selected from the group consisting of:



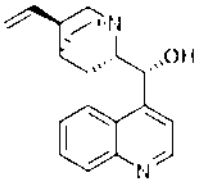
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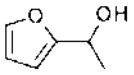
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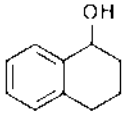
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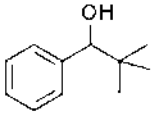
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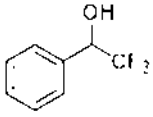
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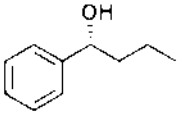
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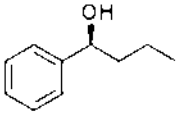
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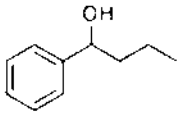
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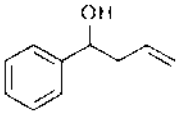
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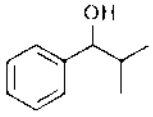
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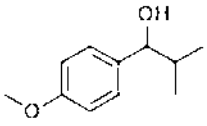
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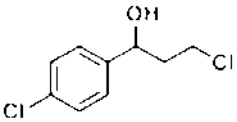
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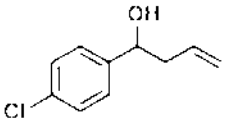
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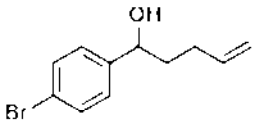
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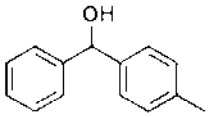
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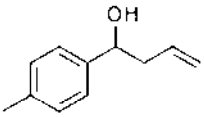
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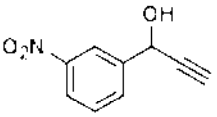
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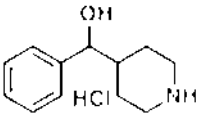
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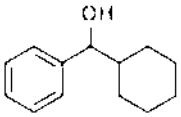
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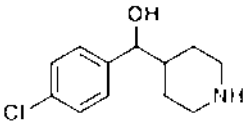
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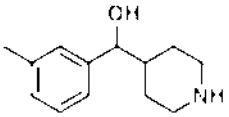
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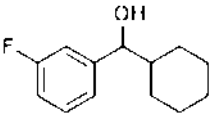
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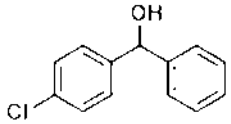
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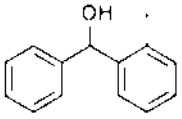
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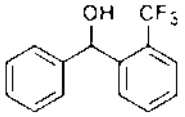
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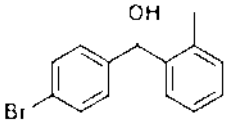
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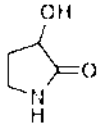
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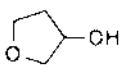
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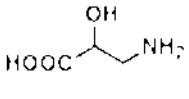
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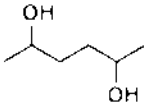
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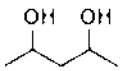
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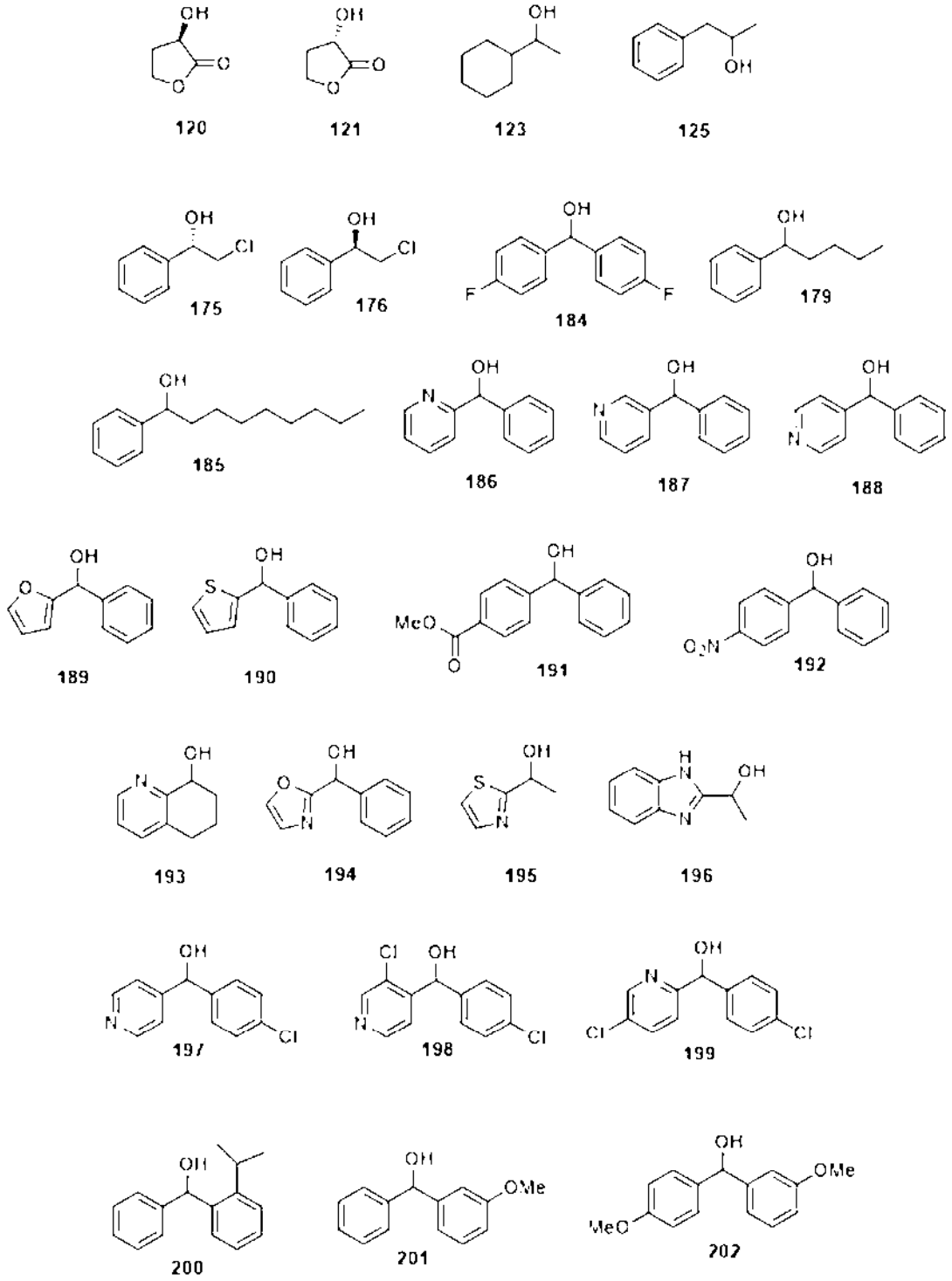
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21. The method of any one of claims 17-20, wherein the secondary alcohol is chiral or non-chiral, wherein optionally the chiral secondary alcohol is present as (S)-enantiomer, (R)-

enantiomer, or a mixture of (S)- and (R)-enantiomers, wherein optionally the chiral secondary alcohol is present as a racemic mixture.

22. The method of any one of claims 17-21, wherein the contacting step is performed at a temperature of 25℃, 30℃, 35℃, 40℃, 45℃, 50℃, 55℃, wherein optionally the temperature is 35℃.

23. The method of any one of claims 17-22, further comprising converting the secondary alcohol into an active pharmaceutical ingredient.

24. A kit for use in the method of any one of claims 17-23, wherein the kit comprises the modified galactose oxidase of any one of claims 1-4.

25. The kit of claim 24, wherein the kit further comprises one or more of the following:
    (a) one or more reaction buffers;
    (b) one or more reaction vessels, wherein optionally the reaction vessel is a HPLC vial;
    (c) one or more reaction solvents, wherein optionally the reaction solvent is 5% v/v dimethyl sulfoxide in 100 mM sodium phosphate buffer with a pH of 7;
    (d) one or more secondary alcohol substrates;
    (e) one or more co-enzymes to break down the by-product of the oxidation reaction, wherein optionally the co-enzyme is a horseradish peroxidase that breaks down the H_2O_2; and
    (f) instructions for performing the method of any one of claims 14-21.