CLAIMS

What is claimed is:

1. A modified galactose oxidase comprising a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 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 24, 26, 28, 45, 49, 65, 69, 104, 114, 117, 153, 157, 172, 173, 175, 193, 194, 195, 197, 198, 202, 214, 217, 218, 220, 224, 226, 239, 240, 250, 259, 265, 268, 269, 284, 294, 313, 314, 325, 341, 346, 348, 352, 353, 356, 389, 401, 429, 447, 485, 487, 537, 538, 546, 559, 576, 599, and 662, 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:

- M353F;
- S314C;
- L537G;
- F217V;
- F217P;
- F217Q;
- F217N;
- F217S;
- N268W;
- E218P;
- F217I;
- F217T;
- F217L;
- F217E;
- F217Y;
- F217A;

- F217C;
- F217D;
- F217G;
- F217H;
- F217K;
- F217M;
- F217R;
- a combination of F217P and E218P;
- a combination of F217P and L537G;
- a combination of F217P, E218P and L537G;
- a combination of F217V and E218P;
- a combination of F217V and L537G;
- a combination of F217V, E218P and L537G;
- a combination of E218P and L537G;
- a combination of F217A and N268W;
- a combination of F217A, N268W and M353F;
- a combination of F217A, N268W and S314C;
- a combination of F217G and N268W;
- a combination of F217G, N268W and M353F;
- a combination of F217A, N268W and A195S;
- a combination of F217A, N268W and V193T;
- a combination of F217A, N268W and N259T;
- a combination of F217A, N268W and Y325F;
- a combination of F217A, N268W and G294A;
- a combination of F217A, N268W and A195T;
- a combination of F217A, N268W and Y352T;
- a combination of F217A, N268W and N341W;
- a combination of F217A, N268W and A197C;
- a combination of F217A, N268W and R240G;
- a combination of F217A, N268W and A198I;
- a combination of F217A, N268W and K348S;
- a combination of F217A, N268W and T202D;
- a combination of F217A, N268W and T202Q;
- a combination of F217A, N268W and T202I;

- a combination of F217A, N268W and T202K;
- a combination of F217A, N268W and P194C;
- a combination of F217A, N268W and A346L;
- a combination of F217A, N268W and K389I;
- a combination of F217A, N268W and Y352W;
- a combination of F217A, N268W and C538K;
- a combination of F217A, N268W and C538R;
- a combination of F217A, N268W and F250E;
- a combination of F217A, N268W and F250Y;
- a combination of F217A, N268W and N356K;
- a combination of F217A, N268W and N356R;
- a combination of F217A, N268W and N356W;
- a combination of F217A, N268W and F487R;
- a combination of F217A, N268W and T429W;
- a combination of F217A, N268W and E218K;
- a combination of F217A, N268W and F313R;
- a combination of F217A, N268W and I284Y;
- a combination of F217A, N268W and A24H;
- a combination of F217A, N268W and I28R;
- a combination of F217A, N268W and I28M;
- a combination of F217A, N268W and S576D;
- a combination of F217A, N268W and I284S;
- a combination of F217A, N268W and F65P;
- a combination of F217A, N268W and T153R;
- a combination of F217A, N268W and A197G;
- a combination of F217A, N268W and T265S;
- a combination of F217A, N268W and S220T;
- a combination of F217A, N268W and N214S;
- a combination of F217A, N268W and I485A;
- a combination of F217A, N268W and N214R;
- a combination of F217A, N268W and D269N;
- a combination of F217A, N268W and I485R;
- a combination of F217A, N268W and I485K;
- a combination of F217A, N268W and D239E;

- a combination of F217A, N268W and T117D;
- a combination of F217A, N268W and S114R;
- a combination of F217A, N268W and S559P;
- a combination of F217A, N268W and S114N;
- a combination of F217A, N268W and Q662P;
- a combination of F217A, N268W and S173R;
- a combination of F217A, N268W and D239P;
- a combination of F217A, N268W and S172E;
- a combination of F217A, N268W and Q662D;
- a combination of F217A, N268W and S173K;
- a combination of F217A, N268W and A195V;
- a combination of F217A, N268W and E218F;
- a combination of F217A, N268W and L226Q;
- a combination of F217A, N268W and I224W;
- a combination of F217A, N268W and A26R;
- a combination of F217A, N268W and I28F;
- a combination of F217A, N268W and E218Y;
- a combination of F217A, N268W and L226V;
- a combination of F217A, N268W and D239Q;
- a combination of F217A, N268W and F546M;
- a combination of F217A, N268W and A24N;
- a combination of F217A, N268W and A26H;
- a combination of F217A, N268W and Y599M;
- a combination of F217A, N268W and G157N;
- a combination of F217A, N268W and Q45D;
- a combination of F217A, N268W and Q45E;
- a combination of F217A, N268W and Q45N;
- a combination of F217A, N268W and E49K;
- a combination of F217A, N268W and N69D;
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- a combination of F217A, N268W and N69L;
- a combination of F217A, N268W and N69M;

- a combination of F217A, N268W and N69T;
- a combination of F217A, N268W and N69W;
- a combination of F217A, N268W and N69Y;
- a combination of F217A, N268W and W104G;
- a combination of F217A, N268W and W104N;
- a combination of F217A, N268W and T175E;
- a combination of F217A, N268W and T175H;
- a combination of F217A, N268W and T175I;
- a combination of F217A, N268W and T175K;
- a combination of F217A, N268W and A401D;
- a combination of F217A, N268W, A195T and Y352W;
- a combination of F217A, N268W, N341W and Y352T;
- a combination of F217A, N268W, A198I and N259T;
- a combination of F217A, N268W, A195S and N341W;
- a combination of F217A, N268W, T153K and A195S;
- a combination of F217A, N268W, I28R and K389I;
- a combination of F217A, N268W, T153R and A195T;
- a combination of F217A, N268W, I28R, T153R, A198I and N341W;
- a combination of F217A, N268W, A195T, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T and K389I;
- a combination of F217A, N268W, I28R, N259T and N341W;
- a combination of F217A, N268W, I28R, A195S and K389I;
- a combination of F217A, N268W, A195T and N341W;
- a combination of F217A, N268W, I28R, A195T, N341W and K389I;
- a combination of F217A, N268W, I28R, A195T, N259T and T447A;
- a combination of F217A, N268W, I28R, A195S, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T, N341W and K389I;

and

- a combination of F217A, N268W, T153R and N341W.

2. The modified galactose oxidase of claim 1, wherein the polypeptide sequence of the modified galactose oxidase has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in the even numbered sequences of SEQ ID NOS: 7-298.

3. The modified galactose oxidase of claim 2, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOS: 7-298.

4. The modified galactose oxidase of claim 1, wherein the mutation is selected from the group consisting of:
(a) S314C;
(b) N268W;
(c) F217A;
(d) a combination of F217A and N268W;
(e) a combination of F217A, N268W and A195S;
(f) a combination of F217A, N268W and N259T;
(g) a combination of F217A, N268W and A195T;
(h) a combination of F217A, N268W and A198I;
(i) a combination of F217A, N268W and T202I;
(j) a combination of F217A, N268W and Y352W;
(k) a combination of F217A, N268W and A24H;
(l) a combination of F217A, N268W and I28R;
(m) a combination of F217A, N268W and D239E;
(n) a combination of F217A, N268W and A24N;
(o) a combination of F217A, N268W and E218K;
(p) a combination of F217A, N268W and E218F;
(q) a combination of F217A, N268W and Y599M;
(r) a combination of F217A, N268W and L226Q;
(s) a combination of F217A, N268W and A195V;
(t) a combination of F217A, N268W, A195T and Y352W;
(u) a combination of F217A, N268W, I28R and K389I;
(v) a combination of F217A, N268W, I28R, A195T, N259T, and K389I; and
(w) a combination of F217A, N268W, I28R, A195T, N259T, N341W, and K389I.

5. The modified galactose oxidase of claim 1 or 4, wherein the modified galactose oxidase comprises the mutations of F217A and N268W.

6. The modified galactose oxidase of claim 5, wherein the polypeptide sequence of the modified galactose oxidase has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:68.

7. The modified galactose oxidase of claim 5 or 6, wherein the modified galactose oxidase further comprises at least one mutation selected from the group consisting of A24H, A24N, A26H, A26R, I28F, I28M, I28R, Q45D, Q45E, Q45N, E49K, F65P, N69D, N69E, N69F, N69H, N69L, N69M, N69T, N69W, N69Y, W104G, W104N, S114N, S114R, T117D, T153K, T153R, G157N, S172E, S173K, S173R, T175E, T175H, T175I, T175K, V193T, P194C, A195SX, A195T, A195V, A197C, A197G, A198I, T202D, T202I, T202K, T202Q, N214R, N214S, E218F, E218K, E218P, E218Y, S220T, I224W, L226Q, L226V, D239E, D239P, D239Q, R240G, F250E, F250Y, N259T, T265S, D269N, I284S, I284Y, G294A, F313R, S314C, Y325F, N341W, A346L, K348S, Y352T, Y352W, M353F, N356K, N356R, N356W, K389I, A401D, T429W, T447A, I485A, I485K, I485R, F487R, L537G, C538K, C538R, F546M, S559P, S576D, Y599M, Q662D, and Q662P, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

8. The modified galactose oxidase of claim 7, wherein the polypeptide sequence of the modified galactose oxidase has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in SEQ ID NOS: 70, 72 or the even numbered sequences of SEQ ID NOS: 77-298.

9. The modified galactose oxidase of claim 8, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NOS: 70, 72 or the even numbered sequences of SEQ ID NOS: 77-298.

10. The modified galactose oxidase of any one of claims 1-9, wherein the modified galactose oxidase comprises one or more of the following properties:

(a) enhanced protein expression 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 of claim 5;

(b) enhanced solubility 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 of claim 5;

(c) enhanced thermal stability 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 of claim 5 at a temperature of at least 50°C;

(d) 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 of claim 5, 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; and

(e) reduced enantioselectivity 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 of claim 5.

11. A polynucleotide sequence encoding the modified galactose oxidase of any one of claims 1-10.

12. The polynucleotide sequence of claim 11, wherein the polynucleotide sequence comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 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 11 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 1-10.

13. The polynucleotide sequence of claim 11 or 12, wherein the polynucleotide is codon optimized, wherein optionally the polynucleotide is codon optimized for protein expression in *Escherichia coli*.

14. The polynucleotide sequence of any one of claims 11-13, 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.

15. The polynucleotide sequence of any one of claims 11-14, wherein the polynucleotide comprises an odd numbered sequence of SEQ ID NOS: 7-298.

16. An expression vector comprising the polynucleotide sequence of any one of claims 11-15.

17. The expression vector of claim 16, 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.

18. A host cell comprising the polynucleotide sequence of any one of claims 11-15 or the expression vector of claim 16 or 17.

19. The host cell of claim 18, 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*.

20. A method of producing a modified galactose oxidase, comprising culturing the host cell of claim 18 or 19 under suitable culture conditions such that the modified galactose oxidase is produced.

21. The method of claim 20, further comprises recovering the modified galactose oxidase from the culture and/or the host cell.

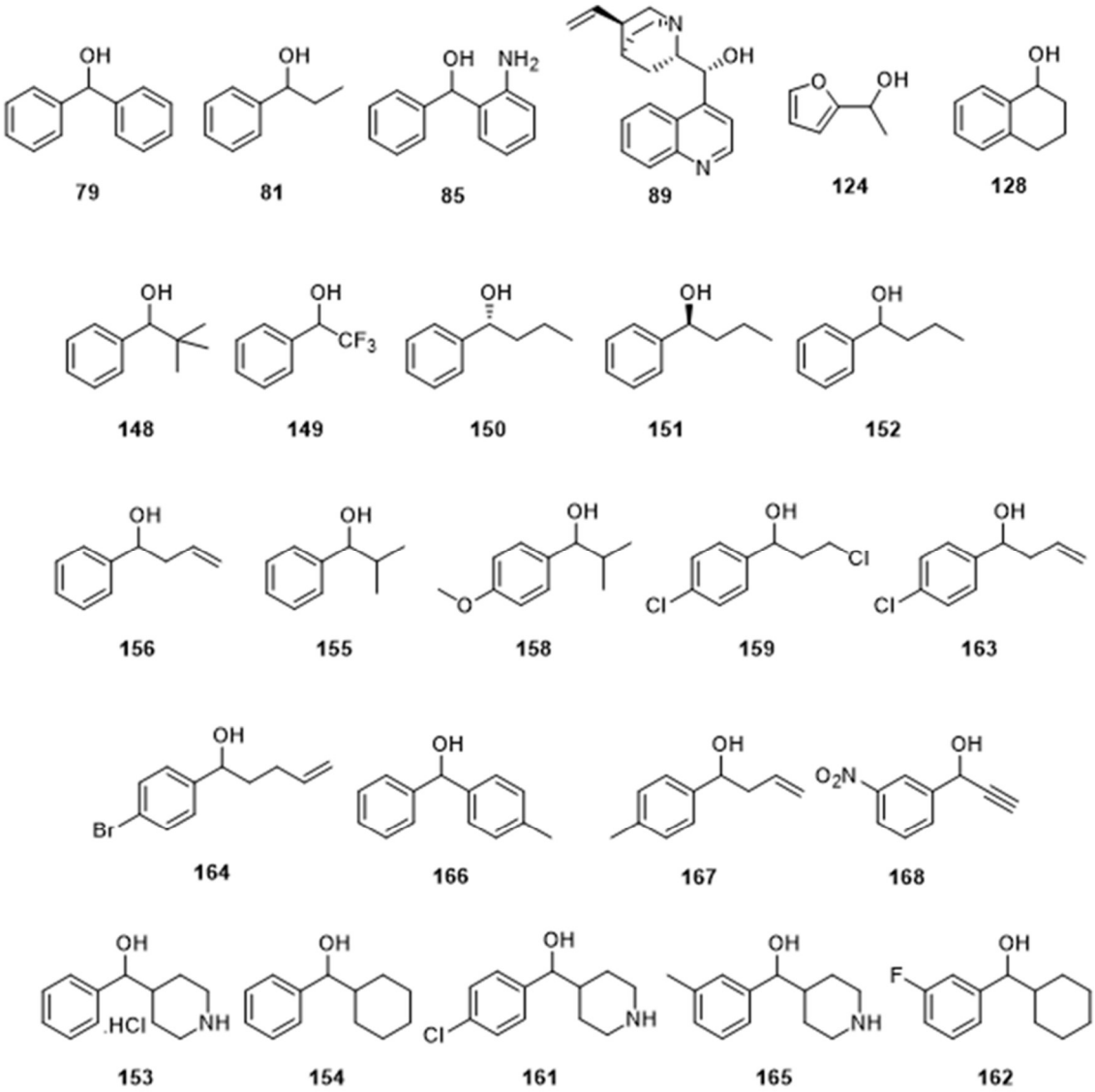
22. The method of claim 20 or 21, further comprises purifying the modified galactose oxidase.

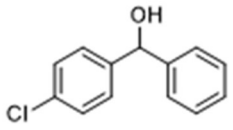
23. A method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase of any one of claims 1-10 under suitable conditions for an oxidation reaction.

24. The method of claim 23, 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.

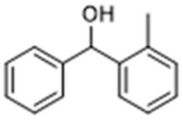
25. The method of claim 23 or 24, wherein the secondary alcohol comprises the formula R^1R^2CHOH, wherein R^1 and R^2 are independently selected from the group consisting of cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl, wherein the cycloalkyl, aryl, heteroaryl, 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.

26. The method of any one of claims 23-25, wherein the secondary alcohol is selected from the group consisting of:

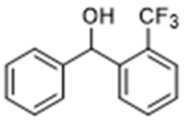




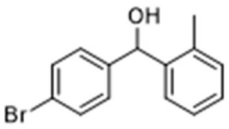
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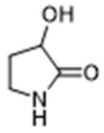
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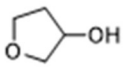
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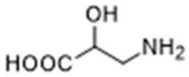
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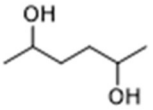
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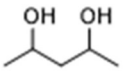
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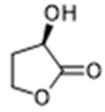
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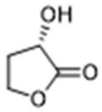
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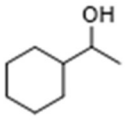
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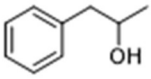
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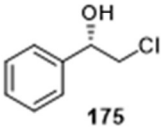
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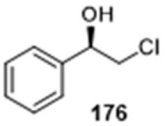
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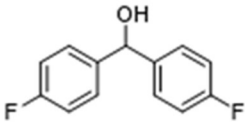
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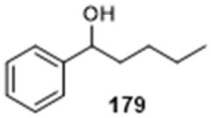
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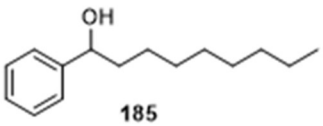
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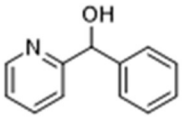
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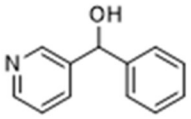
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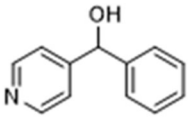
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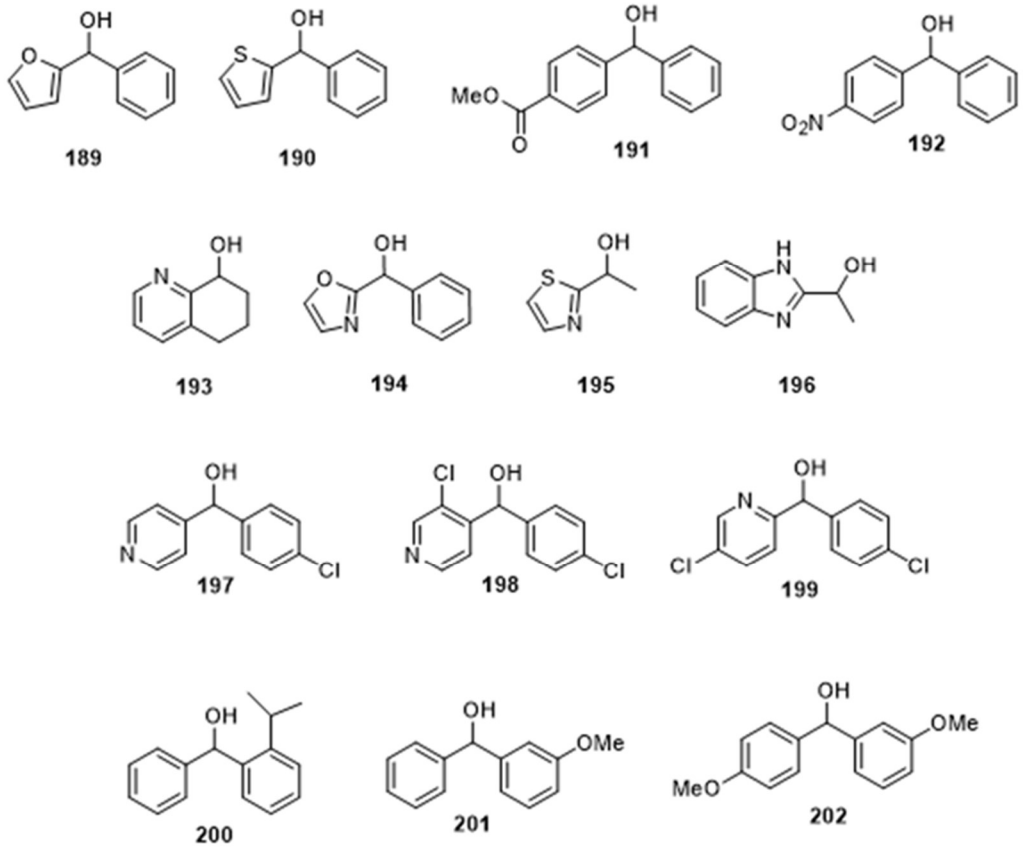
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27. The method of any one of claims 23-26, wherein the secondary alcohol is chiral or non-chiral, wherein optionally the chiral secondary alcohol is present as (S)-enantiomer, (R)-enantiomer, or a mixture of (S)- and (R)-enantiomers, wherein optionally the chiral secondary alcohol is present as a racemic mixture.

28. The method of any one of claims 23-27, wherein the contacting step is performed at a temperature of 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, or 55°C, wherein optionally the temperature is 35°C.

29. The method of any one of claims 23-28, further comprising converting the secondary alcohol into an active pharmaceutical ingredient.

30. A kit for use in the method of any one of claims 23-29, wherein the kit comprises the modified galactose oxidase of any one of claims 1-10.

31. The kit of claim 30, 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 20-27.