High Activity Mutants of Butyrylcholinesterase for Cocaine Hydrolysis

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ABSTRACT

Butyrylcholinesterase (BChE) polypeptide variants of the presently-disclosed subject matter have enhanced catalytic efficiency for (-)-cocaine, as compared to wild-type BChE. Pharmaceutical compositions of the presently-disclosed subject matter include a BChE polypeptide variant having an enhanced catalytic efficiency for (-)-cocaine. A method of the presently-disclosed subject matter for treating a cocaine-induced condition includes administering to an individual an effective amount of a BChE polypeptide variant, as disclosed herein, to lower blood cocaine concentration.
HIGH ACTIVITY MUTANTS OF BUTYRYLCHOLINESTERASE FOR COCAINE HYDROLYSIS

RELATED APPLICATIONS


GOVERNMENT INTEREST

Subject matter described herein was made with government support under Grant Number R01DA013930 awarded by the National Institute on Drug Abuse (NIDA) of the National Institutes of Health (NIH). The government has certain rights in the described subject matter.

TECHNICAL FIELD

The presently disclosed subject matter relates to butyrylcholinesterase variant polypeptides, and in particular, butyrylcholinesterase mutants having amino acid substitutions.

INTRODUCTION

Cocaine abuse is a major medical and public health problem that continues to defy treatment. The disastrous medical and social consequences of cocaine addiction, such as violent crime, loss in individual productivity, illness, and death, have made the development of an effective pharmacological treatment a high priority. However, cocaine mediates its reinforcing and toxic effects by blocking neurotransmitter reuptake and the classical pharmacodynamic approach has failed to yield small-molecule receptor antagonists due to the difficulties inherent in blocking a blocker. An alternative to receptor-based approaches is to interfere with the delivery of cocaine to its receptors and accelerate its metabolism in the body.

The dominant pathway for cocaine metabolism in primates is butyrylcholinesterase (BChE)-catalyzed hydrolysis at the benzoyl ester group (Scheme 1).

Scheme 1. Schematic representation of BChE-catalyzed hydrolysis at the benzoyl ester group.

Only 5% of the cocaine is deactivated through oxidation by the liver microsomal cytochrome P450 system. Cocaine hydrolysis at benzoyl ester group yields ecgonine methyl ester, whereas the oxidation produces norcocaine. The metabolite ecgonine methyl ester is a biologically inactive metabolite, whereas the metabolite norcocaine is hepatotoxic and a local anesthetic. BChE is synthesized in the liver and widely distributed in the body, including plasma, brain, and lung. Extensive experimental studies in animals and humans demonstrate that enhancement of BChE activity by administration of exogenous enzyme substantially decreases cocaine half-life.

Enhancement of cocaine metabolism by administration of BChE has been recognized to be a promising pharmacokinetic approach for treatment of cocaine abuse and dependence. However, the catalytic activity of this plasma enzyme is three orders-of-magnitude lower against the naturally occurring (−)-cocaine than that against the biologically inactive (+)-cocaine enantiomer. (+)-cocaine can be cleared from plasma in seconds and prior to partitioning into the central nervous system (CNS), whereas (−)-cocaine has a plasma half-life of approximately 45-90 minutes (for a relatively low dose of cocaine), long enough for manifestation of the CNS effects which peak in minutes. Under the overdose condition, BChE is saturated with (−)-cocaine and, thus, the plasma half-life of (−)-cocaine will be longer. Hence, BChE mutants with high activity against (−)-cocaine are highly desired for use in humans. Although some BChE mutants with increased catalytic activity over wild-type BChE have previously been generated, there exists a need for mutant BChE with even higher catalytic activity.
SUMMARY

The presently-disclosed subject matter meets some or all of the above-identified needs, as will become evident to those of ordinary skill in the art after a study of information provided in this document.

This Summary describes several embodiments of the presently-disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This Summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, these features can be applied to other embodiments of the presently-disclosed subject matter, whether listed in this Summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

The presently-disclosed subject matter includes butyrylcholinesterase (BChE) polypeptide variants. In some embodiments the amino acid sequence of the BChE polypeptide variant includes an amino acid sequence selected from the group consisting of: SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32, as set forth herein.

The presently-disclosed subject matter further includes a pharmaceutical composition that includes a butyrylcholinesterase polypeptide variant and a suitable pharmaceutical carrier.

The presently-disclosed subject matter further includes a method of treating a cocaine-induced condition, which includes administering to an individual an effective amount of BChE polypeptide variant or a pharmaceutical composition comprising a BChE polypeptide variant, as described herein, to lower blood cocaine concentration. In some embodiments, the BChE polypeptide variant exhibits a one-hundred-fold or more increase in cocaine hydrolysis catalytic efficiency compared to wild-type butyrylcholinesterase.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO: 9 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 10;

SEQ ID NO: 10 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227A, P285G, S287G, A328W, and Y332G;

SEQ ID NO: 11 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 12;

SEQ ID NO: 12 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227A, L286M, S287G, A328W, and Y332G;

SEQ ID NO: 13 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 14;

SEQ ID NO: 14 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, P285Q, S287G, A328W, and Y332G;

SEQ ID NO: 15 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 16;

SEQ ID NO: 16 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, P285I, S287G, A328W, and Y332G;

SEQ ID NO: 17 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 18;

SEQ ID NO: 18 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227G, S287G, A328W, and Y332G;

SEQ ID NO: 19 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 20;

SEQ ID NO: 20 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, P285S, S287G, A328W, and Y332G;

SEQ ID NO: 21 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 22;

SEQ ID NO: 22 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227V, S287G, A328W, and Y332G;

SEQ ID NO: 23 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 24;

SEQ ID NO: 24 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, P285G, S287G, A328W, and Y332G;

SEQ ID NO: 25 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 26;

SEQ ID NO: 26 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227I, S287G, A328W, and Y332G;

SEQ ID NO: 27 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 28;
SEQ ID NO: 28 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227L, S287G, A328W, and Y332G;

SEQ ID NO: 29 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 30;

SEQ ID NO: 30 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, L286M, S287G, A328W, and Y332G;

SEQ ID NO: 31 is a nucleotide sequence encoding a butyrylcholinesterase (BChE) polypeptide variant of SEQ ID NO: 32; and

SEQ ID NO: 32 is an amino acid sequence encoding a BChE polypeptide variant having the following amino acid substitutions, as compared to wild type BChE: A199S, F227A, P285K, S287G, A328W, and Y332G.

**DESCRIPTION OF EXEMPLARY EMBODIMENTS**

The details of one or more embodiments of the presently-disclosed subject matter are set forth in this document. Modifications to embodiments described in this document, and other embodiments, will be evident to those of ordinary skill in the art after a study of the information provided in this document. The information provided in this document, and particularly the specific details of the described exemplary embodiments, is provided primarily for cleanness of understanding and no unnecessary limitations are to be understood therefrom. In case of conflict, the specification of this document, including definitions, will control.

The presently-disclosed subject matter includes butyrylcholinesterase (BChE) polypeptide variants. The BChE polypeptide variants disclosed herein each have enhanced catalytic efficiency for (−)-cocaine, as compared to wild-type BChE. The presently-disclosed subject matter further includes a pharmaceutical composition including a butyrylcholinesterase polypeptide variant, as described herein, and a suitable pharmaceutical carrier. The presently-disclosed subject matter further includes a method of treating a cocaine-induced condition comprising administering to an individual an effective amount of a butyrylcholinesterase polypeptide variant, as disclosed herein, to lower blood cocaine concentration.

In some embodiments, the BChE polypeptide variant is selected from a BChE polypeptide variants set forth in Table 1. Table 1 also includes the SEQ ID NOs associated with the identified BChE polypeptide variants, as well as a summary of the approximate fold increase in catalytic efficiency against (−)-cocaine for the identified BChE polypeptide variants, as compared to wild type BChE.

**TABLE 1**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Amino Acid Substitution</th>
<th>Catalytic Efficiency (k_cat/K_M) against (−)-cocaine</th>
<th>Nucleic Acid Acid</th>
<th>Amino Acid</th>
<th>Amino Acid</th>
<th>NO: NO:</th>
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<td>Number 1</td>
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<td>227</td>
<td>285</td>
<td>286</td>
<td>287</td>
<td>328</td>
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<td>1 A199S</td>
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<td>P285K</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
<td>4060</td>
</tr>
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<td>P285S</td>
<td>S287G</td>
<td>A328W</td>
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</tr>
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<td>F227A</td>
<td>P285Q</td>
<td>S287G</td>
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<td>Y332G</td>
<td>3590</td>
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<td>4 A199S</td>
<td>F227P</td>
<td>—</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
<td>1860</td>
</tr>
<tr>
<td>5 A199S</td>
<td>F227A</td>
<td>P285G</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
<td>2420</td>
</tr>
<tr>
<td>6 A199S</td>
<td>F227A</td>
<td>—</td>
<td>L286M</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
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<tr>
<td>7 A199S</td>
<td>F227A</td>
<td>P285Q</td>
<td>S287G</td>
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<td>Y332G</td>
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<tr>
<td>8 A199S</td>
<td>—</td>
<td>P285I</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
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<td>—</td>
<td>S287G</td>
<td>A328W</td>
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<td>10 A199S</td>
<td>—</td>
<td>P285S</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
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<td>S287G</td>
<td>A328W</td>
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<td>950</td>
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<td>A328W</td>
<td>Y332G</td>
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<td>23</td>
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<td>S287G</td>
<td>A328W</td>
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<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
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<tr>
<td>15 A199S</td>
<td>—</td>
<td>—</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
<td>740</td>
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<tr>
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<td>F227A</td>
<td>P285K</td>
<td>S287G</td>
<td>A328W</td>
<td>Y332G</td>
<td>1540</td>
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</tbody>
</table>

*The approximate ratio of the k_cat/K_M value for the BChE mutant to that for the wild-type BChE against (−)-cocaine.

The terms “polypeptide”, “protein”, and “peptide”, which are used interchangeably herein, refer to a polymer of the protein amino acids, or amino acid analogs, regardless of its size or function. Although “protein” is often used in reference to relatively large polypeptides, and “peptide” is often used in reference to small polypeptides, usage of these terms in the art overlaps and varies. The term “polypeptide” as used herein refers to peptides, polypeptides, and proteins, unless otherwise noted. The terms “protein”, “polypeptide”, and “peptide” are used interchangeably herein when referring to a gene product. Thus, exemplary polypeptides include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing.

The term “variant” refers to an amino acid sequence that is different from the reference polypeptide by one or more amino acids, e.g., one or more amino acid substitutions. For example a butyrylcholinesterase (BChE) polypeptide variant differs from wild-type BChE by one or more amino acid substitutions, i.e., mutations.

The terms “polypeptide fragment” or “fragment”, when used in reference to a reference polypeptide, refers to a polypeptide in which amino acid residues are deleted as compared to the reference polypeptide itself, but where the remaining amino acid sequence is usually identical to the
corresponding positions in the reference polypeptide. Such deletions can occur at the amino-terminus, carboxy-terminus of the reference polypeptide, or alternatively both. A fragment can also be a “functional fragment,” in which case the fragment retains some or all of the activity of the reference polypeptide as described herein. For example, a functional fragment of a particular BCHe polypeptide variant retains some or all of the cocaine hydrolysis activity, i.e., the catalytic efficiency for (−)-cocaine, of the particular BCHe polypeptide variant. In this regard, the term “BCHe polypeptide variant” is inclusive of functional fragments of the BCHe polypeptide variant. Such fragments are typically at least about 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, or 550 amino acids long. One or more residues from about 1 to 67 and/or one or more residues from about 443 to 574 can be removed without substantially affecting the catalytic activity of the BCHe polypeptide variant. As such, the term “BCHe polypeptide variant” is inclusive of functional fragments wherein one or more residues from 1 to 67 and/or one or more residues from 443 to 574 is truncated relative to the full-length BCHe polypeptide variant.

The BCHe polypeptide variant (e.g., SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32) can be formulated in a pharmaceutical composition along with a suitable pharmaceutical carrier known to one skilled in the art.

The present BCHe variant polypeptides can be used in treating a cocaine-induced condition by administering to an individual, an effective amount of a BCHe variant polypeptides, (e.g., SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32), to lower blood cocaine concentration. The BCHe polypeptide variant can be administered in the form of a pharmaceutical composition in which the BCHe polypeptide variant is included with a suitable pharmaceutical carrier. Treatment of a cocaine-induced condition using one of the aforementioned BCHe polypeptide variants can be in a manner that will be understood by those skilled in the art.

The preferred dose for administration of a BCHe polypeptide variant or pharmaceutical composition in accordance with the presently-described subject matter is that amount which will be effective in lowering (−)-cocaine concentration in a patient’s bloodstream, and one would readily recognize that this amount will vary greatly depending on the nature of cocaine consumed, e.g., injected or inhaled, and the condition of a patient. An “effective amount” of butyrylcholinesterase polypeptide variant or pharmaceutical composition to be used in accordance with the presently-disclosed subject matter is intended to mean a nontoxic but sufficient amount of the agent, such that the desired prophylactic or therapeutic effect is produced. Thus, the exact amount of the enzyme or a particular agent that is required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular carrier or adjuvant being used and its mode of administration, and the like. Similarly, the dosing regimen should also be adjusted to suit the individual to whom the composition is administered and will once again vary with age, weight, metabolism, etc. of the individual. Accordingly, the “effective amount” of any particular butyrylcholinesterase polypeptide variant, or pharmaceutical composition thereof, will vary based on the particular circumstances, and an appropriate effective amount may be determined in each case of application by one of ordinary skill in the art using only routine experimentation.

The presently-disclosed subject matter is further illustrated by the following specific but non-limiting examples. The following examples may include compilations of data that are representative of data gathered at various times during the course of development and experimentation related to the presently-disclosed subject matter.

EXAMPLES

Embodiments of the BCHe polypeptide variants of the presently-disclosed subject matter were made and studied using the following experimental procedure.

Site-directed mutagenesis of human BCHe cDNA was performed by the QuikChange method of Branan, J.; Papworth, C.; Greener, A. Methods Mol. Biol. 1996, 57, 5731, incorporated herein by this reference. Mutations were generated from wild-type human BCHe in a pRC/CMV expression plasmid in accordance with Xie, W.; Altamirano, C. V.; Bartels, C. F.; Speirs, R. J.; Cashman, J. R.; Lockridge, O. Mol. Pharmacol. 1999, 55, 53, each of which is incorporated herein by this reference. The expression plasmid pRC/CMV was kindly provided by Dr. O. Lockridge, University of Nebraska Medical Center (Omaha, Nebr.).

Using plasmid DNA as template and primers with specific base-pair alterations, mutations were made by polymerase chain reaction with Pfu DNA polymerase, for replication fidelity. The PCR product was treated with Dpn I endonuclease to digest the parental DNA template. Cloned pfu DNA polymerase and Dpn I endonuclease were obtained from Stratagene (La Jolla, Calif.). Modified plasmid DNA was transformed into Escherichia coli, amplified, and purified.

The DNA sequences of the mutants were confirmed by DNA sequencing. All oligonucleotides were synthesized by the Integrated DNA Technologies, Inc. The QIAprep Spin Plasmid Miniprep Kit and Qiagen plasmid purification kit and QIAquick PCR purification kit were obtained from Qiagen (Santa Clarita, Calif.).

BCHe mutants were expressed in human embryonic kidney cell line 293T/17. Cells were grown to 80%-90% confluence in 6-well dishes and then transfected by Lipofectamine 2000 complexes of 4 µg plasmid DNA per well. Cells were incubated at 37°C in a CO₂ incubator for 24 hours and cells were moved to 60-mm culture vessel and cultured for four more days. The culture medium [10% fetal bovine serum in Dulbecco’s modified Eagle’s medium (DMEM)] was harvested for a BCHe activity assay.

Human embryonic kidney 293T/17 cells were from ATCC (Manassas, Va.). Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Fisher Scientific (Fairlawn, N.J.). Oligonucleotide primers were synthesized by the Integrated DNA Technologies and Analysis Facility of the University of Kentucky. 3',5'-Tetramethylbenzidine (TMB) was obtained from Sigma (Saint Louis, Mo.). Anti-butyrylcholinesterase (mouse monoclonal antibody, # HAI1002-01) was purchased from AntibodyShop (Gentofte, Denmark) and Goat anti-mouse IgG HRP conjugate from Zymed (San Francisco, Calif.).

To measure cocaine and benzoic acid, the product of cocaine hydrolysis by BCHe, sensitive radiometric assays based on toluene extraction of [⁹⁹Tc]−(−)-cocaine labeled on its benzene ring were used in accordance with Zheng, F.; Yang, W.; Ro, M.-C.; Liu, J.; Cho, H.; Gao, D.; Tong, M.; Tai, H.-H.; Woods, J. H.; Zhan, C.-G. “Most Efficient Cocaine Hydrodase Designed by Virtual Screening of Transition States”, J. Am. Chem. Soc. 2008, 130, 12148-12155, which is incorporated herein by this reference. [⁹⁹Tc]−(−)-cocaine (50 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, Mass.).

In brief, to initiate reactions, 100 nCi of [⁹⁹Tc]−(−)-cocaine was mixed with 100 µl of culture medium. Reactions proceeded at room temperature (25°C) with varying concentrations of (−)-cocaine. Reactions were stopped by adding 500 µl of 0.02 M HCl, which neutralized the liberated benzoic acid
while ensuring a positive charge on the residual cocaine. [3H]benzonic acid was extracted by 1 ml of toluene and measured by scintillation counting. Finally, the measured (→)-cocaine concentration-dependent radiometric data were analyzed by using the standard Michaelis–Menten kinetics so that the catalytic efficiency (kcat/Km) was determined, along with the use of an enzyme-linked immunosorbent assay (ELISA) described in by Zheng, F.; Yang, W.; Ko, M.-C.; Liu, J.; Cho, H.; Gao, D.; Tong, M.; Tai, H.-H.; Woods, J. H.; Zhan, C.-G. “Most Efficient Cocaine Hydrolyse Designed by Virtual Screening of Transition States”, J. Am. Chem. Soc. 2008, 130, 12148-12155.

The catalytic efficiency (kcat/Km) of the BChE polypeptide variants of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 are set forth in Table 2.

<table>
<thead>
<tr>
<th>Variant Number</th>
<th>Amino Acid SEQ ID NO</th>
<th>Catalytic Efficiency against (→)-cocaine (M⁻¹ min⁻¹)</th>
<th>Catalytic Efficiency against (→)-cocaine (Approximate Fold Increase)</th>
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<td>1</td>
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<td>3.72 × 10⁶</td>
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<tr>
<td>2</td>
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<td>3.30 × 10⁶</td>
<td>3700</td>
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<td>3</td>
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<td>3.27 × 10⁶</td>
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<td>16</td>
<td>32</td>
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*The approximate ratio of the kcat/Km value for the BChE mutant to that for the wild-type BChE against (→)-cocaine.

The catalytic efficiencies (kcat/Km) of the BChE polypeptide variants were found to be between about 6.74 × 10⁶ and 3.72 × 10⁶ M⁻¹ min⁻¹, which is about 740 to about 4080 times the kcat/Km value (9.11 × 10⁵ M⁻¹ min⁻¹) of the wild-type BChE.

Enzyme-linked immunosorbent assays (ELISA) were performed as follows. The ELISA buffers used were the same as those described in the literature such as Brock, A.; Mortensen, V.; Loft, A. G. R.; Nergaard-Pedersen, B.J. Clin. Chem. Clin. Biochem. 1990, 28, 321-324; and Khattab, A. D.; Walker, C. H.; Johnston, G.; Siddiqui, M. K.; Saphier, P. W. Environmental Toxicology and Chemistry 1994, 13, 161-167, both of which are incorporated herein by reference. The coating buffer was 0.1 M sodium carbonate/bicarbonate buffer, pH 9.5. The diluent buffer (ELISA buffer) was potassium phosphate monobasic/potassium phosphate monohydrate buffer, pH 7.5, containing 0.9% sodium chloride and 0.1% bovine serum albumin. The washing buffer (PBS-T) was 0.01 M potassium monobasic/potassium phosphate monohydrate buffer, pH 7.5, containing 0.05% (v/v) Tween-20. All the assays were performed in triplicate. Each well of an ELISA microtiter plate was filled with 100 μl of the mixture buffer consisting of 20 μl culture medium and 80 μl coating buffer.

The plate was covered and incubated overnight at 4° C to allow the antigen to bind to the plate. The solutions were then removed and the wells were washed four times with PBS-T. The washed wells were filled with 200 μl diluent buffer and kept shaking for 1.5 h at room temperature (25° C). After washing with PBS-T for four times, the wells were filled with 100 μl antibody (1:8000) and were incubated for 1.5 h, followed by washing for four times. Then, the wells were filled with 100 μl goat anti-mouse IgG HRP conjugate complex diluted to a final 1:3000 dilution, and were incubated at room temperature for 1.5 h, followed by washing for four times. The enzyme reactions were started by addition of 100 μl substrate (TMB) solution. The reactions were stopped after 15 min by the addition of 100 μl of 2 M sulfuric acid, and the absorbance was read at 460 nm using a Bio-Rad ELISA plate reader.

While the terms used herein are believed to be well understood by one of ordinary skill in the art, the definitions set forth herein are provided to facilitate explanation of the presently-disclosed subject matter. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the presently-disclosed subject matter belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the presently-disclosed subject matter, representative methods, devices, and materials are now described.

Following long-standing patent law convention, the terms “a,” “an,” and “the” refer to “one or more” when used in this application, including the claims. Thus, for example, reference to “a cell” includes a plurality of such cells, and so forth.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently-disclosed subject matter.

As used herein, the term “about,” when referring to a value or to an amount of mass, weight, time, volume, concentration or percentage is meant to encompass variations of in some embodiments ±50%, in some embodiments ±40%, in some embodiments ±30%, in some embodiments ±20%, in some embodiments ±10%, in some embodiments ±5%, in some embodiments ±1%, in some embodiments ±0.5%, and in some embodiments ±0.1% from the specified amount, as such variations are appropriate to perform the disclosed method.

Throughout this document, various references are mentioned. All such references are incorporated herein by reference.
US 8,206,703 B1

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Val Leu Glu Met Thr Gly Aaa 11e Asp Glu Ala Glu Trp Glu Trp Lye
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<210> SEQ ID NO: 9
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE
<400> SEQUENCE: 9

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caagctcag ttagctcag aacatgttcc ctctttgaca atcatctttg caccagagac
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780
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840
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gacatgcgc actatatttact taacatggga caatttaaas aaccccagat tttgtggtgt
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1020
aacataattgct ctaaatag ctgaagagtg taaaattttag aataactgga
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1260
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1440
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1560
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Glu 1 Asn
Tyr
Leu
Cys 65 Met
Val
Ile
Asp
145 Glu
Trp
Thr
Leu
Gly 225 Asn
Glu
Leu
Ile 305 Val
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Ala
Thr 50 Cys
Trp
Trp
Tyr
Gly 130 Tyr
Ala
Val
Leu
Ser 210 Ser
Arg
Thr
Leu
Phe 290 Leu
Asn
Thr
Gln 35 Lys
Gln
Asn
Ile
Gly 115 Lys
Arg
Pro
Gln
Phe 195 Pro
Ala
Thr
Glu 275
Asn
Gly
Trp
Asn
Pro
Pro 100 Gly
Phe
Val
Gly Lys 180 Gly Gly
Asn
Leu
Ile 260 Glu
Pro
Glu
Asp 90 Asn
Thr
Arg
Leu
Asp 170 Gly
Thr
Arg
Glu 330 Gly
Trp
Lys
Phe
Leu
Ala
Gly 15 Ile
Gln
Asn
Ser
Leu 95 Ile
Val
Ser
Asn 175 Ser
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Gln
Ala 255 Glu
Gly
Pro
Val
Leu 190 Leu
Leu
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Arg
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Met 335
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Trp
Tyr
Met
Pro 160 Gln
Val
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Ser 240 Asn
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Asp Gly
320 Gly
Ile 15
Gly
Arg Gly
Pro
Ala
Gly Tyr
Leu 110 His
Val
Gly
Ala 190 Leu
Leu
Glu
Arg
Leu
Met
Leu
Ala
Ala 320 Gly
Lys
Phe
Trp
Lys Lys 60 Phe
Cys
Thr
Ser
Ile 140 Leu
Gln
Asn
Val
Asn
Leu 220 Leu
Cys Asp
Thr
Thr 300 Gln
Gly
Val
Leu
Lys 45 Tyr
His
Leu
Val
Leu 125 Val
Pro
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Gly 1620 1680 1722

<210> SEQ ID NO 10
<211> LENGTH: 574
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE: OTHER INFORMATION: mutant of human BChE
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Tyr Ala Glu Pro Pro Leu Gly Arg Leu Arg Phe Lys Pro Gln Ser 35 40 45
Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser 50 55 60
Cys Cys Glu Asn Ile Asp Glu Ser Phe Pro Gly Phe His Gly Ser Glu 65 70 75 80
Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Ann 85 90 95
Val Trp Ile Pro Ala Pro Lys Pro Lys Ann Ala Thr Val Leu Ile Trp 100 105 110
Ile Tyr Gly Gly Gly Phe Gin Thr Gly Thr Ser Ser Leu His Val Tyr 115 120 125
Asp Gly Lys Phe Leu Ala Arg Val Gly Arg Val Ile Val Val Ser Met 130 135 140
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro 145 150 155 160
Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gin Gin Leu Ala Leu Gin 165 170 175
Trp Val Gin Lys Asn Ile Ala Ala Phe Gin Gin Asn Pro Lys Ser Val 180 185 190
Thr Leu Phe Gin Glu Ser Ser Gly Ala Ala Ser Val Ser Leu His Leu 195 200 205
Leu Ser Pro Gly Ser His Ser Leu Phe Thr Arg Ala Ile Leu Gin Ser 210 215 220
Gly Ser Ala Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg 225 230 235 240
Asn Arg Thr Leu Asn Leu Ala Lys Leu Thr Gly Cys Ser Arg Glu Ann 245 250 255
Glu Thr Glu Ile Ile Lys Cys Gin Gin Asn Lys Gin Asp Glu Glu Ile 260 265 270
Leu Leu Asn Glu Ala Phe Val Val Gly Thr Gly Thr Gly Leu Gly Val 275 280 285
Asn Phe Glu Pro Thr Val Asp Gin Phe Leu Thr Asp Met Pro Asp 290 295 300
Ile Leu Leu Glu Gin Gin Lys Lys Thr Gin Gin Ile Leu Val Gly 305 310 315 320
Val Asn Lys Gin Gin Lys Gin Thr Trp Phe Leu Val Gly Gly Ala Pro Gly 325 330 335
33 US 8,206,703 B1

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Phe Ser Lys Asp Asn Ser Ile Ile Thr Arg Lys Glu Phe Gln Glu 340 345 350
Gly Leu Lys Ile Phe Phe Pro Gly Val Ser Glu Phe Gly Lys Glu Ser 355 360 365
Ile Leu Phe His Tyr Thr Asp Trp Val Asp Gln Arg Pro Glu Asn 370 375 380
Tyr Arg Glu Ala Leu Gly Asp Val Val Gly Tyr Asn Phe Ile Cys 385 390 395 400
Pro Ala Leu Glu Phe Thr Lys Phe Ser Glu Trp Gly Asn Asn Ala 405 410 415
Phe Phe Tyr Tyr Phe Glu His Arg Ser Ser Lys Leu Pro Trp Pro Glu 420 425 430
Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu 435 440 445
Pro Leu Glu Arg Arg Asp Asn Tyr Thr Lys Ala Glu Glu Ile Leu Ser 450 455 460
Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro 465 470 475 480
Asn Glu Thr Gln Asn Asn Ser Thr Ser Trp Pro Val Phe Lys Ser Thr 485 490 495
Glu Gln Lys Tyr Leu Thr Leu Asn Thr Glu Ser Thr Arg Ile Met Thr 500 505 510
Lys Leu Arg Ala Gln Gln Cys Arg Phe Trp Thr Ser Phe Phe Pro Lys 515 520 525
Val Leu Glu Met Thr Gly Asn Ile Asp Glu Ala Glu Trp Glu Trp Lys 530 535 540
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE

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<210> SEQ ID NO 12
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER:
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<400> SEQUENCE: 12

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Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Lys Pro Gln Ser
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Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser
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Cys Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe His Gly Ser Glu
65                       70                          75                          80
Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Asn
95                       100                         105                         110
Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp
100                      105                         110
Ile Tyr Gly Gly Gly Phe Gly Thr Gly Thr Ser Leu His Val Tyr
115                      120                         125
Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met
130                      135                         140
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro
145                      150                         155                         160
Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gin Gin Leu Ala Leu Gin
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<222> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE
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tcttggatca aasaaccacca gctctctcgg acttctctag atatatttgg ttgcaaaaaa 180
tatagcaaat ttctgtgcta gaaacatag ctaaagtttc cagctttttc tggatcagag 240
atgataagc aacaacactg cctcacgtta gcctgttatt atataaatgt atggatcaca 300
gcaacataac aacaaatagc cactgtatag atagcgttgg ttctctaaact 360
ggaacatacct cttcatatgt ttatgatgcg aatcgttcttg cttggtttga aagagttatt 420
gtagtgcata taagctatat ggtgggtgcc ctagattttct tagctttgccc cggaaactct 480
gagcctccag ggaacatgctgg ctttatttga cacaagtggt ccttcacagtg gtttcaaaaaa 540
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ccctagggc ctcagccctg cttcttattt gttcagccag tcggatgcttg ttctctctag 900
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gtaataaatg atggcttgac atgctttttta gttcaggggt gtttctgtct ccacccagat 1020
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cctcggctg agctcattcc gacacgactg gcagttcttc gccggtgtg gatgtctctct 1260
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tggacagttgg aagcagcatg ccacgctcag aacaaatcaca tgaagactc ggaaatctca 1680
tttaaagct acactagccaa gaaagagat tcgtgtggtgct tc 1722

SEQ ID NO 14
LENGTH: 574
TYPE: PAT
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: mutant of human BChE
SEQUENCE: 14

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Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Gly Lys Pro Glu Ser
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Leu Thr Lys Trp Ser Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser
50 55 60
Cys Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe His Gly Ser Glu
65      70     75     80
Met Trp Asn Pro Asn Thr Asp Ser Glu Asp Cys Leu Tyr Leu Asn
85      90     95
Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp
100     105    110
Ile Tyr Gly Gly Phe Glu Thr Gly Thr Ser Ser Leu His Val Tyr
115     120    125
Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met
130     135    140
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro
145     150    155    160
Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Glu Glu Leu Ala Leu Glu
165     170    175
Trp Val Glu Lys Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val
180     185    190
Thr Leu Phe Gly Glu Ser Ser Gly Ala Ala Ser Val Ser Leu His Leu
195     200    205
Leu Ser Pro Gly Ser His Ser Leu Phe Thr Arg Ala Ile Leu Glu Ser
210     215    220
Gly Ser Phe Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg
225     230    235    240
Asn Arg Thr Leu Asn Leu Ala Lys Leu Thr Gly Cys Ser Arg Glu Asn
245     250    255
Glu Thr Glu Ile Ile Lys Cys Leu Arg Asn Lys Asp Pro Glu Glu Ile
260     265    270
Leu Leu Asn Glu Ala Phe Val Val Pro Tyr Glu Thr Glu Leu Gly Val
275     280    285
Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr Asp Met Pro Asp
290     295    300
Ile Leu Leu Glu Leu Gly Gin Phe Lys Thr Gin Ile Leu Val Gly
305     310    315    320
Val Asn Lys Asp Glu Gly Thr Trp Phe Leu Val Gly Gly Ala Pro Gly
325     330    335
Phe Ser Lys Asp Asn Asn Ser Ile Ile Thr Arg Lys Glu Phe Gin Glu
340     345    350
Gly Leu Lys Ile Phe Phe Pro Gly Val Ser Glu Phe Gly Lys Glu Ser
355     360    365
Ile Leu Phe His Tyr Thr Asp Trp Val Asp Asp Gin Arg Pro Glu Asn
370     375    380
Tyr Arg Glu Ala Leu Gly Asp Val Gly Asp Tyr Asn Phe Ile Cys
395     390    395    400
Pro Ala Leu Glu Phe Thr Lys Phe Ser Glu Thr Gin Asn Ala
405     410    415
Phe Phe Tyr Tyr Phe Gin His Arg Ser Ser Lys Leu Pro Thr Pro Glu
420     425    430
Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu
435     440    445
Pro Leu Glu Arg Arg Asn Tyr Thr Lys Ala Glu Glu Ile Leu Ser
450     455    460
Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro
465     470    475    480
Asn Glu Thr Gin Asn Asn Ser Thr Ser Trp Pro Val Phe Lys Ser Thr
Glu Glu Lys Tyr Leu Thr Leu Aen Thr Glu Ser Thr Arg Ile Met Thr
500  505  510
Lys Leu Arg Ala Gln Gln Cys Arg Phe Trp Thr Ser Phe Pro Lys
515  520  525
Val Leu Glu Met Thr Gly Aen Ile Asp Glu Ala Glu Trp Glu Trp Lys
530  535  540
Ala Gly Phe His Arg Trp Aen Ien Tyr Met Met Asp Trp Lys Aen Glu
545  550  555  560
Phe Aen Asp Tyr Thr Ser Lys Glu Ser Cys Val Gly Leu
565  570

<210> SEQ ID NO 15
<211> LENGTH: 1722
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE

<400> SEQUENCE: 15

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cctcgattca aaaaagcaca gctctttgac caagtcggtgc atatcttggaa tggcaacaaca 180
tatgcaaaat cttggtgca gaaatcctag caaagttcctcgctctgca tggatcagag 240
atggtcggca caacacgctg cctgcagttga gctgtttttg ataatattaag atggattccga 300
gcctcaacc ccaaaaaagg cacatgagg cagtaatttag atggctttttg tttccttaact 360
ngaaccatcg cttctatact cttataattgc aagtttctgg cttggttctga aagagtattat 420
gtaagcttca gtaagcctag ggtgggtgcc cttggttagt tattgctggc ggaaaccctt 480
gagctcccag ggaacattcgg tcctttttttg ctctgactgt gctgttaggt gattcttgact 540
aatatacgcg cctggttccct aamctctaata agtgaacactcg ttccctgctt cgaattccgg 600
gcgcctctcg ctctttcttt cttggaagcc atctcattgt cacagagocc 660
attaagcttca gttggggcgtc tatgcctctctt gggccatttt atctcctata gaaagtctgtg 720
aacacaggt gtaaccttcgc taaaggctat gttggctctta gagaagaatg gactggaataa 780
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gttctttcg gttgcgtctt gttgggttggta cttctttcttt atgtctttgg 1140
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aatgcgtttt gttgggagag atcttttttt gttggttggta cttctttcttt atgtctttgg 1620
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ttaaacgatt acacagtgaa aagagaaggt tgtgtggtgct tc
1722

<210> SEQ ID NO 16
<211> LENGTH: 574
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE

<400> SEQUENCE: 16
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Asn Leu Thr Val Phe Gly Gly Thr Val Thr Ala Phe Leu Gly Ile Pro
20 25 30
Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Lys Pro Gin Ser
35 40 45
Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser
50 55 60
Cys Cys Gln Asn Ile Asp Gin Ser Phe Pro Gly Phe His Gly Ser Glu
65 70 75 80
Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Asn
85 90 95
Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp
100 105 110
Ile Tyr Gly Gly Gly Phe Gin Thr Gly Thr Ser Ser Leu His Val Tyr
115 120 125
Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met
130 135
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro
145 150 155 160
Glu Ala Pro Gly Asn Met Gin Leu Phe Gin Gin Gin Leu Ala Leu Gin
165 170 175
Trp Val Gin Lys Asn Ile Ala Ala Phe Gly Gin Pro Lys Ser Val
180 185 190
Thr Leu Phe Gly Ser Ser Gly Ala Ala Ser Val Ser Leu His Leu
195 200 205
Leu Ser Pro Gin Ser His Ser Leu Phe Thr Arg Ala Ile Leu Gin Ser
210 215 220
Gly Ser Phe Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg
225 230 235 240
Asn Arg Thr Leu Asn Leu Ala Lys Leu Thr Gly Cys Ser Arg Glu Asn
245 250 255
Glu Thr Glu Ile Ile Lys Cys Leu Asn Lys Gly Asp Pro Gin Glu Ile
260 265 270
Leu Leu Asn Glu Ala Phe Val Val Pro Tyr Gly Thr Ile Leu Gly Val
275 280 285
Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr Asp Met Pro Asp
290 295 300
Ile Leu Leu Gly Leu Gly Gin Phe Lys Thr Gin Ile Leu Val Gly
305 310 315 320
Val Asn Lys Arg Glu Gly Thr Trp Phe Leu Val Gly Gly Ala Pro Gly
325 330 335
Phe Ser Lys Asp Asn Asn Ser Ile Ile Thr Arg Lys Glu Phe Gin Glu
340 345 350
Gly Leu Lys Ile Phe Phe Pro Gly Val Ser Glu Phe Gly Lys Glu Ser 355 360 365
Ile Leu Phe His Tyr Thr Asp Trp Val Asp Asp Gln Arg Pro Glu Asn 370 375 380
Tyr Arg Glu Ala Leu Gly Asp Val Val Gly Asp Tyr Asn Phe Ile Cys 385 390 395 400
Pro Ala Leu Glu Phe Thr Lys Phe Ser Glu Thr Gly Asn Asn Ala 405 410 415
Phe Phe Tyr Tyr Phe Glu His Arg Ser Ser Lys Leu Pro Trp Pro Glu 420 425 430
Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu 435 440 445
Pro Leu Glu Arg Arg Asp Asn Tyr Thr Lys Ala Glu Ile Leu Ser 450 455 460
Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro 465 470 475 480
Asn Glu Thr Gln Asn Asn Ser Thr Ser Trp Pro Val Phe Lys Ser Thr 485 490 495
Glu Gln Lys Tyr Leu Thr Leu Asn Thr Glu Ser Thr Arg Ile Met Thr 500 505 510
Lys Leu Arg Ala Gln Gln Cys Arg Phe Thr Ser Phe Phe Pro Lys 515 520 525
Val Leu Glu Met Thr Gly Asn Ile Asp Glu Ala Glu Trp Glu Lys 530 535 540
Ala Gly Phe His Arg Trp Asn Asn Tyr Met Met Asp Trp Lys Asn Gln 545 550 555 560
Phe Asn Asp Tyr Thr Ser Lys Glu Ser Cys Val Gly Leu 565 570

<210> SEQ ID NO 17
<211> LENGTH: 1722
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE

<400> SEQUENCE: 17

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tatgaaatgt tttggtgcca ggacatatgg ccagattttcg caggctccca tggatcagag 240
atggagaccc caaaccagtc ctccagtgaa gcacttttat atctaaatgt atggatctcca 300
gccctcaacc caaaaaattg cactgatttg atatggtttt atggtgctgg ttttcaaat 360
ggaacacatc cttcatcgat ttgtatgggc aaggactctt gttctgtgga aagagttatat 420
gtactgtcaaa tgaactatag ggtggttgcct ctaggtttct tagcctgcg aggaaatctc 480
gaggtctccg ggaacacatgg tttatgtat gtcacagttg ctcctcagtc ggctccttcas 540
aatatagcg ctttgggtgcc aatctctaaa aagttactct ctttgggaa aagttcctga 600
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attctgctaa gttggttccc ttaagcttc tggcgcgttaa catctctttta tgaagcttgg 720
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<210> SEQ ID NO 18
<211> LENGTH: 574
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATUERE: OTHER INFORMATION: mutant of human BChE
<400> SEQUENCE: 18

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Asn Leu Thr Val Phe Gly Gly Thr Val Thr Ala Phe Leu Gly Ile Pro
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Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Pro Gln Ser
35 40 45
Leu Thr Lys Trp Ser Asp Ile Trp Amin Ala Thr Lys Tyr Ala Asn Ser
50 55 60
Cys Cys Gln Amin Ile Amin Gly Ser Phe Pro Gly Phe His Gly Ser Glu
65 70 75 80
Met Trp Asn Pro Asp Thr Asp Leu Ser Glu Amin Cys Leu Tyr Leu Amin
85 90 95
Val Trp Ile Pro Ala Pro Lys Pro Lys Amin Ala Thr Val Leu Ile Trp
100 105 110
Ile Tyr Gly Gly Gly Phe Glu Thr Gly Thr Ser Ser Leu His Val Tyr
115 120 125
Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met
130 135 140
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Pro Gly Aen Pro
145 150 155 160
Glut Amin Pro Gly Met Gly Leu Phe Asp Gln Gln Leu Ala Leu Gln
165 170 175
Trp Val Glu Lys Amin Ile Ala Ala Phe Gly Aamin Pro Lys Ser Val
180 190
Thr Leu Phe Gly Glu Ser Gly Ala Ala Ser Val Ser Leu His Leu
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<210> SEQ ID NO 19
<211> LENGTH: 1722
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<222> OTHER INFORMATION: mutant of human BChE
<400> SEQUENCE: 19

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cattcaccg aaagcaccaga gtcctctgcgc aagtggtctgtg atatggggaa agttcaca 180
tatatgttta ctcttggtt tagatcatttctttgttttcagcgtactt 190
tggtggccat ccacagcactgcag tggcgtggtgcag gcctgtacgtg 240
cggcagcactgcag tggcgtggtgc 260
tggtggccat ccacagcactgcag tggcgtggtgc 280
tggtggccat ccacagcactgcag tggcgtggtgc 300
cggcagcactgcag tggcgtggtgc 310
tggtggccat ccacagcactgcag tggcgtggtgc 330
tggtggccat ccacagcactgcag tggcgtggtgc 350
tggtggccat ccacagcactgcag tggcgtggtgc 370

tgccagcttttttctttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Asn
   85   90  95
Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp
   100 105 110
Ile Tyr Gly Gly Gly Phe Gln Thr Gly Thr Ser Ser Leu His Val Tyr
   115 120 125
Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met
   130 135 140
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro
   145 150 155 160
Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gin Gin Leu Ala Leu Gin
   165 170 175
Trp Val Gln Lys Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val
   180 185 190
Thr Leu Phe Gly Glu Ser Ser Gly Ala Ala Ser Val Ser Leu His Leu
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE

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Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp 105 110
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Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met 130 135 140
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<222> OTHER INFORMATION: mutant of human BChE

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**<210> SEQ ID NO: 24**

**<211> LENGTH: 574**

**<212> TYPE: PRT**

**<213> ORGANISM: Artificial Sequence**

**<220> FEATURE:***

**<223> OTHER INFORMATION: mutant of human BChE**

**<400> SEQUENCE:***

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260 265 270
Leu Leu Asn Glu Ala Phe Val Val Pro Tyr Gly Thr Gly Leu Gly Val
275 280 285
Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr Asp Met Pro Asp
290 295 300
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305 310 315 320
Val Asn Lys Asp Glu Gly Thr Trp Phe Leu Val Gly Gly Ala Pro Gly
325 330 335
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340 345 350
Gly Leu Lys Ile Phe Phe Pro Gly Val Ser Glu Phe Gly Lys Glu Ser
355 360 365
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<210> SEQ ID NO 25
<211> LENGTH: 1722
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE
<400> SEQUENCE: 25

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**<210> SEQ ID NO 26**
**<211> LENGTH: 574**
**<212> TYPE: PRT**
**<213> ORIGIN: Artificial Sequence**
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**<223> OTHER INFORMATION: mutant of human BChE**

**<400> SEQUENCE: 26**

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<211> LENGTH: 574
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutant of human BChE

<400> SEQ断裂: 28

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Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser 50  55  60
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Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met 130  135 140
Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro 145  150 155 160
Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gin Gin Leu Ala Leu Gin 165 170 175
Trp Val Gin Lys Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val 180 185 190
Thr Leu Phe Gly Glu Ser Ser Gly Ala Ala Ala Ser Val Ser Leu His Leu 195 200 205
Leu Ser Pro Gly Ser His Ser Leu Phe Thr Arg Ala Ile Leu Gin Ser 210 215 220
Gly Ser Leu Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg 225 230 235 240
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Glu Thr Glu Ile Ile Lys Cys Leu Arg Asn Lys Asp Pro Gin Glu Ile 260 265 270
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Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr Asp Met Pro Asp 290 295 300
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Val Asn Lys Asp Glu Gly Thr Trp Phe Leu Val Gly Gly Ala Pro Gly 325 330 335
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<210> SEQ ID NO 29
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<222> OTHER INFORMATION: mutant of human BChE

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Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Gly Lys Pro Gln Ser
35  40  45
Leu Thr Lys Trp Ser Asp Ile Thr Asn Ala Thr Lys Tyr Ala Asn Ser
50  55  60
Cys Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe His Gly Ser Glu
65  70  75  80
Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Asn
85  90  95
Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp
100 105 110
Ile Tyr Gly Gly Gly Phe Gln Thr Gly Thr Ser Ser Leu His Val Tyr
115 120 125
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Trp Val Gln Lys Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val
190 195 200 205
Thr Leu Phe Gly Gly Ser Ser Gly Ala Asp Ser Leu His Leu
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Gly Ser Phe Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg
Glu Asp Asp Ile Ile Ile Ala Thr Lys Arg Arg Gly Lys Val Arg Gly Met
1    5    10    15
Asn Leu Thr Val Phe Gly Gly Thr Val Thr Ala Phe Leu Gly Ile Pro
20    25    30
Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Pro Glu Ser
35    40    45
Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser
50    55    60
Cys Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe His Gly Ser Glu
65    70    75    80
Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Asn
95    90    95
Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp 100 105 110
Ile Tyr Gly Gly Gly Phe Gln Thr Gly Thr Ser Ser Leu His Val Tyr 115 120 125
Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met 130 135 140
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Thr Leu Phe Gly Glu Ser Ser Gly Ala Ala Ser Val Ser Leu His Leu 195 200 205
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Ile Leu Leu Glu Leu Gly Gin Phe Lys Thr Gin Ile Leu Val Gly 305 310 315 320
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Gly Leu Lys Ile Phe Phe Pro Gly Val Ser Glu Phe Gly Lys Glu Ser 355 360 365
Ile Leu Phe His Tyr Thr Asp Trp Val Asp Gin Arg Pro Glu Asn 370 375 380
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Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu 435 440 445
Pro Leu Glu Arg Arg Asp Thr Tyr Ala Glu Glu Ile Leu Ser 450 455 460
Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro 465 470 475 480
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Glu Gin Lys Tyr Leu Thr Leu Asn Thr Glu Ser Thr Arg Ile Met Thr 500 505 510
Lys Leu Arg Ala Glu Gin Cys Arg Phe Trp Thr Ser Phe Phe Pro Lys
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Phe Asn Asp Tyr Thr Ser Lys Lys Glu Ser Cys Val Gly Leu
565  570

What is claimed is:
1. A butyrylcholinesterase polypeptide variant comprising the amino acid sequence of SEQ ID NO: 6.
2. A pharmaceutical composition comprising:
a butyrylcholinesterase polypeptide variant comprising the amino acid sequence of SEQ ID NO: 6; and a suitable pharmaceutical carrier.
3. A method of treating a cocaine-induced condition comprising administering to an individual an effective amount of the butyrylcholinesterase polypeptide variant of claim 1 to lower blood cocaine concentration.

4. The method of claim 3, wherein said butyrylcholinesterase polypeptide variant exhibits a one-hundred-fold or more increase in cocaine hydrolysis catalytic efficiency compared to butyrylcholinesterase.

5. A method of treating a cocaine-induced condition comprising administering to an individual an effective amount of the pharmaceutical composition of claim 2 to lower blood cocaine concentration.