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(54) **Title:** MODIFIED LIGAND-GATED ION CHANNELS AND METHODS OF USE

(57) **Abstract:** This document relates to materials and methods for controlling ligand gated ion channel (LGIC) activity. For example, modified LGICs including at least one LGIC subunit having a modified ligand binding domain (LBD) and/or a modified ion pore domain (IPD) are provided. Also provided are exogenous LGIC ligands that can bind to and activate the modified LGIC, as well as methods of modulating ion transport across the membrane of a cell of a mammal, methods of modulating the excitability of a cell in a mammal, and methods of treating a mammal having a channelopathy.

MODIFIED LIGAND-GATED ION CHANNELS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Patent Application Serial No. 62/359,534, filed on July 7, 2016, and claims the benefit of U.S. Patent Application Serial No. 62/486,779, filed on April 18, 2017. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

BACKGROUND

1. Technical Field

This document relates to materials and methods for controlling ligand gated ion channel (LGIC) activity. For example, this document provides modified LGICs including at least one LGIC subunit having a modified ligand binding domain (LBD) and/or a modified ion pore domain (IPD). Also provided are exogenous LGIC ligands that can bind to and activate the modified LGIC. In some cases, a modified LGIC and an exogenous ligand can be used to treat a mammal having a channelopathy (*e.g.*, a neural channelopathy or a muscle channelopathy). In some cases, a modified LGIC and an exogenous LGIC ligand can be used to modulate (*e.g.*, activate or inhibit) ion transport across the membrane of a cell of a mammal. In some cases, a modified LGIC and an exogenous LGIC ligand can be used to modulate (*e.g.*, increase or decrease) the excitability of a cell in a mammal.

2. Background Information

Ion channels mediate ionic flux in cells, which profoundly affects their biological function. A prominent instance of this is in neurons, where ion channels control electrical signaling within between neurons to influence physiology, sensation, behavior, mood, and cognition.

Different LGICs have distinct ligand binding properties as well as specific ion conductance properties (Hille 2001 Ion Channels of Excitable Membranes. pp. 814. Sunderland, MA: Sinauer Associates; Kandel et al 2000 Principles of Neural Science. USA: McGraw-Hill Co. 1414 pp). For example, nicotinic acetylcholine receptors (nAChRs) bind the endogenous ligand acetylcholine (ACh), which activates conductances for cations and typically depolarizes cells, thereby increasing cellular excitability. In contrast, the glycine

receptor (GlyR) binds the endogenous ligand glycine, which activates chloride anion conductance and typically reduces the excitability of cells by hyperpolarization and/or by an electrical shunt of the cellular membrane resistance.

SUMMARY

Levels of endogenous LGIC agonists such as ACh are not readily controlled.

This document provides materials and methods for controlling LGIC activity (*e.g.*, increasing the sensitivity of LGICs to exogenous ligands and/or reducing sensitivity to endogenous ligands such as ACh). For example, this document provides modified LGICs including at least one modified LGIC subunit having a LBD and an IPD, and having at least one modified amino acid (*e.g.*, an amino acid substitution). Also provided are exogenous LGIC ligands that can bind to and activate the modified LGIC. In some cases, a modified LGIC and an exogenous ligand can be used to treat a mammal having a channelopathy (*e.g.*, a neural channelopathy or a muscle channelopathy). In some cases, a modified LGIC and an exogenous LGIC ligand can be used to modulate (*e.g.*, activate or inhibit) ion transport across the membrane of a cell of a mammal. In some cases, a modified LGIC and an exogenous LGIC ligand can be used to modulate (*e.g.*, increase or decrease) the excitability of a cell in a mammal.

Having the ability to control LGIC activity provides a unique and unrealized opportunity to achieve control of ion transport in cells. For example, modified LGICs having increased sensitivity for one or more exogenous LGIC ligands can be used to provide temporal and spatial control of ion transport and/or cellular excitability based on delivery of the exogenous LGIC ligand. For example, modified LGICs with reduced sensitivity for endogenous LGIC ligands prevent unwanted activation of modified LGICs and allow for selective control over the modified LGIC by exogenous ligands. Further, exogenous LGIC ligands having increased potency for a modified LGIC improve selectivity of targeting of the modified LGIC over endogenous ion channels. Thus, the modified LCIGs and exogenous LGIC ligands provided herein are useful to achieve a therapeutic effect while reducing side effects from the small molecules on unintended targets.

As described herein, one or more mutations in a modified LGIC can enhance potency for exogenous LGIC ligands. Mutation of the α7 LBD of α7-GlyR at residue L131 (*e.g.*, substituting Leu with Gly or Ala) increased potency for varenicline (16-fold) and tropisetron

(3.6-fold) while reducing ACh potency (-6.4-fold) relative to α7-GlyR. Mutation of α7 LBD of α7-GlyR at residue G175 (*e.g.*, G175K) or P216 (*e.g.*, P216I) enhanced potency for ACh, nicotine, tropisetron, varenicline, as well as other quinuclidine and tropane agonists. Combining the mutation at residue G175K with mutations that reduce potency of the endogenous agonist ACh (*e.g.* Y115F) produced α7-GlyR Y115F G175K with increased potency for tropisetron (5.5-fold) and reduced potency from ACh (-8-fold). In addition, combining mutations in the α7 LBD at residues 77 (*e.g.*, substituting Trp with Phe or Tyr) and/or 79 (*e.g.*, substituting Gln with Gly, Ala, or Ser) and/or 131 (*e.g.*, substituting Leu with Gly or Ala) and/or141 (*e.g.*, substituting Leu with Phe or Pro) in these chimeric channels with potency enhancing mutations at residues G175 (*e.g.*, G175K) or P216 (*e.g.*, P216I) increase potency for distinct ligands and/or reduce ACh potency. For example, a chimeric α7-GlyR LGIC with a α7 nAChR LBD (α7 LBD) having a mutation at residue 79 (*e.g.*, substituting Gln with Gly), a mutation at residue 115 (*e.g.*, substituting Tyr with Phe), and a mutation at residue 175 (*e.g.*, substituting Gly with Lys) has greater than 100-fold increased sensitivity to an exogenous tropane LGIC ligand compound 723 (a tropane), and reduced ACh sensitivity (-15-fold) relative to the unmodified chimeric α7-GlyR LGIC. Furthermore, a modified LGIC including at least one chimeric LGIC subunit having an α7 nAChR LBD (α7 LBD) having a mutation at residue 79 (*e.g.*, substituting Gln with Ala, Gly, or Ser) and a GlyR IPD having a mutation at residue 298 (*e.g.*, substituting Ala with Gly) has nearly 20-fold increased sensitivity for an exogenous LGIC ligand, such as a quinuclidine or a tropane. Additional mutations at residue 27 (*e.g.*, substituting Arg with Asp) and 41 (*e.g.*, substituting Glu with Arg) of the α7 LBD reduced the association of the modified chimeric LGIC with an unmodified ion channels. Additional mutations at residue 115 (*e.g.*, substituting Tyr with Phe), 139 (*e.g.*, substituting Gln with Gly or Leu), 210 (*e.g.*, substituting Tyr with Phe) 217 (*e.g.*, substituting Tyr with Phe), and/or 219 (*e.g.*, substituting Asp with Ala) of the α7 LBD reduced sensitivity of the chimeric LGIC to the endogenous ligand ACh. These chimeric LGICs allow for highly selective control over cellular function in cells of a mammal while minimizing cross-reactivity with endogenous signaling systems in the mammal.

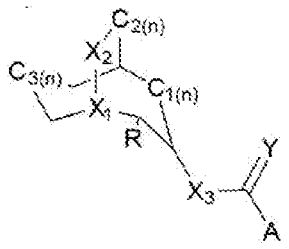
In general, one aspect of this document features a modified LGIC having at least one modified LGIC subunit which includes a LBD having an amino acid modification, and an IPD, where an exogenous LGIC ligand activates the modified LGIC. The modified LGIC can be a chimeric LGIC having a LBD from a first LGIC and an IPD from a second LGIC.

The LBD can be an alpha7 nicotinic acetylcholine receptor (α7-nAChR) LBD. The modified LGIC of claim 3, wherein the at least one modified amino acid in the α7-nAChR LBD comprises an amino acid substitution at an amino acid residue selected from the group consisting of residues 77, 79, 131, 139, 141, 175, and 216 of the α7-nAChR LBD. The amino acid substitution can be at residue 79 of the α7 LBD, and the amino acid substitution can be Q79A, Q79G, or Q79S. For example, the amino acid substitution at residue 79 of the α7 LBD can be Q79G. The IPD can be a serotonin 3 receptor (5HT3) IPD, a glycine receptor (GlyR) IPD, a gamma-aminobutyric acid (GABA) receptor IPD, or an α7-nAChR IPD. The IPD can be a GlyR IPD, and the GlyR IPD can include an amino acid substitution at residue 298 (*e.g.*, a A298G substitution) of the chimeric LGIC. The IPD can be a GABA IPD, and the GABA IPD can include an amino acid substitution at residue 298 (*e.g.*, a W298A substitution) of the modified LGIC. The modified LGIC can be a chimeric LGIC including an α7 LBD having a Q79G amino acid substitution, and a GlyR IPD having a A298G amino acid substitution. The exogenous LGIC ligand can be a synthetic exogenous LGIC ligand selected from the group consisting of a quinuclidine, a tropane, a 9-azabicyclo[3.3.1]nonane, a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine, and a 1,4-diazabicyclo[3.2.2]nonane. When the synthetic exogenous LGIC ligand is a tropane, the tropane can be tropisetron, pseudo-tropisetron, nortropisetron, compound 723, compound 725, compound 737, or compound 745. When the synthetic exogenous LGIC ligand is a quinuclidine, the quinuclidine can be PNU-282987, PHA-543613, compound 0456, compound 0434, compound 0436, compound 0354, compound 0353, compound 0295, compound 0296, compound 0536, compound 0676, or compound 702. When the synthetic exogenous LGIC ligand is a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine, the ligand can be compound 765 or compound 770. When the synthetic exogenous LGIC ligand is a 1,4-diazabicyclo[3.2.2]nonane, the ligand can be compound 773 or compound 774. In some cases, the LBD can be an α7 LBD, and the α7 LBD can also include at least one modified amino acid that confers selective binding to another α7 LBD having the at least one modified amino acid over binding to an unmodified LGIC. The unmodified LGIC can be an endogenous LGIC (*e.g.*, an endogenous α7-nAChR). The at least one modified amino acid in the α7 LBD that confers reduced binding to the unmodified LGIC can include an amino acid substitution at residue 27 (*e.g.*, a R27D substitution) and/or residue 41 (*e.g.*, an E41R substitution). In some cases, the IPD can be a 5HT3 IPD, and the

5HT3 IPD can include at least one modified amino acid that confers increased ion conductance to the modified LGIC. The at least one modified amino acid in the 5HT3 IPD that confers increased ion conductance to the modified LGIC can include an amino acid substitution at an amino acid residue at residue 425 (*e.g.*, a R425Q substitution), 429 (*e.g.*, a R429D substitution), and/or 433 (*e.g.*, a R433A substitution).

In another aspect, this document features a modified LGIC having at least one modified LGIC subunit including a LBD having at least one modified amino acid, and an IPD, where the at least one modified amino acid in the LBD reduces binding with an endogenous LGIC ligand. The modified LGIC can be a chimeric LGIC having a LBD from a first LGIC and an IPD from a second LGIC. The endogenous LGIC ligand can be ACh. The modified LGIC can have an EC50 of greater than 20 μM for Ach. The at least one modified amino acid can include an amino acid substitution at residue 115, 139, 210, 217, and/or 219. When the at least one modified amino acid includes an amino acid substitution at residue 115, the amino acid substitution can be a Y115F substitution. When the at least one modified amino acid includes an amino acid substitution at residue 139, the amino acid substitution can be a Q139G or a Q139L substitution. When the at least one modified amino acid includes an amino acid substitution at residue 210, the amino acid substitution can be a Y210F substitution. When the at least one modified amino acid includes an amino acid substitution at residue 217, the amino acid substitution can be a Y217F substitution. When the at least one modified amino acid includes an amino acid substitution at residue 219, the amino acid substitution can be a D219A substitution.

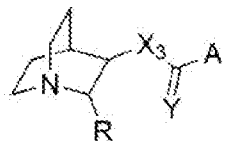
In another aspect, this document features a ligand having increased potency for a modified ligand gated ion channel (LGIC), wherein the ligand comprises Formula I:



where each of X1, X2, and X3 can independently be CH, CH2, O, NH, or NMe; where each n can independently be 0 or 1; where Y = O or S; where A = an aromatic substituent; and

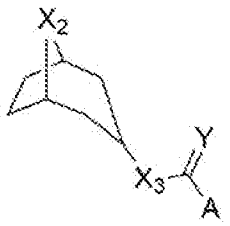
where R= H or pyridinylmethylene. The aromatic substituent can be 1H-indole, 4-(trifluoromethyl) benzene, 2,5-dimethoxy benzene, 4-chloroaniline, aniline, 5-(trifluoromethyl) pyridin-2-yl, 6-(trifluoromethyl) nicotinic, or 4-chloro-benzene.

In some cases, a LGIC ligand can be a quinuclidine and can have a structure shown in Formula II:



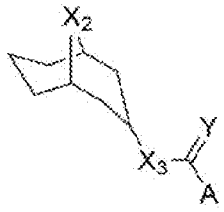
where X3 = O, NH, or CH2; where Y = O or S; where A = an aromatic substituent; and where R = H or pyridinylmethylene. The aromatic substituent can be 1H-indole, 4-(trifluoromethyl) benzene, 4-chloro benzene, 2,5-dimethoxy benzene, 4-(trifluoromethyl) benzene, 4-chloroaniline, aniline, 5-(trifluoromethyl) pyridin-2-yl, 6-(trifluoromethyl) nicotinic, 3-chloro-4-fluoro benzene, or 1H-indole. The quinuclidine can be PNU-282987, PHA-543613, compound 0456, compound 0434, compound 0436, compound 0354, compound 0353, compound 0295, compound 0296, compound 0536, compound 0676, or compound 702.

In some cases, a LGIC ligand can be a tropane and can have a structure shown in Formula III:



where X2 = NH or NMe; where X3 = O, NH, or CH2; where Y = O or S; and where A = an aromatic substituent. The aromatic substituent can be 1H-indole, 7-methoxy-1H-indole, 7-methyl-1H-indole, 5-chloro-1H-indole, or 1H-indazole. The tropane can be tropisetron, pseudo-tropisetron, nortropisetron, compound 723, compound 725, compound 737, or compound 745.

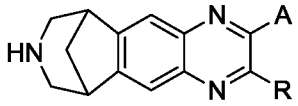
In some cases, a LGIC ligand can be a 9-azabicyclo[3.3.1]nonane and can have a structure shown in Formula IV:



,

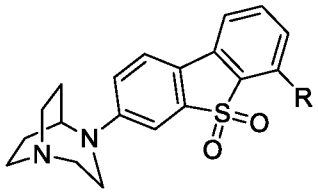
where X1 can be CH, X2 can be NH or NMe, X3 can be O, NH, or CH; Y can be O or S, and A can be an aromatic substituent. The aromatic substituent can be 4-chloro-benzene. The 9-azabicyclo[3.3.1]nonane can be compound 0536.

In another aspect, this document features a ligand having increased potency for a modified ligand gated ion channel (LGIC), where the ligand can be a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine and have a structure shown in Formula V:



where R can be H or CH3, and where A can be H or an aromatic substituent. The 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine can be varenicline, compound 0765, or compound 0770.

In another aspect, this document features a ligand having increased potency for a modified ligand gated ion channel (LGIC), where the ligand can be a 1,4-diazabicyclo[3.2.2]nonane and can have a structure shown in Formula VI:



where R can be H, F, or NO_2. The 1,4-diazabicyclo[3.2.2]nonane can be 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]thiophene 5,5-dioxide, compound 0773, or compound 0774.

In another aspect, this document features methods of treating a channelopathy in a mammal. The methods include, or consist essentially of, administering to a cell in the mammal a modified LGIC, where an exogenous LGIC ligand selectively binds the modified LGIC. The modified LGIC has at least one modified LGIC subunit including a LBD including at least one modified amino acid, and an IPD; and administering the exogenous ligand to the mammal. The channelopathy can be Bartter syndrome, Brugada syndrome,

catecholaminergic polymorphic ventricular tachycardia (CPVT), congenital hyperinsulinism, cystic fibrosis, Dravet syndrome, episodic ataxia, erythromelalgia, generalized epilepsy (*e.g.*, with febrile seizures), familial hemiplegic migraine, fibromyalgia, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, Lambert-Eaton myasthenic syndrome, long QT syndrome (*e.g.*, Romano-Ward syndrome), short QT syndrome, malignant hyperthermia, mucolipidosis type IV, myasthenia gravis, myotonia congenital, neuromyelitis optica, neuromyotonia, nonsyndromic deafness, paramyotonia congenital, retinitis pigmentosa, timothy syndrome, tinnitus, seizure, trigeminal neuralgia, or multiple sclerosis.

In another aspect, this document features methods of modulating ion transport across a cell membrane of a mammal. The methods include, or consist essentially of, administering to the cell a modified LGIC, where an exogenous LGIC ligand selectively binds the modified LGIC. The modified LGIC has at least one modified LGIC subunit including a LBD including at least one modified amino acid, and an IPD; and administering the exogenous ligand to the mammal. The modulating can include activating or inhibiting ion transport. The cell can be a neuron, a glial cell, a myocyte, a stem cell, an endocrine cell, or an immune cell. The administering the modified LGIC to the cell can be an in vivo administration or an ex vivo administration. The administering the modified LGIC to the cell can include administering a nucleic acid encoding the modified LGIC.

In another aspect, this document features methods of modulating the excitability of a cell in a mammal. The methods include, or consist essentially of, administering to the cell from the mammal a modified LGIC, where an exogenous LGIC ligand selectively binds the modified LGIC. The modified LGIC has at least one modified LGIC subunit including a LBD including at least one modified amino acid, and an IPD; and administering the exogenous ligand to the mammal. The modulating can include increasing the excitability of the cell or decreasing the excitability of the cell. The cell can be an excitable cell. The cell can be a neuron, a glial cell, a myocyte, a stem cell, an endocrine cell, or an immune cell. The administering the modified LGIC to the cell can be an in vivo administration or an ex vivo administration. The administering the modified LGIC to the cell can include administering a nucleic acid encoding the modified LGIC.

In another aspect, this document features methods of modulating the activity of a cell in a mammal. The methods include, or consist essentially of, administering to the cell a modified LGIC, where an exogenous LGIC ligand selectively binds the modified LGIC. The

modified LGIC has at least one modified LGIC subunit including a LBD including at least one modified amino acid, and an IPD; and administering the exogenous ligand to the mammal. The modulating can include increasing the activity of the cell or decreasing the activity of the cell. The activity can be ion transport, passive transport, excitation, inhibition, or exocytosis. The cell can be a neuron, a glial cell, a myocyte, a stem cell, an endocrine cell, or an immune cell. The administering the modified LGIC to the cell can be an in vivo administration or an ex vivo administration. The administering the modified LGIC to the cell can include administering a nucleic acid (*e.g.*, via a viral vector such as an adeno-associated virus, a herpes simplex virus, or a lentivirus) encoding the modified LGIC.

In another aspect, this document features a method for identifying a ligand that selectively binds to a modified LGIC. The method includes, or consists essentially of, providing one or more candidate ligands to the modified LGIC described herein, and detecting binding between the candidate ligand and the modified LGIC, thereby identifying a ligand that selectively binds the modified LGIC. The modified LGIC can be a homomeric modified LGIC.

In another aspect, this document features a method for detecting a modified LGIC. The method includes, or consists essentially of, providing one or more modified LGIC subunits described herein, providing an agent that selectively binds the modified LGIC, and detecting binding between the modified LGIC and the agent that selectively binds the modified LGIC, thereby detecting the modified LGIC. The agent that selectively binds the modified LGIC comprises can be antibody, a protein (*e.g.*, bungarotoxin), or a small molecule (*e.g.*, a positron emission tomography (PET) ligand). The agent that selectively binds the modified LGIC can include a detectable label (*e.g.*, a fluorescent label, a radioactive label, or a positron emitting label).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows exemplary amino acid sequences of chimeric LGICs. Mutation of amino acid residue 77 (*e.g.*, W77F or W77Y) resulted in sensitivity to granisetron and tropisetron. Mutation of amino acid residue 79 (*e.g.*, Q79G) was most effective for several agonists. Mutations of amino acid residue 131 (*e.g.*, L131G, L131A, L131M, or L131N) altered sensitivity to varenicline, tropisetron, granisetron, and ACh. Potency was considerably enhanced when LBD mutations were combined with mutation at amino acid residue 298 in the GlyR or GABAC IPD. Potency was also enhanced when α7 nAChR LBD mutations were combined with mutation at amino acid residue G175 and P216. A) An amino acid sequence of α7-5HT3 chimeric receptor (SEQ ID NO:6) including a human α7 nAChR LBD (SEQ ID NO:1) and a murine 5HT3 IPD (SEQ ID NO:3) components. B) An amino acid sequence of α7-GlyR chimeric receptor (SEQ ID NO:7), including a human α7 nAChR LBD (SEQ ID NO:2) and a human GlyR IPD (SEQ ID NO:5) components. C) An amino acid sequence of α7-5HT3 chimeric receptor (SEQ ID NO:8) including human α7 nAChR LBD (SEQ ID NO:1) and a human 5HT3 IPD (SEQ ID NO:4) components. D) An amino acid sequence of α7- $GABA_C$ chimeric receptor (SEQ ID NO:10) including a human α7 nAChR LBD (SEQ ID NO:2) and a human $GABA_C$ IPD (SEQ ID NO:9) components. E) An amino acid sequence of rat nAChR sequence (SEQ ID NO:12).

Figure 2 shows EC50s for tropisetron against a α7-5HT3 chimeric LGIC and variants of the chimeric LGIC with LBD mutations at positions noted in Figure 1. Multiple mutations at Gln79 showed similar or improved potency relative to the unmodified α7-5HT3 channel (arrows).

Figure 3 shows the relative potency of known nAChR agonists for α7-5HT3 chimeric LGICs. A) A graph of EC50s normalized to the unmodified α7-5HT3 chimeric channel (log scale). *$P<0.05$, statistically significant potency changes are noted (ANOVA followed by Dunn's test). B) Chemical structures of known nAChR agonists.

Figure 4 shows the relative potency of known nAChR agonists for α7-GlyR chimeric LGICs. A) A graph of EC50s for Q79 LBD mutants normalized to the unmodified α7-GlyR

chimeric channel (log scale). B) A graph of EC50s for A298G IPD mutation normalized to the unmodified α7-GlyR chimeric channel (log scale). C) A graph of EC50s for α7-GlyR^{A298G} normalized to the unmodified α7-GlyR chimeric channel and compared to the double mutant channel α7Q79G-GlyR^{A298G} (log scale). *P<0.05, statistically significant potency changes are noted (ANOVA followed by Dunn's test).

Figure 5 shows schematic structures of LGIC agonists with substitution patterns most compatible with potency enhancement for α7^{Q79G}-5HT3 and α7^{Q79G}-GlyR^{A298G}. A) A generalized structure showing attributes associated with enhanced potency. B) Specific pharmacophores represented in (A) are quinuclidine, tropane, and 9-azabicyclo[3.3.1]nonane core structures. C) Exemplary synthetic molecules that show high potency for α7^{Q79G}-GlyR^{A298G}, α7^{Q79G,Y115F,G175K}-GlyR, α7^{W77F,Q79G,G175K}-GlyR.

Figure 6 shows mutations that reduce association of chimeric LCIG α7 nAChR LBDs with unmodified LBDs. A) Charge reversal schematic potential configurations of transfecting two epitope tagged (HA and V5) constructs encoding α7-5HT3 (top) or two constructs encoding α7-5HT3-HA and α7^{R21D,E41R}-5HT3-V5 where association between the two different epitope tagged subunits would be unfavored due to charge reversal mutations at the subunit interfaces. B) Whole cell recordings in HEK cells expressing α7^{R21D,E41R}-5HT3 with a V5 epitope tag shows potent responses to PNU-282987. C) Association of α7-5HT3 LGICs with HA and V5 epitope tags in HEK cells was probed by HA immunoprecipitation (left) or total lysate isolation followed by western blotting with either anti-HA (top) or anti-V5 antibodies (bottom). In cells co-expressing channels with the HA and V5 epitopes, anti-HA IP followed by anti-V5 immunoblotting shows the co-immunoprecipation of unmodified channels of each type, but charge reversal mutations in the LBD α7^{R21D,E41R}-5HT3-V5 was not immunoprecipitated. MW of α7-5HT3 is ~48 kD (arrow).

Figure 7 shows that chimeric LGICs can be controlled using an exogenous ligand. Cortical neurons from a mouse brain transduced with α7^{Q79G}-GlyR^{A298G} chimeric LGIC via adeno-associated virus (AAV) vectors fires action potentials in response to 40 pA current injection (PRE) that are potently suppressed by 30 nM tropisetron. After washout (WASH) of tropisetron, neuron firing is restored.

Figure 8 shows activity of agonists on chimeric LGICs with a G175K mutation. A) A graph of EC50s for Q79G G175K LBD mutants against known agonists normalized to the unmodified α7-GlyR chimeric channel (log scale). B) A graph of EC50s for ACh and

tropisetron for channels with mutations in α7-GlyR chimeric LGICs. Mutations that result in channels with high potency for tropisetron and low potency for the endogenous ligand, acetylcholine (ACh) are optimal (grey shading). Unmod.: unmodified α7-GlyR chimeric LGIC. C) Action potentials of cortical neurons from a mouse brain transduced with $\alpha7^{Q79G,Y115F,G175K}$-GlyR chimeric LGIC. Neurons fire in response to current injection (PRE) and are potently suppressed by 100 nM tropisetron. After washout (WASH) of tropisetron, neuron firing is restored.

Figure 9 shows activity of agonists on chimeric LGICs with a L131G mutation. A) A graph of EC50s for L131 LBD mutants against known agonists normalized to the unmodified α7-GlyR chimeric channel (log scale). B) A graph of EC50s for ACh and tropisetron for channels with mutations in $\alpha7^{L131G}$-GlyR chimeric LGICs. C) A graphs showing mutations that result in channels with high potency for varenicline and low potency for the endogenous ligand, acetylcholine (ACh) are optimal (grey shading). Unmod.: unmodified α7-GlyR chimeric LGIC. D) Action potentials of a cortical neuron from a mouse brain transduced with $\alpha7^{L131G,Q139L,Y217F}$-GlyR chimeric LGIC. Neuron fires in response to current injection (PRE) and are potently suppressed by 10 nM varenicline, even with >6-fold greater injected current. After washout (WASH) of tropisetron, neuron firing is restored.

Figure 10 shows chemical structures of LGIC agonists. A) Chemical structures of LGIC agonists with substitution patterns most compatible with potency enhancement for $\alpha7^{Q79G,Y115F,G175K}$-GlyR. B) Chemical structures of LGIC agonists with substitution patterns most compatible with potency enhancement for $\alpha7^{L131G,Q139L,Y217F}$-GlyR or $\alpha7^{L131G,Q139L,Y217F}$-5HT3 HC.

DETAILED DESCRIPTION

This document provides modified LGICs and methods of using them. For example, this document provides modified LGICs including at least one modified LGIC subunit having a LBD and an IPD, and having at least one modified amino acid (*e.g.*, an amino acid substitution). In some cases, a modified LGIC can be a chimeric LGIC. For example, a chimeric LGIC can include a LBD from a first LGIC and an IPD from a second LGIC. In some cases, the modified amino acid can confer pharmacological selectivity to the modified LGIC. For example, the modified amino acid can confer the modified LGIC with selective binding of an exogenous LGIC ligand. For example, the modified amino acid can confer the

modified LGIC with reduced (minimized or eliminated) binding of an unmodified LGIC subunit (an LGIC subunit lacking the modification and/or an endogenous LGIC subunit). For example, the modified amino acid can confer the modified LGIC with reduced (minimized or eliminated) binding of an endogenous LGIC ligand.

Modified LGICs provided herein can be used, for example, in methods for treating channelopathies (*e.g.*, a neural channelopathy or a muscle channelopathy). For example, a modified LGIC, and an exogenous LGIC ligand that can bind to and activate the modified LGIC, can be used to treat a mammal having a channelopathy. In some cases, a modified LGIC and an exogenous LGIC ligand can be used to modulate (*e.g.*, activate or inhibit) ion transport across the membrane of a cell of a mammal. In some cases, a modified LGIC and an exogenous LGIC ligand can be used to modulate (*e.g.*, increase or decrease) the excitability of a cell in a mammal.

Modified LGICs

As used herein a "modified" LGIC is an LGIC that includes at least one LGIC subunit. A modified LGIC subunit can include at least one modified amino acid (*e.g.*, an amino acid substitution) in the LBD and/or at least one modified amino acid (*e.g.*, an amino acid substitution) in the IPD. A modified LGIC subunit described herein can be a modification of an LGIC from any appropriate species (e.g., human, rat, mouse, dog, cat, horse, cow, goat, pig, or monkey). In some cases, a modified LGIC can include at least one chimeric LGIC subunit having a non-naturally occurring combination of a LBD from a first LGIC and an IPD from a second LGIC.

A modified LGIC can be a homomeric (*e.g.*, having any number of the same modified LGIC subunits) or heteromeric (*e.g.*, having at least one modified LGIC subunit and any number of different LGIC subunits). In some cases, a modified LGIC described herein can be a homomeric modified LGIC. A modified LGIC described herein can include any suitable number of modified LGIC subunits. In some cases, a modified LGIC can be a trimer, a tetramer, a pentamer, or a hexamer. For example, a modified LGIC described herein can be a pentamer.

A modified LGIC subunit described herein can be a modification of any appropriate LGIC. The LGIC can conduct anions, cations, or both through a cellular membrane in response to the binding of a ligand. For example, the LGIC can transport sodium (Na+),

potassium (K+), calcium (Ca2+), and/or chloride (Cl–) ions through a cellular membrane in response to the binding of a ligand. Examples of LGICs include, without limitation, Cys-loop receptors (*e.g.*, AChR such as a nAChR (*e.g.*, a muscle-type nAChR or a neuronal-type nAChR), gamma-aminobutyric acid (GABA; such as $GABA_A$ and $GABA_A$-ρ (also referred to as $GABA_C$) receptors, GlyR, GluCl receptors, and 5HT3 receptors), ionotropic glutamate receptors (iGluR; such as AMPA receptors, kainate receptors, NMDA receptors, and delta receptors), ATP-gated channels (*e.g.*, P2X), and phosphatidylinositol 4,5-bisphosphate (PIP2)-gated channels. In cases where a modified LGIC described herein is a chimeric LGIC, the chimeric LGIC can include a LBD selected from any appropriate LGIC and an IPD selected from any appropriate LGIC. In cases where a LGIC includes multiple different subunits (for example, a neuronal-type nAChR includes α4, β2, and α7 subunits), the LBD and/or IPD can be selected from any of the subunits. For example, a LBD from a nAChR can be a α7 LBD. A representative rat α7 nAChR amino acid sequence (including both a LBD and an IPD) is as follows.

SEQ ID NO:12

MGGGRGGIWLALAAALLHVSLQGEFQRRLYKELVKNYNPLERPVANDSQPLTVYFSLSLLQI
MDVDEKNQVLTTNIWLQMSWTDHYLQWNMSEYPGVKNVRFPDGQIWKPDILLYNSADERFDA
TFHTNVLVNASGHCQYLPPGIFKSSCYIDVRWFPFDVQQCKLKFGSWSYGGWSLDLQMQEAD
ISSYIPNGEWDLMGIPGKRNEKFYECCKEPYPDVTYTVTMRRRTLYYGLNLLIPCVLISALA
LLVFLLPADSGEKISLGITVLLSLTVFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVV
TVIVLRYHHHDPDGGKMPKWTRIILLNWCAWFLRMKRPGEDKVRPACQHKPRRCSLASVELS
AGAGPPTSNGNLLYIGFRGLEGMHCAPTPDSGVVCGRLACSPTHDEHLMHGAHPSDGDPDLA
KILEEVRYIANRNRCQDESEVICSEWKFAACVVDPLCLMAFSVFTIICTIGILMSAPNFVEA
VSKDFA

In some cases, a modified LGIC subunit described herein can include a LBD from a α7 nAChR. Examples of α7 nAChR LBDs include, without limitation, a human α7 nAChR LBD having the amino acid sequence set forth in SEQ ID NO:1, a human α7 nAChR LBD having the amino acid sequence set forth in SEQ ID NO:2, and a human α7 nAChR LBD having the amino acid sequence set forth in SEQ ID NO:11. In some cases, a α7 nAChR LBD can be a homolog, orthologue, or paralog of the human α7 nAChR LBD set forth in

SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:11. In some cases, a α7 nAChR LBD can be have at least 75 percent sequence identity (*e.g.*, at least 80%, at least 82%, at least 85%, at least 88%, at least 90%, at least 93%, at least 95%, at least 97% or at least 99% sequence identity) to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:11.

SEQ ID NO:1

MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQPLTVYFSLSLLQI
MDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIWKPDILLYNSADERFDA
TFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEAD
ISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVTFTV

SEQ ID NO:2

MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQPLTVYFSLSLLQI
MDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIWKPDILLYNSADERFDA
TFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEAD
ISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVTFTVTMRRR

SEQ ID NO:11

MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQPLTVYFSLSLLQI
MDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQIWKPDILLYNSADERFDA
TFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEAD
ISGYIPNGEWDLVGIPGKRSERFYECCKEPYPDVTFTVTMRRRTLYY

In some cases, a modified LGIC subunit described herein can include a IPD from a 5HT3 receptor. Examples of 5HT3 IPDs include, without limitation, a murine 5HT3 IPD having the amino acid sequence set forth in SEQ ID NO:3, and a human 5HT3 IPD having the amino acid sequence set forth in SEQ ID NO:4. In some cases, a 5HT3 IPD can be a homolog, orthologue, or paralog of a 5HT3 IPD set forth in SEQ ID NO:3 or SEQ ID NO:4. In some cases, a 5HT3 IPD can be have at least 75 percent sequence identity (*e.g.*, at least 80%, at least 82%, at least 85%, at least 88%, at least 90%, at least 93%, at least 95%, at least 97% or at least 99% sequence identity) to SEQ ID NO:3 of SEQ ID NO:4.

SEQ ID NO:3

IIRRRPLFYAVSLLLPSIFLMVVDIVGFCLPPDSGERVSFKITLLLGYSVFLIIVSDTLPAT
IGTPLIGVYFVVCMALLVISLAETIFIVRLVHKQDLQRPVPDWLRHLVLDRIAWILCLGEQP
MAHRPPATFQANKTDDCSGSDLLPAMGNHCSHVGGPQDLEKTPRGRGSPLPPPREASLAVRG
LLQELSSIRHFLEKRDEMREVARDWLRVGYVLDRLLFRIYLLAVLAYSITLVTLWSIWHYS

SEQ ID NO:4

LFYVVSLLLPSIFLMVMDIVGFYLPPNSGERVSFKITLLLGYSVFLIIVSDTLPATAIGTPL
IGVYFVVCMALLVISLAETIFIVRLVHKQDLQQPVPAWLRHLVLERIAWLLCLREQSTSQRP
PATSQATKTDDCSAMGNHCSHMGGPQDFEKSPRDRCSPPPPPREASLAVCGLLQELSSIRQF
LEKRDEIREVARDWLRVGSVLDKLLFHIYLLAVLAYSITLVMLWSIWQYA

In some cases, a modified LGIC subunit described herein can include an IPD from a GlyR. Examples of GlyR IPDs include, without limitation, a murine GlyR IPD having the amino acid sequence set forth in SEQ ID NO:5. In some cases, a GlyR IPD can be a homolog, orthologue, or paralog of the human GlyR IPD set forth in SEQ ID NO:5. In some cases, a GlyR IPD can be have at least 75 percent sequence identity (*e.g.*, at least 80%, at least 82%, at least 85%, at least 88%, at least 90%, at least 93%, at least 95%, at least 97% or at least 99% sequence identity) to SEQ ID NO:5.

SEQ ID NO:5

MGYYLIQMYIPSLLIVILSWISFWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVK
AIDIWMAVCLLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKEDEAGEGRFNFSAYGMGP
ACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKISRIGFPMAFLIFNMF
YWIIYKIVRREDVHNQ

In some cases, a modified LGIC subunit described herein can include an IPD from a GABA receptor (*e.g.*, $GABA_A$-ρ, also referred to as $GABA_C$). Examples of $GABA_A$-ρ IPDs include, without limitation, a human $GABA_A$-ρ IPD having the amino acid sequence set forth in SEQ ID NO:9. In some cases, a $GABA_A$-ρ IPD can be a homolog, orthologue, or paralog of the human $GABA_A$-ρ IPD set forth in SEQ ID NO:9. In some cases, a $GABA_A$-ρ IPD can be have at least 75 percent sequence identity (*e.g.*, at least 80%, at least 82%, at least 85%, at

least 88%, at least 90%, at least 93%, at least 95%, at least 97% or at least 99% sequence identity) to SEQ ID NO:9.

SEQ ID NO:9

LLQTYFPATLMVMLSWVSFWIDRRAVPARVPLGITTVLTMSTIITGVNASMPRVSYIKAVDI
YLWVSFVFVFLSVLEYAAVNYLTTVQERKEQKLREKLPCTSGLPPPRTAMLDGNYSDGEVND
LDNYMPENGEKPDRMMVQLTLASERSSPQRKSQRSSYVSMRIDTHAIDKYSRIIFPAAYILF
NLIYWSIFS

In calculating percent sequence identity, two sequences are aligned and the number of identical matches of amino acid residues between the two sequences is determined. The number of identical matches is divided by the length of the aligned region (i.e., the number of aligned amino acid residues) and multiplied by 100 to arrive at a percent sequence identity value. It will be appreciated that the length of the aligned region can be a portion of one or both sequences up to the full-length size of the shortest sequence. It also will be appreciated that a single sequence can align with more than one other sequence and hence, can have different percent sequence identity values over each aligned region. The alignment of two or more sequences to determine percent sequence identity can be performed using the computer program ClustalW and default parameters, which calculates the best match between a query and one or more subject sequences, and aligns them so that identities, similarities and differences can be determined. See, *e.g.*, Chenna et al., 2003, Nucleic Acids Res., 31(13):3497-500.

In cases where a modified LGIC subunit described herein is a chimeric LGIC subunit, the chimeric LGIC subunit can include a LBD and IPD from the same species or a LBD and IPD from different species. In some cases, a chimeric LGIC subunit can include a LBD from a human LGIC protein and an IPD from a human LGIC protein. For example, a chimeric LGIC subunit can include a human α7 LBD and a human GlyR IPD. In some cases, a chimeric LGIC subunit can include a LBD from a human LGIC protein and an IPD from a murine LGIC protein. For example, a chimeric LGIC subunit can include a human α7 LBD and a murine 5HT3 IPD.

In cases where a modified LGIC subunit described herein is a chimeric LGIC subunit, the chimeric LGIC subunit can include varied fusion points connecting the LBD and the IPD

such that the number of amino acids in a LBD may vary when the LBD is fused with different IPDs to form a chimeric channel subunit. For example, the length of an α7 nAChR LBD used to form a chimeric LGIC subunit with a 5HTS IPD is different from the length of an α7 nAChR LBD used to form a chimeric LGIC subunit with a GlyR IPD (compare, for example, Figures 1A and 1C to Figure 1B).

A modified LGIC subunit described herein can include a LBD having at least one modified amino acid and/or an IPD having at least one modified amino acid. For example, a modified LGIC subunit described herein can include a α7 LBD having at least 75 percent sequence identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12, and an amino acid substitution at amino acid residue 27, 41, 77, 79, 131, 139, 141, 175, 210, 216, 217, and/or 219. For example, a modified LGIC subunit described herein can include a GlyR IPD having at least 75 percent sequence identity to a sequence set forth in SEQ ID NO:5, and an amino acid substitution at amino acid residue 298 of an α7-GlyR chimeric receptor (*e.g.*, SEQ ID NO:7). For example, a modified LGIC subunit described herein can include a $GABA_C$ IPD having at least 75 percent sequence identity to SEQ ID NO:9, and an amino acid substitution at amino acid residue 298 of an α7-$GABA_C$ chimeric receptor (*e.g.*, SEQ ID NO:10). In some cases, a modified LGIC subunit described herein can include more than one (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more) amino acid modifications. The modification can be an amino acid substitution. In some cases, the modified amino acid can confer pharmacological selectivity to the modified LGIC. For example, the modified amino acid can confer the modified LGIC with selective binding of an exogenous LGIC ligand. For example, the modified amino acid can confer the modified LGIC with reduced (minimized or eliminated) binding of an unmodified LGIC subunit (an LGIC subunit lacking the modification and/or an endogenous LGIC subunit). For example, the modified amino acid can confer the modified LGIC with reduced (minimized or eliminated) binding of an endogenous LGIC ligand.

In some aspects, a modified LGIC subunit described herein can include at least one modified amino acid that confers the modified LGIC with selective binding (*e.g.*, enhanced binding or increased potency) with an exogenous LGIC ligand. The binding with an exogenous LGIC ligand can be selective over the binding with an endogenous LGIC ligand. A modified LGIC subunit with selective binding with an exogenous LGIC ligand can include any appropriate LDB (*e.g.*, a α7 LBD). In some aspects, the modified LGIC subunit can

include a α7 LBD set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12, and the amino acid modification can be a substitution at amino acid residue 77, 79, 131 139, 141, 175, and/or 216. In some cases, the tryptophan at amino acid residue 77 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with a hydrophobic amino acid residue such as phenylalanine (*e.g.*, W77F), tyrosine (*e.g.*, W77Y), or methionine (*e.g.*, W77M). For example, a modified LGIC subunit described herein can include a α7 LBD set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 and having a W77F substitution. In some cases, the glutamine at amino acid residue 79 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an amino acid residue such as alanine (*e.g.*, Q79A), glycine (*e.g.*, Q79G), or serine (*e.g.*, Q79S). For example, a modified LGIC subunit described herein can include a α7 LBD having a Q79G substitution. In some cases, the leucine at amino acid residue 131 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an amino acid residue such as alanine (*e.g.*, L131A), glycine (*e.g.*, L131G), methionine (*e.g.*, L131M), asparagine (e.g., L131N), glutamine (*e.g.*, L131Q), valine (*e.g.*, L131V), or phenylalanine (*e.g.*, L131F). In some cases, the glycine at amino acid residue 175 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an amino acid residue such as lysine (*e.g.*, G175K), alanine (e.g., G175A), phenyalanine (e.g., G175F), histidine (e.g., G175H), methionine (e.g., G175m), arginine (e.g., G175R), serine (e.g., G175S), valine (e.g., G175V). In some cases, the proline at amino acid residue 216 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an amino acid residue such as isoleucine (*e.g.*, P216I). A modified LGIC subunit with selective binding with an exogenous LGIC ligand can include any appropriate IPD (*e.g.*, a GlyR IPD or a $GABA_A$-ρ IPD). In some aspects, the modified LGIC subunit can include a GlyR IPD set forth in SEQ ID NO:5, and the amino acid modification can be a substitution at amino acid residue 298 of an α7-GlyR chimeric receptor (*e.g.*, SEQ ID NO:7). In some cases, the alanine at amino acid residue 298 of SEQ ID NO:7 can be substituted with an amino acid residue such as glycine (*e.g.*, A298G). In some aspects, the modified LGIC subunit can include the a $GABA_A$-ρ IPD set forth in SEQ ID NO:9, and the amino acid modification can be a substitution at amino acid residue 298 of an α7-$GABA_A$-ρ chimeric receptor (*e.g.*, SEQ ID NO:10). In some cases, the tryptophan at amino acid residue 298 of SEQ ID NO:10 can be substituted with an amino acid residue such as alanine (*e.g.*, W298A).

In some cases, a modified LGIC subunit described herein can include more than one (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more) amino acid modifications. For example, a modified LGIC subunit described herein can have at least 75 percent sequence identity to SEQ ID NO:7 and can include a Q79G substitution and a A298G substitution. Additional examples of modifications that can confer the modified LGIC with selective binding of an exogenous LGIC ligand include modifications described elsewhere (see, *e.g.*, US 8,435,762).

A modified LGIC subunit that selectively binds (*e.g.*, enhanced binding or increased potency) an exogenous LGIC ligand over an endogenous (*e.g.*, a canonical) LGIC ligand can also be described as having enhanced potency for an exogenous ligand. In some cases, a modified LGIC subunit described herein that selectively binds an exogenous LGIC ligand can have at least 4 fold (*e.g.*, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 11 fold, at least 12 fold, at least 13 fold, at least 14 fold, at least 15 fold, at least 16 fold, at least 17 fold, at least 18 fold, at least 19 fold, or at least 20 fold) enhanced potency for an exogenous ligand. In some cases, a modified LGIC subunit described herein that selectively binds an exogenous LGIC ligand can have about 4 fold to about 200 fold (*e.g.*, about 4 fold to about 200 fold, about 5 fold to about 180 fold, about 6 fold to about 175 fold, about 7 fold to about 150 fold, about 8 fold to about 125 fold, about 9 fold to about 100 fold, about 10 fold to about 90 fold, about 11 fold to about 75 fold, about 12 fold to about 65 fold, about 13 fold to about 50 fold, about 14 fold to about 40 fold, or about 15 fold to about 30 fold) enhanced potency for an exogenous ligand. For example, a modified LGIC subunit described herein that selectively binds an exogenous LGIC ligand can have about 10 fold to about 100 fold enhanced potency for an exogenous ligand. For example, a modified LGIC subunit described herein that selectively binds an exogenous LGIC ligand can have about 10 fold to about 20 fold enhanced potency for an exogenous ligand.

In some aspects, a modified LGIC subunit described herein can include at least one modified amino acid that confers the modified LGIC with reduced (*e.g.*, minimized or eliminated) binding with an unmodified LGIC subunit. The binding with a modified LGIC subunit having the same modification can be selective over the binding with an unmodified LGIC subunit. An unmodified LGIC subunit can be a LGIC subunit lacking the modification that confers the modified LGIC with reduced binding with an unmodified LGIC subunit or an

unmodified LGIC can be an endogenous LGIC subunit. The modification that confers the modified LGIC with reduced binding with an unmodified LGIC subunit can be a charge reversal modification. A modified LGIC subunit with reduced binding with an unmodified LGIC subunit can include any appropriate LBD (*e.g.*, a α7 LBD). In some aspects, the modified LGIC subunit can include a α7 LBD set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12, and the amino acid modification can be a substitution at amino acid residue 27 and/or 41. For example, the arginine at amino acid residue 27 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an aspartic acid (*e.g.*, R27D). For example, the glutamic acid at amino acid residue 41 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an arginine (*e.g.*, E41R). In some cases, a modified LGIC subunit described herein can include a α7 LBD having a R27D substitution and a E41R.

In some aspects, a modified LGIC subunit described herein can include at least one modified amino acid that confers the modified LGIC with reduced (*e.g.*, minimized or eliminated) binding of an endogenous LGIC ligand. The endogenous LGIC ligand can be ACh. A modified LGIC subunit with reduced binding of an endogenous LGIC ligand can include any appropriate IPD (*e.g.*, a GlyR LBD). For example, the modified LGIC subunit can include a α7 LBD set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12, and the amino acid modification can be a substitution at amino acid residue 115, 131, 139, 210, 217 and/or 219. In some cases, the tyrosine at amino acid residue 115 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with a phenylalanine (*e.g.*, Y115F). In some cases, the leucine at amino acid residue 131 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an amino acid residue such as alanine (*e.g.*, L131A), glycine (*e.g.*, L131G), methionine (*e.g.*, L131M), asparagine (e.g., L131N), glutamine (*e.g.*, L131Q), valine (*e.g.*, L131V), or phenylalanine (*e.g.*, L131F). In some cases, the glutamine at amino acid residue 139 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with a glycine (*e.g.*, Q139G) or a leucine (*e.g.*, Q139L). In some cases, the tyrosine at amino acid residue 210 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with a phenylalanine (*e.g.*, Y210F). In some cases, the tyrosine at amino acid residue 217 of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with a phenylalanine (*e.g.*, Y217F). In some cases, the aspartate at amino acid residue 219 of SEQ

ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12 can be substituted with an alanine (*e.g.*, D219A).

In some aspects, a modified LGIC subunit described herein can include at least one modified amino acid that confers the modified LGIC with increased ion conductance. In some cases, the modified LGIC subunit can include a 5HT3 IPD set forth in SEQ ID NO:3, and the amino acid modification can be a substitution at amino acid residue 425, 429, and/or 433. For example, a modified LGIC subunit described herein can include a 5HT3 IPD having a R425Q substitution, a R429D substitution, and a R433A substitution. In some cases, the modified LGIC subunit can include a 5HT3 IPD set forth in SEQ ID NO:4, and the amino acid modification can be a substitution at amino acid residue 420, 424, and/or 428. For example, a modified LGIC subunit described herein can include a 5HT3 IPD having a R420Q substitution, a R424D substitution, and a R428A substitution.

In some cases, a modified LGIC described herein can include at least one chimeric α7-5HT3 LGIC subunit (SEQ ID NO:6) having a human α7 nAChR LBD (SEQ ID NO:1) with a Q79G amino acid substitution and a Y115F amino acid substitution, and a murine 5HT3 IPD (SEQ ID NO:3).

In some cases, a modified LGIC described herein can include at least one chimeric α7-5HT3 LGIC subunit (SEQ ID NO:6) having a human α7 nAChR LBD (SEQ ID NO:1) with a Q79G amino acid substitution and a Q139G amino acid substitution, and a murine 5HT3 IPD (SEQ ID NO:3).

In some cases, a modified LGIC described herein can include at least one chimeric α7-GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a Q79G amino acid substitution and a Y115F amino acid substitution, and a human GlyR IPD (SEQ ID NO:5) with a A298G amino acid substitution.

In some cases, a modified LGIC described herein can include at least one chimeric α7-GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a Q79G amino acid substitution and a Q139G amino acid substitution, and a human GlyR IPD (SEQ ID NO:5) with a A298G amino acid substitution.

In some cases, a modified LGIC described herein can include at least one chimeric α7-GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a R27D amino acid substitution, a E41R amino acid substitution, a Q79G amino acid

substitution, and a Y115F amino acid substitution, and a human GlyR IPD (SEQ ID NO:5) with a A298G amino acid substitution.

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residue 131 (*e.g.*, L131G, L131A, L131M, or L131N), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 131 (e.g., L131G, L131A, L131M, or L131N) and Y115 (e.g., Y115F), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 131 (*e.g.*, L131G, L131A, L131M, or L131N) and 139 (*e.g.*, Q139L), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 131 (*e.g.*, L131G, L131A, L131M, or L131N) and 217 (*e.g.*, Y217F), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 131 (*e.g.*, L131G, L131A, L131M, or L131N), 139 (*e.g.*, Q139L), and 217 (*e.g.*, Y217F), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- 5HT3 LGIC subunit having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residue 131 (*e.g.*, L131G, L131A, L131M, or L131N), and a human 5HT3 IPD (SEQ ID NO:4).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residue 175 (*e.g.*, G175K), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- 5HT3 LGIC subunit having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution

at amino acid residue 131 (*e.g.*, L131G, L131A, L131M, or L131N) and 139 (*e.g.*, Q139L), and a human 5HT3 IPD (SEQ ID NO:4) with a R420Q substitution, a R424D substitution, and a R428A substitution.

In some cases, a modified LGIC described herein can include at least one chimeric α7- 5HT3 LGIC subunit having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residue 131 (*e.g.*, L131G, L131A, L131M, or L131N) and 139 (*e.g.*, Q139L) and 217 (*e.g.*, Y217F), and a human 5HT3 IPD (SEQ ID NO:4) with a R420Q substitution, a R424D substitution, and a R428A substitution.

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 175 (*e.g.*, G175K) and 115 (*e.g.*, Y115F), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 175 (*e.g.*, G175K) and 115 (*e.g.*, Y115F) and 79 (*e.g.*, Q79G), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 175 (*e.g.*, G175K) and 77 (*e.g.*, W77F) and 79 (*e.g.*, Q79G), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residue 216 (*e.g.*, P216I), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7- GlyR LGIC subunit (SEQ ID NO:7) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residues 216 (*e.g.*, P216I) and 79 (*e.g.*, Q79G), and a human GlyR IPD (SEQ ID NO:5).

In some cases, a modified LGIC described herein can include at least one chimeric α7-GlyR LGIC subunit (SEQ ID NO:10) having a human α7 nAChR LBD (SEQ ID NO:2) with a substitution at amino acid residue 131 (*e.g.*, L131A, L131G, L131M, L131N, L131Q, L131V, or L131F), and a human $GABA_C$ IPD (SEQ ID NO:9).

In cases where a LBD and/or a IPD is a homolog, orthologue, or paralog of a sequence set forth herein (*e.g.*, SEQ ID NOs:1-5 and/or 9), it is understood that reference to a particular modified amino acid residue can shift to the corresponding amino acid in the homolog, orthologue, or paralog. For example, residues 425, 429, and 433 in a murine 5HT3 IPD set forth in SEQ ID NO:3 correspond to residues 420, 424, and 428 in a human 5HT3 IPD set forth in SEQ ID NO:4, and the R425Q, R429D, and R433A substitutions in a murine 5HT3 IPD correspond to R420Q, R424D, and R428A substitutions in a human 5HT3 IPD.

Any method can be used to obtain a modified LGIC subunit described herein. In some cases, peptide synthesis methods can be used to make a modified LGIC subunit described herein. Examples of methods of peptide synthesis include, without limitation, liquid-phase peptide synthesis, and solid-phase peptide synthesis. In some cases, protein biosynthesis methods can be used to make a modified LGIC subunit described herein. Examples of methods of protein biosynthesis include, without limitation, transcription and/or translation of nucleic acids encoding a phosphorylation-mimicking peptide provided herein. Similar modified LGIC subunits (*e.g.*, modified subunits having essentially the same modifications and/or having essentially the same amino acid sequence) will self-assemble through interactions between the LBDs to form a modified LGIC.

This document also provides nucleic acids encoding modified LGIC subunits described herein as well as constructs (*e.g.*, plasmids, non-viral vectors, viral vectors (such as adeno-associated virus, a herpes simplex virus, or lentivirus vectors)) for expressing nucleic acids encoding modified LGIC subunits described herein. Nucleic acids encoding modified LGIC subunits described herein can be operably linked to any appropriate promoter. A promoter can be a native (i.e., minimal) promoter or a composite promoter. A promoter can be a ubiquitous (i.e., constitutive) promoter or a regulated promoter (*e.g.*, inducible, tissue specific, cell-type specific (*e.g.*, neuron specific, muscle specific, glial specific), and neural subtype-specific). Examples of promoters that can be used to drive expression of nucleic acids encoding modified LGIC subunits described herein include, without limitation, synapsin, CAMKII, CMV, CAG, enolase, TRPVl, POMC, NPY, AGRP, MCH, and Orexin promoters. In some cases, a nucleic acid encoding a modified LGIC subunit described herein can be operably linked to a neuron specific promoter.

This document also provides cells (*e.g.*, mammalian cells) having a modified LGIC described herein. Mammalian cells having a modified LGIC described herein can be

obtained by any appropriate method. In some cases, a pre-assembled modified LGIC can be provided to the cell. In some cases, a nucleic acid encoding a modified LGIC subunit described herein can be provided to the cell under conditions in which a modified LGIC subunit is translated and under conditions in which multiple (*e.g.*, three, four, five, six, or more) modified LGIC subunits can assemble into a modified LGIC described herein.

LGIC Ligands

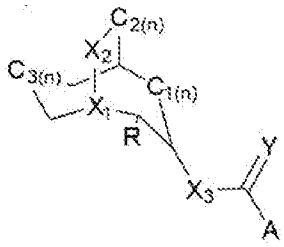
This document also provides LGIC ligands that can bind to and activate modified LGICs described herein. A LGIC ligand that can bind to and activate modified LGICs described herein can be exogenous or endogenous. A LGIC ligand that can bind to and activate modified LGICs described herein can be naturally occurring or synthetic. A LGIC ligand that can bind to and activate modified LGICs described herein can be canonical or non-canonical. A LGIC ligand that can bind to and activate modified LGICs described herein can be an agonist or an antagonist. In some cases, an LGIC ligand is an exogenous LGIC agonist. Examples of LGIC ligands include, without limitation, ACh, nicotine, epibatatine, cytisine, RS56812, tropisetron, nortropisetron, PNU-282987, PHA-543613, compound 0353, compound 0354, compound 0436, compound 0676, compound 702, compound 723, compound 725, granisetron, ivermectin, mequitazine, promazine, varenicline, compound 765, compound 770, 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]thiophene 5,5-dioxide, compound 773, and compound 774 (see, *e.g.*, Figure 3B, Figure 5C, Figure 10A, and Figure 10B).

A LGIC ligand that can bind to and activate modified LGICs described herein can have selective binding (*e.g.*, enhanced binding or increased potency) for a modified LGIC described herein. In some cases, a LGIC ligand that can bind to and activate modified LGICs described herein does not bind to and activate endogenous receptors. A LGIC ligand that selectively binds to and activates a modified LGIC (*e.g.*, a modified LGIC having at least one amino acid modification that confers pharmacological selectivity to the modified LGIC) described herein over an unmodified LGIC ligand can also be described as having enhanced potency for a modified LGIC. In some cases, a modified LGIC subunit described herein that selectively binds an exogenous LGIC ligand can have at least 5 fold (*e.g.*, at least 10 fold, at least 15 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 35 fold, at least 40 fold, at least 45 fold, at least 50 fold, at least 55 fold, at least 60 fold, at least 65 fold, at least 70

fold, at least 75 fold, at least 80 fold, at least 85 fold, at least 95 fold, at least 100 fold, at least 125 fold, at least 150 fold, at least 200 fold, at least 250 fold, or at least 300 fold) enhanced potency for a modified LGIC. For example, a LGIC ligand that selectively binds to and activates a modified LGIC can have about 10 fold to about 300 fold (e.g., about 10 fold to about 250 fold, about 10 fold to about 200 fold, about 10 fold to about 150 fold, about 10 fold to about 100 fold, about 25 fold to about 300 fold, about 50 fold to about 300 fold, about 100 fold to about 300 fold, about 200 fold to about 300 fold, about 25 fold to about 250 fold, about 50 fold to about 200 fold, or about 100 fold to about 150 fold) enhanced potency for a modified LGIC. In some cases, a LGIC ligand that binds to and activates a modified LGIC described herein can have a ligand potency of less than 25 nM (*e.g.*, less than 22 nM, less than 20 nM, less than 17 nM, less than 15 nM, less than 13 nM, less than 12 nM, less than 11 nM, less than 10 nM, less than 5 nM, less, than 2 nM, or less than 1 nM). For example, a LGIC ligand that binds to and activates a modified LGIC described herein can have a ligand potency of less than 15 nM. In some cases, a LGIC ligand can have an EC50 of less than 25 nM (*e.g.*, less than 22 nM, less than 20 nM, less than 17 nM, less than 15 nM, less than 13 nM, less than 12 nM, less than 11 nM, or less than 10 nM) for a modified LGIC subunit described herein. For example, a LGIC ligand (*e.g.*, tropisetron) can have an EC50 of about 11 nM for a modified LGIC subunit described herein (*e.g.*, $\alpha7^{Q79G}$-$GlyR^{A298G}$). For example, a LGIC ligand (*e.g.*, nortropisetron) can have an EC50 of about 13 nM for a modified LGIC subunit described herein (*e.g.*, $\alpha7^{Q79G,Y115F}$-$GlyR^{A298G}$). In some cases, a LGIC ligand can have an EC50 of greater than 20 μM (*e.g.*, greater than 22 μM, greater than 25 μM, greater than 35 μM, greater than 50, greater than 65 μM, greater than 80 μM, or greater than 100 μM) for a modified LGIC subunit described herein. For example, a LGIC ligand (*e.g.*, ACh) can have an EC50 of greater than 100 μM for a modified LGIC subunit described herein (*e.g.*, $\alpha7^{Q79G,Y115F}$-$GlyR^{A298G}$).

In some aspects, a LGIC ligand can be a synthetic ligand that can bind to and activate modified LGICs described herein can be a quinuclidine, a tropane, a 9-azabicyclo[3.3.1]nonane, or a 2-phenyl-7,8,9,10-tetrahydro-6H-6,10-methanoazepino[4,5-g]quinoxaline.

A LGIC ligand that can be to and activate a modified LGIC described herein can have Formula I:



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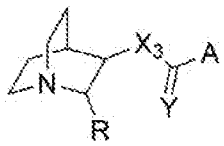
where X1 and X2 can independently be CH, CH2, O, NH, or NMe; each n can independently be 0 or 1; Y can be O or S; A can be an aromatic substituent; and R can be H or pyridinymethylene. Examples of aromatic substituents include, without limitation, 4-chloro-benzene, 1H-indole, 4-(trifluoromethyl) benzene, 4-chloro benzene, 2,5-dimethoxy benzene, 4-chloroaniline, aniline, 5-(trifluoromethyl) pyridin-2-yl, 6-(trifluoromethyl) nicotinic, and 4-chloro-benzene.

A LGIC ligand that can bind to and activate a modified LGIC described herein can be a quinuclidine. A quinuclidine can have the structure of Formula II:



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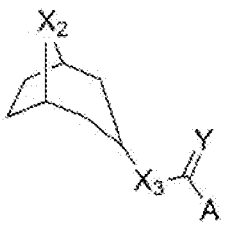
where X3 can be O, NH, or CH2; Y can be O or S; A can be an aromatic substituent; and R can be H or pyridinylmethylene. Examples of aromatic substituents include without limitation, 1H-indole, 4-(trifluoromethyl) benzene, 4-chloro benzene, 2,5-dimethoxy benzene, 4-(trifluoromethyl) benzene, 4-chloroaniline, aniline, 5-(trifluoromethyl) pyridin-2-yl, 6-(trifluoromethyl) nicotinic, 3-chloro-4-fluoro benzene, 4-chloro-benzene, and 1H-indole. Examples of quinuclidines include, without limitation, compounds PNU-282987, PHA-543613, 0456, 0434, 0436, 0354, 0353, 0295, 0296, and 0676 (see, *e.g.*, Figure 5C, Table 3, and Table 6).

A LGIC ligand that can bind to and activate a modified LGIC described herein can be a tropane. A tropane can have the structure of Formula III:



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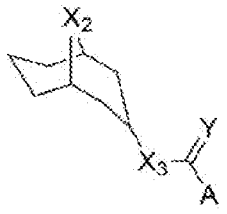
where X2 can be NH or NMe; X3 can be O, NH, or CH2; Y can be O or S; and A can be an aromatic substituent. Example of aromatic substituents include, without limitation, 1H-indole, 7-methoxy-1H-indole, 7-methyl-1H-indole, 5-chloro-1H-indole, and 1H-indazole. Examples of tropanes include, without limitation, tropisetron, pseudo-tropisetron, nortropisetron, compound 737, and compound 745 (see, *e.g.*, Figure 5C, Table 3, and Table 6).

A LGIC ligand that can bind to and activate a modified LGIC described herein can be a 9-azabicyclo[3.3.1]nonane. A 9-azabicyclo[3.3.1]nonane can have the structure of Formula IV:



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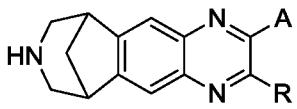
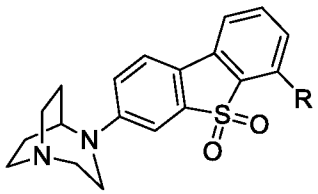
where X1 can be CH, X2 can be NH or NMe, X3 can be O, NH, or CH; Y can be O or S; and A can be an aromatic substituent. An example of an aromatic substituent is, without limitation, 4-chloro-benzene. Examples of 9-azabicyclo[3.3.1]nonanes include, without limitation, compound 0536, compound 0749, compound 0751, compound 0760, and compound 0763 (see, *e.g.*, Figure 5C, Table 3, and Table 6).

In some cases, a LGIC ligand can be an a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine and can have a structure shown in Formula V:



where R = H or CH_3; and where A = H or an aromatic substituent. Examples of 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepines include, without limitation, varenicline, compound 0765, and compound 0770 (see, *e.g.*, Figure 10A, Table 3, and Table 9).

In some cases, a LGIC ligand can be a 1,4-diazabicyclo[3.2.2]nonane and can have a structure shown in Formula VI:



where R = H, F, NO_2. Examples of 1,4-diazabicyclo[3.2.2]nonanes include, without limitation, 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]thiophene 5,5-dioxide, compound 0773, and compound 0774 (see, *e.g.*, Figure 10B, Table 6, and Table 9).

Methods of Using

This document also provides methods of using a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein. A LGIC ligand that can bind to and activate the modified LGIC can be used to activate a modified LGIC with temporal and/or spatial control based on delivery of the ligand.

In some aspects, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be used to identify a ligand that selectively binds to a modified LGIC described herein. For example, such screening methods can include providing one or more candidate ligands to a modified LGIC described herein, and detecting binding between the candidate ligand and the modified LGIC.

Any appropriate method can be used to detect binding between a candidate ligand and the modified LGIC and any appropriate method can be used to detect activity of a modified LGIC. For example, the ability of a ligand to bind to and activate a modified LGIC can be measured by assays including, but not limited to, membrane potential (MP) assay (*e.g.*, a fluorescence MP assay), radioactive binding assays, and/or voltage clamp measurement of peak currents and sustained currents.

In some aspects, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be used to treat a mammal having a channelopathy (*e.g.*, a neural channelopathy or a muscle channelopathy). For example, a mammal having a channelopathy can be treated by administering a modified LGIC described herein, and then administering a LGIC ligand that can bind to and activate the modified LGIC. For example, a mammal having a channelopathy can be treated by administering a modified LGIC described herein (*e.g.*, including at least one chimeric α7-GlyR LGIC subunit (SEQ ID NO:6) having a human α7 nAChR LBD (SEQ ID NO:2) with a R27D amino acid

substitution, a E41R amino acid substitution, a Q79G amino acid substitution, and a Y115F amino acid substitution, and a human GlyR IPD (SEQ ID NO:5) with a A298G amino acid substitution), and then administering tropisetron. For example, a mammal having a channelopathy can be treated by administering a modified LGIC described herein including a modified human α7 nAChR LBD (*e.g.*, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:11, or SEQ ID NO:12) with an L131 amino acid substitution (*e.g.*, L131G, L131A, L131M, or L131N) and, optionally, a Q79S amino acid substitution, a Q139L amino acid substitution, and/or a Y217F amino acid substitution, and then administering varenicline, tropisetron, and/or compound 765.

Any type of mammal can be treated using a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein. For example, humans and other primates such as monkeys can be treated using a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein. In some cases, dogs, cats, horses, cows, pigs, sheep, rabbits, mice, and rats can be treated using a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein.

Any appropriate method can be used to identify a mammal having a channelopathy and/or a mammal at risk of developing a channelopathy. For example, genetic testing can be used to identify a mammal having a channelopathy and/or a mammal at risk of developing a channelopathy.

Once identified as having a channelopathy and/or a mammal at risk of developing a channelopathy, the mammal can be administered or instructed to self-administer a modified LGIC described herein, and then administered or instructed to self-administer a LGIC ligand that can bind to and activate the modified LGIC as described herein. A modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be administered together or can be administered separately.

When treating a mammal having a channelopathy and/or a mammal at risk of developing a channelopathy using the materials and methods described herein, the channelopathy can be any channelopathy. As used herein, a channelopathy can be any disease or disorder caused by aberrant ion channel function and/or aberrant ligand function, or which could be alleviated by modulated ion channel function and/or altered cellular ion flux (e.g., calcium ion flux). A channelopathy can be congenital or acquired. Examples of

channelopathies include, without limitation, Bartter syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), congenital hyperinsulinism, cystic fibrosis, Dravet syndrome, episodic ataxia, erythromelalgia, generalized epilepsy (*e.g.*, with febrile seizures), familial hemiplegic migraine, fibromyalgia, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, Lambert-Eaton myasthenic syndrome, long QT syndrome (*e.g.*, Romano-Ward syndrome), short QT syndrome, malignant hyperthermia, mucolipidosis type IV, myasthenia gravis, myotonia congenital, neuromyelitis optica, neuromyotonia, nonsyndromic deafness, paramyotonia congenital, retinitis pigmentosa, timothy syndrome, tinnitus, seizure, trigeminal neuralgia, and multiple sclerosis. Alternatively, or in addition, the materials and methods described herein can be used in other applications including, without limitation, pain treatment, cancer cell therapies, appetite control, spasticity treatment, muscle dystonia treatment, tremor treatment, and movement disorder treatment.

In some cases, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be used to modulate the activity of a cell. The activity of the cell that is modulated using a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be any cellular activity. Examples of cellular activities include, without limitation, active transport (*e.g.*, ion transport), passive transport, excitation, inhibition, ion flux (*e.g.*, calcium ion flux), and exocytosis. The cellular activity can be increased or decreased. For example, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be used to modulate (*e.g.*, increase) ion transport across the membrane of a cell. For example, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be used to modulate (*e.g.*, increase) the excitability of a cell.

A modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be used to modulate the activity of any type of cell in a mammal. The cell can be a neuron, a glial cell, a myocyte, an immune cell (*e.g.*, neutrophils, eosinophils, basophils, lymphocytes, and monocytes), an endocrine cell, or a stem cell (*e.g.*, an embryonic stem cell). In some cases, the cell can be an excitable cell. The cell can be in vivo or ex vivo.

A modified LGIC described herein can be administered by any appropriate method. A modified LGIC can be administered as modified LGIC subunits or as pre-assembled modified LGICs. A modified LGIC can be administered as a nucleic acid encoding a modified LGIC. A modified LGIC can be administered as a nucleic acid encoding a modified LGIC subunit described herein. For example, a nucleic acid can be delivered as naked nucleic acid or using any appropriate vector (*e.g.*, a recombinant vector). Vectors can be a DNA based vector, an RNA based, or combination thereof. Vectors can express a nucleic acid in dividing cells or non-dividing cells. Examples of recombinant vectors include, without limitation, plasmids, viral vectors (*e.g.*, retroviral vectors, adenoviral vectors, adeno-associated viral vectors, and herpes simplex vectors), cosmids, and artificial chromosomes (*e.g.*, yeast artificial chromosomes or bacterial artificial chromosomes). In some cases, a nucleic acid encoding a modified LGIC subunit described herein can be expressed by an adeno-associated viral vector.

A modified LGIC described herein can be detected (*e.g.*, to confirm its presence in a cell) by any appropriate method. In some cases, an agent that selectively binds a modified LGIC can be used to detect the modified LGIC. Examples of agents that can be used to bind to a modified LGIC described herein include, without limitation, antibodies, proteins (e.g., bungarotoxin), and small molecule ligands (*e.g.*, PET ligands). An agent that selectively binds a modified LGIC can include a detectable label (*e.g.*, fluorescent labels, radioactive labels, positron emitting labels, and enzymatic labels). Methods to detect LGIC expression in a cell can include fluorescence imaging, autoradiography, functional MRI, PET, and SPECT.

A modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be administered to a mammal having a channelopathy and/or at risk of developing a channelopathy as a combination therapy with one or more additional agents/therapies used to treat a channelopathy. For example, a combination therapy used to treat a mammal having a channelopathy as described herein can include administering a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein and treating with acetazolaminde, dichlorphenamide, mexilitine, glucose, calcium gluconate, L-DOPA, muscle stimulation, spinal stimulation, brain stimulation, and/or nerve stimulation.

In embodiments where a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein are used in combination with additional agents/therapies used to treat a channelopathy, the one or more additional agents can be administered at the same time or independently. For example, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein first, and the one or more additional agents administered second, or vice versa. In embodiments where a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein are used in combination with one or more additional therapies used to treat a channelopathy, the one or more additional therapies can be performed at the same time or independently of the administration of a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein. For example, a modified LGIC described herein and a LGIC ligand that can bind to and activate the modified LGIC as described herein can be administered before, during, or after the one or more additional therapies are performed.

In some cases, a modified LGIC described herein and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be formulated into a pharmaceutically acceptable composition for administration to a mammal having a channelopathy or at risk of developing a channelopathy. For example, a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. A pharmaceutical composition can be formulated for administration in solid or liquid form including, without limitation, sterile solutions, suspensions, sustained-release formulations, tablets, capsules, pills, powders, and granules.

Pharmaceutically acceptable carriers, fillers, and vehicles that may be used in a pharmaceutical composition described herein include, without limitation, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based

substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

A pharmaceutical composition containing a modified LGIC described herein and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be designed for oral, parenteral (including subcutaneous, intracranial, intraarterial, intramuscular, intravenous, intracoronary, intradermal, or topical), or inhaled administration. When being administered orally, a pharmaceutical composition containing a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be in the form of a pill, tablet, or capsule. Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that can contain anti-oxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Compositions for inhalation can be delivered using, for example, an inhaler, a nebulizer, and/or a dry powder inhaler. The formulations can be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

A pharmaceutically acceptable composition including a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be administered locally or systemically. In some cases, a composition containing a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be administered systemically by venous or oral administration to, or inhalation by a mammal (*e.g.*, a human). In some cases, a composition containing a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be

administered locally by percutaneous, subcutaneous, intramuscular, intracranial, or open surgical administration (*e.g.*, injection) to a target tissue of a mammal (*e.g.*, a human).

Effective doses can vary depending on the severity of the channelopathy, the route of administration, the age and general health condition of the subject, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents, and the judgment of the treating physician.

The frequency of administration can be any frequency that improves symptoms of a channelopathy without producing significant toxicity to the mammal. For example, the frequency of administration can be from about once a week to about three times a day, from about twice a month to about six times a day, or from about twice a week to about once a day. The frequency of administration can remain constant or can be variable during the duration of treatment. A course of treatment with a composition containing a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can include rest periods. For example, a composition containing a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be administered daily over a two week period followed by a two week rest period, and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents, route of administration, and severity of the channelopathy may require an increase or decrease in administration frequency.

An effective duration for administering a composition containing a therapeutically effective amount of a modified LGIC described herein (*e.g.*, a nucleic acid encoding a modified LGIC described herein) and/or a LGIC ligand that can bind to and activate the modified LGIC as described herein can be any duration that improves symptoms of a channelopathy without producing significant toxicity to the mammal. For example, the effective duration can vary from several days to several weeks, months, or years. In some cases, the effective duration for the treatment of a channelopathy can range in duration from about one month to about 10 years. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the

frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the channelopathy being treated.

In certain instances, a course of treatment and the symptoms of the mammal being treated for a channelopathy can be monitored. Any appropriate method can be used to monitor the symptoms of a channelopathy.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Potency-enhancing ligand binding domain mutations

A screen was performed with a panel of 41 α7-5HT3 chimeric channels having mutant LBDs against a panel of 51 clinically used drugs with chemical similarity to nicotinic receptor agonists. Mutations were at residues highlighted in Figure 1. The screen revealed mutations at Gln^{79} in the α7 nAChR LBD that enhanced potency for the known nAChR agonist tropisetron (Figure 2). These mutations (Q79A, Q79G, Q79S) reduce the size of the amino acid side chain. Some mutant ion channel-ligand combinations gave up to 12-fold improvement in potency (Table 1, Figure 3). Canonical α7 nAChR agonists, ACh, nicotine, epibatidine, and the anti-smoking drug varenicline were not significantly affected by Q79A, Q79G, or Q79S mutations. However, a subset of α7 nAChR agonists showed enhanced potency with some of the mutations. Cytisine, RS56812, tropisetron, nortropisetron, and PNU-282987 showed significantly improved potency for $\alpha7^{Q79G}$-5HT3. Additionally, nortropisetron and PNU-282987 showed a significantly enhanced potency for $\alpha7^{Q79A}$-5HT3 and $\alpha7^{Q79S}$-5HT3, respectively. In general, agonists based on a quinuclidine or tropane pharmacophore with a linked aromatic structure that interacts with the complementary binding face of the ligand binding domain showed improved potency with Gln79 substitution with the smaller amino acid residues Ala, Gly, or Ser. For most agonists, $\alpha7^{Q79G}$-5HT3 was the most preferred mutant chimeric ion channel.

Table 1. Potency of nAChR agonists against chimeric cation channels mutated at Gln79 in HEK cells. Mean EC50, SEM in parentheses (μM).

Agonist	α7-5HT3	$\alpha7^{Q79A}$-5HT3	$\alpha7^{Q79G}$-5HT3	$\alpha7^{Q79S}$-5HT3

Acetylcholine	7.0 (0.8)	9.2 (1.8)	6.7 (0.6)	6.2 (1.4)
Nicotine	3.9 (0.4)	4.1 (1.3)	3.1 (0.5)	2.1 (0.4)
Epibatidine	0.053 (0.006)	0.067 (0.022)	0.050 (0.008)	0.044 (0.006)
Varenicline	0.92 (0.16)	0.76 (0.21)	0.91 (0.12)	0.47 (0.07)
Cytisine	8.2 (0.3)	4.0 (0.9)	1.7 (0.2)	4.4 (1.0)
RS56812	10 (1.8)	6.8 (1.9)	1.4 (0.2)	5.7 (0.8)
Tropisetron	0.24 (0.03)	0.08 (0.02)	0.035 (0.002)	0.11 (0.02)
Nortropisetron	0.061 (0.021)	0.010 (0.002)	0.006 (0.001)	0.019 (0.007)
PNU-282987	0.22 (0.03)	0.037 (0.009)	0.018 (0.003)	0.023 (0.004)

These mutated LBDs were used to generate α7-GlyR chimeric channels having enhanced potency for most of these ligands up to 6-fold (Figure 4A). Like mutations of α7-5HT3, these mutations at Gln79 did not significantly affect potency of ACh, nicotine, epibatidine, varenicline, or cytisine. However, tropisetron, nortropisetron, and RS56812 showed significantly enhanced potency for $\alpha7^{Q79G}$-GlyR. Similar to LBD mutations for α7-5HT3, nortropisetron had significantly enhanced potency for $\alpha7^{Q79A}$-GlyR, and PNU-282987 showed significantly enhanced potency for $\alpha7^{Q79S}$-GlyR. For most agonists, $\alpha7^{Q79G}$-GlyR was the most preferred mutant chimeric ion channel.

Another relationship that was observed in the small molecule screen was that mutations at Trp77 conferred agonist activity for the drug granisetron at the $\alpha7^{W77F}$-5HT3 (EC50: 1.2 μM), $\alpha7^{W77Y}$-5HT3 (EC50: 1.1 μM), and $\alpha7^{W77F}$-GlyR (EC50: 0.66 μM) receptors. Granisetron is a 5HT3 receptor antagonist granisetron, which does not activate α7-5HT3 or α7-GlyR.

These results show that mutation of Q79 (to A, G, or S) in the α7 nAChR LBD enhanced binding of known LGIC ligands to modified LGICs.

Example 2: Potency enhancing ion pore domain mutations

α7-GlyR channels having IPD mutations previously established in full length glycine receptor channels (T258S and A288G, GlyR numbering; equivalent to T268S and A298G for α7-GlyR numbering) were examined for enhanced potency for the allosteric agonist ivermectin. Channels having α7-$GlyR^{T268S}$ were found to have substantial ligand-free open probability, which rendered them unsuitable for ligand-controlled manipulations of cells. Mutations at α7-$GlyR^{A298G}$, which were effective for enhancing ivermectin potency at the full length glycine receptor, led to modest change in open probability in the absence of the ligand; thus this channel was examined for activity against a panel of known agonists. For

canonical agonists ACh, nicotine, and epibatidine, as well as for varenicline and tropisetron, the agonist potency was not significantly enhanced in α7-GlyR^{A298G}. A subset of α7 nAChR agonists did show up to a modest 4-fold increase in potency: RS56812, cytisine, PNU-282987, and nortropisetron were significantly more potent. Therefore, the effect of the IPD A298G mutation improved ligand potency, but depended on ligand structure and was not as effective as mutations in the LBD.

The Q79G mutation in the LBD and the A298G IPD mutation for α7-GlyR was examined (Table 2). The double mutant chimeric channel, α7^{Q79G}-GlyR^{A298G}, led to synergistic enhancement of potency showing up to 18-fold enhancement of potency relative to α7-GlyR to a7 nAChR agonists. The enhancement from this double mutant channel was greater than that from the individual mutations for agonists RS56812, tropisetron, nortropisetron, and PNU-282987. Further underscoring the unexpected structural sensitivity of this combination of mutations, multiple agonists, such as ACh, nicotine, epibatidine, varenicline, and cytisine were not significantly changed between α7-GlyR and α7^{Q79G}-GlyR^{A298G}. Therefore, combination of the LBD mutation Q79G with the IPD mutation A298G led to a synergistic effect where potency for some but not all nicotinic agonists was greatly increased by ~10-20-fold.

Table 2. Potency of nAChR agonists against mutated chimeric chloride channels. Mean EC50 and SEM in parentheses (μM) for agonist activity in HEK cells expressing chimeric channels.

Agonist	**α7 GlyR**	**α7^{Q79A}-GlyR**	**α7^{Q79G}-GlyR**	**α7^{Q79S}-GlyR**	**α7-GlyR^{A298G}**	**α7^{Q79G}-GlyR^{A298G}**
Acetylcholine	6.4 (1.2)	7.6 (1.7)	7.1 (1.2)	4.5 (1.2)	6.4 (1.8)	4.8 (0.5)
Nicotine	5.0 (1.8)	2.6 (0.7)	4.1 (0.3)	1.4 (0.4)	3.1 (1.8)	2.2 (0.6)
Epibatidine	0.062 (0.021)	0.038 (0.005)	0.069 (0.011)	0.024 (0.003)	0.018 (0.001)	0.032 (0.007)
Varenicline	0.62 (0.2)	0.48 (0.08)	1.1 (0.25)	0.28 (0.06)	0.25 (0.04)	0.33 (0.08)
Cytisine	6.4 (2.0)	4.5 (0.6)	5.6 (2.1)	2.5 (0.7)	2.1 (0.28)	2.8 (1.0)
RS56812	6.5 (1.8)	3.5 (0.5)	2.0 (0.15)	2.8 (0.5)	2.3 (0.1)	0.61 (0.14)
Tropisetron	0.15 (0.045)	0.044 (0.008)	0.038 (0.003)	0.040 (0.009)	0.065 (0.026)	0.011 (0.002)
Nortropisetron	0.022 (0.007)	0.004 (0.001)	0.008 (0.003)	0.005 (0.001)	0.005 (0.001)	0.002 (0.001)
PNU-282987	0.13 (0.038)	0.022 (0.004)	0.026 (0.005)	0.014 (0.002)	0.035 (0.005)	0.007 (0.001)

These results show that mutation of Q79 (to A, G, or S) in the α7 nAChR LBD and/or mutation of A298 (to G) in the GlyR IPD further enhanced selective binding of known LGIC ligands to modified LGICs.

Example 3: Molecules exhibiting enhanced potency

Based on the structure activity relationship of known agonists that showed enhanced potency with $\alpha7^{Q79G}$-$GlyR^{A298G}$, a variety of synthetic molecules comprised of either quinuclidine, tropane, or 9-azabicyclo[3.3.1]nonane pharmacophores with one or more aromatic side chain substituents were tested. In addition, the known α7 nAChR agonist PHA-543613 (Walker et al 2006, Wishka et al 2006) was also tested and showed exceptional potency for $\alpha7^{Q79G}$-$GlyR^{A298G}$. These molecules generally showed enhanced potency 10-fold to 100-fold (Table 3), indicating that, for these pharmacophores, a range of structural features were compatible with improved potency for $\alpha7^{Q79G}$-$GlyR^{A298G}$.

These results show that modified LGICs can be activated by synthetic quinuclidine-containing and tropane-containing LGIC ligands.

Table 3. Potency of compounds against chimeric channels. Mean EC50 and SEM in parentheses (μM) for agonist activity in HEK cells expressing chimeric channels. Partial refers to partial agonist activity.

Compound	X_1	X_2	X_3	Y	C_1 n	C_2 n	C_3 n	C-X config	R	A	α7-5HT3 EC_{50} (μM)	α7-GlyR EC_{50} (μM)	$\alpha7^{Q79G}$-$GlyR^{A298G}$ EC_{50} (μM)
PNU-282987	N	CH_2	NH	O	0	1	0	R	H	4-chloro-benzene	0.22	0.13	0.007
Tropisetron	C	NMe	O	O	1	0	0	Endo	H	1H-indole	0.24	0.15	0.011
Pseudo-tropisetron	C	NMe	O	O	1	0	0	Exo	H	1H-indole	2	0.7	<0.2
Nortropisetron	C	NH	O	O	1	0	0	Endo	H	1H-indole	0.061	0.022	0.002
PHA-543613	N	CH_2	NH	O	0	1	0	R	H	furo[2,3]pyridine	0.046	0.039	0.004
0542	C	NMe	NH	S	1	0	0	Endo	H	1H-indole	3.8	0.58	0.072
0026	N	CH_2	O	O	0	1	0	S	H	4-(trifluoromethyl) benzene	--	13.7	1.43
0456	N	CH_2	CH_2-NH	S	0	1	0	mix	H	4-chloro benzene	--	2.8	0.47
0434	N	CH_2	NH	O	0	1	0	mix	pyridin-3-ylmethyl	2,5-dimethoxy benzene	> 10	> 10	0.19
0436	N	CH_2	NH	O	0	1	0	mix	pyridin-3-ylmethyl	4-(trifluoromethyl) benzene	0.84	0.31	0.006
0354	N	CH_2	NH	S	0	1	0	R	H	4-chloroaniline	1.4 partial	1.0	0.03
0353	N	CH_2	NH	O	0	1	0	S	H	aniline	0.65	0.27	0.01
0295	N	CH_2	NH	O	0	1	0	S	H	5-(trifluoromethyl) pyridin-2-yl)	> 100	>100	4.6
0296	N	CH_2	NH	O	0	1	0	S	H	6-(trifluoromethyl) nicotinic	>100	--	0.45
0536	C	NMe	NH	S	1	0	1	Endo	H	4-chloro-benzene	>33	>100	9.1
0676	N	CH_2	NH	O	0	1	0	S	H	1H-indole	0.03	0.018	0.002

Example 4: Mutations that reduce acetylcholine responsiveness

The α7 nAChR has relatively low sensitivity to ACh compared to other nAChR isoforms, and potency enhancing mutations for tropane and quinuclidine ligands did not substantially alter the potency of acetylcholine at these channels. Thus, the chimeric channels were further modified to reduce acetylcholine responsiveness of these channels. Acetylcholine responsiveness was considerably reduced to more than 100 μM in some cases with additional LBD mutations Y115F and Q139G that that only modestly reduced the potency of some agonists for $\alpha7^{Q79G,Y115F}$-5HT3, $\alpha7^{Q79G,Q139G}$-5HT3, $\alpha7^{Q79G,Q139G}$-$GlyR^{A298}$, $\alpha7^{Q79G,Y115F}$-$GlyR^{A298G}$. For example, $\alpha7^{Q79G,Y115F}$-$GlyR^{A298G}$ has an EC50 of 13 nM for nortropisetron and >100 μM for ACh (Table 4).

Table 4. Potency of nAChR agonists against mutated chimeric chloride channels with low acetylcholine responsiveness. Mean EC50 and SEM in parentheses (μM) for activity in HEK cells expressing chimeric channels.

	$\alpha7^{Q79G,Y115F}$-5HT3	**$\alpha7^{Q79G,Q139G}$-5HT3**	**$\alpha7^{Q79G,Y115F}$-$GlyR^{A298G}$**	**$\alpha7^{Q79G,Q139G}$-$GlyR^{A298G}$**	**$\alpha7^{R27D,E41R,Q79G,Y115F}$-$GlyR^{A298G}$**
Acetylcholine	>100	36 (2)	>100	73 (27)	>100
Nicotine	34 (4)	24 (4)	22 (3)	30 (8)	7.5 (1.3)
Tropisetron	0.10 (0.12)	0.31 (0.06)	0.086 (0.043)	0.26 (0.04)	0.035 (0.021)
Nortropisetron	0.028 (0.005)	0.047 (0.013)	0.013 (0.001)	0.031 (0.006)	0.003 (0.001)
PNU-282987	0.35 (0.07)	0.16 (0.04)	0.22 (0.04)	0.18 (0.04)	0.066 (0.010)

These results show that Y115F and/or Q139G mutations in the α7 nAChR LBD reduced binding of the endogenous LGIC ligand Ach to the modified LGIC.

Example 5: Mutations that reduce associations with endogenous receptor subunits

Assembly of α7 nAChRs is based on associations of five homomeric subunits through interactions between the LBDs (Celie et al 2004 Neuron 41: 907-14). To minimize undesired associations with endogenous α7 nAChR subunits and/or unwanted associations of chimeric channels, potential inter-subunit salt bridges were identified by examining the crystal structure of the acetylcholine binding protein and identifying nearby inter-subunit residues with opposite charge that also have homologous ionic amino acids in the α7 nAChR receptor LBD. Charge reversal mutations (switching the acidic member of a potential salt bridge to a

basic residue and its basic partner to an acidic residue) were designed to disrupt inter-subunit interactions with unmodified subunits but preserve interactions between the subunits with charge reversal mutations (Figure 6A). Chimeric LGIC subunits having charge reversal mutations were able to assemble selectively with each other without interacting with unmodified channels, *e.g.* endogenous α7 nAChR. The double mutation of R27D,E41R in the α7 nAChR LBD resulted in functional channels (Figure 6B). Co-expression of these charge reversal channels with α7-5HT3 channels having an unmodified sequence showed that the charge reversal subunits did not co-immunoprecipitate with unmodified channels (Figure 6C). Combination with potency enhancing mutations and acetylcholine blocking mutations to give the chimeric channel $\alpha7^{R27D,E41R,Q79G,Y115F}$-$GlyR^{A298G}$ revealed that some agonists retained high potency for their cognate agonist (Table 4, right column).

These results show that R27D and E41R mutations in α7 nAChR LBD reduced association of the modified LGIC subunits with other modified and/or endogenous LGIC subunits.

Example 6: LBD mutations that increase ligand potency

Mutations in Gly^{175} and Pro^{216} of the α7 nAChR LBD in α7-GlyR chimeric channels were tested. Mutation of Gly^{175} to Lys ($\alpha7^{G175K}$-GlyR) showed increased potency for ACh (5-fold) (Table 5). For $\alpha7^{G175K}$-GlyR, it was also found that nicotine potency was enhanced 10-fold relative to the unmodified α7-GlyR chimeric channel (Table 5). Mutation of Pro^{216} to Ile ($\alpha7^{P216I}$-GlyR) did not substantially alter ACh potency (Table 5). However, $\alpha7^{P216I}$-GlyR showed increased nicotine potency by >4-fold relative to unmodified α7-GlyR (Table 5). These potency enhancing mutations in $\alpha7^{G175K}$-GlyR and $\alpha7^{P216I}$-GlyR also affected potency of several other α7-GlyR agonists up to 30-fold (Table 5). For $\alpha7^{G175K}$-GlyR, greater than 10-fold potency enhancement over α7-GlyR was seen for the clinically used drugs tropisetron, varenicline, cytisine, granisetron, and epibatidine. For $\alpha7^{P216I}$-GlyR, potency enhancement was approximately 3-fold (Table 5).

Table 5. Agonist potency enhancement by G175K and P216I mutations at a7GlyR chimeric channels.

Compound	a7GlyR	α7GlyR G175K	α7GlyR P216I	α7GlyR Y115F G175K	α7GlyR G175K Y210F	α7GlyR W77F G175K	α7GlyR Q79G G175K	α7GlyR W77F Q79G G175K	α7GlyR W77F Q79G Y115F G175K	α7GlyR W77F G175K Y210F	α7GlyR Q79G G175K Y115F	α7GlyR Q79G Y115F G175K Y210F	α7GlyR Q79G Y115F G175K K322L	α7GlyR Y115F G175K L141F	α7GlyR Q79G Q139L G175K
Acetylcholine	6.4 (1.2)	1.2 (0.41)	4.0 (0.5)	52 (6.6)	93 (1.3)	6.8 (1.6)	4.5 (1.3)	41 (3.1)	143 (13)	80 (31)	98 (10)	> 1000	> 200	58	53
Nicotine	5.0 (1.8)	0.5 (0.25)	1.4 (0.1)	4.1 (1.4)	6 (0.5)	1.3 (0.4)	1.1 (0.1)	2.6 (0.7)	6.1 (2.0)	4.2	13 (0.2)	> 100	14.5	3	5.8
Epibatidine	0.062 (0.021)	0.005 (0.001)	0.03 (0.01)	0.036 (0.006)	0.65 (0.11)	0.04 (0)	0.037 (0.013)	2.6 (2.3)	0.33	0.38	0.22 (0.015)	> 10	0.27	0.144	0.144
Varenicline	0.62 (0.2)	0.056 (0.014)	0.18 (0.06)	5.0 (1.7)	4.3 (0.6)	0.57 (0.18)	0.42 (0.1)	3.3 (1.0)	>10	>9	>10	> 30	> 30	> 8.1	0.96
Cytisine	6.4 (2.0)	0.4 (0.05)	1.9 (0.2)	7.1 (1.2)	>10	1.5 (0.6)	2.5 (1.1)	6.9 (1.2)	4.02	5.1	>10	> 30	> 30	4.74	3.24
PNU-282987	0.13 (0.038)	0.005 (0.001)	0.04 (0.004)	0.1 (0.01)	0.7 (0.3)	0.67 (0.35)	0.06 (0.05)	0.5 (0.2)	>1	>40	0.08 (0.01)	>1	0.018	0.51	0.05
Tropisetron	0.15 (0.045)	0.011 (0.002)	0.05 (0.003)	0.027 (0.004)	1.1 (0.2)	0.04 (0.01)	0.01 (0.001)	0.024 (0.004)	0.1 (0.04)	>1	0.027 (0.002)	0.717	0.066	0.117	0.105
Nortropisetron	0.022 (0.007)	0.003 (0.002)	0.006 (0.0004)	0.007 (0.001)	0.28 (0.09)	0.004 (0.001)	0.0008 (0.0001)	0.0026 (0.0004)	0.014	>12	0.012 (0.001)	> 0.3	0.069	0.075	0.001
PHA-543613	0.03 (0.01)	0.001 (0.0001)	0.009 (0.001)	0.02 (0.007)	0.26 (0.08)	0.041 (0.016)	0.003 (0.0004)	0.12 (0.04)	> 0.3	>3	0.036 (0.006)	> 1	0.111	0.057	0.024
Granisetron	>100	3.3 (0.1)	6.1 (0.9)	1.6 (0.6)	1.4 (0.1)	0.18 (0.02)	> 100	1.6 (0.4)	0.2	0.06 (0.01)	6.8 (1.7)	4.8	> 30	0.84	> 30
Ivermectin		nd	nd	nd	nd	nd	nd	nd	nd	nd	0.21	nd	nd	nd	nd

nd = not determined

For use in organisms that produce ACh, it is important to reduce the endogenous ACh potency at these channels comprised of the α7 nAChR LBD. Mutation G175K could be further combined with other mutations that reduced sensitivity to ACh, such as Y115F and Y210F. For $\alpha7^{Y115F,G175K}$-GlyR, high potency for agonists based on tropane or quinuclidine core structures were found for tropisetron, granisetron, nortropisetron, PNU-282987, and PHA-543613, and greatly reduced potency for varenicline and cytisine (Table 5). For $\alpha7^{G175K,Y210F}$-GlyR, potency for most agonists was considerably reduced, however potency enhancement for granisetron was observed (Table 5).

To develop channels with reduced ACh responsiveness but high potency for other agonists, $\alpha7^{G175K}$-GlyR was combined with additional mutations that increase the potency of specific agonists. Combination with W77F reduced ACh potency, and $\alpha7^{W77F,G175K}$-GlyR showed increased potency over α7-GlyR for granisetron, nortropisetron, and tropisetron but not for PNU282-987, varenicline, cytisine, or PHA-543613 (Table 5). Combination of G175K with Q79G reduced ACh potency, and $\alpha7^{Q79G,G175K}$-GlyR showed increased potency for nortropisetron, PHA-543613, and tropisetron (Table 5). However, this potency enhancement was not observed for other agonists, such as PNU282-987, or varenicline. $\alpha7^{G175K,Q139L}$-GlyR reduced ACh potency and increased potency for nortropisetron and tropisetron (Table 5).

Further reductions in ACh potency were achieved while maintaining high potency for with synthetic agonists, including those based on tropane and quinuclidine core structures, by incorporating mutations at W77F, Q79G, L141F, Y115F, G175K, and Y210F in various combinations. $\alpha7^{Q79G,Y115F,G175K}$-GlyR reduced ACh responsiveness while maintaining potent responses to tropisetron (Table 5). These mutations also enhanced responsiveness to other tropane and quinuclidine core structures relative to $\alpha7^{Y115F,G175K}$-GlyR as well as relative to α7-5HT3 (representative of endogenous α7 nAChR activity), especially quinuclidine thioureas 702 and 703 as well as tropane ester 723, 725, 726, 736, 737, 738, and 745 (Table 6). $\alpha7^{Q79G,Y115F,G175K}$-GlyR also showed high sensitivity to ivermectin (Table 5). $\alpha7^{W77F,Q79G,G175K}$-GlyR reduced ACh responsiveness while maintaining high potency responses to tropisetron, and nortropisetron (Table 5). $\alpha7^{W77F,Q79G,G175K}$-GlyR also showed enhanced potency for additional tropane-based core structures, such as compounds 723 and 725, as well as the clinically used drugs mequitazine and promazine (Table 6).

$\alpha7^{W77F,G175K,Y210F}$-GlyR reduced ACh responsiveness but markedly improved potency to granisetron (Table 5). $\alpha7^{L141F,Y115F,G175K}$-GlyR reduced ACh responsiveness while conferring sensitivity to granisetron (Table 5). $\alpha7^{Q79G,Q139L,G175K}$-GlyR reduced ACh responsiveness but showed potent responses to nortropisetron (Table 5).

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Table 6. Potency enhancement of tropane, quniuclidine agonists, 9-azabicyclo[3.3.1]nonane agonists, diazabicyclo[3.2.2]nonane agonists, and promazine by G175K and P216I α7GlyR chimeric channels. Indole and indazole aromatic (A) substituents attached at 3-position.

Agonist class	X_1	X_2	X_3	Y	C_1 n	C_2 n	C_3 n	C-X configuration	R	Aromatic substitution (A)	Compound	α7-5HT3	α7-GlyR	α7GlyR G175K	α7GlyR Q79G G175K	α7GlyR Y115F G175K	α7Gly Q79G G175K Y115F	α7GlyR Q79G G175K Y115F R27D E41R	α7GlyR W77F Q79G G175K
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	3,5-dichloro-aniline	677	10.6	4.4	0.66 (0.06)	0.86 (0.004)	3.7 (0.7)	0.98 (0.09)	0.58 (0.14)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	3,4-dichloro-aniline	682	>100	0.2	0.12 (0.1)	0.013 (0.001)	0.40 (0.01)	0.13 (0.01)	0.06 (0.012)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	4-(trifluoromethoxy)aniline	684	>100	1.6	0.23 (0.02)	0.078 (0.022)	3.0 (0.3)	0.79 (0.04)	0.4 (0.03)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	4-fluoroaniline	699	2.8	3.6	0.26 (0.11)	0.039 (0.009)	2.9	0.52 (0.09)	0.33 (0.1)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	3-chloro-aniline	700	1.8	1.9	0.081 (0.009)	0.012 (0.0002)	1.5	0.21 (0.04)	0.11 (0.02)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	3-chloro-2-fluoroaniline	701	>100	nd	0.47 (0.17)	0.086 (0.014)	5.46	1.0 (0.2)	0.58 (0.03)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	3-chloro-4-fluoroaniline	702	>100	0.9	0.12 (0.004)	0.018 (0.003)	1.6	0.17 (0.03)	0.12 (0.02)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	5-chloro-2-fluoroaniline	703	>100	nd	0.52 (0.08)	0.03 (0.01)	12.7	1.2 (0.06)	1.1 (0.5)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	3-chloro-4-methylaniline	704	0.7	nd	0.062 (0.008)	0.018 (0.002)	0.76 (0.01)	0.24 (0.02)	0.18 (0.06)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	R	H	5-chloro-2-methylaniline	705	>100	nd	9.6	0.67 (0.14)	>10	4.8 (1.4)	4.5 (2.7)	nd
Quinuclidine	N	CH_2	NH	S	0	1	0	S	H	4-(trifluoromethoxy)aniline	713	>100	nd	2.1 (0.2)	0.54 (0.06)	>10	23.9	>10	nd
Tropane	C	NMe	NH	S	1	0	0	Endo	H	1-methyl-1H-indole	622	>100	nd	0.87	1.3 (0.2)	2.5 (0.4)	0.93 (0.02)	1.0 (0.2)	1.7
Tropane	C	NMe	O	O	1	0	0	Endo	H	4-methoxy-1H-indole	721	0.5	nd	0.027 (0)	0.015 (0.003)	0.080 (0.002)	0.020 (0.001)	0.016 (0.001)	0.04
Tropane	C	NMe	O	O	1	0	0	Endo	H	6-methoxy-1H-indole	722	0.5	nd	0.02 (0.001)	0.015 (0)	0.052 (0.008)	0.028 (0.008)	0.016 (0.001)	0.03
Tropane	C	NMe	O	O	1	0	0	Endo	H	7-methoxy-1H-indole	723	12.8	4	0.31 (0.02)	0.02 (0)	0.71 (0.46)	0.07 (0.01)	0.024 (0.003)	0.02

Tropane	C	NMe	O	O	1	0	0	Endo	H	4-methyl-1H-indole	724	1.2	nd	0.036 (0.003)	0.012 (0.002)	0.091 (0.013)	0.02 (0.006)	0.012 (0.002)	0.06
Tropane	C	NMe	O	O	1	0	0	Endo	H	7-methyl-1H-indole	725	12.2	8.1		0.022 (0.02)	0.069 (0.33)	0.042 (0.005)	0.022 (0.0001)	0.024
Tropane	C	NMe	O	O	1	0	0	Endo	H	4-chloro-1H-indole	726	4.2	nd	0.58 (0.24)	0.016 (0.001)	0.51 (0.37)	0.044 (0.006)	0.018 (0)	0.03
Tropane	C	NMe	O	O	1	0	0	Endo	H	5-methoxy-1H-indole	736	0.83	nd	0.2 (0.01)	0.044 (0.002)	0.57 (0.21)	0.078 (0.018)	0.078 (0.024)	0.06
Tropane	C	NMe	O	O	1	0	0	Endo	H	5-chloro-1H-indole	737	1	0.9	0.082 (0.004)	0.013 (0.001)	0.16 (0.03)	0.033 (0.004)	0.016 (0.001)	0.101
Tropane	C	NMe	O	O	1	0	0	Endo	H	6-chloro-1H-indole	738	0.4	nd	0.015 (0)	0.016 (0.002)	0.04 (0.014)	0.025 (0.002)	0.012 (0.001)	0.033
Tropane	C	NMe	O	O	1	0	0	Endo	H	1H-indazole	745	1.2	1.3	0.069	0.026 (0.002)	0.26 (0.03)	0.089 (0.024)	0.043 (0.014)	0.05
9-azabicyclo[3.3.1]nonane	CH	NMe	NH	O	1	0	1	Endo	H	1H-indole	749	6.6	nd	nd	nd	nd	1.3	nd	1.9
9-azabicyclo[3.3.1]nonane	CH	NMe	NH	O	1	0	1	Endo	H	1H-indazole	751	1.8	3.4	nd	nd	nd	3.2	nd	0.7
9-azabicyclo[3.3.1]nonane	CH	NMe	NH	O	1	0	1	Endo	H	7-methoxy-1H-indazole	760	>100	9.8	nd	nd	nd	3	nd	1.3
9-azabicyclo[3.3.1]nonane	CH	NH	O	O	1	0	1	Endo	H	1H-indole	763	1.9	0.17	nd	nd	nd	0.3	nd	0.2
1,4-diazabicyclo[3.2.2]nonane									F	dibenzo[b,d]thiophene 5,5-dioxide	773	0.135	0.001	nd	nd	0.0003	0.00042	nd	0.0014
1,4-diazabicyclo[3.2.2]nonane									NO_2	dibenzo[b,d]thiophene 5,5-dioxide	774	0.03	0.006	nd	nd	0.00078	0.03	nd	0.03
Quinuclidine	N	CH_2	CH_2		0	1	0	R	H	10H-phenothiazine	Mequitazine	>30	nd	nd	nd	nd	>10	nd	0.15
N,N-dimethylpropylamine										10H-phenothiazine	Promazine	>100	nd	nd	nd	nd	>100	nd	1.6

nd = not determined; parentheses: SEM

$\alpha7^{G175K}$-GlyR and $\alpha7^{P216I}$-GlyR along with mutations at Q79G, Y115F, and G175K were also compatible with non-association mutations R27D,E41R as well as the GlyR IPD mutation A298G, which further enhanced ligand potency for granisetron, epibatidine, varenicline, cytisine, PNU-282987, tropisetron, nortropisetron, and PHA-543613 (Table 7). Combination with non-association mutations to form $\alpha7^{R27D,E41R,Q79G,Y115F,G175K}$ further improved the potency for 702, 723, 725, and 726, with low ACh responsiveness (Table 6).

Table 7. Agonist potency enhancement by G175K and A298G mutations at α7GlyR chimeric channels as well as W298A at α7GABAc (also referred to as $GABA_A$-ρ) channels.

Compound	α7GlyR Q79G W77F A298G	α7GlyR Q79G G175K A298G	α7GlyR Q79G A298G G175K Y115F	α7GlyR Q79G A298G P216I	α7GlyR Q79G A298G Y115F K395 K396A	a7GABAc Q79G L141F W298A	α7GlyR Q79G G175K Y115F R27D,E41R	α7GlyR R27D E41R Q79G Y115F
Acetylcholine	45	0.66	31	5	90	52	52 (7.7)	> 500
Nicotine	3.8	0.11	3.3	1.6	16.5	16.2	4.8 (0.4)	> 39.8
Epibatidine	0.37	0.0023	0.011	0.05	0.15	0.42	0.059 (0.03)	0.267
Varenicline	3.66	0.022	2.37	0.18	> 30	6.27	4.9 (0.3)	> 30
Cytisine	14.1	0.134	4.6	5.5	> 30	13.3	4.8 (0.4)	> 30
PNU-282987	1.63	0.00036	0.009	0.25	0.11	0.12	0.05 (0.03)	0.34
Tropisetron	0.018	0.0006	0.0028	0.009	0.021	0.111	0.013 (0.005)	> 0.096
Nortropisetron	0.0024	0.00013	0.0084	0.0012	0.0063	0.009	0.003 (0.001)	0.102
PHA-543613	0.0066	0.00018	0.0039	0.003	0.0408	0.039	0.0054	0.156
Granisetron	1.2	nd	nd	nd	> 30	> 100	2.4 (0.3)	> 30

nd = not determined; parentheses: SEM

Additional amino acid substitutions at Gly^{175} of the α7 nAChR LBD in $\alpha7^{Y115F}$ -GlyR chimeric channels are also enhanced agonist potency. Potency for tropisetron at $\alpha7^{Y115F}$ – GlyR chimeric channels was enhanced with additional mutations, which include G175A (7.1-fold), G175F (2-fold), G175H (2.3-fold), G175K (5.6-fold), G175M (2.6-fold), G175R (5.8-fold), G175S (9.3-fold), G175V (16.7-fold).

Table 8. Agonist potency enhancement by G175 mutations at α7GlyR Y115F chimeric channels.

Compound	a7GlyR	α7GlyR Y115F G175K	α7GlyR Y115F G175A	α7GlyR Y115F G175F	α7GlyR Y115F G175H	α7GlyR Y115F G175M	α7GlyR Y115F G175R	α7GlyR Y115F G175S	α7GlyR Y115F G175V
Acetylcholine	6.4 (1.2)	52 (6.6)	24	67	79	71	29.5	31.5	15
Varenicline	0.62 (0.2)	5.0 (1.7)	5.9	13.6	12.7	14.1	7.6	9.7	4.6
Tropisetron	0.15 (0.045)	0.027 (0.004)	0.021	0.074	0.064	0.057	0.024	0.016	0.009
PHA-543613	0.03 (0.01)	0.02 (0.007)	0.027	0.173	0.12	0.25	0.11	0.12	0.037

nd = not determined; parentheses: SEM

Mutations for Leu^{131} to smaller amino acids were found to reduce the potency of canonical agonists ACh and nicotine, while markedly increasing potency of varenicline, tropisetron and several other agonists. $\alpha7^{L131A}$-GlyR and $\alpha7^{L131G}$-GlyR had reduced ACh responsiveness (6-fold) and enhanced potency for varenicline (8-fold and 17-fold, respectively) and tropisetron (2.5-fold and 3.6-fold, respectively) (Table 9). $\alpha7^{L131G}$-5HT3 HC had reduced ACh responsiveness (5-fold) and enhanced potency for varenicline (16-fold) and tropisetron (2.3-fold) (Figure 9A and Table 9). $\alpha7^{L131G,Q139L}$-GlyR and $\alpha7^{L131G,Y217F}$-GlyR showed similar potency enhancement over α7-GlyR for varenicline (21-fold) but also reduced ACh sensitivity (-11-fold and -13-fold, respectively). $\alpha7^{Q79S,L131G}$-GlyR further improved potency over α7-GlyR for varenicline (89-fold) and tropisetron (15-fold). $\alpha7^{L131G,Q139L,Y217F}$-GlyR showed the greatest improvement in potency over α7-GlyR for varenicline (387-fold) and also showed reduced ACh potency (13-fold) (Figure 9B and Table 9). $\alpha7^{L131G,Q139L,Y217F}$-GlyR also showed extremely high potency for compound 770 (0.001 μM), compound 773 (0.00034 μM), and compound 774 (0.00013 μM) (Figure 10). $\alpha7^{Q79S,L131G,\ Q139L}$-GlyR also improved potency over α7-GlyR for varenicline (31-fold) and tropisetron (3-fold) but reduced ACh potency (9-fold) (Figure 9B and Table 9). $\alpha7^{L131M}$-GlyR, $\alpha7^{L131Q}$-GlyR, and $\alpha7^{L131V}$-GlyR reduced ACh potency but enhanced potency to tropisetron, nortropisetron, PHA-543613, and granisetron (Table 9). $\alpha7^{L131F}$-GlyR was found to substantially reduced ACh potency but did not improve potency for other agonists (Table 8). $\alpha7^{L131G}$-$GABA_C$ substantially reduced ACh potency but did not improve potency for other agonists (Table 9). $\alpha7^{L131G,Q139L,Y217F}$-5HT3 HC (Table 9) improved varenicline potency by 131-fold over a7-5HT3 (Table 1). $\alpha7^{L131G,Q139L,Y217F}$-5HT3 HC also showed high

potency for compound 770 (0.007 μM), compound 773 (0.002 μM), and compound 774 (0.004 μM) (Table 8).

Table 9. Agonist potency enhancement by chimeric channels with L131 mutations.

Compound	a7GlyR	α7GlyR L131A	α7GlyR L131G	α7GlyR L131G Q139L	α7GlyR L131G Y217F	α7GlyR L131G Q139L Y217F	α7GlyR Q79G L131G	α7GlyR Q79S L131G	α7GlyR Q79S L131G Q139L	α7GlyR L131G D219A	α7GlyR L131F	α7GlyR Q79S L131G Q139L Y217F	α7GlyR L131M	α7GlyR Y115F L131M	α7GlyR L131N	α7GlyR L131Q	α7GlyR L131V	α75HT3 L131G HC	α75HT3 L131G Q139L Y217F HC	α7-GABA$_C$ L131G
Acetylcholine	6.4 (1.2)	42 (21)	41 (11)	68	85	83 (20)	>500	21 (3.5)	58	210	92 (32)	67 (3)	29	>500	5 (0.5)	58	16 (5)	35	39	>500
Nicotine	5.0 (1.8)	8.0 (3.2)	15 (3.5)	26	28	55 (18)	>100	8.2 (0.8)	25	36	20 (6.3)	41 (8)	15	nd	nd	13	3.9 (0.7)	15	20	>500
Epibatidine	0.062 (0.021)	0.027	0.009 (0.004)	0.012	0.015	0.021 (0.002)	nd	0.007 (0.001)	0.012	0.16	0.24 (0.05)	0.022 (0.004)	0.042	nd	nd	0.027	0.21 (0.04)	0.009	nd	
Varenicline	0.62 (0.2)	0.082 (0.068)	0.037 (0.026)	0.03	0.03	0.0016 (0.001)	>10	0.007 (0.001)	0.02	0.78	2.6 (1.1)	0.003 (0.001)	0.53	>100	0.069 (0.027)	0.72	0.33 (0.21)	0.04	0.007	0.3
Cytisine	6.4 (2.0)	20.6 (9.4)	13.1 (0.66)	12	30	nd	>30	8.1 (0.3)	10	>30	10.5 (1.8)	nd	7	nd	nd	>30	4.3 (0.7)	11	nd	>500
PNU-282987	0.13 (0.038)	0.055 (0.025)	0.034 (0.008)	0.063	0.054	0.16 (0.03)	0.096	0.006 (0.002)	0.018	0.41	0.20 (0.04)	0.05 (0.01)	0.021	nd	nd	0.048	0.064 (0.018)	0.033	0.015	0.12
Tropisetron	0.15 (0.045)	0.06 (0.021)	0.042 (0.01)	0.13	0.087	0.31 (0.05)	0.09	0.01 (0.003)	0.045	0.36	0.39 (0.2)	0.084 (0.009)	0.024	0.035	0.025 (0.005)	0.048	0.062 (0.013)	0.066	0.04	0.18
Nortropisetron	0.022 (0.007)	0.006 (0.003)	0.004 (0.001)	0.024	0.018	0.047 (0.006)	0.012	0.004 (0.002)	0.006	0.07	0.027 (0.008)	0.014 (0.002)	0.006	nd	nd	0.009	0.003 (0.001)	0.009	nd	0.021
PHA-543613	0.03 (0.01)	0.012 (0.006)	0.008 (0.002)	0.021	0.016	0.045 (0.008)	0.066	0.002 (0.0005)	0.009	0.038	0.04 (0.007)	0.015 (0.001)	0.009	0.028	0.02	0.015	0.011 (0.002)	0.012	0.009	0.027
Granisetron	>100	17.2 (12.8)	6.7 (1.6)	4	4	nd	nd	4.2 (0.8)	nd	>30	>100	nd	4	nd	nd	4	5.4 (1.3)	4	nd	>500
765	> 100	nd	nd	nd	nd	0.031 (0.02)	0.027	0.024	nd	nd	nd	0.034 (0.013)	nd	nd	>10	nd	nd	nd	0.11	nd
770	nd	nd	nd	nd	nd	0.001 (0.0003)	nd	nd	nd	nd	0.034	0.001 (0.0001)	0.03	nd	>10	> 0.3	nd	nd	0.007	nd
773	0.001	nd	0.00013	0.00004	nd	0.00034	0.00004	nd	nd	nd	0.0005	nd	0.00005	nd	0.0004	0.006	nd	nd	0.002	nd
774	0.006	nd	0.00004	0.00004	nd	0.00018	0.00004	nd	nd	nd	0.0013	nd	0.001	nd	0.0006	0.002	nd	nd	0.004	nd

nd = not determined; parentheses: SEM

Example 7: Chimeric LGICs in neurons

AAVs or DNA plasmids containing nucleic acids encoding a $\alpha7^{Q79G}$-$GlyR^{A298G}$ or α7Q79G,Y115F,G175K-GlyR chimeric LGICs were transduced into mouse cortical neurons. A low concentration of tropisetron (30 nM or 100 nM) was administered to mouse cortical neurons. Neuron activity was silenced by application of low concentration of agonist (Figure 7 and Figure 8C).

DNA plasmids containing nucleic acids encoding a α7L131G,Q139L,Y217F-GlyR chimeric LGICs were transfected into mouse cortical neurons. Low concentration of varenicline (10 nM) was administered to mouse cortical neurons. Neuron activity was silenced by application of low concentration of agonist (Figure 9C).

These results show that modified LGIC activity can be controlled in neurons using low concentration of the LGIC ligands tropisetron and varenicline.

Example 8: Chimeric LGICs in therapy

Chemogenetic tools offer an attractive strategy for combined drug and gene therapy. This is because cellular function can be modulated in a consistent manner across different cell types in various indications using the same ion channels and ligands by use of an exogenously delivered ion channel that is selectively engaged by administration of a drug. Identification of ion channels that are gated by well tolerated, clinically used drugs are especially attractive for potentially extending chemogenetics to human therapeutic use.

For the drug tropisetron, we have found that it activates $\alpha7^{Q79G}$-$GlyR^{A298G}$ with an EC50 of 11 nM, which is similar to the reported IC50 of 10 nM tropisetron for its therapeutic target, the 5HT3 receptor (Combrink et al 2009 Pharmacological reports: PR 61: 785-97).

OTHER EMBODIMENTS

It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A modified ligand gated ion channel (LGIC) comprising at least one modified LGIC subunit, said modified LGIC subunit comprising:

a ligand binding domain (LBD) comprising an amino acid modification, and

an ion pore domain (IPD).

2. The modified LGIC of claim 1, wherein the modified LGIC is a chimeric LGIC comprising a LBD from a first LGIC and an IPD from a second LGIC.

3. The modified LGIC of claim 1, wherein the LBD is an alpha7 nicotinic acetylcholine receptor (α7-nAChR) LBD.

4. The modified LGIC of claim 3, wherein the amino acid modification comprises an amino acid substitution at one or more amino acid residues selected from the group consisting of residues 77, 79, 115, 131, 139, 141, 175, 210, 216, 217, and 219 of the α7-nAChR LBD.

5. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 77 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of W77F and W77Y.

6. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 79 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of Q79A, Q79G, and Q79S.

7. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 115 of the α7-nAChR LBD, and wherein the amino acid substitution is a Y115F substitution.

8. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 131 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of L131A, L131G, L131M, and L131N.

9. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 139 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of Q139G and Q139L.

10. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 175 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of G175A, G175F, G175H, G175K, G175M, G175R, G175S, and G175V.

11. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 210 of the α7-nAChR LBD, and wherein the amino acid substitution is a Y210F substitution.

12. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 216 of the α7-nAChR LBD, and wherein the amino acid substitution is a P216I substitution.

13. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 217 of the α7-nAChR LBD, and wherein the amino acid substitution is a Y217F substitution.

14. The modified LGIC of claim 4, wherein the amino acid substitution is at residue 219 of the α7-nAChR LBD, and wherein the amino acid substitution is a D219A substitution.

15. The modified LGIC of claim 4, wherein the α7-nAChR LBD comprises a L131G amino acid substitution, a Q139L amino acid substitution, and a Y217F amino acid substitution.

16. The modified LGIC of claim 4, wherein the α7-nAChR LBD comprises a L131M amino acid substitution and a Y115F amino acid substitution.

17. The modified LGIC of claim 4, wherein the α7-nAChR LBD comprises a W77F amino acid substitution, a Q79G amino acid substitution, and a G175K amino acid substitution.

18. The modified LGIC of claim 4, wherein the α7-nAChR LBD comprises a Q79G amino acid substitution, a Y115F amino acid substitution, and a G175K amino acid substitution.

19. The modified LGIC of claim 4, wherein the α7-nAChR LBD comprises a Y115F amino acid substitution and a G175K amino acid substitution.

20. The modified LGIC of claim 4, wherein the α7-nAChR LBD comprises a Q79G amino acid substitution and a 216I amino acid substitution.

21. The modified LGIC of claim 1, wherein the IPD is an IPD from a receptor selected from the group consisting of a serotonin 3 receptor (5HT3) IPD, a glycine receptor (GlyR) IPD, a gamma-aminobutyric acid (GABA) receptor IPD, and an alpha7 nicotinic acetylcholine receptor (α7-nAChR) IPD.

22. The modified LGIC of claim 21, wherein the IPD comprises an amino acid substitution at residue 298.

23. The modified LGIC of claim 22, wherein the IPD is a GlyR IPD, and wherein the amino acid substitution is an A298G substitution.

24. The modified LGIC of claim 22, wherein the IPD is a GABA IPD, and wherein the amino acid substitution is a W298A substitution.

25. The modified LGIC of claim 1, wherein an exogenous LGIC ligand activates the modified LGIC, and wherein the exogenous LGIC ligand is a synthetic exogenous LGIC ligand selected from the group consisting of a quinuclidine, a tropane, a 9-azabicyclo[3.3.1]nonane, a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine, and a 1,4-diazabicyclo[3.2.2]nonane.

26. The modified LGIC of claim 25, wherein the synthetic exogenous LGIC ligand is a tropane, and wherein the tropane is selected from the group consisting of tropisetron, pseudo-

tropisetron, nortropisetron, compound 723, compound 725, compound 737, and compound 745.

27. The modified LGIC of claim 25, wherein the synthetic exogenous LGIC ligand is a quinuclidine, wherein the quinuclidine is selected from the group consisting of PNU-282987, PHA-543613, compound 0456, compound 0434, compound 0436, compound 0354, compound 0353, compound 0295, compound 0296, compound 0536, compound 0676, and compound 702.

28. The modified LGIC of claim 25, wherein the synthetic exogenous LGIC ligand is a 9-azabicyclo[3.3.1]nonane, and wherein the 9-azabicyclo[3.3.1]nonane is compound 536.

29. The modified LGIC of claim 25, wherein the synthetic exogenous LGIC ligand is a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine, and wherein the 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine is selected from the group consisting of varenicline, compound 765, and compound 770.

30. The modified LGIC of claim 25, wherein the synthetic exogenous LGIC ligand is a 1,4-diazabicyclo[3.2.2]nonane, and wherein the a 1,4-diazabicyclo[3.2.2]nonane is selected from the group consisting of 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]thiophene 5,5-dioxide, compound 773, and compound 774.

31. The modified LGIC of claim 1, wherein the LBD is a α7-nAChR LBD, and wherein the α7-nAChR LBD further comprises at least one modified amino acid that confers selective binding to another α7-nAChR LBD having the at least one modified amino acid over binding to an unmodified LGIC.

32. The modified LGIC of claim 31, wherein the unmodified LGIC is an endogenous LGIC.

33. The modified LGIC of claim 32, wherein the endogenous LGIC is an endogenous α7-nAChR.

34. The modified LGIC of claim 31, wherein the at least one modified amino acid that confers selective binding comprises an amino acid substitution at an amino acid residue at residue 27 and/or residue 41 of the α7-nAChR LBD.

35. The modified LGIC of claim 34, wherein the at least one modified amino acid comprises a R27D substitution and/or a E41R substitution.

36. The modified LGIC of claim 1, wherein the IPD is a murine 5HT3 IPD, and wherein the murine 5HT3 IPD further comprises at least one modified amino acid that confers increased ion conductance to the modified LGIC.

37. The modified LGIC of claim 36, wherein the at least one modified amino acid in the murine 5HT3 IPD that confers increased ion conductance to the modified LGIC comprises an amino acid substitution at an amino acid residue at residue 425, 429, and/or 433 of the murine 5HT3 IPD.

38. The modified LGIC of claim 37, wherein at least one modified amino acid comprises a R425Q substitution, a R429D substitution, and/or a R433A substitution.

39. The modified LGIC of claim 1, wherein the IPD is a human 5HT3 IPD, and wherein the human 5HT3 IPD further comprises at least one modified amino acid that confers increased ion conductance to the modified LGIC.

40. The modified LGIC of claim 39, wherein the at least one modified amino acid in the human 5HT3 IPD that confers increased ion conductance to the modified LGIC comprises an amino acid substitution at an amino acid residue at residue 420, 424, and/or 428 of the human 5HT3 IPD.

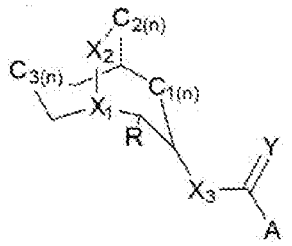
41. The modified LGIC of claim 40, wherein at least one modified amino acid comprises a R420Q substitution, a R424D substitution, and/or a R428A substitution.

42. The modified LGIC of claim 1, wherein the LBD has reduced binding with an endogenous LGIC ligand.

43. The modified LGIC of claim 42, wherein the endogenous LGIC ligand is acetylcholine (ACh).

44. The modified LGIC of claim 43, wherein the modified LGIC has an EC50 of greater than 20 μM for Ach.

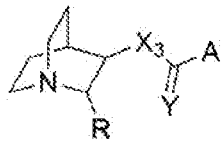
45. A ligand having increased potency for a modified ligand gated ion channel (LGIC), wherein the ligand comprises Formula I:



wherein each of X1, X2, and X3 is independently CH, CH2, O, NH, or NMe;
wherein each n is independently 0 or 1;
wherein Y = O or S;
wherein A = an aromatic substituent; and
wherein R= H or pyridinylmethylene.

46. The ligand of claim 45, wherein the aromatic substituent is selected from the group consisting of 1H-indole, 4-(trifluoromethyl) benzene, 2,5-dimethoxy benzene, 4-chloroaniline, aniline, 5-(trifluoromethyl) pyridin-2-yl, 6-(trifluoromethyl) nicotinic, and 4-chloro-benzene.

47. The ligand of claim 45, wherein the ligand a quinuclidine having Formula II:



wherein X3 = O, NH, or CH2;

wherein Y = O or S;

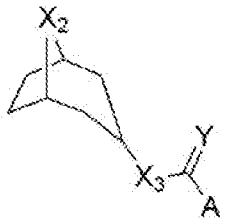
wherein A = an aromatic substituent; and

wherein R = H or pyridinylmethylene.

48. The ligand of claim 47, wherein the aromatic substituent is selected from the group consisting of 1H-indole, 4-(trifluoromethyl) benzene, 4-chloro benzene, 2,5-dimethoxy benzene, 4-(trifluoromethyl) benzene, 4-chloroaniline, aniline, 5-(trifluoromethyl) pyridin-2-yl, 6-(trifluoromethyl) nicotinic, 3-chloro-4-fluoro benzene, and 1H-indole.

49. The ligand of claim 47, wherein the quinuclidine is selected from the group consisting of PNU-282987, PHA-543613, compound 0456, compound 0434, compound 0436, compound 0354, compound 0353, compound 0295, compound 0296, compound 0536, compound 0676, and compound 702.

50. The ligand of claim 45, wherein the ligand is a tropane having Formula III:



wherein X2 = NH or NMe;

wherein X3 = O, NH, or CH2;

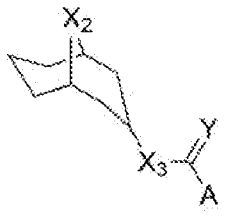
wherein Y = O or S; and

wherein A = an aromatic substituent.

51. The ligand of claim 50, wherein the aromatic substituent is selected from the group consisting of 1H-indole, 1H-indazole, 7-methoxy-1H-indole, 7-methyl-1H-indole, and 5-chloro-1H-indole.

52. The ligand of claim 50 wherein the tropane is selected from the group consisting of tropisetron, pseudo-tropisetron, nortropisetron, compound 723, compound 725, compound 737, and compound 745.

53. The ligand of claim 45, wherein the ligand is a 9-azabicyclo[3.3.1]nonane having Formula IV:



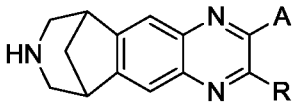
,

wherein X2 = NH or NMe;
wherein X3 = O, NH, or CH;
wherein Y = O or S; and
wherein A = an aromatic substituent.

54. The ligand of claim 54, wherein the aromatic substituent is selected from the group consisting of 4-chloro-benzene, 1H-indole, 1H-indazole, 7-methoxy-1H-indazole.

55. The ligand of claim 54, wherein the 9-azabicyclo[3.3.1]nonane is selected from the group consisting of compound 0536, compound 0749, compound 0751, compound 0760, and compound 0763.

56. A ligand having increased potency for a modified ligand gated ion channel (LGIC), wherein the ligand comprises Formula V:

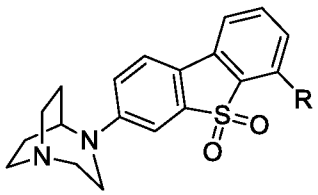


wherein R = H or CH3; and

wherein A = H or an aromatic substituent.

57. The ligand of claim 56, wherein the 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine is selected from the group consisting of varenicline, compound 0765, and compound 0770.

58. A ligand having increased potency for a modified ligand gated ion channel (LGIC), wherein the ligand comprises Formula VI:



wherein R = H, F, or NO_2.

59. The ligand of claim 58, wherein the 1,4-diazabicyclo[3.2.2]nonane is selected from the group consisting of 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]thiophene 5,5-dioxide, compound 0773, and compound 0774.

60. A method of treating a channelopathy in a mammal, the method comprising:

administering to a cell in the mammal a modified ligand gated ion channel (LGIC), wherein an exogenous LGIC ligand selectively binds the modified LGIC, said modified LGIC comprising at least one modified LGIC subunit, said modified LGIC subunit comprising:

a ligand binding domain comprising at least one modified amino acid, and

an ion pore domain; and

administering the exogenous ligand to the mammal.

61. The method of claim 60, wherein the channelopathy is selected from the group consisting of Bartter syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), congenital hyperinsulinism, cystic fibrosis, Dravet syndrome, episodic ataxia, erythromelalgia, generalized epilepsy (*e.g.*, with febrile seizures), familial hemiplegic migraine, fibromyalgia, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, Lambert-Eaton myasthenic syndrome, long QT syndrome (*e.g.*, Romano-Ward syndrome), short QT syndrome, malignant hyperthermia, mucolipidosis type IV, myasthenia gravis, myotonia congenital, neuromyelitis optica, neuromyotonia, nonsyndromic deafness, paramyotonia congenital, retinitis pigmentosa, timothy syndrome, tinnitus, seizure, trigeminal neuralgia, and multiple sclerosis.

62. A method of modulating ion transport across a cell membrane of a mammal, said method comprising:

administering to the cell a modified ligand gated ion channel (LGIC), wherein an exogenous LGIC ligand selectively binds the modified LGIC, said modified LGIC comprising at least one modified LGIC subunit, said modified LGIC subunit comprising:

a ligand binding domain comprising at least one modified amino acid, and

an ion pore domain; and

administering the exogenous ligand to the mammal.

63. The method of claim 62, wherein the modulating comprises activating ion transport.

64. The method of claim 62, wherein the modulating comprises inhibiting ion transport.

65. The method of claim 62, wherein the cell is selected from the group consisting of a neuron, a glial cell, a myocyte, a stem cell, an endocrine cell, and an immune cell.

66. The method of claim 62, wherein the administering the modified LGIC to the cell comprises in vivo administration.

67. The method of claim 62, wherein the administering the modified LGIC to the cell comprises ex vivo administration.

68. A method of modulating the excitability of a cell in a mammal, said method comprising:

administering to the cell from the mammal a modified ligand gated ion channel (LGIC), wherein an exogenous LGIC ligand selectively binds the modified LGIC, said modified LGIC comprising at least one modified LGIC subunit, said modified LGIC subunit comprising:

a ligand binding domain comprising at least one modified amino acid, and

an ion pore domain; and

administering the exogenous ligand to the mammal.

69. The method of claim 68, wherein the modulating comprises increasing the excitability of the cell.

70. The method of claim 68, wherein the modulating comprises decreasing the excitability of the cell.

71. The method of claim 68, wherein the cell is an excitable cell.

72. The method of claim 68, wherein the cell is selected from the group consisting of a neuron, a glial cell, a myocyte, a stem cell, an endocrine cell, and an immune cell.

73. The method of claim 68, wherein the administering the modified LGIC to the cell comprises in vivo administration.

74. The method of claim 68, wherein the administering the modified LGIC to the cell comprises ex vivo administration.

75. A method of modulating the activity of a cell in a mammal, said method comprising:

administering to the cell a modified ligand gated ion channel (LGIC), wherein an exogenous LGIC ligand selectively binds the modified LGIC, said modified LGIC comprising at least one modified LGIC subunit, said modified LGIC subunit comprising:

a ligand binding domain comprising at least one modified amino acid, and

an ion pore domain; and

administering the exogenous ligand to the mammal.

76. The method of claim 75, wherein the modulating comprises increasing the activity of the cell.

77. The method of claim 75, wherein the modulating comprises decreasing the activity of the cell.

78. The method of claim 75, wherein the activity is selected from the group consisting of ion transport, passive transport, excitation, inhibition, and exocytosis.

79. The method of claim 75, wherein the cell is selected from the group consisting of a neuron, a glial cell, a myocyte, a stem cell, an endocrine cell, and an immune cell.

80. The method of claim 75, wherein the administering the modified LGIC to the cell from the mammal comprises in vivo administration.

81. The method of claim 75, wherein the administering the modified LGIC to the cell from the mammal comprises ex vivo administration.

82. The modified LGIC of any one of claims 60, 62, 68, or 75, wherein the modified LGIC is a chimeric LGIC comprising a LBD from a first LGIC and an IPD from a second LGIC.

83. The method of any one of claim 82, wherein the chimeric LGIC is a homomeric chimeric LGIC.

84. The method of any one of claim 60, 62, 68, or 75, wherein the administering the modified LGIC to the cell comprises administering a nucleic acid encoding the modified LGIC.

85. The method of claim 84, wherein the modified LGIC comprises a sequence having at least 85% identity to SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:10.

86. The method of claim 85, wherein the modified LGIC comprises a sequence having at least 90% identity to SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:10.

87. The method of claim 86, wherein the modified LGIC comprises a sequence having at least 95% identity to SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:10.

88. The method of claim 87, wherein the modified LGIC comprises a sequence set forth in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:10.

89. The method of any one of claims 60, 62, 68, or 75, wherein the LBD is an alpha7 nicotinic acetylcholine receptor (α7-nAChR) LBD.

90. The method of claim 89, wherein the at least one modified amino acid in the α7-nAChR LBD comprises an amino acid substitution at least one amino acid residue selected from the group consisting of residues 77, 79, 115, 131, 139, 141, 175, 210, 216, 217, and 219 of the α7-nAChR LBD.

91. The method of claim 90, wherein the amino acid substitution is at residue 77 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of W77F and W77Y.

92. The method of claim 90, wherein the amino acid substitution is at residue 79 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of Q79A, Q79G, and Q79S.

93. The method of claim 90, wherein the amino acid substitution at residue 115 of the α7-nAChR LBD, and wherein the amino acid substitution is a Y115F substitution.

94. The method of claim 90, wherein the amino acid substitution is at residue 131 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of L131A, L131G, L131M, and L131N.

95. The method of claim 90, wherein the amino acid substitution at residue 139 of the α7-nAChR LBD, and wherein the amino acid substitution is a Q139G or a Q139L substitution.

96. The method of claim 90, wherein the amino acid substitution is at residue 175 of the α7-nAChR LBD, and wherein the amino acid substitution is selected from the group consisting of G175A, G175F, G175H, G175K, G175M, G175R, G175S, and G175V.

97. The method of claim 90, wherein the amino acid substitution at residue 210 of the α7-nAChR LBD, and wherein the amino acid substitution is a Y210F substitution.

98. The method of claim 90, wherein the amino acid substitution is at residue 216 of the α7-nAChR LBD, and wherein the amino acid substitution is P216I.

99. The method of claim 90, wherein the amino acid substitution is at residue 217 of the α7-nAChR LBD, and wherein the amino acid substitution is Y217F.

100. The method of claim 90, wherein the amino acid substitution is at residue 219 of the α7-nAChR LBD, and wherein the amino acid substitution is D219A.

101. The method of any one of claims 60, 62, 68, or 75, wherein the IPD comprises at least one modified amino acid.

102. The method of claim 101, wherein the IPD is selected from the group consisting of a serotonin 3 receptor (5HT3) IPD, a glycine receptor (GlyR) IPD, a GABA receptor IPD, and an alpha7 nicotinic acetylcholine receptor (α7-nAChR) IPD.

103. The method of claim 102, wherein the IPD is a GlyR IPD, and wherein the at least one modified amino acid comprises an amino acid substitution at residue 298 of the modified LGIC.

104. The method of claim 103, wherein the amino acid substitution at residue 298 of the modified LGIC is a A298G substitution.

105. The modified LGIC of claim 102, wherein the IPD is a GABA IPD, and wherein the at least one modified amino acid in the GABA IPD comprises an amino acid substitution at residue 298 of the modified LGIC.

106. The modified LGIC of claim 105, wherein the amino acid substitution at residue 298 of the chimeric LGIC is a W298A substitution.

107. The modified LGIC of any one of claims 60, 62, 68, or 75, wherein the exogenous LGIC ligand is a synthetic exogenous LGIC ligand.

108. The method of claim 107, wherein the synthetic exogenous LGIC ligand is selected from the group consisting of a tropane, a quinuclidine, a 9-azabicyclo[3.3.1]nonane, a 1,4-diazabicyclo[3.2.2]nonane, and a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine.

109. The method of claim 108, wherein the synthetic exogenous LGIC ligand is a tropane, and wherein the tropane is selected from the group consisting of tropisetron, pseudo-tropisetron, nortropisetron, compound 723, compound 725, compound 737, and compound 745.

110. The method of claim 108, wherein the synthetic exogenous LGIC ligand is a quinuclidine, and wherein the quinuclidine is selected from the group consisting of PNU-282987, PHA-543613, compound 0456, compound 0434, compound 0436, compound 0354, compound 0353, compound 0295, compound 0296, compound 0536, compound 0676, and compound 702.

111. The method of claim 108, wherein the synthetic exogenous LGIC ligand is a 9-azabicyclo[3.3.1]nonane, and wherein the 9-azabicyclo[3.3.1]nonane is selected from the group consisting of compound 0536, compound 0749, compound 0751, compound 0760, and compound 0763.

112. The method of claim 108, wherein the synthetic exogenous LGIC ligand is a 1,4-diazabicyclo[3.2.2]nonane, and wherein the 1,4-diazabicyclo[3.2.2]nonane is selected from the group of 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]thiophene 5,5-dioxide, compound 0773, and compound 0774.

113. The method of claim 108, wherein the synthetic exogenous LGIC ligand is a 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine, and wherein the 6,7,8,9-tetrahydro-6,10-methano-6H-pyrazino(2,3-h)benzazepine is selected from the group consisting of varenicline, compound 0765, and compound 0770.

114. A method for identifying a ligand that selectively binds to a modified ligand-gated ion channel (LGIC), said method comprising:

providing one or more candidate ligands to the modified LGIC of claim 1; and

detecting binding between the candidate ligand and the modified LGIC, thereby identifying a ligand that selectively binds the modified LGIC.

115. The method of claim 114, wherein the modified LGIC is a chimeric LGIC comprising a LBD from a first LGIC and an IPD from a second LGIC.

116. A method of detecting a modified ligand gated ion channel (LGIC) comprising at least one modified LGIC subunit, said method comprising:

providing one or more modified LGIC subunits of claim 1;

providing an agent that selectively binds the modified LGIC; and

detecting binding between the modified LGIC and the agent that selectively binds the modified LGIC, thereby detecting the modified LGIC.

117. The method of claim 116, wherein the agent that selectively binds the modified LGIC comprises an antibody, a protein, or a small molecule.

118. The method of claim 117, wherein the agent that selectively binds the modified LGIC comprises a detectable label.

119. The method of claim 118, wherein the detectable label comprises a label selected from the group consisting of a fluorescent label, a radioactive label, and a positron emitting label.

120. A mammalian cell comprising the modified LGIC of claim 1.

121. A nucleic acid expression the modified LGIC subunit of claim 1.

122. A homomeric chimeric ligand gated ion channel (LGIC) comprising chimeric LGIC subunits, each chimeric LGIC subunit comprising:

an alpha7 nicotinic acetylcholine receptor ligand binding domain having a Q79G amino acid substitution, and having at least one of a W77F amino acid substitution, Q139G amino acid substitution, a Y115F amino acid substitution, a G175K amino acid substitution, a Y210F amino acid substitution, a P216I amino acid substitution, a R27D amino acid substitution, and a E41R amino acid substitution; and

a glycine receptor ion pore domain;

wherein a ligand selected from the group consisting of tropisetron and granisetron selectively binds the chimeric LGIC, and wherein the chimeric LGIC minimally binds acetylcholine (ACh).

123. A homomeric chimeric ligand gated ion channel (LGIC) comprising chimeric LGIC subunits, each chimeric LGIC subunit comprising:

an alpha7 nicotinic acetylcholine receptor ligand binding domain having a L131G amino acid substitution, and having at least one of a Q79S amino acid substitution, a Q139L amino acid substitution, a Y217F amino acid substitution, a R27D amino acid substitution, and a E41R amino acid substitution; and

an ion pore domain selected from the group consisting of a glycine receptor ion pore domain and a serotonin 3 receptor ion pore domain;

wherein a ligand selected from the group consisting of varenicline and tropisetron selectively binds the chimeric LGIC, and wherein the chimeric LGIC minimally binds acetylcholine (ACh).

124. A method of treating a channelopathy in a mammal, said method comprising:

administering to a cell in the mammal the LGIC of claim 122 or claim 123; and

administering the ligand to the mammal.

125. A method of modulating the excitability of a cell in a mammal, said method comprising:

administering to a cell in the mammal the LGIC of claim 122 or claim 123; and

administering the ligand to the mammal.

126. A method of modulating the activity of a cell in a mammal, said method comprising:

administering to a cell in the mammal the LGIC of claim 122 or claim 123; and

administering the ligand to the mammal.


Signal Peptide 1-22
MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQP
alpha7 nAChR LBD
50
LTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV
alpha7 nAChR LBD
100
RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC
alpha7 nAChR LBD
150
YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVG
alpha7 nAChR LBD
200
IPGKRSERFYECCKEPYPDVTFTVIIRRRPLFYAVSLLLPSIFLMVVDIV
alpha7 nAchR LBD
5HT3a IPD
250
GFCLPPDSGERVSFKITLLLGYSVFLIIVSDTLPATIGTPLIGVYFVVCM
5HT3a IPD
300
ALLVISLAETIFIVRLVHKQDLQRPVPDWLRHLVLDRIAWILCLGEQPMA
5HT3a IPD
350
HRPPATEQANKTDDCSGSDLLPAMGNHCSHVGGPQDLEKTPRGRGSPLPP
5HT3a IPD
400
PREASLAVRGLLQELSSIRHFLEKRDEMREVARDWLRVGYVLDRLLFRIY
5HT3a IPD
450
LLAVLAYSITLVTLWSIWHYS.
5HT3a IPD

FIG. 1A

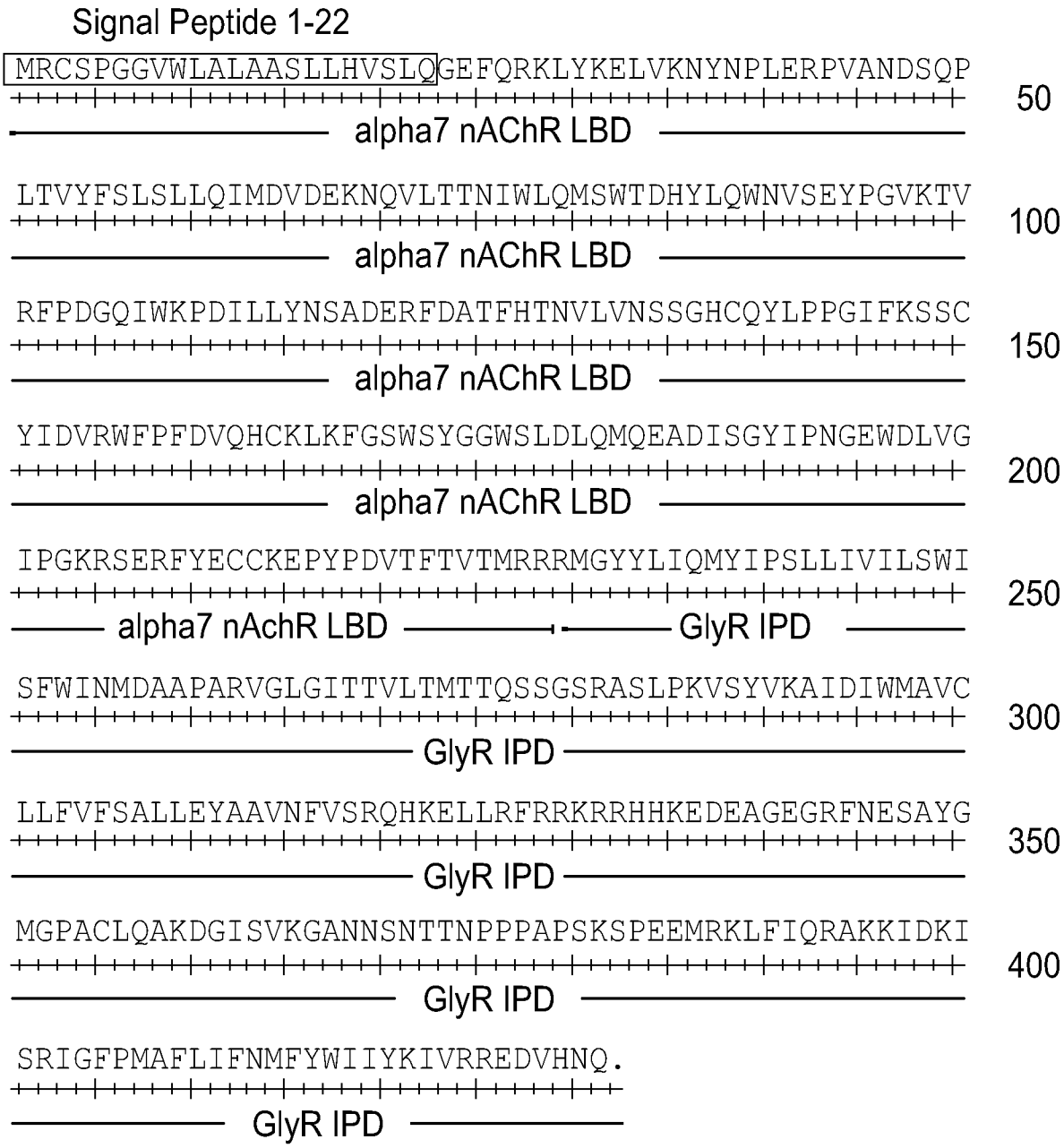

Signal Peptide 1-22
MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQP
alpha7 nAChR LBD
50
LTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV
alpha7 nAChR LBD
100
RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC
alpha7 nAChR LBD
150
YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVG
alpha7 nAChR LBD
200
IPGKRSERFYECCKEPYPDVTFTVTMRRRMGYYLIQMYIPSLLIVILSWI
alpha7 nAchR LBD
GlyR IPD
250
SFWINMDAAPARVGLGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVC
GlyR IPD
300
LLFVFSALLEYAAVNFVSRQHKELLRFRRKRRHHKEDEAGEGRFNESAYG
GlyR IPD
350
MGPACLQAKDGISVKGANNSNTTNPPPAPSKSPEEMRKLFIQRAKKIDKI
GlyR IPD
400
SRIGFPMAFLIFNMFYWIIYKIVRREDVHNQ.
GlyR IPD

FIG. 1B

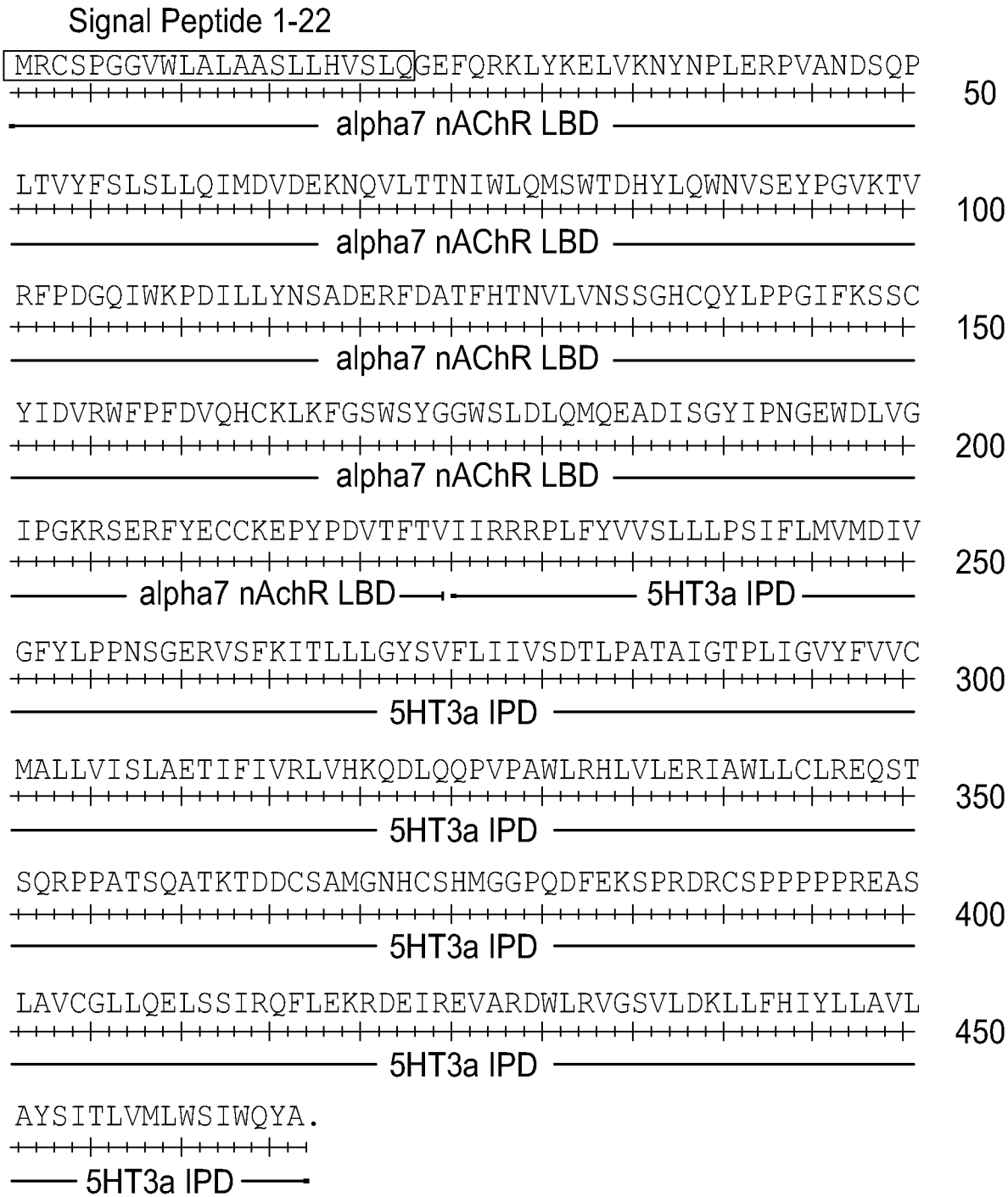

Signal Peptide 1-22
MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQP
50
alpha7 nAChR LBD
LTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV
100
alpha7 nAChR LBD
RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC
150
alpha7 nAChR LBD
YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVG
200
alpha7 nAChR LBD
IPGKRSERFYECCKEPYPDVTFTVIIRRRPLFYVVSLLLPSIFLMVMDIV
250
alpha7 nAchR LBD
5HT3a IPD
GFYLPPNSGERVSFKITLLLGYSVFLIIVSDTLPATAIGTPLIGVYFVVC
300
5HT3a IPD
MALLVISLAETIFIVRLVHKQDLQQPVPAWLRHLVLERIAWLLCLREQST
350
5HT3a IPD
SQRPPATSQATKTDDCSAMGNHCSHMGGPQDFEKSPRDRCSPPPPPREAS
400
5HT3a IPD
LAVCGLLQELSSIRQFLEKRDEIREVARDWLRVGSVLDKLLFHIYLLAVL
450
5HT3a IPD
AYSITLVMLWSIWQYA.
5HT3a IPD

FIG. 1C

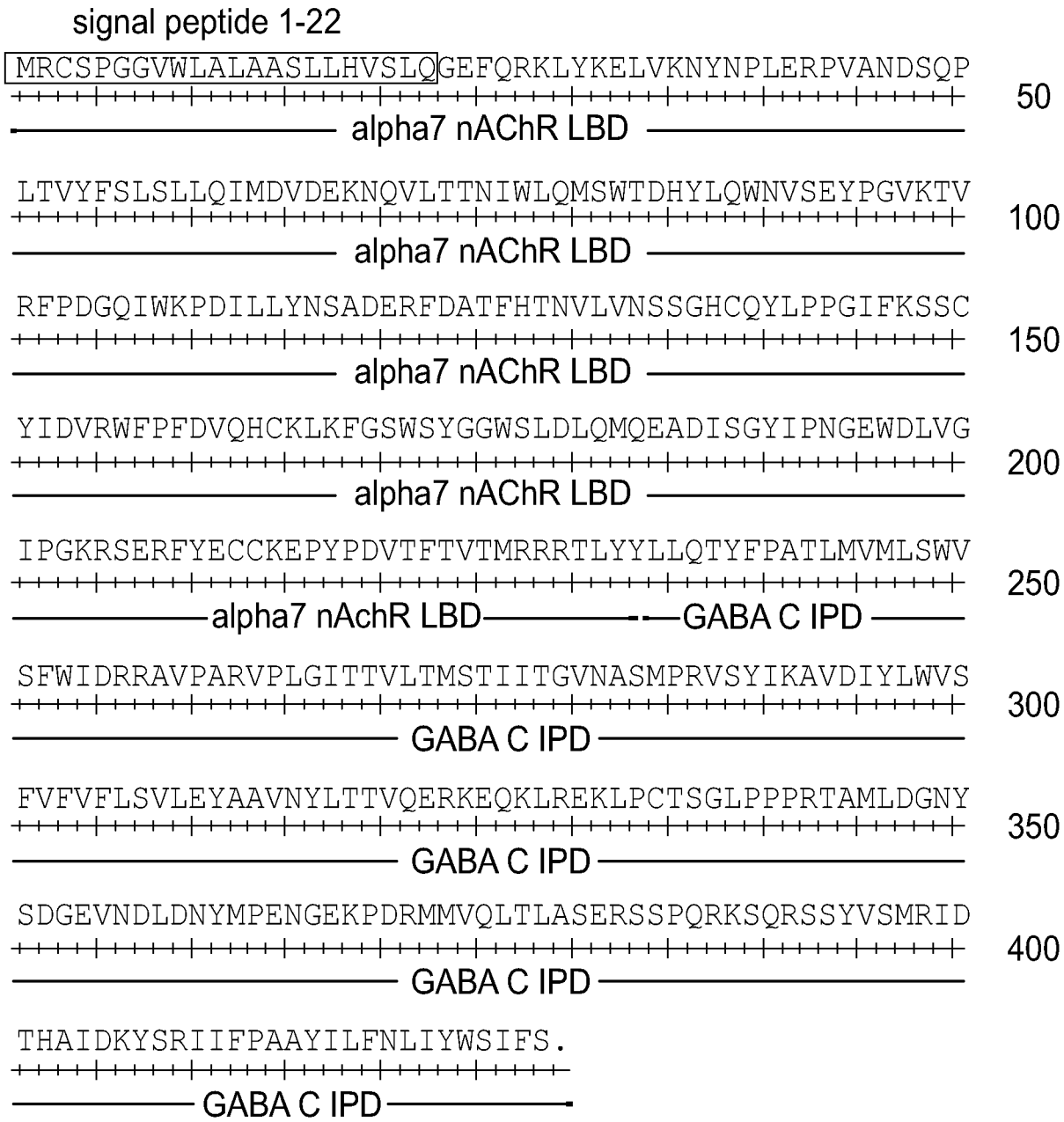

signal peptide 1-22
MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKNYNPLERPVANDSQP
alpha7 nAChR LBD
50
LTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTV
alpha7 nAChR LBD
100
RFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSC
alpha7 nAChR LBD
150
YIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVG
alpha7 nAChR LBD
200
IPGKRSERFYECCKEPYPDVTFTVTMRRRTLYYLLQTYFPATLMVMLSWV
alpha7 nAchR LBD
GABA C IPD
250
SFWIDRRAVPARVPLGITTVLTMSTIITGVNASMPRVSYIKAVDIYLWVS
GABA C IPD
300
FVFVFLSVLEYAAVNYLTTVQERKEQKLREKLPCTSGLPPPRTAMLDGNY
GABA C IPD
350
SDGEVNDLDNYMPENGEKPDRMMVQLTLASERSSPQRKSQRSSYVSMRID
GABA C IPD
400
THAIDKYSRIIFPAAYILFNLIYWSIFS.
GABA C IPD

FIG. 1D

signal peptide 1-22

MGGGRGGIWLALAAALLHVSLQGEFQRRLYKELVKNYNPLERPVANDSQP 50

LTVYFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNMSEYPGVKNV 100

RFPDGQIWKPDILLYNSADERFDATFHTNVGVNASGHCQYLPPGIFKSSC 150

YIDVRWFPFDVQQCKLKFGSWSYGGWSLDLQMQEADISSYIPNGEWDLMG 200

IPGKRNEKFYECCKEPYPDVTYTVTMRRRTLYYGLNLLIPCVLISALALL 250

VFLLPADSGEKISLGITVLLSLTTFMLLVAEIMPATSDSVPLIAQYFAST 300

MIIVGLSVVVTVIVLRYHHHDPDGGKMPKWTRIILLNWCAWFLRMKRPGE 350

DKVRPACQHKPRRCSLASVELSAGAGPPTSNGNLLYIGFRGLEGMHCAPT 400

PDSGVVCGRLACSPTHDEHLMHGAHPSDGDPDLAKILEEVRYIANRNRCQ 450

DESEVICSEWKFAACVVDPLCLMAFSVFTIICTIGILMSAPNFVEAVSKD 500

FA.

FIG. 1E

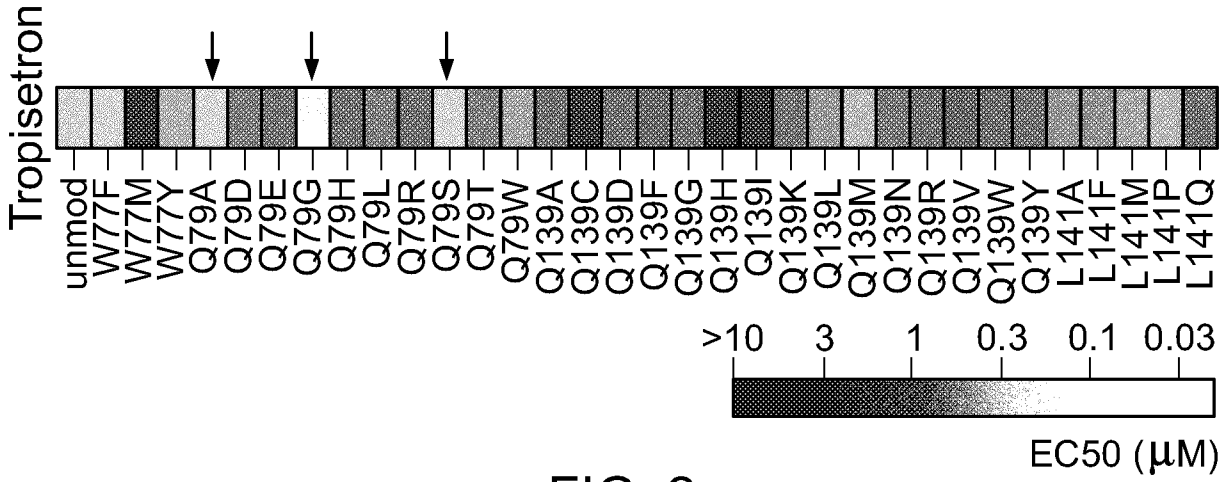

Tropisetron
unmod
W77F
W77M
W77Y
Q79A
Q79D
Q79E
Q79G
Q79H
Q79L
Q79R
Q79S
Q79T
Q79W
Q139A
Q139C
Q139D
Q139F
Q139G
Q139H
Q139I
Q139K
Q139L
Q139M
Q139N
Q139R
Q139V
Q139W
Q139Y
L141A
L141F
L141M
L141P
L141Q
>10
3
1
0.3
0.1
0.03
EC50 (μM)

FIG. 2

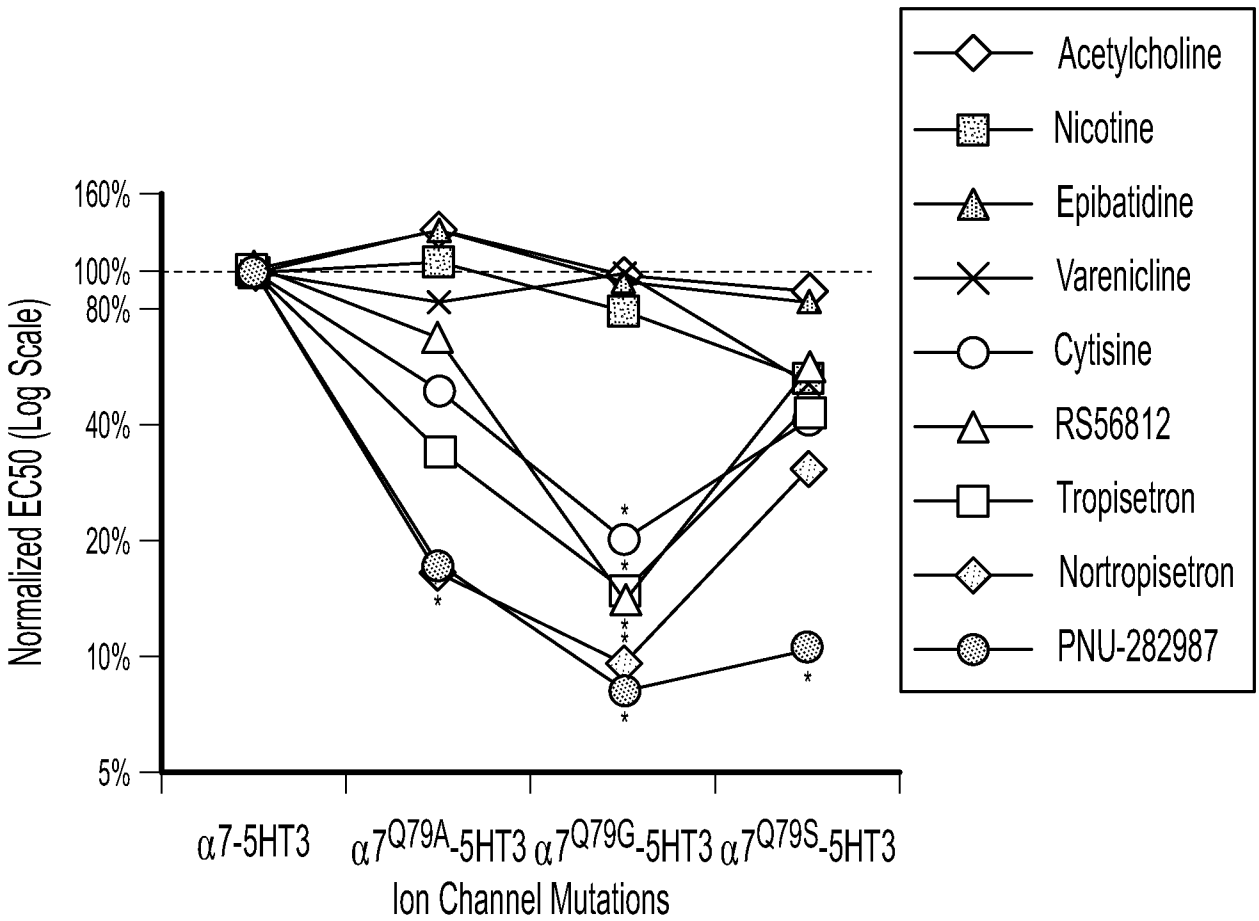

Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
RS56812
Tropisetron
Nortropisetron
PNU-282987
Normalized EC50 (Log Scale)
160%
100%
80%
40%
20%
10%
5%
α7-5HT3
α7Q79A-5HT3
α7Q79G-5HT3
α7Q79S-5HT3
Ion Channel Mutations

FIG. 3A

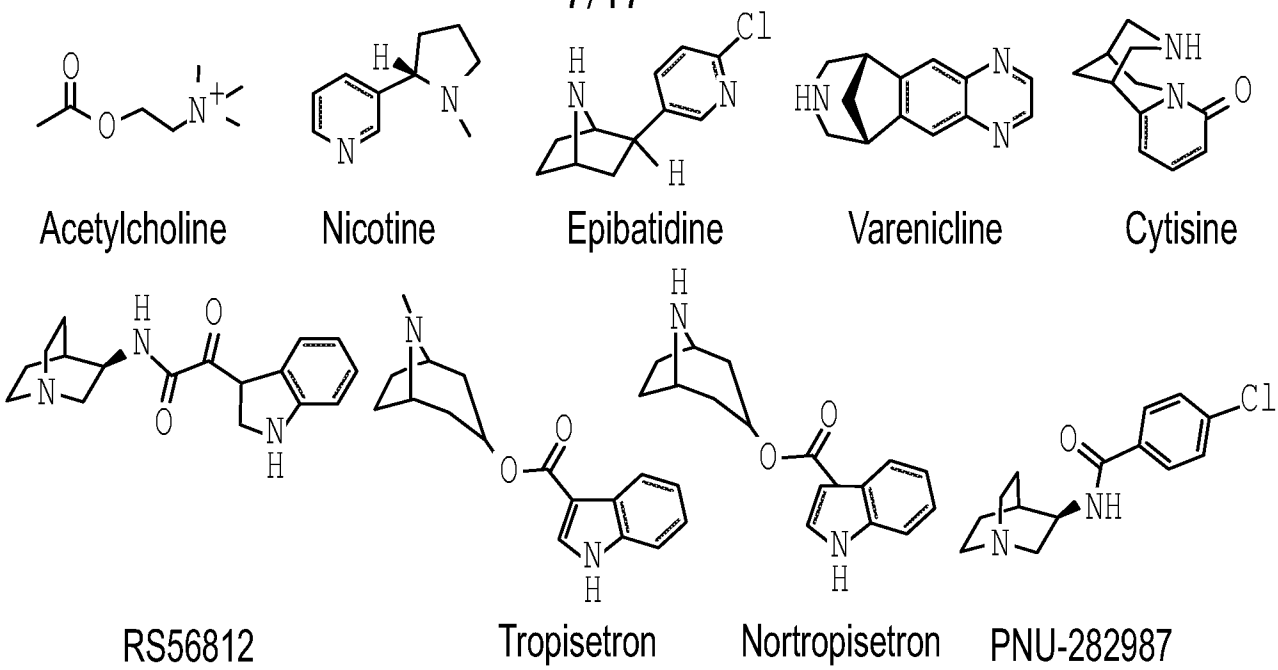

Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
RS56812
Tropisetron
Nortropisetron
PNU-282987

FIG. 3B

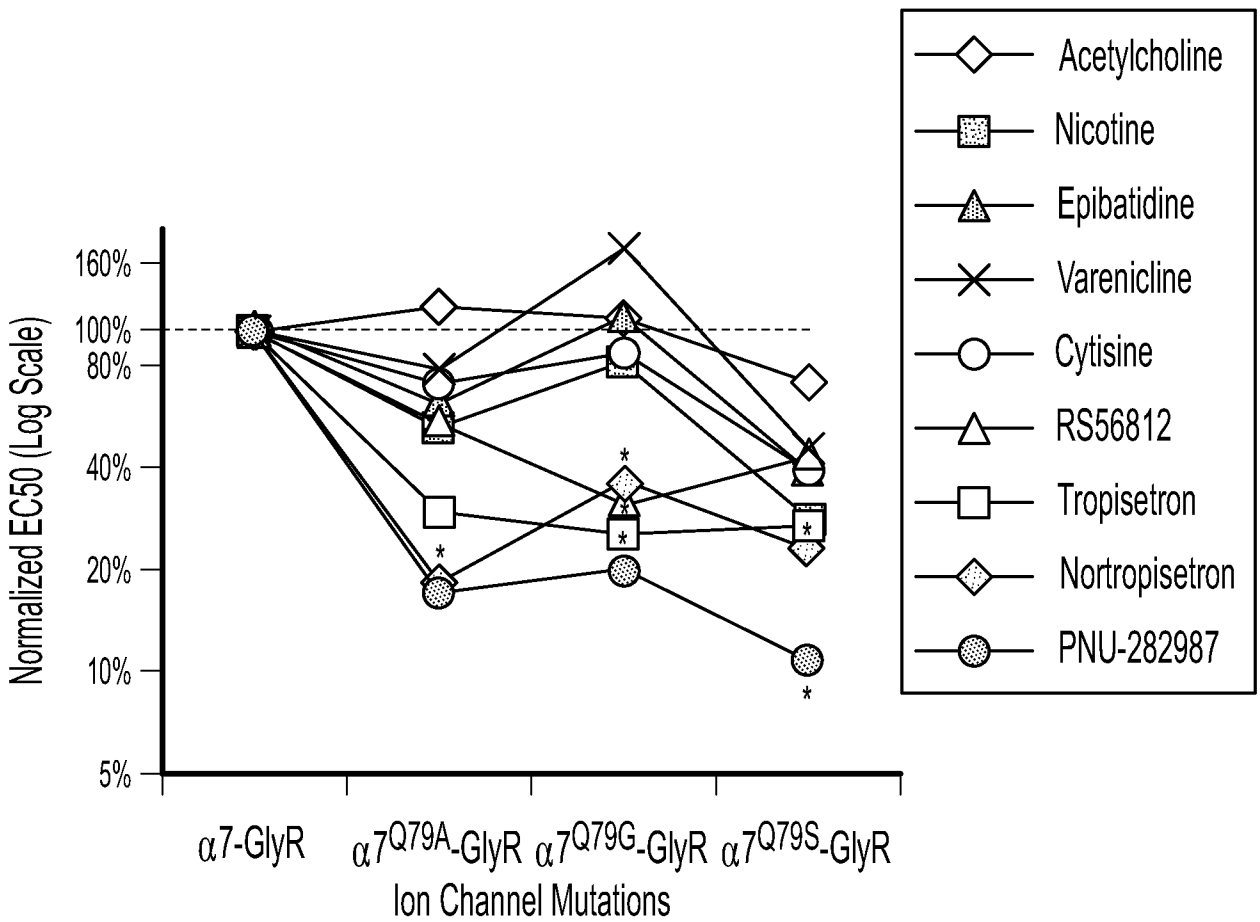

Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
RS56812
Tropisetron
Nortropisetron
PNU-282987
Normalized EC50 (Log Scale)
160%
100%
80%
40%
20%
10%
5%
α7-GlyR
α7Q79A-GlyR
α7Q79G-GlyR
α7Q79S-GlyR
Ion Channel Mutations

FIG. 4A

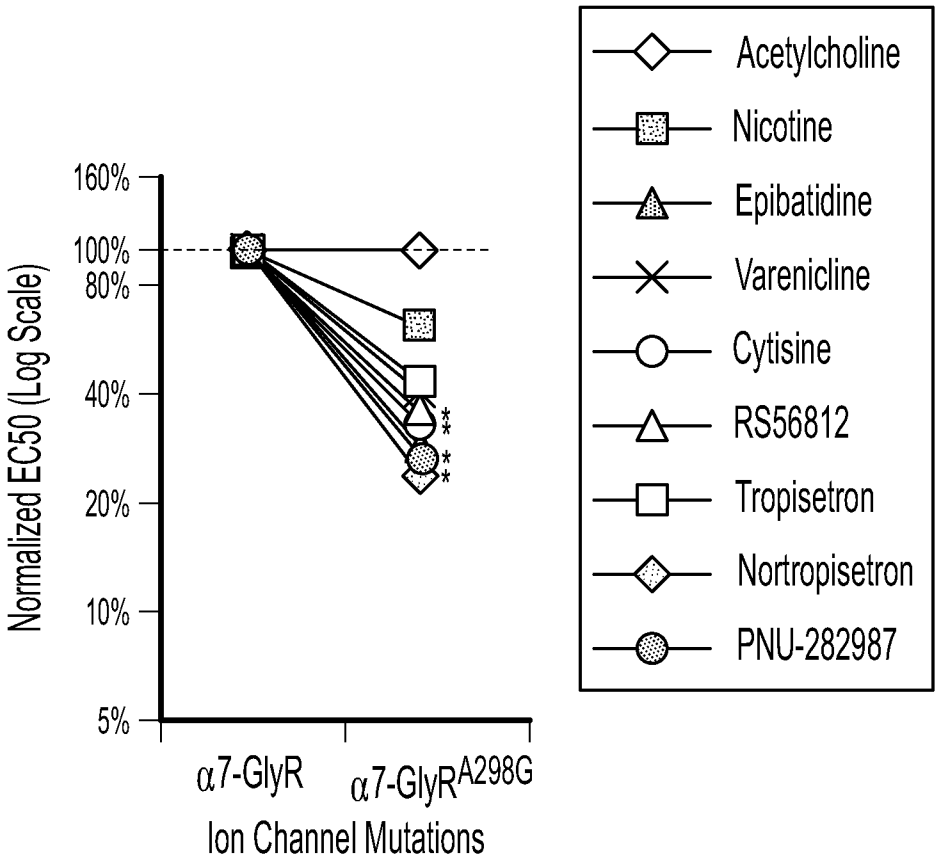

Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
RS56812
Tropisetron
Nortropisetron
PNU-282987
160%
100%
80%
40%
20%
10%
5%
Normalized EC50 (Log Scale)
α7-GlyR
α7-GlyR^A298G
Ion Channel Mutations

FIG. 4B

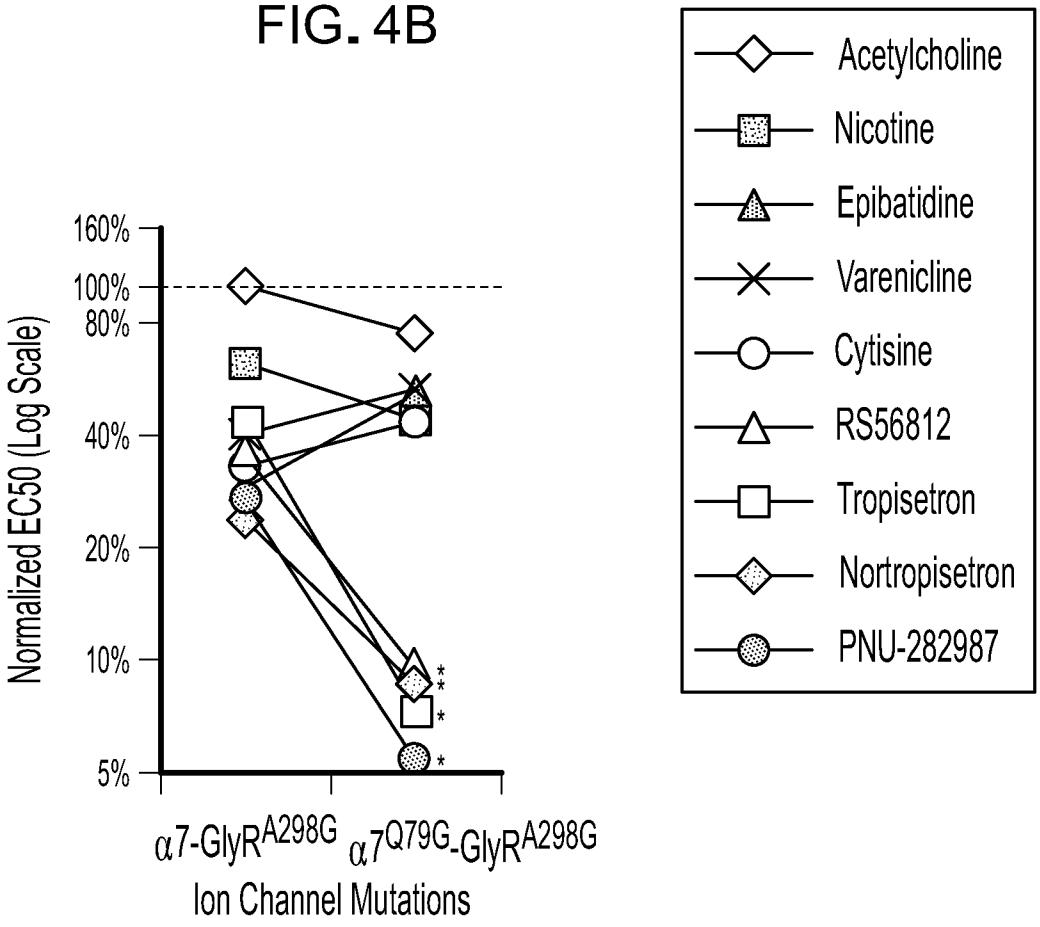
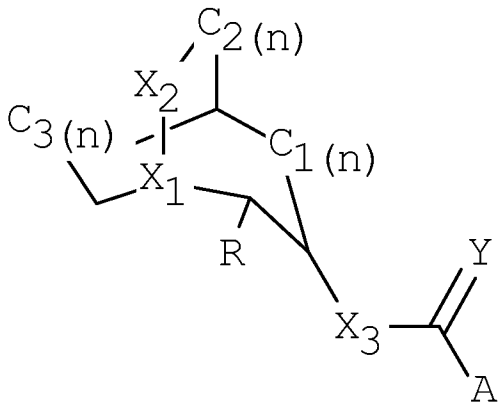
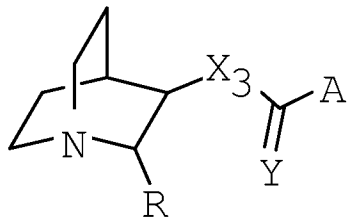

Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
RS56812
Tropisetron
Nortropisetron
PNU-282987
160%
100%
80%
40%
20%
10%
5%
Normalized EC50 (Log Scale)
α7-GlyR^A298G
α7^Q79G-GlyR^A298G
Ion Channel Mutations

FIG. 4C



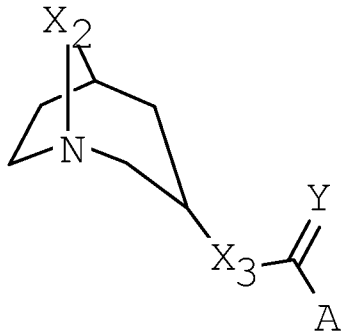
X = CH,CH_2, O, NH, NMe
n = 0 or 1
Y = O, S
A = Aromatic
R = Pyridinylmethylene

FIG. 5A



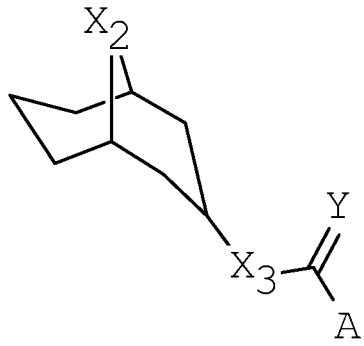
Pharmacophore: Quinuclidine

$C_{1(n)}$ = 0
$C_{2(n)}$ = 1
$C_{3(n)}$ = 0
X_1 = N
X_2 = CH_2
X_3 = O, NH, CH_2
Y = O,S
A = Aromatic
R = Pyridinylmethylene



Pharmacophore: Tropane

$C_{1(n)}$ = 1
$C_{2(n)}$ = 0
$C_{3(n)}$ = 0
X_1 = CH
X_2 = NH, NMe
X_3 = O, NH, CH_2
Y = O,S
A = Aromatic



Pharmacophore: 9-azabicyclo[3.3.1] nonane

$C_{1(n)}$ = 1
$C_{2(n)}$ = 0
$C_{3(n)}$ = 1
X_1 = CH
X_2 = NH, NMe
X_3 = O, NH, CH_2
Y = O,S
A = Aromatic

FIG. 5B

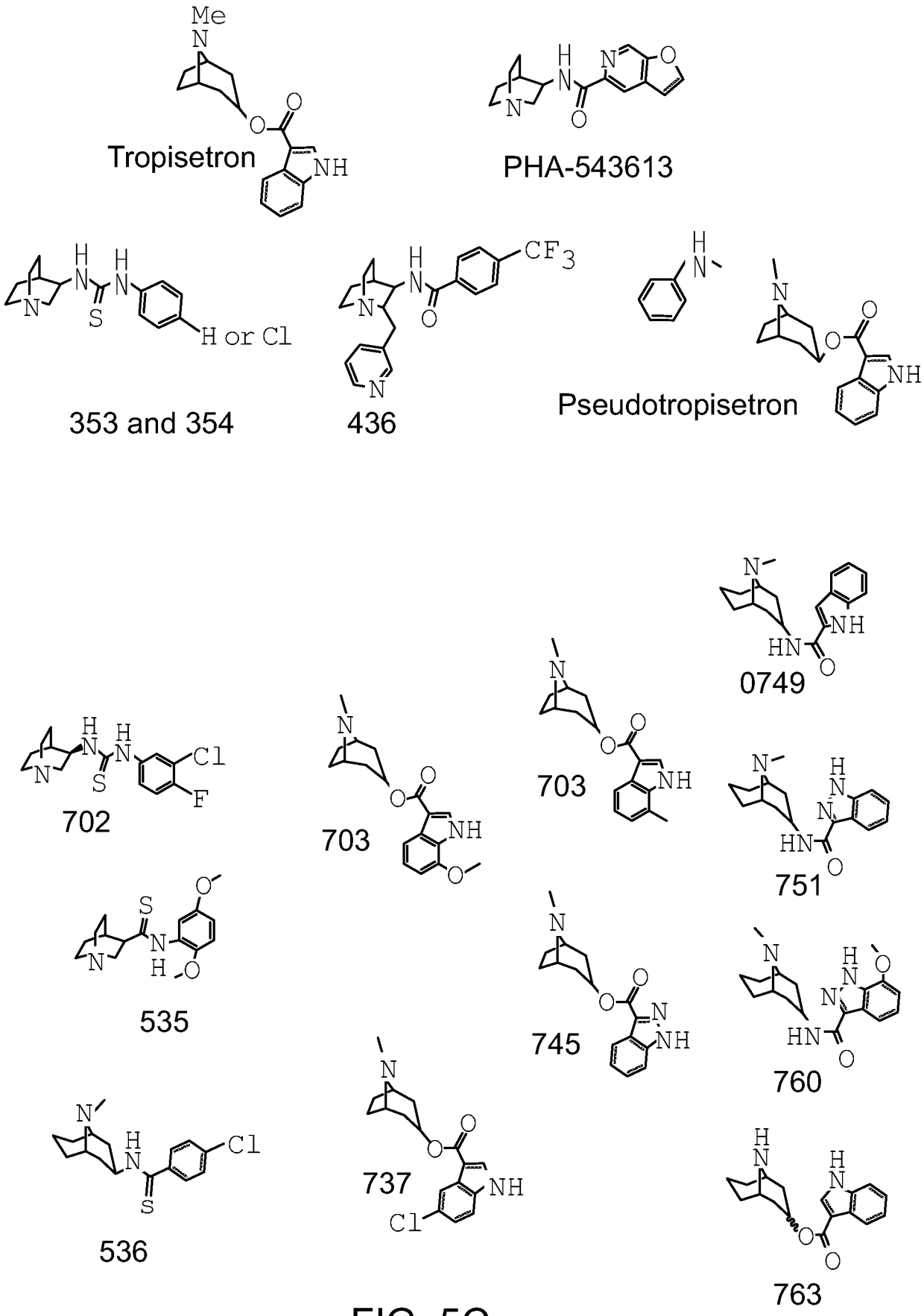

Tropisetron
PHA-543613
H or Cl
353 and 354
436
Pseudotropisetron
0749
702
703
703
751
535
745
760
536
737
763

FIG. 5C

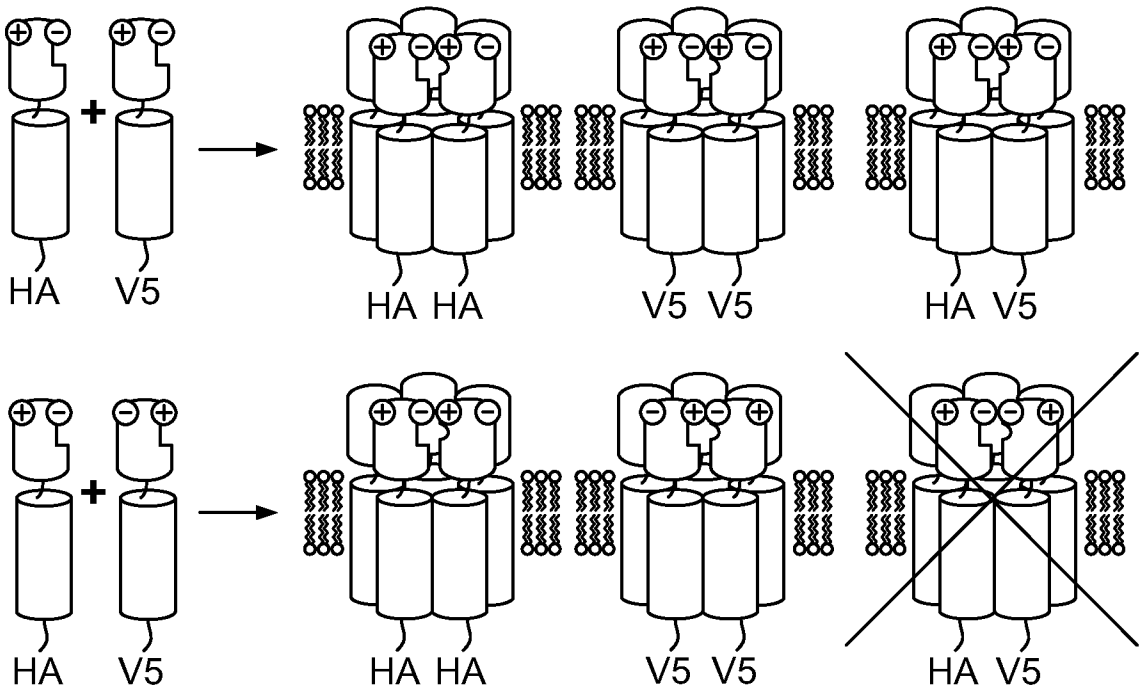

HA
V5
HA HA
V5 V5
HA V5
HA
V5
HA HA
V5 V5
HA V5

FIG. 6A

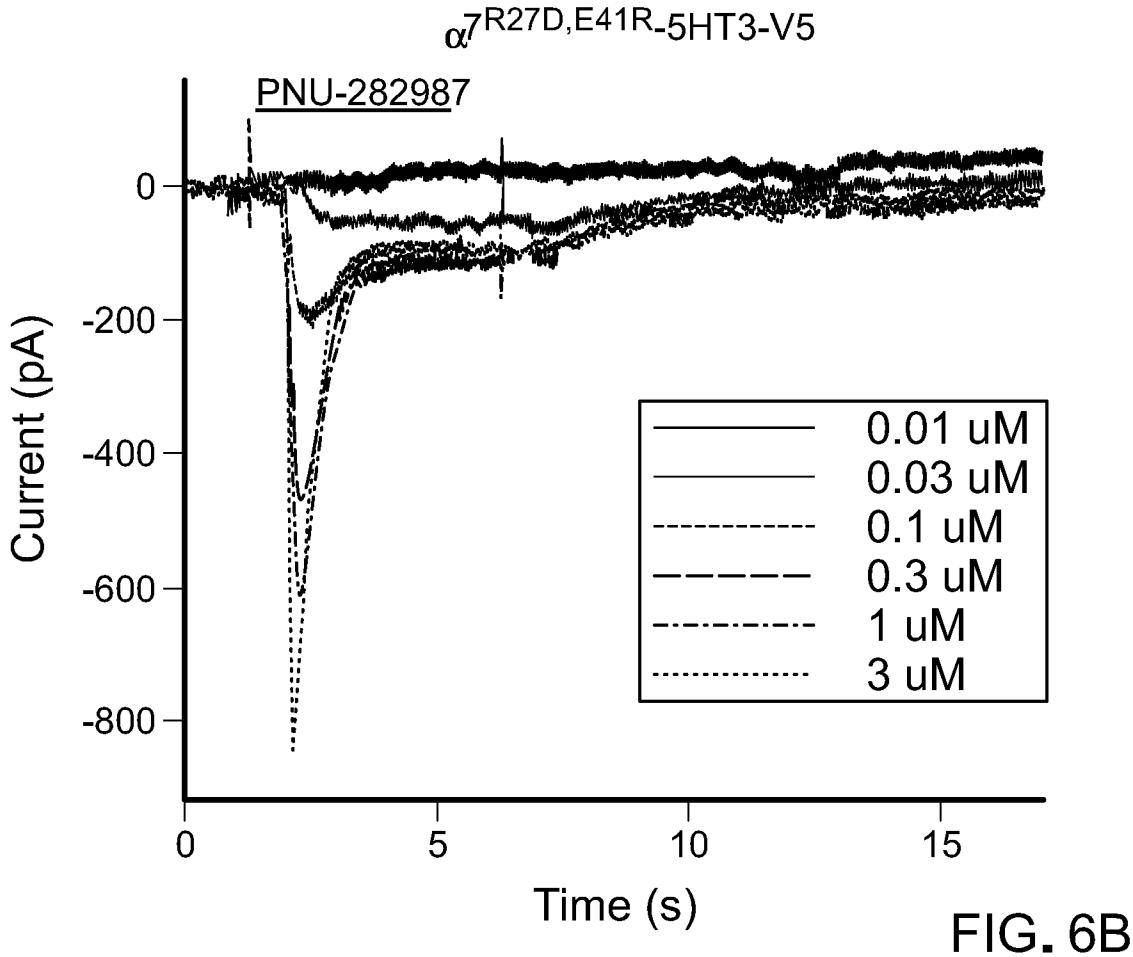

α7R27D,E41R-5HT3-V5
PNU-282987
0
-200
-400
-600
-800
Current (pA)
0
5
10
15
Time (s)
0.01 uM
0.03 uM
0.1 uM
0.3 uM
1 uM
3 uM

FIG. 6B

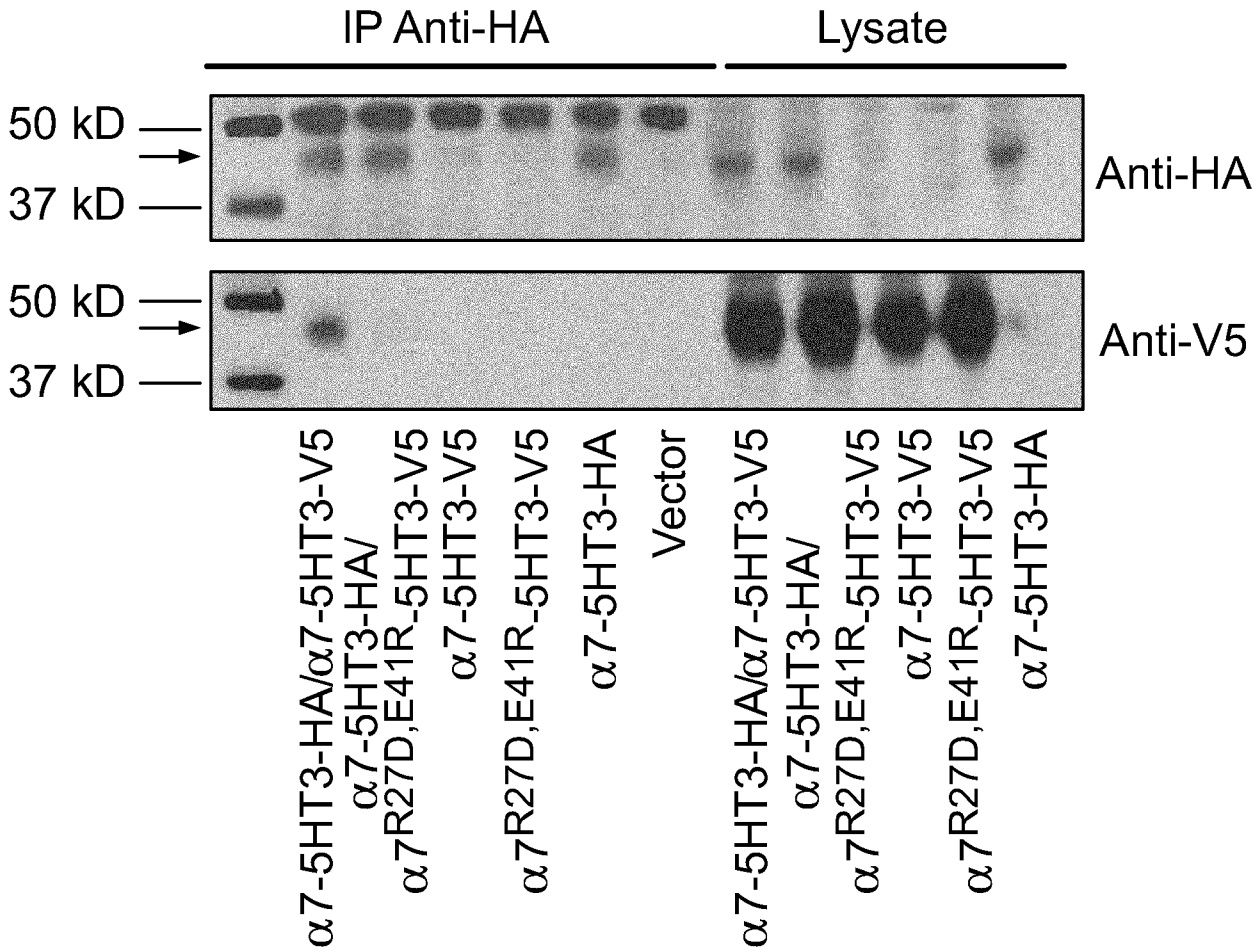

IP Anti-HA
Lysate
50 kD
37 kD
Anti-HA
50 kD
37 kD
Anti-V5
α7-5HT3-HA/α7-5HT3-V5
α7-5HT3-HA/ α7R27D,E41R_5HT3-V5
α7-5HT3-V5
α7R27D,E41R_5HT3-V5
α7-5HT3-HA
Vector
α7-5HT3-HA/α7-5HT3-V5
α7-5HT3-HA/ α7R27D,E41R_5HT3-V5
α7-5HT3-V5
α7R27D,E41R_5HT3-V5
α7-5HT3-HA

FIG. 6C

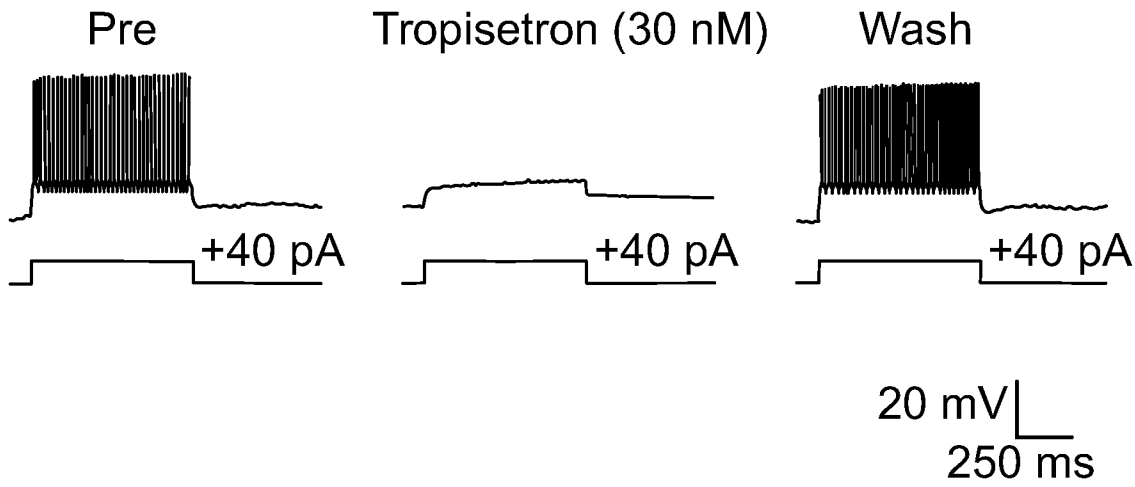

Pre
Tropisetron (30 nM)
Wash
+40 pA
+40 pA
+40 pA
20 mV
250 ms

FIG. 7

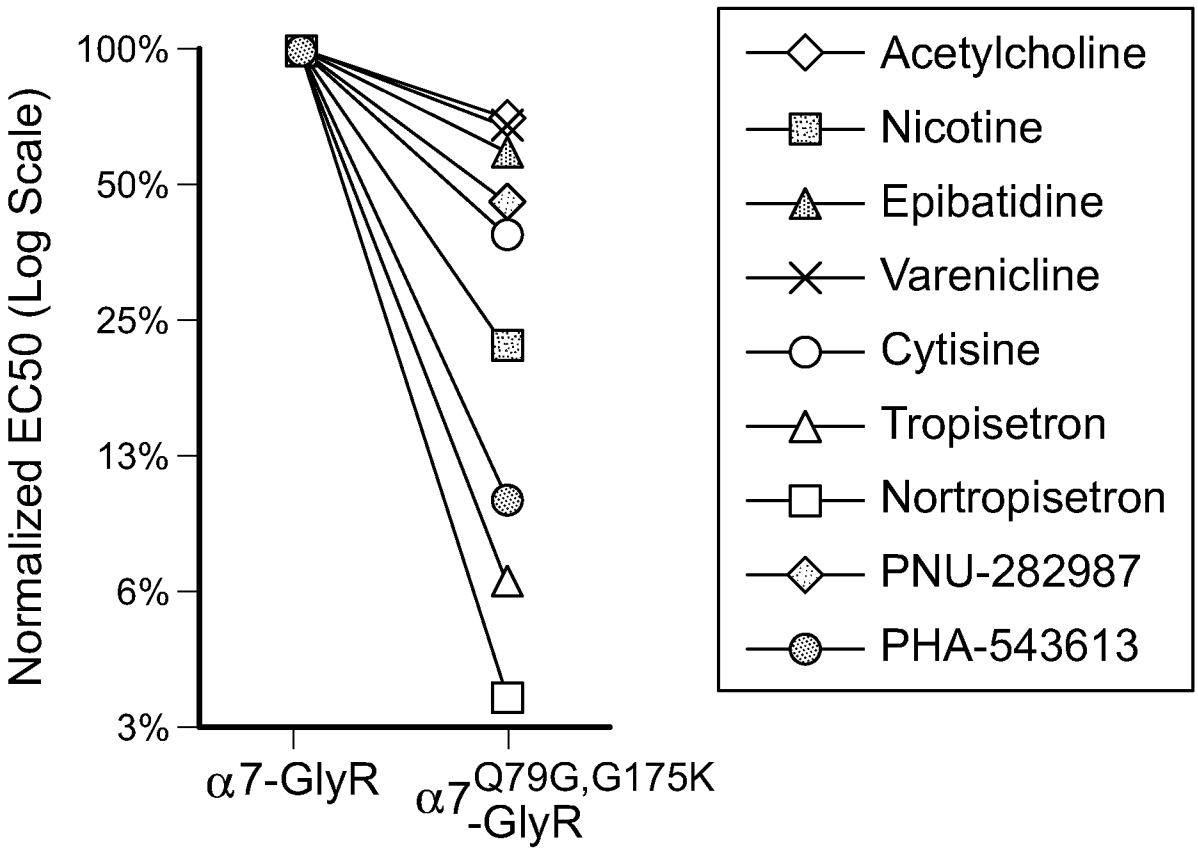

Normalized EC50 (Log Scale)
100%
50%
25%
13%
6%
3%
α7-GlyR
α7Q79G,G175K-GlyR
Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
Tropisetron
Nortropisetron
PNU-282987
PHA-543613

FIG. 8A

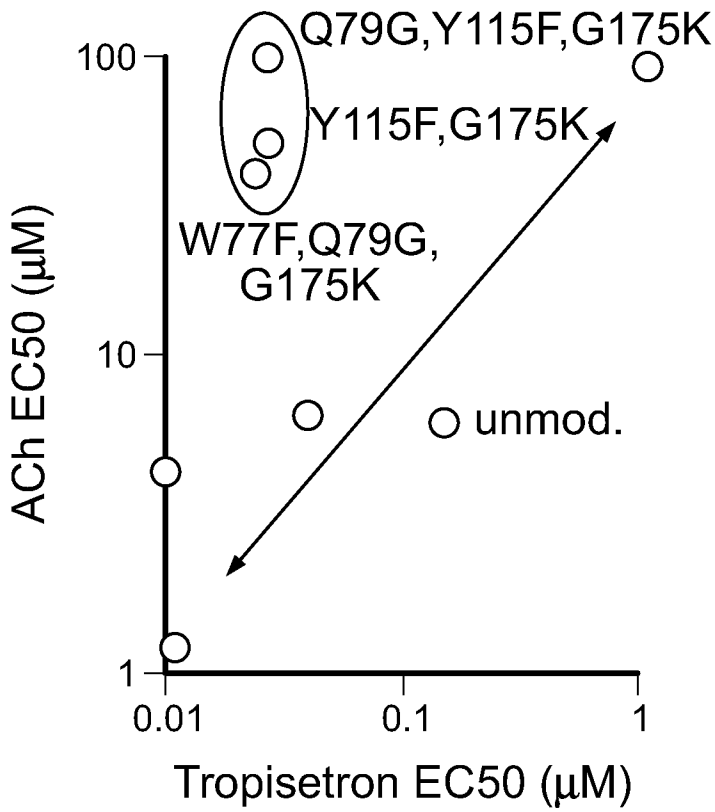

Q79G,Y115F,G175K
Y115F,G175K
W77F,Q79G,
G175K
unmod.
100
10
1
0.01
0.1
1
ACh EC50 (μM)
Tropisetron EC50 (μM)

FIG. 8B

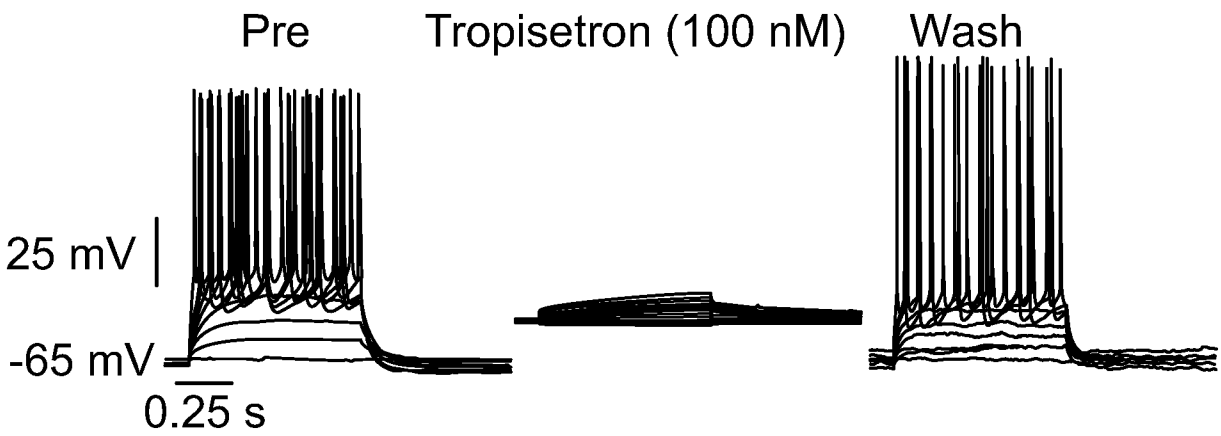

Pre
Tropisetron (100 nM)
Wash
25 mV
-65 mV
0.25 s

FIG. 8C

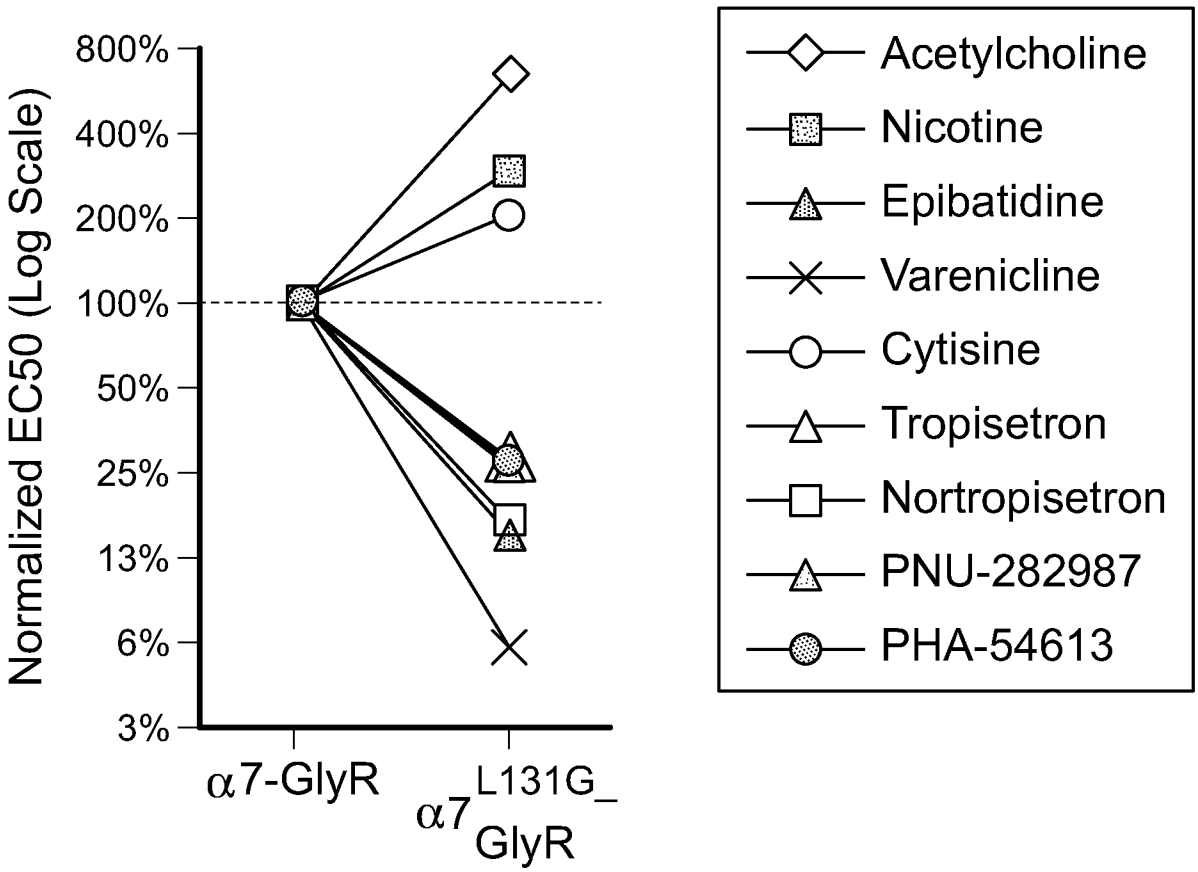

Normalized EC50 (Log Scale)
800%
400%
200%
100%
50%
25%
13%
6%
3%
α7-GlyR
α7 L131G_ GlyR
Acetylcholine
Nicotine
Epibatidine
Varenicline
Cytisine
Tropisetron
Nortropisetron
PNU-282987
PHA-54613

FIG. 9A

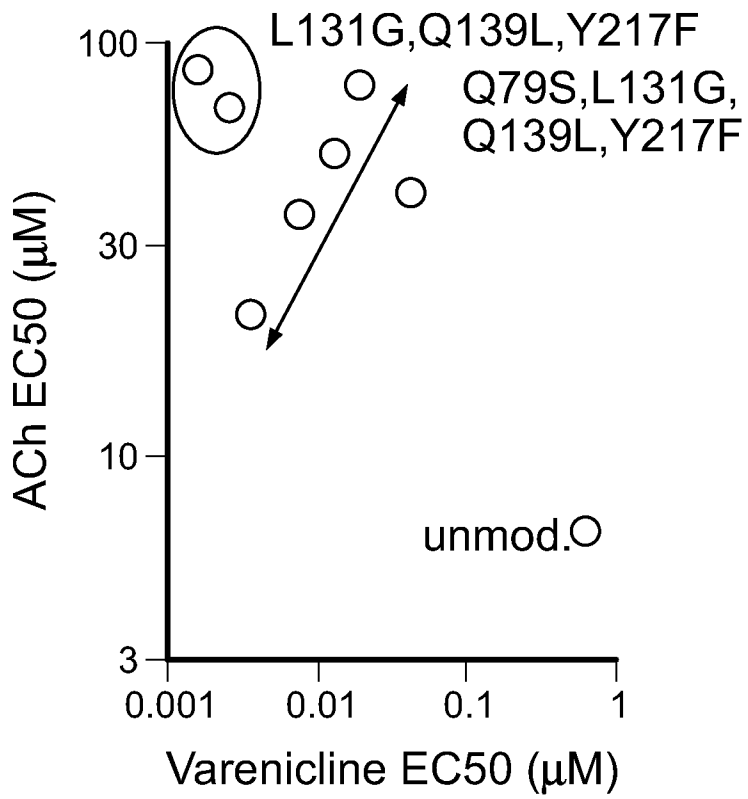

ACh EC50 (μM)
100
30
10
3
0.001
0.01
0.1
1
Varenicline EC50 (μM)
L131G,Q139L,Y217F
Q79S,L131G,
Q139L,Y217F
unmod.

FIG. 9B

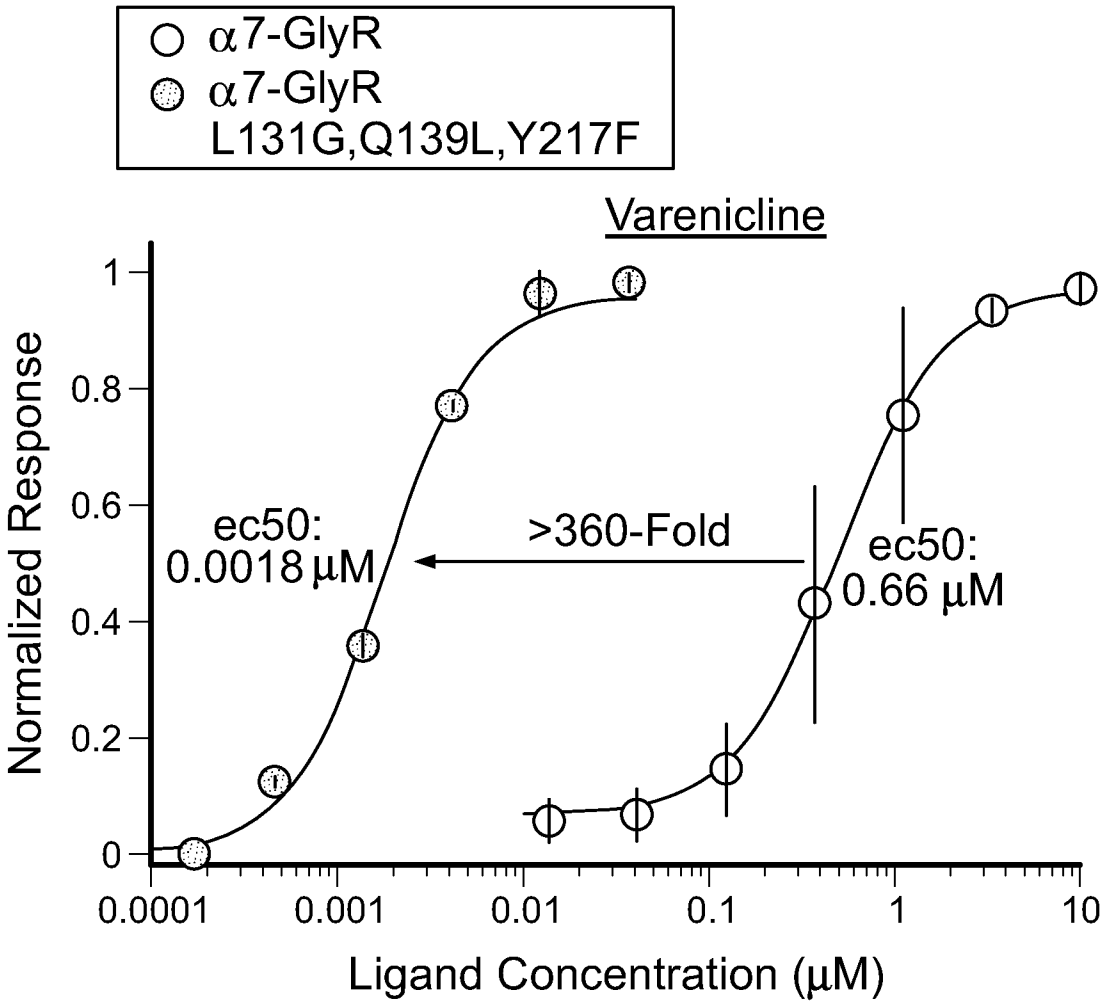

α7-GlyR
α7-GlyR
L131G,Q139L,Y217F
Varenicline
Normalized Response
1
0.8
0.6
0.4
0.2
0
ec50:
0.0018 μM
>360-Fold
ec50:
0.66 μM
0.0001
0.001
0.01
0.1
1
10
Ligand Concentration (μM)

FIG. 9C

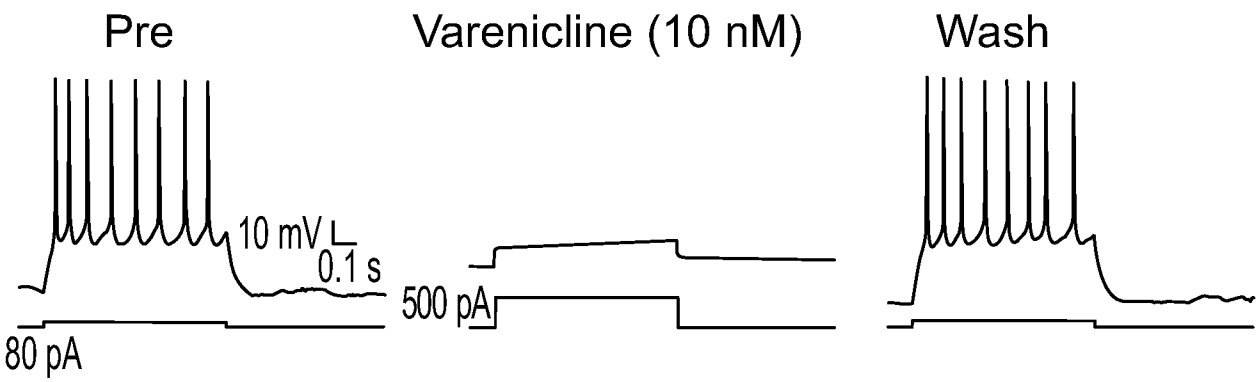
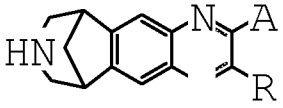
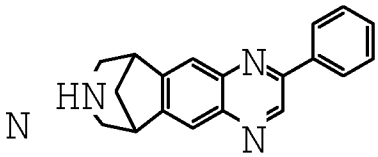

Pre
Varenicline (10 nM)
Wash
10 mV
0.1 s
500 pA
80 pA

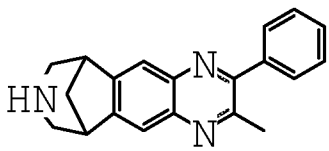
FIG. 9D



R = H, CH3
A = aromatic

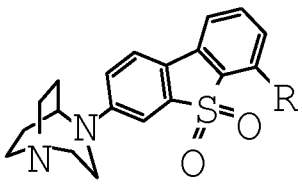


765

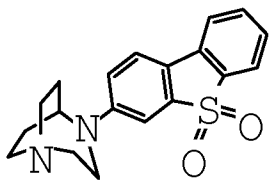


770

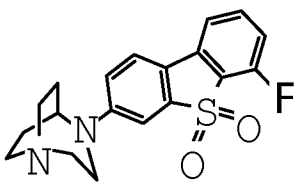
FIG. 10A



R = H, F, NO2



3-(1,4-diazabicyclo[3.2.2] nonan-4-yl)dibenzo[b,d] thiophene 5,5-dioxide



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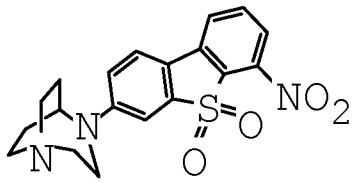

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FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/041147

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/041147

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 60-113, 124-126
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 60-113, 124-126 pertain to a method for treating a human body, and thus relate to a subject matter not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/041147

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/705(2006.01)i, G01N 33/58(2006.01)i, G01N 33/68(2006.01)i, C12N 5/071(2010.01)i, A61K 48/00(2006.01)i, A61K 38/00(2006.01)n

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 14/705; C12N 5/02; C07D 498/02; A61K 38/17; A61K 31/4745; G01N 33/58; G01N 33/68; C12N 5/071; A61K 48/00; A61K 38/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(Kipo internal), STN (Registry, Caplus) & Keywords: ligand gated ion channel, ion pore domain, ligand binding domain, nicotinic acetylcholine receptor, mutation, varenicline, quinuclidine, tropisetron

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010-0130420 A1 (STERNSON, S. et al.) 27 May 2010 See abstract; paragraphs [0003]-[0016], [0030]-[0038], [0046], [0099]-[0110]; claims 1-62; figures 1a-1d, 2; and table 2.	1-7,9,11,21,25,27,31-33,36-39,42-49,114-121
Y		26,28-30,122
A		8,10,12-20,22-24,34,35,40,41,50-59,123
X	CAPPELLI, A. et al., "The interactions of the 5-HT3 receptor with aryl piperazine, tropane, and quinuclidine ligands", Current Topics in Medicinal Chemistry, 2002, Vol. 2, No. 6, pp. 599-624 See abstract; page 599; and chart 1.	45,46,50-55
Y		26,28,122
X	PRICE, K. L. et al., "Varenicline interactions at the 5-HT3 receptor ligand binding site are revealed by 5-HTBP", ACS Chemical Neuroscience, 2015, Vol. 6, No. 7, pp. 1151-1157 See abstract; and page 1151.	56,57
Y		29
X	US 2005-0250808 A1 (XIE, W. et al.) 10 November 2005 See paragraphs [0003], [0004]; and claim 34.	45,46,50-55

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2017 (18.10.2017)	**18 October 2017 (18.10.2017)**

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y		28
X	GAO, Y. et al., "Derivatives of dibenzothiophene for positron emission tomography imaging of α7-nicotinic acetylcholine receptors", Journal of Medicinal Chemistry, 2013, Vol. 56, No. 19, pp. 7574-7589 See abstract; and page 7574.	58,59
Y		30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/041147

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010-0130420 A1	27/05/2010	AU 2009-302217 A1	15/04/2010
		CA 2740125 A1	15/04/2010
		EP 2344636 A2	20/07/2011
		EP 2352490 A1	10/08/2011
		JP 2012-504966 A	01/03/2012
		JP 2012-505243 A	01/03/2012
		JP 5775819 B2	09/09/2015
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		US 9173840 B2	03/11/2015
		WO 2010-042799 A2	15/04/2010
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		CA 2567977 A1	05/01/2006
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		EP 1742944 B1	10/11/2010
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		JP 2011-241227 A	01/12/2011
		US 2009-0118232 A1	07/05/2009
		US 2011-0178075 A1	21/07/2011
		US 7488737 B2	10/02/2009
		US 7902217 B2	08/03/2011
		WO 2006-001894 A1	05/01/2006