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(54) Titre : COMPOSITIONS PHARMACEUTIQUES COMPRENANT DES AGENTS THERAPEUTIQUES OTIQUES ET PROCEDES ASSOCIES

(54) Title: PHARMACEUTICAL COMPOSITIONS AND METHODS RELATED TO OTIC THERAPEUTIC AGENTS

(57) Abrégé/Abstract:
The present disclosure relates to pharmaceutical compositions (e.g., pre-lyophilized pharmaceutical compositions, lyophilized pharmaceutical compositions, and reconstituted solutions) comprising one or more otic therapeutic agents. The present disclosure also relates to methods of preparing the pharmaceutical compositions, and methods of using the pharmaceutical compositions for therapeutic purpose.

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(54) **Title:** PHARMACEUTICAL COMPOSITIONS COMPRISING OTIC THERAPEUTIC AGENTS AND RELATED METHODS

(57) **Abstract:** The present disclosure relates to pharmaceutical compositions (e.g., pre-lyophilized pharmaceutical compositions, lyophilized pharmaceutical compositions, and reconstituted solutions) comprising one or more otic therapeutic agents. The present disclosure also relates to methods of preparing the pharmaceutical compositions, and methods of using the pharmaceutical compositions for therapeutic purpose.

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PHARMACEUTICAL COMPOSITIONS AND METHODS RELATED TO OTIC THERAPEUTIC AGENTS

This application claims priority to U.S. Provisional Patent Application Serial No. 62/739,933, filed October 2, 2018; which is incorporated herein by reference in it entirety.

BACKGROUND

[001] Stem cells exhibit an extraordinary ability to generate multiple cell types in the body. Besides embryonic stem cells, tissue specific stem cells serve a critical role during development as well as in homeostasis and injury repair in the adult. Stem cells renew themselves through proliferation as well as generate tissue specific cell types through differentiation. The characteristics of different stem cells vary from tissue to tissue, and are determined by their intrinsic genetic and epigenetic status. However, the balance between self-renewal and differentiation of different stem cells are all stringently controlled. Uncontrolled self-renewal may lead to overgrowth of stem cells and possibly tumor formation, while uncontrolled differentiation may exhaust the stem cell pool, leading to an impaired ability to sustain tissue homeostasis. Thus, stem cells continuously sense their environment and appropriately respond with proliferation, differentiation or apoptosis. It would be desirable to drive regeneration by controlling the timing and extent of stem cell proliferation and differentiation. Controlling the proliferation with small molecules that are cleared over time would allow for control of the timing and extent of stem cell proliferation and differentiation. Remarkably, tissue stem cells from different tissues share a limited number of signaling pathways for the regulation of their self-renewal and differentiation, albeit in a very context dependent manner. Some of these pathways are the Wnt and GSK3-β pathways.

[002] Lgr5 is expressed across a diverse range of tissues and has been identified as a biomarker of adult stem cells in a variety of tissues such as the gut epithelia (Barker et al. 2007), kidney, hair follicle, and stomach (Barker et al, 2010; Haegebarth & Clevers, 2009). For example, it was first published in 2011, that mammalian inner ear hair cells are derived from $LGR5^+$ cells (Chai et al, 2011, Shi et al. 2012). Lgr5 is a known component of the Wnt/β-catenin pathway, which has been shown to play major roles in differentiation, proliferation, and inducing stem cell characteristics (Barker et al. 2007).

[003] Permanent damage to the hair cells of the inner ear results in sensorineural hearing loss, leading to communication difficulties in a large percentage of the population. Hair cells are the receptor cells that transduce the acoustic stimulus. Regeneration of damaged hair cells would provide an avenue for the treatment of a condition that currently has no therapies other than prosthetic devices. Although hair cells do not regenerate in the mammalian cochlea, new hair cells in lower vertebrates are generated from epithelial cells, called supporting cells, that surround hair cells.

[004] Thus, there remains a need for novel pharmaceutical compoisitions to protect auditory cells before injury and preserve/promote the function of existing cells after injury. There remains a need for for novel pharmaceutical compoisitions to regenerate cochlear supporting cells or hair cells after injury.

[005] In addition to the above reasons for the need of novel pharmaceutical compositions to regenerate cochlea supporting cells or hair cells after injury, there remains a need to be able to provide the novel pharmaceutical compositions in a manner to efficiently facilitate their intended use. For example, manufacturing and storing the pharmaceutical compositions until required poses many challenges, such as those relating to stability of the pharmaceutically active ingredients. For example, gel formulations may pose particular challenges in relation to stability and a dry composition might not be readily reconstituted to form a gel formulation.

SUMMARY

[006] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising a gelling agent.

[007] In some aspects, the present disclosure provides a gel pharmaceutical composition, for example a thermoreversible gel, comprising one or more otic therapeutic agents.

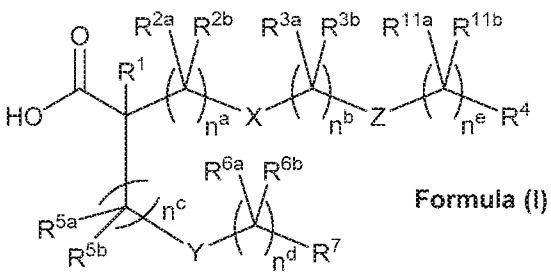
[008] In some aspects, the lyophilized pharmaceutical compositions disclosed herein are reconstituted to form the gel pharmaceutical composition, for example a thermoreversible gel, disclosed herein.

[009] In some aspects, the present disclosure provides, *inter alia*, a lyophilized pharmaceutical composition comprising one or more otic therapeutic agents and a gelling agent.

[010] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising about 50 to about 500 mg of poloxamer and about 50 to about 500 mg of a compound of formula (I), for example valproic acid or a pharmaceutically acceptable salt thereof.

[011] In some aspects, the present disclosure provides a pharmaceutical composition comprising one or more otic therapeutic agents and a gelling agent. For example, a pharmaceutical composition may comprise purified poloxamer and an increased concentration of valproic acid or a pharmaceutically acceptable salt thereof while maintaining suitable gelling characteristics. In a further example, a pharmaceutical composition may comprise an increased concentration of valproic acid or a pharmaceutically acceptable salt thereof and CHIR99021 or a pharmaceutically acceptable salt thereof, wherein the increased concentration of valproic acid or a pharmaceutically acceptable salt thereof increases the level of CHIR99021 or a pharmaceutically acceptable salt thereof in the inner ear.

[012] In some aspects, the present disclosure provides comprising a gelling agent and a compound of formula (I):



Formula (I)

or a pharmaceutically acceptable salt thereof.

[013] In some aspects, the present disclosure provides a pharmaceutical composition comprising a gelling agent, valproic acid or a pharmaceutically acceptable salt thereof at a concentration of greater than about 70 mg/ml, and one or more otic therapeutic agents.

[014] In some aspects, the present disclosure provides a composition that is suitable for intratympanic injection.

[015] In some aspects, the present disclosure provides a pharmaceutical composition comprising a poloxamer, wherein at least 85% by wt.% of the poloxamer has an average molecular weight of greater than about 7250 Da, and valproic acid or a pharmaceutically acceptable salt thereof is present at a concentration of greater than 70 mg/ml.

[016] In some aspects, the present disclosure provides a pharmaceutical composition comprising a poloxamer, wherein less than 20% by wt.% of the poloxamer has an average molecular weight less about 7250 Da, and valproic acid or a pharmaceutically acceptable salt thereof at a concentration of greater than 70 mg/ml.

[017] In some aspects, the present disclosure provides a method for preparing a pharmaceutical composition comprising the steps of: (a) having an aqueous solution comprising a gelling agent; and (b) adding a solution of one or more otic therapeutic agents or a pharmaceutically acceptable salt thereof.

[018] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising a gelling agent and one or more otic therapeutic agents, wherein the composition does not contain an additional bulking agent.

[019] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising a poloxamer and one or more otic agents, wherein the composition does not contain an antioxidant.

[020] In some aspects, the present disclosure provides a method of lyophilizing a pharmaceutical composition.

[021] In some aspects, the present disclosure provides a method of reconstituting a lyophilized pharmaceutical composition.

[022] In some aspects, the present disclosure provides a reconstituted pharmaceutical composition.

[023] In some embodiments, the one or more otic therapeutic agents are one or more hearing loss treatment agents.

[024] In some embodiments, the one or more otic therapeutic agents are modulators of one or more biological pathways and biological targets associated with hearing loss.

[025] In some embodiments, the one or more otic therapeutic agents are hair cell regeneration agents and/or otoprotective agents.

[026] In some embodiments, the one or more otic therapeutic agents are selected from the group consisting of the agents described in Tables 1-13, and pharmaceutical salts thereof.

[027] In some embodiments, the one or more otic therapeutic agents are CHIR99021 or a pharmaceutical acceptable salt thereof, and valproic acid or a pharmaceutical acceptable salt thereof.

[028] In some embodiments, the composition comprises CHIR99021 or a pharmaceutically acceptable salt thereof, valproic acid or a pharmaceutically acceptable salt thereof, and a gelling agent.

[029] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt (e.g., sodium valproate).

[030] In some embodiments, the gelling agent is a thermoreversible gelling agent (e.g., a poloxamer).

[031] In some embodiments, the poloxamer is Poloxamer 407.

[032] In some embodiments, the poloxamer is a purified poloxamer (e.g., purified Poloxamer 407).

[033] In some aspects, the present disclosure provides a method of treating hearing loss, comprising administering to a subject in need thereof a pharmaceutically acceptable amount of a reconstituted solution, wherein the reconstituted solution is prepared by a reconstitution process using the lyophilized pharmaceutical composition of any one of the preceding claims.

[034] In some aspects, the present disclosure provides a pharmaceutical composition, comprising:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.5 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 7.5 wt%.

[035] In some aspects, the present disclosure provides a pharmaceutical composition, comprising:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 1 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 7.5 wt%.

[036] In some aspects, the present disclosure provides a pharmaceutical composition, comprising:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.5 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 7.5 wt%.

[037] In some aspects, the present disclosure provides a method of processing the pharmaceutical composition of the present disclosure to form a lyophilized pharmaceutical composition.

[038] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition being prepared by lyophilizing the pharmaceutical composition of the present disclosure.

[039] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition being prepared by the method of the present disclosure.

[040] In some aspects, the present disclosure provides a reconstituted solution being prepared by adding a diluent to the lyophilized pharmaceutical composition of the present disclosure.

[041] In some aspects, the present disclosure provides a reconstituted solution being prepared by adding a diluent to a lyophilized pharmaceutical composition which is prepared by lyophilizing the pharmaceutical composition of the present disclosure.

[042] In some aspects, the present disclosure provides a reconstituted solution being prepared by adding a diluent to a lyophilized pharmaceutical composition which is prepared by the method of the present disclosure.

[043] In some aspects, the present disclosure provides a reconstituted solution being prepared by adding a diluent to a lyophilized pharmaceutical composition, comprising one or more otic therapeutic agents and a gelling agent.

[044] In some aspects, the present disclosure provides a method of facilitating the generation of a tissue and/or a cell, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure to the tissue and/or the cell.

[045] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing, a disease associated with absence or a lack of a tissue and/or a cell, comprising administering to the subject a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure.

[046] In some aspects, the present disclosure provides a method of increasing a population of vestibular cells in a vestibular tissue, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure.

[047] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing a vestibular condition, comprising administering to the subject a pharmaceutically effective amount of the lyophilized pharmaceutical composition of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure.

[048] In some aspects, the present disclosure provides a method of increasing a population of cochlear cells in a cochlear tissue, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure.

[049] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing a cochlear condition, comprising administering to the subject a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure.

[050] In some aspects, the present disclosure provides a method of increasing a population of cells found in the Organ of Corti, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure to the population.

[051] In some aspects, the present disclosure provides a method of increasing a population of hair cells found in the Organ of Corti, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure to the population.

[052] In some aspects, the present disclosure provides a method of increasing a population of inner hair cells found in the Organ of Corti, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure to the population.

[053] In some aspects, the present disclosure provides a method of increasing a population of outer hair cells found in the Organ of Corti, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure to the population.

[054] In some aspects, the present disclosure provides a method of increasing a population of neuronal cells found in the Organ of Corti, comprising delivering a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure to the population.

[055] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing a hearing condition, comprising administering to the subject a pharmaceutically effective amount of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure.

[056] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in facilitating the generation of a tissue and/or a cell.

[057] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in in treating a subject who has, or is at risk of developing, a disease associated with absence or a lack of a tissue and/or a cell.

[058] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of vestibular cells in a vestibular tissue.

[059] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in treating a subject who has, or is at risk of developing a vestibular condition.

[060] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of cochlear cells in a cochlear tissue.

[061] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in treating a subject who has, or is at risk of developing a cochlear condition.

[062] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of cells found in the Organ of Corti.

[063] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of hair cells found in the Organ of Corti.

[064] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of inner hair cells found in the Organ of Corti.

[065] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of outer hair cells found in the Organ of Corti.

[066] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in increasing a population of neuronal cells found in the Organ of Corti.

[067] In some aspects, the present disclosure provides the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, for use in treating a subject who has, or is at risk of developing a hearing condition.

[068] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for facilitating the generation of a tissue and/or a cell.

[069] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for in treating a subject who has, or is at risk of developing, a disease associated with absence or a lack of a tissue and/or a cell.

[070] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of vestibular cells in a vestibular tissue.

[071] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for treating a subject who has, or is at risk of developing a vestibular condition.

[072] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of cochlear cells in a cochlear tissue.

[073] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for treating a subject who has, or is at risk of developing a cochlear condition.

[074] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of cells found in the Organ of Corti.

[075] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of hair cells found in the Organ of Corti.

[076] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of inner hair cells found in the Organ of Corti.

[077] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of outer hair cells found in the Organ of Corti.

[078] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for increasing a population of neuronal cells found in the Organ of Corti.

[079] In some aspects, the present disclosure provides for the use of the lyophilized pharmaceutical composition, the pharmaceutical composition, or the reconstituted solution of the present disclosure, in the manufacture of a medicament for treating a subject who has, or is at risk of developing a hearing condition.

[080] 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. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents and other references mentioned herein are incorporated by reference for all purposes. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods and examples are illustrative only and are not intended to be limiting. In the case of conflict between the chemical structures and names of the compounds disclosed herein, the chemical structures will control.

[081] Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

[082] Figure 1: Shows an analysis of auditory brainstem responses (ABR) for the treatment in a noise-damage model for induced hearing loss. Treatment with CHIR99021 + VPA leads to hearing improvement in an in vivo noise damage model. (A) Image of injection procedure to transtympanically inject poloxamer into the middle ear of mice.

(B) Animals designated to control and treated groups had elevated thresholds at 24hrs and 5wks after noise exposure compared to pre-exposure baseline. Control n=37 animals, treated n=47 animals. (C) At 5wks after injection, treated animals had significantly lower hearing thresholds relative to control animals for 4 of the 5 frequencies tested. (D) The distribution of individual hearing recoveries was analyzed. Values represent the change in dB needed to elicit an ABR response, with positive values representing further threshold increases (further hearing loss) and negative values representing threshold decreases (improved hearing). The fraction of animals with a given ABR change from 24hr to 5wks are shown for each frequency tested. The treated group had a higher incidence of animals with hearing improvement and the greatest individual recoveries. Values are presented as means ± SE; * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

[083] Figure 2 shows an analysis of hair cell count for treatment in a noise-damage model for induced hearing loss. (A) Low magnification view of a healthy isolated cochlear section showing complete rows of inner hair cells (IHCs) and outer hair cells (OHCs). (B) High magnification view of the region highlighted in a) showing intact IHCs and OHCs in mid frequency regions. (C) Cochleae of vehicle injected animals show widespread hair cell loss throughout the cochlea (apex and mid region shown). (D) High magnification view of the region highlighted in (C) showing substantial absence of hair cells in mid frequency regions, where a single IHC can be seen in the field of view (solid arrow). (E) Cochleae of CV (CHIR99021 and NaVPA) treated animals show a greater overall population of hair cells compared to vehicle treated animals (apex and mid region shown). (F) High magnification view of the region highlighted in (E) showing a complete row of IHCs (solid arrow) and a population of OHCs (open arrow). (G) CV treated cochlea (blue) show significantly more total hair cells, IHCs, and OHCs relative to vehicle treated cochleae (grey). (H) The number of hair cells depicted as the percentage relative to an undamaged healthy cochlea. CV treated cochlea (blue) show significantly higher percentage of total hair cells, IHCs, and OHCs relative to vehicle treated cochleae (grey). Scale bars, 100µm low magnification, 20µm high magnification. Values are presented as box-whisker plots; n=7 animals per group, * = $p<0.05$, ** = $p<0.01$.

[084] Figure 3. Animal model data: significant improvement in thresholds seen at 20kHz and 28.3kHz.

[085] Figure 4. Animal model data: significant improvement in thresholds seen at all frequencies.

[086] Figure 5 Animal model data: significant improvement in thresholds seen at all frequencies.

[087] Figure 6: NaVPA logarithmic mean concentrations.

[088] Figure 7: CHIR99021 logarithmic mean concentrations.

[089] Figure 8: Lyophilized test composition without use of an appropriate lyophilization cycle.

[090] Figure 9: Lyophilized test composition manufactured using the developed lyophilization cycle.

[091] Figure 10. Test composition time course stability.

[092] Figure 11. Solutions of the test composition after time, T.

[093] Figure 12. Reconstituted NaVPA and CHIR99021 assay levels within refrigerated syringes.

[094] Figure 13: The chromatogram P407 Lot GNAC17521C before (red trace) and after purification (blue trace).

[095] Figure 14: High molecular weight (HWM) impurities correspond to a very small percentage by weight. Where present, high molecular weight impurities are observed as a small shoulder eluting before the desired MW peak. The chromatogram illustrates the HMW content for two lots of unpurified P407.

[096] Figure 15: A zoomed in portion of Figure 12.

[097] Figure 16: Molecular weight calibration curve for PEG standards analyzed by SEC.

[098] Figure 17: Cumulative molecular weight distribution.

[099] Figure 18: A typical CAD chromatogram for a blank H_2O injection compared to a 1% P407 sample.

[0100] Figure 19: RPLC-CAD chromatogram of P407 with impurities are divided into "zones" in the chromatogram.

[0101] Figure 20: lyophilized test composition A (entry 2, Table 35).

[0102] Figure 21: lyophilized test composition B (entry 3, Table 35).

[0103] Figure 22 lyophilized test composition C (entry 4, Table 35).

[0104] Figure 23 lyophilized test composition D (entry 5, Table 35).

[0105] Figure 24: lyophilized test composition E (entry 6, Table 35).

[0106] Figure 25: reconstituted compositions A (A1), B (B-1), C (C-1), D (F-1), and E (G-1) from Table 35.

[0107] Figure 26: Aldehyde content in liquid placebo before and after lyophilization.

DETAILED DESCRIPTION

[0108] In some aspects, the present disclosure provides, *inter alia*, a lyophilized pharmaceutical composition comprising one or more otic therapeutic agents (e.g., CHIR99021 and sodium valproate) and a gelling agent (e.g., Poloxamer 407).

[0109] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising one or more otic therapeutic agents (e.g., CHIR99021 or a pharmaceutically acceptable salt thereof and sodium valproate or a pharmaceutically acceptable salt thereof) and a gelling agent (e.g., a poloxamer).

[0110] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising one or more otic therapeutic agents (e.g., LY2090314 or a pharmaceutically acceptable salt thereof and sodium valproate or a pharmaceutically acceptable salt thereof) and a gelling agent (e.g., a poloxamer).

[0111] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising a gelling agent (e.g., a poloxamer) and a compound of formula (I) (e.g., an HDAC inhibitor, such as valproic acid or a pharmaceutically acceptable salt thereof).

[0112] In some aspects, the present disclosure provides a pharmaceutical composition comprising one or more otic therapeutic agents (e.g., CHIR99021 or a pharmaceutically acceptable salt thereof, and valproic acid or a pharmaceutically acceptable salt thereof), wherein the increased concentration of one of the one or more otic therapeutic agents (e.g., valproic acid or a pharmaceutically acceptable salt thereof), increases the level of the other one or more otic therapeutic agents (e.g., CHIR99021 or a pharmaceutically acceptable salt thereof) in the inner ear.

[0113] In some aspects, the present disclosure provides a pharmaceutical composition comprising a gelling agent (e.g., a poloxamer) at a certain purity and one or more otic therapeutic agents (e.g., valproic acid or a pharmaceutically acceptable salt thereof) at a certain concentration.

[0114] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising one or more otic therapeutic agents (e.g., CHIR99021 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof) and a gelling agent (e.g., poloxamer), where the composition does not comprise an additional bulking agent.

[0115] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising one or more otic therapeutic agents (e.g., CHIR99021 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof) and a gelling agent (e.g. poloxamer), where the composition does not comprise an antioxidant.

[0116] In some aspects, the present disclosure provides a method of preparing the pharmaceutical composition of the present disclosure.

[0117] In some aspects, the present disclosure provides a method for preparing a pharmaceutical composition comprising the steps of: (a) having a solution comprising a gelling agent (e.g. a poloxamer) and one or more otic therapeutic agents (e.g. valproic acid or a pharmaceutically acceptable salt thereof); and (b) adding a solution of one or more otic therapeutic agents (e.g. CHIR99021 or a pharmaceutically acceptable salt thereof).

[0118] In some aspects, the present disclosure provides a method for lyophilizing a pharmaceutical composition.

[0119] In some aspects, the present disclosure provides a pharmaceutical composition (e.g., a pre-lyophilized pharmaceutical composition) comprising one or more otic therapeutic agents (e.g., CHIR99021 and sodium valproate) and a gelling (e.g., Poloxamer 407 and other polyethylene oxide-polypropylene oxide block copolymers, including triblock polymers) or other thermoreversible (also called "thermosetting" gelling agents) such as polylactic acid (PLA) – polyethylene oxide block copolymers (including PEO-PLA-PEO triblock copolymers).

[0120] In some aspects, the present disclosure provides a method of processing the pharmaceutical composition of the present disclosure to form a lyophilized pharmaceutical composition (e.g., the pharmaceutical composition of the present disclosure).

[0121] In some aspects, the present disclosure provides a reconstituted solution comprising one or more otic therapeutic agents (e.g., CHIR99021 and sodium valproate) and a gelling (e.g., Poloxamer 407).

[0122] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, CHIR99021 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0123] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, CHIR99021 or a pharmaceutically acceptable salt thereof and 2-hexyl-5-pentynoic acid or a pharmaceutically acceptable salt thereof (e.g. sodium 2-hexyl-5-pentynoic acid).

[0124] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, CHIR99021 or a pharmaceutically acceptable salt thereof and linoleic acid or a pharmaceutically acceptable salt thereof (e.g. sodium lineolate).

[0125] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, LY2090314 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0126] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, AZD1080 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0127] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, GSK3 XXII or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0128] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, Compound I-7 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0129] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, Compound I-1 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0130] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, Compound I-3 or a pharmaceutically acceptable salt thereof and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0131] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407 and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate).

[0132] In some aspects, the present disclosure provides a pharmaceutical composition suitable for intratympanic injection comprising Poloxamer 407, valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate) at a concentration of at least about 120 mg/ml, and CHIR99021 or a pharmaceutically acceptable salt thereof.

[0133] In some aspects, the present disclosure provides a pharmaceutical composition comprising at least 85 wt% .% Poloxamer 407 having an average molecular weight greater than about 7250, and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate) at a concentration of greater than 120 mg/ml, and CHIR99021 or a pharmaceutically acceptable salt thereof.

[0134] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate), and CHIR99021 or a pharmaceutically acceptable salt thereof, wherein in the composition does not comprise an additional bulking agent.

[0135] In some aspects, the present disclosure provides a lyophilized pharmaceutical composition comprising Poloxamer 407, valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate), and CHIR99021 or a pharmaceutically acceptable salt thereof, wherein in the composition does not comprise an antioxidant.

[0136] In some aspects, the present disclosure provides a method for preparing a pharmaceutical composition comprising the steps of: (a) having an aqueous solution comprising Poloxamer 407 and valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate); and (b) adding a solution comprising DMSO and CHIR99021 or a pharmaceutically acceptable salt thereof.

[0137] In some aspects, the present disclosure provides a method for lyophilizing a pharmaceutical composition comprising Poloxamer 407, valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate), and CHIR99021 or a pharmaceutically acceptable salt thereof, wherein the method comprises:

[0138] (a) providing the pharmaceutical composition; (b) lyophilizing the composition by: (i) reducing the temperature in the lyophilizer to -45 °C at a rate of 0.5 °C per minute, and then holding it at -45 °C for 3 hours; (ii) applying a vacuum of 80 mTorr; (iii) increasing the temperature to -30 °C (at a rate of 0.5 °C per minute) and holding it at -30 °C for 15 hours under a vacuum of 80 mTorr; (iv) increasing the temperature to 15 °C (at a rate of 0.5 °C per minute); and/or (v) holding the temperature at 15 °C for 20 hours under a vacuum of 80 mTorr; and (c) obtaining a lyophilized pharmaceutical composition.

[0139] In some aspects, the present disclosure provides a method for lyophilizing a pharmaceutical composition comprising Poloxamer 407, valproic acid or a pharmaceutically acceptable salt thereof (e.g. sodium valproate), and CHIR99021 or a pharmaceutically acceptable salt thereof, wherein the method comprises:

[0140] (a) providing the pharmaceutical composition; (b) lyophilizing the composition by: (i) reducing the temperature in the lyophilizer to about -45 °C at a rate of about 0.5 °C per minute, and then holding it at about -45 °C for about 3 hours; (ii) applying a vacuum of about 80 mTorr; (iii) increasing the temperature to about -30 °C (at a rate of about 0.5 °C per minute) and holding it at about -30 °C for about 15 hours under a vacuum of about 80 mTorr; (iv) increasing the temperature to about 15 °C (at a rate of about 0.5 °C per minute); and/or (v) holding the temperature at about 15 °C for about 20 hours under a vacuum of about 80 mTorr; and (c) obtaining a lyophilized pharmaceutical composition.

[0141] Improved Reconstitution Time

[0142] A way to provide a pharmaceutical composition is in a dry or non-hydrated form, e.g. as a tablet, since this typically renders the pharmaceutically active ingredient(s) in the composition stable for a useful time period that may elapse between the composition being manufactured and to when composition is administered. The pharmaceutically active ingredient(s) is usually stable in the dry composition at varying conditions (temperature, humidity etc.) over the time period that it may be subjected to.

[0143] However, for a pharmaceutical composition that is administered as a solution or a gel, the time between manufacturing to administration poses significant challenges because the pharmaceutically active ingredient(s) in the composition may be not be stable in solution for extended periods of time, and start to degrade, thus creating a

degradation problem. The inventors addressed this degradation problem by lyophilizing the pharmaceutical composition to improve stability, for example for a useful time period between manufacturing and administration.

[0144] The degradation problem can be further exacerbated when the components of the composition are slow to dissolve into the solution (i.e. have poor solubility). For example, with the extended time period time taken to dissolve the components in the solution, degradation can occur. In addition, components can precipitate out of the solution over periods of time. Lyophilization of the composition does not necessarily solve the degradation problem in this scenario where the component(s) also has poor solubility because the composition has two instances, one when the composition is being manufactured and another when the composition is being reconstituted, where the composition is in the form of a solution for an extended period of time, which can lead to degradation of the components. While the long period of time to manufacture the composition may be acceptable since this can be done in a controlled environment, the long period of time taken to reconstitute the lyophilized pharmaceutical composition is not always practical since this typically would occur immediately before the composition is administered in an environment that may vary and cannot be controlled, e.g. in a medical environment. Accordingly, there remains a need to be able to manufacture a lyophilized composition that is stable and reconstitutes on an acceptable time scale.

[0145] The present disclosure offers a solution to the problem described above. Surprisingly, it has been discovered that a lyophilized composition comprising a gelling agent and a salt of an organic acid reconstitutes (i.e. dissolves into solution) more quickly than the time taken to dissolve its constituent parts prior to lyophilization. This means that the composition can be manufactured, lyophilized to produce a stable composition, stored, and then reconstituted quickly prior to administration. It has also been shown that the components of the lyophilized composition are stable for extended periods of time, unlike the composition in solution form. Thus, the present disclosure provides compositions with improved reconstitution time, for example relative to its constituent parts prior to lyophilization. In one embodiment the present disclosure provides compositions with improved reconstitution time relative to its constituent parts without lyophilization (for example as non-lyophilized powders, crystals or other forms).

[0146] The solution to the problem will be illustrated by a non-limiting example. For example, a lyophilized composition comprising a poloxamer and valproic acid or a pharmaceutically acceptable salt thereof can be reconstituted about three times faster than a lyophilized poloxamer alone or powdered poloxamer (i.e. non-lyophilized poloxamer). This result is unexpected and enables the fast reconstitution of pharmaceutical compositions. The fast reconstitution time is especially useful where it is not practical to either freshly prepare the composition, or to wait for long periods time for the composition to reconstitute e.g. because this would lead to the degradation of components of the composition.

[0147] Increased permeation of otic therapeutic agents

[0148] Delivery of a pharmaceutical composition to the inner ear, in particular the cochlea, often relies on diffusion and/or permeation of the pharmaceutical composition into the cochlea (and in particular into the Organ of Corti). Increasing permeation into the cochlea and/or the Organ of Corti is therefore desirable, and it is also desirable to avoid

decomposition of the composition, prior to this point, and/or the otic therapeutic agent(s) precipitating out of solution prior to delivery to the cochlea or Organ of Corti.

[0149] Accordingly, there is a need for pharmaceutical compositions in which the otic agent(s) diffuse and/or permeate into the cochlea (and Organ of Corti) more effectively.

[0150] The present invention offers a solution to the problem described above. Surprisingly, it has been discovered that a pharmaceutical composition comprising high concentrations of an organic acid as defined herein by Formula (I), for example valproic acid, or a pharmaceutically acceptable salt thereof, increases the levels of otic therapeutic agent(s) in the cochlea.

[0151] The solution to the problem will be illustrated by a non-limiting example. For example, a pharmaceutical composition comprising CH99021 or a pharmaceutically acceptable salt thereof and an increased amount of valproic acid or a pharmaceutically acceptable salt thereof, e.g. greater than 100 mg/mL, leads to a non-linear increase in the levels of CH99021 found in the cochlea after administration. For example, a ~50% increase in the amount of valproic acid or a pharmaceutically acceptable salt thereof in the composition can result in far more than a 50% increase of CHIR99021 in the cochlea. The increase of CHIR99021 in the cochlea can be in region of 4-14 fold. Additionally, the increased concentration of valproic acid or a pharmaceutically acceptable salt thereof in the composition can increase the concentration of valproic acid or a pharmaceutically acceptable salt thereof in cochlea by at least an order of magnitude. This result is unexpected and enables the improved delivery of a pharmaceutically active agent(s) to a part of the ear that is difficult to target and difficult to access.

[0152] Purified Poloxamer

[0153] In some instances, the present invention describes a pharmaceutical composition in the form of a solution, which comprises a poloxamer. The poloxamer, when dissolved in the composition at a certain concentration, may impart various properties to the composition, such as a certain viscosity and/or a certain gelation temperature. In some instances, the present invention requires a pharmaceutical composition with a viscosity to form an immobile gel when heated to about body temperature.

[0154] The inclusion of a further component(s) at particular concentration(s) in the composition may perturb the composition's viscosity and/or gelation in a manner such that the ability to form an immobile gel when heated to about body temperature is diminished (for example where the gel is a thermoreversible gel). Therefore, there may be an upper limit of the concentration(s) of the further component(s), e.g. therapeutic component(s), that can be tolerated by the composition while retaining physical properties that are suitable for use. Accordingly, there is a need to provide a pharmaceutical composition with an increased amount of a further component(s), e.g. therapeutic component(s), while maintaining gelling characteristics in order to manufacture pharmaceutical compositions.

[0155] The present invention offers a solution to the problem described above. Surprisingly, it has been discovered that purifying a poloxamer prior to manufacture of a pharmaceutical composition enables an increased concentration of the other component(s) to be tolerated while maintaining the composition's gelling characteristics. For example, the composition comprising purified poloxamer can tolerate increased concentrations of ionic components, such as salts of

organic acids. The increased concentration of component(s) allowed by purifying the poloxamer can allow increased concentrations of therapeutic components to be achieved without adversely affecting other properties of the composition. The purified poloxamer can be prepared or characterized by any of the methods and/or measures set out herein, in any combination, including those disclosed in the numbered embodiments and examples.

[0156] The solution to the problem will be illustrated by a non-limiting example. For example, a pharmaceutical composition comprising Poloxamer 407 will have a certain gelation temperature. In some instances, the composition desirably forms a gel at about body temperature. However, other components in the composition can perturb the temperature that the composition forms a gel. For a particular composition comprising Poloxamer 407, where Poloxamer 407 has not been purified, a concentration of about 80 mg/mL of sodium valproate can be achieved. At concentrations higher than 80 mg/mL, the gelation temperature may be perturbed and the composition's desirable characteristics, such as gelation temperature, diminish. Unexpectedly, for a pharmaceutical composition comprising purified Poloxamer 407, a concentration of greater than about 80 mg/mL of sodium valproate can be achieved, while the desirable gelation temperature is maintained.

[0157] As gel compositions are often not suitable for storage or distribution, the gel compositions may be lyophilized as set out herein. Those lyophilized compositions will therefore have higher concentrations of further component(s), such as therapeutic components, than would otherwise be possible (e.g. with unpurified poloxamer) while retaining favorable gel properties when reconstituted. For example, where the gel contains a given amount of water, the lyophilized composition made from that gel provides a number of benefits. For example, such a lyophilized composition can be reconstituted, for example with the same or similar given amount of water, to provide the compositions disclosed herein that retain their gel properties despite the increased levels of further component(s).

[0158] Therefore, one aspect of the present invention is a composition comprising a poloxamer having an increased amount of VPA, or pharmaceutically acceptable salt thereof, as disclosed herein. In such embodiments, one approach to achieve the increased level of VPA, or pharmaceutically acceptable salts thereof, is to purify the poloxamer as disclosed herein. In these aspects the composition may, for example, be lyophilized or reconstituted with water.

[0159] No additional bulking agent

[0160] An additional bulking agent, such as a polysaccharide, is typically added to a pharmaceutical composition prior to lyophilization in order to help control the morphology of the lyophilized composition. The additional bulking agent, such as a polysaccharide, can be added to a composition before it is lyophilized to impart improved characteristics to the lyophilized product. For example, the characteristics may be the improved morphology of the lyophilized product, in the form of a cake. It is also advantageous if the lyophilized cake is porous, has a large volume, and/or is a fluffy cake. Balanced with the need to provide a suitable lyophilized pharmaceutical composition, there is a need to provide a pharmaceutical composition with minimal components since the compositions are administered to subjects in need thereof.

[0161] The present invention offers a solution to the problem described above. Surprisingly, it has been discovered that a lyophilized composition of the present invention can be successfully lyophilized even when the composition does not comprise an additional bulking agent.

[0162] No antioxidant

[0163] Many pharmaceutical compositions comprise an antioxidant to increase the stability of the composition over an extended period of time. Typically, an antioxidant is required where the composition contains, or degrades over time to produce, a reactive species that may react further with other components, thereby affecting the stability the composition. A species in a composition that contains an aldehyde functional group can be a reactive species, for example reacting through undesired redox pathways, which may cause degradation of the other components. Hence, the inclusion of an antioxidant may increase stability of the composition by inhibiting the redox pathways. Balanced with the need to provide a stable pharmaceutical composition, there is a need to provide a pharmaceutical composition with minimal components since the compositions are administered to subjects in need thereof.

[0164] The present invention offers a solution to the problem described above. Surprisingly, it has been discovered that a lyophilized composition of the present disclosure, that comprises a poloxamer, is stable when the composition does not comprise an antioxidant even though the poloxamer component can degrade to produce aldehydes.

[0165] The solution to the problem will be illustrated by a non-limiting example. For example, compositions of the present disclosure comprise a poloxamer, which may degrade to produce aldehydes. Unexpectedly, when lyophilizing compositions of the present disclosure, it was found that lyophilization removed substantially all of the aldehydes from the composition and/or resulted in a composition that does not produce further aldehydes once lyophilized. This result means that an antioxidant not required in the composition.

[0166] Order of addition of ingredients

[0167] A pharmaceutical composition that is suitable for administration as a solution or a gel typically comprises an aqueous component, such as water. This poses a problem for many pharmaceutically acceptable agents since they can be sparingly soluble in aqueous solutions. Furthermore, the actives can take extended periods of time to dissolve, precipitate out of solution and/or be unstable in solution. Accordingly, there remains a need to provide further methods of making a pharmaceutical composition as an aqueous solution in less time while maintaining the integrity of the components.

[0168] The present disclosure offers a solution to the problem described above. Surprisingly, it has been discovered that adding a pharmaceutically acceptable active(s) in the form of a concentrated solution of a polar aprotic solvent to an aqueous component results in a pharmaceutical composition where the pharmaceutically acceptable agent(s) has been solubilized in the aqueous solution. Crucially, the time taken to form the composition is reduced in comparison to alternative orders of addition, and the time that any potentially unstable components are in solution is minimized.

[0169] The solution to the problem will be illustrated by a non-limiting example. For example, CHIR99021 may exhibit low solubility in aqueous solutions and manufacturing is especially problematic where large quantities of an aqueous solution and long durations of time are required to dissolve CHIR99021 or its salts. However, pre-dissolving

CHIR99021 in a polar aprotic solvent and adding that solution to the aqueous component of the composition successfully solvates CHIR99021 in an aqueous system. This result is unexpected since it occurs on a relatively short timescale, does not lead to precipitation of CHIR99021, is amenable to scale up, and is reproducible. This result is useful since it allows the formation of previously inaccessible compositions.

[0170] Lyophilization method

[0171] Lyophilizing a pharmaceutical composition to produce an acceptable form of the lyophilized product, such as a porous cake, may be challenging. Many factors affect the outcome of the method, and the factors are amenable to a wide range of variation. For example, temperature, rate of temperature change, pressure, and duration at various temperatures and/or pressures all require careful consideration. Thus, obtaining a suitable lyophilized product from a method is no small endeavor and there remains a need to provide more lyophilization methods.

[0172] The present disclosure offers a solution to the problem described above. Surprisingly, it has been discovered that a particular method gives a suitable lyophilized composition in the form of a lyophilized cake. For example, the lyophilization method of the present disclosure is particularly advantageous because it is requires mild conditions, achievable on commercial lyophilizers, which results in a lyophilized product with good characteristics, e.g. the product cake is porous.

Otic Therapeutic Agents

[0173] As used herein, the term "otic therapeutic agent" refers to an agent capable of treating or preventing a disease associated with the ear (e.g., Meniere's disease, hearing loss, a disease of the vesitubular system, vertigo, ear inflammation, or ear infection) or a condition associated with (e.g., resulting into or resulting from) the disease.

[0174] In some embodiments, the otic therapeutic agent is a hearing loss treatment agent.

[0175] As used herein, the term "hearing loss treatment agent" refers to an agent capable for treating or preventing hearing loss or a condition associated with (e.g., causing or developing into or resulting from) hearing loss.

[0176] In some embodiments, the one or more otic therapeutic agents are one or more hearing loss treatment agents.

[0177] In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are modulators of one or more biological pathways and/or biological targets associated with hearing loss. Each of the modulators may independently be an agonist (e.g., activator) or antagonist (e.g., inhibitor) of one or more biological pathways and/or biological targets. In some embodiments, one or more of the modulators are agents that increase or activate the activity of one or more biological pathways and/or biological targets. In some embodiments, one or more of the modulators are agents that decrease or eliminate the activity of one or more biological pathways and/or biological targets.

[0178] In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are selected from the group consisting of Wnt pathway agonists, histone deacetylase (HDAC) inhibitors, Dkk1 inhibitors, Axin inhibitors, SFRP1 inhibitors, bone morphogenetic protein (BMP) inhibitors, beta-catenin agonists, CyclinD1 activators, REST corepressor 1 (CoREST) inhibitors, NOTCH agonists, TGF-beta inhibitors, cAMP response element

binding protein (CREB) activators, cyclin-dependent kinase (CDK) activators, CDK inhibitors, PI3K-AKT activators, PI3K-AKT inhibitors, PTEN inhibitors, ATOH1 agonists, ATOH1 antagonists, POU4F3 agonists, POU4F3 antagonists, GFI1 agonists, GFI1 antagonists, ERK/MAPK agonists, ERK/MAPK antagonists, FGF agonists, FGF antagonists, γ-aminobutyric acids (GABAs), voltage-gated Na+ channel antagonists, inositol, PKC agonists, PKC antagonists, FOXO inhibitors, FOXO agonists, Kv3 channel antagonists, p27Kip1 inhibitors, IL-1β, N-Methyl-D-aspartate (NMDA) receptor antagonists, NADPH quinone oxidoreductase 1, gamma secretase inhibitors, gamma secretase activators, NK1 receptor antagonist, NK1 receptor agonist, AMPA receptor agonist, AMPA receptor antagonist, Toll-Like Receptor (TLR) agonist, Toll-Like Receptor (TLR) antagonist, histamine H4 receptor agonist, H4 receptor antagonist, 5-HT3 receptor agonist, 5-HT3 receptor antagonist, Oct4 activators, Sox2 activators, Sox17 inducers, Klf4 inducers, cMyc activators, Sonic Hedgehog agonists, Sonic Hedgehog antagonists, Epidermal Growth Factor (EGF), Insulin Like Growth Factor (IGF), vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), prostaglandin E (PGE), Brain-derived neurotrophic factor (BDNF), SMAD inhibitors, Sall4 inducers, Gata4 inducers, Gata6 inducers, proteasome inhibitors, retinoic acid receptor agonists, mTOR inhibitors, mTOR activators, Ascorbic acid, 2-phospho-l-ascorbic acid, KDM inhibitors, TTNPB, neurotrophin 3, DNA-modifying enzymes, LSD-1 inhibitors, Nicotinomide, Sirtuin, Histone methyl transferase inhibitors, Histone demethylase inhibitors, Histone Lysine Methyltransferase inhibitors, DNMT inhibitors, p53 inhibitors, p21 inhibitors, AMPK activators, Hippo activators, Hippo inhibitors, YAP/TAZ inhibitors, Mst1/2 inhibitors, CK1 activators, CK1 inhibitors, Noggin, R-spondin 1, BET activators, Sirt1 activators, Sirt1 inhibitors, Sirt2 activators, Sirt2 inhibitors, Sirt3 activators, Sirt3 inhibitors, JMJD3 inhibitors, DMNT inhibitors, Stat3 inhibitors, LSD1 inhibitors, active prostaglandins, cAMP activators, Oxidative phosphorylation uncouplers, arginine methyltransferase inhibitors, ALK4 inhibitors, Peroxisome proliferator-activated receptor gamma activators, EGFR inhibitors, SHH inhibitors, VitD activators, DOT1L inhibitors, Thyroid hormones, E box-dependent transcriptional activators, and protein degradation inhibitors.

[0179] In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are hair cell regeneration agents and/or otoprotective agents.

[0180] In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are selected from the group consisting of the agents described in Tables 1-13, and pharmaceutical salts thereof.

Table 1

AZD1080	Sodium p-aminosalicylate	GP-HL1
GSK XXII	Oxyphenbutazone	QP-HL-3
LY2090314	Deoxycholic acid	SENS-111
DMH1	Metoprine	R-azasetron besylate
Sodium Butyrate	Dasatinib	SPI-1005
Sodium Phenylbutyrate	Terreic Acid	Alpha lipoic acid
Vo-ohpic	PGE2	Ancrod
SF1670	dmPGE2	Zonisamide

Rapamycin	GW9662	2-phospho-l-ascorbic acid
AICAR	BML-284	Vitamin C
Foxy-5	Chloroquine	Oct4
EPZ004777	YH249	Sox2
SGC0944	Carbamazepine	Klf4
Anandamide	Lamotrigine	cMyc
Simvastatin	Portion of Jag-1 residue 188-04	BF844
Pravastatin	N-methylhemeanthidine chloride	EGF
Amino-bisphosphonates	Tubastatin A	Brain-derived neurotrophic factor (BDNF)
A-83-01	Trichostatin A	rosiglitazone
616452	Panobinostat	BIX 01294
SB431542	MS-275	5-azacytidine
XMU-MP-1	Apicidin	Reversine
WAY-262611	Febuxostat	Pumorphamine
Purpurogalin	Phenyl butyrate	SAG
Exifone	MiR-182-5p	Hh-Ag1.5
Gallic acid	AUT1	LDE225
RG108	AUT3	SMER28
WAY 316606	AUT00063	PGD2
HLY78	AM-101	Metformin
Lycorine	OAC2	S1P
D 4476	AM-111	1-AA
PF-4800567	LY3056480	UM17116
(R)-roscovitine	DzNep	16-dimethyl
PF-670464	PD0325901	Forskolin
PF-5006739	PS48	QS11
TA 01	Fructose 2	BIO
(R)-DRF053	6-bisphosphonate	Cyclopamine
TG003	IM-12	Neuropathiazol
IC261	Casin	Pluripotin
(S)-CR8	LY294002	Y-27632
Riluzole	Dorsomorphin	SKL2001
YM298198	LDN1193189	AS1842856
JNJ16259685	Quercetin	Neurotrophin 3
JNJ10198409	Peptide P13	AS 8351
LY 456236	EPI-743	SJ403
Flunarizine	Vincerinone	TC-E 5002
DAPT	EPI-589	Tranylcypromine
LY411575	Sodium Thiosulfate	SU16F
MDL 28170	RO4929097	GSK2879552
SC1	Concanavalin A	GSK-LSD1
SU5402	Ciprofloxacin	CBB1007

Selumetinib (AZD6244)	Dexamethasone	3TFA
Methotrexate	Betamethasone	SP-2509
Pyrimethamine	ORC-13661	Noggin
Trimetrexate	Betahistine	R-spondin 1
Nolatrexed	HPN-07	Valproic acid
Raltitrexed	NHPN-1010	
6-Mercaptopurine	Gacyclidine	
Retinoic acid	BDNF	
TTNPB	N-acetylcysteine	
Azathioprine	PF-04958242	

Table 2

DZNep	AS8351
SC-1	TC-E 5002
CBB1003	DNP
SP-2509	PS48
AS8351	SC-1
TC-E 5002	LY-364947
NSC 636839	XMU-MP-1
AZD5438	IBS008738
Prostaglandin E2	Ethacridine
Forskolin	Metformin
DNP	SGC0946
RSC133	EPZ004777
LY-364947	OAC2
AMI-5	Robotinikin
AMI-1	XMU-MP-1
Compound B4 (TGFb-RI)	Metformin
PS48	SGC0946
5-aza-2'-deoxycytidine	EPZ004777
Rosiglitazone	OAC2
A-83-01	Robotinikin
TTNPB	Cyclopamine
{2-Methyl-4-{{4-methyl-2-[4-(trifluoromethyl)phenyl]-5-thiazolyl}methylthio}phenoxy}-acetic acid	
Gefitinib	3,3,5 Triiodo-L-thyronine
Cyclopamine	IBS008738
Calcitriol	Ethacridine
Prostaglandin E2	CAY10591
Compound B4 (TGFb-RI)	SRT1720
AMI-1	CAY10602
AMI-5	EX-527
Forskolin	AGK2
AZD5438	Tenovin-6

5-aza-2'-deoxycytidine	Sirtinol
RSC133	(+)-JQ1
DZNep	GSK-J4

Table 3

Compound	Target
CHIR-98023	GSK-3β
CHIR-99021	GSK-3β
CHIR-99030	GSK-3β
Hymenialdisine	GSK-3β
debromohymenialdisine	GSK-3β
dibromocantherelline	GSK-3β
Meridianine A	GSK-3β
alsterpaullone	GSK-3β
cazapaullone	GSK-3β
Aloisine A	GSK-3β
NSC 693868 (1*H*-Pyrazolo[3,4-*b*]quinoxalin-3-amine)	GSK-3β
Indirubin-3′-oxime (Indirubin-3′-monoxime; 3-[1,3-Dihydro-3-(hydroxyimino)-2*H*-indol-2-ylidene]-1,3-dihydro-2*H*-indol-2-one)	GSK-3β
A 1070722 (1-(7-Methoxyquinolin-4-yl)-3-[6-(trifluoromethyl)pyridin-2-yl]urea)	GSK-3β
L803	GSK-3β
L803-mts	GSK-3β
TDZD8	GSK-3β
NP00111	GSK-3β
HMK-32	GSK-3β
Manzamine A	GSK-3β
Palinurin	GSK-3β
Tricantin	GSK-3β
IM-12 (3-(4-Fluorophenylethylamino)-1-methyl-4-(2-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione)	GSK-3β
NP031112	GSK-3β
NP00111	GSK-3β
NP031115	GSK-3β
VP 2.51	GSK-3β
VP2.54	GSK-3β

VP 3.16	GSK-3β
VP 3.35	GSK-3β
HLY78 (4-Ethyl-5,6-Dihydro-5-methyl-[1,3]dioxolo[4,5-j]phenanthridine, 4-Ethyl-5-methyl-5,6-dihydro-[1,3]dioxolo[4,5-j]phenanthridine)	Axin
WAY-262611 ((1-(4-(Naphthalen-2-yl)pyrimidin-2-yl)piperidin-4-yl)methanamine))	Dickkopf-1 (DKK1)
BHQ880	DKK1
NCI8642	DKK1
gallocyanine dyes	DKK1
Compounds 3-8 (Moore et al., *J.Med.Chem.*, 2009; 52:105)	secreted frizzled-related protein 1 (sFRP-1)
WAY-316606	sFRP-1

Table 4

Compound	**Target**
A01 (Cao et al., *Scientific Reports*, 2014; 4:4965)	SMAD1/5/8
A17 (Cao et al., *Scientific Reports*, 2014; 4:4965)	SMAD1/5/8

Table 5

Compound	**Target**
Cerivastatin (Baycol; Lipobay)	p27Kip1
Alsterpaullone 2-cyanoethyl	p27Kip1
SJ403	p27Kip1

Table 6

Compound	Target
Compound A (See FIG. 7)	Atoh1
Compound B (See FIG. 7)	Atoh1
Compound C (See FIG. 7)	Atoh1
1-Azakenpaullone (Pyrido[3',2':2,3]azepino[4,5-b]indol-6(5H)-one, 9-bromo-7,12-dihydro-)	Atoh1
2-(N)-benzyl ellipticene	Atoh1

Table 7

Compound	Target
Delta/Serrate/Lag-2 peptide	Notch receptor

Table 8

Compound	Target
Vorinostat (rINN; suberanilohydroxamic acid; suberoylanilide hydroxamic acid; SAHA (suberoyl+anilide+hydroxamic acid abbreviated); *N*-Hydroxy-*N'*-phenyloctanediamide; Zolinza®)	HDAC class I (HDAC1, 2, 3, and 8) and HDAC class II (IIa: HDAC4, 5, 7, and 9; IIb: 6 and 10)
Trichostatin A (TSA; (2*E*,4*E*,6*R*)-7-(4-(Dimethylamino)phenyl)-*N*-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide)	HDAC class I (HDAC1, 2, 3, and 8) and HDAC class II (IIa: HDAC4, 5, 7, and 9; IIb: 6 and 10)
belinostat (PXD101; Beleodaq)	HDAC

Valproic acid (VPA; sodium valproate; Sodium 2-propylpentanoate)	HDAC
FK 228 (Depsipeptide; FR 901228; Romidepsin; Cyclo[(2*Z*)-2-amino-2-butenoyl-L-valyl-(3*S*,4*E*)-3-hydroxy-7-mercapto-4-heptenoyl-D-valyl-D-cysteinyl], cyclic (3-5) disulfide)	HDAC class I (HDAC1, 2, 3, and 8), HDAC4, and HDAC6
Sodium butyrate (Butanoic acid sodium salt; NaB)	HDAC
LMK 235 (*N*-[[6-(Hydroxyamino)-6-oxohexyl]oxy]-3,5-dimethylbenzamide)	HDAC4 and HDAC5
Scriptaid (*N*-Hydroxy-1,3-dioxo-1*H*-benz[de]isoquinoline-2(3*H*)-hexanamide)	HDAC
M 344 (4-(Diethylamino)-*N*-[7-(hydroxyamino)-7-oxoheptyl]benzamide)	HDAC
SBHA (*N*,*N*'-Dihydroxyoctanediamide; suberic bishydroxamate)	HDAC1 and HDAC3
CBHA (*m*-carboxycinnamic acid bishydroxamide)	HDAC1 and HDAC3
HMBA (hexamethylene bisacetamide).	HDAC

Tubacin (*N*-[4-[(2*R*,4*R*,6*S*)-4-[[(4,5-Diphenyl-2-oxazolyl)thio]methyl]-6-[4-(hydroxymethyl)phenyl]-1,3-dioxan-2-yl]phenyl]-*N'*-hydroxyoctanediamide)	HDAC6
Sodium 4-phenylbutyrate (4-PB; sodium pheylbutyrate; 4-Phenylbutyric acid, sodium salt; 4-phenylbutyrate)	HDAC
MC 1568 (3-[5-(3-(3-Fluorophenyl)-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-*N*-hydroxy-2-propenamide)	HDAC class IIa (HDAC4, 5, 7, and 9)
Compound 9 (Mai et al., *J.Med.Chem.*, 2005; 48:3344)	HDAC class IIa (HDAC4, 5, 7, and 9)
Compound 24 (Mai et al., *J.Med.Chem.*, 2005; 48:3344)	HDAC class IIa (HDAC4, 5, 7, and 9)
TC-H 106 (*N1*-(2-Aminophenyl)-*N7*-(4-methylphenyl)heptanediamide; Pimelic Diphenylamide 106)	HDAC class I (HDAC1, 2, 3, and 8)
Pyroxamide (*N*-Hydroxy-*N'*-3-pyridinyloctanediamide)	HDAC1
NCH 51 (PTACH; 2-Methylpropanethioic acid *S*-[7-oxo-7-[(4-phenyl-2-thiazolyl)amino]heptyl] ester)	HDAC
NCH 31	HDAC
PCI 34051 (*N*-Hydroxy-1-[(4-methoxyphenyl)methyl]-1*H*-indole-6-carboxamide)	HDAC8
thiophene benzamide	HDAC1 and HDAC2
KD 5170 (*S*-[2-[6-[[[4-[3-(Dimethylamino)propoxy]phenyl]sulfonyl]amino]-3-pyridinyl]-2-oxoethyl]ethanethioc acid ester)	HDAC class I (HDAC1, 2, 3, and 8) and HDAC class II (IIa: HDAC4, 5, 7, and 9; IIb: 6 and 10)
TCS HDAC6 20b (2-Methylpropanethioic acid-*S*-[(6*S*)-6-[[(1,1-dimethylethoxy)carbonyl]amino]-7-oxo-7-(tricyclo[3.3.1.1^{3,7}]dec-1-ylamino)heptyl] ester)	HDAC6
NSC 3852 (5-Nitroso-8-quinolinol)	HDAC
NSC69603	HDAC

NSC86371	HDAC
NSC305819	HDAC
CI 994 (*N*-acetyldinaline; Acetyldinaline; 4-(Acetylamino)-*N*-(2-aminophenyl)benzamide)	HDAC class I
LAQ824	HDAC class I
LBH589 (panobinostat; Farydak)	pan-HDAC
MS275 (SNDX-275; entinostat)	HDAC1-3
MGCD0103 (mocetinostat)	HDAC1-8 and 11
UF 010 (4-Bromo-*N*'-butylbenzohydrazide)	HDAC1-3
Cpd60	HDAC1-3
Romidepsin	HDAC1 and HDAC2
MS-27-275	HDAC
NaBu (n-butyrate)	HDAC
trapoxin	HDAC
Apicidin (Cyclo[(2*S*)-2-Amino-8-oxodecanoyl-1-methoxy-L-tryptophyl-L-isoleucyl-(2*R*)-2-piperidinecarbonyl])	HDAC
depudesin	HDAC
EX 527 (6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide)	SIRT1
AGK 2 (2-Cyano-3-[[5-(2,5-dichlorophenyl)-2-furanyl]-*N*-5-quinolinyl-2-propenamide)	SIRT2
AK 7 (*N*-(3-Bromophenyl)-3-[(hexahydro-1*H*-azepin-1-yl)sulfonyl]benzamide)	SIRT2
SirReal2 (2-[(4,6-Dimethyl-2-pyrimidinyl)thio]-*N*-[5-(1-naphthalenylmethyl)-2-thiazolyl]acetamide)	SIRT2
Salermide (*N*-[3-[[(2-Hydroxy-1-naphthalenyl) methylene]amino]phenyl]-α-methylbenzeneacetamide)	SIRT1 and SIRT2
Splitomicin (1,2-Dihydro-3*H*-naphtho[2,1-*b*]pyran-3-one)	Sir2p (yeast form of SIRT1)

Table 9

Compound	Target
MG132 (Z-LLL-al, Z-Leu-Leu-Leu-CHO; *N*-[(Phenylmethoxy)carbonyl]-L-leucyl-*N*-[(1*S*)-1-formyl-3-methylbutyl]-L-leucinamide)	proteasome
MG262 (Z-Leu-Leu-Leu-B(OH)2)	proteasome
MG115 (Z-Leu-Leu-Nva-CHO)	proteasome
Z-Leu-Leu-Phe-CHO (Z-LLF-CHO)	proteasome
N-Acetyl-leucyl-leucyl-norleucinal (Ac-Leu-Leu-Nle-CHO)	proteasome
N-acetyl-leucyl-leucyl-methional (Ac-Leu-Leu-Met-CHO)	proteasome
N-benzyloxycarbonyl-isoleucyl-γ-t-butyl-glutamyl-alanyl-leucinal (Z-Ile-Glu(OtBu)-Ala-Leu-CHO)	proteasome
N-benzyloxycarbonyl-leucyl-leucyl-leucinal (Z-Leu-Leu-Leu-CHO),	proteasome
N-benzyloxycarbonyl-leucyl-leucyl-tyrosyl α-keto aldehyde (Z-Leu-Leu-Tyr-COCHO)	proteasome
N-benzyloxycarbonyl-leucyl-leucyl-phenylalanal (Z-Leu-Leu-Phe-CHO)	proteasome
N-benzyloxycarbonyl-leucyl-leucyl-leucyl boronic acid (Z-Leu-Leu-Leu-B(OH)$_2$)	proteasome
Bortezomib (PS-341; Velcade; Neomib; Bortecad)	proteasome
Lactacystin ((2*R*,3*S*,4*R*)-3-Hydroxy-2-[(1*S*)-1-hydroxy-2-methylpropyl]-4-methyl-5-oxo-2-pyrrolidinecarboxy-*N*-acetyl-L-cysteine thioester)	proteasome
Disulfiram (Antabuse and Antabus)	proteasome
Epigallocatechin-3-gallate (Epigallocatechin gallate; EGCG)	proteasome

Salinosporamide A	proteasome
Carfilzomib (Kyprolis)	proteasome
epoxomicin	proteasome
Ixazomib (Ninlaro; MLN2238)	proteasome
ixazomib citrate (MLN9708)	proteasome
PS-341	proteasome
VLX1500 (b-AP15)	proteasome
clasto-Lactacystin beta Lactone	proteasome
Gliotoxin (Aspergillin; (3*R*,5a*S*,6*S*,10a*R*)-2,3,5a,6-Tetrahydro-6-hydroxy-3-(hydroxymethyl)-2-methyl-10*H*-3,10a-epidithiopyrazino[1,2-*a*]indole-1,4-dione)	proteasome
AM 114 (3,5-*Bis*-[benzylidene-4-boronic acid]-1-methylpiperidin-4-one)	proteasome
PSI (*N*-[(Phenylmethoxy)carbonyl]-L-isoleucyl-L-*α*-glutamyl-*tert*-butyl ester-*N*-[(1S)-1-formyl-3-methylbutyl]-L-alaninamide)	proteasome
Oprozomib (ONX 0912)	proteasome
Delanzomib (CEP-18770)	proteasome
BI8622	Huwe1 (E3 ubiquitin ligase)
BI8626	Huwe1 (E3 ubiquitin ligase)

Table 10

Compound	**Target**
MLN4929 (Pevonedistat)	Akt
API-2 (Triciribine; NSC 154020; TCN; 1,5-Dihydro-5-methyl-1-β-D-ribofuranosyl-1,4,5,6,8-pentaazaacenaphthylen-3-amine; Akt/protein kinase B signaling inhibitor-2)	Akt
API-1 (4-Amino-5,8-dihydro-5-oxo-8-β-D-ribofuranosyl-pyrido[2,3-*d*]pyrimidine-6-carboxamide)	Akt

GSK 690693 (4-[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-[(3*S*)-3-piperidinylmethoxy)-1*H*-imidazo[4,5-c]pyridin-4-yl]-2-methyl-3-butyn-2-ol)	Akt
10-DEBC hydrochloride (10-[4'-(*N*,*N*-Diethylamino)butyl]-2-chlorophenoxazine hydrochloride)	Akt
FPA124 (Dichloro[(2*Z*)-2-[(4-oxo-4*H*-1-benzopyran-3-yl)methylene] hydrazinecarbothioamide copper complex)	Akt
SC66 ((2*E*,6*E*)-2,6-*Bis*(4-pyridinylmethylene)cyclohexanone)	Akt
LY 294002 hydrochloride (2-(4-Morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one hydrochloride)	PI3K
wortmannin	PI3K
PI 103	PI3K
Quercetin (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one)	PI3K and PKC
PHT 427 (4-Dodecyl-*N*-1,3,4-thiadiazol-2-yl-benzenesulfonamide)	Akt and PDK1
GSK 2334470 ((3*S*,6*R*)-1-[6-(3-Amino-1*H*-indazol-6-yl)-2-(methylamino)-4-pyrimidinyl]-*N*-cyclohexyl-6-methyl-3-piperidinecarboxamide)	PDK1
Fisetin (2-(3,4-Dihydroxyphenyl)-3,7-dihydroxy-4*H*-1-benzopyran-4-one)	PI3K, Akt
OSU 03012 (2-Amino-*N*-[4-[5-(2-phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]phenyl]acetamide)	Akt and PDK1
PIT 1 (*N*-[[(3-Chloro-2-hydroxy-5-nitrophenyl) amino]thioxomethyl]benzamide)	Akt

Table 11

Compound	Target
AC102 (6-fluoro-9-methyl-β-carboline; 6F9MβC	CREB

Table 12

Compound	Target
LY411575 (LSN-411575; Compound 5; benzeneacetamide; N-[(1s)-2-[[(7s)-6,7-dihydro-5-methyl-6-oxo-5H-dibenz[b,d]azepin-7-yl]amino]-1-methyl-2-oxoethyl]-3,5-difluoro-α-hyroxy-(αS)-); N2-[(2S)-2-(3,5-Difluorophenyl)-2-hydroxyethanoyl]-N1-[(7S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl]-L-alaninamide)	γ-secretase
L-685458 ((5*S*)-(*tert*-Butoxycarbonylamino)-6-phenyl-(4*R*)-hydroxy-(2*R*)-benzylhexanoyl)-L-leucy-L-phenylalaninamide; LY-685458; GSI-X)	γ-secretase
DBZ (Dibenzazepine; YO-01027; GSI-XX, deshydroxy LY-411575; *N*-[(1*S*)-2-[[(7*S*)-6,7-Dihydro-5-methyl-6-oxo-5*H*-dibenz[*b*,*d*]azepin-7-yl]amino]-1-methyl-2-oxoethyl]-3,5-difluorobenzeneacetamide)	γ-secretase
MRK560 (*N*-[*cis*-4-[(4-Chlorophenyl)sulfonyl]-4-(2,5-difluorophenyl)cyclohexyl]-1,1,1-trifluoromethanesulfonamide)	γ-secretase
MRK-003	γ-secretase
MK-0752	γ-secretase

Compound W (CW; 3,5-Bis(4-nitrophenoxy)benzoic acid) (Okochi et al., *J.Biol.Chem.*, 2006; 281:7890; Ford et al., *J Neurosci Meth.*, 2008; 168:465-474)	γ-secretase
Compound E (GSI-XXI) (Olsauskas-Kuprys et al., *Onco Targets Ther.*, 2013; 6:943)	γ-secretase
BMS 2289948 (4-chloro-N-(2,5-difluorophenyl)-N-((1R)-{4-fluoro-2-[3-(1H-imidazol-1-yl)propyl]phenyl}ethyl)benzenesulfonamide hydrochloride)	γ-secretase
BMS-433796 ((*S*)-2-((*S*)-2-(3,5-difluorophenyl)-2-hydroxyacetamido)-*N*-((*S,Z*)-3-methyl-4-oxo-4,5-dihydro-3*H*-benzo[d][1,2]diazepin-5-yl)propanamide)	γ-secretase
IN973	γ-secretase
Flurbiprofen bi((*R*)-Flurbiprofen; tarenflurbil; Flurizan; (*R*)-2-Fluoro-α-methyl[1,1'-biphenyl]-4-acetic acid)	γ-secretase
JLK2, JLK4, JLK6, JLK7 (7-Amino-4-chloro-3-methoxy-1*H*-2-benzopyran)	γ-secretase
Begacestat (GSI-953; 5-Chloro-*N*-[(1*S*)-3,3,3-trifluoro-1-(hydroxymethyl)-2-(trifluoromethyl)propyl]-2-thiophenesulfonamide)	γ-secretase
DFK167	γ-secretase
PF-0308414	γ-secretase

Table 13

Compound	**Target**
TTNBP (RO 13-7410, arotinoid acid, AGN 191183)	RAR
ATRA	RAR
9-cis RA	RAR
CD271 (6-(4-Methoxy-3-tricyclo[3. 3. 1. 1 3,7]dec-1-ylphenyl)-2-naphthalenecarboxylic acid)	RAR

CD336	RAR
CD-394	RAR
CD437 (6-3-(1-adamantyl)-4-hydroXyphenyl)-2 naphthanoic acid) (6-(4-Hydroxy-3-tricyclo[3. 3. 1. 13,7]dec-1-ylphenyl)-2-naphthalenecarboxylic acid)	RAR
CD666 ((E)-4-(1-hydroXy-1-(5,6,7,8-tetrahydro-5,5,8,8 tetramethyl-2-naphthyl)-2-propenyl)benzoic acid)	RAR
CD1530 (4-(6- Hydroxy-7-tricyclo[3. 3. 1. 13,7]dec-1-yl-2-naphthalenyl)benzoic acid)	RAR
CD2019 (6-(3-(1-methylcycloheXyl)-4-methoXyphenyl)-2 naphthanoic acid)	RAR
CD2247	RAR
CD2081	RAR
CD2314	RAR
CD2325 (4-[(E)-2-(3-(1-adamantyl)-4-hydroXyphenyl)-1 propenyl]benZoic acid)	RAR
CD2425	RAR
CD2503	RAR
CD2665	RAR
BMS-270394 (enantiomer of BMS-189961) (3-Fluoro-4-[(R)-2-hydroxy-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-acetylaminol-benzoic acid)	RAR
BMS-189961 (3-Fluoro-4-[2-hydroxy-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-acetylamino]-benzoic acid)	RAR
6-[3-(adamantan-1-yl)-4-(prop-2-ynyloxy)phenyl]naphthalene-2-carboxylic acid	RAR
5-[(E)-3-oxo-3-(5,5,8,8-tetrahydronaphthalene-2-yl)propenyl]thiophene-2-carboxylic acid	RAR
Palovarotene (4[(1E)-2-[5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-3-(1H-pyrazol-1-ylmethyl)-2-naphthalenyl]-ethenyl]-benzoic acid; R667; CLM-001, RG667)	RAR
CH-55 (4-[(E)-3-(3,5-Di-tert-butyl-phenyl)-3-oxo-propenyl]-benzoic acid)	RAR

Docosahexaenoic acid (DHA; (4Z,7Z,10Z,13Z,16Z,19Z)-4,7,10,13,16,19-Docosahexaenoic acid)	RXR
CD 3254 (3-[4-Hydroxy-3-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)phenyl]-2-propenoic acid)	RXR
9 *cis*-RA	RXR
3-*cis*-retinoic acid (Accutane; isotretinoin; 13-*cis*-Retinoic acid)	RXR
LG 100754 ((2*E*,4*E*,6*Z*)-3-Methyl-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-propoxy-3-naphthalenyl)-2,4,6-octatrienoic acid)	RXR
SR 11237 (BMS 649; 4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1,3-dioxolan-2-yl]-benzoic acid)	RXR
Fluorobexarotene (2-Fluoro-4-[1-(5-,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl]benzoic acid)	RXR
LGD1069 (4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid)	RXR
LG100268 (6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic acid)	RXR
LG100754 (2E,4E,6Z)-3-Methyl-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-3-propoxy-2-naphthalenyl)-2,4,6-Octatrienoic acid)	RXR
Compounds 1-11 (Wagner et al., *J.Med.Chem.*, 2009; 52:5950)	RXR
HX 630 (4-(7,8,9,10-Tetrahydro-7,7,10,10-tetramethylbenzo[*b*]naphtho[2,3-*f*][1,4]thiazepin-12-yl-benzoic acid)	RXR
HX 640	RXR
HX 600	RXR

TZ335	RXR
Adapalene (6-(4-Methoxy-3-tricyclo[3.3.1.1^{3,7}]dec-1-ylphenyl)-2-naphthalenecarboxylic acid, 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid; CD-271; Differin)	RXR
Bexarotene (4-[1-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl]benzoic acid, LGD-1069; SR-11247; targretin; TRG)	RXR
Retinoic acid (ATRA; Tretinoin; Vitamin A acid; all-*trans*-Retinoic acid)	RXR
4-[N-methanesulfonyl-N-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)amino]benzoic acid	RXR
6-[N-ethyl-N-(3-isopropoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IP)	RXR
6-[N-ethyl-N-(3-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (NEt-3IB	RXR
PA024	RXR
AGN 194204	RXR
CNX-013-B2	RXR
UAB30	RXR
IRX4204	RXR

HAIR CELL REGENERATION AGENTS

[0181] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following hair cell regeneration agents.

[0182] A hair cell regeneration agent is an agent that promotes regeneration of hair cells. A single agent may be used as a hair cell regeneration agent or a combination of agents may provide the hair cell regenerative function. Thus, in some embodiments, the hair cell regeneration agent is a single agent. In other embodiments the hair cell regeneration agent is a combination of agents. In certain such embodiments, the combination of agents may be compounded together in a single composition. In other embodiments, the combination of agents may be provided to a patient separately.

[0183] A hair cell regeneration agent may promote regeneration of hair cells by stimulating transdifferentiation of supporting cells within the sensory epithelium of cochlea into replacement hair cells. Alternatively, or additionally, a hair cell regeneration agent may activate a proliferative response in the sensory epithelium of the cochlea, thereby providing a new population of cells that can subsequently differentiate into supporting cells.

[0184] In some embodiments, the hair cell regeneration agent stimulates proliferation of cochlear supporting cells in which proliferation is stimulated expresses Lgr5 (Leucine-rich repeat-containing G-protein coupled receptor 5). However the hair cell regeneration agent may also stimulate proliferation of supporting cells with little or no Lgr5

expression. In some embodiments, the hair cell regeneration agent produces an expanded population of cochlea cells. In some embodiments, the expanded cells are enriched for Lgr5 expression (i.e. a greater percentage of the expanded cell population express Lgr5 compared to the starting cell population).

[0185] Lgr5 is a member of GPCR class A receptor proteins that is expressed across a diverse range of tissues such as in the muscle, placenta, spinal cord and brain, and particularly as a biomarker of adult stem cells in certain tissues. Lgr5+ stem cells are the precursors for sensory hair cells that are present in the cochlea. Increasing the population of Lgr5+ cochlear cells is therefore beneficial because it increases the population of precursor cells which may differentiate into sensory hair cells.

[0186] In some embodiments, the hair cell regeneration agent is a Wnt agonist and an epigenetic modulator. Any Wnt agonist and epigenetic modulator described herein may be used.

[0187] In some embodiments, the hair cell regeneration agent is a Wnt agonist and two or more epigenetic modulator. Any Wnt agonist and epigenetic modulator described herein may be used.

[0188] In some embodiments, the hair cell regeneration agent is a Wnt agonist alone. A Wnt agonist may be used alone in line with any of the treatments disclosed herein that relate to Wnt agonists and/or epigenetic modulators in which both the Wnt agonist and epigenetic modulator are administered to the patient. In these embodiments, the epigenetic modulator is not included. Any Wnt agonist described herein may be used. In certain such embodiments, the hair cell regeneration agents is a GSK3 inhibitor. Any GSK3 inhibitor described herein may be used.

[0189] In some embodiments, the hair cell regeneration agent is gamma secretase inhibitor. Suitable gamma secretase inhibitors are described in WO 2018007331 A1; WO 2018111926 A2; WO 2018065340 A1; WO 2018060300 A1; WO 2018011164 A1; WO 2018087018 A1; WO 2018001918 A1; WO 2018118791 A2; WO 2018118782 A2 and WO 2014045156 A1, each of which is incorporated by reference. Any gamma secretase inhibitor described herein may be used.

[0190] In some embodiments, the hair cell regeneration agent is an Atoh1 activator. Suitable Atoh1 activators are described in US 20160030445 A1; WO 2018172997 A1; WO 2016022776 A2; WO 2014145205 A2 and WO 2009100438 A2, each of which is incorporated by reference.

[0191] In some embodiments, the hair cell regeneration agent is a Notch inhibitor. Suitable Notch inhibitors are described in WO2017007702-A1; WO2016056999-A1; WO2014039781A1; WO2014047369A1; WO2014047372A1; WO2014047390A1; WO2014047391A1; WO2014047397A1; WO2014047392A1; WO2014047370A1; WO2014047374A1; WO2013093885A1; WO2013178821A1 and WO2013016081A1, each of which is incorporated by reference.

[0192] In some embodiments, the hair cell regeneration agent is a Wnt agonist and a Notch inhibitor. Any Wnt agonist and Notch inhibitor may be used as described herein. In certain such embodiments the Wnt agonist is a GSK3 inhibitor. Any GSK3 inhibitor described herein may be used.

[0193] In some embodiments, the hair cell regeneration agent is a Wnt agonist and a gamma secretase inhibitor. Any Wnt agonist and gamma secretase inhibitor may be used as described herein. In certain such embodiments, the Wnt agonist is a GSK inhibitor. Any GSK3 inhibitor described herein may be used.

WNT AGONISTS

[0194] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following WNT agonists.

[0195] Provided in one aspect is a Wnt agonist and/or an epigenetic modulator for use in treating sensorineural hearing loss in a human patient, wherein said Wnt agonist and said epigenetic modulator are administered to a human patient. Also provided is a method of treating sensorineural hearing loss in a human patient comprising administering to the patient a Wnt agonist and an epigenetic modulator. A Wnt agonist and/or an epigenetic modulator may be used for treating a patient as described elsewhere herein.

[0196] A Wnt agonist refers to an agent that increases the expression, levels, and/or activity of a Wnt gene, protein, or signaling pathway (e.g. TCF/LEF, Frizzled receptor family, Wif1, Lef1, Axin2, β-catenin) in a cell, for example, a cochlear cell. A Wnt agonist includes a GSK3 inhibitor, such as a GSK3-α or a GSK3-β inhibitor. In some embodiments the Wnt agonist is a GSK inhibitor that inhibits both GSK3-α and GSK3-β.

[0197] The TCF/LEF family is a group of transcription factors that bind to DNA through a high mobility group domain, and which are involved in the Wnt signaling pathway where they recruit the coactivator β-catenin to enhancer elements of targeted genes. Frizzled is a family of G protein-coupled receptor proteins that serves as receptors in the Wnt signaling pathway. Frizzled receptors inhibit intracellular β-catenin degradation and activate TCF/LEF-mediated transcription.

[0198] In some embodiments, the Wnt agonist increases Wnt signaling in a cochlear cell by about or at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500% or more (or at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more) relative to a control, for example relative to a baseline level of activity.

[0199] In some embodiments, the Wnt agonist increases TCF/LEF-mediated transcription in a cochlear cell, for example, by about or at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500% or more (or at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more) relative to a control, for example relative to a baseline level of activity.

[0200] In some embodiments, the Wnt agonist binds and activates a Frizzled receptor family member, for example, by about or at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500% or more (or at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more) relative to a control, for example relative to a baseline level of activity.

[0201] In some embodiments, the Wnt agonist inhibits GSK3 for example, by about or at least about10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500% or more (or at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2,

3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more) relative to a control, for example relative to a baseline level of activity.

[0202] In some embodiments, the Wnt agonist preferentially upregulates Jag-1, Deltex-1 or Hif-1 more than the Wnt agonist upregulates Hes or Hey. In some embodiments, the Wnt agonist increases the expression of Jag-1, Deltex-1 and/or Hif-1 10%, 25%, 50%, 75%, 100%, 125%, 150%, 175%, 200%, 250% or more than it increases the expression or activity of Hes and Hey.

[0203] Exemplary agents having activity as a Wnt agonist are provided in Table 14 and 15 below, including pharmaceutically-acceptable salts thereof.

Table 14

Agent	CAS	GSK-3 alpha	GSK-3 beta	Lgr5+ Assay	Perilymph Conc.	Formul. Conc. Intraymp
CHIR99021	252917-06-9	4.4 nM	6.6 nM	2-6 uM	2-6 uM	4 mM
AZD 1080	612487-72-6	6.9 nM	31 nM	1-5 uM	1-5 uM	1-5 mM
GSK XXII	1195901-31-5	2.3 nM	2.0 nM	0.2-1 uM	0.2-1 uM	0.2-1 mM
LY2090314	603288-22-8	2.1 nM	0.9 nM	5-20 nM	5-20 nM	5-20 uM

Table 15

Class	Agent	CAS
WNT		
ARFGAP1	QS 11	944328-88-5
ARFGAP1	WASP-1, ZINC00087877	352328-82-6
Axin	Cpd1	1357473-75-6
Axin	Cpd2	1228659-47-9
Axin	HLY78	854847-61-3
Axin	SKL2001	909089-13-0
beta-catenin	DCA	56-47-3
Disrupts the Axin Complex	Compound 2	1360540-82-4
Disrupts the Axin Complex	Compound 71	1622429-71-3
Disrupts the Axin Complex	ISX 9	832115-62-5
DKK1 inhibitor	WAY-262611	1123231-07-1
MEK	Radicicol	12772-57-5
MEK	Selumetinib (AZD6244)	606143-52-6
PP2A	IQ 1	331001-62-8
sFRP-1 inhibitor	(Dimethylamino)propyl)-2-ethyl-5-(phenylsulfonyl)benzenesulfonamide	915754-88-0
sFRP-1 inhibitor	Cyclosporine A (CsA)	59865-13-3
sFRP-1 inhibitor	Cyclosporine analogs	

sFRP-1 inhibitor	PSC833 (Valspodar)	121584-18-7
sFRP-1 inhibitor	WAY 316606	915759-45-4
Target Undetermined	Diketones	WO 2016029021 A1; WO 2012024404 A1
Target Undetermined	Diketones	1622429-56-4
Target Undetermined	Diketones	1360540-88-0
Target Undetermined	Diketones	1360540-89-1
Target Undetermined	Diketones	1622429-79-1
Target Undetermined	Diketones	1622429-75-7
Target Undetermined	Diketones	1622429-74-6
Target Undetermined	Diketones	1622430-76-5
Target Undetermined	Diketones	1622430-31-2
Target Undetermined	Diketones	1622430-52-7
Target Undetermined	Diketones	1622429-67-7
Target Undetermined	Diketones	1622429-65-5
Target Undetermined	Diketones	1622429-69-9
van-Gogh-like receptor proteins (Vangl)	Compound 109	1314885-81-8
Wnt Ligand	Wnt-1	Protein
Wnt Ligand	Wnt-10a	Protein
Wnt Ligand	Wnt-10b/12	Protein
Wnt Ligand	Wnt-11	Protein
Wnt Ligand	Wnt-16	Protein
Wnt Ligand	Wnt-2/Irp (Int-I-related protein)	Protein
Wnt Ligand	Wnt-2b/13	Protein
Wnt Ligand	Wnt-3/Int-4	Protein
Wnt Ligand	Wnt-3a	Protein
Wnt Ligand	Wnt-4	Protein
Wnt Ligand	Wnt-5a	Protein
Wnt Ligand	Wnt-5b	Protein
Wnt Ligand	Wnt-6	Protein
Wnt Ligand	Wnt-7a	Protein
Wnt Ligand	Wnt-7b	Protein
Wnt Ligand	Wnt-8a/8d	Protein
Wnt Ligand	Wnt-8b	Protein
Wnt Ligand	Wnt-9a/14	Protein
Wnt Ligand	Wnt-9b/14b/15	Protein
Wnt Related Protein	Norrin	Protein
Wnt Related Protein	R-Spondin 1/2/3/4	Protein
Wnt-3a/Dkk-1	BML-284	853220-52-7
Wnt-3a/Dkk-1	Compound 1	1084833-94-2
Wnt-3a/Dkk-1	Compound 25	1084834-05-8
GSK3 alpha		

CREB knockdown	666-15	1433286-70-4
Isonicotinamides	Compound 29	1772823-37-6
Isonicotinamides	Compound 33	1772823-64-9
Isonicotinamides	Compound 39	1772824-10-8
Maleimide	I5	264217-24-5
Maleimide	Tivantinib	905854-02-6
Organometallic	Compound (R)-DW12	1047684-07-0
Organometallic	Compound 3	1498285-39-4 1498285-48-5
Organometallic	Compound lambda-OS1	1291104-51-2 1292843-11-8
Oxadiazoles	Compound 14d	1374671-64-3
Oxadiazoles	Compound 15b	1374671-66-5
Oxadiazoles	Compound 27	1820758-44-8
Oxindole	AZD1080	612487-72-6
Pyrazole	AT 7519	844442-38-2
Pyrazole	Compound 4a	1627557-91-8
Pyrazole	Compound 4t	1627558-10-4
Pyrazole	Compound 4z	1627558-16-0
Pyrazole	GSK-3b XXII	1195901-31-5
Pyrazolopyridazines	Compound 18	405223-20-3
Pyrazolopyridazines	Compound 19	405223-71-4
Pyrazolopyridines	Compound 14	583038-63-5
Pyrazolopyridines	Compound 23	583038-76-0
Pyrazolopyridines	Pyrazolopyridine 34	583039-27-4
Pyrazolo-tetrahydroquinolinone	BRD1172	1597438-86-2
Pyrazolo-tetrahydroquinolinone	BRD1652	1597438-93-1
Pyrazolo-tetrahydroquinolinone	BRD4003 chiral	1597439-60-5
Pyrazolo-tetrahydroquinolinone	BRD4003 chiral	1597439-59-2
Pyrazolo-tetrahydroquinolinone	Compound 11	1597439-12-7
Pyrazolo-tetrahydroquinolinone	Compound 16	1597440-17-9
Pyrazolo-tetrahydroquinolinone	Compound 8	1597439-01-4
Pyrazolo-tetrahydroquinolinone	Compound 9	1597439-02-5
Triazolpyrimidine	Compound 90	91322-11-1
Triazolpyrimidine	Compound 92	1043429-30-6
Urea	AR-A014418	487021-52-3
GSK3-beta		

Acid	Bikinin	188011-69-0
Acid	Valproic Acid, Sodium Salt	99-66-1
Aloisines	Aloisine A	496864-16-5
Aloisines	Aloisine B	496864-14-3
Aloisines	TWS119	1507095-58-0
Aminopyrimidine	CHIR98014 (CT98014)	252935-94-7
Aminopyrimidine	CHIR98023 (CT98023)	252904-84-0
Aminopyrimidine	CHIR98024 (CT98024)	556813-39-9
Aminopyrimidine	CHIR99021 (CT99021)	252917-06-9
Aminopyrimidine	CT20026	403808-63-9
Aminopyrimidinyl	CGP60474	164658-13-3
Aminopyrimidinyl	GSK-3β Inhibitor XVIII	1139875-74-3
Azaindolylmaleimide	Compound 29	436866-61-4
Azaindolylmaleimide	Compound 46	682807-74-5
Bisindolylmaleimide	Bisindolylmaleimide X HCl	131848-97-0
Bisindolylmaleimide	Compound 5a	436866-54-5
Bisindolylmaleimide	Enzastaurin (LY317615)	170364-57-5
Bisindolylmaleimide	GF109203x	176504-36-2
Bisindolylmaleimide	Ro318220	125314-64-9
Dihydropyridine	ML320	1597438-84-0
Flavone	Flavopiridol	146426-40-6
Furanosesquiterpenes	Palinurin	254901-27-4
Furanosesquiterpenes	Tricantin	853885-55-9
Furopyrimidine	Compound 100	744255-19-4
Halomethylketones	Compound 17	62673-69-2
Halomethylketones	GSK-3β Inhibitor VI	62673-69-2
Halomethylketones	GSK-3β Inhibitor VII	99-73-0
Hymenidin	Hymenidin	107019-95-4
Indirubins	5-Iodo-indirubin-3'-monoxime	331467-03-9
Indirubins	6-Bromoindirubin-3-acetoxime	667463-85-6
Indirubins	GSK-3 Inhibitor IX	667463-62-9
Indirubins	GSK-3 Inhibitor X	740841-15-0
Indirubins	Indirubin	479-41-4
Indirubins	Indirubin-3'-monoxime	160807-49-8
Indirubins	Indirubin-5-sulfonic acid sodium salt	331467-05-1
Inorganic atom	Beryllium	
Inorganic atom	Lithium Chloride	
Inorganic atom	Tungstate	
Inorganic atom	Zinc	
Isoindolone	Staurosporine	62996-74-1
Isonicotinamides	Compound 29	1772823-37-6
Isonicotinamides	Compound 33	1772823-64-9
Isonicotinamides	Compound 39	1772824-10-8

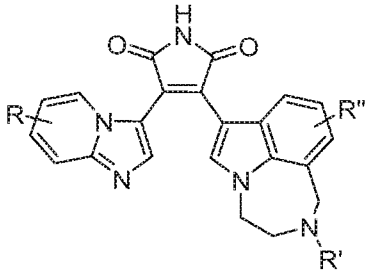
Maleimide	3F8	159109-11-2
Maleimide	603281-31-8	603281-31-8
Maleimide	BIP-135	941575-71-9
Maleimide	Compound 34	396091-16-0
Maleimide	CP21R7	125314-13-8
Maleimide	GSK-3 inhibitor 1	603272-51-1
Maleimide	GSK-3β Inhibitor XI	626604-39-5
Maleimide	I5	264217-24-5
Maleimide	IM-12	1129669-05-1
Maleimide	Isogranulatimide	244148-46-7
Maleimide	KT 5720	108068-98-0
Maleimide	LY2090314	603288-22-8
Maleimide	SB-216763	280744-09-4
Maleimide	SB-415286 (SB-41528)	264218-23-7
Maleimide	TCS 21311	1260181-14-3
Maleimide	Tivantinib	905854-02-6
Manzamines	Manzamine A	104196-68-1
Miscellaneous	AZD2858 (AR28)	486424-20-8
Miscellaneous	CID 755673	521937-07-5
Miscellaneous	Dibromocantharelline	101481-34-9
Miscellaneous	TCS 2002	1005201-24-0
Organometallic	(RRu)-HB1229	
Organometallic	(RRu)-NP549	
Organometallic	Compound (R)-DW12	1047684-07-0
Organometallic	Compound 3	1498285-39-4, 1498285-48-5
Organometallic	Compound lambda-OS1	1291104-51-2, 1292843-11-8
Organometallic	DW12	861251-33-4
Organometallic	HB12	800384-87-6
Organometallic	NP309	937810-13-4
Oxadiazol	Compound 14d	1374671-64-3
Oxadiazol	Compound 15b	1374671-66-5
Oxadiazol	Compound 20x	1005201-80-8
Oxadiazol	GSK-3 Inhibitor II	478482-75-6
Oxadiazol	GSK3 Inhibitor, 2	1377154-01-2
Oxadiazol	TC-G 24	1257256-44-2
Oxindole	AZD1080	612487-72-6
Oxindole	SU9516	77090-84-1
Patent	CN 101341138 B	
Patent	CN 1319968 C	
Patent	CP-70949	
Patent	CT118637	
Patent	EP 1739087 A1	

Patent	EP 1961748 A2	
Patent	EP 2765188 A1	
Patent	GI179186X	
Patent	GW784752X	
Patent	GW784775X	
Patent	US 20070088080 A1	
Patent	US 20100292205 A1	
Patent	US 7514445 B2	
Patent	US 8071591 B2	
Patent	US 8207216 B2	
Patent	US 8686042 B2	
Patent	US 8771754 B2	
Patent	WO 2001085685 A1	
Patent	WO 2003037891 A1	
Patent	WO 2006018633 A1	
Patent	WO 2007102770 A1	
Patent	WO 2008077138 A1	
Patent	WO 2007106537 A2	
Patent	WO 2009017453 A1	
Patent	WO 2010075551 A1	
Patent	WO 2010104205 A1	
Patent	WO 2011089416 A1	
Patent	WO 2013124413 A1	
Patent	WO 2014003098 A1	
Patent	WO 2014013255 A1	
Patent	WO 2014050779 A1	
Patent	WO 2014059383 A1	
Patent	WO 2014083132 A1	
Patent	WO2006100490A1/EP 1863904 A1	
Patent	WO2009017455 A1	
Paullone	Cmpd 17b	408532-42-3
Paullone	Kenpaullone	142273-20-9
Paullones	Alsterpaullone	237430-03-4
Paullones	Alsterpaullone CN Ethyl	852529-97-0
Paullones	Azakenpaullone	676596-65-9
Paullones	Cazpaullone	914088-64-5
Peptide	FRATtide	
Peptide	L803	
Peptides	L803-mts	
Publication	705701	
Publication	708244	
Publication	709125	
Publication	AR79	

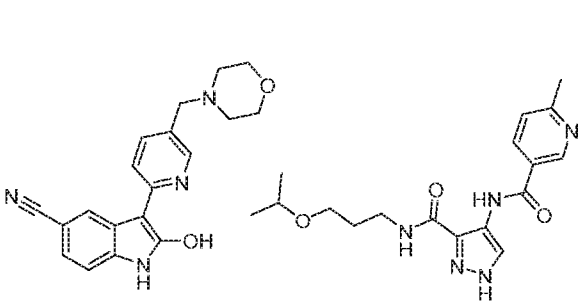
Publication	AZ13282107	No Structure
Publication	AZ13282107	
Publication	CEP-16805	No Structure
Publication	CG-301338	No Structure
Publication	CT73911	
Publication	LY2064827	
Publication	NP-103	No Structure
Publication	SAR 502250	No Structure
Publication	SAR 502250 (Sanofi)	1073653-58-3
Publication	XD-4241	No Structure
Pyrazole	AT 7519	844442-38-2
Pyrazole	Compound 4a	1627557-91-8
Pyrazole	Compound 4t	1627558-10-4
Pyrazole	Compound 4z	1627558-16-0
Pyrazole	GSK-3 Inhibitor XXII	1195901-31-5
Pyrazolone	GSK-3beta Inhibitor XXVI	871843-09-3
Pyrazolopyridazines	Compound 18	405223-20-3
Pyrazolopyridazines	Compound 19	405223-71-4
Pyrazolopyridine	Pyrazolopyridine 18	405221-39-8
Pyrazolopyridine	Pyrazolopyridine 34	583039-27-4
Pyrazolopyridine	Pyrazolopyridine 9	923029-74-7
Pyrazolopyridines	Compound 14	583038-63-5
Pyrazolopyridines	Compound 14	583038-63-5
Pyrazolopyridines	Compound 23	583038-76-0
Pyrazoloquinoxaline	NSC 693868 (Compound 1)	40254-90-8
Pyrazoloquinoxaline	NSC 693868 (Compound 1)	40254-90-8
Pyridinone	Compound 150	1282042-18-5
Pyrrolopyridinyl	Compound 12	2025388-10-5
Pyrrolopyridinyl	Compound 27	2025388-25-2
Pyrroloazepine	Hymenialdisine	82005-12-7
Quinazolin	GSK-3 Inhibitor XIII	404828-08-6
Quinolinecarb	VP0.7	331963-23-6
Quinoline		1132813-46-7
Quinoline		1132812-98-6
Quinoline		950727-66-9
Quinoline		950727-04-5
Quinoline		1132812-98-6
Thiadiazolidindiones	GSK-3β Inhibitor I	327036-89-5
Thiadiazolidindiones	NP031112 (Tideglusib)	865854-05-3
Thiadiazolidindiones	NP031115	1400575-57-6
Triazolpyrimidine	Compound 90	91322-11-1
Triazolpyrimidine	Compound 92	1043429-30-6
Urea	GSK-3β Inh. VIII AR-A014418	487021-52-3

Urea	A-1070722	1384424-80-9

[0204] In some embodiments, an agent having activity as a Wnt agonist is a GSK3 inhibitor. Preferably, the GSK3 inhibitor is AZD1080, GSK3 inhibitor XXII, CHIR99021 or LY2090314. In a preferred embodiment, the Wnt agonist is CHIR99021. In other preferred embodiments, the Wnt agonist and/or GSK3 inhibitor is a substituted 3-Imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-dione. (Formula A.)



Formula A



AZD 1080 GSK3 XXII CHIR 99021 LY 2090314

[0205] The Wnt agonist can be any selected from WO 2018/125746, which is hereby incorporated by reference. In some embodiments, the Wnt agonist can be the compound as defined in claim 1 of WO 2018/125746. In some embodiments, the Wnt agonist can be the compound as defined in claim 12 of WO 2018/125746.

[0206] Exemplary substituted 3-Imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-diones include: 3-(imidazo[1,2-a]pyridin-3-yl)-4-(2-(piperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione; 7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 3-(9-ethynyl-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-amino-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 1-(9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-2-carbonyl)piperidine-4-carbaldehyde; 3-(9-fluoro-2-(4-(hydroxymethyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4,4-difluoropiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(benzo[d]isoxazol-3-yl)-4-(9-fluoro-2-

(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione; N-(7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-9-yl)acetamide; 3-(9-(difluoromethyl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(3,3-difluoropiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-((1R,4R)-2,5-diazabicyclo[2.2.1]heptane-2-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 2-(3,3-difluoropiperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 2-(4,4-difluoropiperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 3-(2-(4,4-difluoropiperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(aminomethyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(hydroxymethyl)piperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 2-(4-(hydroxymethyl)piperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 3-(9-fluoro-2-(3,3,4,4,5,5-hexafluoropiperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(3,3,5,5-tetrafluoropiperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(2,2,6,6-tetrafluoromorpholine-4-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4,4-difluoro-3-hydroxypiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(difluoro(hydroxy)methyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(6,6-difluoro-1,4-oxazepane-4-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-([1,2,4]triazolo[4,3-a]pyridin-3-yl)-4-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(piperidine-1-carbonyl-d10)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl-3,3,4,4-d4)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(4-(2,2,2-trifluoro-1-hydroxyethyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(4-((methylamino)methyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-

a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-((dimethylamino)methyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-aminopiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(4-(methylamino)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(dimethylamino)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-(piperidin-4-ylmethyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide; 9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-(piperidin-4-ylmethyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide; 9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-((1-methylpiperidin-4-yl)methyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide; 3-(9-fluoro-2-((1R,4R)-5-methyl-2,5-diazabicyclo[2.2.1]heptane-2-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(2-methyl-2,8-diazaspiro[4.5]decane-8-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(8-methyl-2,8-diazaspiro[4.5]decane-2-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(imidazo[1,2-a]pyridin-3-yl)-4-(2-(2,2,6,6-tetrafluoromorpholine-4-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione; 3-(2-(6,6-difluoro-1,4-oxazepane-4-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 2-(4-(dimethylamino)piperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 9-cyano-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-((1-methylpiperidin-4-yl)methyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide; 7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(8-methyl-2,8-diazaspiro[4.5]decane-2-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 3-(8,9-difluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; or 3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione (LY20900314).

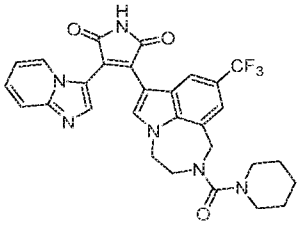
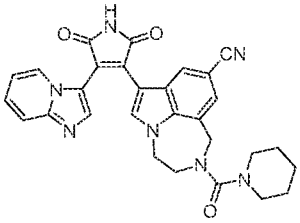
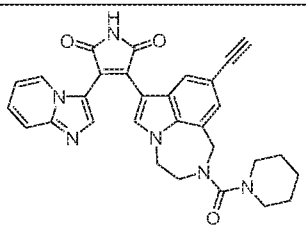
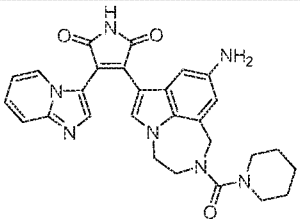
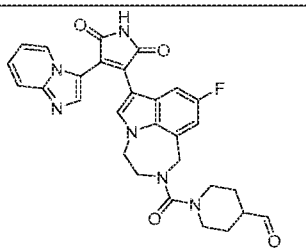
[0207] Preferably, the substituted 3-Imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-dione is: 3-(imidazo[1,2-a]pyridin-3-yl)-4-(2-(piperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione; 7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 3-(9-ethynyl-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(4-(hydroxymethyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4,4-difluoropiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-

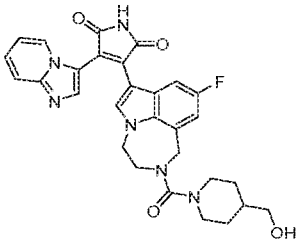
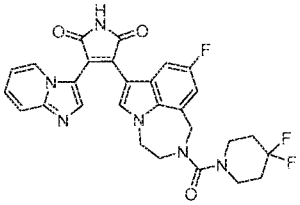
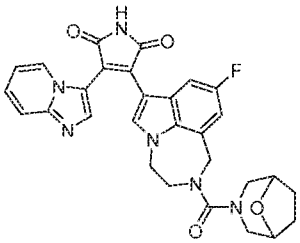
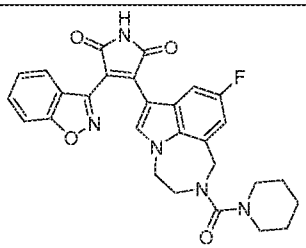
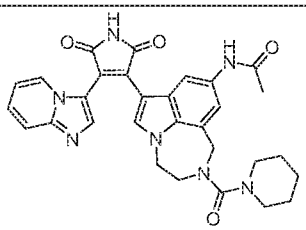
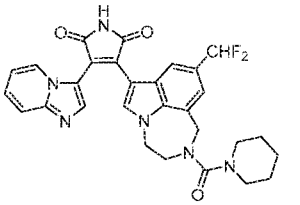
a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-(difluoromethyl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(3,3-difluoropiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 2-(4,4-difluoropiperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile; 3-(2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(hydroxymethyl)piperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(3,3,4,4,5,5-hexafluoropiperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(3,3,5,5-tetrafluoropiperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(2,2,6,6-tetrafluoromorpholine-4-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4,4-difluoro-3-hydroxypiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(difluoro(hydroxy)methyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(6,6-difluoro-1,4-oxazepane-4-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(piperidine-1-carbonyl-d10)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl-3,3,4,4-d4)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(9-fluoro-2-(4-(2,2,2-trifluoro-1-hydroxyethyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-((dimethylamino)methyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(2-(4-(dimethylamino)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-((1-methylpiperidin-4-yl)methyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide; 3-(imidazo[1,2-a]pyridin-3-yl)-4-(2-(2,2,6,6-tetrafluoromorpholine-4-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione; 3-(2-(6,6-difluoro-1,4-oxazepane-4-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; 3-(8,9-difluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione; or 3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione. (LY2090314).

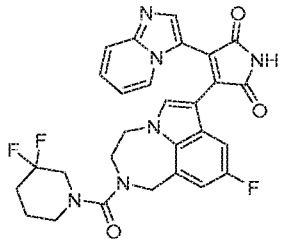
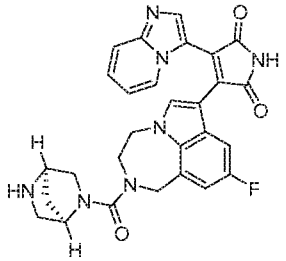
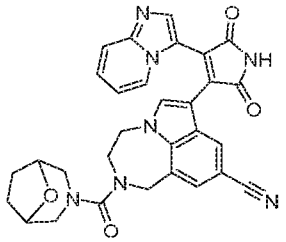
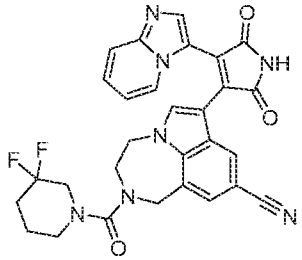
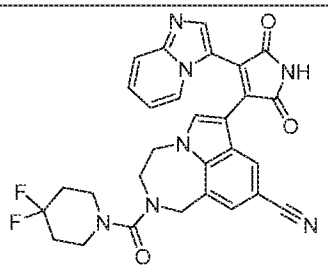
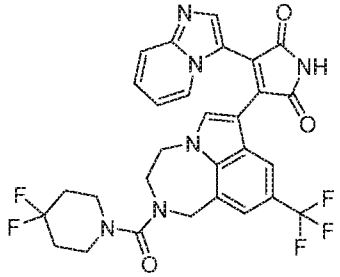
[0208] Most preferably, the substituted 3-Imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-dione is 3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione. (LY2090314).

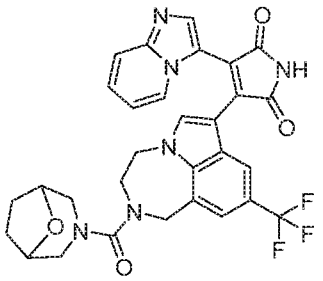
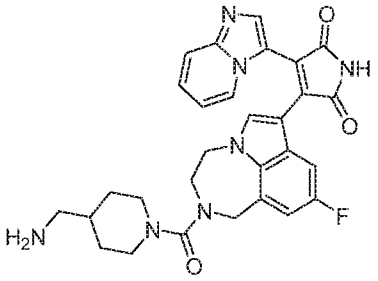
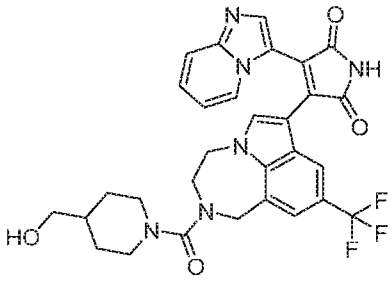
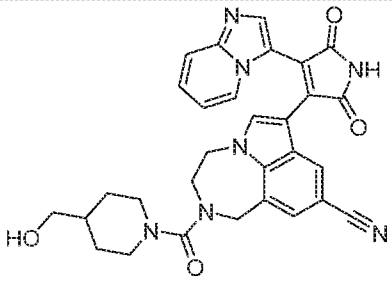
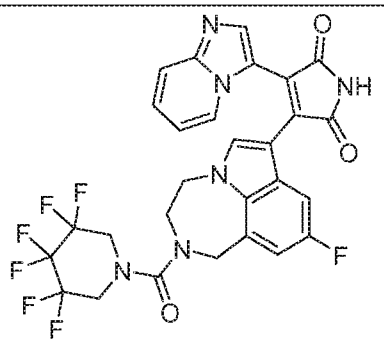
[0209] The structures of the substituted 3-Imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-diones are shown below in Table 16.

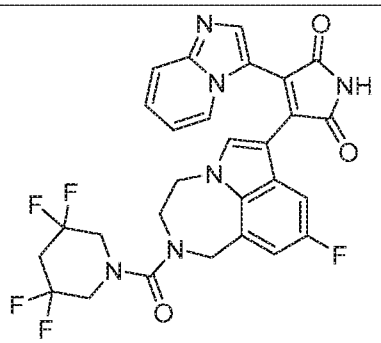
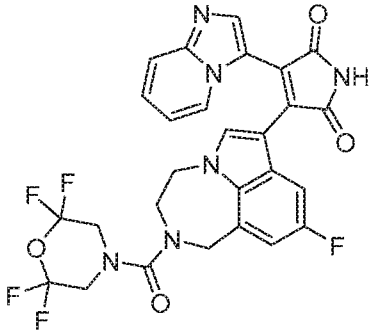
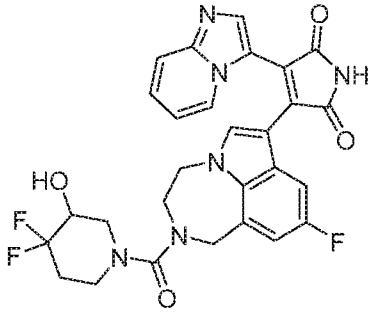
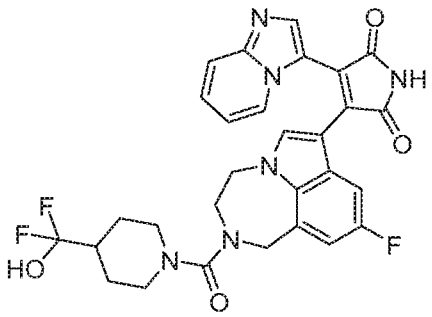
Table 16

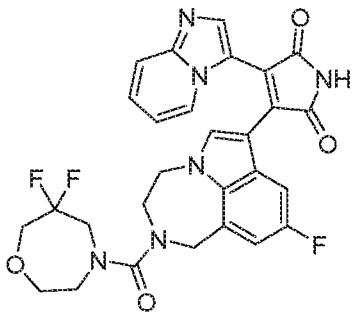
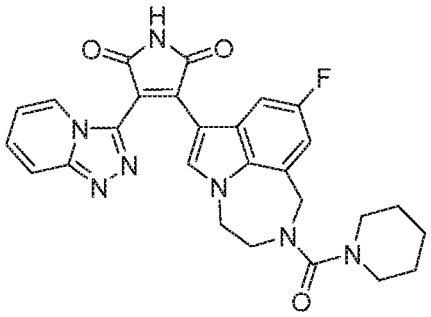
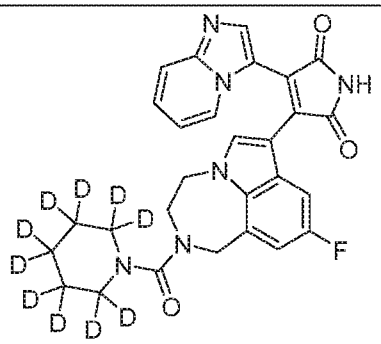
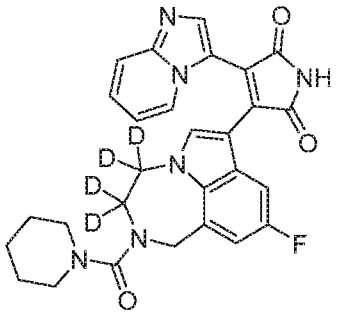
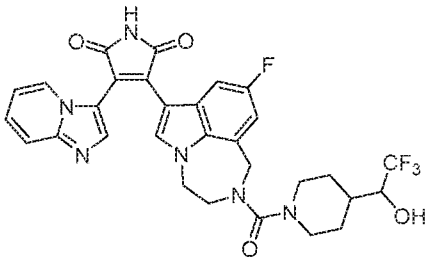
Compound I-1		3-(imidazo[1,2-a]pyridin-3-yl)-4-(2-(piperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione
Compound I-2		7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-3		3-(9-ethynyl-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-4		3-(9-amino-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-5		1-(9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-2-carbonyl)piperidine-4-carbaldehyde

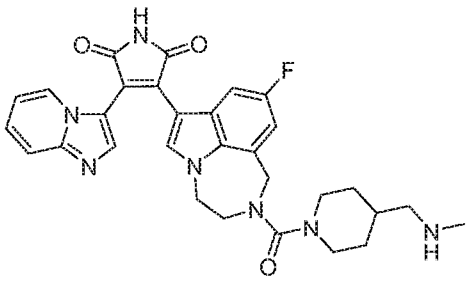
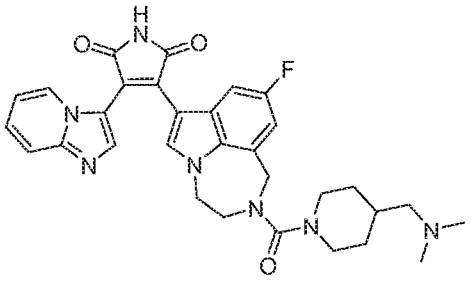
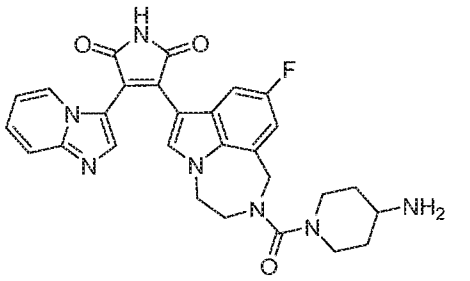
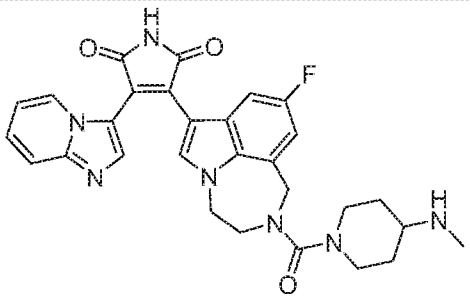
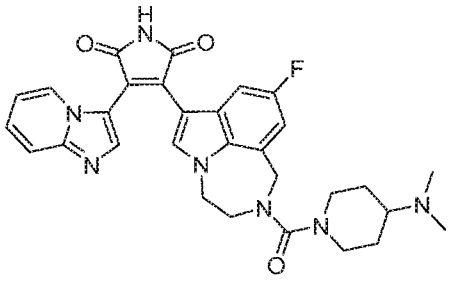
Compound I-6		3-(9-fluoro-2-(4-(hydroxymethyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-7		3-(2-(4,4-difluoropiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-8		3-(2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-9		3-(benzo[d]isoxazol-3-yl)-4-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione
Compound I-10		N-(7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-9-yl)acetamide
Compound I-11		3-(9-(difluoromethyl)-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

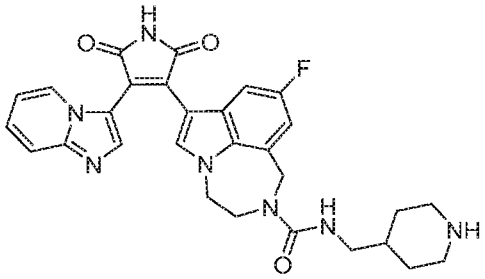
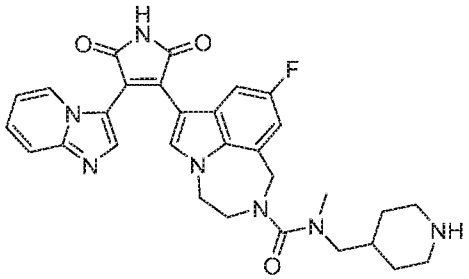
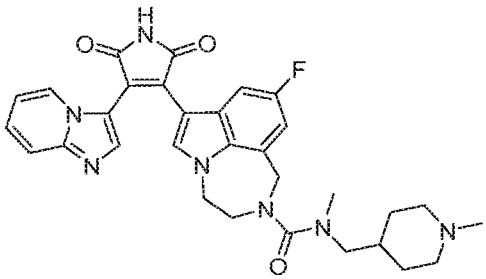
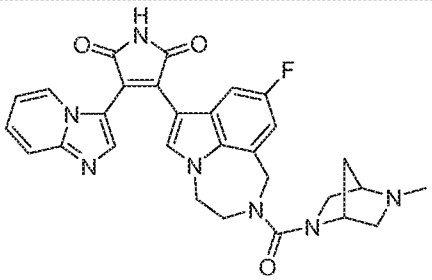
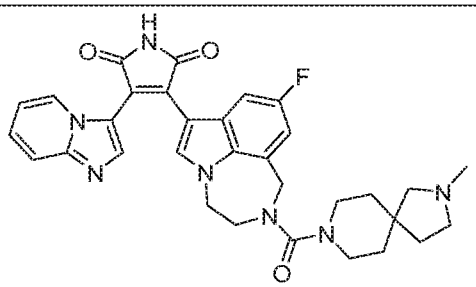
Compound I-12		3-(2-(3,3-difluoropiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-13		3-(2-((1R,4R)-2,5-diazabicyclo[2.2.1]heptane-2-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-14		2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-15		2-(3,3-difluoropiperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-16		2-(4,4-difluoropiperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-17		3-(2-(4,4-difluoropiperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

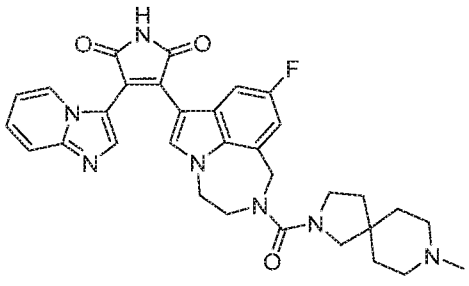
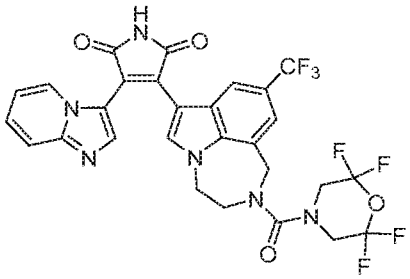
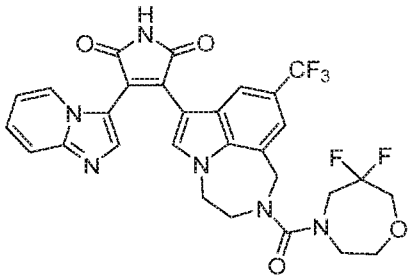
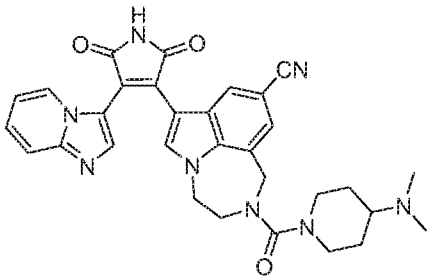
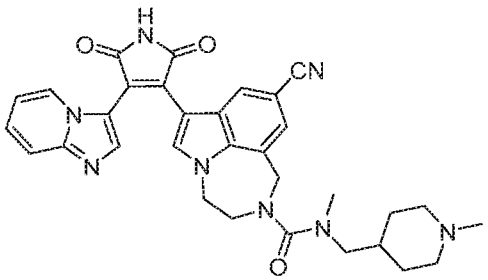
Compound I-18		3-(2-(8-oxa-3-azabicyclo[3.2.1]octane-3-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-19		3-(2-(4-(aminomethyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-20		3-(2-(4-(hydroxymethyl)piperidine-1-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-21		2-(4-(hydroxymethyl)piperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-22		3-(9-fluoro-2-(3,3,4,4,5,5-hexafluoropiperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

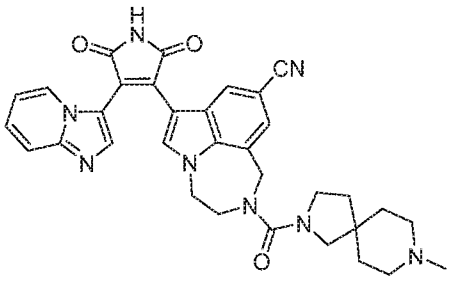
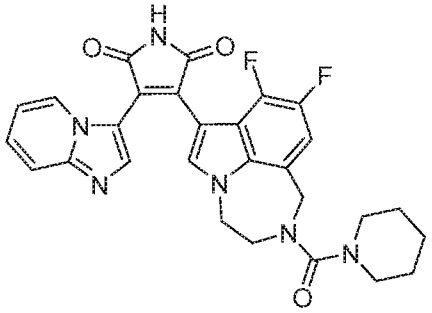
Compound I-23		3-(9-fluoro-2-(3,3,5,5-tetrafluoropiperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
1Compound I-24		3-(9-fluoro-2-(2,2,6,6-tetrafluoromorpholine-4-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-25		3-(2-(4,4-difluoro-3-hydroxypiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-26		3-(2-(4-(difluoro(hydroxy)methyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

Compound I-27		3-(2-(6,6-difluoro-1,4-oxazepane-4-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-28		3-([1,2,4]triazolo[4,3-a]pyridin-3-yl)-4-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione
Compound I-29		3-(9-fluoro-2-(piperidine-1-carbonyl-d10)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-30		3-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl-3,3,4,4-d4)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-31		3-(9-fluoro-2-(4-(2,2,2-trifluoro-1-hydroxyethyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

Compound I-32		3-(9-fluoro-2-(4-((methylamino)methyl)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-33		3-(2-(4-((dimethylamino)methyl)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-34		3-(2-(4-aminopiperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-35		3-(9-fluoro-2-(4-(methylamino)piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-36		3-(2-(4-(dimethylamino)piperidine-1-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

Compound I-37		9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-(piperidin-4-ylmethyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide
Compound I-38		9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-(piperidin-4-ylmethyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide
Compound I-39		9-fluoro-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-((1-methylpiperidin-4-yl)methyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide
Compound I-40		3-(9-fluoro-2-((1R,4R)-5-methyl-2,5-diazabicyclo[2.2.1]heptane-2-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-41		3-(9-fluoro-2-(2-methyl-2,8-diazaspiro[4.5]decane-8-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

Compound I-42		3-(9-fluoro-2-(8-methyl-2,8-diazaspiro[4.5]decane-2-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-43		3-(imidazo[1,2-a]pyridin-3-yl)-4-(2-(2,2,6,6-tetrafluoromorpholine-4-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-1H-pyrrole-2,5-dione
Compound I-44		3-(2-(6,6-difluoro-1,4-oxazepane-4-carbonyl)-9-(trifluoromethyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione
Compound I-45		2-(4-(dimethylamino)piperidine-1-carbonyl)-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-46		9-cyano-7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-N-methyl-N-((1-methylpiperidin-4-yl)methyl)-3,4-dihydro-[1,4]diazepino[6,7,1-hi]indole-2(1H)-carboxamide

Compound I-47		7-(4-(imidazo[1,2-a]pyridin-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-(8-methyl-2,8-diazaspiro[4.5]decane-2-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole-9-carbonitrile
Compound I-48		3-(8,9-difluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indol-7-yl)-4-(imidazo[1,2-a]pyridin-3-yl)-1H-pyrrole-2,5-dione

[0210] In other embodiments, the Wnt agonist and/or GSK3 inhibitor are as described in WO 2018/125746, US 20180214458 and USSN 62/608,663 the contents of which are each incorporated by reference in their entireties for all purposes.

EPIGENETIC MODULATORS

[0211] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following epigenetic modulators.

[0212] Epigenetic modulators included epigenetic modifiers, mediators and modulators. Epigenetic modifiers are genes whose products modify the epigenome directly through DNA methylation, the post-translational modification of chromatin or the alteration of the structure of chromatin. The epigenetic mediators, are often the target of epigenetic modification, although they are rarely mutated themselves. The epigenetic mediators largely overlap with the genes involved in stem cell reprogramming and their role in cancer followed directly from the discovery of their reprogramming role. Epigenetic mediators are those genes whose products are the targets of the epigenetic modifiers. Epigenetic modulators are the genes lying upstream of the modifiers and mediators in signaling and metabolic pathways

[0213] In some embodiments, an agent having activity as an epigenetic modulator is an HDAC inhibitor, a LSD-1 inhibitor, an EZH2 inhibitor, a DOT1L inhibitor, and a KDM inhibitor.

HDAC INHIBITORS

[0214] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following HDAC inhibitors.

[0215] Histone deacetylases (HDAC) are a class of enzymes that remove acetyl groups (O=C-CH_3) from an ε-N-acetyl lysine amino acid on a histone, allowing the histones to wrap the DNA more tightly. This is important because DNA is wrapped around histones, and DNA expression is regulated by acetylation and de-acetylation.

[0216] HDACs are classified in four classes depending on sequence homology to the yeast original enzymes and domain organization. The HDAC classes include HDACI, HDAC IIA, HDAC IIB, HDAC III and HDAC IV.

[0217] Histone deacetylase (HDAC) inhibitors (HDACi, HDIs) are chemical compounds that inhibit histone deacetylases.

[0218] Thus, "HDAC inhibitor" refers to an agent capable of the decreasing the expression or enzymatic activity of HDAC. For example treatment with an HDAC inhibitor results in a decrease in histone deacetylation of a target gene in a cell.

[0219] In certain embodiments, the HDAC inhibitor decreases the expression or enzymatic activity of HDAC by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0220] In certain embodiments, the HDAC inhibitor decreases histone deacetylation of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0221] In some embodiments, the HDAC inhibitor increases expression or activity of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0222] In some embodiments, the HDAC inhibitor decreases expression or enzymatic activity of HDAC by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0223] In some embodiments, the HDAC inhibitor decreases histone deacetylation of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0224] In some embodiments, the HDAC inhibitor increases expression or activity of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

Table 4

Agent	CAS	Chemo-type	Mechanism HDAC Inhib	Class selectivity	HDAC Potenc	Lgr5+ Assay	Perilymph Conc	Formulation Conc
Sodium Valproate	1069-66-5	Acid	1,2,3,8	Class I	39-161uM	100 uM -4 mM	100 uM - 4 mM	100 mM - 4000 mM
2-hexyl-4-pentynoic acid	96017-59-3	Acid	1,2,3,8	Class I	13 uM	100 uM - 4 mM	100 uM - 4 mM	100 mM - 4000 mM
Na		Acid	1,2,3,8	Class I	9-16uM	100	100 uM -	100 mM -

phenylbutyrate	1716-12-7			> Class IIb		uM - 4 mM	4 mM	4000 mM

[1] In various embodiments, the treatments disclosed herein include use an HDAC inhibitor. Exemplary HDAC inhibitors are provided in Table 17.

Table 17

Class	Agent	CAS
Aliphatic Acid	Butyrate	107-92-6
Aliphatic Acid	Phenyl butyrate	1821-12-1
Aliphatic Acid	Valproic Acid	99-66-1
Aliphatic Acid Ester	AN-9	122110-53-6
Amine	932718-22-4	932718-22-4
Benzamide	4SC-202	1186222-89-8
Benzamide	BML-210	537034-17-6
Benzamide	Chidamide	743438-44-0
Benzamide	Entinostat (MS-275)	209783-80-2
Benzamide	HDAC Inhibitor IV	537034-15-4
Benzamide	Mocetinostat (MGCD0103)	726169-73-9
Benzamide	NKL 22	537034-15-4
Benzamide	RGFP109	1215493-56-3
Benzamide	RGFP136	1215493-97-2
Benzamide	RGFP966	1357389-11-7
Benzamide	Tacedinaline	112522-64-2
Benzamide	TC-H 106, HDAC Inhibitor VII	937039-45-7
Cyclic peptide	Apicidin	183506-66-3
Cyclic peptide	Dihydrochlamydocin	52574-64-8
Cyclic peptide	HC Toxin	83209-65-8
Cyclic peptide	Romidepsin	128517-07-7
Cyclic Peptide	Thailandepsin A	1269219-30-8
Cyclic peptide	Trapoxin A	133155-89-2
Epoxide	(−)-Depudecin	139508-73-9
Epoxide	Parthenolide	20554-84-1
Hydroxamate	(S)-HDAC-42	935881-37-1
Hydroxamate	4-(dimethylamino)-N-[6-(hydroxyamino)-6-oxohexyl]-benzamide	193551-00-7
Hydroxamate	4-iodo-SAHA	1219807-87-0
Hydroxamate	4SC-201 (Resminostat)	864814-88-0
Hydroxamate	ACY1215	1316214-52-4
Hydroxamate	APHA Compound 8	676599-90-9
Hydroxamate	BRD9757	1423058-85-8
Hydroxamate	Bufexamac	2438-72-4
Hydroxamate	Butyrylhydroxamic acid	4312-91-8
Hydroxamate	CAY10603	1045792-66-2

Hydroxamate	CBHA	174664-65-4
Hydroxamate	CG200745	936221-33-9
Hydroxamate	CHR-3996	1256448-47-1
Hydroxamate	CUDC-101	1012054-59-9
Hydroxamate	Droxinostat	99873-43-5
Hydroxamate	HDAC Inhibitor II	174664-65-4
Hydroxamate	HDAC Inhibitor VI	926908-04-5
Hydroxamate	HDAC Inhibitor XXIV	854779-95-6
Hydroxamate	HDAC6 Inhibitor III	1450618-49-1
Hydroxamate	HDAC-IN-1	1239610-44-6
Hydroxamate	HNHA	926908-04-5
Hydroxamate	HPOB	1429651-50-2
Hydroxamate	ITF2357	497833-27-9
Hydroxamate	ITF2357 (Givinostat)	497833-27-9
Hydroxamate	LAQ-824	591207-53-3
Hydroxamate	LBH-589 (panobinostat)	404950-80-7
Hydroxamate	LMK235	1418033-25-6
Hydroxamate	M 344	251456-60-7
Hydroxamate	MC 1568	852475-26-4
Hydroxamate	Nexturastat A	1403783-31-2
Hydroxamate	NSC 57457	6953-61-3
Hydroxamate	Oxamflatin	151720-43-3
Hydroxamate	PCI-24781 (Abexinostat)	783355-60-2
Hydroxamate	PCI-34051	950762-95-5
Hydroxamate	PDX-101 (belinostat)	866323-14-0
Hydroxamate	Pyroxamide	382180-17-8
Hydroxamate	SAHA (Zolinza, vorinostat)	149647-78-9
Hydroxamate	SB939 (Pracinostat)	929016-96-6
Hydroxamate	SBHA	38937-66-5
Hydroxamate	Scriptaid	287383-59-9
Hydroxamate	Tefinostat (CHR-2845)	914382-60-8
Hydroxamate	Trichostatin A (TSA)	58880-19-6
Hydroxamate	Tubacin	537049-40-4
Hydroxamate	Tubastatin A	1252003-15-8
Hydroxamate	VAHA	106132-78-9
Ketone	Compound 43	891259-76-0
Ketone - a-ketoamides	436150-82-2	436150-82-2
Ketone - CF3	Compound 27	946499-86-1
Ketone - CF3	Compound 6e	946500-31-8
Ketone - CF3	Compound 6H	946500-39-6
Non classical	Tasquinimod	254964-60-8
Non classical	TMP269	1314890-29-3
Polyketide	Ratjadone A	163564-92-9
Silylalcohol	1587636-32-5	1587636-32-5
Sulphonamide	1587636-33-6	1587636-33-6
Sulphonamide	329967-25-1	329967-25-1

Sulphonyl Urea	960130-17-0	960130-17-0
Thioester	HDAC Inhibitor XXII	848354-66-5
Thioester	KD 5170	940943-37-3
Thioester	PTACH	848354-66-5
Thioester	TCS HDAC6 20b	956154-63-5
Thioketone	SIRT1/2 Inhibitor VII	143034-06-4
Thiol	1368806-68-1	1368806-68-1
Thiol	1428536-05-3	1428536-05-3
Thiol	827036-76-0	827036-76-0
Thiol	828920-13-4	828920-13-4
Thiol	908860-21-9	908860-21-9
Tropones	1411673-95-4	1411673-95-4
Tropones	46189-88-2	46189-88-2

[0225] In some embodiments the HDAC inhibitor is a class I HDAC inhibitor. In these embodiments, the class I HDAC inhibitor may be a short chain carboxylic acid. In a preferred embodiment, the HDAC inhibitor is valproic acid (VPA), 2-hexyl-4-pentynoic acid, or Na phenylbutyrate. More preferably, the HDAC inhibitor is valproic acid (VPA). In certain such embodiments, the HDAC inhibitor is sodium valproate.

[0226] As used herein the terms "valproic acid" and "VPA" are used interchangably to refer to the same compound. Moreover, as used herein the terms "valproic acid" and "VPA" also refer any pharmaceutically acceptable salts thereof.

LSD1 INHIBITORS

[0227] The one or more otic therapeutic agents in any embodiment disclosed herein could be one or more of the following LSD1 inhibitors.

[0228] LSD1 mediated H3K4 demethylation can result in a repressive chromatin environment that silences gene expression. LSD1 has been shown to play a role in development in various contexts. LSD1 can interact with pluripotency factors in human embryonic stem cells and is important for decommissioning enhancers in stem cell differentiation. Beyond embryonic settings, LSD1 is also critical for hematopoietic differentiation. LSD1 is overexpressed in multiple cancer types and recent studies suggest inhibition of LSD1 reactivates the all-trans retinoic acid receptor pathway in acute myeloid leukemia (AML). These studies implicate LSD1 as a key regulator of the epigenome that modulates gene expression through post-translational modification of histones and through its presence in transcriptional complexes.

[0229] Thus, a "LSD1 inhibitor" refers to an agent capable of decreasing the expression or enzymatic activity of LSD1. For example a LSD1 inhibitor results in a decrease in H3K4 demethylation of a target gene in a cell, for instance, in a cochlear cell or a vestibular. cell

[0230] In certain embodiments, a LSD1 inhibitor decreases the expression or enzymatic activity of LSD1 by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0231] In certain embodiments, a LSD1inhibitor decreases H3K4 demethylation by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0232] In some instances, a LSD1 inhibitor decreases H3K4 demethylation by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, or 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0233] In some instances, a LSD1 inhibitor modulates (i.e., increases or decreases) expression or activity of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0234] In some instances, a LSD1 inhibitor modulates (i.e., increases or decreases) expression or enzymatic activity of LSD1 by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0235] In some instances the LSD1 inhibitor is reversible. In other instances the LSD1 inhibitor is irreversible.

[0236] Exemplary agents having activity as a LSD1 inhibitor are provided in Table 18 below, including pharmaceutically-acceptable salts thereof.

Table 18.

Agent	CAS	pKi or IC50	Reversible or Irreversible	Chem o-type	Select KDM1b	Select MAOs A and B	Literat. Cell	Lgr5+ Assay	Perilymph Conc.	Formul. Conc. Intraymp.	Human Plasma Conc	Human Dosage
GSK-2879552	1401966-69-5	1.7 uM (0.11 uM)	Irreversible	Cyproy lamine		20 uM	EC50= 2--240 nM	40 nM - 30 uM	40 nM - 30 µM	40 uM to 30 mM	1-100 nM	1 or 2 mg QD PO
GSK-LSD1	1431368-48-7	16 nM	Irreversible	Cyproylamine	> 1000X	> 1000X		4 nM-50 uM	4 nM-50 uM	4 uM to 50 mM	1-100 nM	10-100 mg PO
Phenelzine sulfate	51-71-8	5.6 uM	Irreversible	Hydrazine		MAO inhibitor 900 nM in Cell	900 nM in Cell		0.1-10 uM	0.1-10 mM	Cmax 10 to 60 ng/mL (73-440 nM)	15-90 mg / day PO
TCP (Tranylcypromin e)	155-09-9	11-477 uM	Irreversible	Cyproylamine	186 uM	1 uM,		0.1-20 uM	0.1-20 uM	0.1-20 mM	Cmax 30-200 ng/ml (225 - 1500nM)	15-150 mg / day PO
CC-90011	2179319-65-2		Reversible	Likely pyrimidinyl								
GCG-11047 (PG-11047)	308145-19-9		Reversible	Polyamine								
IMG-7289	2229826-41-7		Irreversible	Likely Cyproylamine							Cmax 63 ng/ml	80 mg QD PO
INCB059872	1802909-49-4		Irreversible	Likely Cyproylamine								

ORY-1001 (RG6016, RO7051790, Iadademstat)	1431326 -61-2	<20 nM	Irreversible	Cyproylamine	>100 uM	>100 uM >100 uM	0.5-3 nM	Activity				Up to 2 mg
ORY-2001 (Vafidemstat)	1357362 -02-7		Irreversible	Cyproylamine								
Osimertinib (AZD9291)	1421373 -65-0	3.98 uM	Reversible	Pyrimidinyl			43 nM					10-80 mg
SP-2577 (Seclidemstat)	1423715 -37-0		Irreversible	Hydrazone								
	1821307 -10-1											
TCP Trans Chiral	3721-28-6	284 uM	Irreversible	Cyproylamine	137 uM	B: 4.4 uM						
TCP Trans Chiral	3721-26-4	168 uM	Irreversible	Cyproylamine	127 uM	B: 89 uM						
TCP Cis	13531-35-6		Irreversible	Cyproylamine		11 uM 19 uM						
TCP Cis Chiral	69684-88-4		Irreversible	Cyproylamine								
TCP Cis Chiral	69684-89-5		Irreversible	Cyproylamine								
RN-1	1781835 -13-9	70 nM	Irreversible	Cyproylamine		0.51 uM 2.78 uM		FHZ-455				
Compound 1	1221595 -26-1	10 nM	Irreversible	Cyproylamine								
Compound 45	1667721 -01-8	9 nM	Irreversible	Cyproylamine		15 uM >40 uM						

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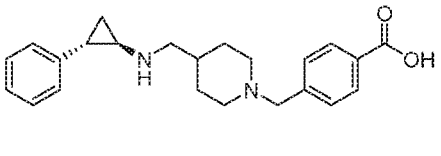
RN-7	1352345-82-4	31 nM	Irreversible	Cyproylamine								
Compound 5A	1613476-09-7	12 nM	Irreversible	Cyproylamine								
Compound 2	1235863-51-0	67 nM	Irreversible	Cyproylamine		>37 uM						
Compound 43	1784703-61-2	610 nM	Irreversible	Cyproylamine								
Compound 12f	1802319-25-0	86 nM	Irreversible	Cyproylamine		460 nM, >70 uM						
T-3775440	1422620-34-5	2.1 nM	Irreversible	Cyproylamine		110 nM, 17 uM						
OG-L002	1357299-45-6	20 nM	Irreversible	Cyproylamine								
S2101	1239262-36-2	990 nM	Irreversible	Cyproylamine								
NCL-1	1196119-03-5	1.6 uM	Irreversible	Cyproylamine								
Compound 9A	2095849-74-2	1.2 uM	Irreversible	Cyproylamine								
Compound 19l	2173543-81-0	0.97 uM	Irreversible	Cyproylamine								
NCD-25	1456972-46-5	480 nM	Irreversible	Cyproylamine								
NCD-38	2078047-42-2	590 nM	Irreversible	Cyproylamine								
Compound 14A	2247939-53-1	2.2 nM	Irreversible	Cyproylamine								
Compound 15A	2247939-55-3	70 nM	Irreversible	Cyproylamine								
Compound 15B	2247939-56-4	11 nM	Irreversible	Cyproylamine								

Compound 4	2226461-60-3	43 nM	Irreversible	Cyproylamine			3.8 uM					
Pargyline	555-57-7	1000 uM	Irreversible	Amino-propyne								
Peptide	945548-35-6		Irreversible	Amino-propyne								
Bizine	1591932-50-1	59 nM	Irreversible	Hydrazine				FHZ-457				
Compound 5a	1990536-90-7	1.4 nM	Reversible	Hydrazone								
Compound 5n	1990537-03-5	1.7 nM	Reversible	Hydrazone								
SP-2509 (HCI-2509)	1423715-09-6	13 nM	Reversible	Hydrazone		>300 uM	350-650 nM	Activity				
LSD1-IN-32	2137044-49-4	83 nM	Reversible	Amide			670 nM					
LSD1-IN-11p	2101951-67-9	20-80 nM	Reversible	Pyrazole			0.52 uM					
Resveratrol	501-36-0	15 uM	Reversible	Resveratrol								
Hydroxylamine	2035912-55-9	121 nM	Reversible	Resveratrol								
Compound 8c	2170023-28-4	283 nM	Reversible	Resveratrol			5 to 9 μM					
CBB-1007	1379573-92-8	2.1 μM	Reversible	Polyamine			IC50 ≤ 5 μM	Activity				
Namoline	342795-11-3	51 μM	Reversible	Benzopyran-4-one								
GSK-354	1841508-96-0	29-80 nM	Reversible	Diphpyridine		A>50uM	1.3 μM					
						B=19uM						
GSK-690	2101305-84-2	37 nM	Reversible	Diphpyridine								

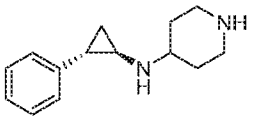
E11	1239589-91-3	243 nM	Reversible	2,4-Quinazolinediamine								
MC2694	1435055-66-5	1 uM	Reversible	2,4-Quinazolinediamine								
Alpha-mangostin	11/1/6147	2.8 uM	Reversible	mangostin								
Compound 12 A	1923750-07-5	0.41 uM	Reversible	Barbituate								
Compound 4	126118-57-8	6.4 uM	Reversible	Purine-2,6-dione								
Compound 10d	2226997-31-3	4 uM	Reversible	Carboxamide								
Compound 90	1884266-15-2	162 nM	Reversible	Carboxamide								
Compound 46	1884266-36-7	8 nM	Reversible	Carboxamide			1-4 uM					
Compound 49	1884266-49-2	7 nM	Reversible	Carboxamide			1-4 uM					
Compound 50	1884266-48-1	8 nM	Reversible	Carboxamide			1-4 uM					
Polymyxin B	1404-26-8	157 nM	Reversible	Polymyxin B								
Polymyxin E	1066-17-7	193 nM	Reversible	Polymyxin E								
Baicalin	21967-41-9	3.0 uM	Reversible	Baicalin								
Compound 16Q	1612870-90-2	9.5 uM	Reversible	Benzenesulfonamide		>500 uM						
LSD1 inhibitor 24	1853269-07-4	1 nM	Reversible	Imidazole								
geranylgeranoic acid	35750-48-2	120 uM	Reversible	Geranyl								

Geranylgeraniol	24034-73-9	80 uM	Reversible	Geranyl								
Thiocarbamate	1430852-56-4	390 nM	Reversible	Thiocarbamate		>1250 uM						
Thiourea	1637373-61-5	650 nM	Reversible	Thiourea		>1250 uM						
Thiourea	2035417-23-1	154 nM	Reversible	Thiourea								
Thienopyrrole	1206028-57-0	2.9 uM	Reversible	Thienopyrrole	>100 uM	>100uM, 57nM						
Thienopyrrole	1884266-15-2	162 nM	Reversible	Thienopyrrole								
Thienopyrrole	1884266-48-1	7.8 nM	Reversible	Thienopyrrole	13 uM	41 uM, 100 uM						
4SC-202	910462-43-0	1-10 uM	Reversible	o-aminoph								25-400 mg /Day
ORY-3001	2179325-30-3											
JL1037												
FLI-06	313967-18-9	92 nM	Inhibits expression of LSD1	Dihydropyridine								
Rhodium Complex 1		40 nM		Rhodium								

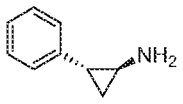
[0237] In some embodiments, an agent having activity as a LSD1 inhibitor is GSK-2879552, GSK-LSD1, Osimertinib (AZD9291), Phenelzine sulfate, Tranylcypromine (TCP), ORY-1001, Seclidemstat (SP-2577), Vafidemstat (ORY-2001), CC-90011, IMG-7289 or, INCB059872. Preferably, the LSD1 inhibitor is GSK-2879552, GSK-LSD1, Phenelzine sulfate or Tranylcypromine (TCP).



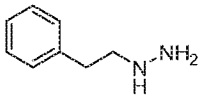
GSK-2879552



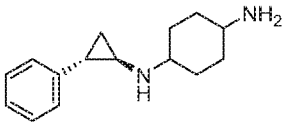
GSK-LSD1



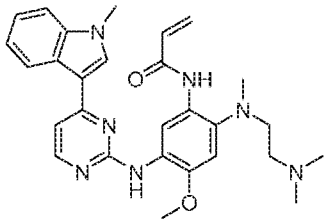
Tranylcypromine (TCP)



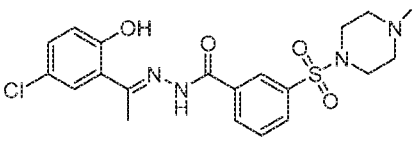
Phenelzine sulfate



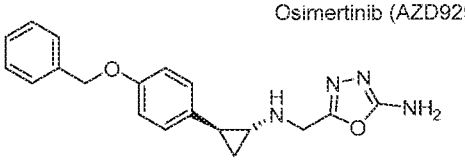
ORY-1001



Osimertinib (AZD9291)



Seclidemstat (SP-2577)



Vafidemstat (ORY-2001)

[0238] More preferably, the LSD1 inhibitor is GSK-2879552, GSK-LSD-1, or Tranylcypromine (TCP).

EZH2 INHIBITORS

[0239] The one or more otic therapeutic agents in any embodiment disclosed herein could be one or more of the following EZH2 inhibitors.

[0240] Enhancer of zeste homolog 2 (EZH2) is a histone-lysine N-methyltransferase enzyme encoded by an EZH2 gene, that participates in histone methylation and, ultimately, transcriptional repression. EZH2 catalyzes the addition of methyl groups to histone H3 at lysine 27, by using the cofactor S-adenosyl-L-methionine. Methylation activity of EZH2 facilitates heterochromatin formation thereby silences gene function. Remodeling of chromosomal heterochromatin by EZH2 is also required during cell mitosis.

[0241] EZH2 is the functional enzymatic component of the Polycomb Repressive Complex 2 (PRC2), which is responsible for healthy embryonic development through the epigenetic maintenance of genes responsible for regulating development and differentiation. EZH2 is responsible for the methylation activity of PRC2, and the complex also contains proteins required for optimal function (EED, SUZ12, JARID2, AEBP2, RbAp46/48, and PCL).

[0242] EZH2 inhibitors are chemical compounds that inhibit histone-lysine N-methyltransferase enzyme encoded by EZH2 gene

[0243] Thus, "EZH2 inhibitor" refers to an agent capable of the decreasing the expression or enzymatic activity of EZH2. For example, an EZH2 inhibitor results in a decrease in histone methylation of a target gene in a cell.

[0244] In certain embodiments, the EZH2 inhibitor decreases the expression or enzymatic activity of EZH2 by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0245] In certain embodiments, the EZH2 inhibitor decreases histone methylation of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0246] In some embodiments, the EZH2 inhibitor increases expression or activity of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0247] In some embodiments, the EZH2 inhibitor decreases expression or enzymatic activity of EZH2 by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0248] In some embodiments, the EZH2 inhibitor decreases histone methylation of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0249] In some embodiments, the EZH2 inhibitor increases expression or activity of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0250] Exemplary EZH2 inhibitors are provided in Table 19.

Table 19

Agent	CAS	pKi or IC50	Enzymatic /Non-enzymatic	Chemo-type	Select vs EZH-1	Lit Cell Poten	Lgr5+ Assay	Perilymph Conc	Formul. Conc. Intraymp	Human In Vivo Conc	Human Dosage
PF-06821497	1844849-10-0	<1 nM	enzymatic	2-Pyridone	70 nM	4-6 nM		1-100 nM	1-100 uM	5-50 nM	75 mg to 625 mg BID PO
CPI-1205	1621862-70-1	2.2 nM	enzymatic	2-Pyridone	24x	32 nM		10-1000 nM	10-1000 uM	25-250 nM	800 mg BID and subsequently TID - PO
Valemetostat (DS-3201b, (R)-OR-S2)	1809336-39-7	2.5 nM	enzymatic	2-Pyridone	8.4 nM	25-250 nM		10-1000 nM	10-1000 uM	25-250 nM	PO starting dose of 100 mg QD with dose escal dep on tox
Tazemetostat (EPZ-6438)	1403254-99-8	2.5 nM	enzymatic	2-Pyridone	35x		0.37-1.1 uM	0.37-1.1 uM	0.1-10 mM	100-800 ng/ml (200-1600 nM)	PO 100 BID to 800 mg BID.
El1	1418308-27-6	13 nM	enzymatic	2-Pyridone	90x		5 uM	1-10 uM	1-10 mM	1-10 uM	(100 to 1000 / daymg PO)
CPI-169		0.24 nM	enzymatic	2-Pyridone	6 nM			1-10 uM	1-10 mM	1-10 uM	100 to 1000 / day mg PO
(R)-OR-S1	1809336-19-3	10 nM	enzymatic	2-Pyridone	7.4 nM						IV 50mg – poor oral bio.
A-395	2089148-72-9	0.3 nM	EED Inhibit	Amino pyrrolidines		90 nM					50mg and 200mg PO

Astemizole	68844-77-9	94 uM	EED Inhibit	Benzimidazole							
Compound 19	2079895-22-8	1.3 uM	EED Inhibit	Imidazole		1.9 uM					
Compound 22	1802175-07-0	2 nM	enzymatic	2-Pyridone							
Compound 24	1659298-29-9	40 nM	enzymatic	2-Pyridone							
Compound 34	2055347-72-1	29 nM	enzymatic	2-Pyridone	>100x						
Compound 41	2055347-94-7	11 nM	enzymatic	2-Pyridone	>100x						
CPI-0169	1450655-76-1	0.24 nM	enzymatic	2-Pyridone	6 nM						
CPI-0169	1802175-07-0	0.24 nM	enzymatic	2-Pyridone	6 nM		1.1 uM				
CPI-0209											
CPI-360	1802175-06-9	0.5 nM	enzymatic	2-Pyridone	~50 nM						
EBI-2511	2098546-05-3	4 nM	enzymatic	2-Pyridone							
EED162	1010897-73-0	30 nM	EED Inhibit	Triazo		80 nM					
EED226	2083627-02-3		EED Inhibit	Triazo			Activity				
EPZ-005687	1396772-26-1	24 nM	enzymatic	2-Pyridone	50X						
EPZ-011989	1598383-40-4	<3 nM	enzymatic	2-Pyridone		94 nM					
GSK126	1346574-57-9	<3 nM	enzymatic	2-Pyridone	150x						
GSK343	1346704-33-3	1.2 nM	enzymatic	2-Pyridone	60x	174 nM					

GSK503	1346572-63-1	<10 nM	enzymatic	2-Pyridone							
GSK926	1346704-13-9	7.9 nM	enzymatic	2-Pyridone		324 nM					
MAK683 (EED162)	1951408-58-4 (likely patent)		EED inhibitor	Triazo							
SHR2554	2098545-98-1		enzymatic	2-Pyridone							
SKLB1049	1826865-42-2	7.2 nM	enzymatic	2-Pyridone		12 uM					
ZLD1039	1826865-46-6	<15 nM	enzymatic	2-Pyridone							
ZLD1122	1826865-51-3	<15 nM	enzymatic	2-Pyridone							
	1404094-15-0	74 nM	enzymatic	2-Pyridone		2510 nM					
	1404094-16-1	14 nM	enzymatic	2-Pyridone		1995 nM					
DZNep	102052-95-9		SAH-hydrolase inhibitor	SAH derived		1 uM	Activity				
Cmpd 44	1378002-93-7	32 nM	SAM Comp	Benzamide		9 uM					
Compound 27	1676100-59-6	270 nM	SAH-hydrolase inhibitor	SAH derived							
Sinefungin	58944-73-3	20 nM	SAH-hydrolase inhibitor	SAH derived		33 nM					

Tanshindiol B	97465-70-8	520 nM	enzymatic	Tanshindiols							
Tanshindiol C	97465-71-9	550 nM	enzymatic	Tanshindiols							
UNC1999	1431612-23-5	10 nM	enzymatic	2-Pyridone	10x	124 nM	Activity				
(-)-Epigallocatechin-3-gallate (EGCG)	989-51-5		enzymatic	a,b-unsat							
Curcumin	458-37-7		enzymatic	a,b-unsat							
MC1945	169903-68-8		enzymatic	a,b-unsat							
MC1947	949090-12-4		Non-enzymatic								
MC1948	949090-20-4		Non-enzymatic								
SAH-EZH2			Non-enzymatic	reactive							
Sulforaphane	4478-93-7		EED Inhibit	Stapled Peptide							

[0251] In some embodiments the EZH2 inhibitor is PF-06821497, CPI-120, Valemetostat, Tazemetostat or E11.

DOT1L INHIBITORS

[0252] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following DOT1L inhibitors.

[0253] DOT1-like (Disruptor of telomeric silencing 1-like), histone H3K79 methyltransferase (S. cerevisiae), also known as DOT1L, is a protein found in humans, as well as other eukaryotes. The methylation of histone H3 lysine 79 (H3K79) by DOT1L which is a conserved epigenetic mark in many eukaryotic epigenomes, increases progressively along the aging process.

[0254] DOT1L inhibitors are chemical compounds that inhibits histone H3K79 methyltransferase .

[0255] Thus, "DOT1L inhibitor" refers to an agent capable of the decreasing the expression or enzymatic activity of DOT1L. For example, an EZH2 inhibitor results in a decrease in histone methylation of a target gene in a cell.

[0256] In certain embodiments, the DOT1L inhibitor decreases the expression or enzymatic activity of DOT1L by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0257] In certain embodiments, the DOT1L inhibitor decreases histone methylation of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0258] In some embodiments, the DOT1L inhibitor increases expression or activity of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0259] In some embodiments, the DOT1L inhibitor decreases expression or enzymatic activity of DOT1L by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0260] In some embodiments, the DOT1L inhibitor decreases histone methylation of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0261] In some embodiments, the DOT1L inhibitor increases expression or activity of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0262] Exemplary DOT1L inhibitors are provided in Table 20.

Table 20

Agent	CAS	pKi or IC50	Chemo-type	Lit Cell	Lgr5+ Assay	Perilymph Conc	Formulation Conc. Intratym	Human In Vivo Conc	Human Dosage
EPZ004777	1338466-77-5	0.3 nM	Adenosine	11 nM	0.6-45uM	0.6-45 uM	0.1-45 mM	0.1-45 uM	10-100 mg/m2 per day IV
Pinometostat (EPZ-5676)	1380288-87-8	0.08 nM	Adenosine	2.7 nM		0.1-10 uM	0.1-10 mM	Total plasma Css 800-1600 ng/mL (1.42-2.94 uM) (1-10 uM)	54-90 mg/m2 per day by continuous IV, Potential for SC dosing
SGC0946	1561178-17-3	0.3 nM	Adenosine	10 nM	0.6-5uM	0.6-5 uM	0.6-5 mM	0.1-5 uM	10-100 mg/m2 per day IV
Bromo-deaza-SAH	1428254-21-0	77 nM	Adenosine						
CN SAH	1985669-27-9	13 nM	Adenosine						
Compound 10	1645266-99-4	29 nM	Adenosine						
Compound 13	1940206-71-2	0.4 nM	Aminopyrimidine						
Compound 7	2088518-50-5	<1 nM	pyrrolopyrimidine						
Compound 8	1940224-84-9	14 nM	Acetylene						
EPZ002696	1381760-94-6	13 nM	Adenosine						
EPZ004450	1380315-97-8	4 nM	Adenosine						
SAH	979-92-0	600 nM	Adenosine						
SYC-522	138176	0.76	Adenosine	6					

	1-52-9	nM		u M					
SYC-687	144050 9-94-3	1.1 nM	Non-Ribose	20 0 n M					
	144051 0-03-1, 144050 9-94-3	1.1 nM	Adenosine	20 0 n M					
Compoun d 21			Peptides						
Compoun d 28			Peptides						
Compoun d 6	167558 -34-1	8.3 uM	triazolothiadiazol						
Compoun d 8H			pyrimidylaminoquin oline						
	116372 9-79-0	1.5 uM	pyrimidine						

[0263] In some embodiments the DOT1L inhibitor is EPZ004777, Pinometostat or SGC0946.

KDM INHIBITORS

[0264] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following KDM inhibitors.

[0265] About 30 JmjC domain-containing proteins have been identified as lysine demethylases in the human genome. Based on histone lysine sites and demethylation states, the JmjC domain-containing protein family is divided into six subfamilies: KDM2, KDM3, KDM4, KDM5, KDM6 and PHF. The JmjC domain-containing proteins belong to the Fe(II) and 2-oxoglutarate (2-OG)-dependent dioxygenases, which demethylate a variety of targets, including histones (H3K4, H3K9, H3K27, H3K36 as well as H1K26) and non-histone proteins. Unlike the LSD family, the JmjC-domain-containing histone demethylases (JHDMs) are able to erase all three kinds of histone lysine-methylation states since the JHDMs do not require protonated nitrogen for demethylation.

[0266] The KDM2 (also named FBXL) subfamily includes two members: KDM2A and KDM2B. KDM4 gene family, first identified in silico, consists of six members, including KDM4A, KDM4B, KDM4C, KDM4D, KDM4E and KDM4F. The KDM5 subfamily contains four enzymes: KDM5A, KDM5B, KDM5C and KDM5D, which specifically remove methyl marks from H3K4me2/3. In the human genome, the KDM6 subfamily is comprised of KDM6A, KDM6B and UTY, which share a well-conserved JmjC histone catalytic domain.

[0267] KDM inhibitors are chemical compounds that inhibits lysine demethylases.

[0268] Thus, "KDM inhibitor" refers to an agent capable of the decreasing the expression or enzymatic activity of KDM. For example, an KDM inhibitor results in a decrease in histone demethylation of a target gene in a cell.

[0269] In certain embodiments, the KDM inhibitor decreases the expression or enzymatic activity of KDM by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0270] In certain embodiments, the KDM inhibitor decreases histone demethylation of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0271] In some embodiments, the KDM inhibitor increases expression or activity of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0272] In some embodiments, the KDM inhibitor decreases expression or enzymatic activity of KDM by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0273] In some embodiments, the KDM inhibitor decreases histone demethylation of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0274] In some embodiments, the KDM inhibitor increases expression or activity of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity. Exemplary KDM inhibitors are provide in Table 21.

Table 21

Agent	CAS	Chemo-type	Covalent or not	Select KDM 1	Select KDM 2	Select KDM 3	Select KDM 4	Select KDM 5	Select KDM 6	Select KDM 7	Select KDM 8	Lit Cell	Lgr5 + Assay	Perilymph Conc	Formulation Conc	Human In Vivo Conc	Human Dosage
AS 8351	796-42-9	Hydrazone											1-3 uM	1-3 uM	1-3 mM	1-3 uM	100-2000mg/day
TC-E 5002	1453071-47-0	Hydroxamate	No		6.8 uM		83 uM	55 uM	>100 uM	200, 1200 nM		16-40 uM	0.12-10uM	0.12-10 uM	0.12-10 mM	0.12-10 uM	100-1000mg/day
EPT-103182							20-50	<1 nM	3K			1.8 nM	1-100 nM	1-100 nM	1-100 uM	5-50 nM	10 mg to 1000 mg/day
Compound 54k	1844064-06-7	pyrimidin-4-one	No				102/31nM	23 nM									
Cmpd 1	1516899-38-9	Cyproamine isonicotinic acid	Yes		220 nM			190 nM		220 nM							
Cmpd 105	1613514-89-8	Isonicotinic acid	No					<100 nM				0.1-1 uM					
Compound 34	1461602-86-7	Isonicotinic acid	No				<100 nM	<100 nM									
Compound 41	1628332-52-4	pyridopyrimidinone	No					<100 nM									
compound 48	1628210-26-3	cyanopyrazole	No					15 nM				340 nM					
Compound 18	1993438-65-5	naphthyridones	No					206 nM				>10 uM					
Compound 33	1613410-75-5	pyrazolylpyridines	No					10 nM				~1nM					
Compound 48	1905482-57-6	Amide	No									1-10 uM					

Compound 48	1905482-57-6	Pyrazole	No														
Compound 49	1905481-35-7	Pyrazole	No														
Compound 50	1905481-36-8	Pyrazole	No														
Compound 6	2169272-46-0	1-H-Indole	No					50 nM									
Compound R-35	1807514-47-1	Triazole	No					65 nM				1.5 uM					
CPI-455	1628208-23-0	cyanopyrazole	No				41 nM	13, 2 nM				90 nM					
CPI-4203	1628214-07-2	cyanopyrazol	No					1.1 uM									
E67-2	1364914-62-4	Quinazoline	No														
GSK-467	1628332-52-4	Pyrazole	No					14 nM									
GSK-J1	1373422-53-7	Acid	No									50 uM					
GSK-J4	1373423-53-0	Ethyl Ester	No					170 nM	28 nM								
KDM5-C49	1596embodiment-16-1	Pyridine	No														
KDM5-C50	1596348-32-1	Pyridine	No														
KDOAM25	2230731-99-2	Amide	No														
N11	1613515-45-9	isonicotinic	No					65 nM				1600					
	1807514-47-1	Amide	No					90 nM									

	1844064-07-8	Pyridopyrimidinone	No					45 nM		960						
Compound 1		Rh Complex														
Compound 15e	1498096-89-6	Hydrazine						X								
Damnacanthal	1596-84-5	Hydrazine			X					X						
JIB-04	99596-05-9	Hydrazine						220 nM								
Methylstat	13108877-95-2	Unsaturnide														
Compound 10r	2098902-68-0	cyanopyrazole	No													
N71			Yes					X								
NSC 6369819								410 nM								

[0275] In some embodiments the KDM inhibitor is AS 8351 or TC-E 5002.

TAZ ACTIVATORS

[0276] The one or more otic therapeutic agents in any embodiment disclosed herein could be one or more of the following TAZ activators.

[0277] TAZ motif (also called WWTR1) a transcriptional coactivator with a PDZ-binding was identified as a 14-3-3-binding protein. It is similar to Yes-associated protein 1 (YAP1) in its molecular structure, which consists of an N-terminal TEAD binding domain, one or two WW domains, and a transcriptional activation domain.

[0278] TAZ is phosphorylated at four sites by large tumor suppressor kinase 1 (LATS1) and LATS2, which are core kinases of the Hippo pathway. Phosphorylated TAZ is trapped by 14-3-3, is recruited from the nucleus to the cytoplasm, and undergoes protein degradation. In this way, the Hippo pathway negatively regulates TAZ.

[0279] In addition to the Hippo pathway, TAZ is regulated by cell junction proteins such as ZO-1, ZO-2, and angiomotin. Recent studies have revealed that TAZ is under the control of the actin cytoskeleton and the mechanical stretch. Moreover, Wnt signaling stabilizes. Conversely, cytoplasmic TAZ binds -catenin and Dishevelled (DVL) and inhibits -catenin nuclear localization and DVL phosphorylation to negatively regulate the Wnt pathway.

[0280] TAZ activators are chemical compounds that stabilize and increase unphosphorylated TAZ levels.

[0281] Thus, "TAZ activator" refers to an agent capable of the increasing the stability or activity of TAZ. For example, an TAZ activator results in a decrease in TAZ phosphorylation and/or TAZ protein degradation .

[0282] In certain embodiments, the TAZ activator increases the stability or activity of TAZ by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0283] In certain embodiments, the TAZ activator increases the expression of a target gene by at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% relative to a control, for example relative to a baseline level of activity.

[0284] In some embodiments, the TAZ activator increases the stability or activity of TAZ by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0285] In some embodiments, increases the expression of a target gene by at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more relative to a control, for example relative to a baseline level of activity.

[0286] Exemplary TAZ Activators are provide in Table 22.

Table 22

Agent	CAS	Chemo-type	Mechanism	Lit Cell	Lgr5+ Assay	Perilymph Conc	Formulation Conc	Human In Vivo Conc	Human Dosage
IBS008738	371128-48-2	Hydrazone	TAZ Activ.		1.1-30 uM	1.1-30 uM	1.1-30 mM		25-500 mg
TM-25659	260553-97-7	AT II	TAZ Activ.	10-100 uM	10-100 uM	10-100 uM	10-100 mM		25-500 mg
TT10	2230640-94-3	Thiazole	TAZ Activ.	1 uM	1-10 uM	1-10 uM	1-10 mM		25-500 mg
IBS003031	381177-81-7	Acridine	YAP Activ.						
TAZ12	371128-48-2	Thiazole	TAZ Activ.						
TM-53	1257247-76-9	AT II	TAZ Activ.	10-100 uM					
TM-54	1257247-77-0	AT II	TAZ Activ.	10-100 uM					
(-)-epicatechin gallate	1257-08-5	Nat Prod							
Ethacridine	1837-57-6	Acridine			Activity				
Ethacridine	1837-57-6	Acridine			Activity				
kaempferol	520-18-3	Nat Prod							
KR 62980	867187-61-9	N-Oxide							
phorbaketal A	1196507-03-5	Nat Prod							

[0287] In some embodiments the TAZ activator is IBS008738, TM-25659 or TT10.

[0288] In some embodiments the agents are a gamma- secretase inhibitor, a Taz activator, a Notch inhibitor, or an ErbB3/HER3 inhibitor.

GAMMA SECRETASE INHIBITORS

[0289] The one or more otic therapeutic agents in any embodiment disclosed herein could be one or more of the following gamma secretase inhibitors.

[0290] Gamma secretase is an internal protease that cleaves within the membrane-spanning domain of its substrate proteins, including amyloid precursor protein (APP) and Notch.

[0291] Sequential cleavages of the APP by β- and γ-secretases generates Aβ. First, APP is proteolytically processed by β-secretase (BACE1) and generates a 12 kDa C-terminal stub of APP (C99); second, C99 is cleaved by γ-secretase to yield two major species of Aβ ending at residue 40 (Aβ40) or 42 (Aβ42).

[0292] Gamma secretase inhibitors may target γ-secretase and reduce Aβ production.

[0293] Exemplary gamma secretase inhibitors are provided in Table 23

Table 23

Agent	CAS	Chemo-type	Lit cell conc	Human Dosage
Semagacestat LY 450139	425386-60-3	Amide	Aβ38, Aβ40, and Aβ42 with IC50=12.0, 12.1, 10.9 nM,	60 mg-140 mg
Begacestat / GSI-953	769169-27-9	Sulphonamide	Lowers Aβ42, Aβ40 (EC50=12.4, 14.8 nM in cells	10 and 50-mg
Avagacestat / BMS-708163	114669 9-66-2	Sulphonamide	IC50 = 0.27 and 0.30 nM for Aβ42 and Aβ40,	25 to 125 mg
EVP-0962				10, 50, 100 or 200 mg
Crenigacestat LY 3039478 (JSMD194)	142143 8-81-4	Amide	IC50 of ~1nM in most of the tumor cell lines	2.5 mg - 100 mg
MK-0572	471905-41-6	Acid	SH-SY5Y cells with an IC50 value of 5 nM	
NIC5-15				800-2000 mg
NGP 555	130463 0-27-0	Heterocycle	10 nM	100 mg, 200 mg, or 400 mg
Nirogacestat PF 03084014	129054 3-63-3	Amide	(IC50 values are 1.2 and 6.2 nM in whole cell and cell-free assays	150 mg
PF-06648671	158772 7-31-8	Amide		300 mg
RO4929097	847925-91-1	Amide		20 mg, 30 mg, 45 mg, 90 mg or140 mg
BMS-905024	140106 6-79-2	Amide		
BMS-932481	126387	Heterocyc	IC50 at 6.6 to reduce Aβ42	

	1-36-8	le		
BMS-986133			IC50 3.5 nM to reduce Aβ42	
BMS 299897	290315-45-6	Sulphonamide	Inhibits Aβ40 and Aβ42 in vitro (IC50 7.4 and 7.9 nM	
BPN-15606	1914989-49-3	Heterocycle	IC50 of 7 nM and 17 nM to reduce Aβ42 and Aβ40 cells	
Carprofen	53716-49-7	Acid	76 uM	
CHF5022	749269-77-0	Acid		
CHF5074	749269-83-8	Acid	reduces Aβ42 and Aβ40 secretion, IC50 3.6, 18.4 μM	
Compound E	209986-17-4	Amide		
Compound W	173550-33-9	Acid	neuronal culture (IC50 115, 200 nM for total Aβ, Aβ42	
DAPT	208255-80-5	Amide	neuronal culture (IC50 115, 200 nM for total Aβ, Aβ42	
DBZ	209984-56-5	Amide		
E-2012	870843-42-8	Unsaturamide		
EVP-A			IC50 reduction of Aβ40 and Aβ42 0.24 μM and 0.14 μM,	
EVP-B			IC50 reduction of Aβ40 and Aβ42 0.24 μM and 0.14 μM,	
EVP-0015962	1447811-26-8	Acid		
Flurizan	51543-40-9	Acid		
GSI-136	443989-01-3	Sulphonamide		
Indomethacin	53-86-1	Acid	25-50 uM	
JLK 6	62252-26-0	Aniline	30 μM	
JNJ-40418677	1146594-87-7	Acid	0.18 - 0.2 μM,	
L-685,458	292632-98-5	Peptide	48 -67 nM	
LY 411575	209984-57-6	Amide		
Deshydroxy LY-411575	209984-56-5	Amide		
MDL 28170	88191-84-8	Amide		
MRK 560	677772-84-8	Sulphonamide	0.65 nM	
MW167				
NMK-T-057				

Suldinac sulfide	2004-67-4	Acid	34 uM	

NOTCH INHIBITORS

[0294] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following Notch inhibitors.

[0295] Exemplary Notch inhibitors are provided in Table 24

Table 24

Agent	CAS
3H4MB	1958071-88-9
BMS-871	15894631-89-9
EDD3	25279-15-6
ELN-46719	1576239-16-1
FLI-06	313967-28-9
IMR-1	310456-65-6
JLK6	62252-26-0
TAPI-1	171235-71-5
Natural Products	
Honokiol	
epigallocatechin-3-gallate (EGCG)	
3,5-bis(2,4-difluorobenzylidene)-4-piperidone (DiFiD)	
curcumin	
3,3'-diindolylmethane (DIM)	
resveratrol	
Antibodies	
MEDI0639	

ERBB3/HER3 INHIBITORS

[0296] The one or more otic therapeutic agents in any embodiment disclosed could be one or more of the following ErbB3/HER3 inhibitors.

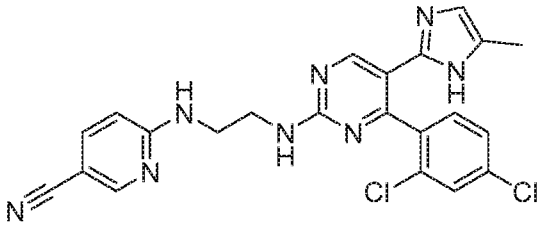
[0297] Exemplary ErbB3/HER3 inhibitors are provided in Table 25.

[0298] Table 25

Agent	CAS	HER 3 pKi or IC50	HER1	HER 2	HER4
Bosutinib / SKI-606	380843-75-4	0.77 nM		2500 nM	26 nM
Dasatinib / KIN001-5	302962-49-8	18 nM		1400 nM	55 nM
Sapitinib / AZD8931 /	848942-61-0	4 nM	4 nM	3 nM	
Vandetanib		180 nM		2600 nM	480 nM
WS3	1421227-52-2	74 nM			
WS6	1421227-53-3	280 nM			
Afatinib	850140-72-6			14 nM	
Erlotinib	183321-74-6	1100 nM		2900 nM	230 nM
Gefitinib	184475-35-2	790 nM		3500 nM	410 nM
KIN001-51					
KIN001-111					
Lapatinib	231277-92-2	5500 nM		7 nM	54 nM
Neratinib	698387-09-6			59 nM	
poziotinib	1092364-38-9		3 nM	5 nM	23 nM
TX2-121-1	1603845-42-6				
WS1	936099-44-4	3.8 uM			
AV-203					
Duligotuzumab					
Elgemtumab LJM716/					
GSK2849330					
KTN3379 / CDX-3379					
Lumretuzumab RG7116					
Patritumab / U3--128 7					
Seribantumab / MM-121					
U3--1402					
MEHD7945A / Duligotumab					
MCLA-128					
MM-111					
MM-141 / Istiratumab					

[0299] In some embodiments the ErbB3/HER3 inhibitors is WS3 or WS6.

[0300] In some embodiments, at least one hearing loss treatment agent is CHIR99021:



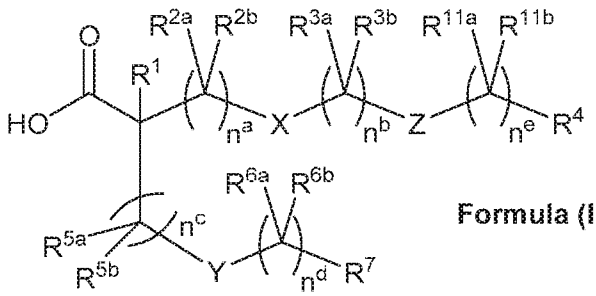
(CHIR99021),

or a pharmaceutical acceptable salt thereof.

[0301] Pharmaceutically acceptable salts include, for example salts formed by reacting any of the weakly basic active agents described herein, such as CHIR99021, with a pharmaceutically acceptable acid known in the art. A non-limiting list of suitable acid salts include hydrochloride, hydrobromide, citrate, malate, mesylate, phosphate, tartrate, hydrochloride, tosylate, glucuronate, ethanesulfonate, fumarate, sulfate, napthalene-2-sulfonate, ascorbate, oxalate, napthalene-1,5-disulfonate, malonate, aminosalicylate, benzenesulfonate, isethionate, genistate, 1-hydroxy-2-napthoate, dichloroacetate, cyclamate, and ethane-1,2-disulfonate.

[0302] In some embodiments, the composition of the present disclosure may comprise a compound of formula (I) or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of formula (I) may also be an otic therapeutic agent. In some embodiments, wherein the compound of formula (I) is an otic therapeutic agent, it may be included in compositions of the present disclosure that comprise one or more otic therapeutic agents. In some embodiments, the compound of formula (I) may also be a hearing loss treatment agent. In some embodiments, the compound of formula (I) may be an HDAC inhibitor. In some embodiments, the compound of formula (I) or a pharmaceutically acceptable salt thereof is included in lyophilized pharmaceutical compositions of the present disclosure. In some embodiments, the compound of formula (I) or a pharmaceutically acceptable salt thereof is included in reconstituted pharmaceutical compositions of the present disclosure.

[0303] A compounds of formula (I), or a pharmaceutically acceptable salt has the following structure:



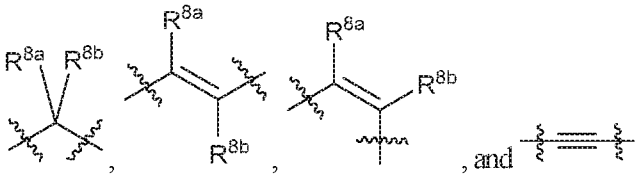
Formula (I)

wherein:

R^1 is selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{2a} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{2b} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

X is selected from  , and , or is not present;

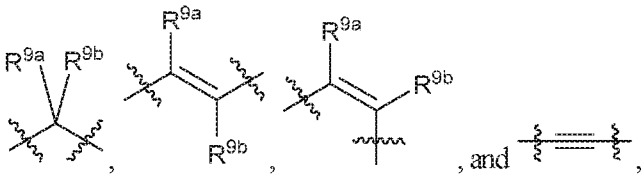
R^{3a} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{3b} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{4} is selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{5a} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{5b} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

Y is selected from  , and , or is not present;

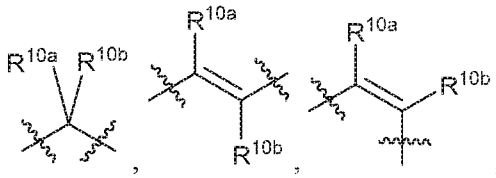
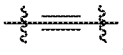
R^{6a} is selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{6b} is selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

each R^{7} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{8a} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{8b} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

Z is selected from  , and  , or is not present;

R^{10a} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{10b} is independently selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{11a} is selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

R^{11b} is selected from H, alkyl, alkoxy, halo, cycloalkyl, alkenyl, alkynyl, carbocyclyl, and aryl;

n^{a} is selected from 0, 1, 2, 3, 4, 5, 6, 7, and 8;

n^{b} is selected from 0, 1, 2, 3, and 4;

n^{c} is selected from 0, 1, and 2;

n^{d} is selected from 0, 1, and 2; and;

n^e is selected from 0, 1, 2, 3, 4, 5, and 6.

[0304] In some embodiments, R^1 is H. In some embodiments, R^1 is alkyl. In some embodiments, R^1 is alkoxy. In some embodiments, R^1 is halo. In some embodiments, R^1 is cycloalkyl. In some embodiments, R^1 is alkenyl. In some embodiments, R^1 is alkynyl. In some embodiments, R^1 is carbocyclyl. In some embodiments, R^1 is aryl.

[0305] In some embodiments, R^{2a} is H. In some embodiments, R^{2a} is alkyl. In some embodiments, R^{2a} is alkoxy. In some embodiments, R^{2a} is halo. In some embodiments, R^{2a} is cycloalkyl. In some embodiments, R^{2a} is alkenyl. In some embodiments, R^{2a} is alkynyl. In some embodiments, R^{2a} is carbocyclyl. In some embodiments, R^{2a} is aryl. In some embodiments, R^{2b} is H. In some embodiments, R^{2b} is alkyl. In some embodiments, R^{2b} is alkoxy. In some embodiments, R^{2b} is halo. In some embodiments, R^{2b} is cycloalkyl. In some embodiments, R^{2b} is alkenyl. In some embodiments, R^{2b} is alkynyl. In some embodiments, R^{2b} is carbocyclyl. In some embodiments, R^{2b} is aryl.

[0306] In some embodiments, R^{3a} is H. In some embodiments, R^{3a} is alkyl. In some embodiments, R^{3a} is alkoxy. In some embodiments, R^{3a} is halo. In some embodiments, R^{3a} is cycloalkyl. In some embodiments, R^{3a} is alkenyl. In some embodiments, R^{3a} is alkynyl. In some embodiments, R^{3a} is carbocyclyl. In some embodiments, R^{3a} is aryl. In some embodiments, R^{3b} is H. In some embodiments, R^{3b} is alkyl. In some embodiments, R^{3b} is alkoxy. In some embodiments, R^{3b} is halo. In some embodiments, R^{3b} is cycloalkyl. In some embodiments, R^{3b} is alkenyl. In some embodiments, R^{3b} is alkynyl. In some embodiments, R^{3b} is carbocyclyl. In some embodiments, R^{3b} is aryl.

[0307] In some embodiments, R^4 is H. In some embodiments, R^4 is alkyl. In some embodiments, R^4 is alkoxy. In some embodiments, R^4 is halo. In some embodiments, R^4 is cycloalkyl. In some embodiments, R^4 is alkenyl. In some embodiments, R^4 is alkynyl. In some embodiments, R^4 is carbocyclyl.

[0308] In some embodiments, R^4 is aryl.

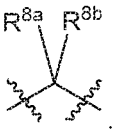
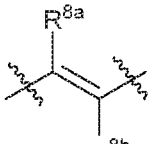
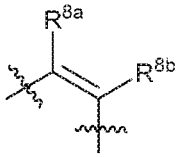
[0309] In some embodiments, R^{5a} is H. In some embodiments, R^{5a} is alkyl. In some embodiments, R^{5a} is alkoxy. In some embodiments, R^{5a} is halo. In some embodiments, R^{5a} is cycloalkyl. In some embodiments, R^{5a} is alkenyl. In some embodiments, R^{5a} is alkynyl. In some embodiments, R^{5a} is carbocyclyl. In some embodiments, R^{5a} is aryl.

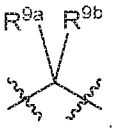
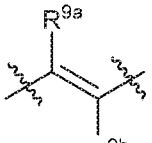
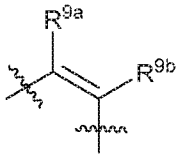
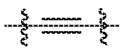
[0310] In some embodiments, R^{5b} is H. In some embodiments, R^{5b} is alkyl. In some embodiments, R^{5b} is alkoxy. In some embodiments, R^{5b} is halo. In some embodiments, R^{5b} is cycloalkyl. In some embodiments, R^{5b} is alkenyl. In some embodiments, R^{5b} is alkynyl. In some embodiments, R^{5b} is carbocyclyl. In some embodiments, R^{5b} is aryl.

[0311] In some embodiments, R^{6a} is H. In some embodiments, R^{6a} is alkyl. In some embodiments, R^{6a} is alkoxy. In some embodiments, R^{6a} is halo. In some embodiments, R^{6a} is cycloalkyl. In some embodiments, R^{6a} is alkenyl. In some embodiments, R^{6a} is alkynyl. In some embodiments, R^{6a} is carbocyclyl. In some embodiments, R^{6a} is aryl.

[0312] In some embodiments, R^{6b} is H. In some embodiments, R^{6b} is alkyl. In some embodiments, R^{6b} is alkoxy. In some embodiments, R^{6b} is halo. In some embodiments, R^{6b} is cycloalkyl. In some embodiments, R^{6b} is alkenyl. In some embodiments, R^{6b} is alkynyl. In some embodiments, R^{6b} is carbocyclyl. In some embodiments, R^{6b} is aryl.

[0313] In some embodiments, R^7 is H. In some embodiments, R^7 is alkyl. In some embodiments, R^7 is alkoxy. In some embodiments, R^7 is halo. In some embodiments, R^7 is cycloalkyl. In some embodiments, R^7 is alkenyl. In some embodiments, R^7 is alkynyl. In some embodiments, R^7 is carbocyclyl. In some embodiments, R^7 is aryl.

[0314] In some embodiments, X is  . In some embodiments, X is  . In some embodiments, X is  . In some embodiments, X is  . In some embodiments, X is not present.

[0315] In some embodiments, Y is  . In some embodiments, Y is  . In some embodiments, Y is  . In some embodiments, Y is  . In some embodiments, Y is not present.

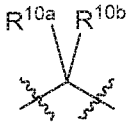
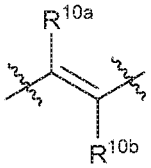
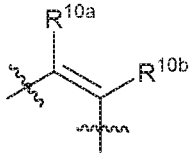
[0316] In some embodiments, R^{8a} is H. In some embodiments, R^{8a} is alkyl. In some embodiments, R^{8a} is alkoxy. In some embodiments, R^{8a} is halo. In some embodiments, R^{8a} is cycloalkyl. In some embodiments, R^{8a} is alkenyl. In some embodiments, R^{8a} is alkynyl. In some embodiments, R^{8a} is carbocyclyl. In some embodiments, R^{8a} is aryl.

[0317] In some embodiments, R^{8b} is H. In some embodiments, R^{8b} is alkyl. In some embodiments, R^{8b} is alkoxy. In some embodiments, R^{8b} is halo. In some embodiments, R^{8b} is cycloalkyl. In some embodiments, R^{8b} is alkenyl. In some embodiments, R^{8b} is alkynyl. In some embodiments, R^{8b} is carbocyclyl. In some embodiments, R^{8b} is aryl.

[0318] In some embodiments, R^{9a} is H. In some embodiments, R^{9a} is alkyl. In some embodiments, R^{9a} is alkoxy. In some embodiments, R^{9a} is halo. In some embodiments, R^{9a} is cycloalkyl. In some embodiments, R^{9a} is alkenyl. In some embodiments, R^{9a} is alkynyl. In some embodiments, R^{9a} is carbocyclyl. In some embodiments, R^{9a} is aryl.

[0319] In some embodiments, R^{9b} is H. In some embodiments, R^{9b} is alkyl. In some embodiments, R^{9b} is alkoxy. In some embodiments, R^{9b} is halo. In some embodiments, R^{9b} is cycloalkyl. In some embodiments, R^{9b} is

alkenyl. In some embodiments, R^{9b} is alkynyl. In some embodiments, R^{9b} is carbocyclyl. In some embodiments, R^{9b} is aryl.

[0320] In some embodiments, Z is  . In some embodiments, Z is  . In some embodiments, Z is  . In some embodiments, Z is  . In some embodiments, Z is not present.

[0321] In some embodiments, R^{10a} is H. In some embodiments, R^{10a} is alkyl. In some embodiments, R^{10a} is alkoxy. In some embodiments, R^{10a} is halo. In some embodiments, R^{10a} is cycloalkyl. In some embodiments, R^{10a} is alkenyl. In some embodiments, R^{10a} is alkynyl. In some embodiments, R^{10a} is carbocyclyl. In some embodiments, R^{10a} is aryl.

[0322] In some embodiments, R^{10b} is H. In some embodiments, R^{10b} is alkyl. In some embodiments, R^{10b} is alkoxy. In some embodiments, R^{10b} is halo. In some embodiments, R^{10b} is cycloalkyl. In some embodiments, R^{10b} is alkenyl. In some embodiments, R^{10b} is alkynyl. In some embodiments, R^{10b} is carbocyclyl. In some embodiments, R^{10b} is aryl.

[0323] In some embodiments, R^{11a} is H. In some embodiments, R^{11b} is alkyl. In some embodiments, R^{11a} is alkoxy. In some embodiments, R^{11a} is halo. In some embodiments, R^{11a} is cycloalkyl. In some embodiments, R^{11a} is alkenyl. In some embodiments, R^{11a} is alkynyl. In some embodiments, R^{11a} is carbocyclyl. In some embodiments, R^{11a} is aryl.

[0324] In some embodiments, R^{11b} is H. In some embodiments, R^{11b} is alkyl. In some embodiments, R^{11b} is alkoxy. In some embodiments, R^{11b} is halo. In some embodiments, R^{11b} is cycloalkyl. In some embodiments, R^{11b} is alkenyl. In some embodiments, R^{11b} is alkynyl. In some embodiments, R^{11b} is carbocyclyl. In some embodiments, R^{11b} is aryl.

[0325] In some embodiments, n^a is 0. In some embodiments, n^a is 1. In some embodiments, n^a is 2. In some embodiments, n^a is 3. In some embodiments, n^a is 4. In some embodiments, n^a is 5. In some embodiments, n^a is 6. In some embodiments, n^a is 7. In some embodiments, n^a is 8.

[0326] In some embodiments, n^b is 0. In some embodiments, n^b is 1. In some embodiments, n^b is 2. In some embodiments, n^b is 3. In some embodiments, n^b is 4.

[0327] In some embodiments, n^c is 0. In some embodiments, n^c is 1. In some embodiments, n^c is 2.

[0328] In some embodiments, n^d is 0. In some embodiments, n^d is 1. In some embodiments, n^d is 2.

[0329] In some embodiments, n^e is 0. In some embodiments, n^e is 1. In some embodiments, n^e is 2. In some embodiments, n^e is 3. In some embodiments, n^e is 4. In some embodiments, n^e is 5. In some embodiments, n^e is 6.

[0330] In some embodiments, R^1 is Me. In some embodiments, R^{2a} is Me. In some embodiments, R^{2b} is Me. In some embodiments, R^{3a} is Me. In some embodiments, R^{3b} is Me. In some embodiments, R^4 is Me. In some embodiments, R^{5a} is Me. In some embodiments, R^{5b} is Me. In some embodiments, R^{6a} is Me. In some embodiments, R^{6b} is Me. In some embodiments, R^7 is Me. In some embodiments, R^{8a} is Me. In some embodiments, R^{8b} is Me. In some embodiments, R^{9a} is Me. In some embodiments, R^{9b} is Me. In some embodiments, R^{10a} is Me. In some embodiments, R^{10b} is Me. In some embodiments, R^{11a} is Me. In some embodiments, R^{11b} is Me.

[0331] In some embodiments, R^1 is F. In some embodiments, R^{2a} is F. In some embodiments, R^{2b} is F. In some embodiments, R^{3a} is F. In some embodiments, R^{3b} is F. In some embodiments, R^4 is F. In some embodiments, R^{5a} is F. In some embodiments, R^{5b} is F. In some embodiments, R^{6a} is F. In some embodiments, R^{6b} is F. In some embodiments, R^7 is F. In some embodiments, R^{8a} is F. In some embodiments, R^{8b} is F. In some embodiments, R^{9a} is F. In some embodiments, R^{9b} is F. In some embodiments, R^{10a} is F. In some embodiments, R^{10b} is F. In some embodiments, R^{11a} is F. In some embodiments, R^{11b} is F.

[0332] In some embodiments, R^1 is alkyl. In some embodiments, R^{2a} is alkyl. In some embodiments, R^{2b} is alkyl. In some embodiments, R^{3a} is alkyl. In some embodiments, R^{3b} is alkyl. In some embodiments, R^4 is alkyl. In some embodiments, R^{5a} is alkyl. In some embodiments, R^{5b} is alkyl. In some embodiments, R^{6a} is alkyl. In some embodiments, R^{6b} is alkyl. In some embodiments, R^7 is alkyl. In some embodiments, R^{8a} is alkyl. In some embodiments, R^{8b} is alkyl. In some embodiments, R^{9a} is alkyl. In some embodiments, R^{9b} is alkyl. In some embodiments, R^{10a} is alkyl. In some embodiments, R^{10b} is alkyl. In some embodiments, R^{11a} is alkyl. In some embodiments, R^{11b} is alkyl.

[0333] In some embodiments, alkyl is methyl. In some embodiments, alkyl is ethyl. In some embodiments, alkyl is n-propyl. In some embodiments, alkyl is iso-propyl. In some embodiments, alkyl is n-butyl. In some embodiments, alkyl is sec-butyl. In some embodiments, alkyl is iso-butyl. In some embodiments, alkyl is tert-butyl.

[0334] In some embodiments, alkoxy is methoxy. In some embodiments, alkoxy is ethoxy. In some embodiments, alkoxy is n-propoxy. In some embodiments, alkoxy is iso-propoxy. In some embodiments, alkoxy is n-butoxy. In some embodiments, alkoxy is sec-butoxy. In some embodiments, alkoxy is iso-butoxy. In some embodiments, alkoxy is tert-butoxy.

[0335] In some embodiments, halo is F. In some embodiments, halo is Cl. In some embodiments, halo is Br. In some embodiments, halo is I.

[0336] In some embodiments, cycloalkyl is cyclopropyl. In some embodiments, cycloalkyl is cyclobutyl. In some embodiments, cycloalkyl is cyclopentyl. In some embodiments, cycloalkyl is cyclohexyl.

[0337] In some embodiments, aryl is phenyl. In some embodiments, aryl is tolyl. In some embodiments, aryl is xylyl.

[0338] In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with methyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with ethyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted

with n-propyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b},is further substituted with iso-propyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with n-butyl.

[0339] In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with sec-butyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with iso-butyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with tert-butyl.

[0340] In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with methoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with ethoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with n-propoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b}, is further substituted with iso-propoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with n-butoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with sec-butoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with iso-butoxy. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with tert-butoxy.

[0341] In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with F. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with Cl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with Br. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with I.

[0342] In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with cycloalkyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with alkenyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with alkynyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with carbocyclyl. In some embodiments, one of R^1, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^4, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^7, R^{8a}, R^{8b}, R^{9a}, R^{9b}, R^{10a}, R^{10b}, R^{11a}, and R^{11b} is further substituted with aryl.

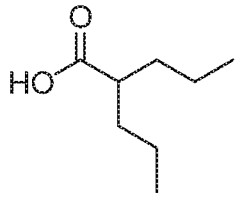
[0343] In some embodiments, the compound of formula (I) is valproic acid or a pharmaceutically acceptable salt thereof.

[0344] In some embodiments, the compound of formula (I) is 2-(prop-2-yn-1-yl)-octanoic acid or a pharmaceutically acceptable salt thereof.

[0345] In some embodiments, the compound of formula (I) is linoleic acid or a pharmaceutically acceptable salt thereof.

[0346] In some embodiments, the compound of formula (I) is phenylbutyric acid or a pharmaceutically acceptable salt thereof.

[0347] In some embodiments, at least one hearing loss treatment agent is valproic acid:



(valproic acid),

or a pharmaceutical acceptable salt thereof (e.g., sodium valproate). A non-limiting list of other suitable valproate salts includes potassium valproate, lithium valproate, etc. A further non-limiting list of other suitable of valproate salts includes sodium valproate, valproate semisodium, magnesium divalproate (magnesium valproate), calcium divalproate (calcium valproate). Valproic acid is also referred to as VPA. Sodium valproate is also referred to as NaVPA.

[0348] In some embodiments, at least one hearing loss treatment agent is CHIR99021 or a pharmaceutical acceptable salt thereof, and at least one hearing loss treatment agent is valproic acid or a pharmaceutical acceptable salt thereof (e.g., sodium valproate).

[0349] In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are CHIR99021 or a pharmaceutical acceptable salt thereof, and valproic acid or a pharmaceutical acceptable salt thereof (e.g., sodium valproate).

[0350] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium valproate.

[0351] In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are CHIR99021 and sodium valproate.

[0352] In some embodiments the at least one otic therapeutic agent is LY2090314 or a pharmaceutically acceptable salt thereof.

[0353] In some embodiments, at least one hearing loss treatment agent is LY2090314 or a pharmaceutical acceptable salt thereof.

[0354] In some embodiments, at least one hearing loss treatment agent is LY2090314 or a pharmaceutical acceptable salt thereof, and at least one hearing loss treatment agent is valproic acid or a pharmaceutical acceptable salt thereof (e.g., sodium valproate).

In some embodiments, the one or more otic therapeutic agents (e.g., hearing loss treatment agents) are LY2090314 and sodium valproate.

Gelling Agents

[0355] As used herein, the term "gelling agent" refers to an agent capable of imparting a gel-like or thickening quality to the pharmaceutical composition or reconstituted solution of the present disclosure upon being subjected to a gelling condition (e.g., a particular temperature or temperature range, the presence of an ion, a pH value or range, or a concentration of gelling agent that causes the gelling agent to undergoing a change or transition from low viscosity to high viscosity, or the reverse). In some embodiments, the gelling condition is a particular temperature (e.g., about 26 °C, about 27 °C, about 28 °C, about 29 °C, about 30 °C, about 31 °C, about 32 °C, about 33 °C, about 34 °C, about 35 °C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, or about 40 °C). In some embodiments, the gelling condition is a particular temperature range (e.g., about 26 °C or higher, about 27 °C or higher, about 28 °C or higher, about 29 °C or higher, about 30 °C or higher, about 31 °C or higher, about 32 °C or higher, about 33 °C or higher, about 34 °C or higher, about 35 °C or higher, about 36 °C or higher, about 37 °C or higher, about 38 °C or higher, about 39 °C or higher, or about 40 °C or higher). In some embodiments, the gelling agent provides a viscosity of between about 1,000 and 10,000,000 centipoise, between about 5,000 and 5,000,000 centipoise, or between about 100,000 and 4,000,000 centipoise, to the pharmaceutical composition or reconstituted solution of the present disclosure. In some embodiments, the gelling agent provides a viscosity of between about 50,000 and 2,000,000 centipoise to the pharmaceutical composition or reconstituted solution of the present disclosure.

[0356] In some embodiments, prior to gelling (e.g., at ambient temperature (e.g., between about 20 °C and about 26 °C)), the gelling agent provides a viscosity of less than about 100,000 centipoise, less than about 50,000 centipoise, 20,000 centipoise, less than about 10,000 centipoise, less than about 8,000 centipoise, less than about 7,000 centipoise, less than about 6,000 centipoise, less than about 5,000 centipoise, less than about 4,000 centipoise, less than about 3,000 centipoise, less than about 2,000 centipoise, or less than about 1,000 centipoise to the pharmaceutical composition or reconstituted solution of the present disclosure.

[0357] In some embodiments, upon gelling (e.g., at the temperature of a human body (e.g., between about 35 °C to about 39 °C, between about 36 °C to about 38 °C, or at about 37 °C)), the gelling agent provides a viscosity of greater than about 1,000 centipoise, greater than about 5,000 centipoise, greater than about 10,000 centipoise, greater than about 20,000 centipoise, greater than about 50,000 centipoise, greater than about 60,000 centipoise, greater than about 70,000 centipoise, greater than about 80,000 centipoise, greater than about 90,000 centipoise, or greater than about 100,000 centipoise.

[0358] In some embodiments, upon gelling (e.g., at the temperature of a human body (e.g., between about 36 °C to about 39 °C, or at about 37 °C)), the viscosity of the pharmaceutical composition or reconstituted solution of the present disclosure, as measured in units of centipoise, being about 2 fold or greater, about 5 fold or greater, about 10

fold or greater, about 20 fold or greater, about 50 fold or greater, about 60 fold or greater, about 7 fold or greater, about 80 fold or greater, about 90 fold or greater, about 100 fold or greater as compared to the viscosity of the pharmaceutical composition or reconstituted solution prior to gelling (e.g., at ambient temperature (e.g., at about 25 °C)).

[0359] It is understood that the gelling condition (e.g., gelling temperature) of the pharmaceutical composition or reconstituted solution of the present disclosure may be measured with a variety of techniques in the art. In some embodiment, the gelling temperature is determined using a commercially available rheomoeter having a parallel plate geometry (e.g., with plate distance ranging from 0.5 mm to 1.0 mm). In some embodiments, the analysis is performed over a continuous temperature range (e.g., 15 °C to 40 °C) at a constant rate (e.g., 2 to 3 °C/min) and a deformation frequency of 0.74 Hz to 1 Hz. The gelation temperature is determined at the temperature whereby the shear storage modulus (G') and the shear loss modulus (G'') are equal.

[0360] In some embodiments, the gelling agent comprises acacia, alginic acid, bentonite, poly(acrylic acid) (Carbomer), carboxymethyl cellulose, ethylcellulose, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, magnesium aluminum silicate (Veegum), methylcellulose, poloxamer, hyaluronic acid sodium, polylacticglycolic acid sodium, chitosan, polyvinyl alcohol, sodium alginate, tragacanth, xanthan gum, or any combination thereof. In some embodiment, the gelling agent comprises poloxamer. In some embodiments, the gelling agent comprises hyaluronic acid. In some embodiments, the gelling agent is hyaluronic acid. In some embodiments the hyaluronic has a MW average of between 7.0 x 10^5 Daltons and 8.5 10^5 Daltons. In some embodiments the hyaluronic has a MW average of 8.23 x 10^5 Daltons. In some embodiments, the hyaluronic acid is 'HA1M' provided by Lifecore Bio. In some embodiments the hyaluronic acid is a 0.5-5% aq. solution. In some embodiments the hyaluronic acid is a 1-3% aq. solution. In some embodiments, the hyaluronic acid has an average MW of 8.23 x 10^5 Daltons and is prepared as a 1-3% aq. solution.

[0361] In some embodiments, the gelling agent comprises acacia. In some embodiments, the gelling agent comprises alginic acid. In some embodiments, the gelling agent comprises bentonite. In some embodiments, the gelling agent comprises poly(acrylic acid) (Carbomer). In some embodiments, the gelling agent comprises carboxymethyl cellulose. In some embodiments, the gelling agent comprises ethylcellulose. In some embodiments, the gelling agent comprises gelatin. In some embodiments, the gelling agent comprises hydroxyethyl cellulose. In some embodiments, the gelling agent comprises hydroxypropyl cellulose. In some embodiments, the gelling agent comprises magnesium aluminum silicate (Veegum). In some embodiments, the gelling agent comprises methylcellulose. In some embodiments, the gelling agent comprises poloxamer. In some embodiments, the gelling agent comprises hyaluronic acid sodium. In some embodiments, the gelling agent comprises hyaluronic acid. In some embodiments, the gelling agent comprises polylacticglycolic acid sodium. In some embodiments, the gelling agent comprises chitosan. In some embodiments, the gelling agent comprises polyvinyl alcohol. In some

embodiments, the gelling agent comprises sodium alginate. In some embodiments, the gelling agent comprises tragacanth. In some embodiments, the gelling agent comprises xanthan gum. In some embodiments, the gelling agent comprises a cellulosic derivative (e.g., carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and/or methylcellulose).

[0362] In some embodiments, the gelling agent is a thermoreversible gelling agent.

[0363] As used herein, the term "thermoreversible" refers to a capability of being reversible by the application of heat. The "thermoreversible gelling agent" refers to an agent capable of reversibly imparting a gel-like or thickening quality to the pharmaceutical composition or reconstituted solution of the present disclosure upon application of heat

[0364] In some embodiments, the thermoreversible gelling agent comprises a poloxamer.

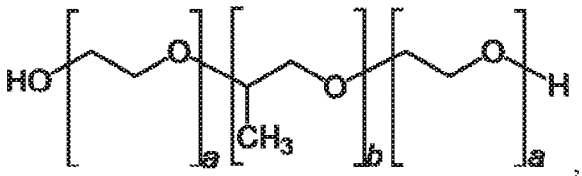
[0365] In some embodiments, poloxamer forms a thermoreversible gel. For example, with the application of heat to a solution of a poloxamer, the viscosity of the solution increases. The viscosity of the solution can increase to the extent that the solution forms a gel. In some embodiments, the solution of poloxamer forms a gel at about body temperature (37 °C). In some embodiments, the solution of poloxamer forms an immobile gel at about body temperature. In preferred such embodiments, the solution of poloxamer is a composition comprising further components, such as one or more otic therapeutic agents and/or valproic acid or a pharmaceutically acceptable salt thereof.

[0366] In certain embodiments it can be useful for a thermoreversible gelling agent disclosed herein to be a gel when at body temperature but a liquid when below body temperature. For example, it may be a liquid in order for it to be injected into the ear (for example the middle ear). Thermoreversible gelling agents are known in the art, for example those polymers that reversibly impart a gel-like or thickening quality upon application of heat disclosed in Shalaby et al. Water-Soluble Polymers, ACS Symposium Series, American Chemical Societry, 1991 (Chapter 33). Those include those polymers that have those properties are also disclosed in Molyneaux, P. "Water-Soluble Polymers: Properties and Behavior", CRC Press, Vol. I, p.58, Vol. II, p.86, New York, 1982; Prasad, K.N., Luong, T.T., Florence, A.T., Paris, J., Vaution, C., Seiller, M. and Puisieux, F., J. Colloid Interface Sci., 69, 225(1979); A.V. Kabanov et al. / Journal of Controlled Release 82 (2002) 189–212; Peppas and Khare, Advanced Drug Delivery Reviews, 11 (1993) 1-35; US6316011B1; US 4474751; US4478822; US6346272 and US4188373. Any thermoreversible gelling agent disclosed in these references, and in particular those that are a gel when at body temperature but a liquid when below body temperature, can be used as a gelling agent in all aspects and options disclosed herein.

[0367] It is undersood that the gelling agent (e.g., the thermoreversible gelling agent) may also be a bulking agent of the pharmaceutical composition or reconstituted solution of the present disclosure. In some embodiments, a poloxamer (e.g., poloxamer 407) is the gelling agent and/or the bulking agent of the pharmaceutical composition or

reconstituted solution of the present disclosure. Poloxomers are a general class of commercially available and pharmaceutically acceptable triblock copolymers of polyethylene oxide-polypropylene oxide-polyethylene oxide which exhibit relatively low viscosity at low temperatures (e.g., room tempature or below) but much high viscosities at elevated temperatures (e.g., body temperatures of approximately 37°C) whereby compositions containing such thermoreversible gelling agents effectively solidify in place. Other thermoreversible gelling agents such as polyethylene oxide – polylactic acid- polyethylene oxide polymers are also suitable in various embodiments of the present invention.

[0368] Poloxamers are a general class of commercially available triblock copolymers that in certain embodiments can be used as the gelling agent. More specifically, such poloxamers can comprise a central hydrophobic chain of polyoxypropylene (poly(propylene oxide) or PPO) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide) or PEG). This forms an A-B-A structure, shown below:



, where a = 2–130 and b = 15–70.

[0369] In some embodiments, a is 10–120. In some embodiments, a is 20–120. In some embodiments, a is 30–120. In some embodiments, a is 40–120. In some embodiments, a is 50–120. In some embodiments, a is 60–120. In some embodiments, a is 70–120. In some embodiments, a is 80–120. In some embodiments, a is 90–120. In some embodiments, a is 100–120. In some embodiments, a is 110–120. In some embodiments, a is 10–110. In some embodiments, a is 20–110. In some embodiments, a is 30–110. In some embodiments, a is 40–110. In some embodiments, a is 50–110. In some embodiments, a is 60–110. In some embodiments, a is 70–110. In some embodiments, a is 80–110. In some embodiments, a is 90–110. In some embodiments, a is 100–110. In some embodiments, a is 10–100. In some embodiments, a is 20–100. In some embodiments, a is 30–100. In some embodiments, a is 40–100. In some embodiments, a is 50–100. In some embodiments, a is 60–100. In some embodiments, a is 70–100. In some embodiments, a is 80–100. In some embodiments, a is 90–100. In some embodiments, a is 95–105. In some embodiments, a is 95–115. In some embodiments, a is 85–105. In some embodiments, a is 85–115. In some embodiments, b is 25–70. In some embodiments, b is 35–70. In some embodiments, b is 45–70. In some embodiments, b is 55–70. In some embodiments, b is 60–70. In some embodiments, b is 65–70. In some embodiments, b is 56 +/- 10%, and each a is 101 +/- 10%. In some embodiments, b is 61 +/- 15%, and each a is 101 +/- 10%. In some embodiments, b is 70 +/- 20%, and each a is 101 +/- 20%. In some embodiments, b is 56 +/- 10%, and each a is 100 +/- 10%. In some embodiments, b is 61 +/- 15%, and each a is 100 +/- 10%. In some embodiments, b is 70 +/- 20%, and each a is 100 +/- 10%.

[0370] In certain embodiments, Poloxamers are also known by the tradenames of: Synperonics, Pluronics, and Kolliphor. For the generic term poloxamer, these copolymers are commonly named with the letter P (for

poloxamer) followed by three digits: the first two digits multiplied by 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit multiplied by 10 gives the percentage polyoxyethylene content (e.g. P407 = poloxamer with a polyoxypropylene molecular mass of 4000 g/mol and a 70% polyoxyethylene content). For the Pluronic and Synperonic tradenames, coding of these copolymers starts with a letter to define its physical form at room temperature (L = liquid, P = paste, F = flake (solid)) followed by two or three digits. The first digit (two digits in a three-digit number) in the numerical designation, multiplied by 300, indicates the approximate molecular weight of the hydrophobe; and the last digit x 10 gives the percentage polyoxyethylene content (e.g., L61 indicates a polyoxypropylene molecular mass of 1800 g/mol and a 10% polyoxyethylene content). In the example given, poloxamer 181 (P181) = Pluronic L61 and Synperonic PE/L 61 .

[0371] For Poloxamer 407 (P407), the approximate lengths of the two PEG blocks is about 100 repeat units while the approximate length of the propylene glycol block is about 56-67 repeat units (where about is +/- 10%). P407 is also known by the BASF trade name Pluronic F127 or by the Croda trade name Synperonic PE/F 127.

[0372] Poloxamers can also be composed of a central hydrophilic chain of polyoxyethylene (poly(ethylene oxide) or PEG) flanked by two hydrophobic chains of polyoxypropylene (poly(propylene oxide)). This forms an analogous B-A-B structure. Other PPO-PEG block copolymers exist, such as those that comprise four PPO-PEO chains, which extend outward from an amine-terminated central chain (e.g. N-CH_2-CH_2-N), and in certain embodiments the disclosed compositions can comprise one or more of such four block polymers. (either in addition to or instead of the poloxamers otherwise disclosed herein).

[0373] In some embodiments, the poloxamer (e.g., poloxamer 407) is the gelling agent and the bulking agent of the pharmaceutical composition or reconstituted solution of the present disclosure. In some embodiments, the presence of the poloxamer (e.g., poloxamer 407) in the pharmaceutical composition (e.g., the lyophilized pharmaceutical composition) alleviates the need for any other excipient (e.g., additional bulking agent). Such alleviation may provide one or more advantages to the pharmaceutical composition (e.g., enhanced stability and/or reduced reconstitution time).

[0374] In some embodiments, the pharmaceutical composition of the present disclosure does not comprise an additional bulking agent.

[0375] In some embodiments, the lyophilized pharmaceutical composition of the present disclosure does not comprise an additional bulking agent.

[0376] In some embodiments, the reconstituted lyophilized pharmaceutical composition of the present disclosure does not comprise an additional bulking agent.

[0377] Several parameters may be used to characterize the poloxamers that feature in the compositions of the present disclosure, such as the percentage PEO in the polymer and/or average molecular weight and/or levels of

purity. It will be appreciated that these parameters may be combinable and any number of different parameters may be used to described the poloxamer.

[0378] In some embodiments, the poloxamer is purified. In some embodiments, the poloxamer is not purified. In some embodiments, the poloxamer (e.g., Poloxamer 407) has an average molecular weight of about 7.25 KDa or greater, about 9 kDa or greater, about 9.2 kDa or greater, about 9.4 kDa or greater, about 9.6 kDa or greater, about 9.8 kDa or greater, about 10 kDa or greater, about 10.2 kDa or greater, about 10.4 kDa or greater, about 10.6 kDa or greater, about 10.8 kDa or greater, about 11 kDa or greater, about 11.2 kDa or greater, about 11.4 kDa or greater, about 11.6 kDa or greater, about 11.8 kDa or greater, about 12 kDa or greater, or about 12.1 kDa or greater.In some embodiments, the poloxamer comprises at least 50% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 55% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 60% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 65% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 66% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 67% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 68% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 69% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises at least 70% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises 60-80% polyethylene oxide by molecular mass. In some embodiments, the poloxamer comprises 65-75% polyethylene oxide by molecular mass.

[0379] In some embodiments, the poloxamer has an average molecular weight of about 7250 to about 17350 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 8000 to about 17000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 8000 to about 16000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 9000 to about 16000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 9000 to about 15000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 9800 to about 14600 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 10000 to about 14000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 10500 to about 14000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 10500 to about 13500 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 11000 to about 14000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 11000 to about 13500 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 11500 to about 14000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 11500 to about 13000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 12000 to about 14000 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 12000 to about 13000

Daltons. In some embodiments, the poloxamer has an average molecular weight of about 10500 to about 12500 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 10500 to about 11500 Daltons. In some embodiments, the poloxamer has an average molecular weight of about 11500 to about 12500 Daltons.

[0380] In some embodiments, at least 85% by wt. of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 86% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 87% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 88% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 89% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 90% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 91% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 92% by weight of the poloxamer has an average molecular weight of about 7250 to about 17350 Da. In some embodiments, at least 86% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da. In some embodiments, at least 87% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da. In some embodiments, at least 88% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da. In some embodiments, at least 89% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da. In some embodiments, at least 90% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da. In some embodiments, at least 91% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da. Related to the embodiments in this paragraph, the poloxamer may have the following properties. In some embodiments, the poloxamer has a peak molecular weight of about 12,000 to about 12,500 Da. In some embodiments, the poloxamer has a number average molecular weight of about 11,500 to about 12,000 Da. In some embodiments, the poloxamer has a weight average molecular weight of about 11,750 to about 12,250 Da. In some embodiments, the poloxamer has a polydispersity index of about 1.02.

[0381] In some embodiments, less than 19% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 18% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 17% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 16% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 15% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 14% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 13% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 12% by weight of

the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 11% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 10% by weight of the poloxamer has an average molecular weight less about 7250 Da. In some embodiments, less than 9% by weight of the poloxamer has an average molecular weight less about 7250 Da. Related to the embodiments in this paragraph, the poloxamer may have the following properties. In some embodiments, the poloxamer has a peak molecular weight of about 5,000 to about 5,500 Da. In some embodiments, the poloxamer has a number average molecular weight of about 5,000 to about 5,500 Da. In some embodiments, the poloxamer has a weight average molecular weight of about 5,000 to about 5,500 Da. In some embodiments, the poloxamer has a polydispersity index of about 1.02.

[0382] In some embodiments, the entire poloxamer distribution has a number average molecular weight of about 10,800 to about 11,200 Da. In some embodiments, the poloxamer distribution has a weight average molecular weight of about 11,500 to about 11,700 Da. In some embodiments, the poloxamer distribution is from 0 to about 16,600 Da. In some embodiments, the poloxamer has a polydispersity index of about less than 1.07.

[0383] In some embodiments, the poloxamer is selected from the group consisting of Poloxamer 101, Poloxamer 105, Poloxamer 108, Poloxamer 122, Poloxamer 123, Poloxamer 124, Poloxamer 181, Poloxamer 182, Poloxamer 183, Poloxamer 184, Poloxamer 185, Poloxamer 188, Poloxamer 212, Poloxamer 215, Poloxamer 217, Poloxamer 231, Poloxamer 234, Poloxamer 235, Poloxamer 237, Poloxamer 238, Poloxamer 282, Poloxamer 284, Poloxamer 288, Poloxamer 331, Poloxamer 333, Poloxamer 334, Poloxamer 335, Poloxamer 338, Poloxamer 401, Poloxamer 402, Poloxamer 403, and Poloxamer 407.

[0384] In some embodiments, the poloxamer is Poloxamer 188 or Poloxamer 407.

[0385] In some embodiments, the poloxamer is Poloxamer 407.

[0386] In some embodiments, the poloxamer comprises Poloxamer 407. In some embodiments, the Poloxamer 407 is at least 10% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 20% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 30% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 40% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 50% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 60% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 70% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 75% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 80% by weight of the poloxamer. In some embodiments, the Poloxamer 407 is at least 90% by weight of the poloxamer. In some embodiments, the poloxamer is Poloxamer 407.

[0387] In some embodiments, the poloxamer is purified Poloxamer 407.

[0388] In some embodiments, the poloxamer is a purified poloxamer (e.g., purified Poloxamer 407). In such embodiments, the solubility of the otic agent(s) may be usefully increased.

[0389] In some embodiments, the purified poloxamer (e.g., purified Poloxamer 407) has an average molecular weight of about 9 kDa or greater, about 9.2 kDa or greater, about 9.4 kDa or greater, about 9.6 kDa or greater, about 9.8 kDa or greater, about 10 kDa or greater, about 10.2 kDa or greater, about 10.4 kDa or greater, about 10.6 kDa or greater, about 10.8 kDa or greater, about 11 kDa or greater, about 11.2 kDa or greater, about 11.4 kDa or greater, about 11.6 kDa or greater, about 11.8 kDa or greater, about 12 kDa or greater, or about 12.1 kDa or greater.

[0390] In some embodiments, the purified poloxamer (e.g., purified Poloxamer 407) has a reduced level of polymer chains with molecular weight below 9 kDa as compared to the unpurified poloxamer (e.g., unpurified Poloxamer 407). In some embodiments, the polymer chains with molecular weight below 7250 Da may be regarded as impurities.

[0391] In some embodiments, the purified poloxamer (e.g., purified Poloxamer 407) has about 99% or less, about 98% or less, about 95% or less, about 90% or less, about 80% or less, about 70% or less, about 60% or less, about 50% or less, about 40% or less, about 30% or less, about 20% or less, or about 10% or less of polymer chains with molecular weight below 9 kDa as compared to the unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0392] In some embodiments, the purified poloxamer (e.g., purified Poloxamer 407) contains less than about 15% by weight of polymer having a molecular weight below about 9 kDa (e.g., PEO homopolymer or PEO-PPO copolymer), for example less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.9%, less than about 0.8%, less than about 0.7%, less than about 0.6%, less than about 0.5%, less than about 0.4%, less than about 0.3%, less than about 0.2%, or less than about 0.1%, (by weight) of polymer with a molecular weight below about 9 kDa, inclusive of all ranges between any of these values.

[0393] In some embodiments, the purified poloxamer (e.g., purified Poloxamer 407) is prepared by liquid-liquid extraction or size exclusion chromatography.

[0394] General guidelines on purifying polymers are available, *e.g.*, in US Patent No. 6,977,045, Fakhari *et al.* (*Heliyon* 3:e00390 (2017)), and PCT Application Publication No. WO/2017/108457, each of which is incorporated herein by reference. The liquid-liquid extraction procedure involves the fractionation of the poloxamer (e.g., Poloxamer 407) between two aqueous phases containing with different salt concentration. In some embodiments, one or more inpurities preferentially partition into the aqueous phase with high salt concentration, and the purified poloxamer (e.g., Poloxamer 407) remains in the aqueous phase with low salt concentration. The size exclusion chromatography provides separation based on hydrodynamic radius. The fractions containing purified poloxamer (e.g., Poloxamer 407) with the desired molecular weight range are collected.

[0395] In some embodiments, about 10% or more, about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, about 95%

or more, about 98% or more, or about 99% or more of the one or more impurities having molecular weights below 9 kDa are removed from the poloxamer (e.g., Poloxamer 407) during the purification.

[0396] In some embodiments, about 10% or more, about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, about 95% or more, about 98% or more, or about 99% or more of the one or more diblock copolymers (e.g., PEO-PPO), single block polymers (e.g., PEO), and/or aldehydes are removed from the poloxamer (e.g., Poloxamer 407) during the purification.

[0397] In some embodiments, about 10% by weight or more, about 20% by weight or more, about 30% by weight or more, about 40% by weight or more, about 50% by weight or more, about 60% by weight or more, about 70% by weight or more, about 80% by weight or more, about 90% by weight or more, about 95% by weight or more, about 98% by weight or more, or about 99% by weight or more of the one or more diblock copolymers (e.g., PEO-PPO), single block polymers (homopolymers) (e.g., PEO), and/or aldehydes are removed from the poloxamer (e.g., Poloxamer 407) during the purification.

Other Aspects of the Lyophilized Pharmaceutical Compositions

[0398] In some embodiments, the lyophilized pharmaceutical composition is in the form of a lyophilized cake.

[0399] In some embodiments, lyophilization of the pharmaceutical composition of the present disclosure may substantially remove all volatile components from the composition. For example, water may be substantially removed by lyophilization. For example, DMSO may be substantially removed by lyophilization. In some embodiments, the lyophilized composition is substantially free from water and/or DMSO. In some embodiments, the lyophilized composition contains less than about 5% by weight of water and/or DMSO. In some embodiments, the lyophilized composition contains less than about 4% by weight of water and/or DMSO. In some embodiments, the lyophilized composition contains less than about 3% by weight of water and/or DMSO. In some embodiments, the lyophilized composition contains less than about 2% by weight of water and/or DMSO. In some embodiments, the lyophilized composition contains less than about 1% by weight of water and/or DMSO.

[0400] In some embodiments, the lyophilized pharmaceutical composition has a higher stability to oxygen and/or light as compared to a comparable pharmaceutical composition comprising one or more solvents.

[0401] In general, where a composition with a property is compared to a composition with or without a feature to demonstrate that property, the comparative composition is an otherwise identical composition. This applies throughout the disclosure. For example, the paragraph above can be read as: the lyophilized pharmaceutical composition has a higher stability to oxygen and/or light, as compared to an otherwise identical pharmaceutical composition comprising one or more solvents.

[0402] In some embodiments, the lyophilized composition comprises at least about 1% by weight of CHIR99021 or a pharmaceutically acceptable salt thereof. In some embodiments, the lyophilized composition comprises about 1% by weight to about 2% by weight of CHIR99021. In some embodiments, the lyophilized composition comprises at least about 30% by weight of valproic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the lyophilized composition comprises at least about 40% by weight of valproic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the lyophilized composition comprises about 30% by weight to about 50% by weight of valproic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the lyophilized composition comprises at least about 50% by weight of poloxamer. In some embodiments, the lyophilized composition comprises at least about 60% by weight of poloxamer. In some embodiments, the lyophilized composition comprises about 50% by weight to about 70% by weight of poloxamer. In some embodiments, the lyophilized composition comprises about 1.5% to about 2% by weight of CHIR99021, about 42.5% by weight to about 47.5% by weight of sodium valproate, and the remaining percentage is Poloxamer 407.

[0403] In some embodiments, the level of an impurity present in the lyophilized pharmaceutical composition is less than about 10000 parts per million (ppm), less than about 1000 ppm, less than about 100 ppm, less than about 10 ppm, less than about 1 ppm, or less than about 0.1 ppm.

[0404] In some embodiments, the total level of all the impurities present in the lyophilized pharmaceutical composition is less than about 10000 parts per million (ppm), less than about 1000 ppm, less than about 100 ppm, less than about 10 ppm, less than about 1 ppm, or less than about 0.1 ppm.

[0405] In some embodiments, the impurity is a residual solvent. In some embodiments, the impurity is selected from the group consisting of 1-acetate-2-formate-1,2-propanediol, acetic acid, formic acid, formaldehyde, acetaldehyde, and propionaldehyde.

[0406] In some embodiments, the level of polyethylene oxide presented in the lyophilized pharmaceutical composition is below about 3 %, below about 2 %, below about 1 %, below about 0.5 %, or below about 0.1 %, as measured by high-performance liquid chromatography (HPLC).

[0407] In some embodiments, the total level of one or more impurities with cLog P of about 1 or less presented in the lyophilized pharmaceutical composition is from about 30 % to about 35 %, from about 25 % to about 29 %, from about 20 % to about 25 %, from about 15 % to about 19 %, from about 10 % to about 14 %, from about 5 % to about 9 %, or from about 0 % to about 4 %, as measured by high-performance liquid chromatography (HPLC).

[0408] In some embodiments, the total level of one or more impurities having a boiling point of about 220 °C or less presented in the lyophilized pharmaceutical composition is from about 35 % to about 40 %, from about 30 % to about 34 %, from about 25 % to about 29 %, from about 20 % to about 25 %, from about 15 % to about 19 %, from about 10 % to about 14 %, from about 5 % to about 9 %, or from about 0 % to about 4 %, as measured by high-performance liquid chromatography (HPLC).

[0409] In some embodiments, the lyophilized pharmaceutical composition comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the level of the one or more otic therapeutic agents (e.g., hearing loss treatment agents) presented in the lyophilized pharmaceutical composition is about 1.5 fold or higher, about 1.8 fold or higher, about 2 fold or higher, about 2.5 fold or higher, about 3 fold or higher, about 5 fold or higher, or about 10 fold or higher as compared to a comparable lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0410] In some embodiments, the lyophilized pharmaceutical composition comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the dissolved concentration of the one or more otic therapeutic agents (e.g., hearing loss treatment agents) presented in the lyophilized pharmaceutical composition is about 1.5 fold or higher, about 1.8 fold or higher, about 2 fold or higher, about 2.5 fold or higher, about 3 fold or higher, about 5 fold or higher, or about 10 fold or higher as compared to an otherwise identical lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the otherwise identical lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0411] In some embodiments, the lyophilized pharmaceutical composition comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the lyophilized pharmaceutical composition has lower batch-to-batch variability of one or more gelation properties (e.g., gelation temperature, viscosity, and/or stability) as compared to a comparable lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0412] In some embodiments, the lyophilized pharmaceutical composition comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the lyophilized pharmaceutical composition has a lower gelation temperature, a narrower temperature range for gelation, and/or a higher viscosity as compared to a comparable lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0413] In some embodiments, the lyophilized pharmaceutical composition comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the lyophilized pharmaceutical composition has a reduced degradation rate as compared to a comparable lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0414] In some embodiments, the lyophilized pharmaceutical composition comprises one or more of a bulking agent (e.g., purified Poloxamer 407); a stabilizing agent; a tonicity-adjusting agent; and a soothing agent.

[0415] In some embodiments, the lyophilized pharmaceutical composition is prepared by lyophilizing the pharmaceutical composition of the present disclosure.

[0416] In some embodiments, the lyophilized pharmaceutical composition is prepared by the method of the present disclosure.

[0417] In some embodiments, the lyophilized pharmaceutical composition is suitable for preparing a reconstituted solution by a reconstitution process.

[0418] In some embodiments, the reconstitution process is less than about 1 hour. In some embodiments, the reconstitution process is less than about 30 minutes.

[0419] In some embodiments, the reconstituted solution is suitable for injection (e.g., intratympanic injection).

[0420] In some embodiments, the reconstituted solution maintains one or more rheometric properties of a pre-lyophilized solution which is used for preparing the lyophilized pharmaceutical composition.

[0421] In some embodiments, the reconstituted solution has a reduced degradation rate as compared to a reconstituted solution prepared from a comparable lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407). In some embodiments, the reconstituted solution maintains one or more rheometric properties of a pre-lyophilized solution which is used for preparing the lyophilized pharmaceutical composition, when the reconstituted solution is prepared at the same solids content as the pre-lyophilized solution.

Other Aspects of the Pharmaceutical Compositions

[0422] In some embodiments, the pharmaceutical composition is a pre-lyophilized pharmaceutical composition.

[0423] In some embodiments, the pharmaceutical composition may be formed by reconstituting the lyophilized compositions disclosed herein, for example to form an aqueous composition, for example a thermoreversible gel. It will be appreciated that components of the composition will have a certain concentration when the composition is aqueous (e.g. prior to lyophilization) which will change when the composition is lyophilized since, for example, water is removed. However, for ease, it may be convenient to refer to the components of the lyophilized form by reference to their concentration when aqueous since this may be how the composition is initially produced. Reconstitution of the lyophilized composition may substantially restore a component's concentration to that in the composition prior to lyophilization.

[0424] In some embodiments, the composition comprises a gelling agent and a compound of formula (I) (as described above and in the numbered embodiments).

[0425] In some embodiments, the pharmaceutical composition comprises a gelling agent, valproic acid or a pharmaceutically acceptable salt thereof at a concentration of greater than about 70 mg/ml, and one or more otic therapeutic agents.

[0426] In some embodiments, the pharmaceutical composition comprising a poloxamer, wherein at least 85% by weight of the poloxamer has an average molecular weight of greater than about 7250 Da, and valproic acid or a pharmaceutically acceptable salt thereof at greater than 70 mg/mL.

[0427] In some embodiments, the pharmaceutical composition comprises a poloxamer, wherein less than 20% by wt.% of the poloxamer has an average molecular weight less about 7250 Da, and valproic acid or a pharmaceutically acceptable salt thereof at greater than 70 mg/mL.

[0428] In some embodiments, the composition is suitable for intratympanic injection.

[0429] In some embodiments, the gelling agent is a poloxamer (as described above and in the numbered embodiments). In some embodiments, the poloxamer comprises purified poloxamer. In some embodiments, the poloxamer comprises purified poloxamer the poloxamer is purified poloxamer. In some embodiments, the poloxamer is defined as above (as defined above and in the numbered embodiments). In some embodiments, the compositions comprises one or more otic therapeutic agents (as defined above and in the numbered embodiments). In other embodiments, the composition gelling agent comprises a hyaluronic acid. In other embodiments, the composition gelling agent comprises a cellulosic derivative.

[0430] In some embodiments, the one or more otic therapeutic agents include a GSK3 inhibitor.

[0431] In some embodiments, the one or more otic therapeutic agents include an HDAC inhibitor.

[0432] In some embodiments, the one or more otic therapeutic agents are selected from the tables above

[0433] In some embodiments, the one or more otic therapeutic agents include CHIR99021 or a pharmaceutically acceptable salt thereof. In some embodiments, the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is less than about 10 mg/mL. In some embodiments, the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is less than about 7.5 mg/mL. In some embodiments, the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 3 to about 7 mg/mL. In some embodiments, the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 4 to about 6 mg/mL. In some embodiments, the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 1 to about 5 mg/mL. In some embodiments, the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 2 to about 4 mg/mL. In some embodiments the one or more otic therapeutic agents are one or more hearing loss treatment agents.

[0434] In some embodiments, the one or more otic therapeutic agents include valproic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the one or more otic therapeutic agents include valproic acid or a pharmaceutically acceptable salt thereof and CHIR99021 or a pharmaceutically acceptable salt thereof.

[0435] In some embodiments, the composition comprises a compound of formula (I) (as described above and in the numbered embodiments). In some embodiments, the compound of formula (I) and/or the one or more otic therapeutic agents are valproic acid or a pharmaceutically acceptable salt thereof.

[0436] In some embodiments, the pharmaceutically acceptable salt of valproic acid is sodium valproate. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is greater than about 100 mg/ml. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 100 to about 500 mg/mL. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 100 to about 350 mg/mL. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 110 to about 160 mg/ml. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 130 to about 140 mg/ml. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 125 to about 145 mg/ml. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 128 to about 138 mg/ml. In some embodiments, the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 133 mg/ml.

[0437] However, in other embodiments, the compound of formula (I) and/or the one or more otic therapeutic agents is not valproic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of formula (I) and/or the one or more otic therapeutic agents includes 2-(prop-2-yn-1-yl)-octanoic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of formula (I) and/or the one or more otic therapeutic agents includes phenylbutyric acid or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of formula (I) and/or the one or more otic therapeutic agents includes linoleic acid or a pharmaceutically acceptable salt thereof.

[0438] In other embodiments, the one or more otic therapeutic agents can be different. In other embodiments, the one or more otic therapeutic agents do not include CHIR99021 or a pharmaceutically acceptable salt thereof. In some embodiments the one or more otic therapeutic agents includes LY2090314 or a pharmaceutically acceptable salt thereof. In some embodiments the one or more otic therapeutic agents includes AZD1080 or a pharmaceutically acceptable salt thereof. In some embodiments the one or more otic therapeutic agents includes GSK3 XXII or a pharmaceutically acceptable salt thereof. In some embodiments the one or more otic therapeutic agents includes Compound I-7 or a pharmaceutically acceptable salt thereof. In some embodiments the one or more otic therapeutic agents includes Compound I-1 or a pharmaceutically acceptable salt thereof.

[0439] As described above, the composition may comprise a poloxamer. While the poloxamer may vary (PEO content, purity, molecular weight range), the poloxamer may comprise the following weight percentage of the composition. In some embodiments, the concentration of poloxamer is about 2% to about 50% w/v. In some embodiments, the concentration of poloxamer is about 2% to about 40% w/v. In some embodiments, the concentration of poloxamer is about 2% to about 30% w/v. In some embodiments, the concentration of poloxamer

is about 2% to about 20% w/v. In some embodiments, the concentration of poloxamer is about 10% to about 20% w/v. In some embodiments, the concentration of poloxamer is about 12.5% to about 17.5% w/v. In some embodiments, the concentration of poloxamer is about 13 % to about 17.5% w/v. In some embodiments, the concentration of poloxamer is about 13 % to about 17% w/v. In some embodiments, the concentration of poloxamer is about 13.5 % to about 17% w/v. In some embodiments, the concentration of poloxamer is about 13.5 % to about 16.5% w/v. In some embodiments, the concentration of poloxamer is about 14% to about 16.5% w/v. In some embodiments, the concentration of poloxamer is about 14% to about 16% w/v. In some embodiments, the concentration of poloxamer is about 15% to about 17.5% w/v.

[0440] In some embodiments, the disclosure relates to a method for preparing a pharmaceutical composition (for example the compositions described above or by the numbered embodiments) comprising the steps of: (a) having an aqueous solution comprising a gelling agent; and (b) adding a solution of one or more otic therapeutic agents or a pharmaceutically acceptable salt thereof.

[0441] In some embodiments, the aqueous solution further comprises valproic acid or a pharmaceutically acceptable salt thereof to the first solution. In some embodiments, the one or more otic therapeutic agents is CHIR99021 or a pharmaceutically acceptable salt thereof. In some embodiments, the one or more otic therapeutic agents is LY2090314 or a pharmaceutically acceptable salt thereof. In some embodiments, in step (b), the solution comprises a polar aprotic solvent. In some embodiments, in step (b), the polar aprotic solvent comprises DMSO. In some embodiments, in step (b), the polar aprotic solvent is DMSO. In some embodiments, in step (b), the polar aprotic solvent comprises dimethylformamide. In some embodiments, in step (b), the polar aprotic solvent comprises dimethylacetamide. In some embodiments, in step (b), the polar aprotic solvent comprises *N*-methyl-2-pyrrolidone. The method of any preceding embodiment, wherein the gelling agent comprises a poloxamer.

[0442] In some embodiments, the pharmaceutical composition is suitable for preparing the lyophilized pharmaceutical composition of the present disclosure (e.g., by a lyophilization process disclosed herein).

[0443] In some embodiments, the pre-lyophilized pharmaceutical composition comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.5 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 7.5 wt%.

[0444] In some embodiments, the pre-lyophilized pharmaceutical composition comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.5 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 25 wt%.

[0445] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt (e.g., sodium valproate).

[0446] In some embodiments, the pre-lyophilized pharmaceutical composition described in "Other Aspects of the Pharmaceutical Compositions" (or any embodiments described above), the pharmaceutically acceptable salt of valproic acid is a sodium salt (e.g., sodium valproate).

[0447] Any individual component of a composition may be present at a given concentration. Concentration can have the units of percent weight per volume (w/v) which can also be expressed as g/mL.

[0448] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 0.05 mg/ml to about 5 mg/ml, from about 0.25 mg/ml to about 2.5 mg/ml, from about 0.5 mg/ml to about 1.75 mg/ml, or from about 1.45 mg/ml to about 1.65 mg/ml. In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof is about 1.55 mg/ml.

[0449] In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 2.5 mg/ml to about 200 mg/ml, from about 5 mg/ml to about 100 mg/ml, from about 15 mg/ml to about 50 mg/ml, or from about 43 mg/ml to about 46 mg/ml. In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 44.5 mg/ml.

[0450] In some embodiments, the concentration of poloxamer 407 in the pre-lyophilized pharmaceutical composition ranges from about 2.5 wt% to about 12.5 wt%, from about 5 wt% to about 11 wt%, from about 6 wt% to about 10 wt%, or from about 7 wt% to about 8.5 wt%. In some embodiments, the concentration of poloxamer 407 is about 8 wt%.

[0451] In some embodiments, the concentration of DMSO in the pre-lyophilized pharmaceutical composition ranges from about 0.5 wt% to about 5 wt%, from about 1 wt% to about 4 wt%, from about 1.5 wt% to about 3.5 wt%, or from about 2 wt% to about 3 wt%. In some embodiments, the concentration of DMSO is about 2.5 wt%.

[0452] In some embodiments, the concentration of DMSO in the composition is about less than 5 wt%, as described above. However, in other embodiments, it will be appreciated that the concentration of DMSO may be less than about 25 wt%. In some embodiments, the concentration of DMSO is about less than 25 wt%. In some embodiments, the concentration of DMSO is about less than 20 wt%. In some embodiments, the concentration of DMSO is about less than 15 wt%. In some embodiments, the concentration of DMSO is about less than 10 wt%.

In some embodiments, the concentration of DMSO is about less than 5 wt%. In some embodiments, wherein the concentration of DMSO is about 25 to about 15 wt%. In some embodiments, wherein the concentration of DMSO is about 20 to about 10 wt%. In some embodiments, wherein the concentration of DMSO is about 15 to about 5 wt%. In some embodiments, wherein the concentration of DMSO is about 10 to about 5 wt%.

[0453] In some embodiments, the weight ratio between CHIR99021 or the pharmaceutically acceptable salt thereof and valproic acid or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 1:5 to about 1:10, from about 1:10 to about 1:50, from about 1:20 to about 1:35, from about 1:25 to about 1:31, or from about 1:27 to about 1:29. One skilled in the art will understand that the weight ratio of CHIR99012 and valproic acid (or pharmaceutically acceptable salts thereof) will be substantially unchanged in the lyophilized and reconstituted pharmaceutical composition.

[0454] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the pre-lyophilized pharmaceutical composition ranges from about 1:5 to about 40:1, from about 1:2 to about 15:1, from about 1:1 to about 8:1, from about 2:1 to about 4:1, or from about 2.5:1 to about 3.5:1. In some embodiments, the weight ratio between poloxamer 407 and the DMSO is about 3:1.

[0455] In some embodiments, the weight ratio between CHIR99021 and poloxamer 407 in the pre-lyophilized pharmaceutical composition is about 0.02:1; the weight ratio between CHIR99021 and the DMSO is about 0.06:1; the weight ratio between valproic acid sodium salt and poloxamer 407 is about 0.54:1; and/or the weight ratio between valproic acid sodium salt and the DMSO is about 3.2:1.

[0456] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 1.45 mg/ml to about 1.65 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 43 mg/ml to about 46 mg/ml; the concentration of poloxamer 407 ranges from about 7 wt% to about 8.5 wt%; and the concentration of DMSO ranges from about 2 wt% to about 3 wt%.

[0457] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition is about 1.55 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 44.5 mg/ml; the concentration of poloxamer 407 is about 8 wt%; and the concentration of DMSO is about 2.5 wt%.

[0458] In some embodiments, the pre-lyophilized pharmaceutical composition comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 1 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 7.5 wt%.

[0459] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt (e.g., sodium valproate).

[0460] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt in the pre-lyophilized pharmaceutical composition thereof ranges from about 0.05 mg/ml to about 10 mg/ml, from about 0.25 mg/ml to about 2.5 mg/ml, from about 0.5 mg/ml to about 1.75 mg/ml, from about 0.85 mg/ml to about 1.15 mg/ml. In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof is about 1.05 mg/ml.

[0461] In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 2.5 mg/ml to about 200 mg/ml, from about 5 mg/ml to about 100 mg/ml, from about 15 mg/ml to about 50 mg/ml, from about 28 mg/ml to about 31 mg/ml. In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 29.5 mg/ml.

[0462] In some embodiments, the concentration of poloxamer 407 in the pre-lyophilized pharmaceutical composition ranges from about 2.5 wt% to about 12.5 wt%, from about 5 wt% to about 11 wt%, from about 11 wt% to about 10 wt%, from about 7 wt% to about 8.5 wt%. In some embodiments, the concentration of poloxamer 407 is about 7.5 wt%.

[0463] In some embodiments, the concentration of DMSO in the pre-lyophilized pharmaceutical composition ranges from about 0.5 wt% to about 5 wt%, from about 1 wt% to about 4 wt%, from about 1.5 wt% to about 3.5 wt%, from about 2 wt% to about 3 wt%. In some embodiments, the concentration of DMSO is about 2.5 wt%.

[0464] In some embodiments, the weight ratio between CHIR99021 or the pharmaceutically acceptable salt thereof and valproic acid or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 1:5 to about 1:10, from about 1:10 to about 1:50, from about 1:20 to about 1:35, from about 1:25 to about 1:31, or from about 1:27 to about 1:29. One skilled in the art will understand that the weight ratio of CHIR99012 and valproic acid (or pharmaceutically acceptable salts thereof) will be substantially unchanged in the lyophilized and reconstituted pharmaceutical composition.

[0465] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the pre-lyophilized pharmaceutical composition ranges from about 1:5 to about 40:1, from about 1:2 to about 15:1, from about 1:1 to about 8:1, from about 2:1 to about 4:1, from about 2.5:1 to about 3.5:1. In some embodiments, the weight ratio between poloxamer 407 and the DMSO is about 3:1.

[0466] In some embodiments, the weight ratio between CHIR99021 and poloxamer 407 in the pre-lyophilized pharmaceutical composition is about 0.016:1; the weight ratio between the CHIR99021 and the DMSO is about 0.06:1; the weight ratio between valproic acid sodium salt and poloxamer 407 is about 0.42:1; and/or the weight ratio between valproic acid sodium salt and the DMSO is about 1.5:1.

[0467] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof ranges from about 0.95 mg/ml to about 1.15 mg/ml in the pre-lyophilized pharmaceutical composition; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 28 mg/ml to about 31 mg/ml; the concentration of poloxamer 407 ranges from about 7 wt% to about 8.5 wt%; and the concentration of DMSO ranges from about 2 wt% to about 3 wt%.

[0468] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition is about 1.05 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 29.5 mg/ml; the concentration of poloxamer 407 is about 7.5 wt%; and the concentration of DMSO is about 2.5 wt%.

[0469] In some embodiments, the pre-lyophilized pharmaceutical composition comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.025 mg/ml to about 25 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.5 mg/ml to about 500 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 1 wt% to about 25 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 7.5 wt%.

[0470] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt (e.g., sodium valproate).

[0471] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 0.05 mg/ml to about 5 mg/ml, from about 0.25 mg/ml to about 2.5 mg/ml, from about 0.5 mg/ml to about 1.75 mg/ml, or from about 0.6 mg/ml to about 0.75 mg/ml. In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof ranges is about 0.7 mg/ml.

[0472] In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 2.5 mg/ml to about 200 mg/ml, from about 5 mg/ml to about 100 mg/ml, from about 15 mg/ml to about 50 mg/ml, or from about 18 mg/ml to about 21 mg/ml. In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 19.5 mg/ml.

[0473] In some embodiments, the concentration of poloxamer 407 in the pre-lyophilized pharmaceutical composition ranges from about 2.5 wt% to about 12.5 wt%, from about 5 wt% to about 11 wt%, from about 6 wt% to about 10 wt%, or from about 7 wt% to about 8.5 wt%. In some embodiments, the concentration of poloxamer 407 is about 7.5 wt%.

[0474] In some embodiments, the concentration of DMSO in the pre-lyophilized pharmaceutical composition ranges from about 0.5 wt% to about 5 wt%, from about 1 wt% to about 4 wt%, from about 1.5 wt% to about 3.5 wt%, or from about 2 wt% to about 3 wt%. In some embodiments, the concentration of DMSO is about 5 wt%.

[0475] In some embodiments, the weight ratio between CHIR99021 or the pharmaceutically acceptable salt thereof and valproic acid or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 1:5 to about 1:10, from about 1:10 to about 1:50, from about 1:20 to about 1:35, from about 1:25 to about 1:31, or from about 1:27 to about 1:29. One skilled in the art will understand that the weight ratio of CHIR99012 and valproic acid (or pharmaceutically acceptable salts thereof) will be substantially unchanged in the lyophilized and reconstituted pharmaceutical composition.

[0476] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the pre-lyophilized pharmaceutical composition ranges from about 1:5 to about 40:1, from about 1:2 to about 15:1, from about 1:1 to about 8:1, from about 2:1 to about 4:1, from about 2.5:1 to about 3.5:1.

[0477] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the pre-lyophilized pharmaceutical composition is about 3:1; the weight ratio between the CHIR99021 and poloxamer 407 is about 0.013:1; the weight ratio between CHIR99021 and the DMSO is about 0.06:1; the weight ratio between valproic acid sodium salt and poloxamer 407 is about 0.23:1; and/or the weight ratio between valproic acid sodium salt and the DMSO is about 1.8:1.

[0478] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition ranges from about 0.6 mg/ml to about 0.75 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 18 mg/ml to about 21 mg/ml; the concentration of poloxamer 407 ranges from about 7 wt% to about 8.5 wt%; and the concentration of DMSO ranges from about 2 wt% to about 3 wt%.

[0479] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the pre-lyophilized pharmaceutical composition is about 0.7 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 19.5 mg/ml; the concentration of poloxamer 407 is about 7.5 wt%; and the concentration of DMSO is about 2.5 wt%.

[0480] In some embodiments, the pre-lyophilized pharmaceutical composition comprises one or more of water or a buffering agent; a bulking agent; a stabilizing agent (e.g., purified Poloxamer 407); a tonicity-adjusting agent; and a soothing agent.

Methods of Preparing Lyophilized Pharmaceutical Compositions

[0481] In some aspects, the present disclosure provides a method of preparing a lyophilized pharmaceutical composition of the present disclosure.

[0482] In some aspects, the present disclosure provides a method of processing the pharmaceutical composition of the present disclosure to form a lyophilized pharmaceutical composition (e.g., the pharmaceutical composition of the present disclosure).

[0483] In embodiments, the method involves a lyophilization process.

[0484] In embodiments, the disclosure relates to a method of lyophilizing a pharmaceutical composition as described by the pharmaceutical composition above and the numbered embodiments, wherein the method comprises: (a) providing a pharmaceutical composition; (b) lyophilizing the composition by: (i) reducing the temperature in the lyophilizer to -45 °C at a rate of 0.5 °C per minute, and then holding it at -45 °C for 3 hours; (ii) applying a vacuum of 80 mTorr; (iii) increasing the temperature to -30 °C (at a rate of 0.5 °C per minute) and holding it at -30 °C for 15 hours under a vacuum of 80 mTorr; (iv) increasing the temperature to 15 °C (at a rate of 0.5 °C per minute); and/or (v) holding the temperature at 15 °C for 20 hours under a vacuum of 80 mTorr; and (d) obtaining a lyophilized pharmaceutical composition. In some embodiments, the composition is subjected to a temperature of at least -50 °C prior to lyophilization. In some embodiments, the method can be varied by any one or more of the numbered embodiments below.

[0485] In embodiments, the disclosure relates to a method of lyophilizing a pharmaceutical composition as described by the pharmaceutical composition above and the numbered embodiments, wherein the method comprises: (a) providing a pharmaceutical composition; (b) lyophilizing the composition by: (i) reducing the temperature in the lyophilizer to about -45 °C at a rate of about 0.5 °C per minute, and then holding it at about -45 °C for about 3 hours; (ii) applying a vacuum of about 80 mTorr; (iii) increasing the temperature to about -30 °C (at a rate of about 0.5 °C per minute) and holding it at about -30 °C for about 15 hours under a vacuum of about 80 mTorr; (iv) increasing the temperature to about 15 °C (at a rate of about 0.5 °C per minute); and/or (v) holding the temperature at about 15 °C for about 20 hours under a vacuum of about 80 mTorr; and (d) obtaining a lyophilized pharmaceutical composition. In some embodiments, the composition is subjected to a temperature of at least about -50 °C prior to lyophilization. In some embodiments, the method can be varied by any one or more of the numbered embodiments below.

[0486] In some embodiments, the pharmaceutical composition is sterilized prior to the lyophilization process. In some embodiments, the pharmaceutical composition is sterilized through filtration (e.g., a sterile filtration) using a filter, for example a microporous membrane.

[0487] In some embodiments, the filter comprises a nylon, polycarbonate, cellulose acetate, polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE), polyethersulfone (PES), or any combination thereof.

[0488] In some embodiments, the filter is a polyethersulfone (PES) membrane filter or a polytetrafluoroethylene (PTFE) membrane filter. In some embodiments, the filter has a pore size of about 0.01 μm, about 0.02 μm, about 0.05 μm, about 0.08 μm, about 0.1 μm, about 0.2 μm, about 0.3 μm, about 0.4 μm, about 0.5 μm, or about 1 μm.

[0489] In some embodiments, one or more of microorganisms (e.g., bacteria, mold, or yeast) and particles are substantially removed from the pharmaceutical composition by the filtration.

[0490] In some embodiments, the method comprises the steps of:

i) cooling the pharmaceutical composition at a first temperature below 0 °C for a first period of time;

ii) removing one or more solvents from the resulting mixture of step (i) at a second temperature below 0 °C, and at a reduced pressure below 760 Torr, for a second period of time.

[0491] In some embodiments, the method comprises one or more steps selected from:

0a) dispensing the pharmaceutical composition in a sterile vial;

ia) cooling the pharmaceutical composition at a rate ranging from about 0.1 °C per minute to about 5 °C per minute to the first temperature ranging from about -20 °C to about -80 °C;

ib) holding the pharmaceutical composition at the first temperature for the first period of time ranging from about 1 hour to about 6 hours;

iia) subjecting the pharmaceutical composition to the reduced pressure ranging from about 1 mTorr to 1000 mTorr and warming the pharmaceutical composition at a rate ranging from about 0.1 °C per minute to about 5 °C per minute to the second temperature ranging from about -10 °C to -50 °C;

iib) holding the pharmaceutical composition at the second temperature and under the reduced pressure or the second period of time ranging from about 10 hours to about 30 hours;

iiia) filling the sterile vial with nitrogen; and

iiib) capping and crimping the sterile vial.

[0492] In some embodiments, the pharmaceutical composition comprises the one or more otic therapeutic agents (e.g., hearing loss treatment agents) and the poloxamer. In some embodiments, the pharmaceutical composition comprises the one or more otic therapeutic agents (e.g., hearing loss treatment agents) and poloxamer 407. In some embodiments, the pharmaceutical composition comprises the one or more otic therapeutic agents (e.g., hearing loss treatment agents) and purified poloxamer 407.

[0493] In some embodiments, the pharmaceutical composition comprises CHIR99021, valproic acid sodium salt, the poloxamer, DMSO, and water. In some embodiments, the pharmaceutical composition comprises CHIR99021, valproic acid sodium salt, poloxamer 407, DMSO, and water. In some embodiments, the pharmaceutical composition comprises CHIR99021, valproic acid sodium salt, purified poloxamer 407, DMSO, and water.

[0494] In some embodiments, the method comprises one or more steps selected from:

0a) dispensing the pharmaceutical composition in a sterile vial;

ia) cooling the pharmaceutical composition at a rate of about 0.5 °C per minute to the first temperature of about -45 °C;

ib) holding the pharmaceutical composition at the first temperature for the first period of time of about 3 hours;

iia) subjecting the pharmaceutical composition to the reduced pressure of about 80 mTorr to 1000 mTorr and warming the pharmaceutical composition at a rate of about 0.5 °C per minute to the second temperature of about -30 °C;

iib) holding the pharmaceutical composition at the second temperature and under the reduced pressure for the second period of time ranging from about 10 hours to about 15 hours;

iic) warming the pharmaceutical composition at a rate of about 0.5 °C per minute to 20 °C;

iid) holding the pharmaceutical composition at 20 °C and under the reduced pressure for 20 hours,

iiia) filling the sterile vial with nitrogen; and

iiib) capping and crimping the sterile vial.

Other Aspects of the Reconstituted Solutions

[0495] In some embodiments, the reconstituted solution is prepared by adding a diluent to the lyophilized pharmaceutical composition of the present disclosure.

[0496] In some embodiments, the disclosure relates to a method for reconstituting a lyophilized pharmaceutical composition (as described above or in the numbered embodiments), the method comprising: (a) providing the lyophilized pharmaceutical composition of any preceding embodiment; (b) reconstituting the lyophilized pharmaceutical composition with a pharmaceutically acceptable diluent; and (c) obtaining a reconstituted pharmaceutical composition.

[0497] In some embodiments, reconstituting the lyophilized pharmaceutical composition comprises dissolving the lyophilized pharmaceutical composition in the pharmaceutically acceptable diluent. In some embodiments, dissolving the lyophilized pharmaceutical composition in the pharmaceutically acceptable diluent takes less than about 1 hour. In some embodiments, dissolving the lyophilized pharmaceutical composition in the pharmaceutically acceptable diluent takes less than about 45 minutes. In some embodiments, dissolving the lyophilized pharmaceutical composition in the pharmaceutically acceptable diluent takes less than about 30 minutes. In some embodiments, dissolving the lyophilized pharmaceutical composition in the pharmaceutically acceptable diluent takes less than about 15 minutes. In some embodiments, dissolving the lyophilized pharmaceutical composition in the pharmaceutically acceptable diluent takes less than about 10 minutes.

[0498] In some embodiments, a reconstituted pharmaceutical composition can be obtained by the method for reconstituting a lyophilized pharmaceutical composition.

[0499] In some embodiments, a reconstituted pharmaceutical composition comprises the lyophilized composition of the present disclosure and a diluent.

[0500] In some embodiments, the composition reconstitutes in less about 1 hour. In some embodiments, the composition reconstitutes in less than about 45 minutes. In some embodiments, the composition reconstitutes in less

than about 30 minutes. In some embodiments, the composition reconstitutes in less than about 15 minutes. In some embodiments, the composition reconstitutes in less than about 10 minutes.

[0501] In some embodiments, the lyophilized pharmaceutical composition is prepared by lyophilizing the pharmaceutical composition of the present disclosure.

[0502] In some embodiments, the lyophilized pharmaceutical composition is prepared by the method of the present disclosure.

[0503] In some embodiments, the lyophilized pharmaceutical composition comprises one or more otic therapeutic agents (e.g., hearing loss treatment agents) and a gelling agent.

[0504] In some embodiments, the diluent comprises water and dimethyl sulfoxide (DMSO).

[0505] In some embodiments, the concentration of DMSO in the diluent ranges from about 1% w/w to about 15% w/w, from about 2% w/w to about 12% w/w, from about 3% w/w to about 10% w/w, from about 4% w/w to about 9% w/w, from about 5% w/w to about 8% w/w, from about 5.5% w/w to about 7.5% w/w, from about 5.8% w/w to about 7% w/w, from about 6% w/w to about 6.8% w/w, or from about 6.2% w/w to about 6.6% w/w. In some embodiments, the concentration of DMSO in the diluent is about 6.4% w/w. In some embodiments, the diluent is 6.4 w/w% DMSO in water.

[0506] In some embodiments, the amount of the diluent added during the reconstitution ranges from about 1 μL to about 6 μL, from about 2 μL to about 5 μL, from about 2.5 μL to about 4.5 μL, from about 2.8 μL to about 4 μL, from about 3 μL to about 3.8 μL, or from about 3.2 μL to about 3.6 μL per mg of the lyophilized pharmaceutical composition. In some embodiments, the amount of the diluent added during the reconstitution is about 3.4 μL per mg of the lyophilized pharmaceutical composition.

[0507] In some embodiments, the amount of the diluent added during the reconstitution is about 20 grams, about 30 grams, about 40 grams, about 50 grams, about 60 grams, about 70 grams, about 80 grams, about 90 grams, about 100 grams, about 120 grams, about 150 grams, about 200 grams, about 300 grams, about 500 grams, about 800 grams, or about 1000 grams.

[0508] In some embodiments, the amount of the diluent added during the reconstitution is about 0.1mL – about 1.5mL, about 0.3 mL – about 1.3 mL, about 0.5 mL – about 1.1 mL or about 0.7 mL – about 0.9 mL. In some embodiments, the amount of the diluent added during the reconstitution is about 0.85mL.

[0509] In some embodiments, the diluent is sparged with nitrogen for about 10 seconds to about 30 minutes, from about 20 seconds to about 20 minutes, from about 30 seconds, to about 10 minutes, from about 40 seconds to about 5 minutes, from about 50 seconds to about 3 minutes, or from about 1 minute to about 2 minutes prior to being added to the lyophilized pharmaceutical composition.

[0510] In some embodiments, the diluent is sterile filtered (e.g., using a PES 0.2 μm filter and/or a 10 mL syringe) prior to being added to the lyophilized pharmaceutical composition.

[0511] In some embodiments, upon addition, the mixture of the lyophilized pharmaceutical composition and the diluent is held at temperature lower than ambient temperature for a period time, thereby forming the reconstituted solution. In various embodiments, the reconstitution process is conducted without any agitation of the mixture of the lyophilized pharmaceutical composition and the diluent (e.g., shaking, sonication, or vortexing). In some embodiments, the reconstitution process comprises gently rotating the container (e.g., the vial) to mix the lyophilized pharmaceutical composition and the diluent, and/or gently tapping the container (e.g., the vial) until the lyophilized pharmaceutical composition and the diluent form a homogeneous solution.

[0512] In some embodiments, the mixture of the lyophilized pharmaceutical composition and the diluent is held at a temperature ranging from about -10 °C to about 20 °C, from about -5 °C to about 15 °C, from about 0 °C to about 10 °C, from about 1 °C to about 9 °C, or from about 2 °C to about 8 °C. In some embodiments, the mixture of the lyophilized pharmaceutical composition and the diluent is held at a temperature ranging from about 5-8 °C.

[0513] In some embodiments, the mixture of the lyophilized pharmaceutical composition and the diluent is held for a period of time (e.g., reconstitution time) being about 6 hours or less, about 3 hours or less, about 2 hours or less, about 1 hours or less, about 50 minutes or less, about 40 minutes or less, about 30 minutes or less, about 20 minutes or less, or about 10 minutes or less. In certain embodiments, the mixture of the lyophilized pharmaceutical composition and the diluent is held for 20 minutes.

[0514] In some embodiments, the reconstitution process comprises addition of the diluent to the lyophilized pharmaceutical composition and storing the vial at 2-8 °C. In some embodiments, the reconstitution process comprises addition of the diluent to the lyophilized pharmaceutical composition and storing the vial at 2-8 °C and gently tapping the container (e.g., the vial) until the lyophilized pharmaceutical composition and the diluent form a homogeneous solution. In some embodiments, the reconstitution process comprises addition of the diluent to the lyophilized pharmaceutical composition and storing the vial at 2-8 °C and gently tapping the container (e.g., the vial) until the lyophilized pharmaceutical composition and the diluent form a homogeneous solution without sonication or vortexing (for example in order to avoid poloxamer degradation or drug precipitation). In some embodiments, the reconstitution process comprises addition of about 0.85 mL of diluent to the lyophilized pharmaceutical composition and storing the vial at 2-8 °C and gently tapping the container (e.g., the vial) until the lyophilized pharmaceutical composition and the diluent form a homogeneous solution without sonication or vortexing. In some embodiments, the reconstitution process comprises addition of about 0.85 mL of diluent to the lyophilized pharmaceutical composition and storing the vial at 2-8 °C and gently tapping the container (e.g., the vial) until the lyophilized pharmaceutical composition and the diluent form a homogeneous solution without sonication or vortexing where the diluent is 6.4 w/w% DMSO in water. In some embodiments any of the reconsistution processes can be used to measure improved reconstitution time, for example the improvements discussed herein e.g. relative to non-lyophilized solid forms. In certain embodiments, the improvement in reconstitution time disclosed herein is

specifically measured using a reconstitution process in which about 0.85 mL of diluent is added to the lyophilized pharmaceutical composition, the vial is stored at 2-8 °C and gently tapped until the lyophilized pharmaceutical composition and the diluent form a homogeneous solution without sonication or vortexing, where the diluent is 6.4 w/w% DMSO in water. Improvements could be observed after a fixed reconstitution time, e.g. 20 minutes.

[0515] In some embodiments, the reconstituted solution is a clear solution at ambient temperature (e.g., between 20 °C and 26 °C).

[0516] In some embodiments, the reconstituted solution is suitable for injection at ambient temperature (e.g., between 20 °C and 26 °C).

[0517] In some embodiments, the reconstituted solution has a gelation temperature being higher than ambient temperature (e.g., between 20 °C and 26 °C; preferably 25 °C) and being lower than the temperature of human body (e.g., between 36 °C and 39 °C; preferably 37 °C).

[0518] In some embodiments, the reconstituted solution has a gelation temperature range of about 2 °C or about 3 °C.

[0519] In some embodiment, the reconstituted solution is stable upon storage of at a temperature ranging from about -10 °C to about 20 °C, from about -5 °C to about 15 °C, from about 0 °C to about 10 °C, from about 1 °C to about 9 °C, or from about 2 °C to about 8 °C.

[0520] In some embodiment, the reconstituted solution is stored for about 10 minutes or longer, about 20 minutes or longer, about 30 minutes or longer, about 40 minutes or longer, about 50 minutes or longer, about 1 hour or longer, about 2 hours or longer, about 3 hours or longer, about 4 hours or longer, about 5 hours or longer, or about 6 hours or longer prior to use.

[0521] In some embodiment, about 0.1% or less, about 0.09% or less, about 0.08% or less, about 0.07% or less, about 0.06% or less, about 0.05% or less, about 0.04% or less, about 0.03% or less, about 0.02% or less, or about 0.01% or less of one or more otic therapeutic agents (e.g., CHIR99021 and/or sodium valproate) degrades during the storage.

[0522] In some embodiments, the reconstituted solution has a pH value ranging from about 4 to about 13, from about 5 to about 12, from about 6 to about 11, from about 6.5 to about 10.5, or from about 7 to about 10.

[0523] In some embodiments, the reconstituted solution is suitable for injection at ambient temperature (e.g., between 20 °C and 26 °C) through a needle (e.g., a needle having an inner diameter of about 3.81 mm or less, about 3.43 mm or less, about 3.00 mm or less, about 2.69 mm or less, about 2.39 mm or less, about 2.16 mm or less, about 1.80 mm or less, about 160 mm or less, about 1.37 mm or less, about 1.19 mm or less, about 1.07 mm or less, about 0.84 mm or less, about 0.69 mm or less, about 0.60 mm or less, about 0.51 mm or less, about 0.41 mm or less, about 0.34 mm or less, about 0.31 mm or less, or about 0.26 mm or less).

[0524] In some embodiments, the reconstituted solution is formulated for injection in a volume of about 1 ml or less, about 900 µl or less, about 800 µl or less, about 700 µl or less, about 600 µl or less, about 500 µl or less, about 400 µl or less, about 300 µl or less, about 200 µl or less, or about 100 or less.

[0525] In some embodiments, the reconstituted solution comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.05 mg/ml to about 50 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 1 mg/ml to about 1000 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 2 wt% to about 50 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 15 wt%.

[0526] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt. In some embodiments, the pharmaceutically acceptable salt of valproic acid is sodium valproate.

[0527] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 0.1 mg/ml to about 10 mg/ml, from about 0.5 mg/ml to about 5 mg/ml, from about 1 mg/ml to about 3.5 mg/ml, or from about 2.9 mg/ml to about 3.3 mg/ml. In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof is about 3.1 mg/ml.

[0528] In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 5 mg/ml to about 400 mg/ml, from about 10 mg/ml to about 200 mg/ml, from about 30 mg/ml to about 100 mg/ml, or from about 86 mg/ml to about 92 mg/ml. In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 89 mg/ml.

[0529] In some embodiments, the concentration of poloxamer 407 in the reconstituted solution ranges from about 5 wt% to about 25 wt%, from about 10 wt% to about 22 wt%, from about 12 wt% to about 20 wt%, or from about 14 wt% to about 17 wt%. In some embodiments, the concentration of poloxamer 407 is about 16 wt%.

[0530] In some embodiments, the concentration of DMSO in the reconstituted solution ranges from about 1 wt% to about 10 wt%, from about 2 wt% to about 8 wt%, from about 3 wt% to about 7 wt%, or from about 4 wt% to about 6 wt%. In some embodiments, the concentration of DMSO is about 5 wt%.

[0531] In some embodiments, the weight ratio between CHIR99021 or the pharmaceutically acceptable salt thereof and valproic acid or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 1:5 to about 1:10, from about 1:10 to about 1:50, from about 1:20 to about 1:35, from about 1:25 to about 1:31, or from about 1:27 to about 1:29.

[0532] In some embodiments, the weight ratio between poloxamer 407 and the DMSO ranges in the reconstituted solution from about 1:5 to about 40:1, from about 1:2 to about 15:1, from about 1:1 to about 8:1, from about 2:1 to

about 4:1, or from about 2.5:1 to about 3.5:1. In some embodiments, the weight ratio between poloxamer 407 and the DMSO is about 3:1.

[0533] In some embodiments, the weight ratio between CHIR99021 and poloxamer 407 in the reconstituted solution is about 0.02:1; the weight ratio between CHIR99021 and the DMSO is about 0.06:1; the weight ratio between valproic acid sodium salt and poloxamer 407 is about 0.54:1; and/or the weight ratio between valproic acid sodium salt and the DMSO is about 3.2:1.

[0534] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 2.9 mg/ml to about 3.3 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 86 mg/ml to about 92 mg/ml; the concentration of poloxamer 407 ranges from about 14 wt% to about 17 wt%; and the concentration of DMSO ranges from about 4 wt% to about 6 wt%.

[0535] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 3.2 mg/ml to about 3.3 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 87 mg/ml to about 90 mg/ml; the concentration of poloxamer 407 ranges from about 14 wt% to about 16 wt%; and the concentration of DMSO ranges from about 4 wt% to about 5 wt%.

[0536] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution is about 3.1 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 89 mg/ml; the concentration of poloxamer 407 is about 16 wt%; and the concentration of DMSO is about 5 wt%.

[0537] In some embodiments, the reconstituted solution comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.05 mg/ml to about 50 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 1 mg/ml to about 1000 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 2 wt% to about 50 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 15 wt%.

[0538] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt. In some embodiments, the pharmaceutically acceptable salt of valproic acid is sodium valproate.

[0539] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 0.1 mg/ml to about 10 mg/ml, from about 0.5 mg/ml to about 5 mg/ml, from about 1 mg/ml to about 3.5 mg/ml, from about 1.9 mg/ml to about 2.3 mg/ml. In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof is about 2.1 mg/ml.

[0540] In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 5 mg/ml to about 400 mg/ml, from about 10 mg/ml to about 200 mg/ml, from about 30 mg/ml to about 100 mg/ml, from about 56 mg/ml to about 62 mg/ml. In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 59 mg/ml.

[0541] In some embodiments, the concentration of poloxamer 407 in the reconstituted solution ranges from about 5 wt% to about 25 wt%, from about 10 wt% to about 22 wt%, from about 12 wt% to about 20 wt%, from about 14 wt% to about 17 wt%. In some embodiments, the concentration of poloxamer 407 is about 15 wt%.

[0542] In some embodiments, the concentration of DMSO in the reconstituted solution ranges from about 1 wt% to about 10 wt%, from about 2 wt% to about 8 wt%, from about 3 wt% to about 7 wt%, from about 4 wt% to about 6 wt%. In some embodiments, the concentration of DMSO is about 5 wt%.

[0543] In some embodiments, the weight ratio between CHIR99021 or the pharmaceutically acceptable salt thereof and valproic acid or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 1:5 to about 1:10, from about 1:10 to about 1:50, from about 1:20 to about 1:35, from about 1:25 to about 1:31, or from about 1:27 to about 1:29.

[0544] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the reconstituted solution ranges from about 1:5 to about 40:1, from about 1:2 to about 15:1, from about 1:1 to about 8:1, from about 2:1 to about 4:1, from about 2.5:1 to about 3.5:1. In some embodiments, the weight ratio between poloxamer 407 and the DMSO is about 3:1.

[0545] In some embodiments, the weight ratio between CHIR99021 and poloxamer 407 in the reconstituted solution is about 0.016:1; the weight ratio between the CHIR99021 and the DMSO is about 0.06:1; the weight ratio between valproic acid sodium salt and poloxamer 407 is about 0.42:1; and/or the weight ratio between valproic acid sodium salt and the DMSO is about 1.5:1.

[0546] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 1.9 mg/ml to about 2.3 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 56 mg/ml to about 62 mg/ml; the concentration of poloxamer 407 ranges from about 14 wt% to about 17 wt%; and the concentration of DMSO ranges from about 4 wt% to about 6 wt%.

[0547] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution is about 2.1 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 59 mg/ml; the concentration of poloxamer 407 is about 15 wt%; and the concentration of DMSO is about 5 wt%.

[0548] In some embodiments, the reconstituted solution comprises:

i) CHIR99021 or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 0.05 mg/ml to about 50 mg/ml;

ii) valproic acid or a pharmaceutically acceptable salt thereof being present at a concentration ranging from 1 mg/ml to about 1000 mg/ml;

iii) poloxamer 407 being present at a concentration ranging from 2 wt% to about 50 wt%; and

iv) dimethyl sulfoxide (DMSO) being present at a concentration below 15 wt%.

[0549] In some embodiments, the pharmaceutically acceptable salt of valproic acid is a sodium salt (e.g., sodium valproate).

[0550] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 0.1 mg/ml to about 10 mg/ml, from about 0.5 mg/ml to about 5 mg/ml, from about 1 mg/ml to about 3.5 mg/ml, or from about 1.2 mg/ml to about 1.5 mg/ml. In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof ranges is about 1.4 mg/ml.

[0551] In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 5 mg/ml to about 400 mg/ml, from about 10 mg/ml to about 200 mg/ml, from about 30 mg/ml to about 100 mg/ml, or from about 36 mg/ml to about 42 mg/ml. In some embodiments, the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 39 mg/ml.

[0552] In some embodiments, the concentration of poloxamer 407 in the reconstituted solution ranges from about 5 wt% to about 25 wt%, from about 10 wt% to about 22 wt%, from about 12 wt% to about 20 wt%, or from about 14 wt% to about 17 wt%. In some embodiments, the concentration of poloxamer 407 is about 15 wt%.

[0553] In some embodiments, the concentration of DMSO in the reconstituted solution ranges from about 1 wt% to about 10 wt%, from about 2 wt% to about 8 wt%, from about 3 wt% to about 7 wt%, or from about 4 wt% to about 6 wt%. In some embodiments, the concentration of DMSO is about 5 wt%.

[0554] In some embodiments, the weight ratio between CHIR99021 or the pharmaceutically acceptable salt thereof and valproic acid or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 1:5 to about 1:10, from about 1:10 to about 1:50, from about 1:20 to about 1:35, from about 1:25 to about 1:31, or from about 1:27 to about 1:29.

[0555] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the reconstituted solution ranges from about 1:5 to about 40:1, from about 1:2 to about 15:1, from about 1:1 to about 8:1, from about 2:1 to about 4:1, from about 2.5:1 to about 3.5:1.

[0556] In some embodiments, the weight ratio between poloxamer 407 and the DMSO in the reconstituted solution is about 3:1; the weight ratio between the CHIR99021 and poloxamer 407 is about 0.013:1; the weight ratio between CHIR99021 and the DMSO is about 0.06:1; the weight ratio between valproic acid sodium salt and

poloxamer 407 is about 0.23:1; and/or the weight ratio between valproic acid sodium salt and the DMSO is about 1.8:1.

[0557] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution ranges from about 1.2 mg/ml to about 1.5 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof ranges from about 36 mg/ml to about 42 mg/ml; the concentration of poloxamer 407 ranges from about 14 wt% to about 17 wt%; and the concentration of DMSO ranges from about 4 wt% to about 6 wt%.

[0558] In some embodiments, the concentration of CHIR99021 or the pharmaceutically acceptable salt thereof in the reconstituted solution is about 1.4 mg/ml; the concentration of valproic acid or the pharmaceutically acceptable salt thereof is about 39 mg/ml; the concentration of poloxamer 407 is about 15 wt%; and the concentration of DMSO is about 5 wt%.

[0559] In some embodiments, the reconstituted solution comprises, in addition to the active agents, one or more of water or a buffering agent; a bulking agent (e.g., purified Poloxamer 407); a stabilizing agent; a tonicity-adjusting agent; and a soothing agent.

[0560] In some embodiments, the reconstituted solution comprises, in addition to the active agents, purified poloxamer (e.g., purified Poloxamer 407), and wherein the reconstituted solution has a higher stability to oxygen and/or light as compared to a comparable reconstituted solution without (e.g., purified Poloxamer 407). In some embodiments, the comparable reconstituted solution comprises unpurified Poloxamer (e.g., unpurified Poloxamer 407).

[0561] In some embodiments, the level of an impurity present in the reconstituted solution is less than about 10000 parts per million (ppm), less than about 1000 ppm, less than about 100 ppm, less than about 10 ppm, less than about 1 ppm, or less than about 0.1 ppm.

[0562] In some embodiments, the impurity is selected from the group consisting of 1-acetate-2-formate-1,2-propanediol, acetic acid, formic acid, formaldehyde, acetaldehyde, and propionaldehyde.

[0563] In some embodiments, the level of polyethylene oxide present in the reconstituted solution is below about 3 %, below about 2 %, below about 1 %, below about 0.5 %, or below about 0.1 %, as measured by high-performance liquid chromatography (HPLC).

[0564] In some embodiments, the total level of one or more impurities with cLog P of about 1 or less present in the reconstituted solution is from about 30 % to about 35 %, from about 25 % to about 29 %, from about 20 % to about 25 %, from about 15 % to about 19 %, from about 10 % to about 14 %, from about 5 % to about 9 %, or from about 0 % to about 4 %, as measured by high-performance liquid chromatography (HPLC).

[0565] In some embodiments, the total level of one or more impurities having a boiling point of about 220 °C or less present in the reconstituted solution is from about 35 % to about 40 %, from about 30 % to about 34 %, from

about 25 % to about 29 %, from about 20 % to about 25 %, from about 15 % to about 19 %, from about 10 % to about 14 %, from about 5 % to about 9 %, or from about 0 % to about 4 %, as measured by high-performance liquid chromatography (HPLC).

[0566] In some embodiments, the reconstituted solution comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the level of the one or more otic therapeutic agents (e.g., hearing loss treatment agents) present in the reconstituted solution is about 1.5 fold or higher, about 1.8 fold or higher, about 2 fold or higher, about 2.5 fold or higher, about 3 fold or higher, about 5 fold or higher, or about 10 fold or higher as compared to a comparable reconstituted solution without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable reconstituted solution comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0567] In some embodiments, the reconstituted solution comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the reconstituted solution has lower batch-to-batch variability of one or more gelation properties (e.g., gelation temperature, viscosity, and/or stability) as compared to a comparable reconstituted solution without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable reconstituted solution comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0568] In some embodiments, the reconstituted solution comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the reconstituted solution has a lower gelation temperature, a narrower gelation temperature range, a more sustained release of the hearing loss treatment agent, and/or a higher viscosity as compared to a reconstituted solution without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable reconstituted solution comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0569] In some embodiments, the reconstituted solution comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the reconstituted solution has a lower gelation temperature than the gelation temperature of an otherwise identical composition with unpurified poloxamer rather than purified poloxamer, wherein the temperature is about 1 °C lower, about 2 °C lower, about 3 °C lower, about 4 °C lower, about 5 °C lower, about 6 °C lower, about 7 °C lower, about 8 °C lower, about 9 °C lower, about 10 °C lower, about 11 °C lower, about 12 °C lower, or about 13 °C lower than the gelation temperature of an otherwise identical reconstituted solution with unpurified poloxamer (e.g., unpurified Poloxamer 407) rather than purified poloxamer as described herein. In other embodiments, the reconstituted solution comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the reconstituted solution has a narrower gelation temperature range compared to the gelation temperature range of an otherwise identical composition with unpurified poloxamer rather than purified poloxamer. The gelation temperature range is the range of temperatures over which the formulation transitions from being a fluid to being a gel. Composition with unpurified poloxamer generally transition from a fluid to a gel over a range of about 10 °C, whereas otherwise identical compositions with purified poloxamer (e.g., purified Poloxamer 407) transition from a fluid to a gel over a range of about 2 °C to about 3 °C.

[0570] In some embodiments, the reconstituted solution comprises purified poloxamer (e.g., purified Poloxamer 407), and wherein the reconstituted solution has a reduced degradation rate as compared to a comparable reconstituted solution without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable reconstituted solution comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0571] In some embodiments, the reconstituted solution is suitable for injection (e.g., intratympanic injection).

[0572] In some embodiments, the reconstituted solution maintains one or more rheometric properties of a pharmaceutical composition which is used for preparing the lyophilized pharmaceutical composition.

[0573] In some embodiments, the reconstituted solution has a reduced degradation rate as compared to a reconstituted solution prepared from a comparable lyophilized pharmaceutical composition without purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the comparable lyophilized pharmaceutical composition comprises unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0574] In some embodiments, the reconstituted solution comprises one or more of water or a buffering agent; a bulking agent (e.g., purified Poloxamer 407); a stabilizing agent; a tonicity-adjusting agent; and a soothing agent.

Other Components

[0575] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure comprises water.

[0576] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure comprises a buffering agent. The buffer controls the pH of the reconstituted solution to a range of from about 4 to about 13, from about 5 to about 12, from about 6 to about 11, from about 6.5 to about 10.5, or from about 7 to about 10.

[0577] Examples of the buffering agent include, but are not limited to, citrate buffering agents, acetate buffering agents, phosphate buffering agents, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, amino-sulfonate buffers (*e.g.*, HEPES), magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and/or combinations thereof. Lubricating agents may be selected from the non-limiting group consisting of magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl

behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and combinations thereof.

[0578] In some embodiments, the buffering agent comprises phosphate buffered saline, TRIS, tris acetate, tris HCl-65, sodium citrate, histidine, arginine, sodium phosphate, tris base-65, hydroxyethyl starch, or any combination thereof.

[0579] As discussed above, a poloxamer can be used in certain embodiments as a gelling agent. An aldehyde is a compound containing a functional group with the structure −CHO, consisting of a carbon double-bonded to oxygen with the carbon atom also bonded to a hydrogen atom. Aldehydes, including formaldehyde, acetaldehyde, and propionaldehyde, are potential impurities and degradation products of poloxamers and may be formed e.g. when the poloxamer is present in a gel. Lyophilization beneficially removes aldehydes present in the test composition. Lyophilized compositions disclosed herein can also be more stable than the gel form, for example in relation to the levels of aldehyde present over time.

[0580] In some embodiments, lyophilization removes aldehydes from the compositions of the present disclosure.

[0581] In some embodiments, preservatives such as antioxidants are not required in the lyophilized compositions of the present disclosure, for example because of the low levels of aldehydes present.

[0582] In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 1, about 2, about 3, about 4, about 5 or about 10 ppm (μg/g). In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 10 ppm (μg/g). In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 5 ppm (μg/g). In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 4 ppm (μg/g). In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 3 ppm (μg/g). In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 2 ppm (μg/g). In some embodiments of the lyophilized pharmaceutical composition, the concentration of aldehydes is less than about 1 ppm (μg/g).

[0583] In some embodiments, the aldehydes are volatile aldehydes.

[0584] In some embodiments, the aldehydes comprise molecules where each individual molecule has a molecular weight of less than 300 Da. In some embodiments, the aldehydes comprise molecules where each individual molecule has a molecular weight of less than 200 Da. In some embodiments, the aldehydes comprise molecules where each individual molecule has a molecular weight of less than 100 Da.

[0585] In some embodiments, the aldehydes comprise formaldehyde, acetaldehyde, and/or propionaldehyde.

[0586] Some examples of antioxidants include, but are not limited to, RRR-Alpha-Tocopherol, d-Alpha tocopherol; d-alpha tocopheryl acetate; dl-alpha tocopheryl acetate; d-alpha tocopheryl acid succinate; dl-alpha

tocopheryl acid succinate; beta tocopherol; delta tocopherol; gamma tocopherol; tocopherols excipient, Ascorbic Acid; Ascorbyl palmitate; erythorbic acid; sodium ascorbate; sodium erythorbate; butylated hydroxytoluene; Butylated Hydroxyanisole; Anhydrous citric acid; fumaric acid; malic acid; sodium citrate; dihydrate; tartaric acid; Citric Acid Monohydrate; Edetic Acid; Dipotassium edetate; disodium edetate; edetate calcium disodium; sodium edetate; trisodium edetate; propyl gallate; Methionine; Monothioglycerol; Pentetic Acid; Potassium Metabisulphite; Potassium bisulfite; sodium metabisulfite; Propionic Acid; Calcium propionate; sodium propionate; Dodecyl gallate; ethyl gallate; octyl gallate; Sodium Formaldehyde Sulfoxylate; Anhydrous Sodium Sulphite; Sodium Thiosulphate; Sulfur Dioxide; Vitamin E Polyethylene Glycol Succinate.

[0587] In some embodiments, the pharmaceutical composition of the present disclosure does not comprise an antioxidant.

[0588] In some embodiments, the lyophilized pharmaceutical composition of the present disclosure does not comprise an antioxidant.

[0589] In some embodiments, the reconstituted lyophilized pharmaceutical composition of the present disclosure does not comprise an antioxidant.

[0590] In some embodiments, the pharmaceutical composition of the present disclosure does not comprise an antioxidant and has a concentration of aldehydes which is less than about 1, about 2, about 3, about 4, about 5 or about 10 ppm (μg/g).

[0591] In some embodiments, the lyophilized pharmaceutical composition of the present disclosure does not comprise an antioxidant and has a concentration of aldehydes which is less than about 1, about 2, about 3, about 4, about 5 or about 10 ppm (μg/g).

[0592] In some embodiments, the reconstituted pharmaceutical composition of the present disclosure does not comprise an antioxidant and has a concentration of aldehydes which is less than about 1, about 2, about 3, about 4, about 5 or about 10 ppm (μg/g).

[0593] In some embodiments, the the pharmaceutical composition or reconstituted solution of the present disclosure comprises a bulking agent.

[0594] In some embodiments, the bulking agent comprises poloxamer (e.g., poloxamer 407), mannitol, sucrose, maltose, trehalose, dextrose, sorbitol, glucose, raffinose, glycine, histidine, polyvinylpyrrolidone (e.g., polyvinylpyrrolidone K12 or polyvinylpyrrolidone K17), lactose, or any combination thereof.

[0595] In some embodiments, the poloxamer (e.g., poloxamer 407) is the gelling agent and/or the bulking agent. In some embodiments, the poloxamer (e.g., poloxamer 407) is the gelling agent and the bulking agent.

[0596] In some embodiments, where the composition comprises a gelling agent (such as poloxamer, e.g. Poloxamer 407), the composition does not comprise an additional bulking agent (such as mannitol, sucrose, maltose,

trehalose, dextrose, sorbitol, glucose, raffinose, glycine, histidine, polyvinylpyrrolidone (e.g., polyvinylpyrrolidone K12 or polyvinylpyrrolidone K17), lactose, or any combination thereof).

[0597] A bulking agent can positively enhance the lyophilization process, leading to an improved dried/lyophilized product in terms of appearance and characteristics.

[0598] However, a solution of poloxamer 407 can be lyophilized in the absence of a bulking agent to form a porous cake of substantial volume (e.g. see Figure 9) and not a flat sheet of dried mass (e.g. see Figure 10). The same effect was noted when a molecule such as sodium valproate (NaVPA) was added to poloxamer 407 solution. A polymeric lyophilized cake mass produced in this way (e.g. see Step 7 of Example 10) reconstituted well and retained the rheological properties similar to the pre-lyophilized solution.

[0599] In some embodiments, the pharmaceutical composition of the present disclosure does not comprise bulking agent in addition to the gelling agent.

[0600] In some embodiments, the lyophilized pharmaceutical composition of the present disclosure does not comprise a bulking agent in addition to the gelling agent.

[0601] In some embodiments, the reconstituted lyophilized pharmaceutical composition of the present disclosure does not comprise a bulking agent in addition to the gelling agent.

[0602] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure comprises a stabilizing agent. In some embodiments, the stabilizing agent comprises Polyethylene Glycol, saccharides, ascorbic acid, acetylcysteine, bisulfite, metabisulfite, monothioglycerol, inositol, oleic acid, or any combination thereof.

[0603] In some embodiments, the stabilizing agent comprises a cryoprotectant. In some embodiments, the cryoprotectant is a polyol (e.g., a diol or a triol such as propylene glycol (i.e., 1,2-propanediol), 1,3-propanediol, glycerol, (+/-)-2-methyl-2,4-pentanediol, 1,6-hexanediol, 1,2-butanediol, 2,3-butanediol, ethylene glycol, or diethylene glycol), a nondetergent sulfobetaine (e.g., NDSB-201 (3-(1-pyridino)-1-propane sulfonate), an osmolyte (e.g., L-proline or trimethylamine N-oxide dihydrate), a polymer (e.g., polyethylene glycol 200 (PEG 200), PEG 400, PEG 600, PEG 1000, PEG 3350, PEG 4000, PEG 8000, PEG 10000, PEG 20000, polyethylene glycol monomethyl ether 550 (mPEG 550), mPEG 600, mPEG 2000, mPEG 3350, mPEG 4000, mPEG 5000, polyvinylpyrrolidone (e.g., polyvinylpyrrolidone K 15), pentaerythritol propoxylate, or polypropylene glycol P 400), an organic solvent (e.g., dimethyl sulfoxide (DMSO) or ethanol), a sugar (e.g., D-(+)-sucrose, D-sorbitol, trehalose, D-(+)-maltose monohydrate, meso-erythritol, xylitol, myo-inositol, D-(+)-raffinose pentahydrate, D-(+)-trehalose dihydrate, or D-(+)-glucose monohydrate), or a salt (e.g., lithium acetate, lithium chloride, lithium formate, lithium nitrate, lithium sulfate, magnesium acetate, sodium chloride, sodium formate, sodium malonate, sodium nitrate, sodium sulfate, or any hydrate thereof) or any combination thereof.

[0604] In some embodiments, the stabilizing agent comprises a salt. In some embodiment, the salt is selected from the group consisting of lithium salts (e.g., lithium acetate, lithium chloride, lithium formate, lithium nitrate, lithium sulfate, or any hydrate thereof), magnesium salts (e.g., magnesium acetate or a hydrate thereof), and sodium salts (e.g., sodium chloride, sodium formate, sodium malonate, sodium nitrate, sodium sulfate, or any hydrate thereof). For another example, the formulation comprises one or more sodium salts. For yet another example, the formulation comprises sodium chloride.

[0605] In some embodiment, the stabilizing agent comprises a surfactant. In some embodiments, the surfactant comprises one or more anionic surfactants (e.g., 2-acrylamido-2-methylpropane sulfonic acid, ammonium lauryl sulfate, ammonium perfluorononanoate, docusate, disodium cocoamphodiacetate, magnesium laureth sulfate, perfluorobutanesulfonic acid, perfluorononanoic acid, perfluorooctanesulfonic acid, perfluorooctanoic acid, potassium lauryl sulfate, sodium alkyl sulfate, sodium dodecyl sulfate, sodium dodecylbenzenesulfonate, sodium laurate, sodium laureth sulfate, sodium lauroyl sarcosinate, sodium myreth sulfate, sodium nonanoyloxybenzenesulfonate, sodium pareth sulfate, sodium stearate, or sulfolipid), one or more cationic surfactants (e.g., behentrimonium chloride, benzalkonium chloride, benzethonium chloride, benzododecinium bromide, bronidox, carbethopendecinium bromide, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cetylpyridinium chloride, didecyldimethylammonium chloride, dimethyldioctadecylammonium bromide, dimethyldioctadecylammonium chloride, domiphen bromide, lauryl methyl gluceth-10 hydroxypropyl dimonium chloride, octenidine dihydrochloride, olaflur, n-oleyl-1,3-propanediamine, pahutoxin, stearalkonium chloride, tetramethylammonium hydroxide, or thonzonium bromide), one or more zwitterionic surfactants (e.g., cocamidopropyl betaine, cocamidopropyl hydroxysultaine, dipalmitoylphosphatidylcholine, egg lecithin, hydroxysultaine, lecithin, myristamine oxide, peptitergents, or sodium lauroamphoacetate), and/or one or more non-ionic surfactants (e.g., alkyl polyglycoside, cetomacrogol 1000, cetostearyl alcohol, cetyl alcohol, cocamide dea, cocamide mea, decyl glucoside, decyl polyglucose, glycerol monostearate, igepal ca-630, isoceteth-20, lauryl glucoside, maltosides, monolaurin, mycosubtilin, narrow-range ethoxylate, nonidet p-40, nonoxynol-9, nonoxynols, np-40, octaethylene glycol monododecyl ether, n-octyl beta-d-thioglucopyranoside, octyl glucoside, oleyl alcohol, peg-10 sunflower glycerides, pentaethylene glycol monododecyl ether, polidocanol, α-tocopheryl polyethylene glycol succinate (TPGS), poloxamer (e.g., poloxamer 407), polyethoxylated tallow amine, polyglycerol polyricinoleate, polysorbate (e.g., polysorbate 20, polysorbate 40, polysorbate 60, or polysorbate 80), sorbitan, sorbitan monolaurate, sorbitan monostearate, sorbitan tristearate, stearyl alcohol, surfactin, triton x-100).

[0606] In some embodiments, the the pharmaceutical composition or reconstituted solution of the present disclosure comprises a tonicity-adjusting agent.

[0607] In some embodiments, the tonicity-adjusting agent comprises NaCl, dextrose, dextran, ficoll, gelatin, mannitol, sucrose, glycine, glycerol, or any combination thereof.

[0608] In some embodiments, the the pharmaceutical composition or reconstituted solution of the present disclosure comprises a soothing agent. In some embodiments, the soothing agent comprises lidocaine

[0609] In addition to these components, the pharmaceutical composition or reconstituted solution of the present disclosure may include any substance useful in pharmaceutical compositions. In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure may include one or more pharmaceutically acceptable excipients or accessory ingredients such as, but not limited to, one or more solvents, dispersion media, diluents, dispersion aids, suspension aids, granulating aids, disintegrants, fillers, glidants, liquid vehicles, binders, surface active agents, isotonic agents, thickening or emulsifying agents, buffering agents, lubricating agents, oils, preservatives, and other species. Excipients such as waxes, butters, coloring agents, coating agents, flavorings, and perfuming agents may also be included. Pharmaceutically acceptable excipients are well known in the art (see for example Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro; Lippincott, Williams & Wilkins, Baltimore, MD, 2006).

[0610] Examples of diluents may include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, and/or combinations thereof. Granulating and dispersing agents may be selected from the non-limiting list consisting of potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM®), sodium lauryl sulfate, quaternary ammonium compounds, and/or combinations thereof.

[0611] Surface active agents and/or emulsifiers may include, but are not limited to, natural emulsifiers (e.g., acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g., bentonite [aluminum silicate] and VEEGUM® [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g., stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g., carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g., carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g., polyoxyethylene sorbitan monolaurate [TWEEN®20], polyoxyethylene sorbitan [TWEEN® 60], polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40],

sorbitan monostearate [SPAN®60], sorbitan tristearate [SPAN®65], glyceryl monooleate, sorbitan monooleate [SPAN®80]), polyoxyethylene esters (e.g., polyoxyethylene monostearate [MYRJ® 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g., CREMOPHOR®), polyoxyethylene ethers, (e.g., polyoxyethylene lauryl ether [BRIJ® 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLURONIC®F 68, POLOXAMER® 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, and/or combinations thereof.

[0612] A binding agent may be starch (e.g., cornstarch and starch paste); gelatin; sugars (e.g., sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol); natural and synthetic gums (*e.g.,* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (VEEGUM®), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; and combinations thereof, or any other suitable binding agent.

[0613] Examples of preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Examples of antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Examples of antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Examples of antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Examples of alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, benzyl alcohol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Examples of acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroascorbic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated

hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS®, PHENONIP®, methylparaben, GERMALL® 115, GERMABEN®II, NEOLONE™, KATHON™, and/or EUXYL®.

[0614] Examples of buffering agents include, but are not limited to, citrate buffering agents, acetate buffering agents, phosphate buffering agents, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, amino-sulfonate buffers (*e.g.*, HEPES), magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and/or combinations thereof. Lubricating agents may be selected from the non-limiting group consisting of magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and combinations thereof.

[0615] Examples of oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils as well as butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, simethicone, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or

[0616] As used herein, the term "pharmaceutically acceptable salt" takes its normal meaning in the art. In certain embodiments it refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds provided herein include those derived from suitable

inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, besylate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, lactic acid, trifluoracetic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

[0617] The salts can be prepared in situ during the isolation and purification of the disclosed compounds, or separately, such as by reacting the free base or free acid of a parent compound with a suitable base or acid, respectively. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal and alkaline earth metal. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Further pharmaceutically acceptable salts include, when appropriate, potassium, sodium, calcium, and magnesium salts.

[0618] "Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to ten carbon atoms (e.g., C_{1-10} alkyl). Whenever it appears herein, a numerical range such as "1 to 10" refers to each integer in the given range; e.g., "1 to 10 carbon atoms" means that the alkyl group can consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 10 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated. In some embodiments, "alkyl" can be a C_{1-6} alkyl group. In some embodiments, alkyl groups have 1 to 10, 1 to 8, 1 to 6, or 1 to 3 carbon atoms. Representative saturated straight chain alkyls include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, and -n-hexyl; while saturated branched alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, and the like. The alkyl is attached to the parent molecule by a single bond. Unless stated otherwise in the specification, an alkyl group is optionally substituted by one or more of substituents which independently

include: alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, or halo. In a non-limiting embodiment, a substituted alkyl can be selected from fluoromethyl, difluoromethyl, trifluoromethyl, 2-fluoroethyl, 3-fluoropropyl, hydroxymethyl, 2-hydroxyethyl, 3-hydroxypropyl, benzyl, and phenethyl.

[0619] "Alkenyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, and having from two to ten carbon atoms (i.e., C_{2-10} alkenyl). Whenever it appears herein, a numerical range such as "2 to 10" refers to each integer in the given range; e.g., "2 to 10 carbon atoms" means that the alkenyl group can consist of 2 carbon atoms, 3 carbon atoms, etc., up to and including 10 carbon atoms. In certain embodiments, an alkenyl comprises two to eight carbon atoms. In other embodiments, an alkenyl comprises two to six carbon atoms (e.g., C_{2-6} alkenyl). The alkenyl is attached to the parent molecular structure by a single bond, for example, ethenyl (i.e., vinyl), prop-1-enyl (i.e., allyl), but- 1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like. The one or more carbon-carbon double bonds can be internal (such as in 2-butenyl) or terminal (such as in 1-butenyl). Examples of C_{2-4} alkenyl groups include ethenyl (C_2), 1-propenyl (C_3), 2-propenyl (C_3), 1-butenyl (C_4), 2-butenyl (C_4), 2-methylprop-2-enyl (C_4), butadienyl (C_4) and the like. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkenyl groups as well as pentenyl (C_5), pentadienyl (C_5), hexenyl (C_6), 2,3-dimethyl-2-butenyl (C_6) and the like. Additional examples of alkenyl include heptenyl (C_7), octenyl (C_8), octatrienyl (C_8) and the like. Unless stated otherwise in the specification, an alkenyl group can be optionally substituted by one or more substituents which independently include: alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, or halo.

[0620] "Alkynyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one triple bond, having from two to ten carbon atoms (i.e., C_{2-10} alkynyl). Whenever it appears herein, a numerical range such as "2 to 10" refers to each integer in the given range; e.g., "2 to 10 carbon atoms" means that the alkynyl group can consist of 2 carbon atoms, 3 carbon atoms, etc., up to and including 10 carbon atoms. In certain embodiments, an alkynyl comprises two to eight carbon atoms. In other embodiments, an alkynyl has two to six carbon atoms (e.g., C_{2-6} alkynyl). The alkynyl is attached to the parent molecular structure by a single bond, for example, ethynyl, propynyl, butynyl, pentynyl, 3-methyl-4-pentenyl, hexynyl, and the like. Unless stated otherwise in the specification, an alkynyl group can be optionally substituted by one or more substituents which independently include: alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, and halo.

[0621] "Alkoxy" refers to the group -O-alkyl, including from 1 to 10 carbon atoms of a straight, branched, saturated cyclic configuration and combinations thereof, attached to the parent molecular structure through an oxygen. Examples include methoxy, ethoxy, propoxy, isopropoxy, butoxy, t-butoxy, pentoxy, cyclopropyloxy, cyclohexyloxy and the like. "Lower alkoxy" refers to alkoxy groups containing one to six carbons. In some embodiments, C_{1-4} alkoxy is an alkoxy group which encompasses both straight and branched chain alkyls of from 1

to 4 carbon atoms. Unless stated otherwise in the specification, an alkoxy group can be optionally substituted by one or more substituents which independently include: alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, and halo.

[0622] "Aryl" refers to a radical with 6 to 14 ring atoms (e.g., C_{6-14} aromatic or C_{6-14} aryl) which has at least one ring having a conjugated pi electron system which is carbocyclic (e.g., phenyl, fluorenyl, and naphthyl). In some embodiments, the aryl is a C_{6-10} aryl group. For example, bivalent radicals formed from substituted benzene derivatives and having the free valences at ring atoms are named as substituted phenylene radicals. In other embodiments, bivalent radicals derived from univalent polycyclic hydrocarbon radicals whose names end in"-yl" by removal of one hydrogen atom from the carbon atom with the free valence are named by adding "-idene" to the name of the corresponding univalent radical, e.g., a naphthyl group with two points of attachment is termed naphthylidene. Whenever it appears herein, a numerical range such as "6 to 14 aryl" refers to each integer in the given range; e.g., "6 to 14 ring atoms" means that the aryl group can consist of 6 ring atoms, 7 ring atoms, etc., up to and including 14 ring atoms. The term includes monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of ring atoms) groups. Polycyclic aryl groups include bicycles, tricycles, tetracycles, and the like. In a multi-ring group, only one ring is required to be aromatic, so groups such as indanyl are encompassed by the aryl definition. Non-limiting examples of aryl groups include phenyl, phenalenyl, naphthalenyl, tetrahydronaphthyl, phenanthrenyl, anthracenyl, fluorenyl, indolyl, indanyl, and the like. Unless stated otherwise in the specification, an aryl moiety can be optionally substituted by one or more substituents which independently include: alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, and halo. When "aryl" is "tolyl" this term includes any of o-tolyl, m-tolyl, and p-tolyl groups. In other words, "tolyl" includes any of the three isomeric univalent aromatic radicals derived from toluene. When "aryl" is "xylyl" this term includes the univalent radicals, of formula $(CH_3)_2C_6H_3$- derived from the three isomers of xylene: ortho-, meta- and para- (di-methyl benzene).

[0623] "Cycloalkyl" and "carbocyclyl" each refer to a monocyclic or polycyclic radical that contains only carbon and hydrogen, and can be saturated or partially unsaturated. Partially unsaturated cycloalkyl groups can be termed "cycloalkenyl" if the carbocycle contains at least one double bond, or "cycloalkynyl" if the carbocycle contains at least one triple bond. Cycloalkyl groups include groups having from 3 to 13 ring atoms (i.e., C_{3-13} cycloalkyl). Whenever it appears herein, a numerical range such as "3 to 10" refers to each integer in the given range; e.g., "3 to 13 carbon atoms" means that the cycloalkyl group can consist of 3 carbon atoms, 4 carbon atoms, 5 carbon atoms, etc., up to and including 13 carbon atoms. The term "cycloalkyl" also includes bridged and spiro-fused cyclic structures containing no heteroatoms. The term also includes monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of ring atoms) groups. Polycyclic aryl groups include bicycles, tricycles, tetracycles, and the like. In some embodiments, "cycloalkyl" can be a C_{3-8} cycloalkyl radical. In some embodiments, "cycloalkyl" can be a C_{3-5} cycloalkyl radical. Illustrative examples of cycloalkyl groups include, but are not limited to the following moieties: C_{3-6} carbocyclyl groups include, without limitation, cyclopropyl (C_3), cyclobutyl (C_4), cyclopentyl (C_5),

cyclopentenyl (C_5), cyclohexyl (C_6), cyclohexenyl (C_6), cyclohexadienyl (C_6) and the like. Examples of C_{3-7} carbocyclyl groups include norbornyl (C_7). Examples of C_{3-8} carbocyclyl groups include the aforementioned C_{3-7} carbocyclyl groups as well as cycloheptyl(C_7), cycloheptadienyl (C_7), cycloheptatrienyl (C_7), cyclooctyl (C_8), bicyclo[2.2.1]heptanyl, bicyclo[2.2.2]octanyl, and the like. Examples of C_{3-13} carbocyclyl groups include the aforementioned C_{3-8} carbocyclyl groups as well as octahydro-1H indenyl, decahydronaphthalenyl, spiro[4.5]decanyl and the like. Unless stated otherwise in the specification, a cycloalkyl group can be optionally substituted by one or more substituents which independently include: alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, and halo. The terms "cycloalkenyl" and "cycloalkynyl" mirror the above description of "cycloalkyl" wherein the prefix "alk" is replaced with "alken" or "alkyn" respectively, and the parent "alkenyl" or "alkynyl" terms are as described herein. For example, a cycloalkenyl group can have 3 to 13 ring atoms, such as 5 to 8 ring atoms. In some embodiments, a cycloalkynyl group can have 5 to 13 ring atoms.

[0624] As used herein, a "covalent bond" or "direct bond" refers to a single bond joining two groups.

[0625] "Halo", "halide", or, alternatively, "halogen" means fluoro, chioro, bromo or iodo. The terms "haloalkyl," "haloalkenyl," "haloalkynyl" and "haloalkoxy" include alkyl, alkenyl, alkynyl and alkoxy structures that are substituted with one or more halo groups or with combinations thereof. For example, the terms "fluoroalkyl" and "fluoroalkoxy" include haloalkyl and haloalkoxy groups, respectively, in which the halo is fluorine, such as, but not limited to, trifluoromethyl, difluoromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and the like. Each of the alkyl, alkenyl, alkynyl and alkoxy groups are as defined herein and can be optionally further substituted as defined herein.

Methods of Use

[0626] In certain embodiments, the present disclosure relates to inducing, promoting, or enhancing the growth, proliferation or regeneration of inner ear tissue, particularly inner ear supporting cells and hair cells by using the composition disclosed herein. Some embodiments relate to methods for controlled proliferation of stem cells comprising an initial phase of inducing stemness while inhibiting differentiation and a subsequent phase of differentiation of the stem cells into tissue cells.

[0627] When cochlear supporting cell or vestibular supporting cell populations are treated with a hair cell regeneration agent in accordance to the methods of the disclosure, whether the population is in vivo or in vitro, the treated supporting cells exhibit stem-like behavior in that the treated supporting cells have the capacity to proliferate and differentiate and, more specifically, differentiate into cochlear hair cells or vestibular hair cells. In some instances, an agent induces and maintains the supporting cells to produce daughter stem cells that can divide for many generations and maintain the ability to have a high proportion of the resulting cells differentiate into hair cells. In certain embodiments, the proliferating stem cells express stem cell marker(s) selected from one or more of Lgr5,

Sox2, Opeml, Phex, lin28, Lgr6, cyclin D1, Msx1, Myb, Kit, Gdnf3, Zic3, Dppa3, Dppa4, Dppa5, Nanog, Esrrb, Rex1, Dnmt3a, Dnmt3b, Dnmt3l, Utf1, Tcl1, Oct4, Klf4, Pax6, Six2, Zic1, Zic2, Otx2, Bmi1, CDX2, STAT3, Smad1, Smad2, smad2/3, smad4, smad5, and smad7. Preferably, the proliferating stem cells express stem cell marker(s) selected from one or more of Lgr5, the

[0628] In some embodiments, the methods may be used to maintain, or even transiently increase stemness (i.e., self-renewal) of a pre-existing supporting cell population prior to significant hair cell formation. In some embodiments, the pre-existing supporting cell population comprises inner pillar cells, outer pillar cells, inner phalangeal cells, Deiter cells, Hensen cells, Boettcher cells, and/or Claudius cells. Morphological analyses with immunostaining (including cell counts) and lineage tracing across a Representative Microscopy Samples may be used to confirm expansion of one or more of these cell-types. In some embodiments, the pre-existing supporting cells comprise Lgr5+ cells. Morphological analyses with immunostaining (including cell counts) and qPCR and RNA hybridization may be used to confirm Lgr5 upregulation amongst the cell population.

[0629] Advantageously, methods described herein can achieve these goals without the use of genetic manipulation. Germ-line manipulation used in many academic studies is not a therapeutically desirable approach to treating hearing loss. In general, the therapy preferably involves the administration of a small molecule, peptide, antibody, or other non-nucleic acid molecule or nucleic acid delivery vector unaccompanied by gene therapy. In certain embodiments, the therapy involves the administration of a small organic molecule. In some instances, hearing protection or restoration is achieved through the use of a (non-genetic) therapeutic that is injected in the middle ear and diffuses into the cochlea.

[0630] The cochlea relies heavily on all present cell types, and the organization of these cells is important to their function. Supporting cells play an important role in neurotransmitter cycling and cochlear mechanics. Thus, maintaining a rosette patterning within the organ of Corti may be important for function. Cochlear mechanics of the basilar membrane activate hair cell transduction. Due to the high sensitivity of cochlear mechanics, it is also desirable to avoid masses of cells. In all, maintaining proper distribution and relation of hair cells and supporting cells along the basilar membrane, even after proliferation, is likely a desired feature for hearing as supporting cell function and proper mechanics is necessary for normal hearing.

[0631] In some embodiments the hearing loss treated by using a composition as disclosed herein is senorineural hearing loss or hidden hearing loss.

[0632] Sensorineural hearing loss accounts for approximately 90% of hearing loss and it often arises from damage or loss of hair cells in the cochlea. There are numerous causes of hair cell damage and loss, and the agents and treatments described herein may be used in the context of sensorineural hearing loss arising from any cause of hair cell damage or loss. For example, hair cells may be damage and loss may be induced by noise exposure, leading to noise-induced sensorineural hearing loss. Thus, in some embodiments sensorineural hearing loss is noise-induced

sensorineural hearing loss. Noise-induced sensorineural hearing loss can be a result of chronic noise exposure or acute noise exposure. Ototoxic drugs, for example cisplatin and its analogs, aminoglycoside antibiotics, salicylate and its analogs, or loop diuretics, can also cause sensorineural hearing loss. In some embodiments sensorineural hearing loss is drug-induced sensorineural hearing loss. Infection may damage cochlear hair cells, and may be a cause of sudden sensorineural hearing loss. In some embodiments sensorineural hearing loss is sudden sensorineural hearing loss (SSNHL). Sudden sensorineural hearing can also be idiopathic. Hair cells can also be lost or damaged over time as part of the ageing process in humans. In some embodiments, sensorineural hearing loss is age-related sensorineural hearing loss (also known as presbycusis).

[0633] In some aspects, the present disclosure provides a method of facilitating the regeneration of a tissue and/or a cell, comprising delivering a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the tissue and/or the cell.

[0634] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing, a disease associated with absence or a lack of a tissue and/or a cell, comprising administering to the subject a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure.

[0635] In some aspects, the present disclosure provides a method of increasing a population of vestibular cells in a vestibular tissue, comprising delivering a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0636] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing a vestibular condition, comprising administering to the subject a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure.

[0637] In some aspects, the present disclosure provides a method of increasing a population of cochlear cells in a cochlear tissue, comprising delivering a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0638] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing a cochlear condition, comprising administering to the subject a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure.

[0639] In some aspects, the present disclosure provides a method of increasing a population of cells found in the Organ of Corti, comprising delivering a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0640] In some aspects, the present disclosure provides a method of increasing a population of hair cells found in the Organ of Corti, comprising deliverying a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0641] In some aspects, the present disclosure provides a method of increasing a population of inner hair cells found in the Organ of Corti, comprising deliverying a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0642] In some aspects, the present disclosure provides a method of increasing a population of outer hair cells found in the Organ of Corti, comprising deliverying a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0643] In some aspects, the present disclosure provides a method of increasing a population of neuronal cells found in the Organ of Corti, comprising deliverying a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure to the population.

[0644] In some aspects, the present disclosure provides a method of treating a subject who has, or is at risk of developing a hearing condition, comprising administering to the subject a pharmaceutically effective amount of the pharmaceutical composition or the reconstituted solution of the present disclosure.

[0645] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in facilitating the generation of a tissue and/or a cell.

[0646] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in treating a subject who has, or is at risk of developing, a disease associated with absence or a lack of a tissue and/or a cell.

[0647] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of vestibular cells in a vestibular tissue.

[0648] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in treating a subject who has, or is at risk of developing a vestibular condition.

[0649] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of cochlear cells in a cochlear tissue.

[0650] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in treating a subject who has, or is at risk of developing a cochlear condition.

[0651] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of cells found in the Organ of Corti.

[0652] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of hair cells found in the Organ of Corti.

[0653] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of inner hair cells found in the Organ of Corti.

[0654] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of outer hair cells found in the Organ of Corti.

[0655] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in increasing a population of neuronal cells found in the Organ of Corti.

[0656] In some aspects, the present disclosure provides the pharmaceutical composition or the reconstituted solution of the present disclosure for use in treating a subject who has, or is at risk of developing a hearing condition.

[0657] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for facilitating the generation of a tissue and/or a cell.

[0658] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for in treating a subject who has, or is at risk of developing, a disease associated with absence or a lack of a tissue and/or a cell.

[0659] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of vestibular cells in a vestibular tissue.

[0660] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for treating a subject who has, or is at risk of developing a vestibular condition.

[0661] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of cochlear cells in a cochlear tissue.

[0662] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for treating a subject who has, or is at risk of developing a cochlear condition.

[0663] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of cells found in the Organ of Corti.

[0664] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of hair cells found in the Organ of Corti.

[0665] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of inner hair cells found in the Organ of Corti.

[0666] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of outer hair cells found in the Organ of Corti.

[0667] In some aspects, the present disclosure provides for use of the pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for increasing a population of neuronal cells found in the Organ of Corti.

[0668] Use of the lyophilized pharmaceutical composition or the reconstituted solution of the present disclosure in the manufacture of a medicament for treating a subject who has, or is at risk of developing a hearing condition.

[0669] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure is delivered extratympanically (i.e., onto the eardrum).

[0670] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure is delivered intratympanically (i.e., into the middle ear).

[0671] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure is delivered continuously.

[0672] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure is delivered as a bolus injection.

[0673] In some embodiments, about about 1 ml or less, about 900 µl or less, about 800 µl or less, about 700 µl or less, about 600 µl or less, about 500 µl or less, about 400 µl or less, about 300 µl or less, about 200 µl or less, or about 100 or less of the pharmaceutical composition or reconstituted solution is injected.

[0674] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 10 mg/kg, from about 0.001 mg/kg to about 10 mg/kg, from about 0.005 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.05 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg, from about 2 mg/kg to about 10 mg/kg, from about 5 mg/kg to about 10 mg/kg, from about 0.0001 mg/kg to about 5 mg/kg, from about 0.001 mg/kg to about 5 mg/kg, from about 0.005 mg/kg to about 5 mg/kg, from about 0.01 mg/kg to about 5 mg/kg, from about 0.05 mg/kg to about 5 mg/kg, from about 0.1 mg/kg to about 5 mg/kg, from about 1 mg/kg to about 5 mg/kg, from about 2 mg/kg to about 5 mg/kg, from about 0.0001 mg/kg to about 2.5 mg/kg, from about 0.001 mg/kg to about 2.5 mg/kg, from about 0.005 mg/kg to about 2.5 mg/kg, from about 0.01 mg/kg to about 2.5 mg/kg, from about 0.05 mg/kg to about 2.5 mg/kg, from about 0.1 mg/kg to about 2.5 mg/kg, from about 1 mg/kg to about 2.5 mg/kg, from about 2 mg/kg to about 2.5 mg/kg, from about 0.0001 mg/kg

to about 1 mg/kg, from about 0.001 mg/kg to about 1 mg/kg, from about 0.005 mg/kg to about 1 mg/kg, from about 0.01 mg/kg to about 1 mg/kg, from about 0.05 mg/kg to about 1 mg/kg, from about 0.1 mg/kg to about 1 mg/kg, from about 0.0001 mg/kg to about 0.25 mg/kg, from about 0.001 mg/kg to about 0.25 mg/kg, from about 0.005 mg/kg to about 0.25 mg/kg, from about 0.01 mg/kg to about 0.25 mg/kg, from about 0.05 mg/kg to about 0.25 mg/kg, or from about 0.1 mg/kg to about 0.25 mg/kg of a therapeutic and/or prophylactic (*e.g.*, an mRNA) in a given dose, where a dose of 1 mg/kg (mpk) provides 1 mg of a therapeutic and/or prophylactic per 1 kg of subject body weight. In some embodiments, a dose of about 0.001 mg/kg to about 10 mg/kg of a therapeutic and/or prophylactic (*e.g.*, mRNA) of a LNP may be administered. In some embodiments, a dose of about 0.005 mg/kg to about 2.5 mg/kg of a therapeutic and/or prophylactic may be administered. In some embodiments, a dose of about 0.1 mg/kg to about 1 mg/kg may be administered. In some embodiments, a dose of about 0.05 mg/kg to about 0.25 mg/kg may be administered. A dose may be administered one or more times per day, in the same or a different amount, to obtain a desired level of mRNA expression and/or therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered, for example, three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In some embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). In some embodiments, a single dose may be administered, for example, prior to or after a surgical procedure or in the instance of an acute disease, disorder, or condition.

[0675] In some embodiments, the administration of the pharmaceutical composition or reconstituted solution results in a plasma concentration for the one or more otic therapeutic agents (e.g., CHIR99021 and sodium valproate) having a maximum plasma concentration at at time ranging from 10 minutes to about 3 hours, from about 20 minutes to about 2 hours, or form about 30 minutes to about 1 hour.

Definitions

[0676] Articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or all, of the group members are present in, employed in, or otherwise relevant to a given product or process.

[0677] As used herein, the terms "approximately" and "about," as applied to one or more values of interest, refer to a value that is similar to a stated reference value. In some embodiments, the term "approximately" or "about" refers

to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value). In some embodiments, the term "approximately" or "about" refers to +/- 10% of the recited value. In some embodiments, when used in the context of an amount of a given compound in a lipid component of a LNP, "about" may mean +/- 10% of the recited value.

[0678] As used herein, the expressions "one or more of A, B, or C," "one or more A, B, or C," "one or more of A, B, and C," "one or more A, B, and C," "selected from the group consisting of A, B, and C", "selected from A, B, and C", and the like are used interchangeably and all refer to a selection from a group consisting of A, B, and/or C, i.e., one or more As, one or more Bs, one or more Cs, or any combination thereof, unless indicated otherwise.

[0679] As used herein, the term "bulking agent" refers to an agent that adds bulk to a pharmaceutical composition and/or modifies one or more the properties of the pharmaceutical composition (e.g., the appearance of the cake, the porosity, drug stability, and/or the reconstitution time).

[0680] As used herein, the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the terms "consisting essentially of" and "consisting of" are thus also encompassed and disclosed. Throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where methods or processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions is immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

[0681] As used herein, the term "comparable pharmaceutical composition" refers to a pharmaceutical composition with comparable parameters, as of the pharmaceutical composition being compared (e.g., the one or more otic therapeutic agents (e.g., hearing loss treatment agents) and gelling agents therein, and/or the concentration of the one or more otic therapeutic agents (e.g., hearing loss treatment agents) and gelling agents). In some embodiments, the "comparable pharmaceutical composition" comprises a poloxamer (e.g., Poloxamer 407) with lower purity as compared to pharmaceutical composition being compared. In some embodiments, the "comparable pharmaceutical composition" does not comprise a purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the "comparable pharmaceutical composition" comprise a unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0682] As used herein, the term "comparable reconstituted solution" refers to a reconstituted solution with comparable parameters as of the reconstituted solution being compared (e.g., the one or more otic therapeutic agents (e.g., hearing loss treatment agents) and gelling agents therein, and/or the concentration of the one or more otic

therapeutic agents (e.g., hearing loss treatment agents) and gelling agents). In some embodiments, the "comparable reconstituted solution" comprises a poloxamer (e.g., Poloxamer 407) with lower purity as compared to reconstituted solution being compared. In some embodiments, the "comparable reconstituted solution" does not comprise a purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the "comparable reconstituted solution" comprise a unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0683] In some embodiments, the "comparable reconstituted solution" is pepared from a pharmaceutical composition comprising a poloxamer (e.g., Poloxamer 407) with lower purity as compared to pharmaceutical composition used for preparing the reconstituted solution being compared. In some embodiments, the "comparable reconstituted solution" is pepared from a pharmaceutical composition not comprising a purified poloxamer (e.g., purified Poloxamer 407). In some embodiments, the "comparable reconstituted solution" is pepared from a pharmaceutical composition comprising a unpurified poloxamer (e.g., unpurified Poloxamer 407).

[0684] As used herein, the term "impurity" refers to a compound that is undersirable for the pharmaceutical composition. In some mebodiments, the impurity is selected from solvents, 1-acetate-2-formate-1,2-propanediol, acetic acid, fomic acid, formaldehyde, acetaldehyde, propionaldehyde, low MW poloxamers, and degradants from CHIR99021 and valproic acid.

[0685] As used herein, the term "soothing agent" refers to an agent capable of mitigating the discomfort from administration of the formulation to patients.

[0686] As used herein, the term "stabilizing agent" refers to an agent capable of maintaining the one or more desirable properties of the pharmaceutical composition (e.g., reduced suspetability to degradation by heat, light, or air).

[0687] As used herein, the term "unpurified poloxamer" refers to a poloxamer not being purified (e.g., by the process disclosed herein). In some embodiments, the unpurified poloxamer (e.g., unpurified Poloxamer 407) has an average molecular weight of about 12 kDa or lower, about 11 kDa or lower, about 10 kDa or lower, about 9 kDa or lower, about 8 kDa or lower, or about 7 kDa or lower. In some embodiments, the unpurified poloxamer (e.g., unpurified Poloxamer 407) is not purified by any liquid-liquid extraction or size exclusion chromatography.

[0688] As used herein, the term "purified poloxamer" may in some embodiments refer to poloxamer that is at least 85% by weight poloxamer that has a molecular weight of at least 7250 Da. Purified poloxamer can in some embodiments be prepared by following the method of: A. Fakhari, M Corcoran, A Schwarz, Thermogelling Properties of Purified Poloxamer 407, Heliyon (2017), 3(8), e00390. Many further options for how the purified poloxamer can be defined are set out herein, including in the numbered clauses and embodiments.

[0689] It is to be understood that the present disclosure provides methods for preparing any of the pharmaceutical compositions and reconstituted solutions described herein. The present disclosure also provides detailed methods for

preparing various pharmaceutical compositions and reconstituted solutions following the procedures described in the Examples.

[0690] It is to be understood that, throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where methods or processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions is immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

[0691] It is to be understood that, unless otherwise stated, any description of a method of treatment includes use of the compounds to provide such treatment or prophylaxis as is described herein, as well as use of the compounds to prepare a medicament to treat or prevent such condition. The treatment includes treatment of human or non-human animals including rodents and other disease models.

[0692] As used herein, the term "sterile" refers to solutions, products, equipment, or glass ware that are treated and / or handled to be free from bacteria or other living microorganisms.

[0693] As used herein, the term "subject" is interchangeable with the term "subject in need thereof", both of which refer to a subject having a disease or having an increased risk of developing the disease. A "subject" includes a mammal. The mammal can be *e.g.*, a human or appropriate non-human mammal, such as primate, mouse, rat, dog, cat, cow, horse, goat, camel, sheep or a pig. The subject can also be a bird or fowl. In one embodiment, the mammal is a human. A subject in need thereof can be one who has been previously diagnosed or identified as having an imprinting disorder. A subject in need thereof can also be one who has (e.g., is suffering from) an imprinting disorder. Alternatively, a subject in need thereof can be one who has an increased risk of developing such disorder relative to the population at large (*i.e.*, a subject who is predisposed to developing such disorder relative to the population at large). A subject in need thereof can have a refractory or resistant imprinting disorder (i.e., an imprinting disorder that doesn't respond or hasn't yet responded to treatment). The subject may be resistant at start of treatment or may become resistant during treatment. In some embodiments, the subject in need thereof received and failed all known effective therapies for an imprinting disorder. In some embodiments, the subject in need thereof received at least one prior therapy. In a preferred embodiment, the subject has an imprinting disorder.

[0694] As used herein, the term "sterilization" refers to process for ensuring the removal of undesired contamination including bacteria, mold and yeast and particles using e.g., a 0.2-micron filter. Filter materials used in the sterilization of liquids include, but are not limited to, nylon, polycarbonate, cellulose acetate, polyvinylidene fluoride (PVDF), and polyethersulfone (PES).

[0695] As used herein, the term "tonicity" refers to a measured level of effective osmolarity. In some embodiments, the tonicity refers to a measured level of the effective osmotic pressure gradient, as defined by the water potential of two solutions separated by a semipermeable membrane.

[0696] As used herein, the term "tonicity-adjusting agent" refers to an agent capable of changing the tonicity of the pharmaceutical composition or solution to a desired level.

[0697] As used herein, the term "treating" or "treat" describes the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of a compound of the present disclosure, or a pharmaceutically acceptable salt, polymorph or solvate thereof, to alleviate the symptoms or complications of a disease, condition or disorder, or to eliminate the disease, condition or disorder. The term "treat" can also include treatment of a cell *in vitro* or an animal model.

[0698] It is to be understood that a compound of the present disclosure, or a pharmaceutically acceptable salt, polymorph or solvate thereof, can or may also be used to prevent a relevant disease, condition or disorder, or used to identify suitable candidates for such purposes.

[0699] As used herein, the term "preventing," "prevent," or "protecting against" describes reducing or eliminating the onset of the symptoms or complications of such disease, condition or disorder.

[0700] It is to be understood that one skilled in the art may refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. (2005); Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (3rd edition), Cold Spring Harbor Press, Cold Spring Harbor, New York (2000); Coligan *et al.*, *Current Protocols in Immunology*, John Wiley & Sons, N.Y.; Enna *et al.*, *Current Protocols in Pharmacology*, John Wiley & Sons, N.Y.; Fingl *et al.*, *The Pharmacological Basis of Therapeutics* (1975), *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 18th edition (1990). These texts can, of course, also be referred to in making or using an aspect of the disclosure.

[0701] As used herein, the term "pharmaceutical composition" is a formulation containing one or more otic therapeutic agents (e.g., hearing loss treatment agents) of the present disclosure in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler or a vial. The quantity of active ingredient (*e.g.*, a formulation of the disclosed compound or salt, hydrate, solvate or isomer thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, inhalational, buccal, sublingual, intrapleural, intrathecal,

intranasal, and the like. Dosage forms for the topical or transdermal administration of a compound of this disclosure include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, the active compound is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

[0702] As used herein, the term "pharmaceutically acceptable" refers to those compounds, anions, cations, materials, compositions, carriers, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0703] As used herein, the term "pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient.

[0704] It is to be understood that a pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), and transmucosal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0705] It is to be understood that a compound or pharmaceutical composition of the disclosure can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, a compound of the disclosure may be injected into the blood stream or body cavities or taken orally or applied through the skin with patches. The dose chosen should be sufficient to constitute effective treatment but not so high as to cause unacceptable side effects. The state of the disease condition (*e.g.*, imprinting disorders, and the like) and the health of the patient should preferably be closely monitored during and for a reasonable period after treatment.

[0706] As used herein, the term "therapeutically effective amount", refers to an amount of a pharmaceutical agent to treat, ameliorate, or prevent an identified disease or condition, or to exhibit a detectable therapeutic or inhibitory effect. The effect can be detected by any assay method known in the art. The precise effective amount for a subject will depend upon the subject's body weight, size, and health; the nature and extent of the condition; and the

therapeutic or combination of therapeutics selected for administration. Therapeutically effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician. In a preferred aspect, the disease or condition to be treated is an imprinting disorder.

[0707] It is to be understood that, for any compound, the therapeutically effective amount can be estimated initially either in cell culture assays, *e.g.*, of neoplastic cells, or in animal models, usually rats, mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic/prophylactic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The dosage may vary within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0708] Dosage and administration are adjusted to provide sufficient levels of the active agent(s) or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[0709] The pharmaceutical compositions containing active compounds of the present disclosure may be manufactured in a manner that is generally known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and/or auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[0710] General guidelines for the formulation and manufacture of pharmaceutical compositions and agents are available, for example, in Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro; Lippincott, Williams & Wilkins, Baltimore, MD, 2006. Conventional excipients and accessory ingredients may be used in any pharmaceutical composition.

[0711] In some embodiments, the pharmaceutical composition or reconstituted solution of the present disclosure is refrigerated or frozen for storage and/or shipment (e.g., being stored at a temperature of 4 °C or lower, such as a temperature between about -150 °C and about 0 °C or between about -80 °C and about -20 °C (e.g., about -5 °C, -10

°C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C). In some embodiments, the present disclosure also relates to a method of increasing stability of the pharmaceutical composition or reconstituted solution and by storing the pharmaceutical composition or reconstituted solution at a temperature of 4 °C or lower, such as a temperature between about -150 °C and about 0 °C or between about -80 °C and about -20 °C, e.g., about -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C).

[0712] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol and sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0713] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0714] Oral compositions generally include an inert diluent or an edible pharmaceutically acceptable carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents,

and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0715] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0716] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or patches, thin films, tablets to be used for buccal or sublingual application or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, creams, gels, patches or microneedle delivery systems as generally known in the art.

[0717] The active compounds can be prepared with pharmaceutically acceptable carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylacticglycolic acid and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0718] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

[0719] In therapeutic applications, the dosages of the pharmaceutical compositions used in accordance with the disclosure vary depending on the agent, the age, weight, and clinical condition of the recipient patient, and the

experience and judgment of the clinician or practitioner administering the therapy, among other factors affecting the selected dosage. Generally, the dose should be sufficient to result in slowing, and preferably regressing, the symptoms of the imprinting disorder and also preferably causing complete regression of the imprinting disorder. Dosages can range from about 0.01 mg/kg per day to about 5000 mg/kg per day. In preferred aspects, dosages can range from about 1 mg/kg per day to about 1000 mg/kg per day. In an aspect, the dose will be in the range of about 0.1 mg/day to about 50 g/day; about 0.1 mg/day to about 25 g/day; about 0.1 mg/day to about 10 g/day; about 0.1 mg to about 3 g/day; or about 0.1 mg to about 1 g/day, in single, divided, or continuous doses (which dose may be adjusted for the patient's weight in kg, body surface area in m^2, and age in years). An effective amount of a pharmaceutical agent is that which provides an objectively identifiable improvement as noted by the clinician or other qualified observer. Improvement in survival and growth indicates regression. As used herein, the term "dosage effective manner" refers to amount of an active compound to produce the desired biological effect in a subject or cell.

[0720] It is to be understood that the pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0721] It is to be understood that, for the compounds of the present disclosure beingcapable of further forming salts, all of these forms are also contemplated within the scope of the claimed disclosure.

[0722] As used herein, the term "pharmaceutically acceptable salts" refer to derivatives of the compounds of the present disclosure wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines, alkali or organic salts of acidic residues such as carboxylic acids, and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include, but are not limited to, those derived from inorganic and organic acids selected from 2-acetoxybenzoic, 2-hydroxyethane sulfonic, acetic, ascorbic, benzene sulfonic, benzoic, bicarbonic, carbonic, citric, edetic, ethane disulfonic, 1,2-ethane sulfonic, fumaric, glucoheptonic, gluconic, glutamic, glycolic, glycollyarsanilic, hexylresorcinic, hydrabamic, hydrobromic, hydrochloric, hydroiodic, hydroxymaleic, hydroxynaphthoic, isethionic, lactic, lactobionic, lauryl sulfonic, maleic, malic, mandelic, methane sulfonic, napsylic, nitric, oxalic, pamoic, pantothenic, phenylacetic, phosphoric, polygalacturonic, propionic, salicylic, stearic, subacetic, succinic, sulfamic, sulfanilic, sulfuric, tannic, tartaric, toluene sulfonic, and the commonly occurring amine acids, *e.g.*, glycine, alanine, phenylalanine, arginine, etc.

[0723] Other examples of pharmaceutically acceptable salts include hexanoic acid, cyclopentane propionic acid, pyruvic acid, malonic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo-[2.2.2]-oct-2-ene-1-

carboxylic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, muconic acid, and the like. The present disclosure also encompasses salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. In the salt form, it is understood that the ratio of the compound to the cation or anion of the salt can be 1:1, or any ration other than 1:1, e.g., 3:1, 2:1, 1:2, or 1:3.

[0724] It is to be understood that all references to pharmaceutically acceptable salts include solvent addition forms (solvates) or crystal forms (polymorphs) as defined herein, of the same salt.

[0725] It is to be understood that the compounds of the present disclosure can also be prepared as esters, for example, pharmaceutically acceptable esters. For example, a carboxylic acid function group in a compound can be converted to its corresponding ester, *e.g.*, a methyl, ethyl or other ester. Also, an alcohol group in a compound can be converted to its corresponding ester, *e.g.*, acetate, propionate or other ester.

[0726] In certain embodiments, it is to be understood that the compounds of the present disclosure can be a prodrug (that may include an ester) of any compound disclosed herein.

[0727] In certain embodiments, it is to be understood that the compounds of the present disclosure can also be prepared as co-crystals with other compounds.

[0728] The compounds, or pharmaceutically acceptable salts thereof, are administered orally, nasally, transdermally, pulmonary, inhalationally, buccally, sublingually, intraperitoneally, subcutaneously, intramuscularly, intravenously, rectally, intrapleurally, intrathecally and parenterally. In one embodiment, the compound is administered orally. One skilled in the art will recognize the advantages of certain routes of administration.

[0729] The dosage regimen utilizing the compounds is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

[0730] Techniques for formulation and administration of the disclosed compounds of the disclosure can be found in *Remington: the Science and Practice of Pharmacy*, 19th edition, Mack Publishing Co., Easton, PA (1995). In an embodiment, the compounds described herein, and the pharmaceutically acceptable salts thereof, are used in pharmaceutical preparations in combination with a pharmaceutically acceptable carrier or diluent. Suitable pharmaceutically acceptable carriers include inert solid fillers or diluents and sterile aqueous or organic solutions. The compounds will be present in such pharmaceutical compositions in amounts sufficient to provide the desired dosage amount in the range described herein.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 157

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 157

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS:

1. A lyophilized pharmaceutical composition comprising a poloxamer and valproic acid or a pharmaceutically acceptable salt thereof.

2. A pharmaceutical composition comprising a poloxamer, valproic acid or a pharmaceutically acceptable salt thereof at a concentration of greater than about 100 mg/mL, and CHIR99021 or a pharmaceutically acceptable salt thereof.

3. A method for preparing a pharmaceutical composition comprising the steps of:
(a) having an aqueous solution comprising a poloxamer and valproic acid or a pharmaceutically acceptable salt thereof; and
(b) adding a solution of CHIR99021 or a pharmaceutically acceptable salt thereof.

4. The method of claim 3, wherein the CHIR99021 or a pharmaceutically acceptable salt thereof is dissolved in a solution comprising dimethyl sulfoxide (DMSO).

5. A composition of claims 1 or 2, or the method of claims 3 or 4, wherein the composition is suitable for intratympanic injection.

6. The composition or method of any preceding claim, wherein in the composition the concentration of valproic acid or a pharmaceutically acceptable salt thereof is greater than about 100 mg/mL.

7. The composition or method of any preceding claim, wherein in the composition the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 100 to about 500 mg/mL.

8. The composition or method of any preceding claim, wherein in the composition the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 100 to about 350 mg/mL.

9. The composition or method of any preceding claim, wherein in the composition the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 125 to about 140 mg/mL.

10. The composition or method of any preceding claim, wherein in the composition the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 133 mg/mL.

11. The composition or method of any preceding claims, wherein in the composition the concentration of valproic acid or a pharmaceutically acceptable salt thereof is about 130 mg/mL.

12. The composition or method of any preceding claim, wherein in the composition the poloxamer is a thermoreversible gel.

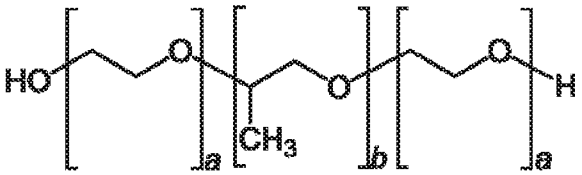
13. The composition or method of any preceding claim, wherein in the composition the poloxamer is a gel at about body temperature.

14. The composition or method of any preceding claim, wherein in the composition the poloxamer is an immobile gel at about body temperature.

15. The composition of claims 13 or 14, wherein body temperature is 37 °C.

16. The composition or method of any preceding claim, wherein in the composition the poloxamer comprises at least 60% polyethylene oxide by molecular mass.

17. The composition or method of any preceding claim, wherein in the composition the poloxamer comprises:



where *a* is about 80 to about 120, and *b* is about 50 to about 70.

18. The composition or method of any preceding claim, wherein in the composition the poloxamer has a number average molecular weight of about 10,800 to about 11,200 Da.

19. The composition or method of any preceding claim, wherein in the composition the poloxamer has a weight average molecular weight of about 11,500 to about 11,700 Da.

20. The composition or method of any preceding claim, wherein in the composition the poloxamer has a polydispersity index of about 1.02 to about 1.08.

21. The composition or method of any preceding claim, wherein in the composition the poloxamer ranges from about 7,250 to about 16,600 Da.

22. The composition or method of any preceding claim, wherein in the composition at least 85% by weight of the poloxamer has an average molecular weight of greater than about 7,250 Da.

23. The composition or method of any preceding claim, wherein in the composition at least 87% by weight of the poloxamer has an average molecular weight of greater than about 7,250 Da.

24. The composition or method of any preceding claim, wherein in the composition at least 90% by weight of the poloxamer has an average molecular weight of greater than about 7,250 Da.

25. The composition or method of any of claims 22-24, wherein in the composition the poloxamer has a peak molecular weight of about 12,000 to about 12,500 Da.

26. The composition or method of any of claims 22-25, wherein in the composition the poloxamer has a number average molecular weight of about 11,500 to about 12,000 Da.

27. The composition or method of any of claims 22-26, wherein in the composition the poloxamer has a weight average molecular weight of about 11,750 to about 12,250 Da.

28. The composition or method of any of claims 22-27, wherein in the composition the poloxamer has a polydispersity index of about 1.02.

29. The composition or method of any preceding claim, wherein in the composition less than 20% by weight of the poloxamer has an average molecular weight less about 7,250 Da.

30. The composition or method of any preceding claim, wherein in the composition less than 15% by weight of the poloxamer has an average molecular weight less about 7,250 Da.

31. The composition or method of any preceding claim, wherein in the composition less than 10% by weight of the poloxamer has an average molecular weight less about 7,250 Da.

32. The composition or method of any of claims 29-31, wherein in the composition the poloxamer distribution has a peak molecular weight of about 5,000 to about 5,500 Da.

33. The composition or method of any of claims 29-32, wherein in the composition the poloxamer distribution has a number average molecular weight of about 5,000 to about 5,500 Da.

34. The composition or method of any of claims 29-33, wherein in the composition the poloxamer has a weight average molecular weight of about 5,000 to about 5,500 Da.

35. The composition or method of any of claims 29-34, wherein in the composition the poloxamer has a polydispersity index of about 1.02.

36. The composition or method of any preceding claim, wherein in the composition the concentration of poloxamer is about 10% to about 20% w/v.

37. The composition or method of any preceding claim, wherein in the composition the concentration of poloxamer is about 12.5% to about 17.5% w/v.

38. The composition or method of any preceding claim, wherein in the composition the concentration of poloxamer is about 14.5% to about 16.5% w/v.

39. The composition or method of any preceding claim, wherein in the composition the concentration of poloxamer is about 15.5% w/v.

40. The composition or method of any preceding claim, wherein in the composition the average molecular weight is the weight average molecular weight.

41. The composition or method of any preceding claim, wherein in the composition the average molecular weight is the number average molecular weight.

42. The composition or method of any preceding claim, wherein in the composition the average molecular weight is the peak molecular weight.

43. The composition or method of any preceding claim, wherein in the composition the poloxamer comprises Poloxamer 407.

44. The composition or method of any preceding claim, wherein in the composition Poloxamer 407 is at least 50% by weight of the poloxamer.

45. The composition or method of any preceding claim, wherein in the composition Poloxamer 407 is at least 75% by weight of the poloxamer.

46. The composition or method of any preceding claim, wherein in the composition the poloxamer is Poloxamer 407.

47. The composition or method of any preceding claim, wherein in the composition the poloxamer is purified poloxamer.

48. The composition or method of any preceding claim, wherein in the composition the pharmaceutically acceptable salt of valproic acid is sodium valproate.

49. The composition or method of any preceding claim, wherein the composition further comprise one or more otic therapeutic agents.

50. The composition or method of any preceding claim, wherein in the composition the concentration of the one or more otic agents is less than about 7.5 mg/mL.

51. The composition of any preceding claim, wherein the one or more otic therapeutic agents includes CHIR99021 or a pharmaceutically acceptable salt thereof.

52. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is less than about 7.5 mg/mL.

53. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 3 to about 7 mg/mL.

54. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 4 to about 6 mg/mL.

55. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 5.1 mg/mL.

56. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 1 to about 5 mg/mL.

57. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 2 to about 4 mg/mL.

58. The composition or method of any preceding claim, wherein in the composition the concentration of CHIR99021 or a pharmaceutically acceptable salt thereof is about 3.1 mg/mL.

59. The composition or method of any preceding claim, wherein in the composition the CHIR99021 or a pharmaceutically acceptable salt thereof is CHIR99021.

60. The composition or method of any of claims 1-50, wherein in the composition the one or more otic therapeutic agents includes LY2090314 or a pharmaceutically acceptable salt thereof.

61. The composition or method of any of claims 1-50, wherein in the composition the one or more otic therapeutic agents includes GSK3 XXII or a pharmaceutically acceptable salt thereof.

62. The composition or method of any of claims 1-50, wherein in the composition the one or more otic therapeutic agents includes Compound I-7 or a pharmaceutically acceptable salt thereof.

63. The composition or method of any preceding claim, wherein the composition further comprises dimethyl sulfoxide (DMSO).

64. The composition or method of any preceding claim, wherein in the composition the concentration of DMSO is less than about 25% by weight.

65. The composition or method of any preceding claim, wherein in the composition the composition is a lyophilized composition.

66. The composition or method of any preceding claim, wherein the composition comprises about 100 to about 200 mg of poloxamer.

67. The composition or method of any preceding claim, wherein the composition comprises about 100 to about 200 mg of sodium valproate.

68. The lyophilized composition of any preceding claim, wherein the composition comprises about 0.01 to about 2% by weight CHIR99021.

69. The lyophilized composition of any preceding claim, wherein the composition comprises about 30 to about 50% by weight sodium valproate.

70. The lyophilized composition of any preceding claim, wherein the composition comprises about 50 to about 70% by weight poloxamer 407.

71. The lyophilized composition of any preceding claim, wherein the composition comprises about 0.01 to about 2% by weight CHIR99021, about 42.5 to about 47.5% by weight sodium valproate, and the remaining percent by weight is Poloxamer 407.

72. The lyophilized composition of any preceding claim, wherein the poloxamer is purified poloxamer.

73. The composition or method of any preceding claim, wherein the composition the composition does not comprise an additional bulking agent.

74. The composition or method of any preceding claim, wherein in the composition the composition does not comprise an antioxidant.

75. The lyophilized pharmaceutical composition of any preceding claim, wherein the concentration of aldehydes is less than about 5 ppm.

76. The lyophilized composition of any preceding claim, wherein the aldehydes are volatile aldehydes.

77. The lyophilized composition of any preceding claim, wherein the aldehydes are selected from the group of formaldehyde, acetaldehyde, and/or propionaldehyde.

78. A method for lyophilizing a pharmaceutical composition, wherein the method comprises:
(a) providing a pharmaceutical composition;
(b) lyophilizing the composition by:
(i) reducing the temperature in the lyophilizer to -45 °C at a rate of 0.5 °C per minute, and then holding it at -45 °C for 3 hours;
(ii) applying a vacuum of 80 mTorr;
(iii) increasing the temperature to -30 °C (at a rate of 0.5 °C per minute) and holding it at -30 °C for 15 hours under a vacuum of 80 mTorr;
(iv) increasing the temperature to 15 °C (at a rate of 0.5 °C per minute); and/or
(v) holding the temperature at 15 °C for 20 hours under a vacuum of 80 mTorr; and
(c) obtaining a lyophilized pharmaceutical composition.

79. The method of claim 78, wherein the pharmaceutical composition of step (a) is a composition of any preceding claim.

80. A reconstituted pharmaceutical composition comprising a lyophilized pharmaceutical composition of any preceding claim and a diluent.

81. The composition of claim 80, wherein the lyophilized pharmaceutical composition is dissolved in the diluent.

82. A method of treating a subject who has, or is at risk of developing a hearing condition, comprising administering to the subject a pharmaceutically effective amount of the pharmaceutical, lyophilized or reconstituted composition of any preceding claim.

83. The method of claim 82, wherein the hearing condition is sensorineural hearing loss.

84. A pharmaceutical, lyophilized or reconstituted composition of any preceding claim for use in therapy.

85. The pharmaceutical, lyophilized or reconstituted composition of any preceding claim, for use in treating a subject who has, or is at risk of developing a hearing condition.

86. The composition for use according to claim 85, wherein the hearing condition is sensorineural hearing loss.

87. Use of the pharmaceutical, lyophilized or reconstituted composition of any preceding claim, in the manufacture of a medicament for treating a subject who has, or is at risk of developing a hearing condition.

88. The use of claim 87, wherein the hearing condition is sensorineural hearing loss.

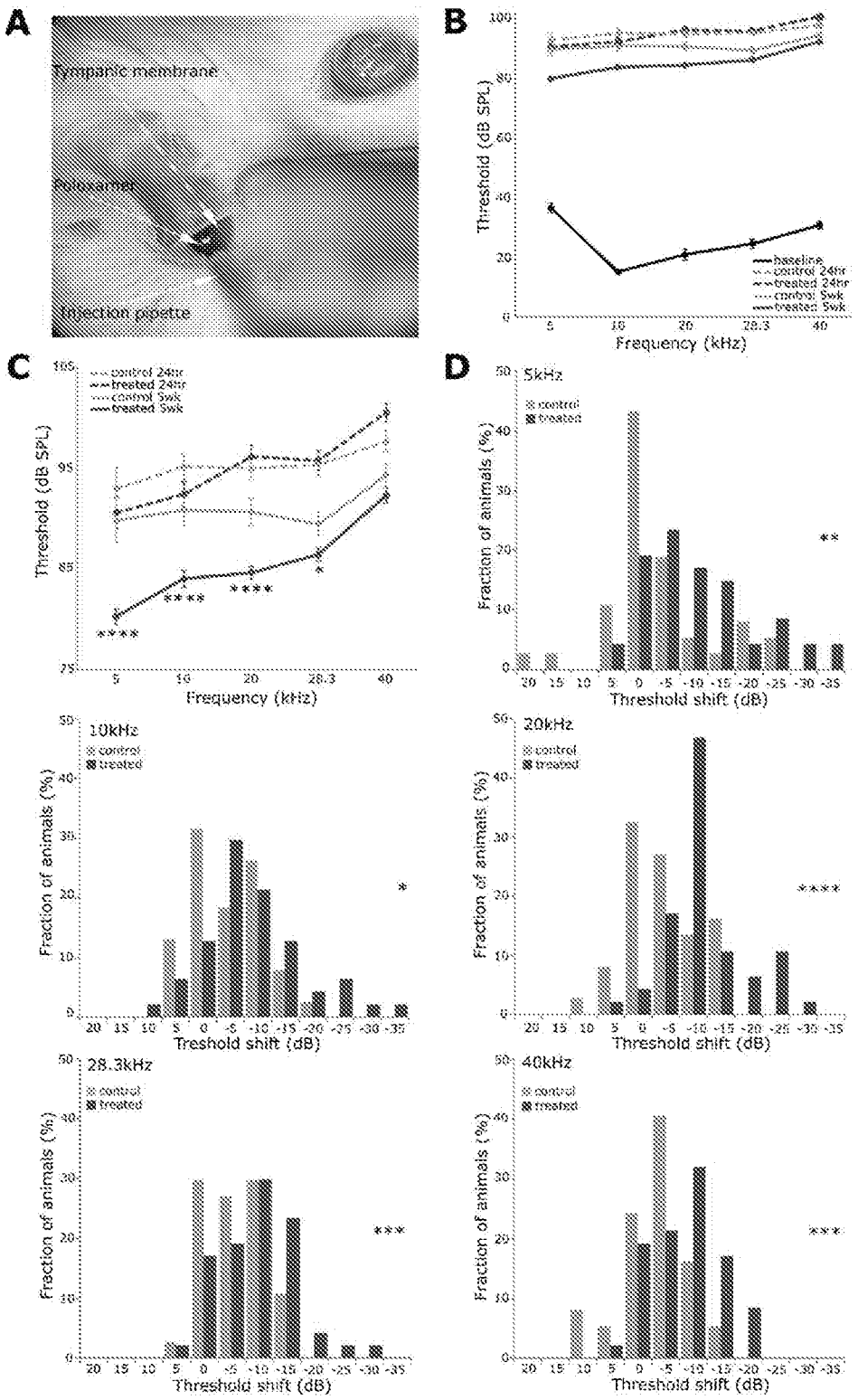

A
Tympanic membrane
Pinna artery
Injection pipette
B
Threshold (dB SPL)
Frequency (kHz)
baseline
control 24hr
treated 24hr
control 5wk
treated 5wk
C
Threshold (dB SPL)
Frequency (kHz)
D
5kHz
10kHz
20kHz
28.3kHz
40kHz
control
treated
Fraction of animals (%)
Threshold shift (dB)

Figure 1

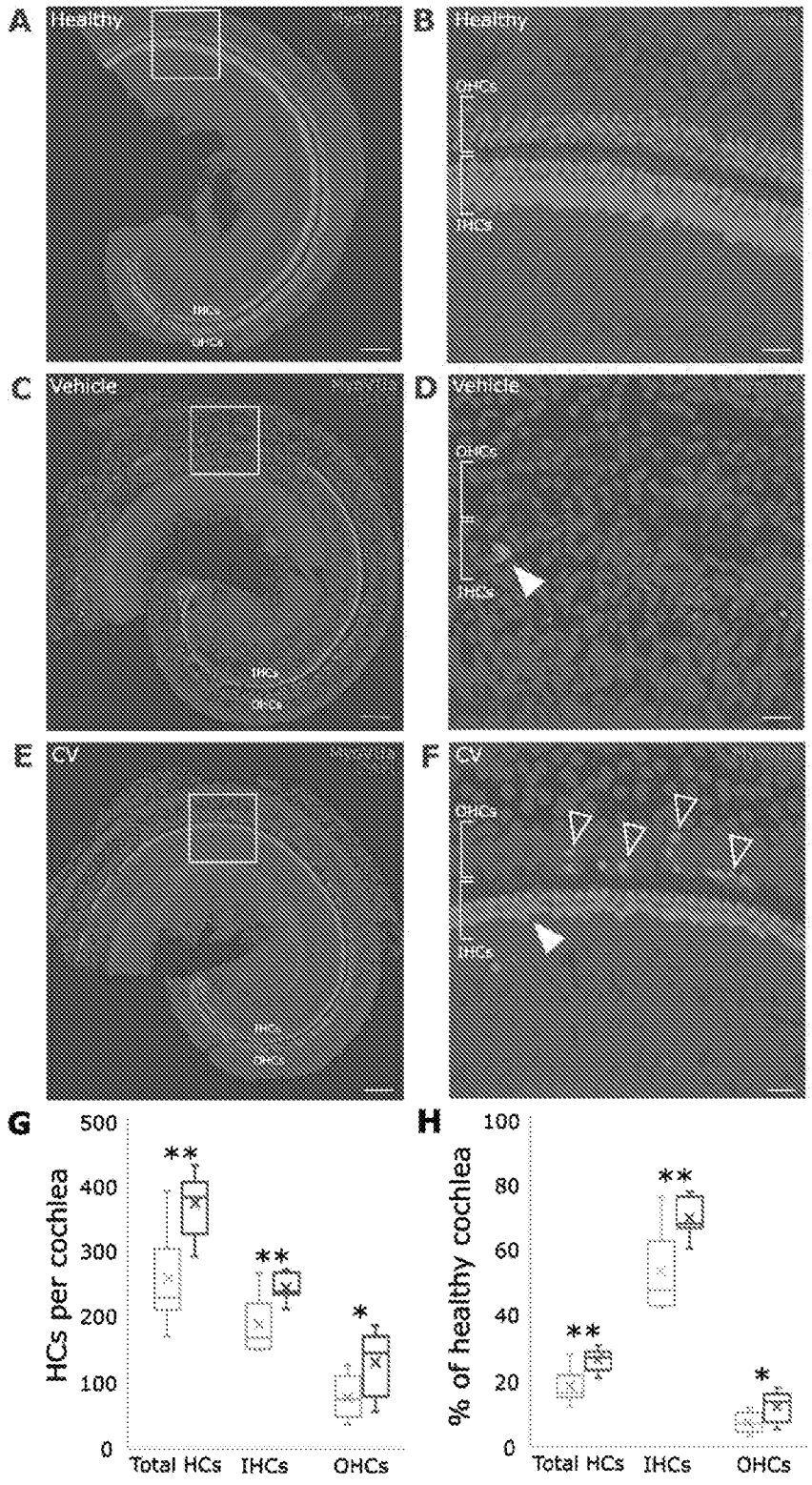

A
Healthy
B
Healthy
C
Vehicle
D
Vehicle
E
F
G
HCs per cochlea
500
400
300
200
100
0
Total HCs
IHCs
OHCs
H
% of healthy cochlea
100
80
60
40
20
0
Total HCs
IHCs
OHCs

Figure 2

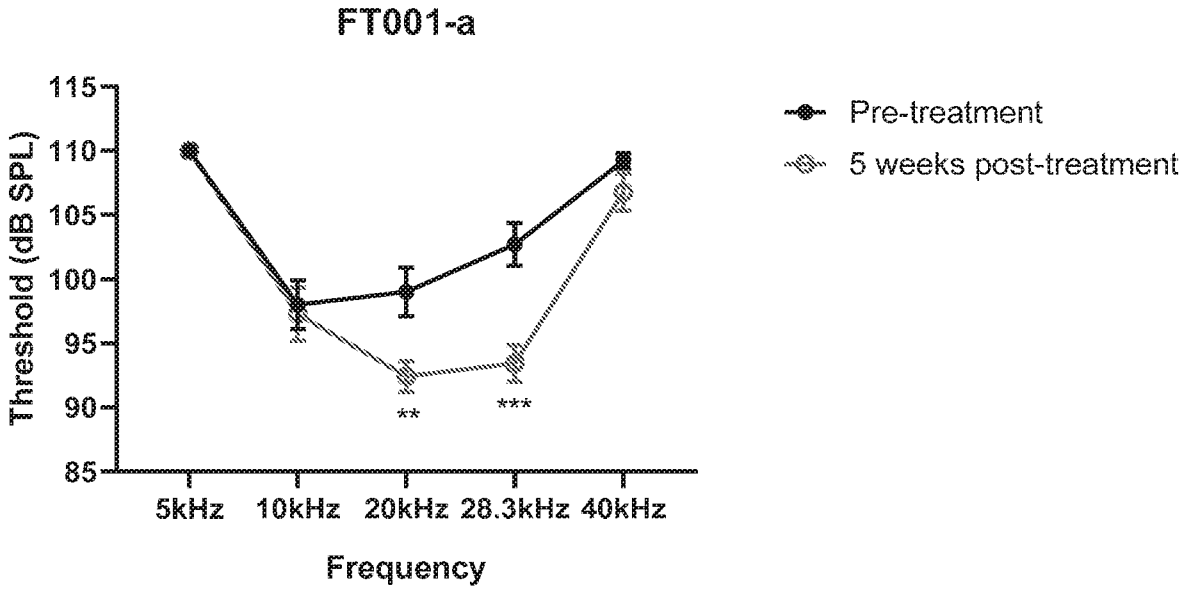


Figure 3

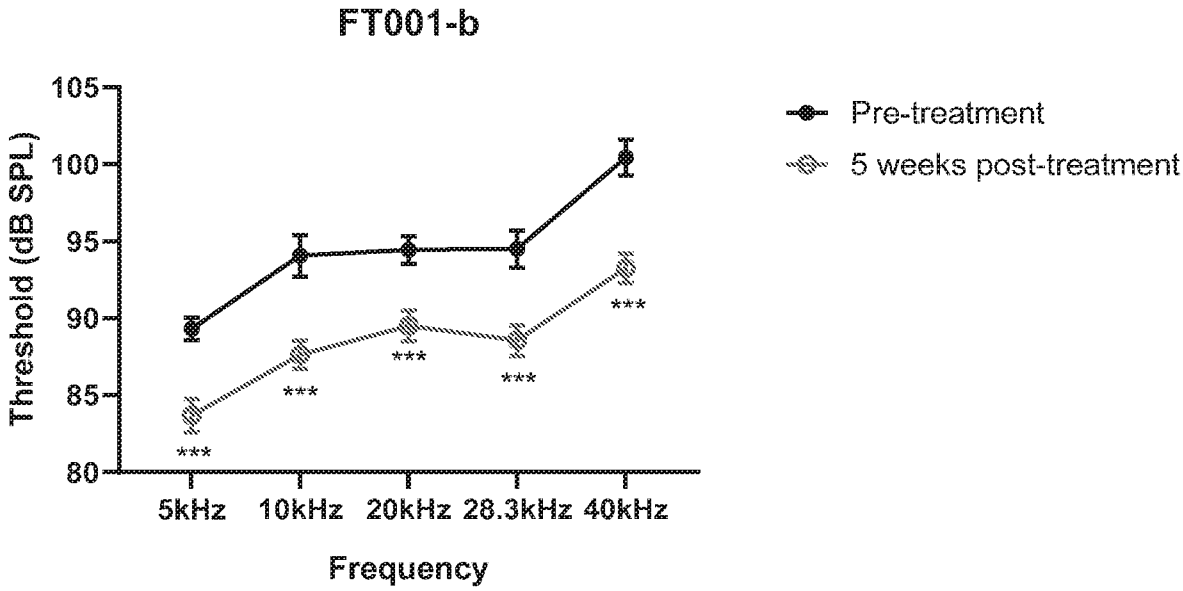


Figure 4

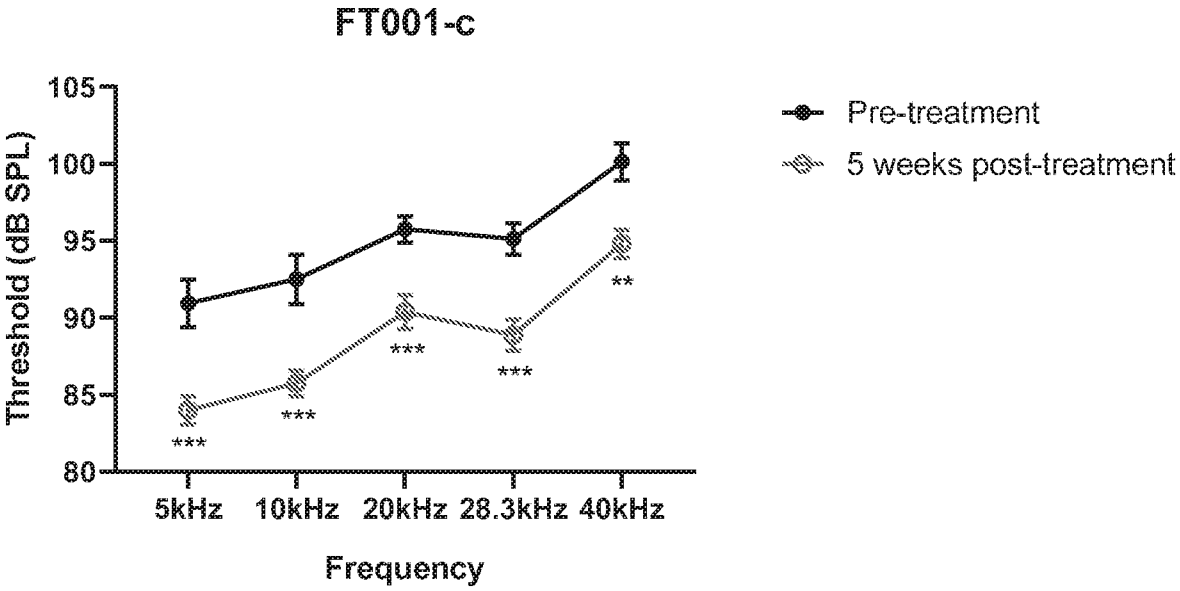

FT001-c
Threshold (dB SPL)
105
100
95
90
85
80
***
***
***
***
**
5kHz
10kHz
20kHz
28.3kHz
40kHz
Frequency
Pre-treatment
5 weeks post-treatment

Figure 5

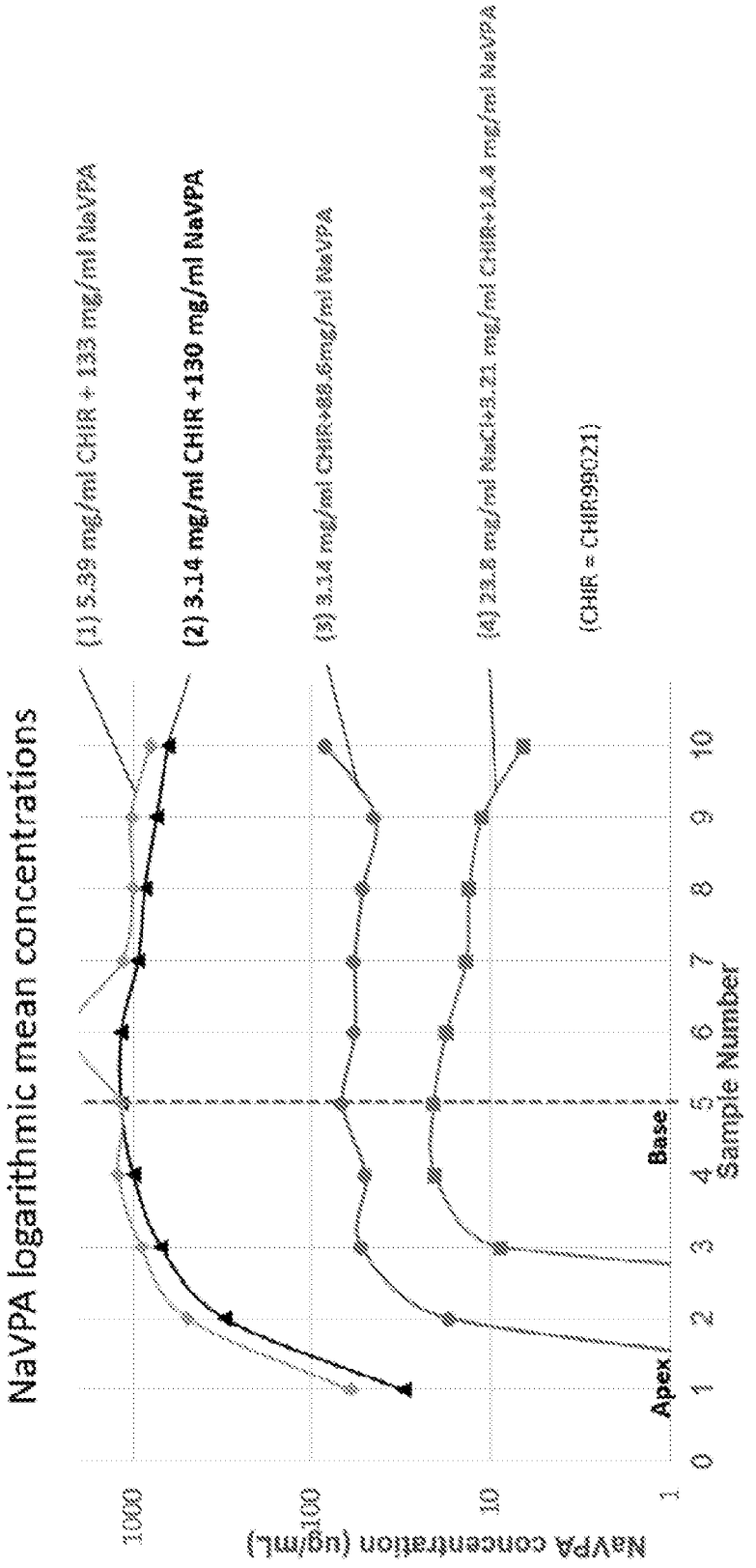

NaVPA logarithmic mean concentrations
(1) 3.39 mg/ml CHIR + 133 mg/ml NaVPA
(2) 3.14 mg/ml CHIR +130 mg/ml NaVPA
(3) 3.14 mg/ml CHIR+88.6mg/ml NaVPA
(4) 23.8 mg/ml NaCl+3.21 mg/ml CHIR+14.4 mg/ml NaVPA
(CHIR = CHIR99021)
NaVPA concentration (ug/mL)
1000
100
10
1
Apex
Base
0 1 2 3 4 5 6 7 8 9 10
Sample Number

Figure 6

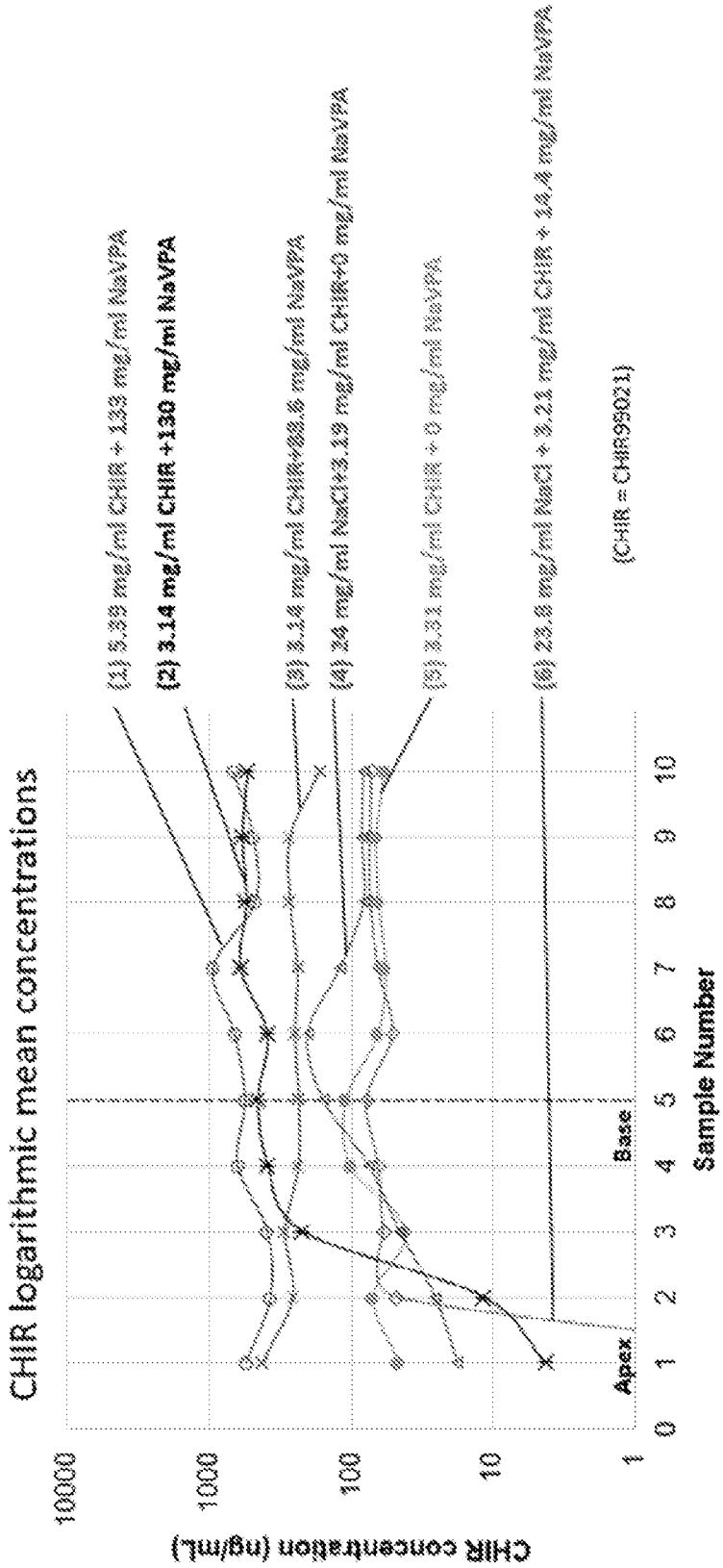

CHIR logarithmic mean concentrations
(1) 3.33 mg/ml CHIR + 133 mg/ml NaVPA
(2) 3.14 mg/ml CHIR +130 mg/ml NaVPA
(3) 3.14 mg/ml CHIR+88.6 mg/ml NaVPA
(4) 24 mg/ml NaCl+3.19 mg/ml CHIR+0 mg/ml NaVPA
(5) 3.31 mg/ml CHIR + 0 mg/ml NaVPA
(6) 23.6 mg/ml NaCl + 3.21 mg/ml CHIR + 14.4 mg/ml NaVPA
(CHIR = CHIR99021)
CHIR concentration (ng/mL)
10000
1000
100
10
1
Apex
Base
0 1 2 3 4 5 6 7 8 9 10
Sample Number

Figure 7

Figure 8

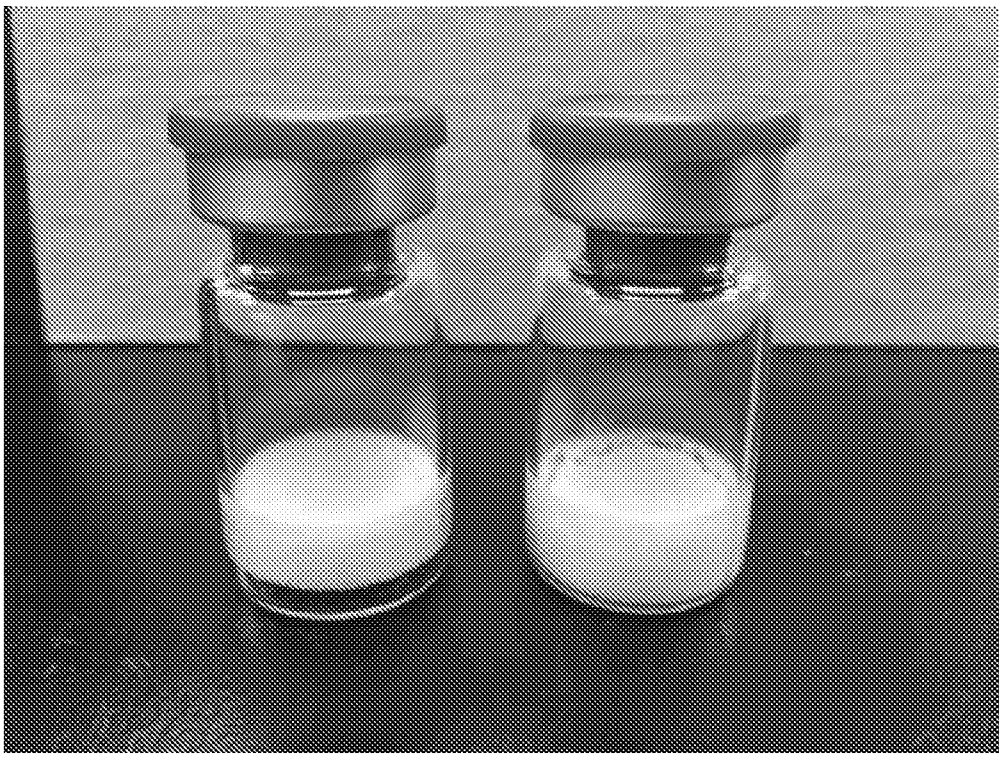


Figure 9

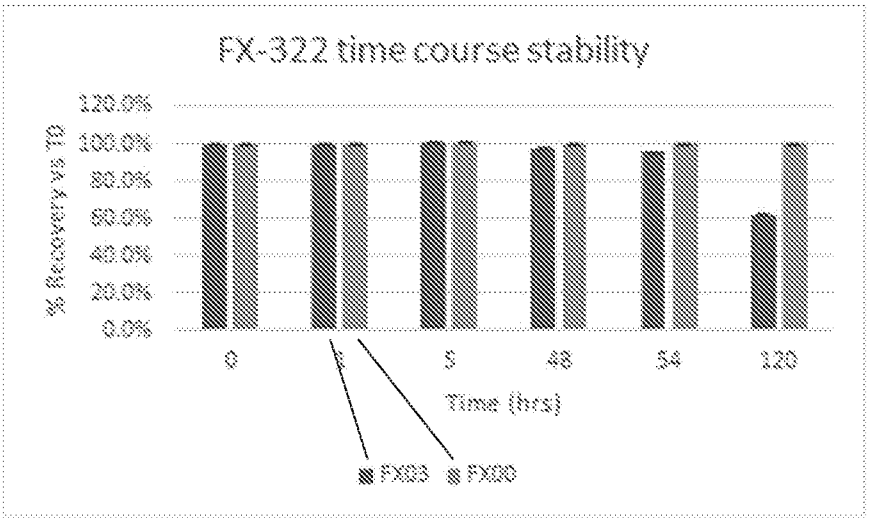


Figure 10

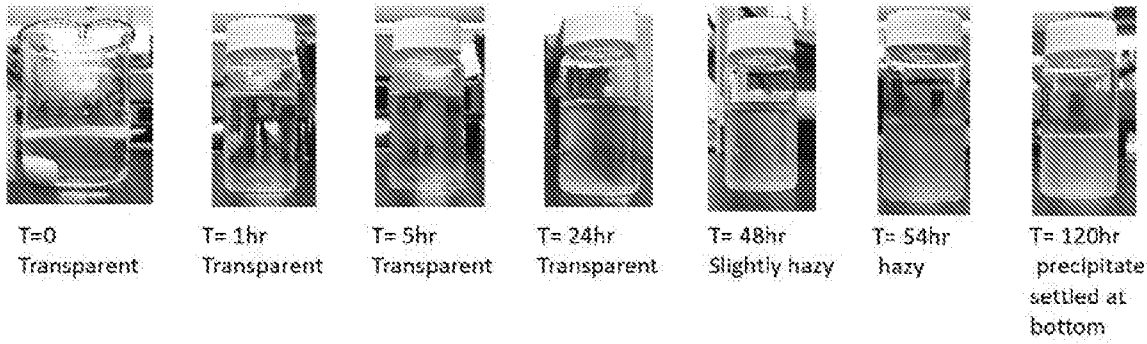


Figure 11

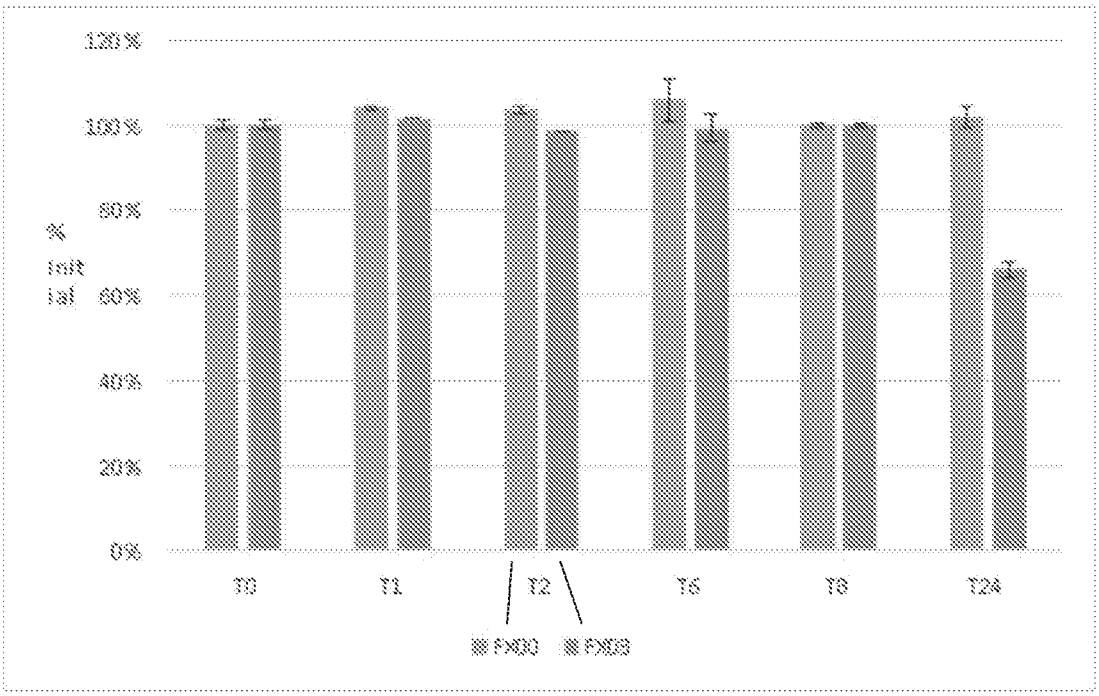


Figure 12

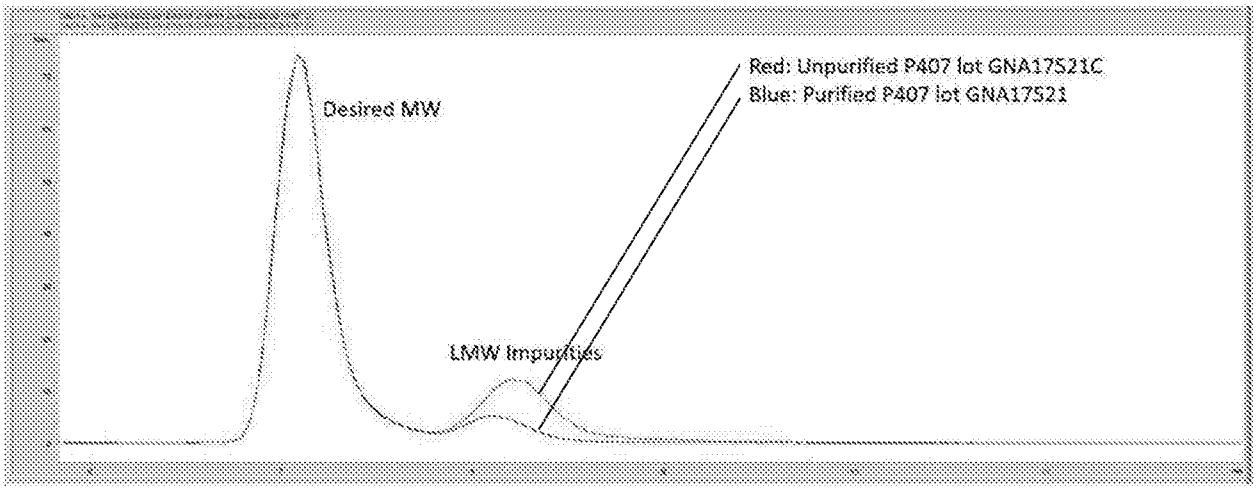


Figure 13

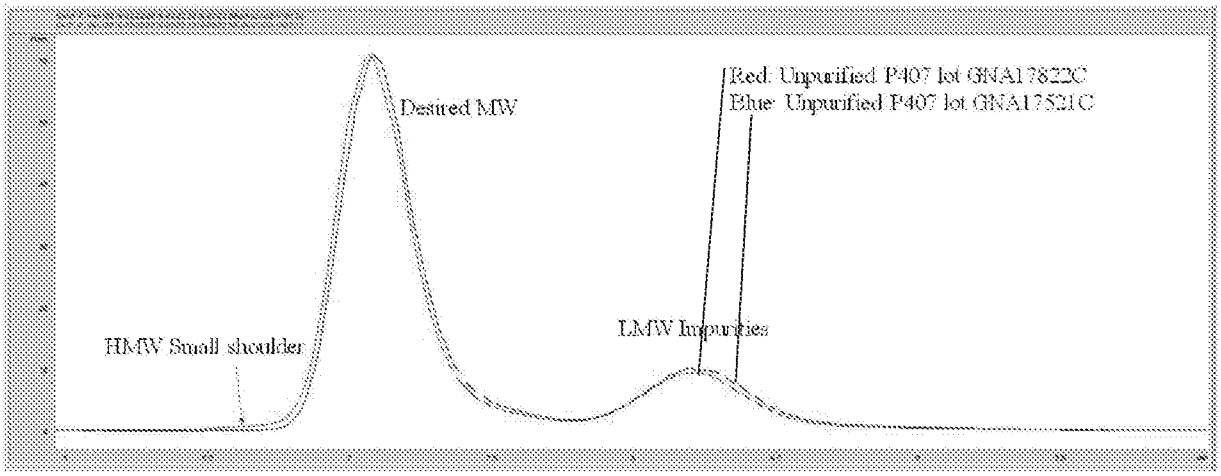


Figure 14

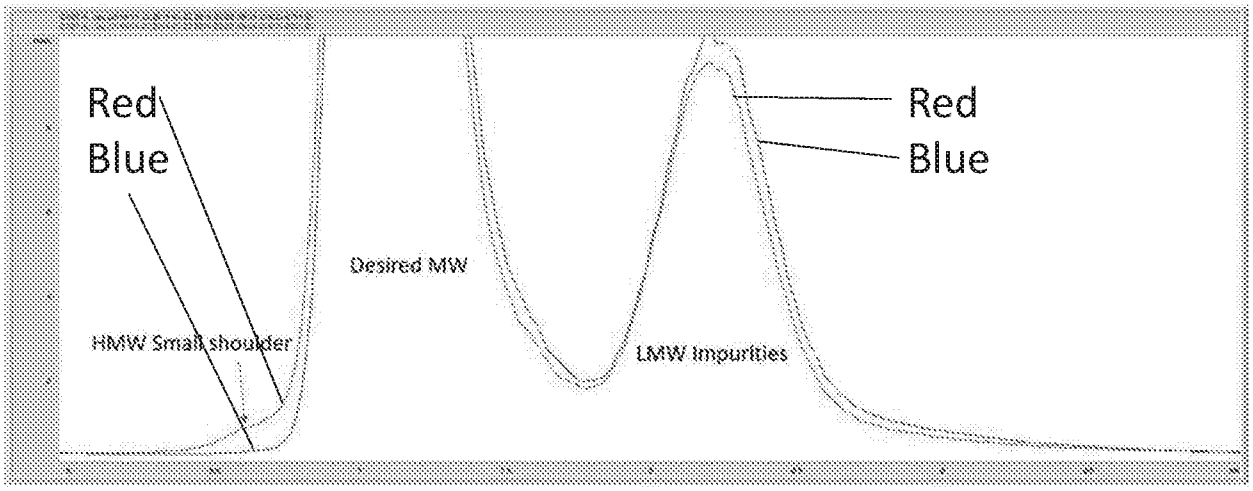


Figure 15

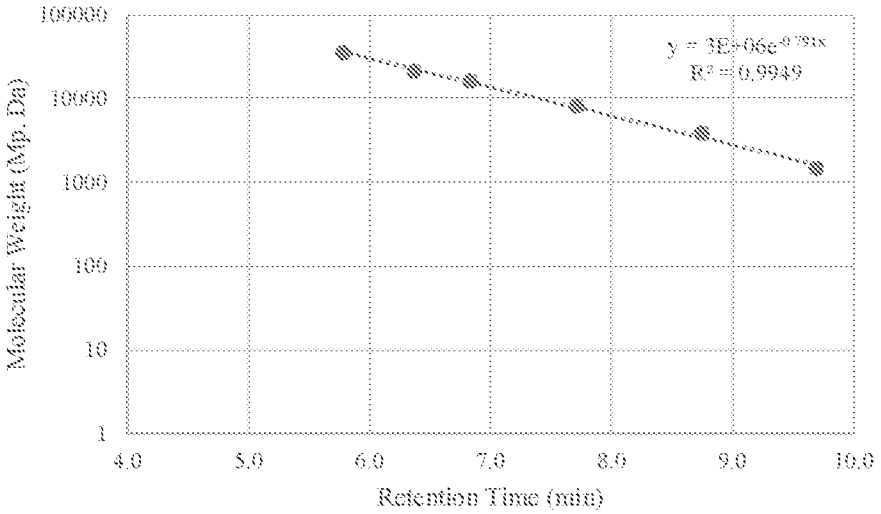


Figure 16

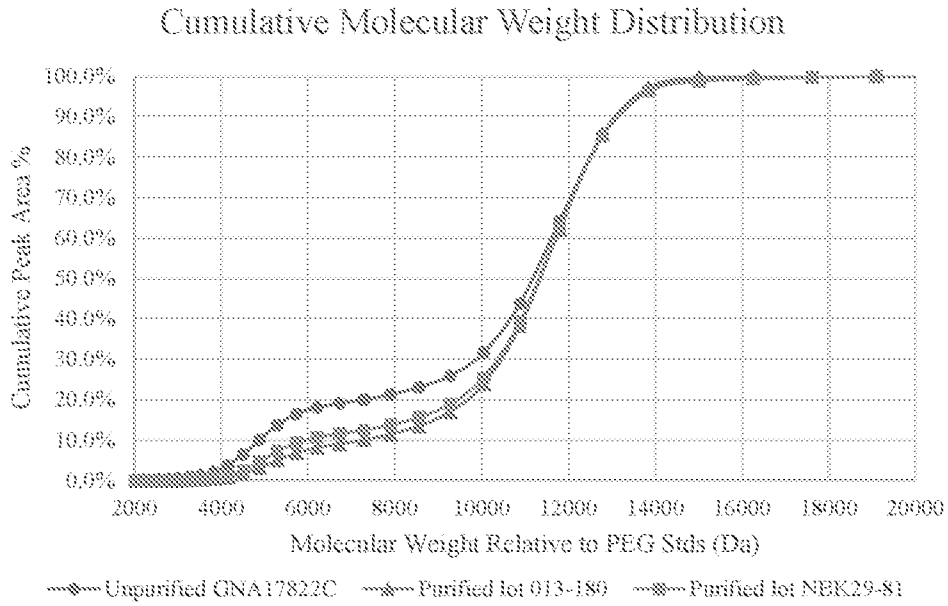


Figure 17

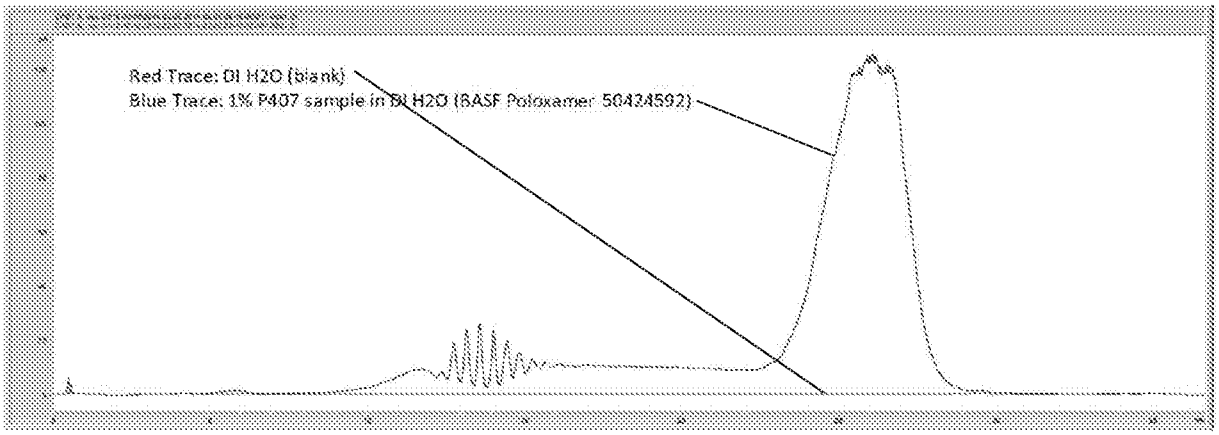


Figure 18

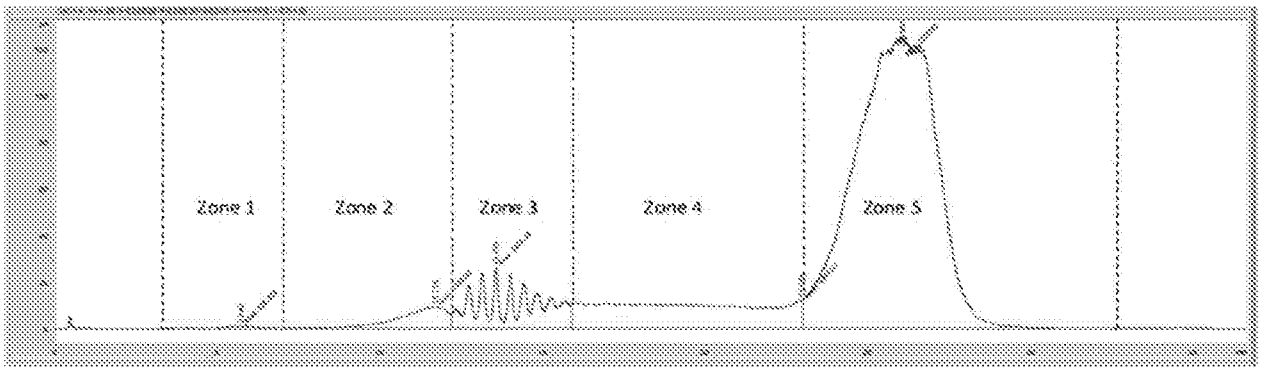


Figure 19

Figure 20

Figure 21

Figure 22

Figure 23

Figure 24

Figure 25

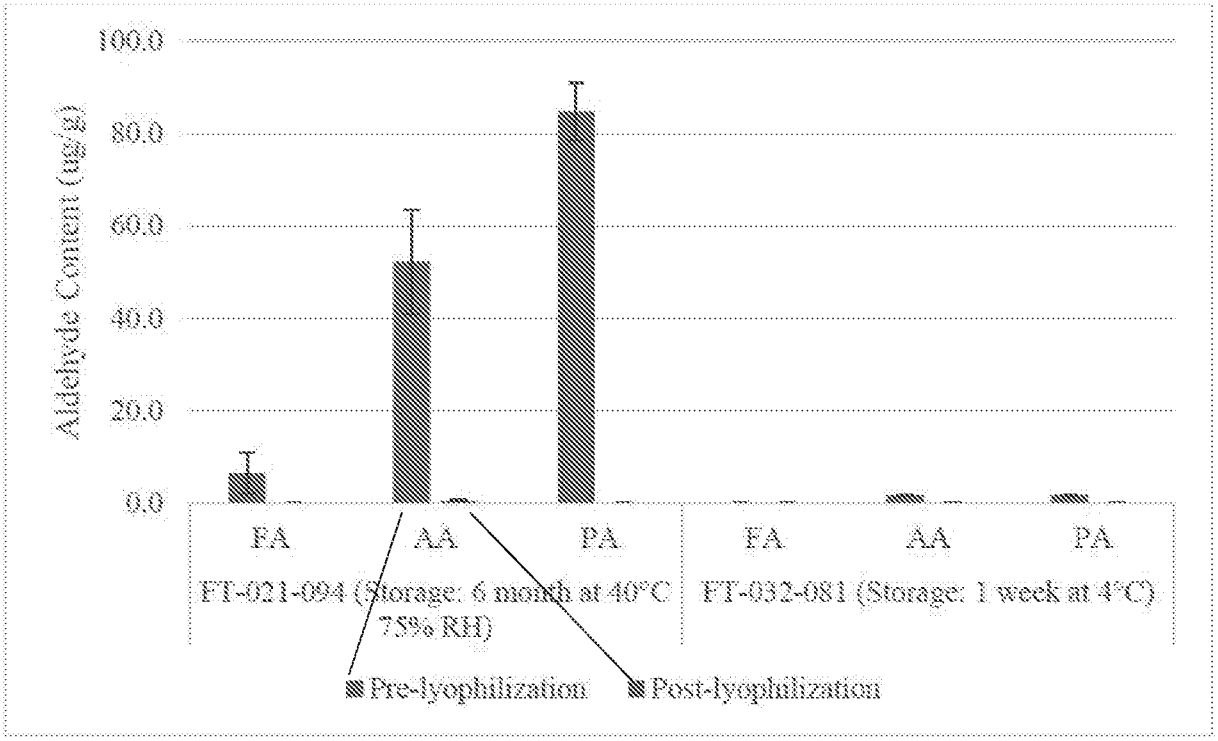

100.0
80.0
60.0
40.0
20.0
0.0
Aldehyde Content (ug/g)
FA
AA
PA
FA
AA
PA
FT-021-094 (Storage: 6 month at 40°C 75% RH)
FT-032-081 (Storage: 1 week at 4°C)
Pre-lyophilization
Post-lyophilization

Figure 26