epilepsy and amyotrophic lateral sclerosis. Hyperexcitation of the nerve is also reported in neurodegenerative diseases including Alzheimer's disease (Non Patent Literature 5) and Parkinson's disease (Non Patent Literature 6) and autism spectrum disorders (Non Patent Literature 7). Therefore, an agent that suppresses hyperexcitation of the nerve can be a therapeutic drug for these diseases.

[0004]

Patent Literature 1 describes a 2,3-diaminoquinazolinone derivative having a Kv7 activation effect, but the chemical structure differs from that of a compound represented by formula (1) described below.

[Citation List]

[Patent Literature]

[0005]

[PTL 1] International Publication No. WO 2008/142140

[Non Patent Literature]

[0006]

[NPL 1] Pacico, N. et al. PLoS One, 2014, 9(1), e84755.
[NPL 2] Shibuya, K. et al. Experimental Neurology, 2011, 232(2), 149-153.
[NPL 3] Kanai, K. et al. Brain, 2006, 129 (Pt4), 953-962.
[NPL 4] Wainger, B. J. et al. Cell Reports, 2014, 7(10), 1-11.
[NPL 5] Palop, J. J. et al. Neuron, 2007, 55, 697-711
[NPL 6] Basso, M. A. et al. The Journal of Neuroscience, 1996, 16, 7318-7330
[NPL 7] Takarae, Y. et al. Brain Science, 2017, 7, 129

[Summary of Invention]

[Solution to Problem]

[0007]

The present disclosure provides a 2-aminoquinazolinone derivative and a pharmaceutically acceptable salt thereof, as well as a nerve hyperexcitation suppressing agent comprising said compound or the like as an active

Reference Examples and Examples do not necessarily follow the IUPAC nomenclature.

[0185]

The following abbreviations may be used in the Reference Examples, Examples, and Tables in the Examples to simplify the descriptions herein. As abbreviations used for a substituent, Me refers to methyl, Ms refers to methanesulfonyl, Ph refers to phenyl, and TFA refers to trifluoroacetic acid. As symbols used for NMR, s refers to singlet, d refers to doublet, dd refers to double doublet, t refers to triplet, td refers to 3 doublet, q refers to quartet, m refers to multiplet, br refers to broad, brs refers to broad singlet, brm refers to broad multiplet, and J refers to a coupling constant.

[0186]

High performance liquid chromatography-mass spectrometer; measurement conditions of LCMS are as follows. The observed mass spectrometry value [MS (m/z)] is indicated by MH^+, and time of retention is indicated by Rt (min). The measurement conditions used for measurement are described for each of the actual measurement values.

[0187]

Measurement condition A

Detector: Agilent 1200 series, Agilent 6110 Quadrupole LC/MS

Column: SunFire C18 (3 × 30 mm, 2.5 μm)

Solvent:

Solution A: 0.01% TFA/H_2O, solution B: 0.01% TFA/MeCN

Gradient Condition:

0.0-0.2 minutes; A/B = 95:5

0.2-1.5 minutes; A/B = 95:5 to 5:95 (linear gradient)

1.5-2.8 minutes; A/B = 5:95

Flow rate; 1.5 ml/minute

UV: 254 nm

Column temperature: 50°C

tissue was dispersed by incubating for 5 minutes at 37°C in a 0.3 mg/mL papain (Sigma-Aldrich, cat# P4762), 0.1 mg/mL DNase I (Roche, cat# 11284932001), and 5 mM magnesium chloride solution. The dispersion reaction was suspended by adding a medium comprising 10% fetal bovine serum. After washing with HBSS, the tissue was physically dispersed by pipetting. Cell clumps were removed through a 70 μm cell strainer (Becton Dickinson, cat# 352350) to obtain a neuron suspension. The suspension was centrifuged for 4 minutes at 1000 rpm to remove the supernatant. After resuspending the cells in a small amount of HBSS, the cells were counted. The neurons were diluted in a medium to achieve a density of 3×10^4 cells per well and seeded on a 384-well plate (Corning, cat# 356697) coated with poly-D-lysine. As the medium, Neurobasal Electro medium (Thermo Fisher Scientific, cat# A14098-01) comprising GlutaMAX (Thermo Fisher Scientific, cat# 35050061), penicillin/streptomycin (Thermo Fisher Scientific, cat# 15140-122), and 2% B27 Electro Supplement (Thermo Fisher Scientific, cat# A14097-01) was used. The seeded cells were cultured for 15 to 17 days in a 37°C incubator under 5% CO_2. On the day after seeding the cells, the culture solution was exchanged with a fresh medium comprising 3 μM cytarabine (Sigma-Aldrich, cat# C1768). 2/3 of the medium was exchanged with a fresh medium (cytarabine free) thereafter once every 3 to 4 days.

[0206]

(2) Fluorescent calcium probe treatment, addition of compound, and evaluation of intracellular calcium concentration

The entire culture solution was removed on day 15 to day 17 of culture. 30 μL of medium for measurement comprising a fluorescent calcium probe (Molecular Device, product name: FLIPR Calcium 6 Assay Bulk Kit, cat# R8191) was added. The culture was left standing for 2 to 4 hours and then subjected to measurement. As the medium for

measurement, 20 mM Hepes (Thermo Fisher Scientific, cat# 15630-080) and 0.1% bovine serum albumin (Sigma-Aldrich, cat# A9576)-containing Hank's buffer (Thermo Fisher Scientific, cat# 14065-056) was used.

[0207]

The test compounds were serially diluted with a dimethyl sulfoxide (DMSO) solution so that the final concentration would be 0.1 to 30 μM. First, the compounds were diluted to a 333-fold concentration of the final concentration with DMSO, and then diluted with a medium for measurement to prepare a concentrate with a 5-fold concentration of the final concentration.

[0208]

The intensity of fluorescence of a calcium probe was measured over time with FDSS7000EX (Hamamatsu Photonics) to evaluate the change in intracellular calcium concentrations. Compounds were added using FDSS7000EX. After 120 seconds from adding 10 μL of test compounds, 10 μL of 4-aminopyridine (final concentration of 100 μM) (FUJIFILM Wako Pure Chemical, cat# 016-02781) was added for an addition 6 minutes and 30 seconds measurement of fluorescence intensity. The frequency of calcium oscillation induced by 4-aminopyridine was quantified as an indicator of nerve excitation. With 100% as the mean frequency of wells treated with DMSO as a control, the inhibitory activity (%) at each serial dilution concentration of the test compounds was determined, and the 50% inhibitory concentration (IC50) or inhibition rate (%) at a certain concentration was determined for each test compound. Table 2 shows inhibitory activity data for representative compounds.

[Table 2-3]

46	9.2	93	6.6	140	1%@30μM	187	0.6
47	2.5	94	0.3	141	19%@30μM	188	1.2

[0209]

As shown in these tables, the compound of the invention had inhibitory activity in a nerve hyperexcitation suppression test using rat primary culture.

[0210]

Test Example 2: Hyperexcitation suppression test using motor neurons induced to differentiate from iPS cells derived from amyotrophic lateral sclerosis patient

(1) Induction of differentiation from iPS cells to motor neurons

An iPS cell line from ALS patients (clone name: CiRA00123, obtained from the Center for iPS Cell Research and Application, Kyoto University) was induced to differentiate into motor neurons. The cells of the patients were confirmed to have a mutation that replaces the 337th methionine residue in the amino acid sequence of TAR DNA-binding protein 43 (TDP-43) with a valine residue. Mitomycin treated SNL cells (Cell Biolabs, cat# CBA-316) were used as feeder cells for seeding iPS cells. SNL cells were treated with mitomycin as follows. First, a 10 cm petri dish (Iwaki, cat# 3020-100) was treated with 0.1% gelatin (FUJIFILM Wako Pure Chemical, cat# 190-15805) for more than 1 hour in a 37°C incubator under 5% CO_2. The gelatin was then removed by aspiration. 1 to 2 × 10^6 thawed SNL cells were seeded using a medium for SNL cells [DMEM (Sigma-Aldrich, cat# D6429), penicillin/streptomycin (Thermo Fisher Scientific, cat# 15140-122), and fetal bovine serum (Thermo Fisher Scientific, cat# 10437-028)]. The cells were diluted 8 to 16-fold every 3 to 4 days, and passaged and grown to the required number of cells. Subsequently, 2 to 4 × 10^6 SNL cells were seeded on a 0.1% gelatin treated 15 cm petri dish (Iwaki, cat# 3030-150) and

cultured to 80 to 90% confluence. Mitomycin C (Kyowa Kirin, YJ code: 4231400D1031) diluted to 0.4 mg/mL with the medium for SNL cells was then added so that the final concentration would be 6.2 μg/mL. After leaving the culture standing for 2 hours and 15 minutes in a 37°C incubator under 5% CO_2, the medium was removed, and the cells were washed once with PBS. 2.5% trypsin/EDTA (Thermo Fisher Scientific, cat# 15090-046) was diluted with PBS (final concentration of 0.25%) and then added to cells. After leaving the cells standing for 1 minute at room temperature, the cells were recovered in a tube. After centrifugation, the cells were suspended in CELLBANKER® (Zenoaq Resource, cat# CB011) and cryopreserved. Differentiation of iPS cells was induced as follows. First, 0.1% gelatin was added to a 10 cm petri dish (Iwaki, cat# 3020-100) for treatment for over an hour in a 37°C incubator under 5% CO_2. SNL cells treated with mitomycin were suspended using a medium for SNL cells. 1.5×10^6 cells were seeded on the 10 cm petri dish and cultured for 2 to 3 days. The medium for SNL cells was subsequently removed. After washing with PBS, iPS cells suspended in a medium for primate ES/iPS cells (ReproCELL, cat# RCHEMD001B) comprising penicillin/streptomycin and Y-27632 (Tocris, cat# 1254) were seeded. The medium was exchanged every day, from two days after seeding until the start of differentiation induction. Y-27632 was then added to the cell culture supernatant for exposure to a concentration of 10 μM for more than 1 hour. The culture supernatant was removed and the cells were washed with phosphate buffer (PBS) (Nacalai Tesque, cat# 14249-24). A CTK solution (ReproCELL, product name: Cell dissociation solution, cat# RCHETP002) was then added and reacted for 1 minute at room temperature. The CTK solution was removed, and the cells were washed twice with PBS. 1 mL of medium for primate ES/iPS cells (ReproCELL, cat# RCHEMD001B) comprising penicillin/streptomycin was then added. The

[0214]

The intensity of fluorescence of a calcium probe was measured over time with FDSS7000EX (Hamamatsu Photonics) to evaluate the change in intracellular calcium concentration. Compounds were added using FDSS7000EX. After 120 seconds from adding 10 µL of test compounds, 10 µL of 4-aminopyridine (final concentration: 100 µM) (FUJIFILM Wako Pure Chemical, cat# 016-02781) was added for an additional 5 minute measurement of fluorescence intensity. The frequency of calcium oscillation induced by 4-aminopyridine was quantified as an indicator of nerve excitation. With 100% as the mean frequency of wells treated with DMSO as a control, the inhibitory activity (%) at each serial dilution concentration of the test compounds was determined, and the 50% inhibitory concentration (IC50) was determined for each test compound. Table 3 shows inhibitory activity data for representative compounds.