Neuroprotection of SAK3 on scopolamine-induced cholinergic dysfunction in human neuroblastoma SH-SY5Y cells

Nopparat Suthprasertporn · Nopparada Mingchinda · Kohji Fukunaga · Wipawan Thangnipon

Received: 5 September 2019 / Accepted: 30 December 2019 / Published online: 14 January 2020
© Springer Nature B.V. 2020

Abstract Alzheimer's disease (AD) is the most common type of senile dementia. A number of factors have been proposed regarding pathology of AD, such as presence of β -amyloid, and cholinergic and oxidative stress. SAK3 (ethyl 8'-methyl-2',5-dioxo-2-piperidin-1-ylspiro[cyclopentene-3,3'-imidazo[1,2-a]pyridine]-1-carboxylate) reduces β -amyloid deposition and improves cognitive functions in amyloid precursor protein knock-in mice. Scopolamine is used to induce in cell lines a cholinergic deficit that mimics AD. In order to evaluate the possible neuroprotective properties of SAK3, human neuroblastoma SH-SY5Y cells were pretreated with the compound (25–100 nM) and further incubated in the presence of scopolamine (2 mM). SAK3 inhibited scopolamine-induced cellular apoptosis (morphologically and by determination of pro- and anti-apoptotic factor levels), increase in ROS levels, decrease in choline acetyltransferase level, phosphorylation of NF- κ B, activation of Akt, JNK and p38 intracellular signaling pathways, and

elevation of proinflammatory cytokines IL-1 β and IL-6, but not enhanced level of β -site amyloid precursor protein cleaving enzyme 1 (BACE1). These results indicate SAK3 possessed protective properties against cholinergic deficit associated with anti-oxidant, anti-apoptotic and anti-inflammatory activities, suggesting that SAK3 might be a potential agent in the development of AD drug therapeutics.

Keywords Alzheimer's disease · Acetylcholine · Apoptosis · Neuroblastoma SH-SY5Y cell line · Oxidative stress · SAK3 · Scopolamine

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder affecting more than 10 million individuals and predicted to increase in global prevalence (Selkoe and Hardy 2016). Alzheimer's disease results in dysfunction of cognitive functions such as memory (Rojsanga et al. 2012) and is one of the major causes of dementia, requiring a better understanding of its etiology and improvement in treatment. Possible explanations of AD involve the role of β -amyloid and oxidative stress (Du et al. 2018). β -Amyloid accumulates in neurons associated with cognitive functions as a result of amyloid precursor protein (APP) defect, leading to elevated levels of A β ₄₂. In addition, levels of neurotransmitter acetylcholine are

N. Suthprasertporn · N. Mingchinda · W. Thangnipon (✉)
Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhonpathom 73170, Thailand
e-mail: wipawan.tha@mahidol.ac.th

K. Fukunaga
Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

abnormally depleted due to reduced activity of cholineacetyltransferase (ChAT) (Rinne et al. 2003). For this reason, physicians currently manage AD by treating with such drugs as donepezil, which inhibit acetylcholine-degrading enzymes (Pudlo et al. 2014). However, other investigators argue Alzheimer's disease pathology is mediated by oxidative stress (Mamelak et al. 2010; Venkatesan et al. 2016) caused by presence of excess amounts of reactive oxygen species (ROS) damaging biological molecules in the nucleus and mitochondria (Wang et al. 2014).

Scopolamine, a muscarinic receptor antagonist, induces cognitive impairments by its ability to debilitate neurotransmission in cholinergic system and increases oxidative stress (Budzynska et al. 2014). Consequently, scopolamine induces cognitive deficits in both human participants and rats (Alvarez-Jimenez et al. 2016; Budzynska et al. 2014). This muscarinic receptor antagonist is widely used to create AD model in cellular studies of potential novel treatments.

Oxidative stress as an etiology of Alzheimer's disease has led to research in discovering potential antioxidants capable of protecting against oxidant-mediated neurotoxicity. Plant polyphenol *N*-transferuloyltyramine (NTF) from *Polyalthia suberosa* (Thangnipon et al. 2012) and phenylethanoid glycoside acteoside (verbascoside) from *Verbascum sinuatum* (Wang et al. 2009), which have protective effects against β -amyloid toxicity in human neuroblastoma SH-SY5Y cell culture through scavenging ROS and reducing apoptosis (Puangmalai et al. 2017; Thangnipon et al. 2013). *N*-benzylcinnamide (PT-3) exhibits neuroprotective effect against oxidative stress in both SH-SY5Y and rat cortical cell cultures (Puangmalai et al. 2017; Thangnipon et al. 2013). Phytochemical 4-allylguaiacol (eugenol) derived from plants such as basil exerts a neuroprotective effect on HT22 hippocampal cell cultures in a scopolamine mice model of memory deficit through antioxidant scavenging properties and suppressing expression of TrKA and TrKB responsible for β -amyloid deposition (Lim et al. 2018). A phenolic compound aloe-emodin antagonizes scopolamine-induced memory deficit by inhibiting acetylcholinesterase and is able to reduce ROS and H_2O_2 -mediated cytotoxicity in rat PC12 pheochromocytoma cells (Tao et al. 2014).

SAK3 (ethyl 8'-methyl-2',5'-dioxo-2-piperidin-1-ylspiro[cyclopentene-3,3'-imidazo[1,2-a]pyridine]-1-carboxylate) is a derivative of spiroidmidazopyridine,

an enhancer of T-type voltage-gated Ca^{2+} channels (T-VGCCs) (Izumi et al. 2018), which primarily function to preserve learning and memory by acting on the hippocampus (Moriguchi et al. 2012; Yabuki et al. 2017a). Recently, SAK3 was implicated as a potential drug alongside phenolic compounds (Izumi et al. 2018). Although there are structural differences between phenolic compounds and SAK3, the latter also is able to reduce β -amyloid deposition and improved cognitive functions in genetically modified mice with APP (Izumi et al. 2018). In addition, SAK3 increased levels of calcium/calmodulin-dependent protein kinase II (CAMKII) autophosphorylation responsible for hippocampal memory processes such as long-term potentiation (LTP) in hippocampal neural cell culture derived from APP knock-in mouse (Izumi et al. 2018; Zhang et al. 2015). In addition, another T-VGCCs enhancer compound, ST101, increases CAMKII autophosphorylation and enhances T-VGCC currents in neuro2A neuroblastoma (N2A) cell culture expressing human $Ca_v3.1$ ($\alpha 1G$) (Moriguchi et al. 2012).

Thus, we investigated the potential neuroprotective effects of SAK3 treatment of SH-SY5Y cells following induction of neurotoxicity by scopolamine. The study should help to expand the armory of compounds with anti-neurotoxicity properties in vitro, which may lead to development of lead compounds in the treatment of the debilitating pathology of Alzheimer's disease.

Materials and methods

Chemicals and reagents

SAK3 was kindly provided by K. Fukunaga (Department of Pharmacology, Tohoku University, Japan). Media and supplementary compounds for cell culture were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA); scopolamine hydrobromide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA), and Bradford reagent from Sigma Aldrich (St. Louis, MO, USA); rabbit anti-Bax, -Bcl-2, -activated caspase-3, -phospho-p38, -p38, -phospho-JNK, -JNK, -phospho-ERK1/2, -ERK1/2, -phospho-Akt, -Akt, -phospho-NF- κ B p65 (Ser536), -NF- κ B, -actin antibodies, and horseradish peroxidase

(HRP)-conjugated goat anti-rabbit IgG antibodies from Cell Signaling Technology, Inc. (Danvers, MA, USA); rabbit anti-BACE1 and mouse anti-ChAT antibodies from Merck Millipore (Billerica, MA, USA); rabbit anti-IL-1 β and mouse anti-IL-6 antibodies from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA); and (HRP)-conjugated goat anti-mouse IgG antibodies from Zymed (Carlsbad, CA, USA).

Cell culture and treatment

Human neuroblastoma SH-SY5Y cells were obtained from the American Type Culture Collection (ATCC CRL-2266). Under the experimental conditions used, SH-SY5Y cells developed more neuron-like morphology with elongated processes (Suthprasertporn et al. 2019), whereas in an undifferentiated form, SH-SY5Y cells are characterized morphologically by neuroblast-like, non-polarized cell bodies with few processes (Kovalevich and Langford 2013). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μ g/ml streptomycin at 37 °C in a humidified incubator under 5% CO₂ atmosphere (Tiong et al. 2010). Media were changed every 2 days and sub cultured once they reached 80–90% cell confluency. Cells were used between passages 30 to 40 in all experiments.

SH-SY5Y cells (2×10^5 /ml and 4×10^5 /ml) were placed in 96-well and 24-well plate respectively. Cells were pretreated with or without 5, 10, 25, 50, and 100 nM SAK3 in distilled water for 3 h, then culture medium was removed and cells were incubated with the same SAK3-containing media with and without 2 mM scopolamine hydrobromide in serum-free DMEM for 24 h (Puangmalai et al. 2017).

Cell viability assay

Viability of SH-SY5Y cells were determined by a colorimetric MTT assay (Dokumaci and Aycan 2019). After treatments, cells were incubated with 0.5 mg/ml MTT at 37 °C in the dark for 3 h under 5% CO₂ humidified atmosphere, then DMSO was added to dissolve blue formazan crystals and A_{570 nm} measured at with a microplate reader (SpectraMax[®] 180; Molecular Devices, Sunnyvale, CA, USA).

Measurement of intracellular ROS production

Intracellular ROS levels were measured using a DCFH-DA assay, in which non-fluorescent DCFH-DA is converted into fluorescent DCF when oxidized by intracellular ROS (Thangnipon et al. 2013). Cells were incubated with 50 μ M DCFH-DA in absolute ethanol in the dark for 45 min at room temperature. DCF fluorescence was measured using a multi-detection microplate reader with 485 nm excitation and 530 nm emission filters (DTX880; Beckman Coulter, Wals, Austria).

Western blot analysis

SH-SY5Y cells were lysed in ice-cold lysis buffer containing 50 mM Tris, 150 mM NaCl, 0.5% Sodium Deoxycholate, 1 mM EDTA, 0.1% SDS, 10% Triton X-100, 10 mM PMSF, 1:100 protease inhibitor. Equal amounts of protein (30 μ g) from each sample were separated by 10% SDS-PAGE, transferred onto a polyvinylidene fluoride (PVDF) membrane, which was then incubated with 5% non-fat milk in Tris-buffered saline solution (pH 7.4) containing 0.1% Tween-20 (TBS-T) at room temperature for 1 h, followed by primary antibodies (anti-BACE1 (1:500 dilution in TBS-T), anti-Bax (1:1000), anti-Bcl-2 (1:1000), anti-activated caspase-3 (1:1000), anti-phospho-p38 (1:1000), anti-p38 (1:1000), anti-phospho-JNK (1:1000), anti-JNK (1:1000), anti-phospho-ERK1/2 (1:1000), anti-ERK1/2 (1:1000), anti-phospho-Akt (1:1000), anti-Akt (1:1000), anti-phospho-NF- κ B p65 (Ser536) (1:2000), anti- NF- κ B (1:2000), anti-IL-1 β (1:500), anti-IL-6 (1:1000) and anti- β -actin (1:2500) as loading control) overnight at 4 °C and then with appropriate secondary HRP-conjugated antibodies (1: 1000) for 1 h at room temperature. Membrane was then treated with enhanced chemical luminescence (ECL) reagent (Bio-Rad, Hercules, CA, USA) and immunoreactive protein bands were recorded on Hyperfilm[™] (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Densitometric analysis was performed using Image J software (National Institutes of Health, Bethesda, MA, USA).

Statistical analysis

Statistical analysis was performed using GraphPad Prism v6.01 software (San Diego, CA, USA). Data

were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Results are presented as mean $\pm$ standard error of the mean (SEM) of three independent experiments. A p value < 0.05 is considered statistically significant.

Results

SAK3 effects on cytotoxicity, ROS production, and apoptosis of scopolamine-treated SH-SY5Y cells

Treatment of SH-SY5Y cells with 2 mM scopolamine significantly decreases cell viability. Cell viability was partially restored by SAK3 (pre-treatment and together with scopolamine) in a dose-dependent manner (10–100 nM) (Fig. 1a). SAK3 pretreatment had no effect on SH-SY5Y cell viability. In addition, exposure of SH-SY5Y cells to 2 mM scopolamine resulted in an increase of $118 \pm 1\%$ (p -value < 0.001) in intracellular ROS level over untreated

control cells (Fig. 1b). However, the accumulation of ROS in the cells pretreated with SAK3 (5–200 nM) was significantly reduced (at 5 nM, p -value < 0.01 ; 10 nM, p -value < 0.01 ; 25 nM, p -value < 0.001 ; 50 nM, p -value < 0.001 ; 100 nM, p -value < 0.001 ; and 200 nM, p -value < 0.001 .) in a dose-dependent manner compared to treatment with scopolamine alone. In addition, exposure of SH-SY5Y cells to 2 mM scopolamine resulted in an increase of $118 \pm 1\%$ (p -value < 0.001) in intracellular ROS level over untreated control cells (Fig. 1b). However, the accumulation of ROS in the cells pretreated with SAK3 (5–200 nM) was significantly reduced (for 5 nM, p -value < 0.01 ; 10 nM, p -value < 0.01 ; 25 nM, p -value < 0.001 ; 50 nM, p -value < 0.001 ; 100 nM, p -value < 0.001 ; and 200 nM, p -value < 0.001) in a dose-dependent manner, compared to scopolamine treatment alone. SAK3 treatment alone at any concentration does not significantly alter ROS generation compared to the control. An inverted phase-contrast microscopy revealed that live cells presented with cell shrinkage and disruption of

Fig. 1 Effect of SAK3 on scopolamine induced cytotoxicity and ROS production in SH-SY5Y cells. **a** Cell viability of scopolamine-treated cells determined by MTT assay was significantly increased by 5–200 nM SAK3 treatment. **b** ROS levels of scopolamine-treated cells determined by ROS assay were significantly reduced by 1–1000 nM SAK3 pre-treatment. Morphological changes were analyzed after 24 h with phase

contrast microscope. **c** Untreated control cells; **d** cells treated with scopolamine and **e** cells treated with SAK3 and scopolamine. Arrows indicate apoptotic cells that shrunken with condensed cytoplasm and the arrowheads indicate the recovery of impaired neurites. * p -value < 0.05 , *** p -value < 0.001 compared to control group; # p -value < 0.05 , ### p -value < 0.001 compared to scopolamine-treated group

Fig. 2 Effect of SAK3 on scopolamine-induced cell apoptosis in SH-SY5Y cells. Cells were treated with SAK3 and scopolamine described in legend to Fig. 1. Western blot analysis was performed to identify the protein levels of **a** Bax, **b** activated caspase-3, and **c** Bcl-2. The results represent mean $\pm$ SEM

neurites of SH-SY5Y cells following scopolamine exposure, which were prevented by SAK3 pre-treatment (Fig. 1c–e). The protective role of SAK3 against scopolamine-induced SH-SY5Y cell cytotoxicity was confirmed by SAK3 inhibition (25–100 nM) of proapoptotic Bax and activated caspase-3 levels upregulated in the presence of this muscarinic receptor antagonist, and conversely, SAK3 elevation threshold (25 nM) of anti-apoptosis Bcl-2 level over control that was depressed by scopolamine (Fig. 2a–c).

SAK3 effects on β -site amyloid precursor protein cleaving enzyme 1 (BACE1) level of scopolamine-treated SH-SY5Y cells

BACE1, identified to be the major β -secretase in neurons, is required for the production of β -amyloid 42 which is one of the major pathogenic molecules of

from three independent experiments. ** p -value < 0.01, *** p -value < 0.001 compared to control group; # p -value < 0.05, ## p -value < 0.01, ### p -value < 0.001 compared to scopolamine-treated group

AD (Chatila et al. 2018). Western blotting was used to demonstrate that exposure to scopolamine significantly increases BACE1 $139 \pm 5\%$ (p -value < 0.05) compared to untreated control, but this is not significantly affected by 25–100 nM SAK3 treatments (Fig. 3a).

SAK3 effects on ChAT levels of scopolamine-treated SH-SY5Y cells

As reduced ChAT level is one of the characteristics of AD cells (Nunes-Tavares et al. 2012), scopolamine-exposed SH-SY5Y cells were evaluated for ChAT content using Western blotting. Scopolamine causes a significant decrease in ChAT level (p -value < 0.001) compared to control cells, and 25–100 nM SAK3 treatments were able to restore to control level in a dose dependent manner (Fig. 3b).

Fig. 3 Effects of SAK3 on scopolamine-induced activation of BACE1 and ChAT levels in SH-SY5Y cells. Cells were treated with SAK3 and scopolamine described in legend to Fig. 1. The results represent mean $\pm$ SEM from three independent

experiments. Western blot analysis was performed to identify the protein levels of **a** BACE1 and **b** ChAT. * p -value < 0.05 , *** p -value < 0.001 compared to control group; ### p -value < 0.001 compared to scopolamine-treated group

SAK3 effects on intracellular signaling pathways and protein expression of scopolamine-treated SH-SY5Y cells

Scopolamine activated p38 and JNK pathways (Puangmalai et al. 2017) and in addition Akt pathway, demonstrated by elevation in their respective phospho-forms in western blotting (Fig. 4a, b, d), which were reduced to control levels by SAK3 either in a dose-dependent manner (as in Akt) or at a threshold level (p38 and JNK). ERK 1/2 pathway was not affected by scopolamine or SAK3 (Fig. 4c).

Regulation of a number of receptor-mediated target gene expression requires phosphorylation of cytoplasmic transcription factor NF- κ B (Liu et al. 2017), a phenomenon observed with SH-SY5Y cells exposed to scopolamine (Fig. 5a) together with enhanced levels of IL-1 β and IL-6 (Fig. 5b and c). NF- κ B is a key transcription factor and is required for induction of inflammatory genes, including IL-1 β and IL-6 (Liu et al. 2017). In addition, SAK3 reversed these effects in a dose dependent manner (for IL-1 β) or at a threshold level (NF- κ B and IL-6).

Discussion

The effects of scopolamine, a muscarinic receptor antagonist, on human neuroblastoma SH-SY5Y cells

were consistent with those previously reported (Puangmalai et al. 2017), except that in the present study scopolamine was able to activate Akt signalling pathway, not observed in the previous report. We surmise this might in part be due to difference in quality of the reagent(s) used, but, nevertheless, the discrepancy does not alter the conclusions reached in the present work. Although both SAK3 and *N*-benzylcinnamide (Puangmalai et al. 2017) showed a neuroprotective effect on SH-SY5Y cells in culture, they manifested different effects on Akt activation level. As the two compounds have different chemical structures (SAK3 being an analog of spiroindolizopyridine) and given the complexity of AD pathophysiology, it should not be surprising the two chemical agents can have different biochemical effects on the same cell type but ultimately producing similar phenotype. Further studies on the exact mechanisms of these two and other neuroprotectants are clearly warranted, in particular to discover the common pathway(s) (if any) through which they exhibit their respective properties.

Scopolamine has been shown to significantly increase Akt phosphorylation levels of rat frontal cortex through induction of mammalian target of rapamycin complex 1 signaling pathway (Voleti et al. 2013). Administration of Akt inhibitor decreases hippocampal neuronal HT22 cell death induced by tumor necrosis factor α and the pan-caspase inhibitor

Fig. 4 Effect of SAK3 on scopolamine-induced activation of MAPK and Akt in SH-SY5Y cells. Cells were treated with SAK3 and scopolamine described in legend to Fig. 1. The results represent mean $\pm$ SEM from three independent experiments. Western blot analysis was performed to identify the

protein levels of **a** p-p38, **b** p-JNK, **c** p-ERK1/2, and **d** p-Akt. * p -value < 0.05 , ** p -value < 0.01 compared to control group; # p -value < 0.05 , ## p -value < 0.01 , ### p -value < 0.001 compared to scopolamine-treated group

N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Liu et al. 2014). SAK3 treatment was shown not to alter ERK1/2 phosphorylation levels induced in mice by a transient brain ischemia (Yabuki et al. 2017a). In addition, we found scopolamine stimulated phosphorylation of NF- κ B, the form capable of translocation to the nuclear compartment for activation of its target genes, as well as scopolamine-induced increase in levels of BACE1 and the inflammatory response cytokines IL-1 β and IL-6.

All these responses in SH-SY5Y cells to scopolamine were effectively nullified by SAK3, except for the increase in BACE1 levels. Izumi et al. (2018) found that SAK3 had no effect on scopolamine-

stimulated BACE1 expression indicating that SAK3 treatment have no effect on APP processing and A β degradation. Moreover, they found that SAK3 is able to reverse down-regulation of SGK1 gene expression, which is related to inflammation and cell death (Baban et al. 2014), in APP^{NL-F} knock-in mice. Inhibition of SGK1 by EMD638683 significantly enhances production of IL-6, IL-12 and TNF in lipopolysaccharide-induced human monocytes (Zhou et al. 2015) and, so, it might not be unexpected that levels of IL-1 β and IL-6 were elevated in scopolamine-treated SH-SY5Y cells. In further studies, the role of scopolamine on SH-SY5Y in SGK1 activity should be explored. It is worth noting that Yabuki et al. (2017b), using a patch

Fig. 5 Effects of SAK3 on scopolamine-induced inflammatory response in SH-SY5Y cells. Cells were treated with SAK3 and scopolamine described in legend to Fig. 1. The results represent mean $\pm$ SEM from three independent experiments. Western blot analysis was performed to identify the protein levels of

a NF- κ B, **b** IL-1 β , and **c** IL-6. ** p -value < 0.01, *** p -value < 0.001 compared to control group; # p -value < 0.05, ### p -value < 0.01, #### p -value < 0.001 compared to scopolamine-treated group

clamp method, demonstrated the ability of SAK3 to cross the blood–brain barrier employing neuro2A cells expressing Cav3.1 (a T-type calcium channel). More recently, Izumi et al. (2018) employed ultra-performance liquid chromatography–tandem mass spectrometry to detect the presence of SAK3 in murine brain following oral administration.

In conclusion, these results suggest the neuroprotective effects of SAK3 on scopolamine-induced cholinergic dysfunction might be linked to its anti-oxidant, anti-apoptotic and anti-inflammatory activities, thus providing further insight into avenues leading to development of potential therapeutic for the treatment of Alzheimer's disease.

Acknowledgements This research was partially supported by a joint Mahidol University and the Thailand Research Fund (TRF) Grant (IRG5780009). The authors are grateful to Prof. Prapon Wilairat, Mahidol University for critical reading of the manuscript.

Compliance with ethical standards

conflict of interest The authors declare no conflicts of interest.

References

Alvarez-Jimenez R, Groeneveld GJ, van Gerven JM, Goulouze SC, Baakman AC, Hay JL, Stevens J (2016) Model-based

- exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. *Br J Clin Pharmacol* 82:1011–1021
- Baban B, Liu JY, Mozaffari MS (2014) SGK-1 regulates inflammation and cell death in the ischemic-reperfused heart: pressure-related effects. *Am J Hypertens* 27:846–856
- Budzynska B, Boguszewska-Czubar A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A, Musik I, Biala G (2014) Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. *Psychopharmacology* 232:931–942
- Chatila ZK, Kim E, Berle C, Bylykbashi E, Rompala A, Oram MK, Gupta D, Kwak SS, Kim YH, Kim DY, Choi SH, Tanzi RE (2018) BACE1 regulates proliferation and neuronal differentiation of newborn cells in the adult hippocampus in mice. *eNeuro*. <https://doi.org/10.1523/ENEURO.0067-18.2018>
- Dokumaci AH, Yerer Aycan MB (2019) Vildagliptine Protects SH-SY5Y human neuron-like cells from A β 1-42 induced toxicity, in vitro. *Cytotechnology* 71:635–646
- Du X, Wang X, Geng M (2018) Alzheimer's disease hypothesis and related therapies. *Transl Neurodegener* 7:1–7
- Izumi H, Shinoda Y, Saito T, Saido TC, Sato K, Yabuki Y, Matsumoto Y, Kanemitsu Y, Tomioka Y, Abolhassani N, Nakabeppu Y, Fukunaga K (2018) The Disease-modifying drug candidate, SAK3 improves cognitive impairment and inhibits amyloid β deposition in APP knock-in mice. *Neuroscience* 377:87–97
- Kovalevich J, Langford D (2013) Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods Mol Biol* 1078:9–21
- Lim H, Kim B, Kim Y, Jeong S (2018) Phytochemical allyl-guaiacol exerts a neuroprotective effect on hippocampal cells and ameliorates scopolamine-induced memory impairment in mice. *Behav Brain Res* 339:261–268
- Liu Q, Qiu J, Liang M, Golinski J, van Leyen K, Jung JE, You Z, Lo EH, Degterev A, Whalen MJ (2014) Akt and mTOR mediate programmed necrosis in neurons. *Cell Death Dis* 5:e1084
- Liu T, Zhang L, Joo D, Sun SC (2017) NF- κ B signaling in inflammation. *Signal Transduct Target Ther* 2:1–9
- Mamelak M, Delaney S, Yang K, O'Brien P (2010) Ketone bodies, sodium oxybate and Alzheimer's disease: oxidative stress and neuroprotection. *Alzheimer's Dementia* 6:S389
- Moriguchi S, Shioda N, Yamamoto Y, Tagashira H, Fukunaga K (2012) The T-type voltage-gated calcium channel as a molecular target of the novel cognitive enhancer ST101: enhancement of long-term potentiation and CaMKII autophosphorylation in rat cortical slices. *J Neurochem* 121:44–53
- Nunes-Tavares N, Santos LE, Stutz B, Brito-Moreira J, Klein WL, Ferreira ST, de Mello FG (2012) Inhibition of choline acetyltransferase as a mechanism for cholinergic dysfunction induced by amyloid- β peptide oligomers. *J Biol Chem* 287:19377–19385
- Puangmalai N, Thangnipon W, Soi-ampornkul R, Suwanna N, Tuchinda P, Nobsathian S (2017) Neuroprotection of *N*-benzylcinnamide on scopolamine-induced cholinergic dysfunction in human SH-SY5Y neuroblastoma cells. *Neural Regen Res* 12:1492–1498
- Pudlo M, Luzet V, Ismaïli L, Tomassoli I, Iutzeler A, Refouvet B (2014) Quinolone-benzylpiperidine derivatives as novel acetylcholinesterase inhibitor and antioxidant hybrids for Alzheimer Disease. *Bioorg Med Chem* 22:2496–2507
- Rinne J, Kaasinen V, Jarvenpää T, Nagren K, Roivainen A, Yu M (2003) Brain acetylcholinesterase activity in mild cognitive impairment and early Alzheimer's disease. *Neurol Neurosurg Psychiatry* 74:113–115
- Rojsanga P, Boonyarat C, Utsintong M, Nemečz Á, Yamauchi J, Talley T, Olson AJ, Matsumoto K, Vajragupta O (2012) The effect of crebanine on memory and cognition impairment via the α -7 nicotinic acetylcholine receptor. *Life Sci* 91:107–114
- Selkoe D, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* 8:595–608
- Suthpraserporn N, Suwanna N, Thangnipon W (2019) Protective effects of diethylpropionitrile against hydrogen peroxide-induced damage in human neuroblastoma SH-SY5Y cells. *Drug Chem Toxicol*. <https://doi.org/10.1080/01480545.2019.1658768>
- Tao L, Xie J, Wang Y, Wang S, Wu S, Wang Q, Ding H (2014) Protective effects of aloe-emodin on scopolamine-induced memory impairment in mice and H₂O₂-induced cytotoxicity in PC12 cells. *Bioorg Med Chem Lett* 24:5385–5389
- Thangnipon W, Suwanna N, Kitiyanant N, Soi-ampornkul R, Tuchinda P, Munyoo B, Nobsathian S (2012) Protective role of *N*-trans-feruloyltyramine against β -amyloid peptide-induced neurotoxicity in rat cultured cortical neurons. *Neurosci Lett* 513:229–232
- Thangnipon W, Puangmalai N, Chinchalongporn V, Jantrachotechachawan C, Kitiyanant N, Soi-Ampornkul R, Tuchinda P, Nobsathian S (2013) *N*-benzylcinnamide protects rat cultured cortical neurons from β -amyloid peptide-induced neurotoxicity. *Neurosci Lett* 556:20–25
- Tiong CX, Lu M, Bian JS (2010) Protective effect of hydrogen sulphide against 6-OHDA-induced cell injury in SH-SY5Y cells involves PKC/PI3K/Akt pathway. *Br J Pharmacol* 161:467–480
- Venkatesan R, Subedi L, Yeo EJ, Kim SY (2016) Lactucopicrin ameliorates oxidative stress mediated by scopolamine-induced neurotoxicity through activation of the NRF2 pathway. *Neurochem Int* 99:133–146
- Voleti B, Navarria A, Liu RJ, Banasr M, Li N, Terwilliger R, Sanacora G, Eid T, Aghajanian G, Duman RS (2013) Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. *Biol Psychiatry* 74:742–749
- Wang H, Xu Y, Yan J, Zhao X, Sun X, Zhang Y, Guo J, Zhu C (2009) Acteoside protects human neuroblastoma SH-SY5Y cells against β -amyloid-induced cell injury. *Brain Res* 1283:139–147
- Wang X, Wang W, Li L, Perry G, Lee H, Zhu X (2014) Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochim Biophys Acta* 1842:1240–1247
- Yabuki Y, Jing X, Fukunaga K (2017a) The T-type calcium channel enhancer SAK3 inhibits neuronal death following transient brain ischemia via nicotinic acetylcholine receptor stimulation. *Neurochem Int* 108:272–281
- Yabuki Y, Matsuo K, Izumi H, Haga H, Yoshida T, Wakamori M, Kakei A, Sakimura K, Fukuda T, Fukunaga K (2017b) Pharmacological properties of SAK3, a novel T-type

voltage-gated Ca^{2+} channel enhancer. *Neuropharmacology* 117:1–13

Zhang H, Wu L, Pchitskaya E, Zakharova O, Saito T, Saido T, Bezprozvanny I (2015) Neuronal store-operated calcium entry and mushroom spine loss in amyloid precursor protein knock-in mouse model of Alzheimer's disease. *J Neurosci* 35:13275–13286

Zhou H, Gao S, Duan X, Liang S, Scott DA, Lamont RJ, Wang H (2015) Inhibition of serum- and glucocorticoid-inducible

kinase 1 enhances TLR-mediated inflammation and promotes endotoxin-driven organ failure. *FASEB J*. 29:3737–3749

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.