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ITH12410/SC058: A New Neuroprotective Compound with Potential in the Treatment of Alzheimer's Disease

Alejandro Romero,[†,∥] Javier Egea,[†] Gema C. González-Muñoz,[§] Mª Dolores Martín de Saavedra,[†,⊥] Laura del Barrio,[†,#] María Isabel Rodríguez-Franco,[§] Santiago Conde,[§] Manuela G. López,[†,‡] Mercedes Villarroya,*[,†] and Cristóbal de los Ríos*[,†,‡]

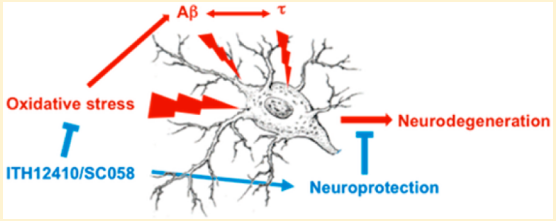
[†]Instituto Teófilo Hernando, Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, C/Arzobispo Morcillo 4, 28029 Madrid, Spain

[‡]Servicio de Farmacología Clínica, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, C/Diego de León 62, 28006 Madrid, Spain

[§]Instituto de Química Médica (IQM-CSIC), C/Juan de la Cierva 3, 28006 Madrid, Spain

Supporting Information

ABSTRACT: The neuroprotective profile of the dibenzothiadiazepine ITH12410/SC058 (2-chloro-5,6-dihydro-5,6-diacetyldibenzo[*b*,*f*]-[1,4,5]thiadiazepine) against several neurotoxicity models related to neurodegenerative diseases is herein described. ITH12410/SC058 protected SH-SY5Y cells against the loss of cell viability elicited by amyloid beta peptide and okadaic acid, a selective inhibitor of phosphoprotein phosphatase 2A that induces neurofibrillary tangle formation. Furthermore, ITH12410/SC058 is neuroprotective against several in vitro models of oxidative stress, that is, H_2O_2 exposure or incubation with rotenone plus oligomycin A in SH-SY5Y cells, and oxygen and glucose deprivation followed by reoxygenation in rat hippocampal slices. By contrast, ITH12410/SC058 was unable to significantly protect SH-SY5Y neuroblastoma cells against the toxicity elicited by Ca^{2+} overload. Our results confirm the hypothesis that the dibenzothiadiazepine ITH12410/SC058 features its neuroprotective actions in a multitarget fashion, and is a promising drug for the treatment of neurodegenerative diseases.



KEYWORDS: *Dibenzothiadiazepine, neurodegenerative diseases, oxidative stress, Alzheimer's disease, amyloid beta, neurofibrillary tangles, neuroprotective drugs*

It is widely accepted that neurodegenerative diseases involve multifactorial pathogenic mechanisms, whereby a set of physiological events are leading neurons either cooperatively or independently to cell death.[1] Thus, drugs acting on the central nervous system that have been designed to interact with a specific biological target have commonly failed in clinical trials. Because of this failure, a growing tendency to look for single molecules capable to interact with more than one target has emerged,[2,3] featuring neuroprotective profiles in both in vitro and in vivo models against toxic stimuli mimicking neurodegeneration. These findings have supported the rising of the multitarget drug therapeutic strategy for neurodegenerative diseases.[4−6] Our research group has a long-standing interest in the search of novel compounds with neuroprotective effects and therapeutic potential in neurodegenerative diseases like Alzheimer's disease (AD).[7,8] The design of such drugs focuses on the physiopathological events that lead to AD, which include amyloid beta (*Aβ*) and tau (*τ*) neurotoxicity, failure of cholinergic neurotransmission, mitochondrial dysfunction, and oxidative imbalance with increased free radical production.[9] In addition, our research group has studied their relationship with Ca^{2+} homeostasis, a key factor in cell death or survival phenomena.[10] In this line, *Aβ* neurotoxicity can be caused by a dysregulation of cellular Ca^{2+} homeostasis,[11] and that antioxidants and free radical suppressors prevent Ca^{2+} overload induced by *Aβ*.[12] Therefore, following a multitarget approach, we centered our attention in certain families of compounds that showed interesting pharmacological profiles, for example, some *N*-acylaminophenothiazine derivatives described as neuroprotective agents against several toxic stimuli such as mitochondrial free radicals, Ca^{2+} overload, *Aβ*, and *τ*-hyperphosphorylation.[13] Related to this family, we have described the synthesis and biological evaluation of dibenzothiadiazepine derivatives, which showed good neuroprotection profile against mitochondrial oxidative stress, a good blood-brain barrier penetration, measured in in vitro PAMPA experiments, capture of reactive oxygen species (ROS) capacity, and a Ca^{2+} channel modulating activity,[14] among which the most interesting was compound **2**, first synthesized by Corral et al.,[15] that now we have named as ITH12410/SC058 [2-chloro-5,6-dihydro-5,6-diacetyldibenzo[*b*,*f*][1,4,5]thiadiazepine; Figure 1].

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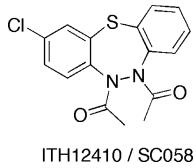

Figure 1. Chemical structure of ITH12410/SC058.

Here we present a thorough study aimed at deepening the neuroprotective profile of ITH12410/SC058 in SH-SY5Y human neuroblastoma cells and in rat hippocampal slices stressed with different toxic stimuli related to AD. We present that ITH12410/SC058 behaves as a neuroprotectant, mainly against oxidative-stress-induced neurodegeneration, able to counteract Aβ and τ hyperphosphorylation-induced neuronal death.

We have previously described that ITH12410/SC058 protected SH-SY5Y cells against cell death induced by the stressor cocktail rotenone 30 μM and oligomycin A 10 μM (R/O) at concentrations of 0.3−3 μM, measured as lactate dehydrogenase (LDH) release.[14] Subjecting SH-SY5Y neuroblastoma cells to R/O is described as a good experimental model of mitochondria-triggered oxidative stress. The cocktail of R/O blocks the complexes I and V of the electron transport chain, respectively, which provokes free radical production and blockade of the ATP synthesis.[16] However, cell death monitored by the method of LDH release only affords information regarding cell necrosis. We have now assessed the effect of ITH12410/SC058 regarding cell viability with the alternative method of the MTT dye.[17] Mitochondrial succinate dehydrogenases from healthy cells reduce MTT to a colored dye. Apoptotic or necrotic cells cannot carry out this chemical modification. Thus, we test if our compound would protect against both apoptotic and necrotic death. In these experiments, SH-SY5Y human neuroblastoma cells were treated with 0.1−3 μM ITH12410/SC058 24 h before and during the additional 24 h incubation period with R/O. Cells exposed to R/O in the absence of the compound showed a viability of 56% with respect to control, nonlesioned cells. Figure 2 shows

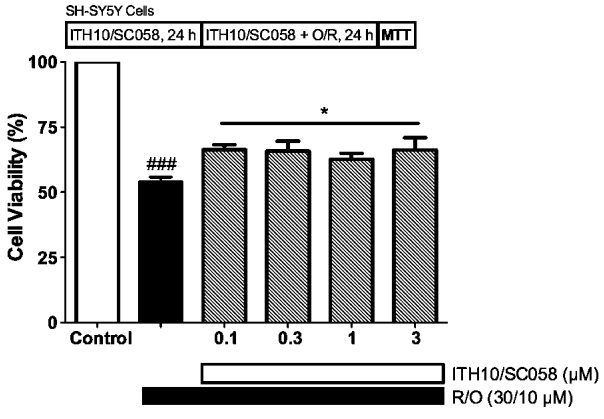

Figure 2. Protection by ITH12410/SC058 against the R/O-evoked toxic stimulus in SH-SY5Y neuroblastoma cells. Cell viability was evaluated by MTT reduction (ordinate); data were normalized (% control, white column; cells only incubated with cell culture medium). Data expressed are the mean ± SEM of triplicates of five different cell line batches: $^{###}p < 0.001$, comparing control and R/O-damaged cells; $^{*}p < 0.05$, comparing with R/O-damaged cells without drug incubation.

pooled results of five different experiments in triplicate. ITH12410/SC058 exerted a sustained improvement of cell viability, of around a 25%, between 0.1 and 3 μM, where no significant differences were observed among all doses used.

In parallel, we assessed the effect of ITH12410/SC058 against an exogenous source of free radicals, by exposing SH-SY5Y cells to 100 μM H_2O_2. SH-SY5Y human neuroblastoma cells were treated with ITH12410/SC058 at the concentrations of 0.3, 1, and 3 μM for 24 h before the incubation of cells with H_2O_2 and maintained during the additional 24 h exposure to H_2O_2. Cells exposed to H_2O_2 in the absence of ITH12410/SC058 showed a viability of about 70% with respect to control cells, as measured with the MTT reduction method. Figure 3

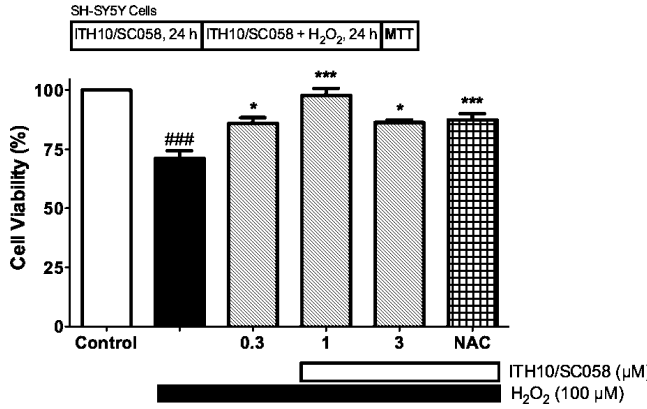

Figure 3. Protection by ITH12410/SC058 against the H_2O_2-evoked toxic stimulus in SH-SY5Y neuroblastoma cells. Cell viability was evaluated by MTT reduction (ordinate); data were normalized (% control, white column; cells only incubated with cell culture medium). *N*-Acetylcysteine at the concentration of 1 mM was used as reference antioxidant. Data expressed are the mean ± SEM of triplicates of six different cell line batches: $^{###}p < 0.001$, comparing control and H_2O_2-damaged cells; $^{***}p < 0.001$, $^{*}p < 0.05$, comparing with H_2O_2-damaged cells without drug incubation.

shows the pooled results of six different experiments in triplicate, where ITH12410/SC058 protected cells drastically, and a maximal protection of 92% was reached at 1 μM. In this model, *N*-acetylcysteine, used at the concentration of 1 mM, was included as a reference antioxidant.

The clear neuroprotective properties as antioxidant of ITH12410/SC058 in neuroblastoma cells motivated us to study it in a more complex preparation. Thus, rat hippocampal slices were subjected to oxygen and glucose deprivation (OGD) followed by reoxygenation, which is an acute model of lesion produced by free radical generation during the reoxygenation period.[18] The lesion generated in this tissue simulates what can occur in cerebral ischemia and Alzheimer's disease patients, where the hippocampus is profoundly affected, causing cardinal symptoms of short-term memory loss.[19] Thus, 15 min of OGD, followed by 2 h of reoxygenation produced a cell viability decrease of about 40% respect to control slices. In this model, ITH12410/SC058 was tested at concentrations ranging from 0.3 to 10 μM. ITH12410/SC058 was able to increase the viability of the tissue in a concentration-dependent fashion, and a maximal protection of 73% was reached at 3 μM. This protection was lost at higher concentrations (10 μM) (Figure 4); what could be due to this high concentration is out of the therapeutic range of the compound, presumably because it is gaining affinity toward other biological targets, and these new

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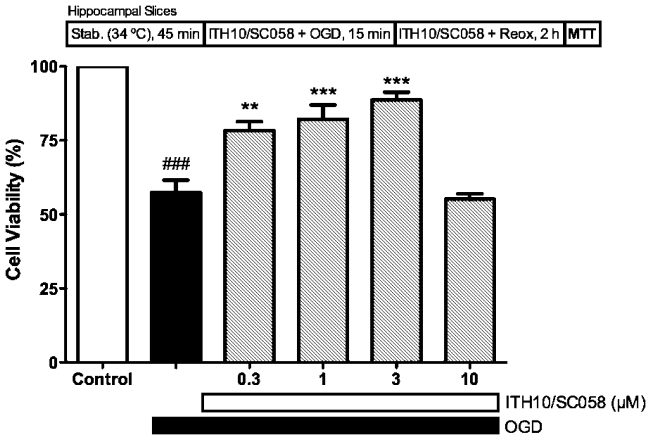

Figure 4. Protection by ITH12410/SC058 against the cytotoxic effects of oxygen and glucose deprivation (OGD) plus reoxygenation in rat hippocampal slices. Tissue viability was evaluated by MTT reduction (ordinate); data were normalized (% control, white column; cells only incubated with cell culture medium). Data expressed are the mean ± SEM of quadruplicates of three different rats: $^{###}p < 0.001$, comparing control and OGD-lesioned cells; $***p < 0.001$, $**p < 0.01$, comparing with OGD-damage tissue without drug incubation.

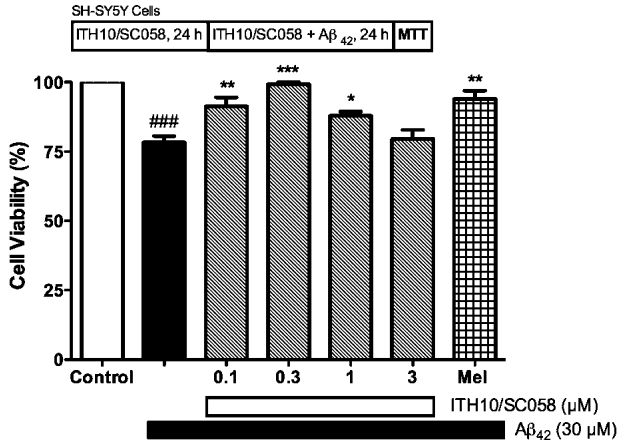

Figure 5. Protection by ITH12410/SC058 against $A\beta_{1-42}$-evoked toxic stimulus in SH-SY5Y neuroblastoma cells. Cell viability was evaluated by MTT reduction (ordinate); data were normalized (% control, white column; cells only incubated with cell culture medium). Data expressed are the mean ± SEM of triplicates of three different cell batches: $^{###}p < 0.001$, comparing control and Aβ-damaged cells; $***p < 0.001$, $**p < 0.01$, $*p < 0.05$, comparing with Aβ-damaged cells without drug incubation.

interactions trigger biological responses that are counteracting the neuroprotective effect observed at lower concentrations.

In summary, these three models of oxidative stress confirm this compound as an efficient antioxidant, which, at low micromolar concentrations, could rescue from death the neurons that become vulnerable due to an oxidative imbalance. Otherwise, the Aβ-elicited neurotoxicity has also been associated with the genesis of free radical production at the mitochondrial level. In fact, Aβ targets the mitochondrial permeability transition pore (mPTP), causing its opening and leading to an excessive free radical production.[20] Taking into account such observations, we tested if the antioxidant properties of ITH12410/SC058 could counteract the Aβ-induced neuronal damage. SH-SY5Y cells were exposed to ITH12410/SC058 at concentrations of 0.1−3 μM for 24 h before incubation with $A\beta_{1-42}$ (30 μM) and during its exposure. Cells treated with $A\beta_{1-42}$ in the absence of ITH12410/SC058 showed a viability of about 78% with respect to control cells. ITH12410/SC058 protected cells from 0.1 μM, and a maximum of 95% was reached at 0.3 μM, with respect to cells only treated with Aβ (Figure 5). Unlike what was observed in Figures 2 and 3, ITH12410/SC058 tested at the concentration of 3 μM lost the neuroprotective effect against Aβ. A possibility exists that some other biological targets overexpressed by the exposure to Aβ would be gaining affinity for ITH12259/SC058 at this concentration and thus reducing its protective profile. Melatonin (10 nM), which exhibits neuroprotective effects in SH-SY5Y cells exposed to Aβ,[21] afforded a 72% protection.

The 1−42 peptide is one of the most common forms of Aβ in amyloid deposits found in AD patients brain;[22−24] hence, the potent neuroprotective action of ITH12410/SC058 against this Aβ form toxicity deserves attention, and it seems that this action could be due in part to its apparent antioxidant profile. Nevertheless, the real mechanism of the Aβ-induced neurotoxicity in AD patients remains unclear, and the influence of the oxidative state imbalance, together with an enhanced Ca^{2+} entry into excitable cells[11] and a sensitization of the mPTP opening by increased Ca^{2+} concentrations at the mitochondrial matrix[25] could be implicated. For this reason, we tested if the ITH12410/SC058-evoked neuroprotection was also derived from a down-regulation of the toxic signal exerted by Ca^{2+} overload. Ca^{2+} overload-induced cell damage was generated by a mild depolarizing stimulus of 20 mM K^+ plus 0.3 μM FPL64176, which augments the opening time of L-type Ca^{2+} channels.[26] SH-SY5Y cells were exposed to ITH12410/SC058 at concentrations from 0.3 to 10 μM for 24 h before incubation with FPL64176 and maintained during the 24-h period of the toxic exposure. Cells treated with 20 mM K^+ plus FPL64176 in the absence of ITH12410/SC058 showed a viability of 65% with respect to control cells. ITH12410/SC058 protected cells up to a 20% at 1 μM (Figure 1 in the Supporting Information), but this protection was not statistically significant with respect to cells treated with the toxic stimulus in the absence of ITH12410/SC058. The L-type calcium channel blocker nimodipine, used as standard, afforded a protection of 32% at the concentration of 3 μM. Although ITH12410/SC058 was described to block by 20% the cytosolic Ca^{2+} increase stimulated by high extracellular K^+ concentration at 3 μM,[14] this activity does not seem to be sufficient to preserve cell viability of SH-SY5Y cells suffering Ca^{2+} overload when this is elicited by the selective opening of L-type voltage-dependent Ca^{2+} channels, by applying 20 mM K^+ plus FPL64176. Indeed, L-type Ca^{2+} channels are the subtype most directly related to the Ca^{2+} overload-induced cell death scenario.[27] Hence, we propose that the effect of ITH12410/SC058 on the Aβ-triggered neurotoxicity is mainly due to its antioxidant activity.

On the other hand, hyperphosphorylation of τ leading to neurofibrillary tangle (NFT) formation is the other principal hallmark to explain the neurodegeneration in AD.[28] We therefore studied the effect of ITH12410/SC058 against an in vitro model of τ hyperphosphorylation. Taking into account that the main phosphatase enzyme dephosphorylating τ is the phosphoprotein phosphatase 2A (PP2A),[29] a reliable model of τ hyperphosphorylation is the administration to neuronal cultures of its selective inhibitor okadaic acid (OA),[30] used in both in vivo[31] and in vitro experiments, such as neuroblastoma cells.[32,33] Cells exposed to 30 nM OA in the absence of

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ITH12410/SC058 showed a viability of 58% with respect to control cells, measured with the MTT reduction method. Figure 6 shows pooled results of four different experiments in triplicate.

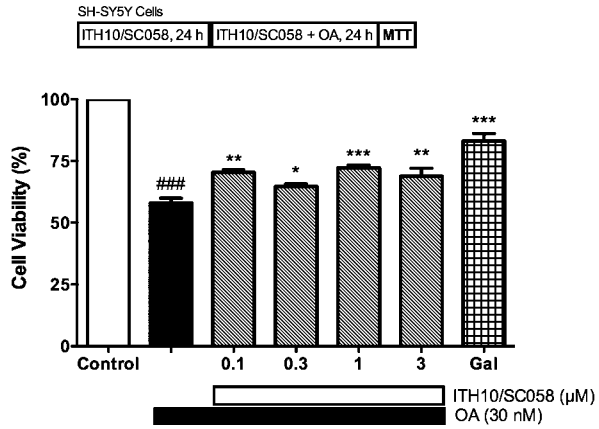

Figure 6. Protection by ITH12410/SC058 against the okadaic acid (OA)-evoked toxic stimulus in SH-SY5Y neuroblastoma cells. Cell viability was evaluated by the MTT reduction (ordinate); data were normalized (% control, white column; cells only incubated with cell culture medium). Data expressed are the mean ± SEM of triplicates of four different cell batches: $^{###}p < 0.001$, comparing control and OA-damaged cells; $***p < 0.001$, $** p < 0.01$, $*p < 0.05$, comparing with OA-damaged without drug incubation.

ITH12410/SC058 protected cells moderately, and the maximum protection of 34% was reached at 1 μM. In this toxicity model, 0.3 μM galantamine, included as reference compound,[33] afforded a protection of 60%. Although ITH12410/SC058 only exerted a mild neuroprotection against OA, the fact that the neuroprotective actions of ITH12410/SC058 include targeting of the two most highlighted hallmarks of AD, that is, hyperphosphorylation of τ and amyloidogenesis, makes these compounds of special interest for further preclinical studies on this neurodegenerative disease.

In summary, the compound ITH12410/SC058 presents a neuroprotective profile that could fit the concept of a multitarget drug[34] because of its ability to participate in the regulation of various physiological events occurring in AD, that is, Aβ, τ hyperphosphorylation, and free radicals of mitochondrial and exogenous origin. Because ITH12410/SC058 targets all these models, it seems that this benzothiadiazepine derivative may behave as an efficacious multitarget neuroprotective compound. The results of this work make this compound a promising candidate to further investigate its preclinical pharmacological properties for the treatment of AD.

METHODS

MTT Assay. The viability of cells and tissue preparations, defined as their mitochondrial activity, was assessed by a colorimetric assay with the dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), as described previously.[17] SH-SY5Y neuroblastoma cells were seeded into 48-well plates, and the dye MTT was injected to wells (5 mg mL^{-1}). After dye incubation at 37 °C for 2 h in the absence of light, cells were lysed. Mitochondrial succinate dehydrogenase cleaves the tetrazolium heterocycle of MTT, producing a precipitated, blue-colored formazan derivative, which is dissolved by adding 200 μL of dimethyl sulfoxide, generating a blue solution with an intensity measurable in a spectrophotometric plate reader at the wavelength of 540 nm (FLUOstar Optima, BMG, Germany). Data are expressed as the percentage of the chemical reduction of the MTT, considering the value obtained in nondamaged, nonincubated cells in each individual experiment as 100% of cell viability. In this paper, data are expressed as the percentage of protection by a certain treatment; as an example, 50% decrease in the reduction of the MTT would mean a 50% cell death, so counteracting such loss of cell viability by 25% because of drug incubation, would mean neuroprotection of 50%.

Data Analysis. Data are expressed as the mean ± standard error (SEM). The statistical differences were calculated by one-way ANOVA followed by Newman-Keuls post hoc test. Statistical differences were taken as significant when $p \leq 0.05$. All statistical analyses were performed using Prism software (GraphPad) version 5.0 for Mac (OS X).

ASSOCIATED CONTENT

Supporting Information

Cell viability experiments using Ca^{2+} overload as cell death model, reagents used, experimental details for the culture of SH-SY5Y neuroblastoma cells and preparation of rat hippocampal slices, and cell incubation with compound solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*Mailing address: Instituto Teofilo Hernando, Instituto de Investigación Sanitaría, Hospital Universitario de la Princesa, Diego de León, 62, 28006 Madrid, Spain. E-mail: cristobal.delosrios@uam.es. Phone: + 34-914972765. Fax: + 34-914973453.

*Mailing address: Instituto Teofilo Hernando, Instituto de Investigación Sanitaría, Hospital Universitario de la Princesa, Diego de León, 62, 28006 Madrid, Spain. E-mail: mercedes.villarroya@uam.es. Phone: + 34-914972765. Fax: + 34-914973453.

Present Addresses

[‖]A.R.: Departamento de Toxicología y Farmacología, Facultad de Veterinaria, Universidad Complutense, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain.

[⊥]M.D.M.d.S.: Department of Physiology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA.

[#]L.d.B.: Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL, USA.

Author Contributions

A.R. carried out neuroprotection experiments against rotenone and oligomycin A, Aβ, and OA; J.E. conducted neuroprotection experiments against H_2O_2 and 20 mM K^+ plus FPL and the experiments using hippocampal slices; G.C.G.-M. synthesized ITH12410/SC058, M.D.M.d.S. and L.d.B. collaborated in neuroprotection experiments; M.I.R.-F. and S.C. oversaw the chemical synthesis; M.G.L. oversaw experiments using hippocampal slices; and C.d.l.R. and M.V. oversaw the neuroprotection experiments in neuroblastoma cells and wrote the paper.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Aβ, amyloid beta peptide; AD, Alzheimer's disease; DMSO, dimethyl sulfoxide; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAC, *N*-acetylcysteine; mPTP, mitocondrial permeability transition pore; NFT, neurofibrilary tangles; OGD, oxygen and glucose deprivation; PP2A, phosphoprotein phosphatase 2A; OA, okadaic acid; R/O, rotenone plus oligomycin A; ROS, reactive oxygen species; SEM, standard error of the mean; τ, tau protein

■ REFERENCES

(1) Carreiras, M. C., Mendes, E., Perry, M. J., Francisco, A. P., and Marco-Contelles, J. (2013) The multifactorial nature of Alzheimer's disease for developing potential therapeutics. *Curr. Top. Med. Chem. 13*, 1745−1770.

(2) Cavalli, A., Bolognesi, M. L., Capsoni, S., Andrisano, V., Bartolini, M., Margotti, E., Cattaneo, A., Recanatini, M., and Melchiorre, C. (2007) A small molecule targeting the multifactorial nature of Alzheimer's disease. *Angew. Chem., Int. Ed. Engl. 46*, 3689−3692.

(3) Rodriguez-Franco, M. I., Fernandez-Bachiller, M. I., Perez, C., Hernandez-Ledesma, B., and Bartolome, B. (2006) Novel tacrine-melatonin hybrids as dual-acting drugs for Alzheimer disease, with improved acetylcholinesterase inhibitory and antioxidant properties. *J. Med. Chem. 49*, 459−462.

(4) Leon, R., and Marco-Contelles, J. (2011) A step further towards multitarget drugs for Alzheimer and neuronal vascular diseases: targeting the cholinergic system, amyloid-beta aggregation and Ca^{2+} dyshomeostasis. *Curr. Med. Chem. 18*, 552−576.

(5) Cavalli, A., Bolognesi, M. L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M., and Melchiorre, C. (2008) Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem. 51*, 347−372.

(6) Espinoza-Fonseca, L. M. (2006) The benefits of the multi-target approach in drug design and discovery. *Bioorg. Med. Chem. 14*, 896−897.

(7) Arce, M. P., Rodriguez-Franco, M. I., Gonzalez-Munoz, G. C., Perez, C., Lopez, B., Villarroya, M., Lopez, M. G., Garcia, A. G., and Conde, S. (2009) Neuroprotective and cholinergic properties of multifunctional glutamic acid derivatives for the treatment of Alzheimer's disease. *J. Med. Chem. 52*, 7249−7257.

(8) Lorrio, S., Romero, A., Gonzalez-Lafuente, L., Lajarin-Cuesta, R., Martinez-Sanz, F. J., Estrada, M., Samadi, A., Marco-Contelles, J., Rodriguez-Franco, M. I., Villarroya, M., Lopez, M. G., and de los Rios, C. (2013) PP2A ligand ITH12246 protects against memory impairment and focal cerebral ischemia in mice. *ACS Chem. Neurosci. 4*, 1267−1277.

(9) Butterfield, D. A., Reed, T., Newman, S. F., and Sultana, R. (2007) Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radical Biol. Med. 43*, 658−677.

(10) Fernandez-Morales, J. C., Arranz-Tagarro, J. A., Calvo-Gallardo, E., Maroto, M., Padin, J. F., and Garcia, A. G. (2012) Stabilizers of neuronal and mitochondrial calcium cycling as a strategy for developing a medicine for Alzheimer's disease. *ACS Chem. Neurosci. 3*, 873−883.

(11) Barger, S. W., Horster, D., Furukawa, K., Goodman, Y., Krieglstein, J., and Mattson, M. P. (1995) Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca^{2+} accumulation. *Proc. Natl. Acad. Sci. U.S.A. 92*, 9328−9332.

(12) Huang, H. M., Ou, H. C., and Hsieh, S. J. (2000) Antioxidants prevent amyloid peptide-induced apoptosis and alteration of calcium homeostasis in cultured cortical neurons. *Life Sci. 66*, 1879−1892.

(13) Gonzalez-Munoz, G. C., Arce, M. P., Lopez, B., Perez, C., Romero, A., del Barrio, L., Martin-de-Saavedra, M. D., Egea, J., Leon, R., Villarroya, M., Lopez, M. G., Garcia, A. G., Conde, S., and Rodriguez-Franco, M. I. (2011) N-acylaminophenothiazines: neuroprotective agents displaying multifunctional activities for a potential treatment of Alzheimer's disease. *Eur. J. Med. Chem. 46*, 2224−2235.

(14) González-Muñoz, G. C., Arce, M. P., Perez, C., Romero, A., Villarroya, M., López, M. G., Conde, S., and Rodríguez-Franco, M. I. (2014) Dibenzo[1,4,5]thiadiazepine: a hardly-known heterocyclic system with neuroprotective properties of potential usefulness in the treatment of neurodegenerative disease. *Eur. J. Med. Chem. 81*, 350−358.

(15) Corral, C., Lissavetzky, J., and Quintanilla, G. (1982) New method for the synthesis of chloro-substituted dibenzo[*b*,*f*][1,4,5]-thiadiazepines and their 5,6-dihydro derivatives. *J. Org. Chem. 47*, 2214−2215.

(16) Egea, J., Rosa, A. O., Cuadrado, A., Garcia, A. G., and Lopez, M. G. (2007) Nicotinic receptor activation by epibatidine induces heme oxygenase-1 and protects chromaffin cells against oxidative stress. *J. Neurochem. 102*, 1842−1852.

(17) Denizot, F., and Lang, R. (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods 89*, 271−277.

(18) Egea, J., Rosa, A. O., Sobrado, M., Gandia, L., Lopez, M. G., and Garcia, A. G. (2007) Neuroprotection afforded by nicotine against oxygen and glucose deprivation in hippocampal slices is lost in alpha7 nicotinic receptor knockout mice. *Neuroscience 145*, 866−872.

(19) De Lacoste, M. C., and White, C. L., 3rd. (1993) The role of cortical connectivity in Alzheimer's disease pathogenesis: a review and model system. *Neurobiol. Aging 14*, 1−16.

(20) Hensley, K., Carney, J. M., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R. A., and Butterfield, D. A. (1994) A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A. 91*, 3270−3274.

(21) Masilamoni, J. G., Jesudason, E. P., Dhandayuthapani, S., Ashok, B. S., Vignesh, S., Jebaraj, W. C., Paul, S. F., and Jayakumar, R. (2008) The neuroprotective role of melatonin against amyloid beta peptide injected mice. *Free Radical Res. 42*, 661−673.

(22) Younkin, S. G. (1998) The role of A beta 42 in Alzheimer's disease. *J. Physiol. (Paris) 92*, 289−292.

(23) Selkoe, D. J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev. 81*, 741−766.

(24) Mayeux, R., Honig, L. S., Tang, M. X., Manly, J., Stern, Y., Schupf, N., and Mehta, P. D. (2003) Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. *Neurology 61*, 1185−1190.

(25) Gandhi, S., Wood-Kaczmar, A., Yao, Z., Plun-Favreau, H., Deas, E., Klupsch, K., Downward, J., Latchman, D. S., Tabrizi, S. J., Wood, N. W., Duchen, M. R., and Abramov, A. Y. (2009) PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol. Cell 33*, 627−638.

(26) Zheng, W., Rampe, D., and Triggle, D. J. (1991) Pharmacological, radioligand binding, and electrophysiological characteristics of FPL 64176, a novel nondihydropyridine Ca^{2+} channel activator, in cardiac and vascular preparations. *Mol. Pharmacol. 40*, 734−741.

(27) Cano-Abad, M. F., Villarroya, M., Garcia, A. G., Gabilan, N. H., and Lopez, M. G. (2001) Calcium entry through L-type calcium channels causes mitochondrial disruption and chromaffin cell death. *J. Biol. Chem. 276*, 39695−39704.

(28) Iqbal, K., and Grundke-Iqbal, I. (2005) Pharmacological approaches of neurofibrillary degeneration. *Curr. Alzheimer Res. 2*, 335−341.

(29) Liu, F., Grundke-Iqbal, I., Iqbal, K., and Gong, C. X. (2005) Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur. J. Neurosci. 22*, 1942−1950.
(30) Medina, M., Avila, J., and Villanueva, N. (2013) Use of okadaic acid to identify relevant phosphoepitopes in pathology: A focus on neurodegeneration. *Mar. Drugs 11*, 1656−1668.
(31) Tian, Q., Lin, Z. Q., Wang, X. C., Chen, J., Wang, Q., Gong, C. X., and Wang, J. Z. (2004) Injection of okadaic acid into the meynert nucleus basalis of rat brain induces decreased acetylcholine level and spatial memory deficit. *Neuroscience 126*, 277−284.
(32) Uberti, D., Rizzini, C., Spano, P. F., and Memo, M. (1997) Characterization of tau proteins in human neuroblastoma SH-SY5Y cell line. *Neurosci. Lett. 235*, 149−153.
(33) de Los Rios, C., Egea, J., Marco-Contelles, J., Leon, R., Samadi, A., Iriepa, I., Moraleda, I., Galvez, E., Garcia, A. G., Lopez, M. G., Villarroya, M., and Romero, A. (2010) Synthesis, inhibitory activity of cholinesterases, and neuroprotective profile of novel 1,8-naphthyridine derivatives. *J. Med. Chem. 53*, 5129−5143.
(34) Bolognesi, M. L., Matera, R., Minarini, A., Rosini, M., and Melchiorre, C. (2009) Alzheimer's disease: New approaches to drug discovery. *Curr. Opin. Chem. Biol. 13*, 303−308.