Supporting Information

ITH10/SC058: a new neuroprotective compound with potential for the treatment of Alzheimer's disease

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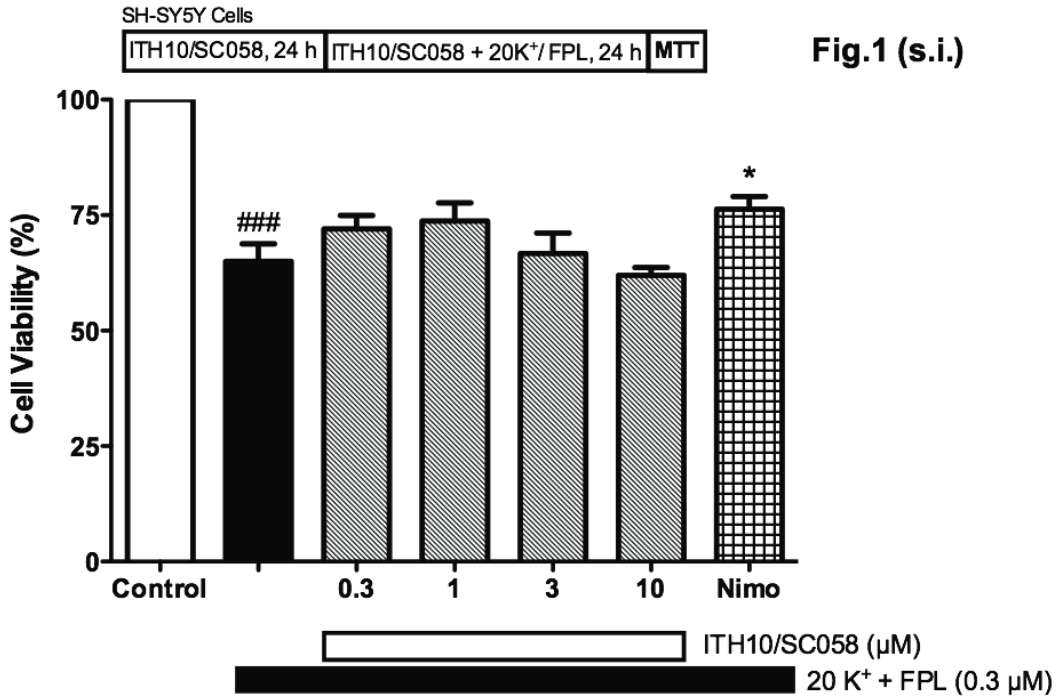

Figure 1. Protection by ITH10/SC058 against 20 K^+ + FPL64176 (FPL)-evoked cell damage in SH-SY5Y neuroblastoma cells. Cell viability was evaluated by the MTT reduction (ordinate), data were normalized (% control, white column; cells incubated with only cell culture medium). Data expressed are the mean ± SEM of triplicates of three different cell batches: $^{###}$ $p < 0.001$, is comparing control and K^+-damaged cells; * $p < 0.05$, is comparing to K^+-damaged cells without drug incubation.

Reagents. Aβ, *N*-acetylcysteine, 3-(4,5-dimethylthiazol-2-il)-2,5-diphenyltetrazole bromide (MTT), FPL64176, melatonin, nimodipine, okadaic acid, rotenone, oligomycin A were purchased from Sigma Aldrich (Madrid, Spain). Galantamine was purchased from Tocris House (Bristol, U.K.). ITH12410/SC058 [2-chloro-5,6-dihydro-5,6-diacetyldibenzo[*b*,*f*][1,4,5]thiadiazepine was synthesized according to the literature,[1] showing a purity higher than 95% (HPLC-MS).[2]

Culture of SH-SY5Y neuroblastoma cells. SH-SY5Y cells treated similarly to what was previously described.[3] Neuroblastoma cells were kept in a 1:1 F-12 nutrient mixture (Ham12) (Sigma-Aldrich, Madrid, Spain) and Eagle's minimum essential medium (EMEM) cocktail, adding 15 non-essential aminoacids, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS) inactivated by heat, 100 IU mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (Invitrogen, Madrid, Spain). They were seeded into flasks that contained supplemented medium and kept at 37 ºC in a moistured atmosphere of 5% CO_2 and 95% air. For assays, neuroblastoma cells were sub-cultured in 48-well plates, with a density of 1×10^5 cells per well. Cells were incubated with the drugs before confluence in F12-EMEM with 1% FBS. All cells were at a low passage number (<13).

Neuronal viability experiments in rat hippocampal subjected to oxygen and glucose deprivation plus reoxygenation (OGD). Experiments were carried out in hippocampal slices extracted from brains of 2-month-old Sprague–Dawley rats (275–325 g weight), upon the European Union Council Directive issued for these purposes and approved by the Ethics Committee of the Facultad de Medicina, UAM, Spain. Efforts to reduce the number of animals and their suffering were made. We followed the protocol reported by Egea et al with some modifications.[3] Rats were decapitated under sodium pentobarbital anesthesia (60 mg kg^{-1}, ip). Forebrains were removed from the skull and placed into chilled Krebs-bicarbonate dissection buffer (pH 7.4), which contained: NaCl 120 mM, KCl 2 mM, $CaCl_2$ 0.5 mM, $NaHCO_3$ 26 mM, $MgSO_4$ 10 mM, KH_2PO_4 1.18 mM, glucose 11 mM, and sucrose 200 mM. Hippocampi were dissected, and 300 μm thick slices prepared with McIlwain tissue chopper, allowing recovering for 45 min in Krebs-bicarbonate buffer at 34 ºC. Slices subjected to OGD were incubated in a glucose-free Krebs solution, equilibrated with a 95% N_2 and 5% CO_2 gas mixture.

Glucose was replaced by 2-deoxyglucose. After OGD period, slices were taken to an oxygenated normal Krebs solution containing glucose (re-oxygenation period). Experiments were performed at 37 ºC. Control and neurotoxicity samples were included in experiments. To perform them, we followed the protocols schemed on top of figures and briefly described in their footnotes. Hippocampal slices were incubated with MTT (0.5 mg mL^{-1}) in Krebs-bicarbonate solution for 30 min at 37 ºC. Viability of the slices was determined by the capability to reduce MTT.[4] Formazan production was measured as described above.

Cell incubation with compound solutions. To prepare stock solutions of the reagents, they were dissolved in dimethylsulfoxide except for galantamine, which was dissolved in water, at the concentration of 10^{-2} M. All solutions were stored in aliquots at -20 ºC. Once defrosted for a given experiment, the aliquot was discarded. The final concentrations of DMSO used (always < 0.1%) did not cause cell toxicity.

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2. 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.

3. Gonzalez-Lafuente, L., Egea, J., Leon, R., Martinez-Sanz, F. J., Monjas, L., Perez, C., Merino, C., Garcia-De Diego, A. M., Rodriguez-Franco, M. I., Garcia, A. G., Villarroya, M., Lopez, M. G., and de Los Rios, C. (2012) Benzothiazepine CGP37157 and its isosteric 2'-methyl analogue provide neuroprotection and block cell calcium entry. *ACS Chem. Neurosci. 3*, 519-529.

4. 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.