Supplementary Data

A Novel Drug Candidate for Alzheimer’s Disease Treatment: gx-50 Derived from Zanthoxylum Bungeanum

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UPLC/Q-TOF-MS ANALYSIS

UPLC/Q-TOF MS was used to detect gx-50 content in brain tissue. Compared with the saline treated control (Supplementary Figure 1), gx-50 was detected in the gx-50 treated Tg mice of having been injected for two months after one week from the last injection. It suggests that gx-50 can accumulate in the brain tissue. The pharmacokinetic experiment showed that gx-50 was metabolized quickly in vivo. However, the result of UPLC/Q-TOF MS displayed that gx-50 could accumulate in the brain and take the neuroprotective effect.

VIDEO SUPPLEMENT

Neurons from different groups of Aβ, gx-50, Aβ plus gx-50 treatment, were incubated with 2 μM Fluo-4 AM for 15 min. In the Aβ or gx-50 treated group, cells were only treated with 10 μM Aβ42 or 5 μM gx-50 separately. In the Aβ plus gx-50 treated group, cells were pretreated with gx-50 10 min before Aβ42 addition. A Leica laser confocal scanning microscope was used to evaluate the intracellular calcium content ([Ca2+]i) by monitoring Fluo-4 fluorescence. The sequential dynamical monitoring was also recorded as a video.

Neuronal [Ca2+]i assay by confocal microscopy

As shown in video 1, Aβ42 exposure (40 s) induced a transient [Ca2+]i increase that reached its peak immediately (55 s), as indicated by a bright green fluorescence. Subsequently, the signal was maintained at this peak level until the end of observation (5 min 5 s). As shown in video 2, gx-50 alone evoked a weak fluorescence, which suggested it almost had no effect on [Ca2+]i.

As shown in video 3, pretreatment with gx-50 10 min before Aβ42 exposure resulted in a transient [Ca2+]i increase (35 s) that reached its peak (1 min) and then dropped gradually and was maintained at weaker fluorescence level until the end of observation (5 min 5 s). Comparatively, gx-50 played a significant role in the inhibition of [Ca2+]i increase induced by Aβ.
Supplementary Figure 1. Tic chromatograms and mass spectra of gx-50 by UPLC–UV-Q-TOF. A) Primary reference standard with 312 m/z of gx-50. B) Residual gx-50 in the brain tissue after the MWM test.