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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 $A\beta_{42}$ or 5 μM gx-50 separately. In the Aβ plus gx-50 treated group, cells were pretreated with gx-50 10 min before $A\beta_{42}$ addition. A Leica laser confocal scanning microscope was used to evaluate the intracellular calcium content ($[Ca^{2+}]i$) by monitoring Fluo-4 fluorescence. The sequential dynamical monitoring was also recorded as a video.

Neuronal $[Ca^{2+}]i$ assay by confocal microscopy

As shown in video 1, $A\beta_{42}$ exposure (40 s) induced a transient $[Ca^{2+}]i$ 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 $[Ca^{2+}]i$.

As shown in video 3, pretreatment with gx-50 10 min before $A\beta_{42}$ exposure resulted in a transient $[Ca^{2+}]i$ 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 $[Ca^{2+}]i$ increase induced by Aβ.

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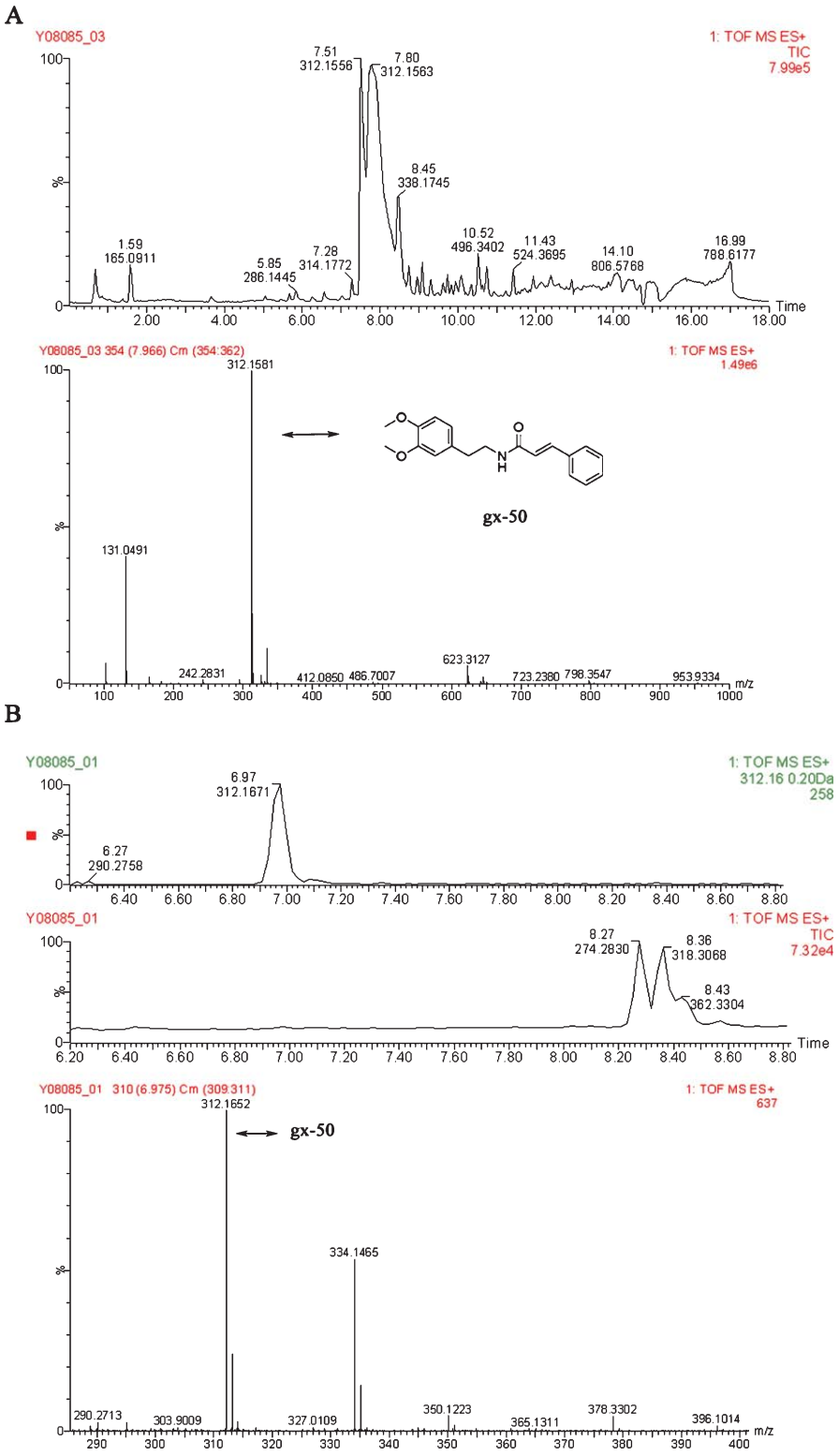

Supplementary Figure 1. Tic chromatograms and mass spectra of gx-50 by UPLC–UV-Q-TOF. A) Primary reference standard with 312 *m/z* of gx-50. B) Residual gx-50 in the brain tissue after the MWM test.