Identification of a novel small-molecule Keap1-Nrf2 PPI inhibitor with cytoprotective effects on LPS-induced cardiomyopathy

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Chemistry

General: commercially available reagents were used without further purification. Organic solvents were evaporated with reduced pressure using a Buchi R-100 rotary evaporator. Reactions were monitored by TLC using Yantai Jiangyou (China) GF254 silica gel plates. Silica gel column chromatography was performed on silica gel (300-400 mesh) from Qingdao Haiyang Ltd. (China). NMR spectra were measured on Bruker Avance 600 spectrometer. Chemical shifts were expressed in δ (ppm) and coupling constants (J) in Hz using solvent signals as internal standards (CDCl3, δH, 7.26 ppm; δC, 77.0 ppm). ESI-MS was recorded on an Agilent 6460 Triple Quadr LC/MS, and HR-ESI-MS spectrum were recorded on an Agilent Q-TOF 6520.

Purities of synthetic compounds were analyzed on an Agilent 1260 HPLC using a ZDRBAX SB-C18 column (4.6×150 mm), with UV detection at 254 nm and a linear A-B gradient (A: MeOH; B: H2O) at a flow rate of 1 mL/min.
Figure S1  $^1$H NMR spectrum of 3

Figure S2  MS spectrum of 3
Figure S3  IR spectrum of 3

Figure S4  $^1$H NMR spectrum of ZJ01
Figure S5  $^{13}$C NMR spectrum of ZJ01

Figure S6  ESI-MS spectrum of ZJ01
Figure S7  HR-ESIMS spectrum of ZJ01

Figure S8  IR spectrum of compound ZJ01
Figure S9  HPLC chromatogram (70–100% MeOH-H2O in 20 minutes) of ZJ01

Figure S10  1H NMR spectrum of ZJ02
Figure S11  $^{13}$C NMR spectrum of ZJ02

Figure S12  ESI-MS spectrum of ZJ02
Figure S13  HR-ESIMS spectrum of ZJ02
Figure S14  Equilibrium binding curve fit of ZJ01

Figure S15  Sensorgrams and equilibrium binding curve fit of S47 in SPR assay

Figure S16  (A) Putative binding mode of ZJ01 (blue) and ZJ02 (purple) to Keap1; (B) Schematic diagram showing interactions between ZJ02 and Keap1.
Figure S17  Effect of S47 on the distribution of Nrf2 in H9c2 cells. H9c2 cells were treated with different concentrations of ZJ01 for 6 h. Immunofluorescence staining analysis of Nrf2 location. Nuclei were counterstained with DAPI.
**Figure S18** Effects of S47 on the expression of pro-inflammatory cytokines and ROS in LPS-treated H9c2 cells. H9c2 cells were stimulated with 1 μg/ml LPS and treated with or without 8 μM S47 for 6 h. (A) The expression of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 were determined by RT-PCR; (B) The intracellular ROS level was examined by DCHF. **p < 0.01 vs. control, #p < 0.05 vs. LPS group. n = 3.

**Figure S19** Effect of S47 on the Nrf2 nuclear accumulation in vivo. C57BL/6 mice were treated intraperitoneally with different concentrations of S47 for 12 hours. The nuclear and non-nuclear Nrf2 protein levels of cardiomyocytes were determined by western blotting technology.