Supplementary File

Materials and Methods

Biological Assay

Cell Culture

Normal murine embryonic liver BNL CL.2 cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in CO₂ incubator with a humidified atmosphere of 95% air and 5% CO₂ at 37 °C.

Cell Cytotoxicity Assay Using MTT Assay

The effects of norcantharidin (2), compounds 9 and 18 on the cell viability were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, murine embryonic liver cells BNL CL.2 (2.5 × 10⁴ cells/well) were seeded in 96-well culture plates. After 24 h incubation to allow cell attachment, the cell were incubated with or without various concentrations of norcantharidin (2), compounds 9 and 18 for 24 h and 48 h. Ten microliter of MTT (5 mg/mL) was then added to each well, and the plates were incubated for an additional 2.5 h at 37 °C. The formazan crystals formed by MTT metabolism were solubilized by 150 μL DMSO to each well. The absorbance at 540 nm was measured with a microplate ELISA reader (Molecular Devices spectramax 340 PC). Values represent the mean ± SD form at least two independent experiments.

Figure S1. The effects of norcantharidin (2), compounds 9 and 18 on cell proliferative activity of normal cell line BNL CL.2 (murine embryonic liver cells) for (A) 24 h and (B) 48 h (n = 3).
Figure S1. Cont.

Figure S2. (A) $^1$H-NMR spectra of norcantharidin (2); (B) $^{13}$C-NMR spectra of norcantharidin (2).
Figure S2. Cont.

Figure S3. (A) $^1$H-NMR spectra of compound 5; (B) $^{13}$C-NMR spectra of compound 5.
Figure S3. Cont.

Figure S4. (A) $^1$H-NMR spectra of compound 6; (B) $^{13}$C-NMR spectra of compound 6.
Figure S4. Cont.

Figure S5. (A) $^1$H-NMR spectra of compound 7; (B) $^{13}$C-NMR spectra of compound 7.
Figure S5. Cont.

Figure S6. (A) $^1$H-NMR spectra of compound 8; (B) $^{13}$C-NMR spectra of compound 8.
Figure S6. Cont.

Figure S7. (A) $^1$H-NMR spectra of compound 9; (B) $^{13}$C-NMR spectra of compound 9.
Figure S7. Cont.

Figure S8. (A) $^1$H-NMR spectra of compound 10; (B) $^{13}$C-NMR spectra of compound 10.
Figure S8. Cont.

Figure S9. (A) $^1$H-NMR spectra of compound 11; (B) $^{13}$C-NMR spectra of compound 11.
Figure S9. Cont.

Figure S10. (A) $^1$H-NMR spectra of compound 12; (B) $^{13}$C-NMR spectra of compound 12.
Figure S10. Cont.

Figure S11. (A) $^1$H-NMR spectra of compound 14; (B) $^{13}$C-NMR spectra of compound 14.
Figure S11. Cont.

Figure S12. (A) $^1$H-NMR spectra of compound 15; (B) $^{13}$C-NMR spectra of compound 15.
Figure S12. Cont.

Figure S13. (A) $^1$H-NMR spectra of compound 16; (B) $^{13}$C-NMR spectra of compound 16.
Figure S13. Cont.

(B)

Figure S14. (A) $^1$H-NMR spectra of compound 17; (B) $^{13}$C-NMR spectra of compound 17.

(A)
Figure S14. Cont.

(B)

Figure S15. (A) $^1$H-NMR spectra of compound 18; (B) $^{13}$C-NMR spectra of compound 18.
Figure S15. Cont.

(B)

Figure S16. (A) $^1$H-NMR spectra of compound 19; (B) $^{13}$C-NMR spectra of compound 19.
Figure S16. Cont.

Figure S17. (A) $^1$H-NMR spectra of compound 20; (B) $^{13}$C-NMR spectra of compound 20.
Figure S17. Cont.

Figure S18. (A) $^1$H-NMR spectra of compound 21; (B) $^{13}$C-NMR spectra of compound 21.
Figure S18. Cont.

Figure S19. (A) $^1$H-NMR spectra of compound 22; (B) $^{13}$C-NMR spectra of compound 22.
Figure S19. Cont.

(B)

Figure S20. (A) $^1$H-NMR spectra of compound 23; (B) $^{13}$C-NMR spectra of compound 23.
Figure S20. Cont.

Figure S21. (A) $^1$H-NMR spectra of compound 24; (B) $^{13}$C-NMR spectra of compound 24.
Figure S21. Cont.

Figure S22. (A) $^1$H-NMR spectra of compound 25; (B) $^{13}$C-NMR spectra of compound 25.
Figure S22. Cont.

Figure S23. (A) $^1$H-NMR spectra of compound 26; (B) $^{13}$C-NMR spectra of compound 26.
Figure S23. Cont.

Figure S24. (A) $^1$H-NMR spectra of compound 27; (B) $^{13}$C-NMR spectra of compound 27.
Figure S24. Cont.