Methods

Morphological evaluation. A549 cells ($5x10^5$) were seeded in 6-well plates overnight and then treated with 1, 2.5 and 5 μg/ml bruceine D for 48 h. Images of the cells in culture at each time point were captured using an inverted phase contrast microscope. For Hoechst 33342 staining, the cells were washed twice with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 30 min at room temperature and washed again with PBS, then stained with 10 μg/ml Hoechst 33342 (Invitrogen; Thermo Fisher Scientific, Inc.) for 15 min in the dark. Stained cells were examined with a fluorescence microscope (Carl Zeiss GmbH).

Results

Morphological observation was conducted to explore whether the cytotoxic effect was associated with the apoptotic process. As shown in Fig. S1A, following treatment with bruceine D, the viability of A549 cells decreased rapidly, and the majority of the cells were shrunken and detached from the substratum of the culture dish. In addition, Hoechst 33342-stained nuclei of A549 cells treated with bruceine D displayed stereotypical apoptotic morphology characterized by chromatin condensation, nuclear fragmentation and apoptotic bodies. By contrast, in control cultures, the nuclei of A549 cells were only lightly stained, and no apoptotic nuclei were identified (Fig. S1B). These results suggest that treatment with bruceine D induces apoptosis in A549 cells.

Figure S1. ^{13}C-NMR spectrum of bruceine D.

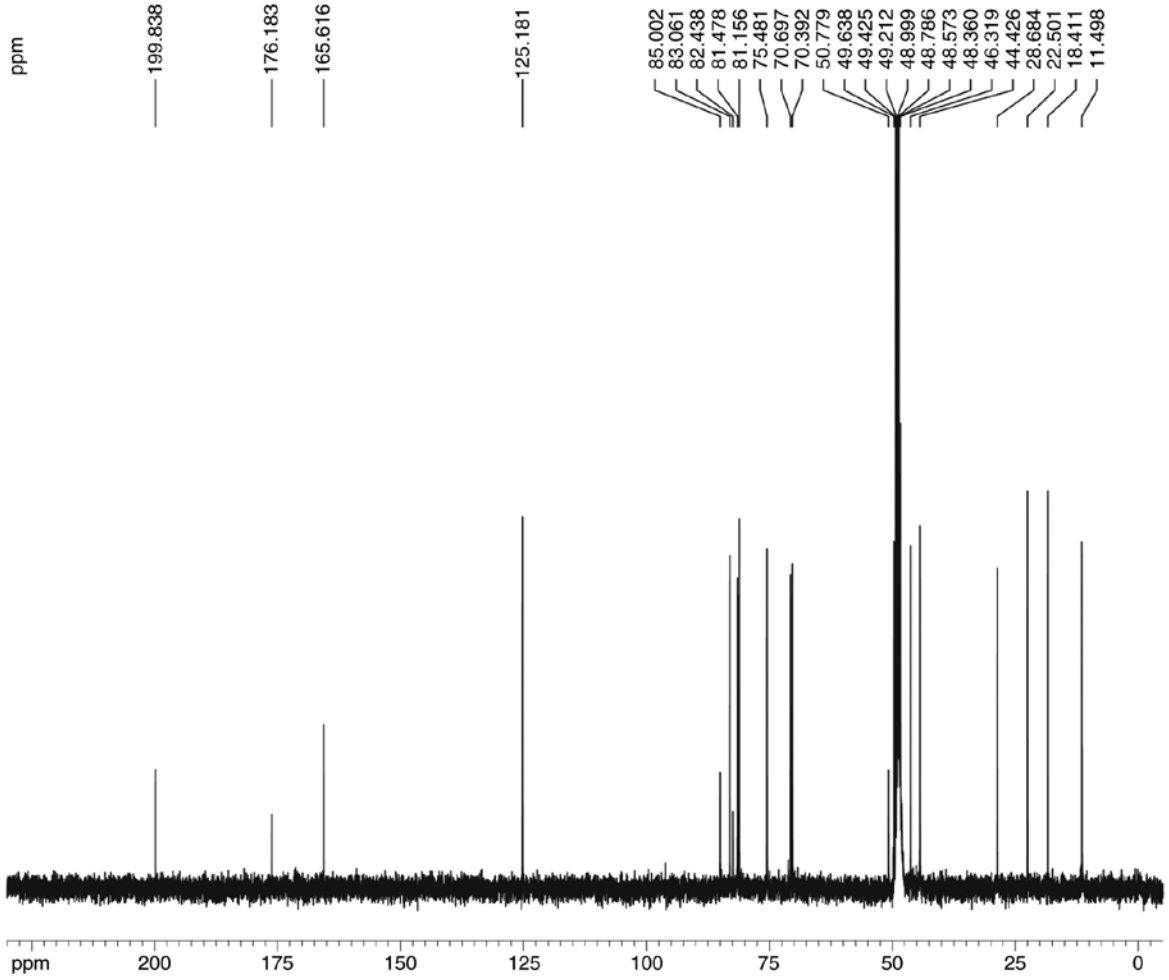

Figure S2. [1]H-NMR spectrum of bruceine D.

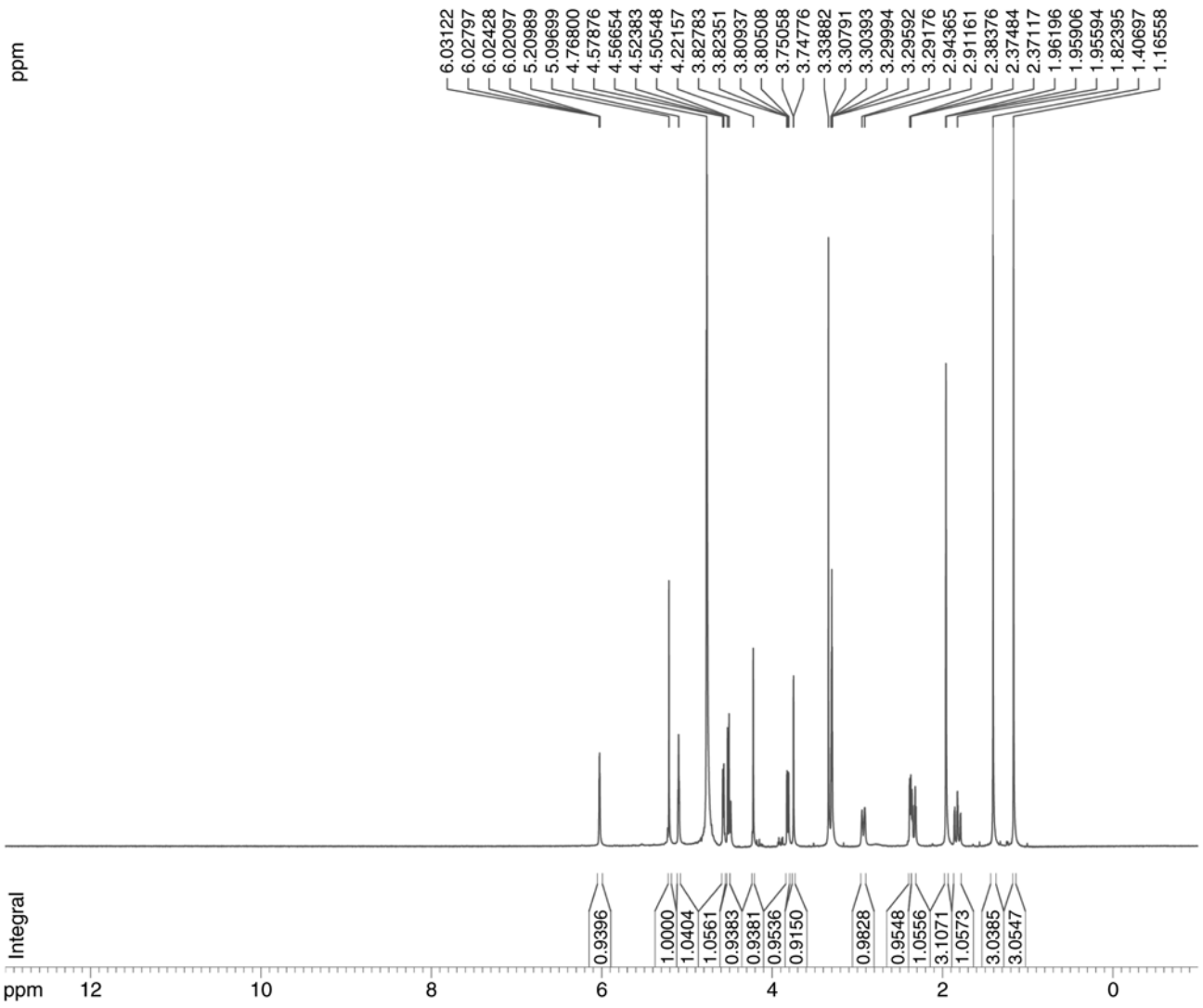

Figure S3. Representative HPLC-DAD chromatogram of (A) bruceine D sample isolated and (B) bruceine D standard.

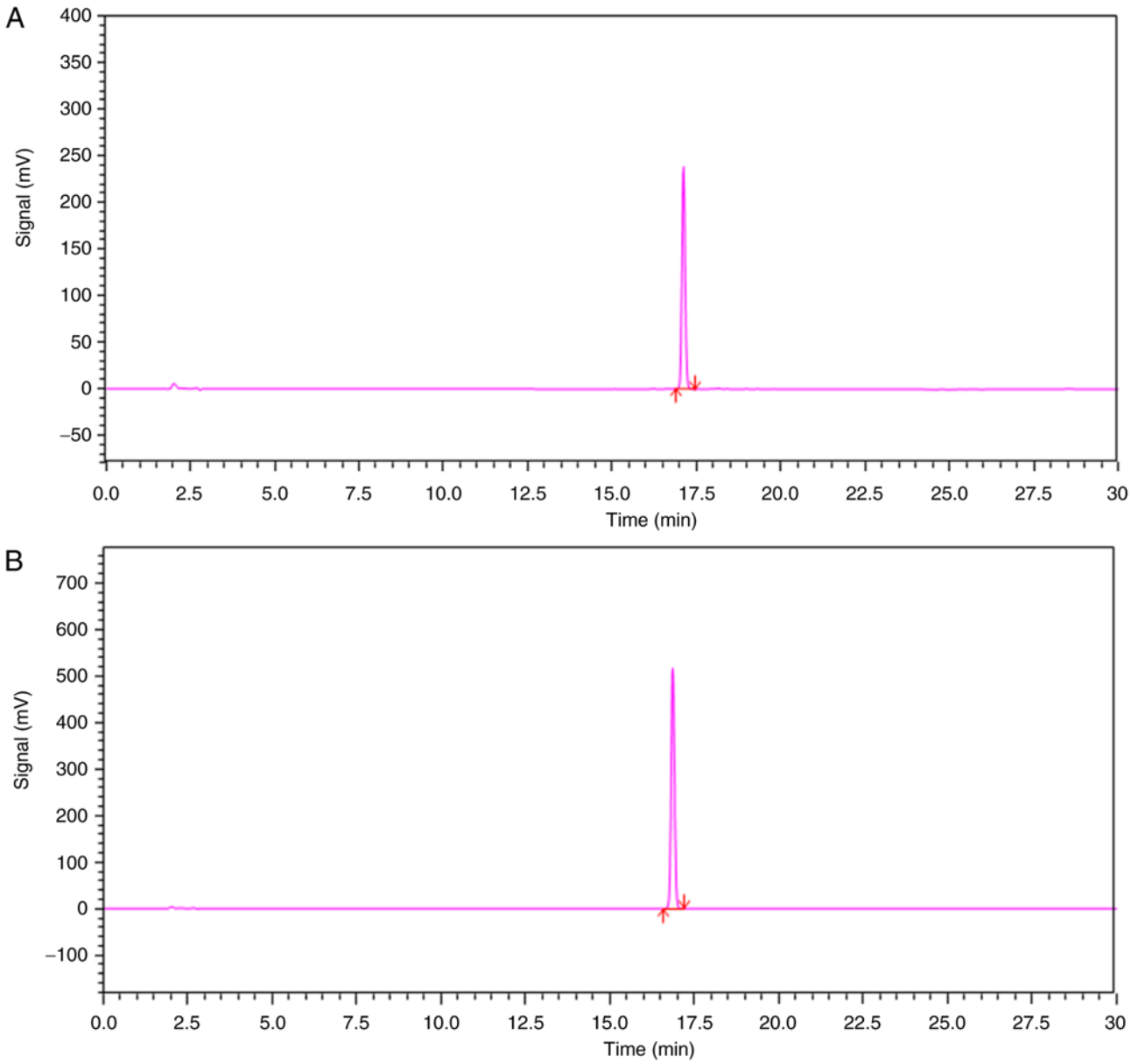

Figure S4. Effect of bruceine D on the morphology of A549 cells. (A) Morphological changes of A549 cells following treatment with bruceine D (magnification, x200). (B) Cell apoptosis observed with Hoechst 33342 staining (magnification, x200). Nuclear condensations (white arrows) were observed in the cells, indicating rapoptosis.