

Supporting Information

Lissoclibadin 1, a Polysulfur Aromatic Alkaloid from the Indonesian Ascidian *Lissoclinum* cf. *badium*, Induces Caspase-dependent Apoptosis in Human Colon Cancer Cells and Suppresses Tumor Growth in Nude Mice

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Contents

Figure S1. Effects of lissoclibadin 1 (**1**) against four human cancer cell lines: HCT-15, HeLa-S3, MCF-7, and NCI-H28.

Each cell line (5×10^4 cells/mL) was precultured for 24 h, and compound **1** in DMSO or DMSO alone (final conc. 0.1%) was added at the indicated concentrations for 24 h. Cells were observed using a light microscope.

Figure S2. Effects of pan-caspase, caspase-8, and caspase-9 inhibitors on morphological changes of HCT-15 cells induced by lissoclibadin 1 (**1**)

HCT-15 cells (5×10^4 cells/mL) were precultured for 24 h in 1 mL. Each caspase inhibitor was added at 50 μ M and incubated for 30 min. Lissoclibadin 1 in DMSO (5.0 μ M) or DMSO alone (final conc. 0.1%) was then added and the cells were incubated for the indicated time. Cell morphology was observed using a light microscope.

Figure S3. HCT-15 tumor-bearing nude mice administered solvent (vehicle control) (left), 25 mg/kg of lissoclibadin 1 (**1**) (middle), and 25 mg/kg of irinotecan (right). Pictures were taken on Day 20.

Figure S4. ^1H NMR spectrum of lissoclibadin 1 (**1**).

Figure S5. ^1H NMR spectrum of lissoclibadin 3 (**2**).

Figure S6. ^1H NMR spectrum of lissoclibadin 4 (**3**).

Figure S7. ^1H NMR spectrum of lissoclibadin 7 (**4**).

Figure S8. ^1H NMR spectrum of lissoclibadin 8 (**5**).

Figure S9. ^1H NMR spectrum of lissoclibadin 14 (**6**).

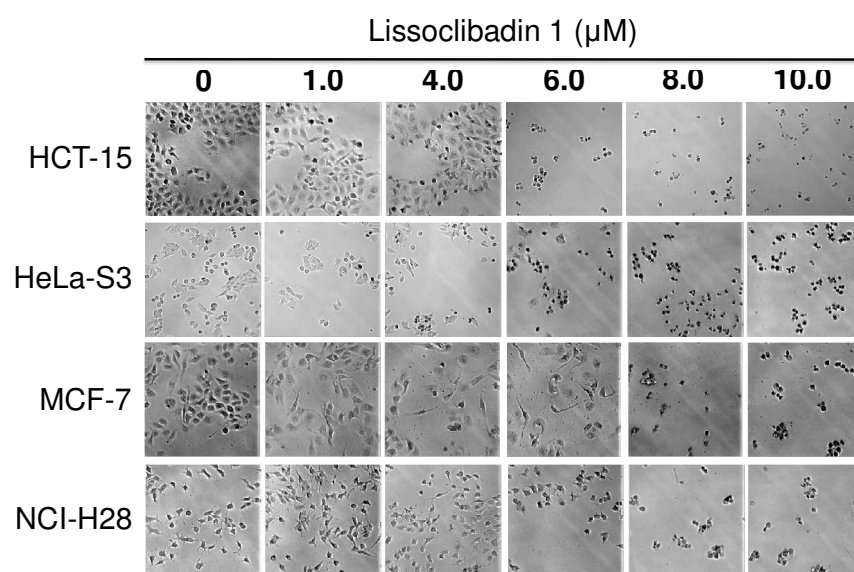


Figure S1

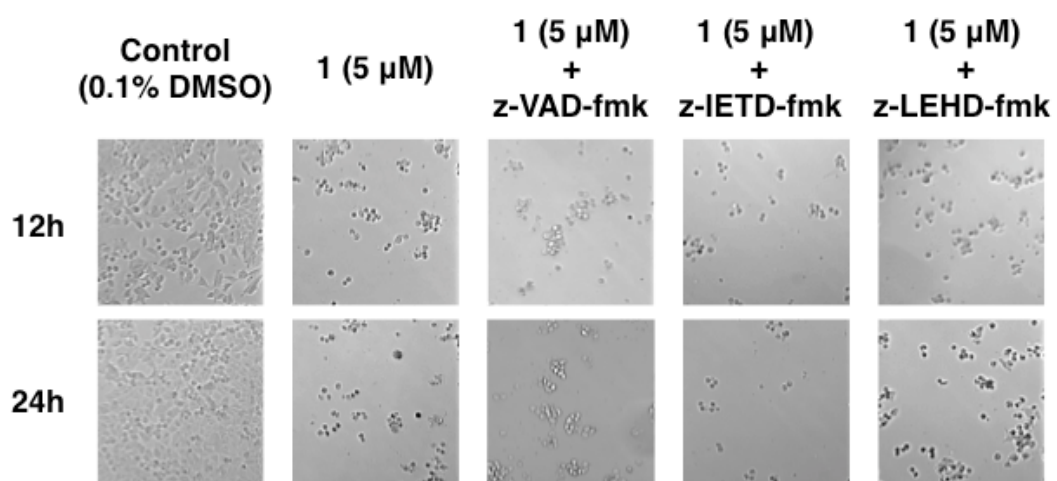


Figure S2



Figure S3

Table S1. Average Tumor Volume For Each Treatment Group At Day 0, 24, and 28

Day	Tumor volume (mm ³)			
	Vehicle	Irinotecan (25mg/kg)	Lissoclibadin 1 (5mg/kg)	Lissoclibadin 1 (25mg/kg)
0	58.51 ± 12.18	56.06 ± 8.937	56.63 ± 5.387	56.54 ± 14.07
14	713.4 ± 164.8	57.82 ± 15.64	719.2 ± 218.1	218.5 ± 89.45
28	1512 ± 299.3	141.1 ± 87.53	1780 ± 516.8	638.7 ± 566.5

The tumor-bearing mice were injected intratumorally with one dose of irinotecan and two doses of lissoclibadin 1. Tumor volume was calculated as following formula: length (mm) × width (mm) × depth (mm) × $\pi/6$. Each value indicates the mean ± SD (n = 6).

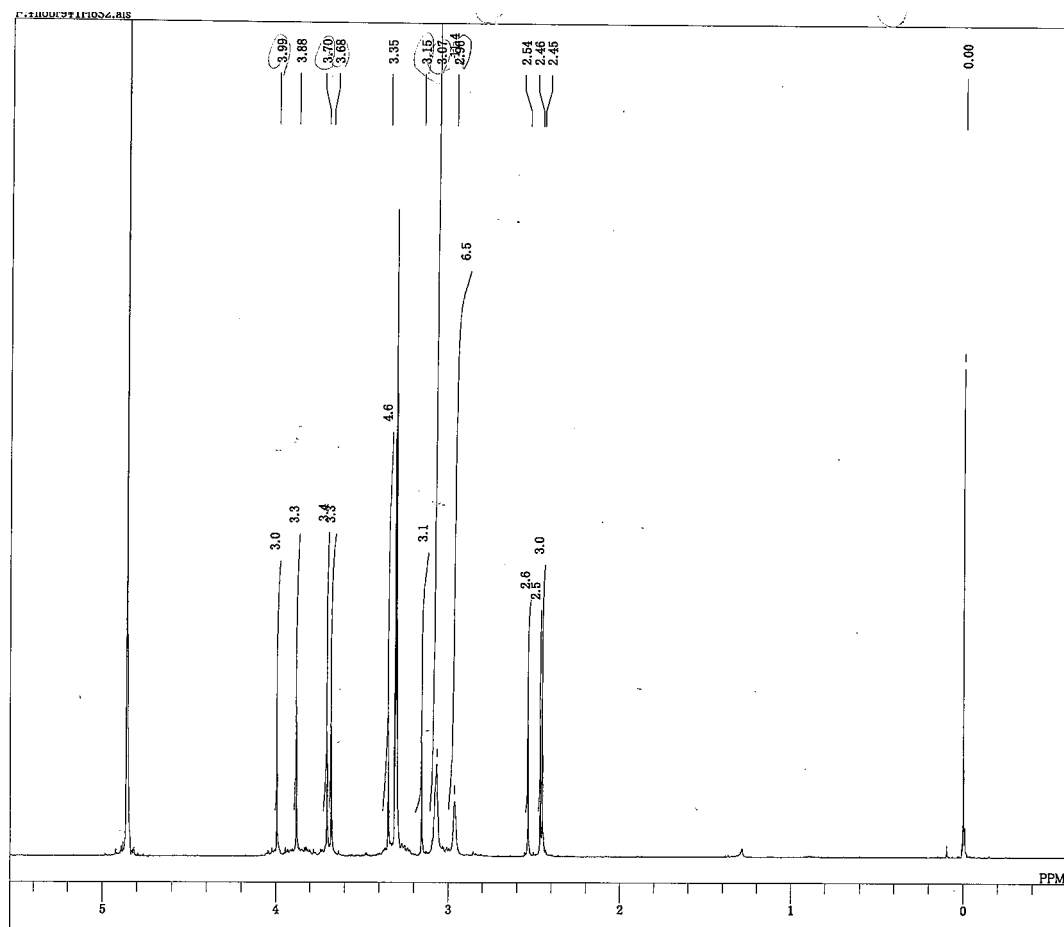


Figure S4

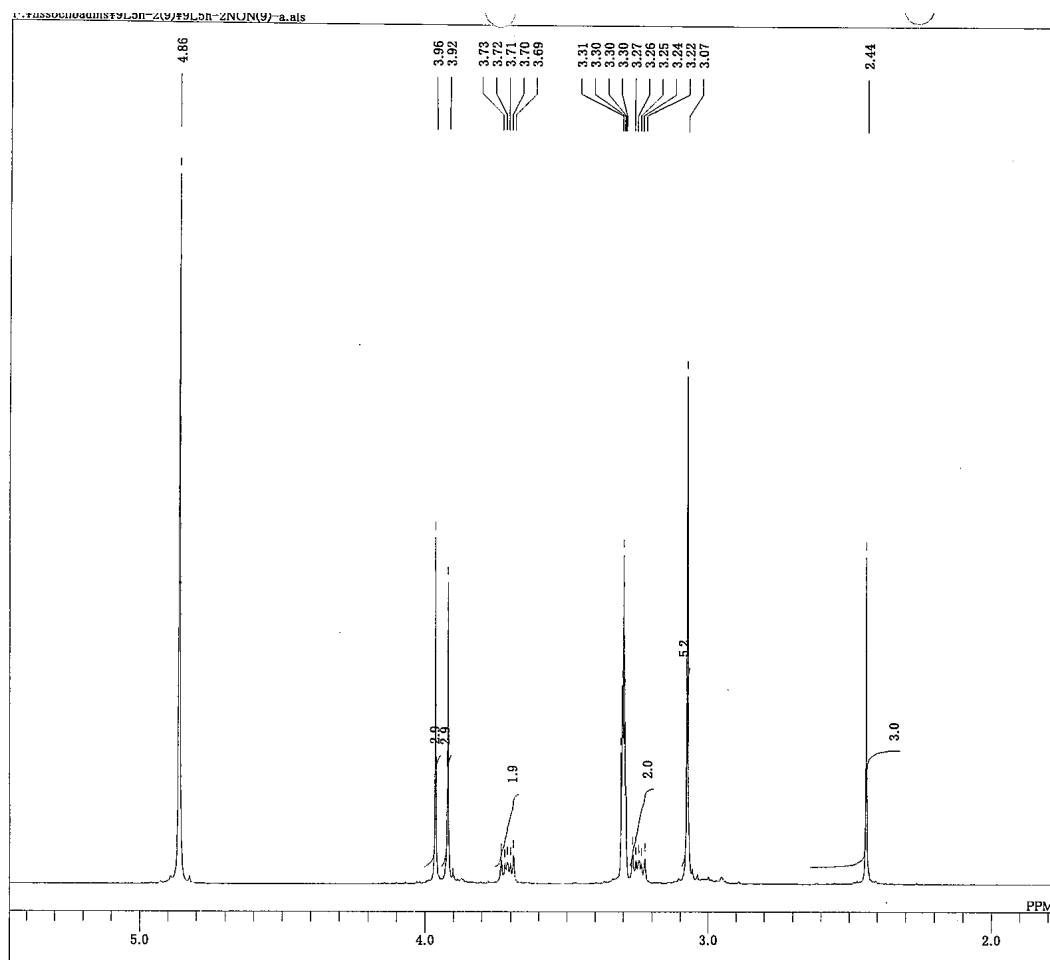


Figure S5

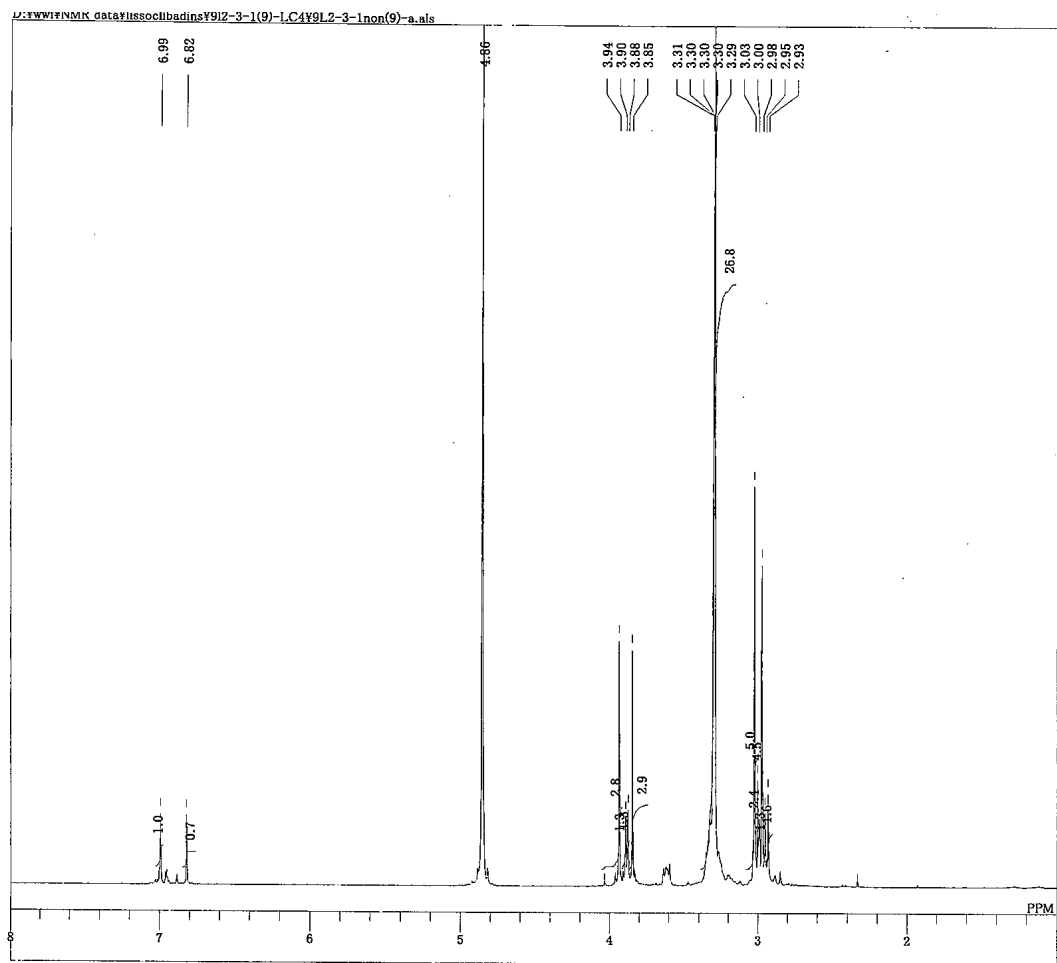


Figure S6

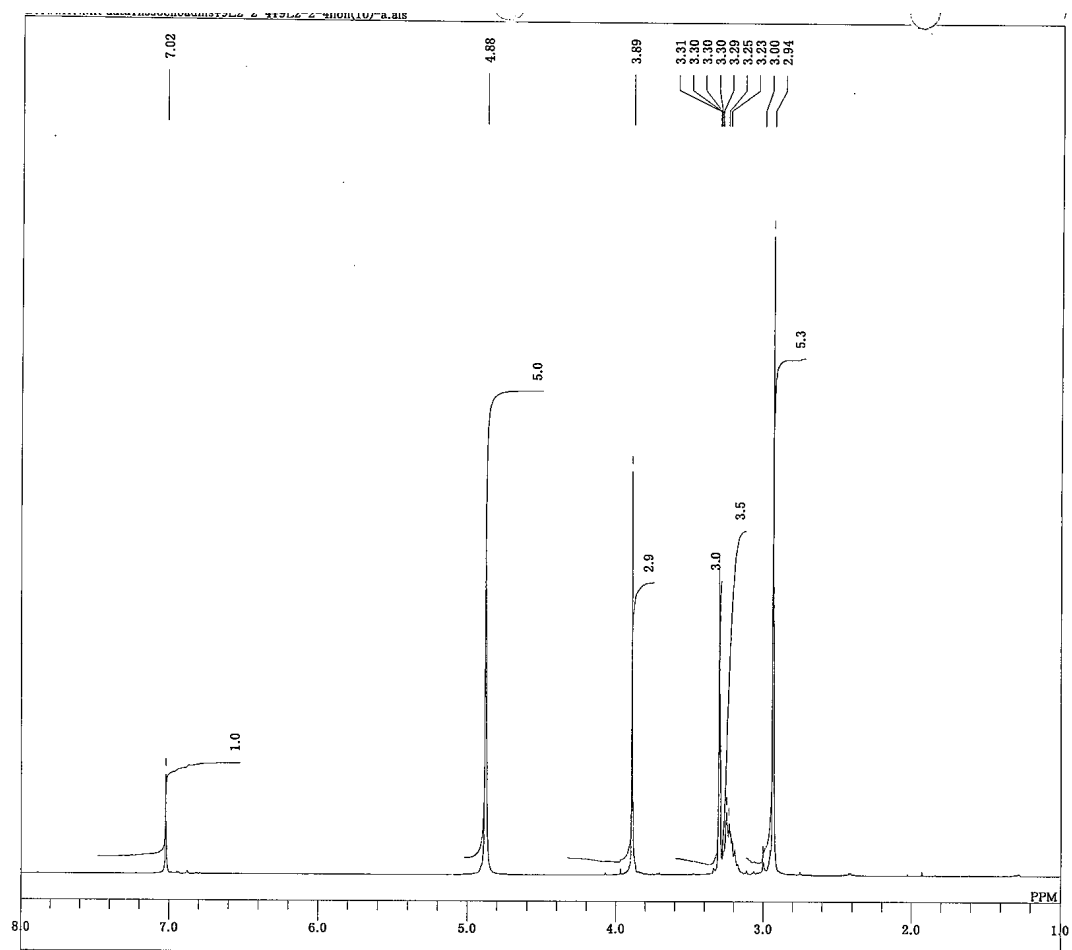


Figure S7

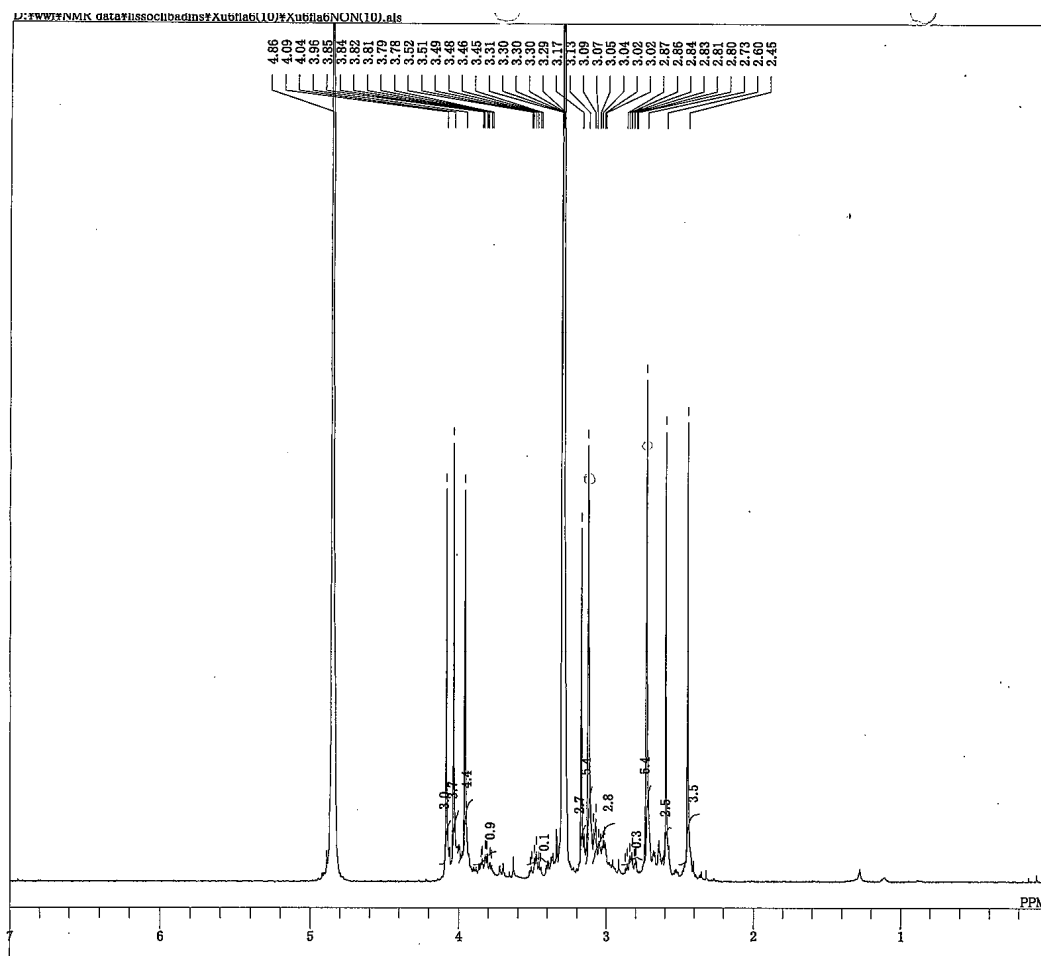


Figure S8

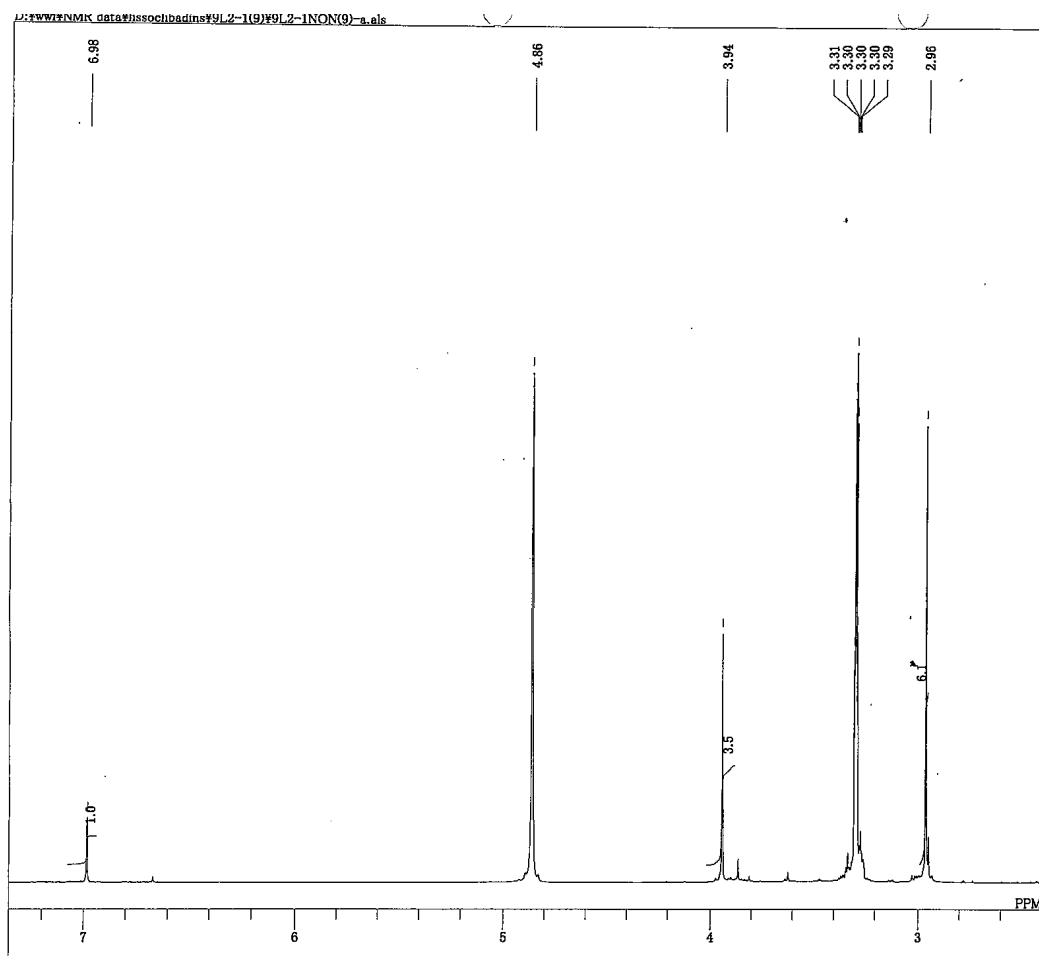


Figure S9