Marine sponge *Hymeniacidon* sp. amphilectane metabolites potently inhibit rat brain microglia thromboxane B2 generation

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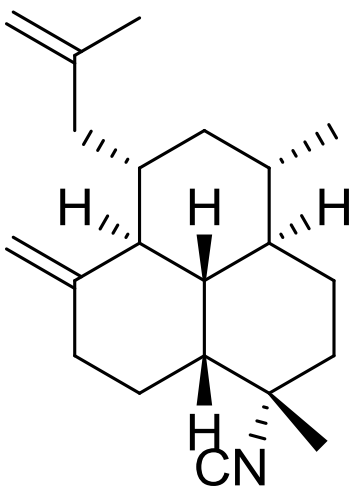
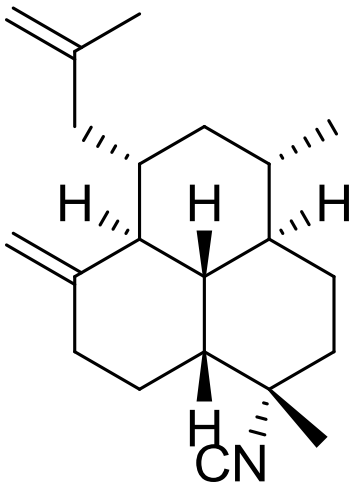
Supporting Material

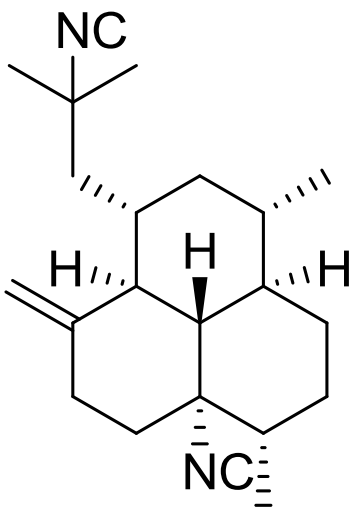
^{1}H-NMR (500 MHz) of compound 1 in $CDCl_3$

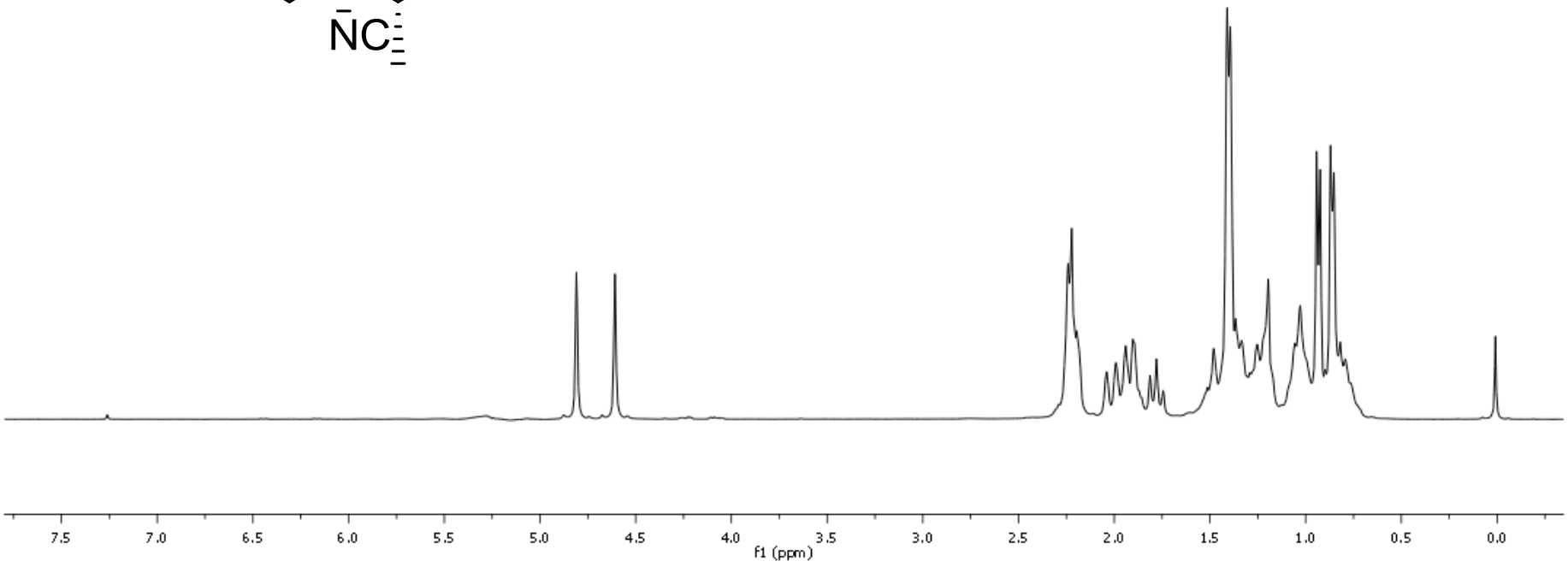


^{13}C-NMR (125 MHz) spectrum of compound 1 in $CDCl_3$

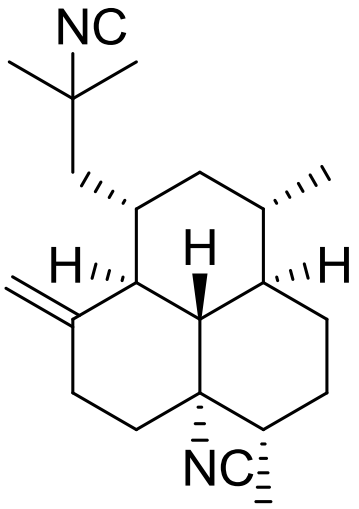


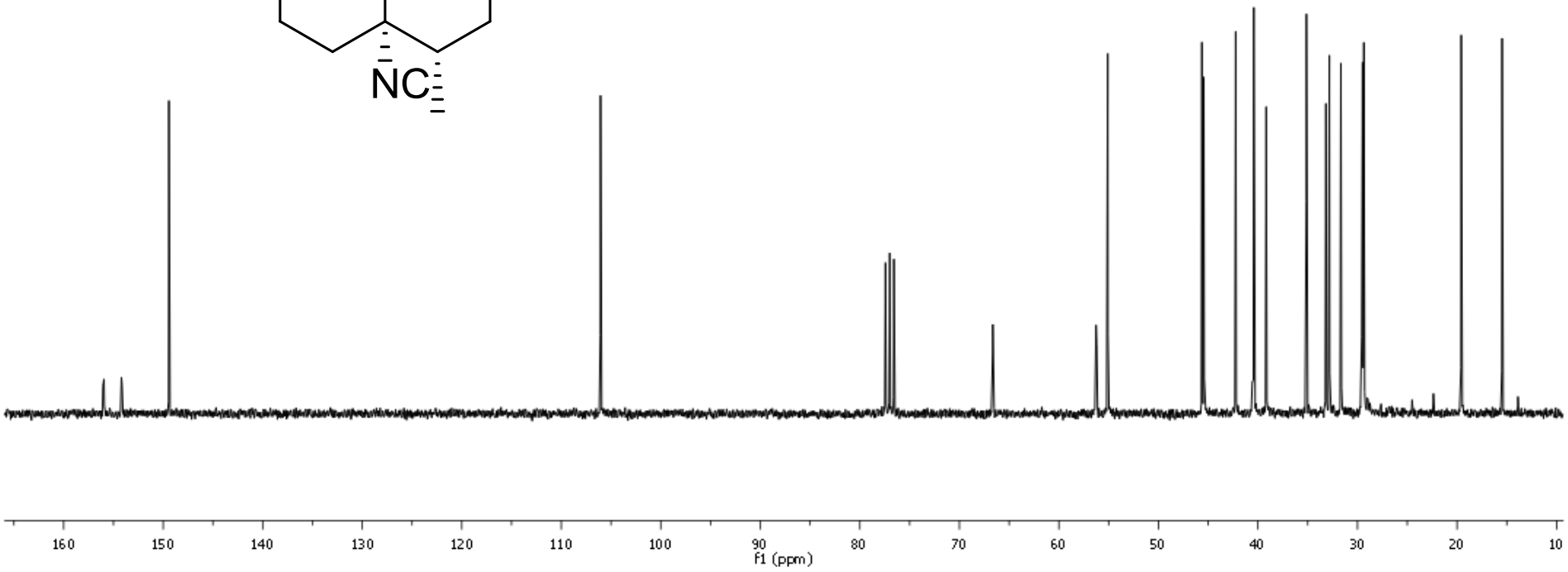
^{1}H-NMR (500 MHz) of compound 2 in $CDCl_3$



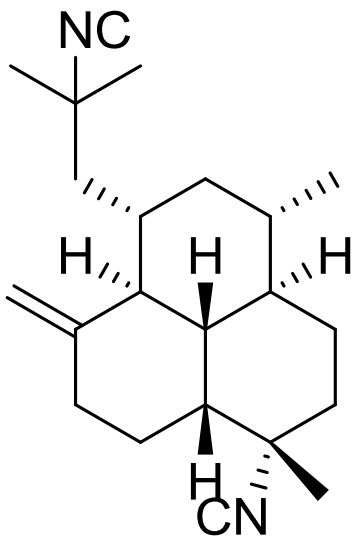


^{13}C-NMR (125 MHz) spectrum of compound 2 in $CDCl_3$

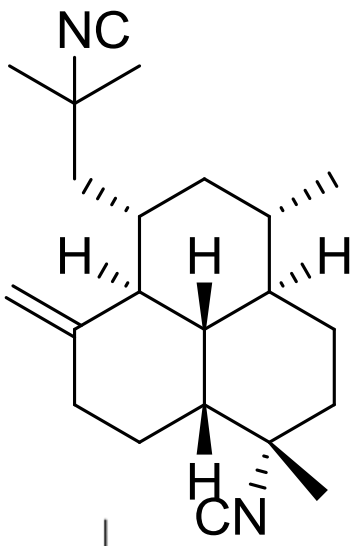


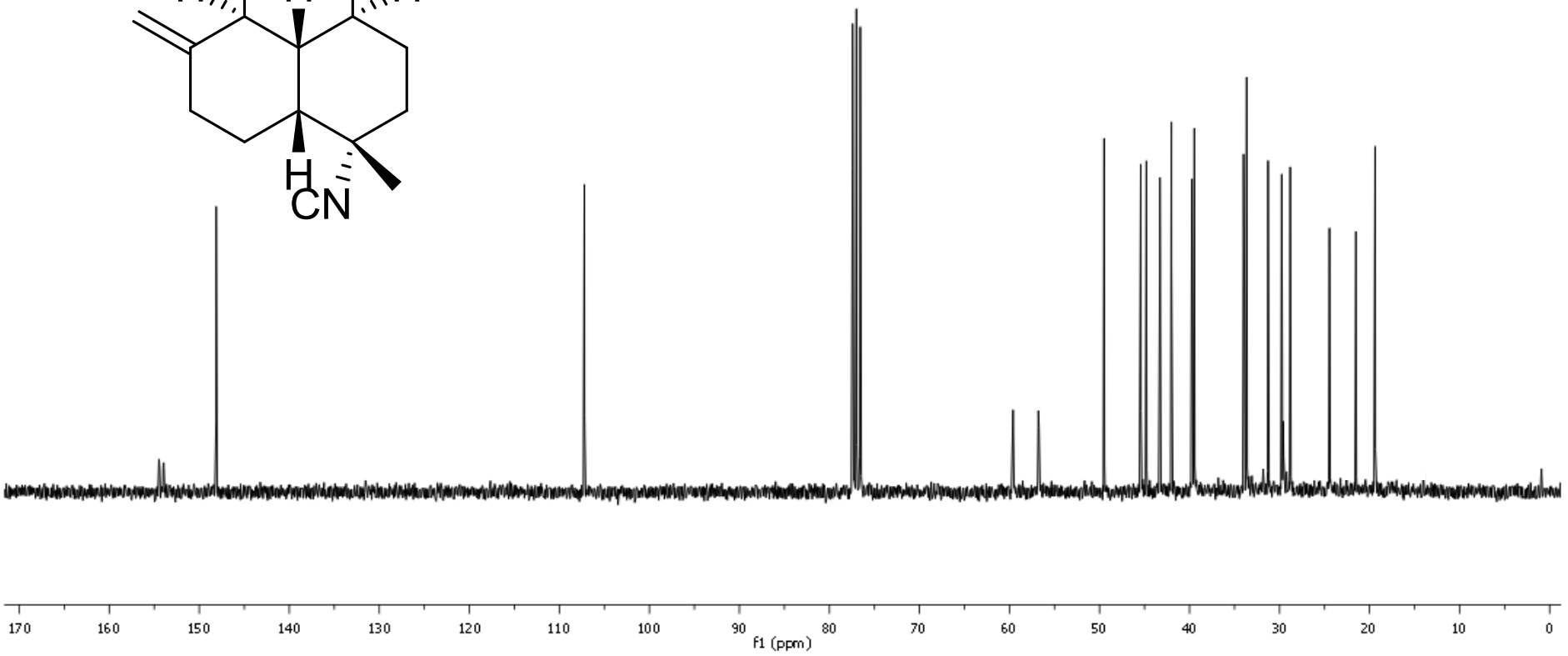


^{1}H-NMR (500 MHz) of compound 3 in $CDCl_3$

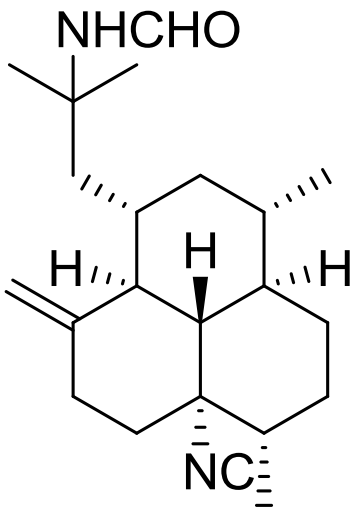


^{13}C-NMR (125 MHz) spectrum of compound 3 in $CDCl_3$

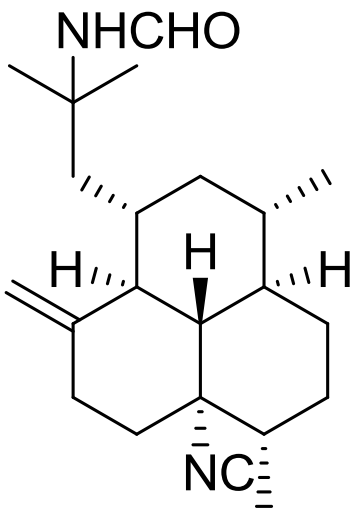




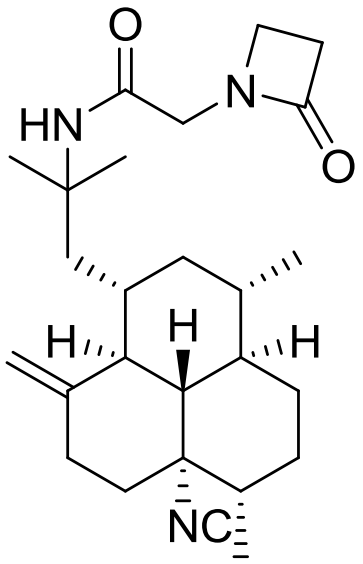
^{1}H-NMR (500 MHz) of compound 4 in $CDCl_3$



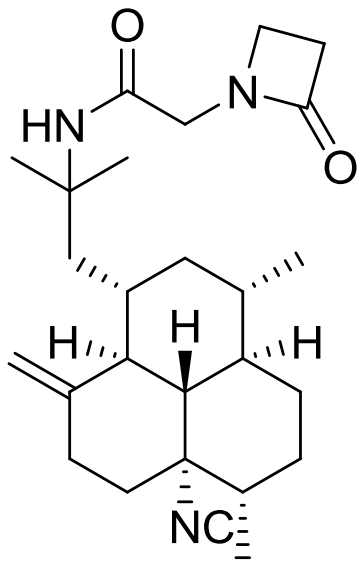
^{13}C-NMR (125 MHz) spectrum of compound 4 in $CDCl_3$



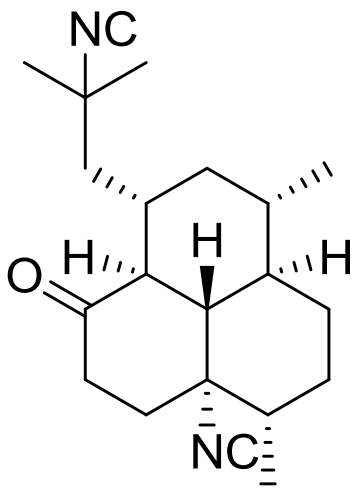
^{1}H-NMR (500 MHz) of compound 5 in $CDCl_3$



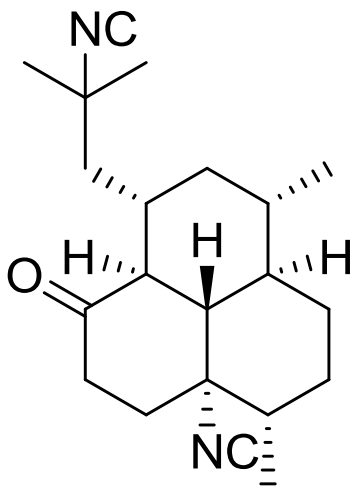
^{13}C-NMR (125 MHz) spectrum of compound 5 in $CDCl_3$



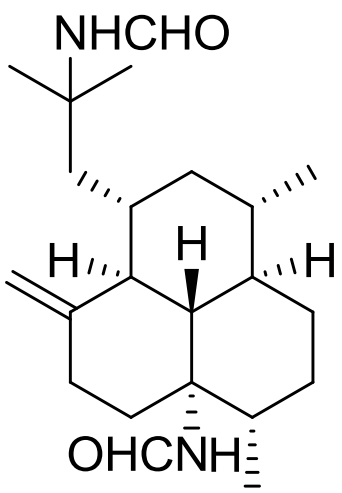
^{1}H-NMR (500 MHz) of compound 6 in $CDCl_3$



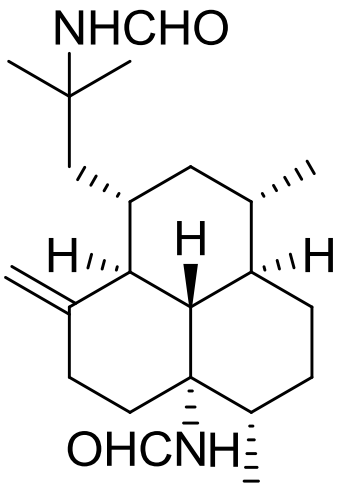
^{13}C-NMR (125 MHz) spectrum of compound 6 in $CDCl_3$



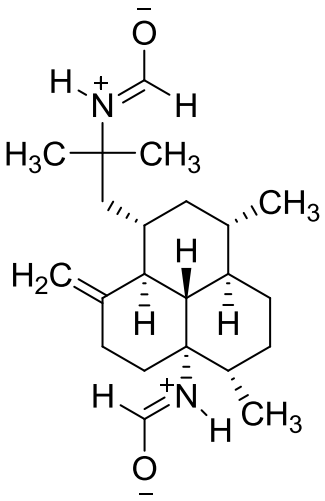
^{1}H-NMR (500 MHz) of compound 7 in $CDCl_3$



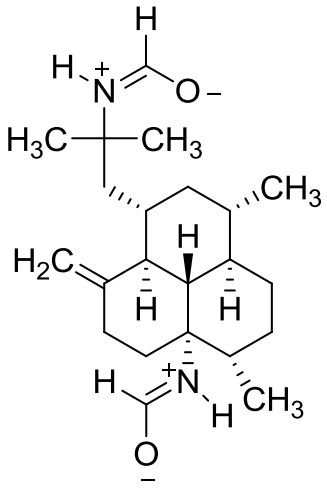
^{13}C-NMR (125 MHz) spectrum of compound 7 in $CDCl_3$



Expansion of the ^{13}C-NMR (125 MHz) spectrum of compound 7 in $CDCl_3$



7a



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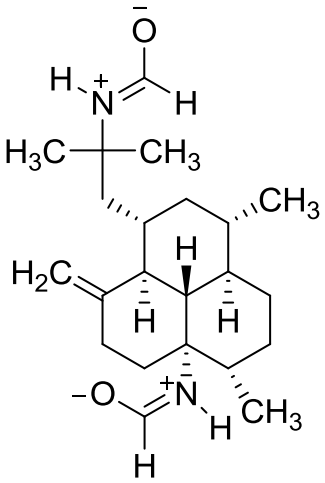

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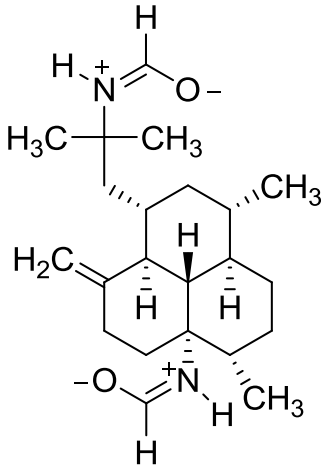
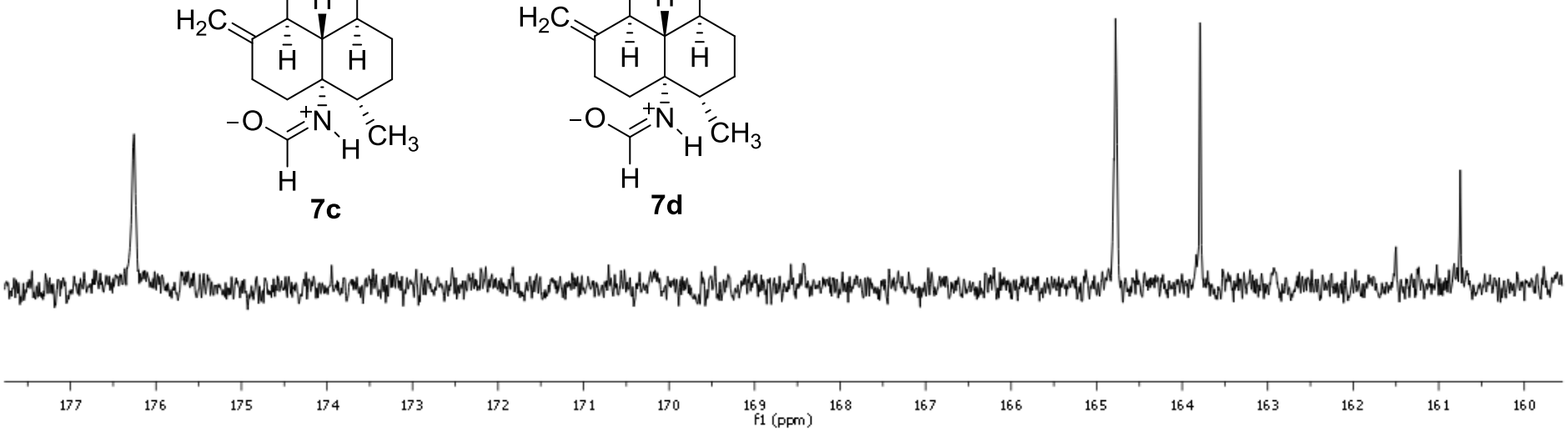


7d



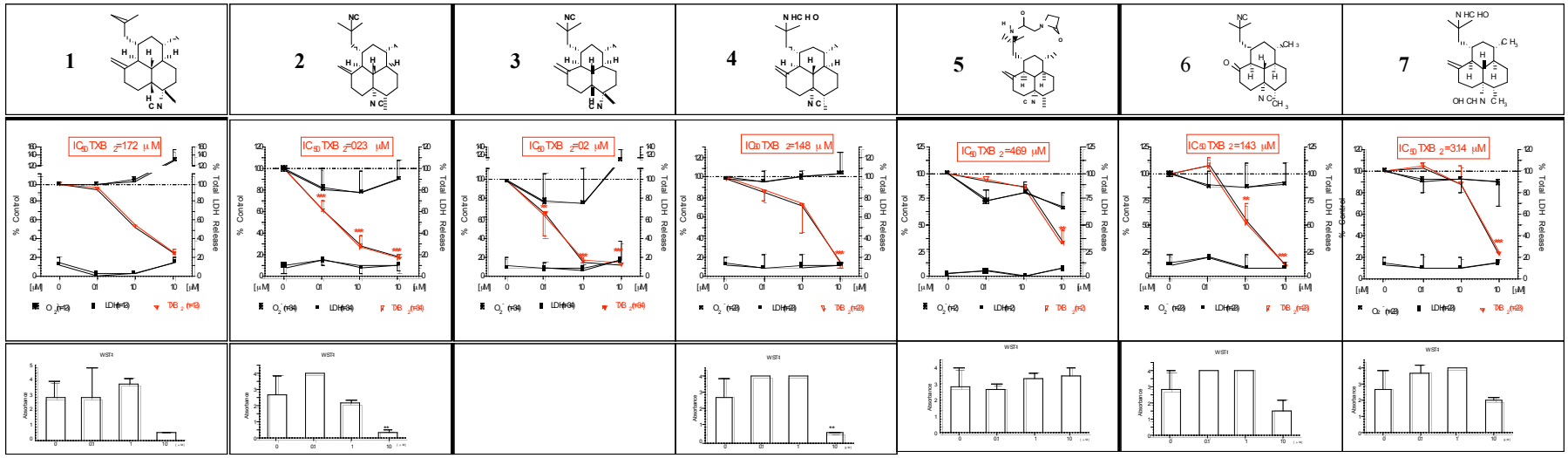


Figure 2. Differential effects of *Hymeniacidon* sp. amphilectane metabolites on PMA (1 μM) -stimulated O_2^- and TXB_2 generation by LPS-activated rat neonatal microglia. (A) Rat neonatal microglia (200,000 cells/well) were activated with LPS (0.3 ng/mL) for 17 h. Compounds **1-7** were added 15 min before stimulation with PMA (1 μM). After 70 min, PMA-triggered O_2^-, TXB_2 and LDH release (*short-term* viability) were measured as described in Methods. Data are expressed as percentage of control O_2^- and TXB_2 release triggered by PMA. Data show mean ± SEM of 2-4 independent experiments. ** P <0.01, *** P <0.001. (B) *Long-term* cell viability (2.5-19 h) was determined by the WST-1 assay as described in Methods. Briefly, rat neonatal microglia (10,000 cells/well) plated in LPS-free 96-well cell culture clusters, were treated with compounds **1-7** (0.1 μM –10μM) or vehicle (DMSO). After 18 h, 20 μL of the tetrazolium salt WST-1was added, plates were incubated at 36° C for 2 h, and reduction of the WST-1 reagent to formazan was measured at 450 nM. Data for compounds **1-7** are from one representative experiment in triplicate. * P <0.05, ** P <0.01.