Supplementary Information

Conyza canadensis: Green Extraction Method of Bioactive Compounds and Evaluation of Their Antifungal Activity

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Instrumentation

Flash chromatography

Flash chromatography was performed using a Biotage® Isolera Four system (Uppsala, Sweden) equipped with a SNAP® cartridge (KP-Sil 100 g, 39 × 157 mm, 45-50 µm) and a UV detection system (254 and 280 nm) on a Biotage® SNAP cartridge (KP-Sil 100 g, 39 × 157 mm, 45-50 µm) under a mobile phase flow rate of 25 mL min^{-1} using a *n*-hexane:ethyl acetate gradient: 98:2 v/v over 264 mL, 98:2 → 80:20 v/v over 1320 mL and 80:20 v/v over 264 mL. The maximum fraction volume was 17 mL and the fractions were collected in 16 × 150 mm test tubes.

NMR spectrometer

^{1}H and ^{13}C NMR spectra, as well as ^{1}H-^{13}C HSQC spectra, were recorded on either a Bruker AVANCE 400 MHz spectrometer or a Bruker AVANCE 500 MHz spectrometer (Rheinstetten, Germany). ^{1}H NMR spectra were acquired using 33 K data points, relaxation delay of 1.0 s and 16 scans. For compound **1**: spectral width 4185 Hz and acquisition time 3.91 s. For compound **2**: spectral width 10,302 Hz and acquisition time 1.59 s. ^{13}C NMR spectra were acquired using 131 K data points, relaxation delay of 2 s and 3,072 scans. For compound **1**: spectral width 24,671 Hz and acquisition time 0.66 s. For compound **2**: spectral width 29,762 Hz and acquisition time 0.55 s. Gradient HSQC experiment was recorded using the pulse sequence of Bruker software. ^{1}H (F2) dimension were acquired using 2048 K data points. For compound **1**: spectral width of 4185 Hz, acquisition time 0.24 s, relaxation delay 1.5 s and 16 scans. For compound **2**: spectral width of 5721 Hz, acquisition time 0.18 s, relaxation delay and 2 s and 8 scans. ^{13}C (F1) dimension were acquired using 256 K data points. For compound **1**: spectral width 19,750 Hz, acquisition time 0.0065 s, relaxation delay 1.5 s. For compound **2**: spectral width 24,038 Hz, acquisition time 0.0053 s, relaxation delay 2.0 s.

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Gas chromatographers

Fractions and purified compounds were analyzed by GC-MS on an Agilent 7890A gas chromatograph (Santa Clara, CA) coupled to a 5975C inert XL MSD detector (single quadrupole) with electron ionization (70 eV) and equipped with an Agilent HP-5ms capillary column (30 m × 0.250 mm × 0.25 µm) operating at the following conditions: injector temperature, 240 °C; column temperature, 120-240 °C at 8 °C min^{-1}; carrier gas, helium (1 mL min^{-1}); injection volume, 1 µL (split 1:100). The MS mass ranged from 40 to 400 *m/z*, with a filament delay of 5 min, 150, 230 and 200 ºC for a quadrupole temperature of 150 °C, a source temperature of 230 °C, and a transfer line temperature of 200 °C.

Compounds identity was further confirmed by analysis on a Shimadzu GC-2010 Plus gas chromatograph (Kyoto, Japan) coupled to a TQ8040a detector (triple quadrupole with electron ionization (70 eV) and equipped with a Restek Rtx-5MS capillary column (30 m × 0.250 mm × 0.25 µm) operating at the following conditions: injector temperature, 240 °C; column temperature, 115 °C held for 19 minutes; carrier gas, nitrogen (2 mL min^{-1}); injection volume, 2 µL (splitless). The MS mass ranged from 40 to 400 *m/z*, with a filament delay of 3 min, a source temperature of 230 °C, and a transfer line temperature of 200 °C. The system was operated at SIM/Scan mode with collision energy of 12 V. For each compound, the precursor ion was its own molecular ion: *m/z* 162 (compound **1**) and *m/z* 160 (compound **2**).

Quantitation of bioactive compounds in the extracts was performed by GC-FID on an Agilent 7890A gas chromatograph (Santa Clara, CA) equipped with an Agilent HP-5 (30 m × 0.320 mm × 0.25 µm) capillary column operating at the following conditions: 200, 300 and 115 ºC for injector, detector and column temperature, respectively, held for 19 minutes and then 115-200 °C at 20 °C min^{-1}; carrier gas, nitrogen (2 mL min^{-1}); injection volume, 1 µL (split 1:50).

Spectroscopic data

(4*Z*)-Lachnophyllum lactone (**1**)

Brownish oil (338 mg); molecular formula $C_{10}H_{10}O_2$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (t, 3H, *J* 5.2 Hz, CH_3), 1.62 (sex, 2H, *J* 7.2 Hz, CH_2), 2.43 (td, 2H, *J* 7.2, 2.4 Hz, CH_2), 5.31 (t, 1H, *J* 2.4 Hz, CH), 6.22 (d, 1H, *J* 5.2 Hz, CH), 7.37 (d, 1H, *J* 5.2 Hz, CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.9, 156.0, 142.6, 120.1, 104.6, 95.1, 74.8, 22.1, 21.8, 13.5; MS/MS (EI, 70 eV) *m/z* (%) 162.0 [M^+] (precursor ion), 147.0 (23) [$M - CH_3$]$^+$, 133.0 (25) [$M - C_2H_5$]$^+$, 119.1 (31) [$M - C_3H_7$]$^+$, 105.1 (14) [$M - C_2H_5 - CO$]$^+$, 91.1 (47) [$M - C_3H_7 - CO$]$^+$, 82.0 (100) [$C_4H_2O_2$]$^+$.

(4*Z*,8*Z*)-Matricaria lactone (**2**)

Brownish oil (53 mg); molecular formula: $C_{10}H_8O_2$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, 1H, *J* 5.0 Hz, CH), 6.25 (dd, 1H, *J* 5.4, 0.5 Hz, CH), 6.16 (dq, 1H, *J* 10.5, 7.0 Hz, CH), 5.72 (dm, 1H, *J* 10.5 Hz, CH), 5.48 (d, 1H, *J* 2.5 Hz, CH), 1.97 (dd, 3H, *J* 7.0, 1.5 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 155.9, 142.4, 141.8, 120.4, 109.9, 99.4, 94.6, 88.0, 16.5; MS/MS (EI, 70 eV)

m/z (%) 160.1 $[M^+]$ (precursor ion), 145.0 (7) $[M - CH_3]^+$, 131.0 (100) $[M - C_2H_5]^+$, 103.0 (37) $[M - C_2H_5 - CO]^+$, 82.0 (18) $[C_4H_2O_2]^+$, 78.1 (58) $[M - C_4H_2O_2]^+$.

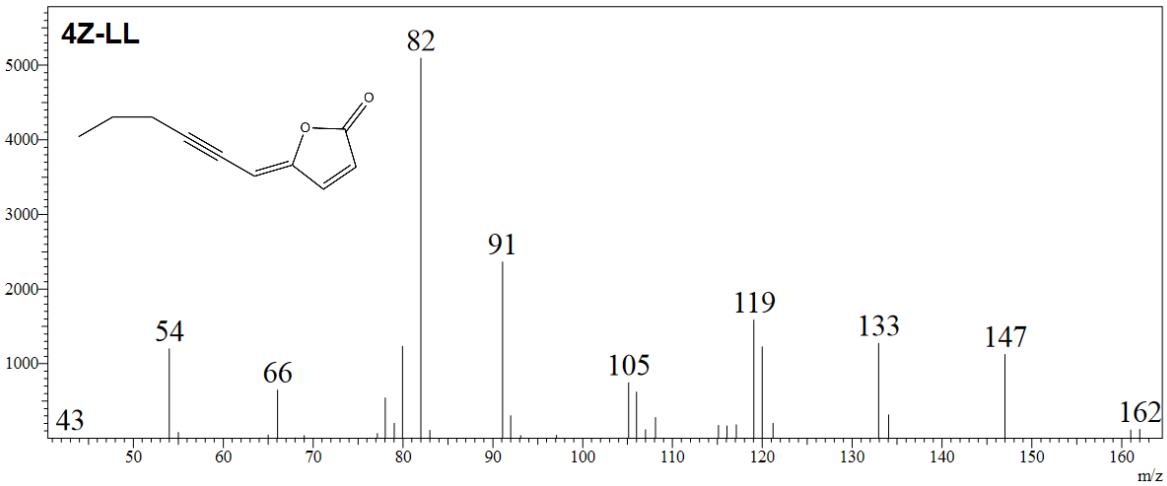

Figure S1. MS^2 spectrum of ion $[M^+]$ (*m/z* 162) for compound **1**.

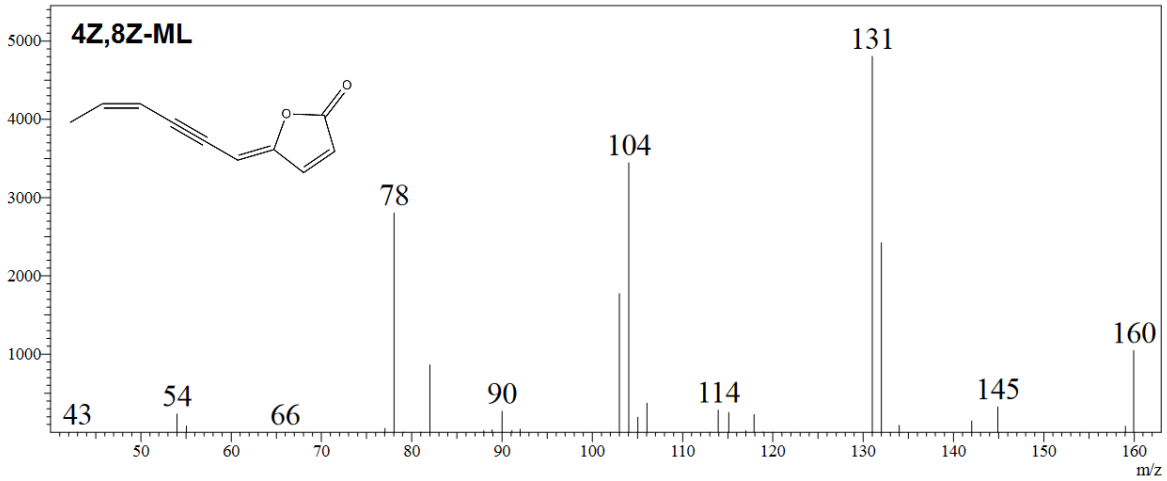

Figure S2. MS^2 spectrum of ion $[M^+]$ (*m/z* 162) for compound **2**.

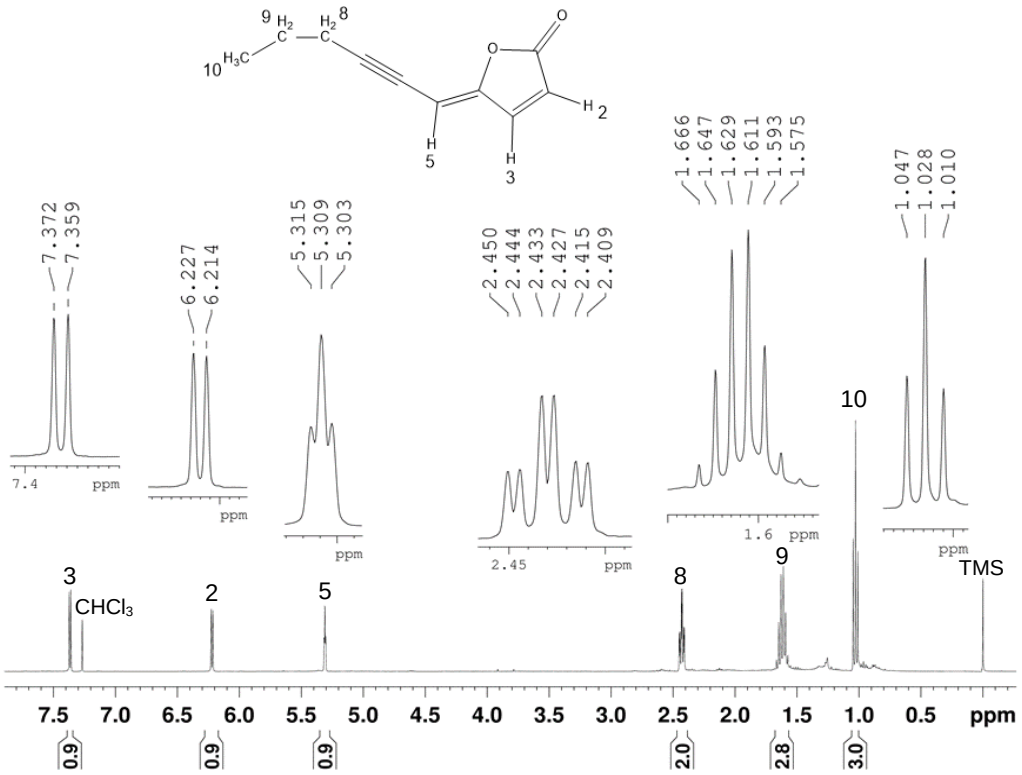

Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1**.

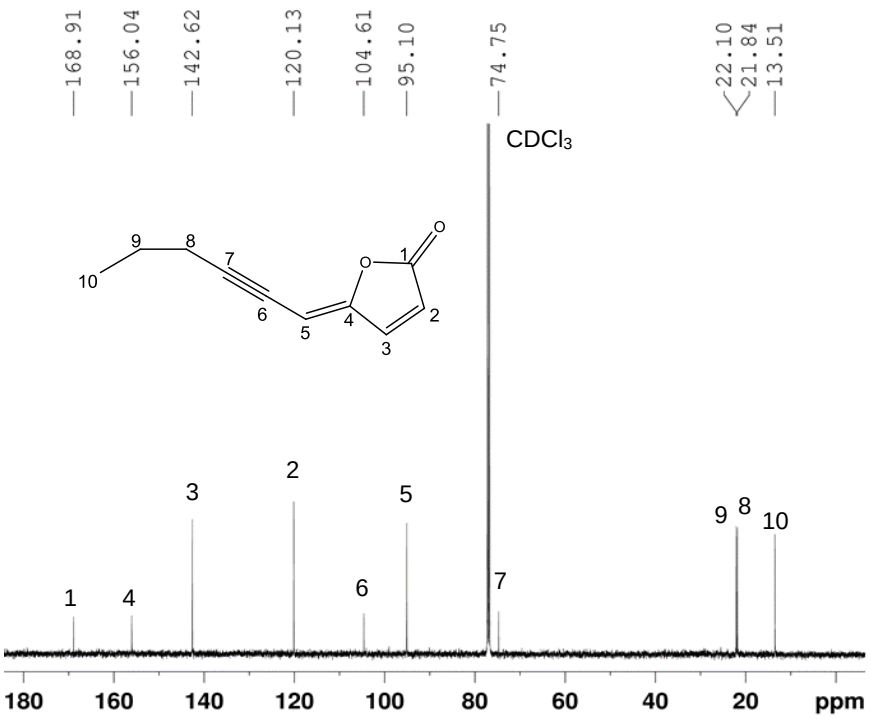

Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **1**.

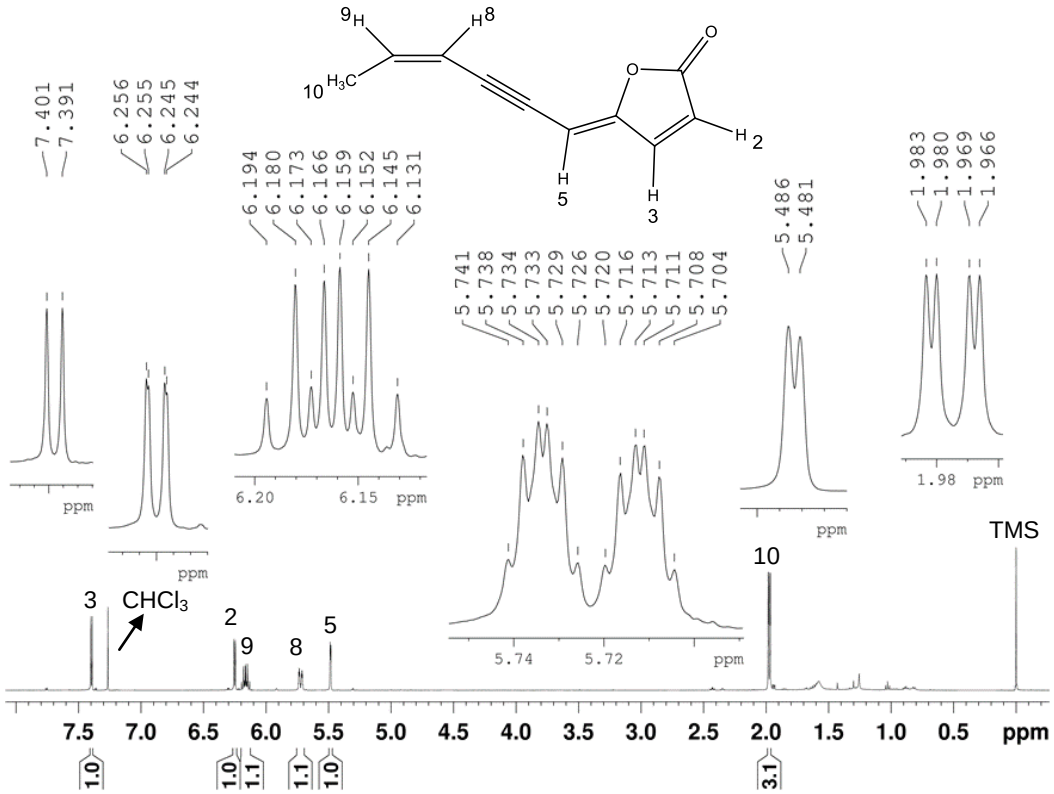

Figure S5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **2**.

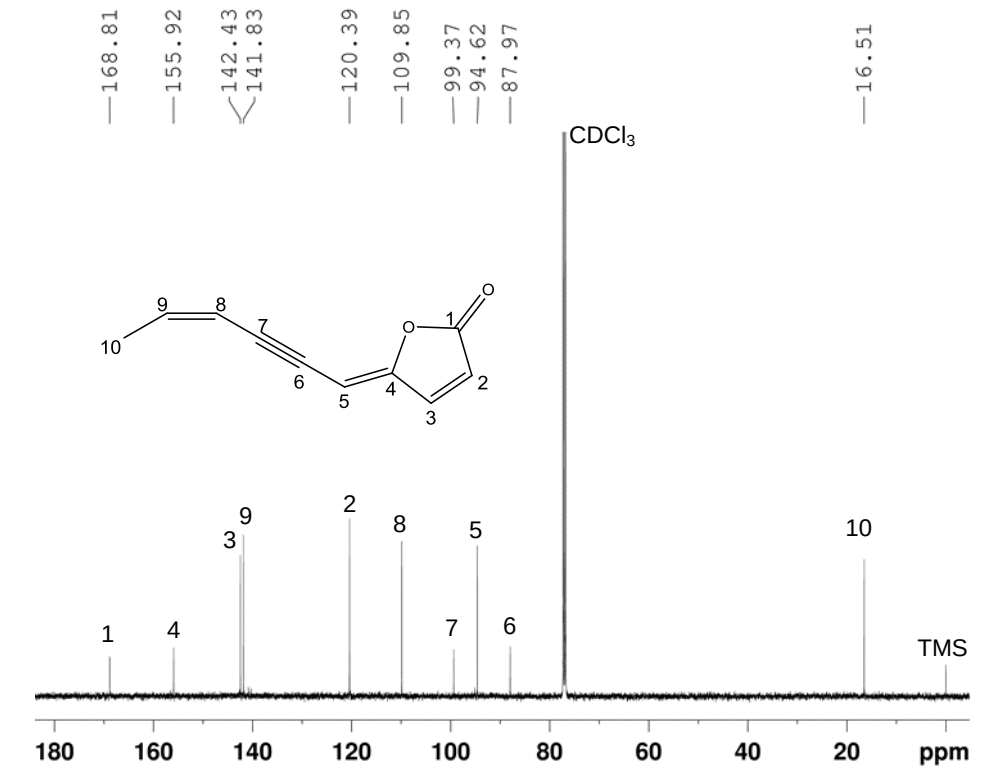

Figure S6. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of compound **2**.

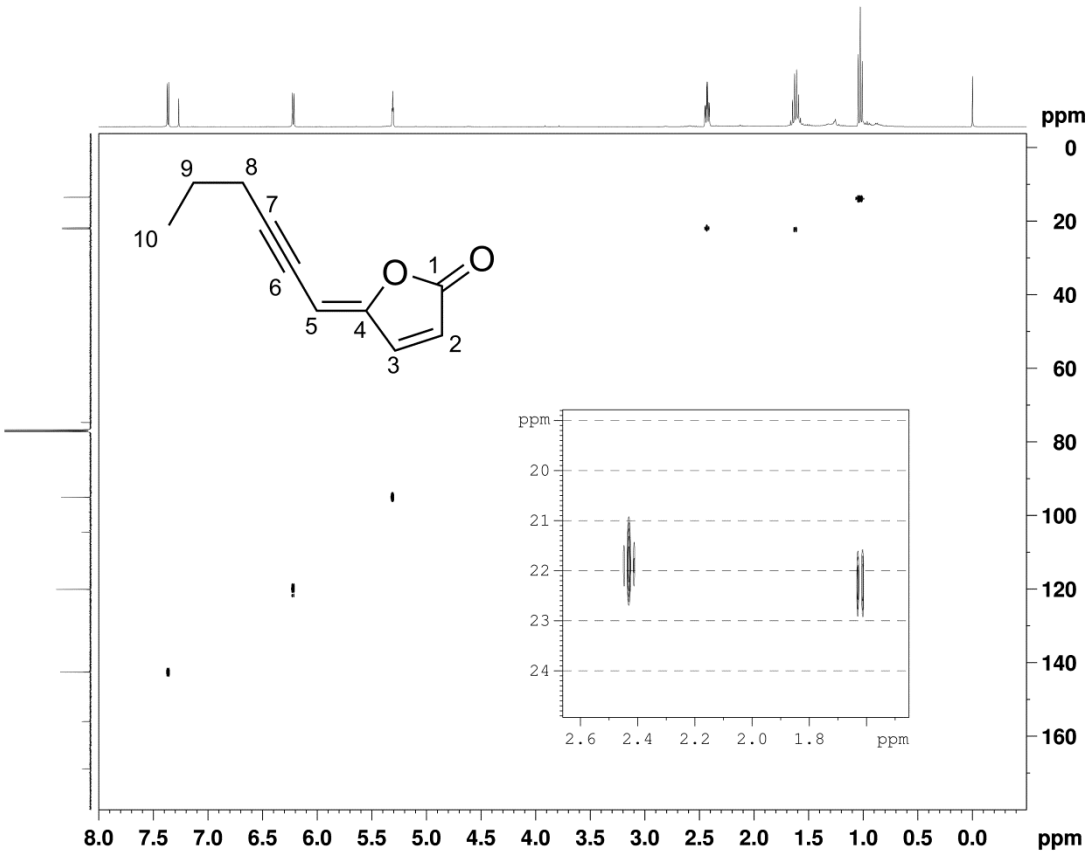

Figure S7. ^{1}H-^{13}C HSQC NMR spectrum (400 MHz, $CDCl_3$), reference TMS for compound **1**.

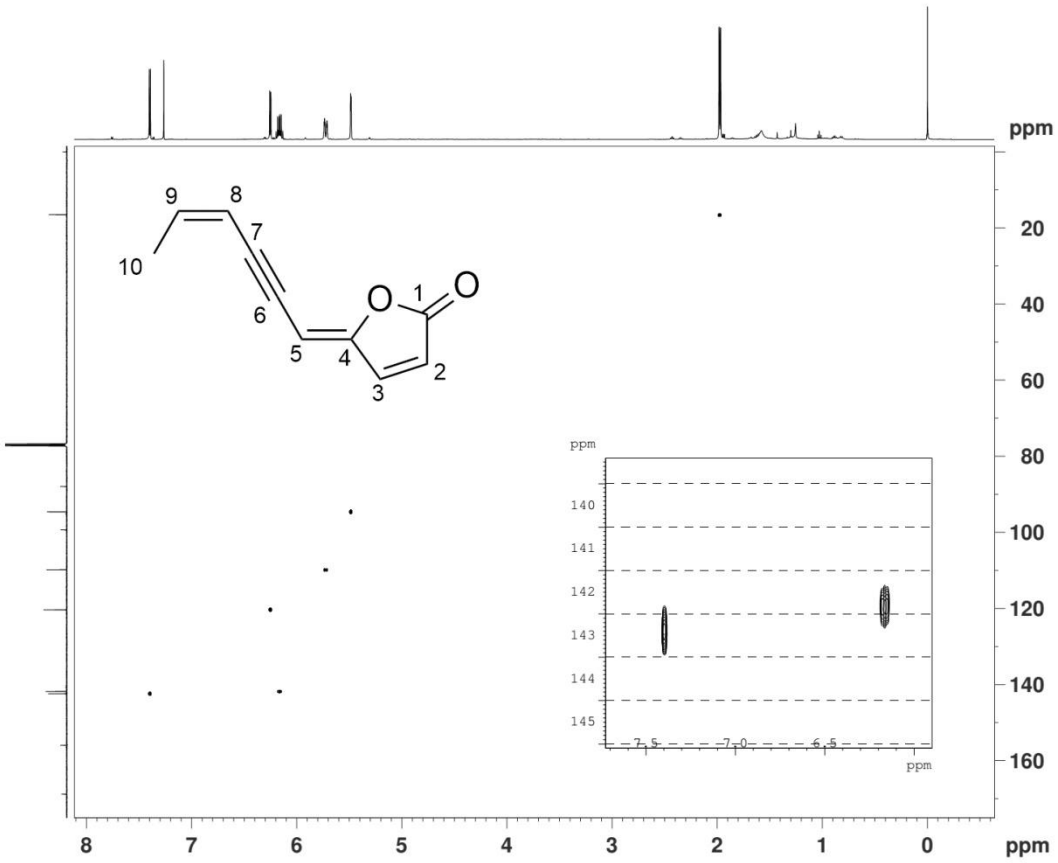

Figure S8. ^{1}H-^{13}C HSQC NMR spectrum (400 MHz, $CDCl_3$), reference TMS, for compound **2**.