Supplementary Materials: Toxicity of Piperine Amide Analogs toward the Tomato Pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae) and Risk Assessment for Two Predators

Elba Pereira, Elizeu Farias, Arthur Ribeiro, Elson Alvarenga, Alex Aguiar, Jhulyana Ferreira and Marcelo Picanço

1. General techniques

The progress of the reactions was monitored by visualizing thin-layer chromatography (TLC) plates in an ultraviolet chamber with a lamp irradiating at 254 nm (Spectroline, model CM-10, Westbury, NY, USA). All compounds were purified by column chromatography on silica gel (70–230 mesh). Melting points were obtained on an MQAPF-301 melting point apparatus (Microquímica, São Paulo, SP, Brazil) and were not corrected. Infrared (IR) spectra were acquired using a Varian 660-IR spectrometer (Varian Inc., Palo Alto, CA, USA) equipped with GladiATR, using the thin-film solid method. Nuclear magnetic resonance (NMR) experiments were recorded on a Varian Mercury 300 spectrometer (Varian Inc.) with $CDCl_3$ as solvent. The ^1H NMR chemical shifts were reported using the signal from residual $CHCl_3$ as reference (δ = 7.27 ppm). 13C NMR chemical shifts were reported using the signal from $CDCl_3$ as reference (δ = 77.0 ppm). Electron impact (70 eV) mass spectra were recorded using a Shimadzu GC-MS-QP5050A (Shimadzu, Milan, Italy).

2. Experimental procedures

Preparation of hexa-2,4-dienoic acid

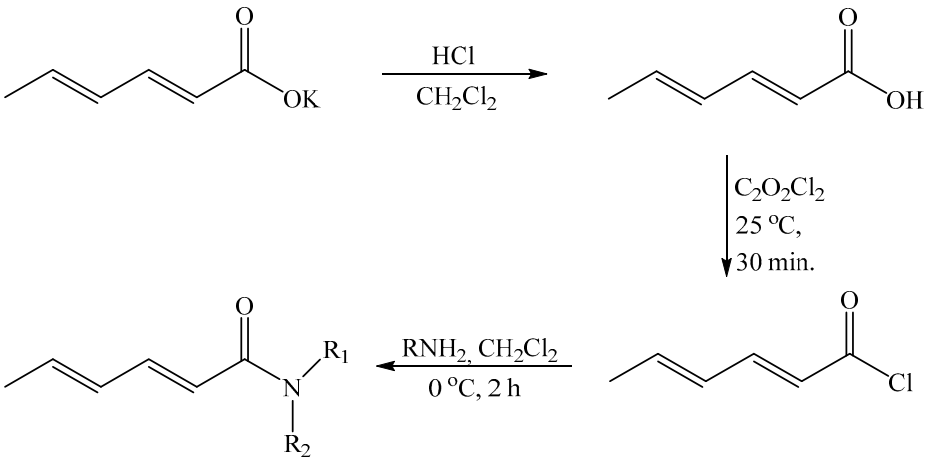
Potassium sorbate (10 g, 67.5 mmol), CH2Cl2 (100 mL) and 1 mol/L HCl solution (100 mL) were added to a 1000 mL separatory funnel. The mixture was stirred for 10 minutes, the organic and aqueous phases were separated, and the aqueous phase extracted with CH2Cl2 (4 × 70 mL). Anhydrous MgSO4 was used to remove residual water from the organic phase and the solvent was removed under reduced pressure to give (2E,4E)-hexa-2,4-dienoic acid (9.0 g, 80 mmol) as a white crystalline solid (mp 133–134°C) in 80% yield. IR (thin-film, cm-1): 3018, 2568, 1691, 1634, 1610. 1H NMR (300 MHz, CDCl3) δ (ppm): 1.80 (3H, d, 15 Hz, H-6), 5.77 (1H, d, 15 Hz, H-2), 6.17–6.25 (2H, m, H-4 and H-5), 7.30–7.38 (1H, m, H-3). 13C NMR (75 MHz, CDCl3) δ (ppm): 18.9 (C-6), 118.3 (C-2), 129.9 (C-4), 141.0 (C-5), 147.5 (C-3), 173.1(C = O).

Step I: Conversion of (2E,4E)-hexa-2,4-dienoic acid into the corresponding acid chloride

(2E,4E)-Hexa-2,4-dienoic acid (0.5 g, 4.46 mmol) was added in a flask containing anhydrous CH2Cl2 (10.0 mL). Subsequently, oxalyl chloride (C2Cl2O2) (1.0 mL, 11.5 mmol) was added forming the acid chloride in situ. The solvent and excess oxalyl chloride were removed under reduced pressure on a rotary evaporator leading to the production of a green oil.

Step II: General procedure for obtaining the amides [1 to 10]

The oil obtained in the step I was dissolved in dry CH2Cl2 (5 mL) and transferred to a round bottom flask. Next, 11.5 mmol of each amine were added to this solution. The reaction mixture was continuously stirred and inert atmosphere at 0 °C for 2 h. Thereafter the excess solvent was removed on a rotary evaporator under reduced pressure. The residue obtained was purified on a silica gel column using a mixture of hexane and ethyl acetate to give the pure amides [1–10] (Scheme 1).

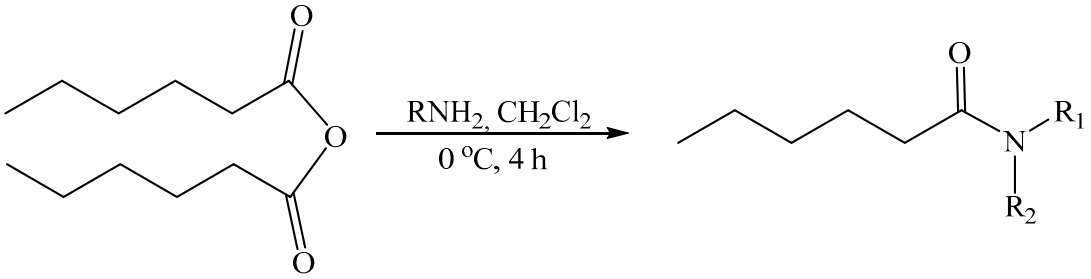


1 R_1 = cyclohexyl R_2 = H
2 R_1 = benzyl; R_2 = H
3 R_1 = phenethyl; R_2 = H
4 R_1 = phenyl; R_2 = H
5 R_1 = phenyl; R_2 = phenyl
6 R_1 = isopropyl; R_2 = isopropyl
7 R_1 = ethyl; R_2 = ethyl
8 NR_1R_2 = pyrrolidine
9 R1 = hexyl; R2 = H
10 NR_1R_2 = piperidine

Scheme 1. Synthesis of the unsaturated amides (**1–10**) from potassium sorbate.

Step III: General procedure for obtaining the amides [11–19]

Hexanoic anhydride (0.4 g, 1.87 mmol) dissolved in anhydrous CH2Cl2 (10.0 mL) was added to a flask and then the corresponding amine (1.87 mmol) was added. The solution was stirred under nitrogen atmosphere for 4 h at 0°C. After this time, the NaHCO3 solution was added to neutralize the acid formed in the reaction. The organic and aqueous phases were separated using a separatory funnel. Magnesium sulfate ($MgSO_4$) was added to the organic phase to remove residual water, then the solvent and excess amine were removed on a rotary evaporator. The obtained residue was purified on a silica gel column to obtain the amides [11–19] (Scheme 2).



11 R_1 = phenyl; R_2 = H
12 R_1 = benzyl; R_2 = H
13 R_1 = cyclohexyl; R_2 = H
14 NR_1R_2 = pyrrolidine
15 R_1 = pentyl; R_2 = H
16 R_1 = phenethyl; R_2 = H
17 R_1 = 3-nitrophenyl; R_2 = H
18 R_1 = 4-chlorophenyl; R_2 = H
19 R_1 = 3-chlorophenyl; R_2 = H

Scheme 2. Synthetic route used for the preparation of the saturated amides from hexanoic anhydride.

Compound 3: (2E,4E)-N-phenethylhexa-2,4-dienamide

Aspect: white solid (mp 98.0–99.3 °C), 88 % yield. IR (thin-film solid, cm-1): 3301, 3064, 2929, 1652, 1625, 1608, 1537. 1H NMR (300 MHz, CDCl3) δ (ppm): 1.55 (2H, m, H-2′), 1.81 (3H, d, 3J = 6.0 Hz, H-6), 5.15-5.25 (2H, m, H-1′), 5.74 (1H, d, 3J = 15.0 Hz H-2), 5.93 (1H, sl, NH), 5.99-6.17 (2H, m, H-4 and H-5), 7.14-7.32 (6H, m, H-4′, H-5′, H-6′, H-7′, H-8′, and H-3). 13C NMR (75 MHz, CDCl3) δ (ppm): 18.8 (C-6), 21.9 (C-2′), 48.9 (C-1′), 121.7 (C-2), 126.5 (C-6′), 127.5 (C-5′ e C-7′), 128.8 (C4′ e C-8′), 129.8 (C-3′ e C-4), 138.0 (C-5), 141.7 (C-3), 143.5 (C-3′), 165.7 (C = O). MS (EI, 70 eV) m/z (%): 215 (25.5, [M+]), 120 (90), 105 (35.5), 95 (100), 77 (29.3), 67 (65.8), 51 (17.0).

Compound 9: (2E,4E)-N-hexylhexa-2,4-dienamide

Aspect: white solid (mp 73.3–74.0 °C), 80% yield. IR (thin-film solid, cm-1): 3267, 2955, 2864, 1654, 1612, 1587, 998. 1H NMR (300 MHz, CDCl3) δ (ppm): 1.51–1.66 (8H, m, H-2′, H-3′, H-4′ and H-5′), 1.82 (6H, dd, 3J = 6.6 Hz, 4J = 0.9 Hz, H-6 and H-6′), 3.53 (2H, tl, 3J = 4.8 Hz, H-1′), 6.06–6.25 (3H, m, H-2, H-4 and H-5), 7.17–7,.26 (1H, dd, 3J = 15.0 Hz, 3J = 9.9 Hz, H-3). 13C NMR (75 MHz, CDCl3) δ (ppm): 14.2 (C-6′), 18.8 (C-6), 22.8 (C-5′) 26.8 (C-4′), 29.8 (C-3′), 31.7 (C-2′), 39.9 (C-1′), 121.6 (C-2), 129.8 (C-4), 138.2 (C-5), 141.5 (C-3), 166.8 (C = O). MS (EI, 70 eV) m/z (%): 179 (21.6), 164 (10.9), 95 (45.9), 86 (30.9), 84 (100), 67 (37.5), 51 (28.4).

Compound 19: N-(3-chlorophenyl)hexanamide

Aspect: yellow solid (mp 58.9–59.7 °C), 79% yield. IR (thin-film solid, cm-1): 3278, 2955, 2926, 2859, 1659, 1592, 1519, 744. 1H NMR (300 MHz, CDCl3) δ (ppm): 0.91 (3H, t, 3J = 6.0 Hz, H-6), 1.32–1.40 (4H, m, H-4, H-5), 1.75 (2H, quit, 3J = 7.2 Hz, H-3), 2.33 (2H, t, 3J = 7.5 Hz, H-2), 7.05 (1H, dt, 3J = 7.8 Hz, 4J = 1.5 Hz, H-4′), 7.26 (1H, dt, 3J = 7.2 Hz, 4J = 1,5 Hz, H-3′), 7.37 (1H, dd, 3J = 2.8 Hz, 4J = 1.2 Hz, H-2′), 7.63 (1H, sl, NH), 8.38 (1H, d, 3J= 7.8 Hz, H-6′). 13C NMR (75 MHz, CDCl3) δ (ppm): 14.1 (C-6), 22.6 (C-5), 25.5 (C-4), 31.5 (C-3), 38.2 (C-2), 121.8 (C-2′), 124.9 (C-4′), 129.1 (C-6′), 134.8 (C-1′ e C-5′), 178.5 (C = O). MS (EI, 70 eV) m/z (%): 225 (7.1, [M+]), 129 (31.9), 127 (100), 99 (10), 83 (10.6), 71 (16.2), 55 (14.3).

4. Supplementary Information

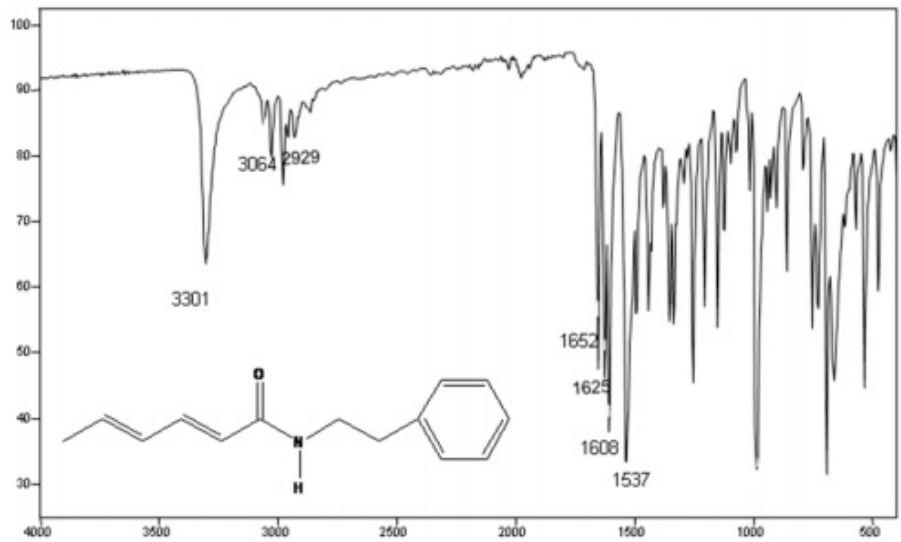


Figure S1. IR spectrum of (2*E*,4*E*)-*N*-phenethylhexa-2,4-dienamide (**3**).

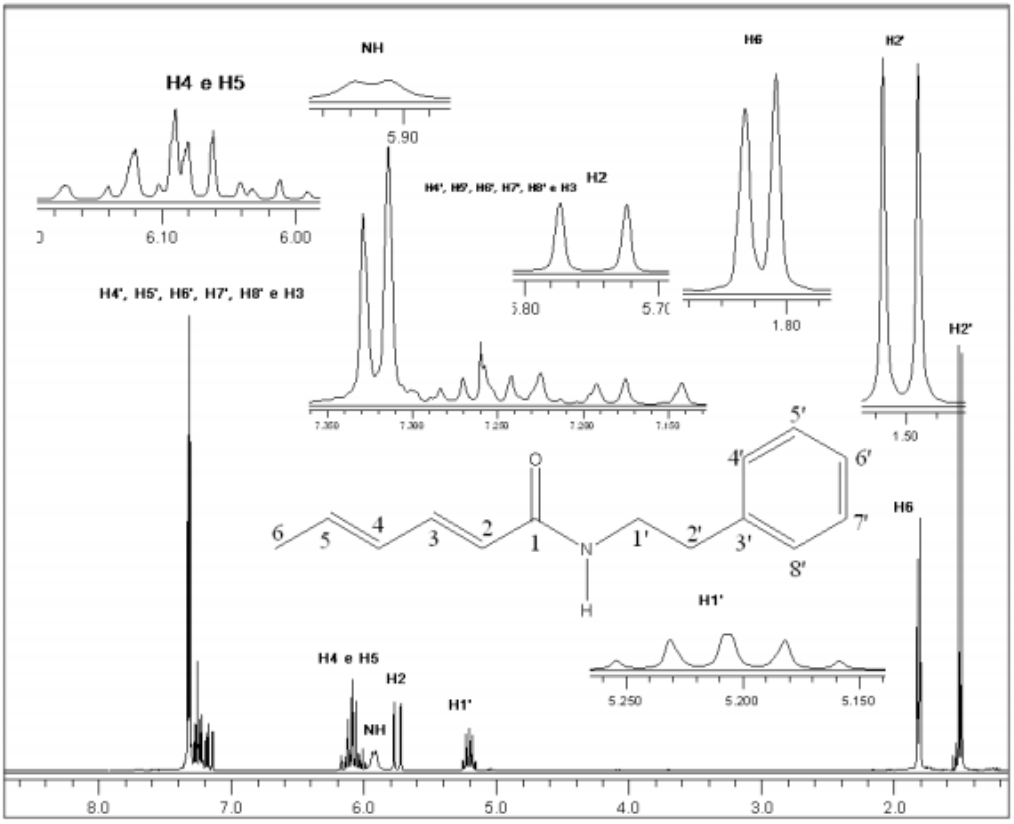


Figure S2. 1H NMR spectra (300 MHz, CDCl3) of (2E,4E)-N-phenethylhexa-2,4-dienamide (3).

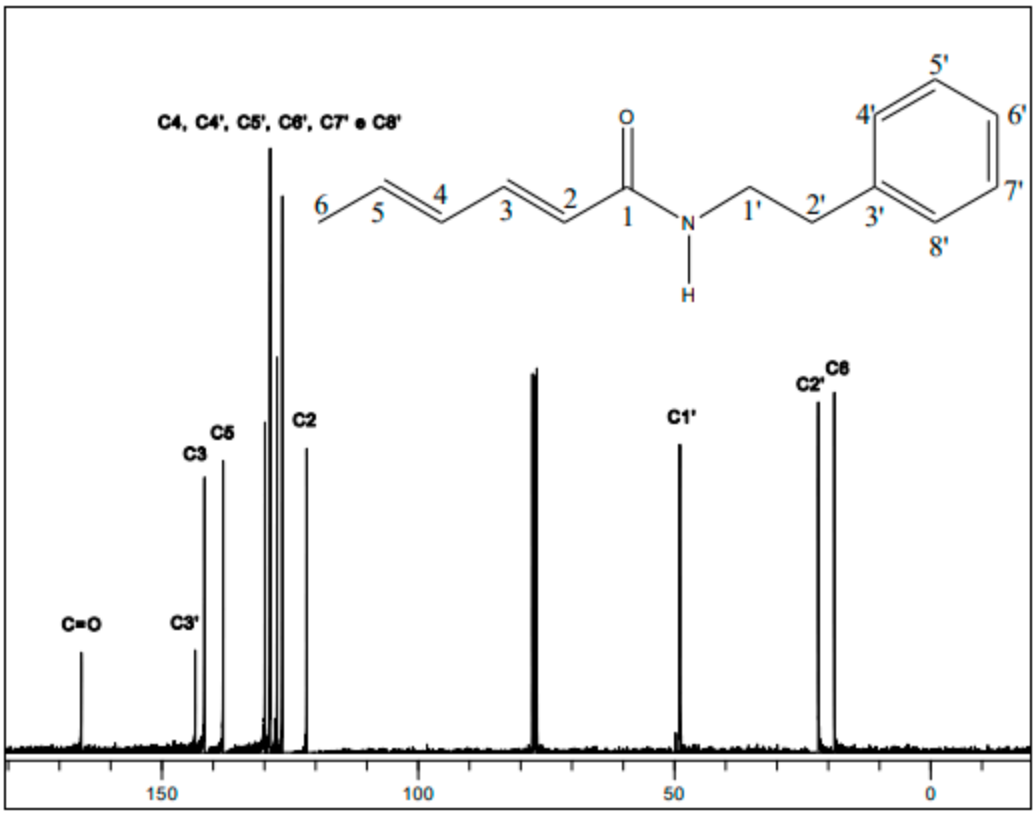


Figure S3. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of (2*E*,4*E*)-*N*-phenethylhexa-2,4-dienamide (**3**).

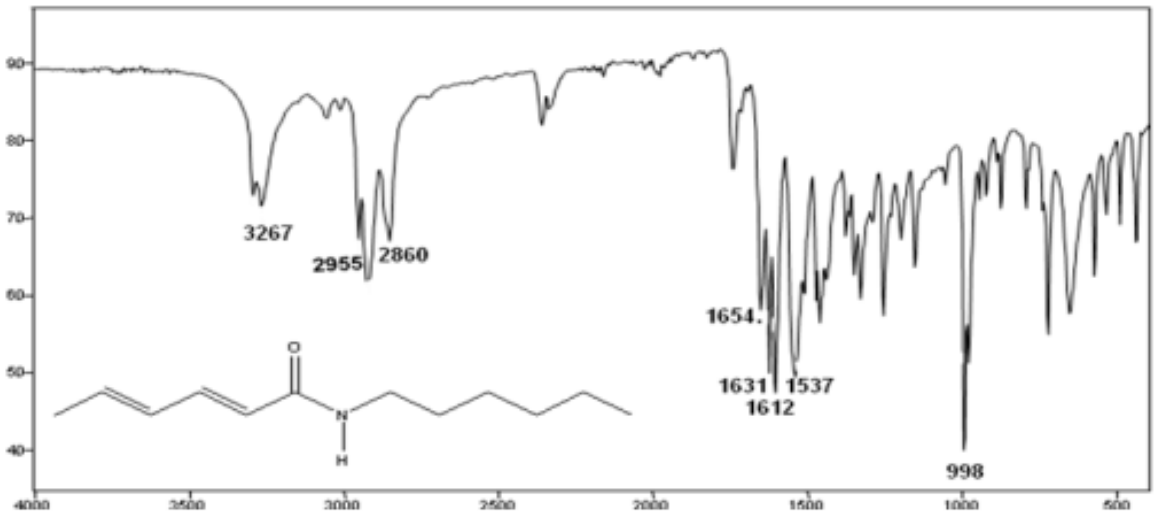


Figure S4. IR spectrum of (2*E*,4*E*)-*N*-hexylhexa-2,4-dienamide (**9**).

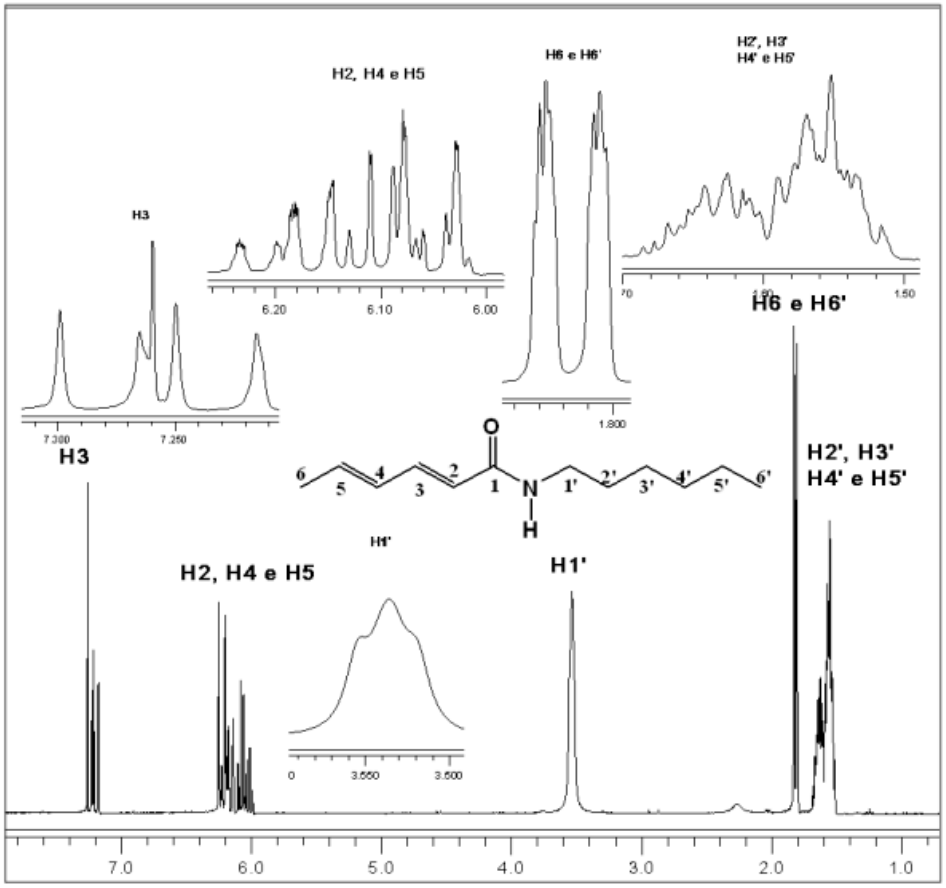


Figure S5. 1H NMR spectrum (300 MHz, CDCl3) of (2E,4E)-N-hexylhexa-2,4-dienamide (9).

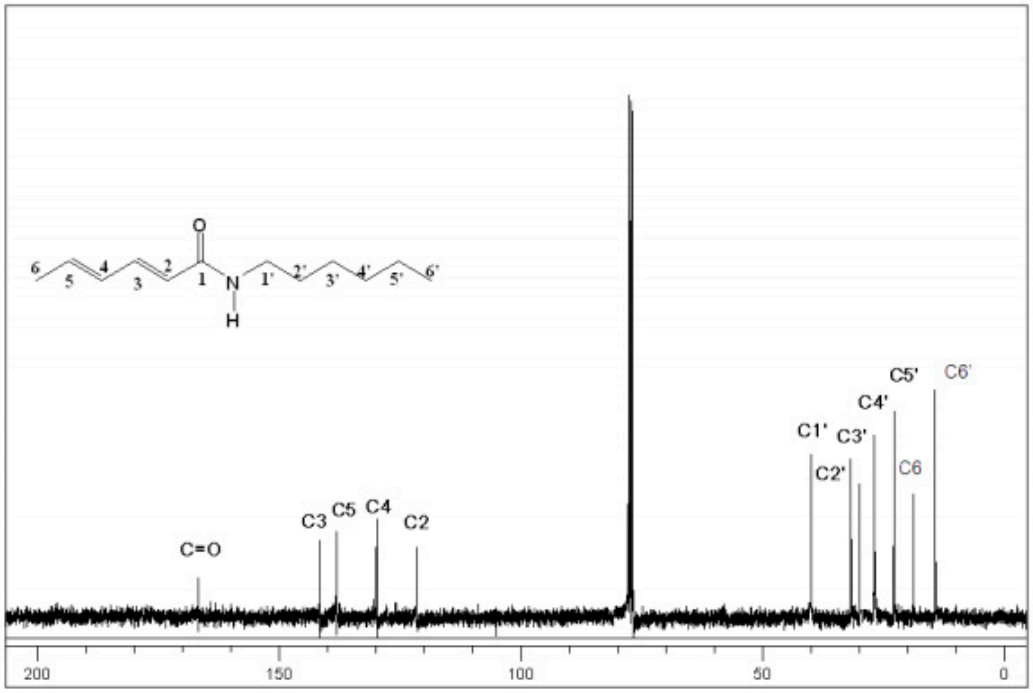


Figure S6. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of (2*E*,4*E*)-*N*-hexylhexa-2,4-dienamide (**9**).

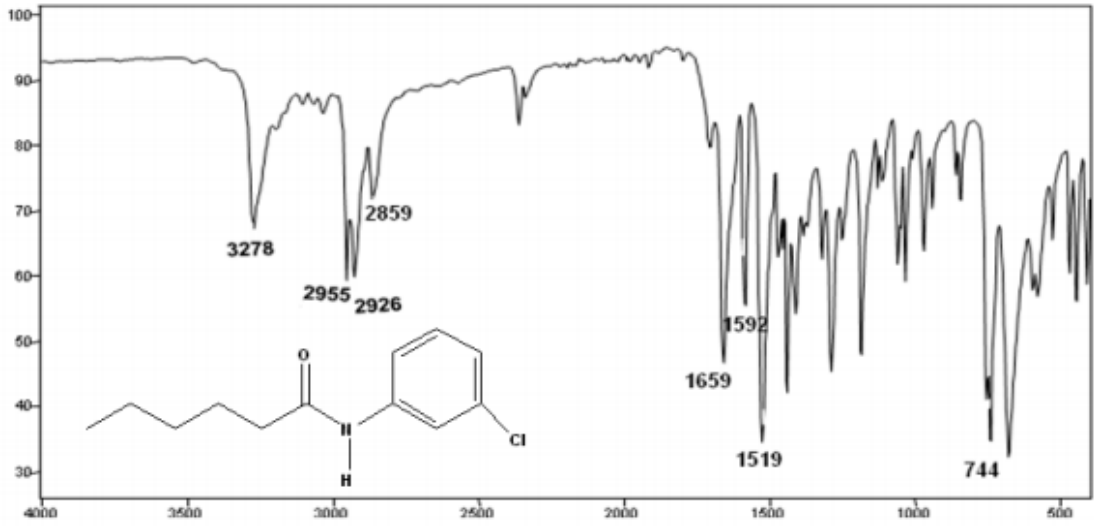


Figure S7. IR spectrum of *N*-(3-chlorophenyl)hexanamide (**19**).

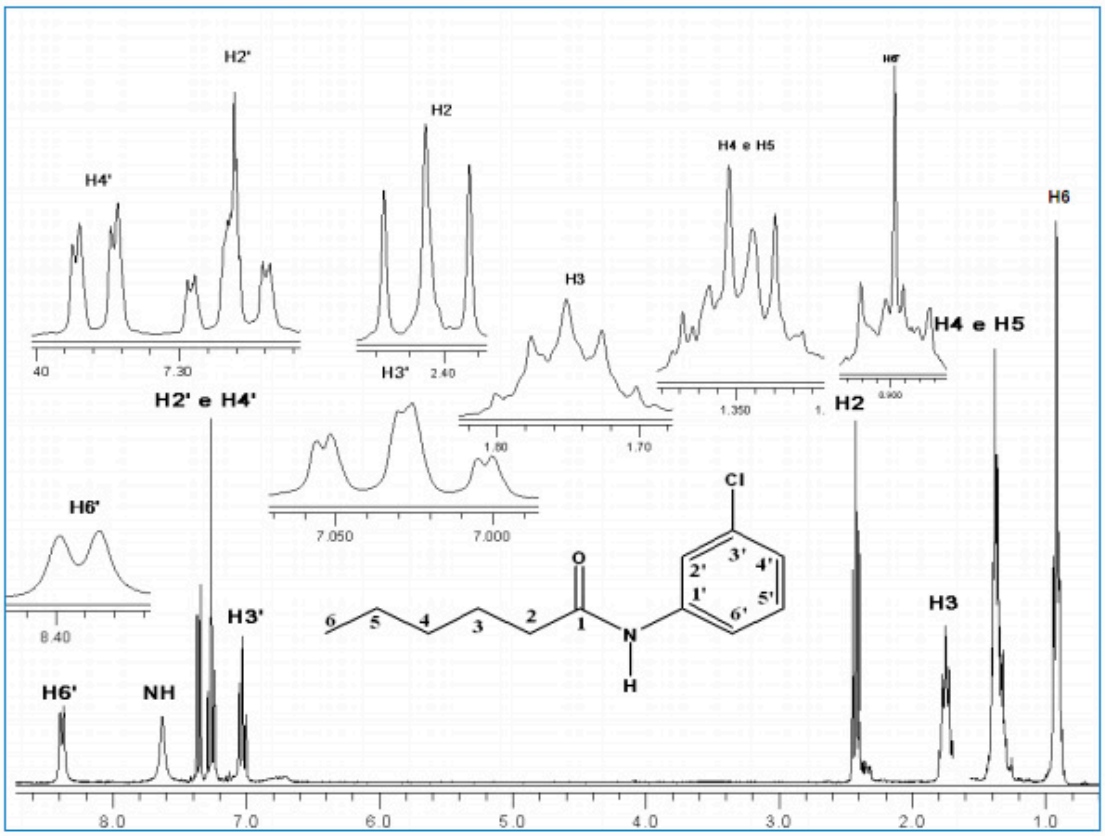


Figure S8. ^{1}H NMR spectra (300 MHz, $CDCl_3$) of *N*-(3-chlorophenyl)hexanamide (**19**).

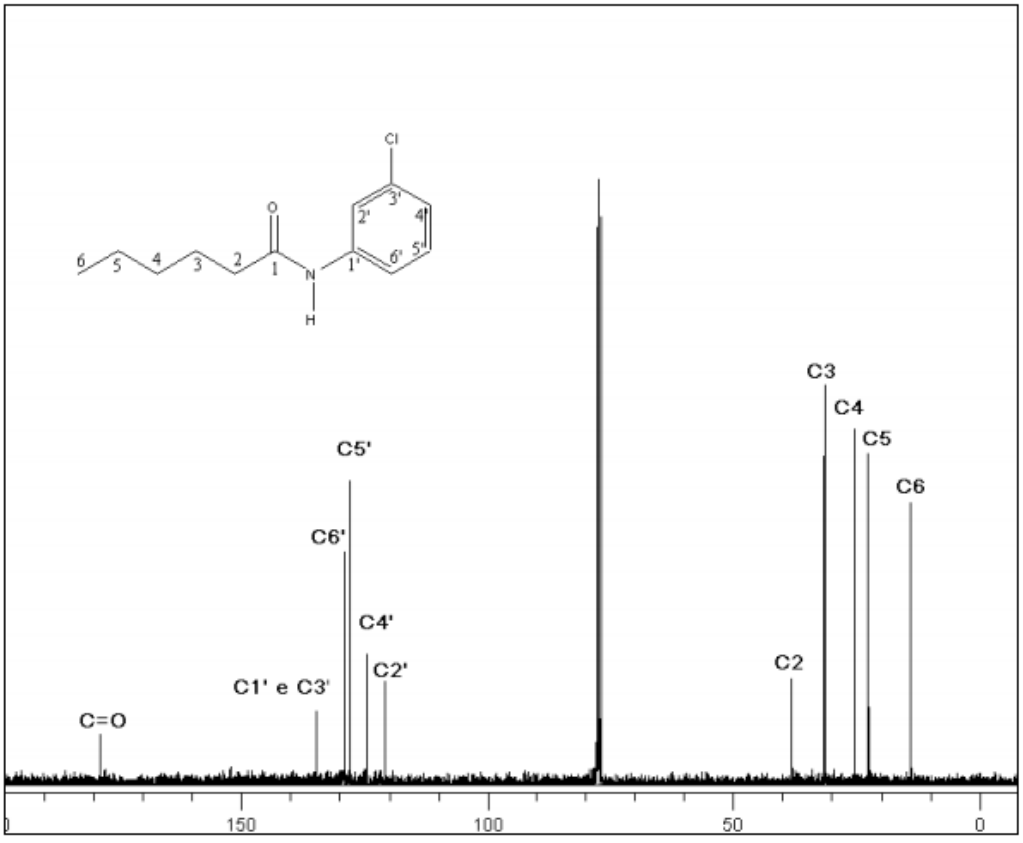


Figure S9. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of *N*-(3-chlorophenyl)hexanamide (**19**).