SUPPLEMENTARY INFORMATION

Interrupting Specific Hydrogen Bonds between ELF3 and MED23 as an alternative drug resistance-free strategy for HER2-overexpressing cancers

Soo-Yeon Hwang,[a] Seojeong Park,[a] Hyunji Jo,[a] Seung Hee Seo,[a] Kyung-Hwa Jeon,[a] Seojeong Kim,[a] Ah-Reum Jung,[a] Chanju Song,[a] Misun Ahn,[a] Soo Yeon Kwak,[c] Hwa-Jong Lee,[a] Motonari Uesugi,[b] Younghwa Na,[c,*] and Youngjoo Kwon[a,*]

[a]*College of Pharmacy & Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 03760, Korea.*

[b]*Institute for Chemical Research and Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Uji, Kyoto 611-0011*

[c]*College of Pharmacy, CHA University, Pocheon, 487-010, Korea*

*To whom correspondence should be addressed.

Y. Na: Phone: 82-31-8017-9896. Fax: 82-31-8017-9420. E-mail: yna7315@cha.ac.kr, Y. Kwon: Phone: 82-2-3277-4653. Fax: 82-2-3277-3051. E-mail: ykwon@ewha.ac.kr.

Contents

Experimental

1. Chemistry general

Chemicals and reagents used were obtained from Aldrich Chemical Co. and others were from company like TCI. Melting points were measured without correction in open capillaries with Barnstead Electrothermal melting point apparatus, Manual MELTEMP(Model No: 1202D). Chromatographic separations were monitored by thin-layer chromatography using a commercially available pre-coated Merck Kieselgel 60 F254 plate (0.25 mm) and detected by visualizing under UV at 254 and 365 nm. Silicagel column chromatography was carried out with Merck Kieselgel 60 (0.040-0.063 mm). All solvents used for chromatography were directly used without distillation. The purity was assessed by HPLC (Shimadzu LC-20AD) analysis under the following conditions; column, SunFire C18 (4.6 mm × 150 mm, 5 mm); mobile phase, condition : A (water) and B (acetonitrile) using a linear gradient of 50-70% B in 0-15 min, 70% B in 15-20 min,100% B in 20-25 min and 50% B in 25-30 min, flow rate; 1.0 mL/min; detection, diode array detector (Shimadzu Spd-M20A). The purity of compound is described as percent (%) and retention time was given in minutes. NMR spectra were recorded on Varian AS 400 (1H NMR at 400 MHz and 13C NMR at 100 MHz) with tetramethylsilane as an internal standard. Chemical shift (d) values are expressed in ppm and coupling constant (J) values in hertz (Hz). The melting points were measured on Gallenkamp Melting Point Apparatus without correction.

2. General synthetic methods for chalcone analogues

To a reaction mixture of acetophenone derivative and benzaldehyde derivative (1.0 equiv.) in EtOH (10 mL) was added 50% NaOH (4.0 equiv.). The reaction mixture was stirred at room temperature (24 h) and then water was added. Solid formed was filtered and washed with H_2O

and then dried under vacuum. The solid was purified by silica gel column chromatography (eluent: ethyl acetate : *n*-hexane) to give the desired product.

2.1 3-Furan-2-yl-1-(3-hydroxyphenyl)-propenone (1)

Following the general method, 3-hydroxyacetophenone (1.00 g, 7.34 mmol), furfural (0.61 mL, 7.34 mmol) and 50% NaOH (2.35 mL, 29.35 mmol) were used. Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **1** (1.45 g, 92.1%) as a brown solid. m.p. 155 - 156 ℃; R_f 0.54 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 4.46 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.37 (d, *J* = 4.8 Hz, 1H), 6.57 (s, 1H), 6.91 (ddd, *J* = 8.0, 1.2, 0.8 Hz, 1H), 7.15 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.25 (d, *J* = 15.6 Hz, 2H), 7.32 (d, *J* = 8.8 Hz, 2H), 7.39 (d, *J* = 8.8 Hz, 1H), 9.04 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) : 112.5, 115.0, 115.9, 120.2, 120.4, 129.4, 130.2, 139.2, 144.8, 151.4, 157.6, 189.6 ppm.

2.2 1-(3-Hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (2)

Following the general method, 3-hydroxyacetophenone (1.00 g, 7.34 mmol), 4-methoxy benzaldehyde (0.89 mL, 7.34 mmol) and 50% NaOH (2.35 mL, 29.35 mmol) were used. Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **2** (2.40 g, 98.9%) as a yellow solid. m.p. 120 - 121 ℃; R_f 0.57 (ethyl acetate : *n*-Hexane = 1 : 1); HPLC: R_T 6.03 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.86 (s , 3H), 6.05 (s, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.09 (ddd, *J* = 8.0, 2.4, 0.8 Hz, 1H), 7.37 (ddd, *J* = 8.0, 8.0, 2.4 Hz, 1H), 7.38 (d, *J* = 15.6 Hz, 1H), 7.54-7.55 (m, 2H), 7.60 (dd, *J*= 8.8 Hz, 2H), 7.79 (d, *J*= 16.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 55.7, 114.7, 115.3, 119.9, 120.2, 121.1, 127.8, 130.1, 130.6, 140.2, 145.4, 156.5, 162.0, 190.7 ppm.

2.3 1-(3-Hydroxyphenyl)-3-(4-methoxynaphthalen-1-yl)-propenone (3)

Following the general method, 3-hydroxyacetophenone (1.00 g, 7.34 mmol), 4-methoxy-1-naphthaldehyde (1.37 g, 7.34 mmol) and 50% NaOH (2.35 mL, 29.35 mmol) were used. The purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **3** (1.56 g, 69.8%) as an orange solid. m.p. 189 - 190 ℃; R_f 0.63 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 10.34 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.99 (s, 3H), 4.73 (s, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 7.03 (ddd, *J*= 8.0, 2.4, 0.8 Hz, 1H), 7.26 (dd, *J* = 8.0, 5.6 Hz, 1H), 7.44-7.49 (m, 4H), 7.53 (ddd, *J* = 8.0, 8.0, 1.6 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.51 (d, *J* = 15.2 Hz, 1H), 8.98 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 55.8, 103.9, 115.3, 119.7, 120.4, 122.4, 122.5, 122.7, 123.1, 123.9, 124.6, 125.3, 126.2, 127.5, 137.9, 157.7, 190.4 ppm.

2.4 3-(4-Chlorophenyl)-1-(3-hydroxyphenyl)-propenone (4)

Following the general method, 3-hydroxyacetophenone (1.00 g, 7.34 mmol), 4-chloro benzaldehyde (1.03 g, 7.34 mmol) and 50% NaOH (2.35 mL, 29.35 mmol) were used. Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **4** (0.99 g, 52.6%) as a pale yellow solid. m.p. 160 - 162 ℃; R_f 0.76 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 8.35 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.01 (ddd, *J* = 8.4, 0.8, 0.8 Hz, 1H), 7.24 (d, *J* = 15.6 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.39 (d, *J* = 8.8 Hz, 2H), 7.41 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 16.0 Hz, 1H), 9.02 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 115.2, 119.5, 120.4, 122.7, 129.1, 129.5,129.6, 133.4, 136.1, 139.2, 142.8, 157.7, 190.1 ppm.

2.5 3-(4-Hydroxyphenyl)-1-(3-hydroxyphenyl)-propenone (5)

Following the general method, 3-hydroxyacetophenone (1.00 g, 7.34 mmol), 4-hydroxy benzaldehyde (0.90 g, 7.34 mmol) and 50% NaOH (2.35 mL, 29.35 mmol) were used. Purification was conducted with eluent (ethyl acetate: *n*-hexane = 1 : 3) to give compound **5** (1.38 g, 78.5%) as a pale yellow solid. m.p. 199 - 200 ℃; R_f 0.45 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 2.80 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.80 (d, *J* = 8.4 Hz, 2H), 6.98 (ddd, *J* = 8.0, 2.4, 0.8 Hz, 1H), 7.23 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.25 (d, *J* = 15.6 Hz, 1H), 7.37-7.41 (m, 2H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 15.6 Hz, 1H), 8.80 (s, 1H), 9.20 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 115.3, 116.2, 119.2, 119.6, 120.0, 126.4, 129.5, 130.4, 140.0, 145.0, 157.6, 160.1, 190.7 ppm.

2.6 1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)-propenone (6)

Following the general method, 2-hydroxyacetophenone (1.00 mL, 6.50 mmol), 4-methoxy benzaldehyde (0.78 mL, 6.50 mmol) and 50% NaOH (2.08 mL, 26.00 mmol) were used. Purification was conducted with eluent (ethyl acetate: *n*-hexane = 1 : 3) to give compound **6** (0.62 g, 37.4%) as a yellow solid. m.p. 92 - 93 ℃; R_f 0.45 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 14.91 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.87 (s, 3H), 6.94 (ddd, *J* = 8.8, 8.4, 1.2 Hz, 1H), 6.60 (d, *J* = 8.8 Hz, 2 H), 7.03 (d d , *J* = 8.0, 1.2 Hz, 2H), 7.49 (ddd, *J* = 8.8, 8.4, 1.2 Hz, 1H), 7.55 (d, *J* = 15.6 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 15.6 Hz, 2H), 7.93 (dd, *J* = 8.8 1.6 Hz, 1H), 12.93 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 55.7, 114.7, 117.8, 118.8, 119.0, 120.3, 127.6, 129.7, 130.8, 136.4, 145.6, 162.3, 163.8, 193.9 ppm.

2.7 1-(2-Hydroxyphenyl)-3-(4-methoxynaphthalen-1-yl)-propenone (7)

Following the general method, 2-hydroxyacetophenone (1.00 mL, 6.50 mmol), 4-methoxy-1-naphthaldehyde (1.21 mL, 6.50 mmol) and 50% NaOH (2.08 mL, 26.00 mmol) were used.

Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **7** (0.57 g, 28.9%) as an orange solid. m.p. 158 - 160 ℃; R_f 0.57 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 20.25 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.08 (s, 3H), 6.89 (d, *J*= 8.0 Hz, 1H), 6.95 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.51 (ddd, *J* = 8.8, 8.4, 0.8 Hz, 1H), 7.55 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.64 (ddd, *J* = 8.8, 8.0, 1.2 Hz, 1H), 7.69 (d, *J* = 15.4 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.76 (d, *J* = 15.4 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 56.0, 104.0, 118.9, 119.0, 120.1, 120.4, 123.0, 123.3, 124.5, 125.9, 126.0, 126.8, 128.0, 129.8, 133.1, 136.4, 142.6, 158.4, 163.9, 193.8 ppm.

2.8 3-Furan-2-yl-1-(2-hydroxyphenyl)-propenone (8)

Following the general method, 2-hydroxyacetophenone (1.00 mL, 6.50 mmol), furfural (0.53 mL, 6.50 mmol) and 50% NaOH (2.08 mL, 26.00 mmol) were used. Purification was conducted with eluent (ethyl acetate: *n*-hexane = 1 : 3) to give compound **8** (0.78 g, 56.1%) as a yellow solid. m.p. 108 - 109 ℃; R_f 0.78 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 10.43 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.54 (dd, *J* = 8.4, 0.8 Hz, 1H), 6.78 (s , 1H), 6.94 (ddd, *J*= 8.0, 1.4, 0.8 Hz, 1H), 7.02 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.49 (ddd, *J*= 8.0, 8.4, 0.8 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 15.2 Hz, 1H), 7.68 (d, *J* = 15.2 Hz, 1H), 7.92 (dd, *J* = 8.0, 1.6 Hz, 1H), 12.88 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 113.1, 117.3, 117.9, 118.8, 119.1, 120.3, 129.9, 131.4, 136.5, 145.6, 151.8, 136.8, 193.6 ppm.

2.9 3-(4-Hydroxyphenyl)-1-(2-hydroxyphenyl)-propenone (9)

Following the general method, 2-hydroxyacetophenone (1.00 mL, 6.50 mmol), 4-hydroxy benzaldehyde (0.80 mL, 6.50 mmol) and 50% NaOH (2.08 mL, 26.00 mmol) were used.

Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **9** (0.46 g, 29.8%) as a yellow solid. m.p. 165 - 166 ℃; R_f 0.80 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 6.93 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.75 (d, J = 8.8 Hz, 2H), 6.79 (ddd, J = 8.0, 8.0, 1.2 Hz, 1H), 6.83 (dd, J = 8.0, 1.2 Hz, 1H), 7.33 (ddd, J = 8.0, 8.0, 1.2 Hz, 1H), 7.37 (d, J = 15.2 Hz, 1H), 7.40 (d, J= 8.8, 2H), 7.72 (d, J = 15.2, 1H), 7.79 (dd, J = 8.0, 1.2, 1H), 9.40 (s, 1H), 12.84 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 116.3, 117.8, 118.0, 118.8, 119.0, 120.3, 127.9, 129.8, 131.0, 136.4, 145.4, 158.3, 163.8, 193.9 ppm.

2.10 3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)-propenone (10)

Following the general method, 2-hydroxyacetophenone (1.00 mL, 6.50 mmol), 4-chlorobenzaldehyde (0.91 mL, 6.50 mmol) and 50% NaOH (2.08 mL, 26.00 mmol) were used. Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **10** (1.28 g, 76.2%) as a yellow solid. m.p. 154 - 155 ℃; R_f 0.78 (ethyl acetate : *n*-hexane = 1 : 1); HPLC: R_T 16.86 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.95 (ddd, J = 8.8, 8.4, 0.8 Hz, 1H), 7.04 (dd, J = 8.4, 1.2 Hz, 1H), 7.40 (d, J = 8.4, Hz, 2H), 7.51 (ddd, J = 8.8, 8.4, 1.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 15.6 Hz, 1H), 7.87 (d, J = 15.6 Hz, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 12.74 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 118.9, 119.1, 120.2, 120.8, 129.6, 129.8, 130.0, 133.3, 136.8, 137.1, 144.2, 163.9, 193.7 ppm.

2.11 1-(2-Hydroxyphenyl)-3-thiophen-2-yl-propenone (11)

Following the general method, 2-hydroxyacetophenone (1.00 mL, 6.50 mmol), thiophene-2-carboxaldehyde (0.60 mL, 6.50 mmol) and 50% NaOH (2.08 mL, 26.00 mmol) were used. Purification was conducted with eluent (ethyl acetate : *n*-hexane = 1 : 3) to give compound **11** (0.86 g, 57.5%) as an orange solid. m.p. 101 - 102 ℃; R_f 0.85 (ethyl acetate : *n*-hexane = 1 : 1);

HPLC: R_T 12.35 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.95 (ddd, *J* = 8.0, 8.4, 1.2 Hz, 1H), 7.03 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.12 (d, *J* = 8.4, Hz, 1H), 7.41 (dd, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 15.2 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.50 (ddd, *J* = 8.4, 8.4, 1.2 Hz, 1H) 7.89 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.04 (d, *J* = 15.2 Hz, 1H), 12.84 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 118.8, 119.1, 120.2, 128.7, 129.8, 132.9, 136.6, 138.1, 140.4, 163.8, 193.4 ppm.

3. General synthetic method for *N*-acetylpyrazoline analogues

The reaction mixture of chalcone analogue and hydrazine·H_2O (65%) (4.0 equiv.) in AcOH (10 mL) was refluxed (2 h) and then cooled to room temperature. The reaction mixture was poured into ice and kept overnight at room temperature. Solid formed was filtered and washed with water. Solid was dried under vacuum and then purified by silica gel column chromatography (eluent : MeOH : chloroform) to give desired product.

3.1 1-[5-Furan-2-yl-3-(3-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (12)

Following the general method, Compound **1** (0.28 g, 1.31 mmol) and hydrazine H_2O (65%) (0.25 mL, 5.24 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **12** (0.40 g, 11.5%) as a beige solid. m.p. 163 - 164 ℃; R_f 0.45 (MeOH : chloroform = 1 : 9); HPLC: R_T 2.95 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.27 (s, 3H), 3.29 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.51 (dd, *J* = 17.6, 12.0 Hz, 1H), 5.55 (dd, *J* = 11.6, 4.4 Hz, 1H), 6.22 (d, *J* = 8.4 Hz, 2H), 6.83 (ddd, *J* = 8.8, 8.8, 1.2 Hz, 1H), 7.09 - 7.17 (m, 3H), 7.23 (d, *J* = 2.4 Hz, 1H), 9.27 (s, 1H) : ^{13}C-NMR ($CDCl_3$, 100 MHz) 21.9, 53.2, 107.2, 110.5, 113.4, 117.8, 117.9, 129.7, 132.3, 141.9, 152.3, 154.5, 157.6, 168.6 ppm.

3.2 1-[3-(3-Hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (13)

Following the general method, compound **2** (0.30 g, 1.18 mmol) and hydrazine H_2O(65%) (0.23 mL, 4.72 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **13** (0.18 g, 48.1%) as a pale orange solid. m.p. 209 - 210 ℃; R_f 0.19 (MeOH : chloroform = 1 : 9); HPLC: R_T 3.58 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.28 (s, 3H), 3.01 (dd, *J* = 17.6, 4.4 Hz, 1H), 3.60 (dd, *J* = 17.6, 11.6 Hz, 1H), 3.67 (s, 3H), 5.41 (dd, *J* = 12.0, 4.8 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 2H), 6.83 (ddd, *J* = 8.0, 1.6, 1.6 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 2H), 7.08 - 7.13 (m, 2H), 7.15 (d, *J* = 8.0 Hz, 1H), 8.89 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 21.9, 42.4, 55.2, 59.3, 113.4, 114.1, 117.8, 117.8, 126.8, 129.7, 132.5, 134.1, 154.2, 157.5, 158.9, 168.6 ppm.

3.3 1-[3-(3-Hydroxyphenyl)-5-(4-methoxynaphthalen-1-yl)-4,5-dihydropyrazol-1-yl]-ethanone (14)

Following the general method, compound **3** (0.10 g, 0.34 mmol) and hydrazine H_2O(65%) (0.06 mL, 1.31 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **14** (46 mg, 29.1%) as a yellow solid. m.p. 280 - 282 ℃; R_f 0.16 (MeOH : chloroform = 1 : 9); HPLC: R_T 6.33 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.37 (s, 3H), 2.95 (dd, *J* = 17.2, 4.4 Hz, 1H), 3.75 (dd, *J* = 17.2, 11.6 Hz, 1H), 3.83 (s, 3H), 6.06 (dd, *J* = 11.6, 4.4 Hz, 1H), 6.59 (d, *J*= 8.0 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 7.09 - 7.10 (m, 1H), 7.37 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.45 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.89 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 21.9, 42.2, 55.4, 103.1, 113.3, 117.7, 122.6, 122.9, 124.9, 126.1, 126.7, 127.9, 129.5, 130.3, 136.4, 154.8, 154.9, 157.4, 168.7 ppm.

3.4 1-[5-(4-Chlorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (15)

Following the general method, Compound **4** (0.30 g, 1.16 mmol) and hydrazine H_2O(65%) (0.22 mL, 4.64 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **15** (0.29 g, 97.5%) as a yellow solid. m.p. 170 - 172 ℃; R_f 0.15 (MeOH : chloroform = 1 : 9); HPLC: R_T 3.40 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.21 (s, 3H), 2.91 (dd, *J* = 17.6, 4.4 Hz, 1H), 4.57 (dd, *J* = 17.6, 12.4 Hz, 1H), 5.35 (dd, *J* = 11.6, 4.8 Hz, 1H), 6.75 (dd, *J* = 8.0, 2.4 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 2H), 7.06 (dd, *J* = 8.0, 2.4 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 8.91 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.0, 42.2, 55.3, 113.6, 118.1, 118.1, 124.6, 124.7, 126.8, 129.8, 132.4, 144.4, 154.4, 157.8, 169.0 ppm.

3.5 1-[5-(4-Hydroxyphenyl)-3-(3-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (16)

Following the general method, compound **5** (0.30 g, 1.25 mmol) and hydrazine H_2O(65%) (0.24 mL, 5.00 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **16** (0.19 g, 51.6%) as a pale yellow solid. m.p. 220 - 221 ℃; R_f 0.13 (MeOH : chloroform = 1 : 9); HPLC: R_T 2.32 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.19 (s, 3H), 2.92 (dd, *J* = 8.8, 1.2 Hz, 1H), 3.50 (dd, *J* = 18.0, 12.0 Hz, 1H), 5.30 (dd, *J* = 15.6, 4.4 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 2H), 6.74 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 7.07 - 7.09 (m, 1H), 8.55 (s, 1H), 8.87 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 21.8, 42.3, 59.2, 113.2, 115.5, 117.6, 117.6, 126.6, 129.5, 132.5, 132.6, 154.0, 156.5, 157.4, 168.3 ppm.

3.6 1-[5-Furan-2-yl-3-(2-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (17)

Following the general method, compound **8** (0.80 g, 3.73 mmol) and hydrazine H_2O(65%) (0.74 mL, 14.9 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 :

15) to give compound **17** (0.91 g, 90.3%) as a pale pink solid. m.p. 132 - 133 ℃; R_f 0.10 (MeOH : chloroform = 1 : 9); HPLC: R_T 5.92 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.36 (s, 3H), 3.58 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.69 (dd, *J* = 17.6, 3.2 Hz, 1H), 5.67 (dd, *J* = 11.6, 4.8 Hz, 1H), 6.32 - 6.37 (m, 2H), 6.96 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 7.31 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.37 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 10.19 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.3, 38.8, 52.1, 108.4, 110.9, 115.3, 117.3, 120.0, 128.6, 132.5, 142.4, 151.5, 156.7, 157.9, 168.1 ppm.

3.7 1-[3-(2-Hydroxyphenyl)-5-(4-methoxynaphthalen-1-yl)-4,5-dihydropyrazol-1-yl]-ethanone (18)

Following the general method, compound **7** (0.30 g, 3.94 mmol) and hydrazine H_2O(65%) (0.19 mL, 3.94 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **18** (91 mg, 21.8%) as a yellow solid. m.p. 203 - 204 ℃; R_f 0.17 (MeOH : chloroform = 1 : 9); HPLC: R_T 11.52 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.50 (s, 3H), 3.27 (dd, *J* = 17.6, 4.4 Hz, 1H), 3.97 (s, 3H), 4.04 (dd, *J* = 19.2, 8.8 Hz, 1H), 6.22 (dd, *J* = 11.6, 4.8 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.87 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.06 (d, *J* = 8.8 Hz, 1H), 7.14 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 2H), 7.33 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.52 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 7.60 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 10.35 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.9, 43.4, 56.3, 103.9, 116.0, 117.8, 120.5, 122.7, 123.2, 124.0, 125.9, 127.2, 127.7, 128.0, 129.2, 131.1, 133.0, 156.1, 157.9, 158.5, 168.6 ppm.

3.8 1-[5-(4-Hydroxyphenyl)-3-(2-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (19)

Following the general method, Compound **9** (0.30 g, 1.25 mmol) and hydrazine H_2O(65%) (0.24 mL, 4.99 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **19** (0.22 g, 60.4%) as a yellow solid. m.p. 260 - 262 ℃; R_f 0.20 (MeOH : chloroform = 1 : 9); HPLC: R_T 3.64 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.20 (s, 3H), 3.13 (dd, *J* = 18.0, 4.4 Hz, 1H), 3.68 (dd, *J* = 18.0, 12.0 Hz, 1H), 5.33 (dd, *J* = 11.6, 4.4 Hz, 1H), 6.63 (d, *J* = 8.0 Hz, 2H), 6.78 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 2H) 7.09 (d, *J*= 8.0 Hz, 1H), 7.18 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 8.72 (s, 1H), 10.12 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 21.8, 42.5, 57.7, 115.0, 115.5, 116.6, 119.4, 126.5, 128.3, 131.7, 131.8, 156.2, 156.7, 157.3, 167.1 ppm.

3.9 1-[5-(4-Chlorophenyl)-3-(2-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (20)

Following the general method, compound **10** (0.30 g, 1.16 mmol) and hydrazine H_2O(65%) (0.22 mL, 4.64 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **20** (0.34 g, 94.4%) as a yellow solid. m.p. 143 - 144 ℃; R_f 0.42 (MeOH : chloroform = 1 : 9); HPLC: R_T 9.46 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.39 (s, 3H), 3.27 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.87 (dd, *J* = 18.0, 12.0 Hz, 1H), 5.53 (dd, *J* = 6.8, 4.8 Hz, 1H), 6.94 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 2H), 7.20 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.37 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 2H), 10.20 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.3, 42.8, 58.1, 115.2, 117.4, 120.0, 127.3, 128.6, 129.4, 132.7, 134.0, 139.9, 156.4, 158.0, 168.0 ppm.

3.10 1-[3-(2-Hydroxyphenyl)-5-thiophen-2-yl-4,5-dihydropyrazol-1-yl]-ethanone (21)

Following the general method, compound **11** (0.30 g, 1.25 mmol) and hydrazine H_2O(65%) (0.24 mL, 4.99 mmol) were used. Purification was conducted with eluent (MeOH : chloroform

= 1 : 15) to give compound **21** (0.17 g, 48.9%) as a yellow solid. m.p. 120 - 122 ℃; R_f 0.76 (MeOH : chloroform = 1 : 9); HPLC: R_T 6.37 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.37 (s, 3H), 3.49 (dd, *J* =17.6, 4.0 Hz, 1H), 3.83 (dd, *J* = 17.6, 11.2 Hz, 1H), 5.89 (dd, *J* = 11.6, 4.0 Hz, 1H), 6.94 (d, *J*= 8.0 Hz, 2H), 7.05 (d, *J*= 8.0 Hz, 2H), 7.21 (d, *J* = 6.8 Hz, 1H), 7.27 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 1H), 7.37 (ddd, *J* = 8.8, 8.4, 1.2 Hz, 1H), 10.20 (s, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.3, 42.6, 54.0, 115.3, 117.4, 120.0, 125.2, 125.3, 127.1, 128.6, 132.6, 143.7, 156.5, 158.0, 168.1 ppm.

3.11 1-[3-(2-Hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-ethanone (22)

Following the general method, compound **6** (0.30 g, 1.18 mmol) and hydrazine H_2O(65%) (0.23 mL, 4.72 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **22** (0.34 g, 93.9%) as a pale yellow solid. m.p. 139 - 140 ℃; R_f 0.24 (MeOH : chloroform = 1 : 9); HPLC: R_T 6.87 min (purity: 99.9%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.37 (s, 3H), 3.30 (dd, *J* = 17.6, 4.4 Hz, 1H), 3.78 (s, 3H), 3.82 (dd, *J* = 18.0, 6.0 Hz, 1H), 5.53 (dd, *J* = 11.6, 4.8 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.94 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 1H), 7.36 (ddd, *J* = 8.0, 8.0, 0.8 Hz, 1H), 10.29 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 22.3, 42.3, 55.5, 58.2, 114.6, 115.4, 117.3, 120.0, 127.2, 128.6, 132.5, 133.6, 156.6, 157.9, 159.5, 168.0 ppm.

4. General synthetic method for *N*-propionylpyrazoline analogues

The reaction mixture of chalcone analogue and hydrazine·H_2O (65%) (4.0 equiv.) in propionic acid (10mL) was refluxed (2 h) and then cooled to room temperature. The reaction mixture was poured into ice and kept overnight at room temperature. Solid formed was filtered and washed

with water. Solid was dried under vacuum and then purified by silica gel column chromatography (eluent : MeOH : chloroform) to give desired product.

4.1 1-[3-(3-Hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-propan-1-one (23)

Following the general method, compound **6** (0.30 g, 0.98 mmol) and hydrazine H_2O(65%) (0.23 mL, 4.72 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **23** (93.2 mg, 24.4%) as a white color solid. m.p. 233 - 234 ℃; R_f 0.19 (MeOH : chloroform = 1 : 9); HPLC: R_T 5.59 min (purity: 98.6%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.05 (t, *J* = 7.6, 3H), 2.38 (s, 3H), 2.66 (dd, *J* = 14.8, 4.6 Hz, 2H), 2.99 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.57 (dd, *J* = 18.0, 12.0 Hz, 1H), 5.38 (dd, *J* = 11.6, 4.8 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 2H), 6.80 (ddd, *J* = 8.0, 1.6, 1.2 Hz, 1H), 7.03 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.14 (ddd, *J* = 8.0, 1.6, 1.2 Hz, 1H), 8.84 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 8.9, 27.5, 55.2, 59.4, 113.4, 114.1, 117.7, 117.8, 126.9, 129.6, 132.7, 134.4, 153.8, 157.5, 158.8, 172.0 ppm.

4.2 1-[3-(3-Hydroxyphenyl)-5-thiophen-2-yl-4,5-dihydropyrazol-1-yl]-propan-1-one (24)

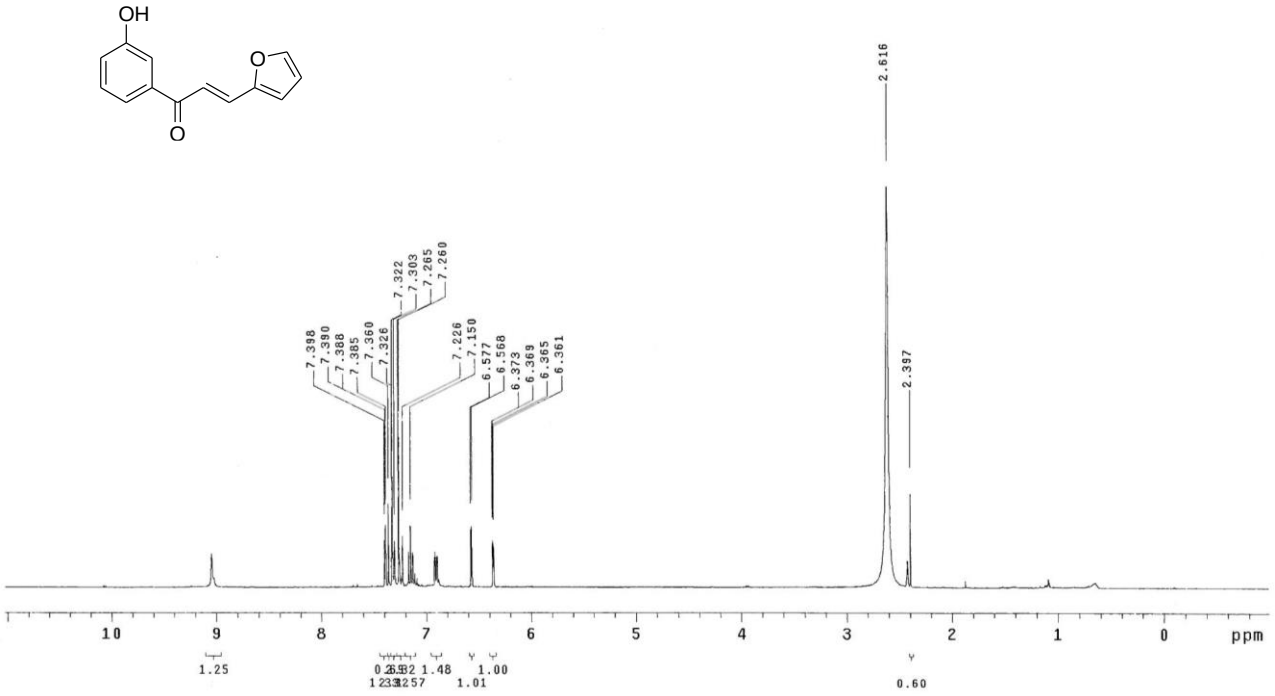
Following the general method, Compound **11** (0.30 g, 1.30 mmol) and hydrazine H_2O(65%) (0.25 mL, 5.21 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **24** (63.6 mg, 16.3%) as an orange color solid. m.p. 183 - 184 ℃; R_f 0.36 (MeOH : chloroform = 1 : 9); HPLC: R_T 5.21 min (purity: 98.1%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.08 (t, *J* = 7.6 Hz, 3H), 2.63 - 2.70 (m, 2H), 3.19 (dd, *J* = 17.6, 4.0 Hz, 1H), 5.33 (s, 1H), 5.77 (dd, *J* = 11.2, 4.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 2H), 6.90 - 6.91 (m, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 9.17 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 8.5, 26.9, 41.4, 49.0, 54.7, 112.7, 117.2, 123.7, 123.9, 126.2, 129.2, 131.8, 144.3, 153.4, 157.1, 171.2 ppm.

4.3 1-[5-(4-Chlorophenyl)-3-(3-hydroxyphenyl)-4,5-dihydropyrazol-1-yl]-propan-1-one (25)

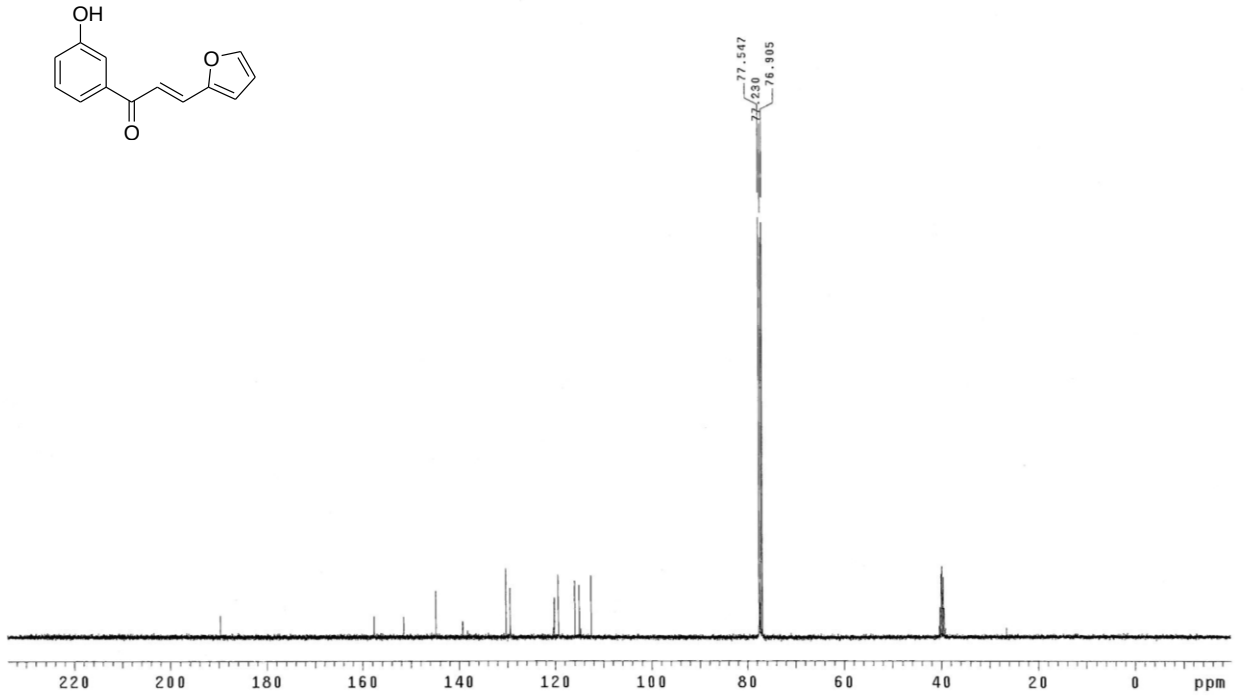
Following the general method, **compound 4** (0.30 g, 1.16 mmol) and hydrazine H_2O(65%) (0.22 mL, 4.64 mmol) were used. Purification was conducted with eluent (MeOH : chloroform = 1 : 15) to give compound **25** (0.12 g, 31.8%) as a white color solid. m.p. 206 - 207 ℃; R_f 0.26 (MeOH : chloroform = 1 : 9); HPLC: R_T 10.74 min (purity: 99.6%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.02 (t, *J* = 7.6, Hz, 3H), 2.64 (dd, *J* = 14.8, 7.6 Hz, 2H), 2.93 (dd, *J* = 17.6, 4.8 Hz, 1H), 3.58 (dd, *J* = 17.6, 1.2 Hz, 1H), 5.36 (dd, *J* = 12.4, 4.8 Hz, 1H), 6.78 (ddd, J = 8.0, 1.2, 1.2 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 7.03 - 7.06 (m, 1H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 8.89 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 8.8, 27.4, 59.3, 113.3, 117.6, 117.7, 127.0, 128.8, 129.6, 132.2, 133.0, 140.6, 153.7, 157.5, 172.0 ppm.

^{1}H NMR and ^{13}C NMR Spectra of all the final compounds

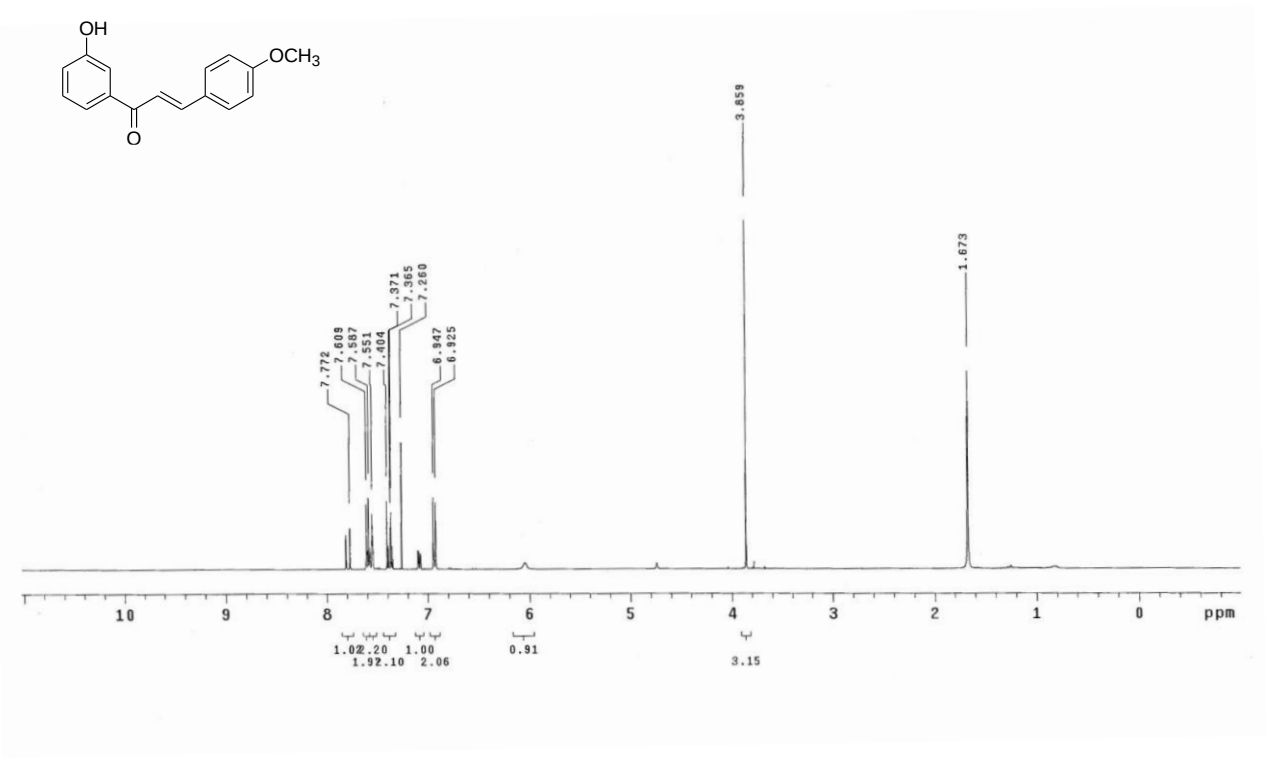
^{1}H NMR Spectrum of compound **1**



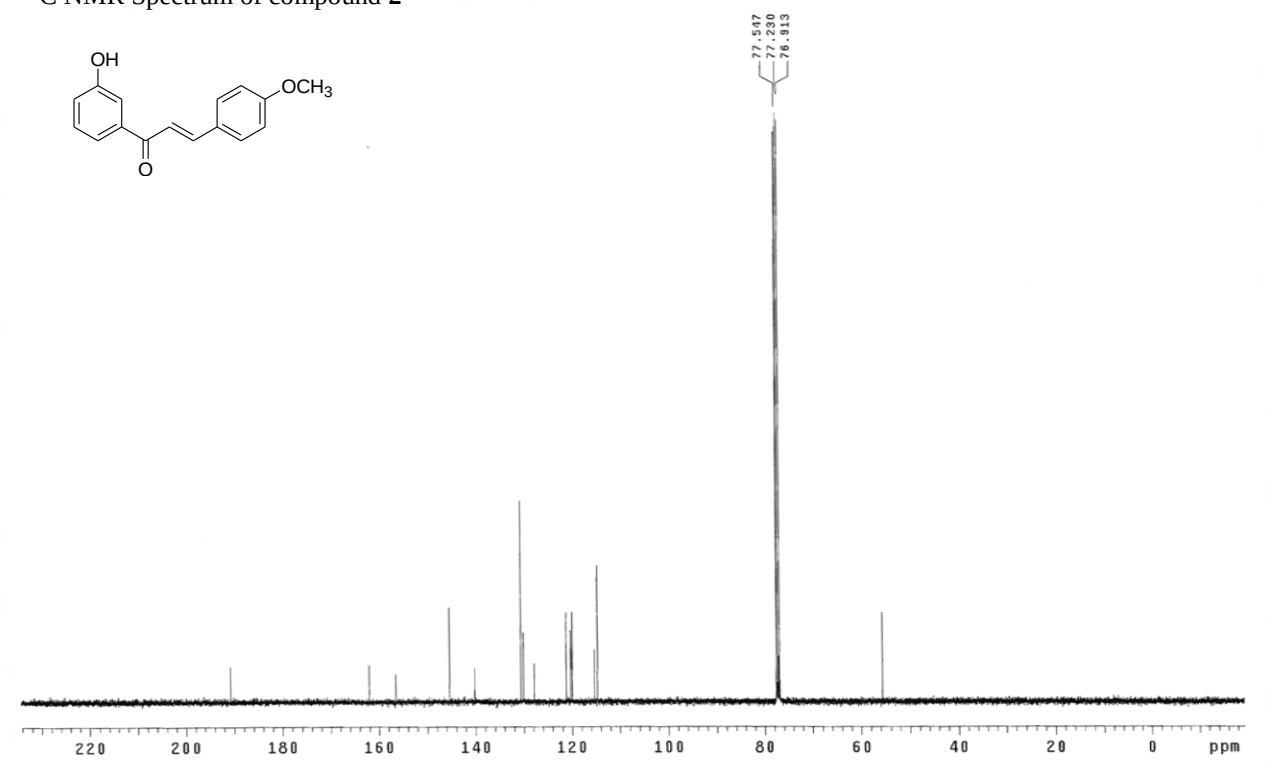
^{13}C NMR Spectrum of compound **1**



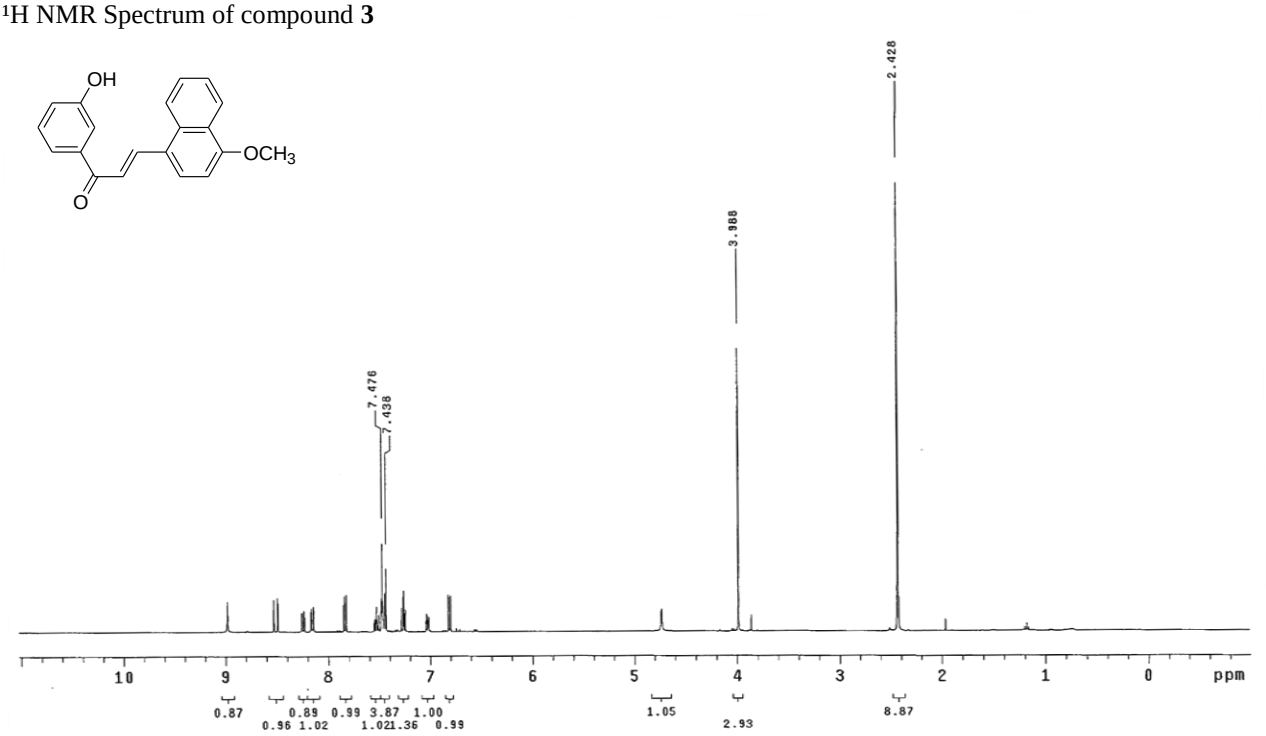
[1]H NMR Spectrum of compound **2**



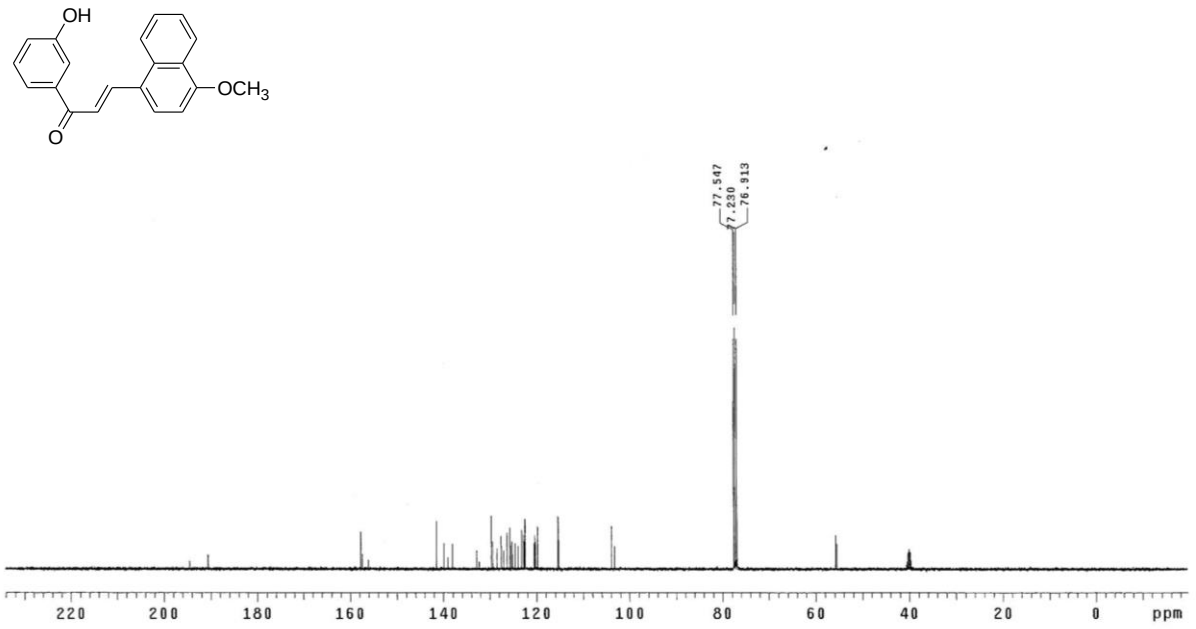
[13]C NMR Spectrum of compound **2**



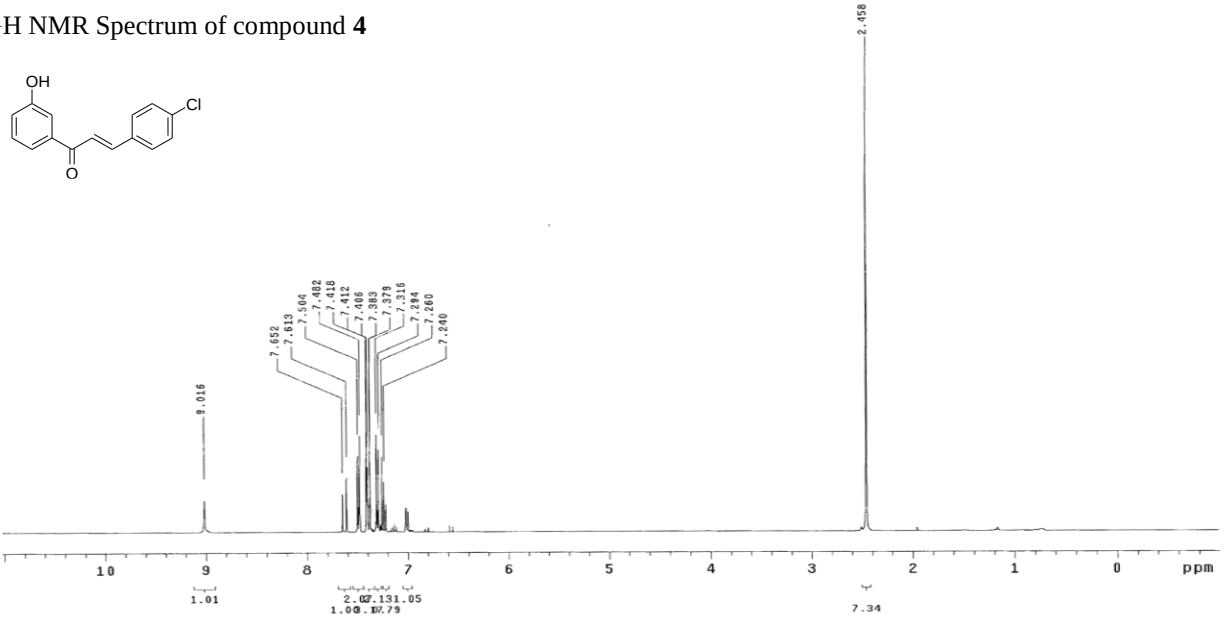
[1]H NMR Spectrum of compound **3**



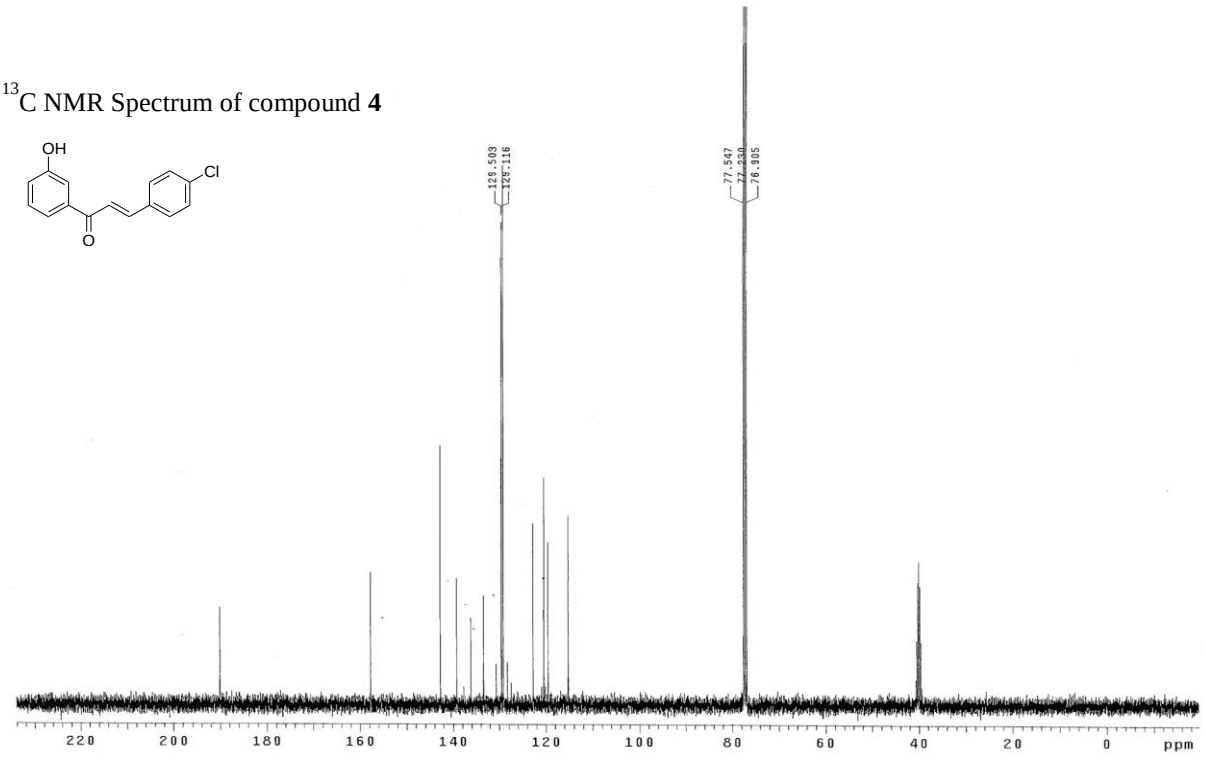
[13]C NMR Spectrum of compound **3**



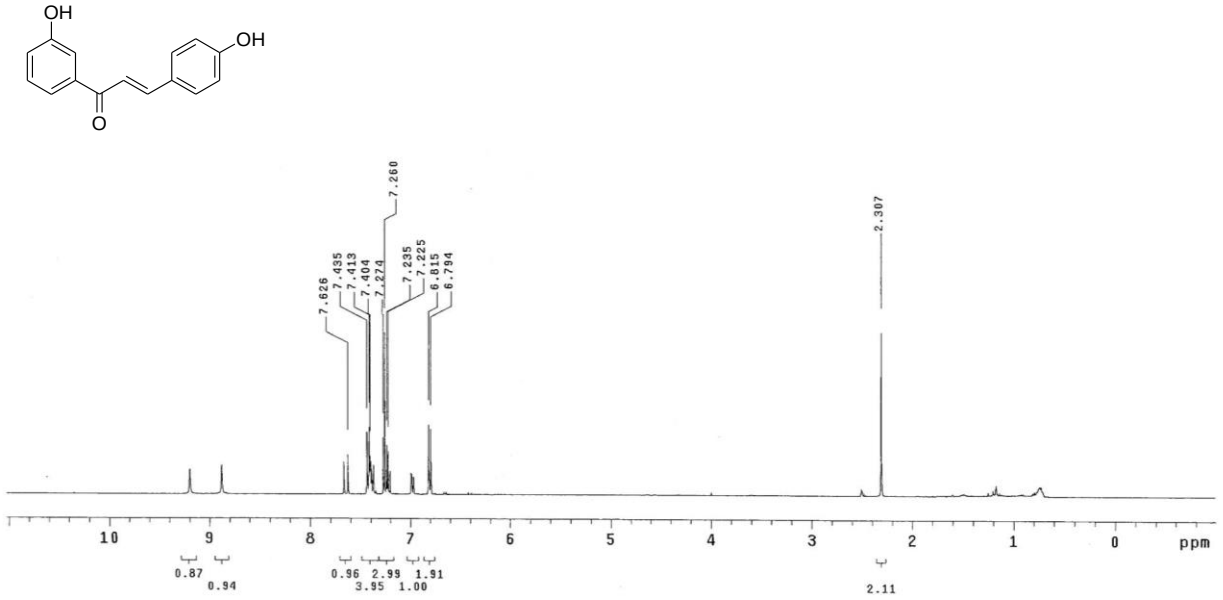
^{1}H NMR Spectrum of compound **4**



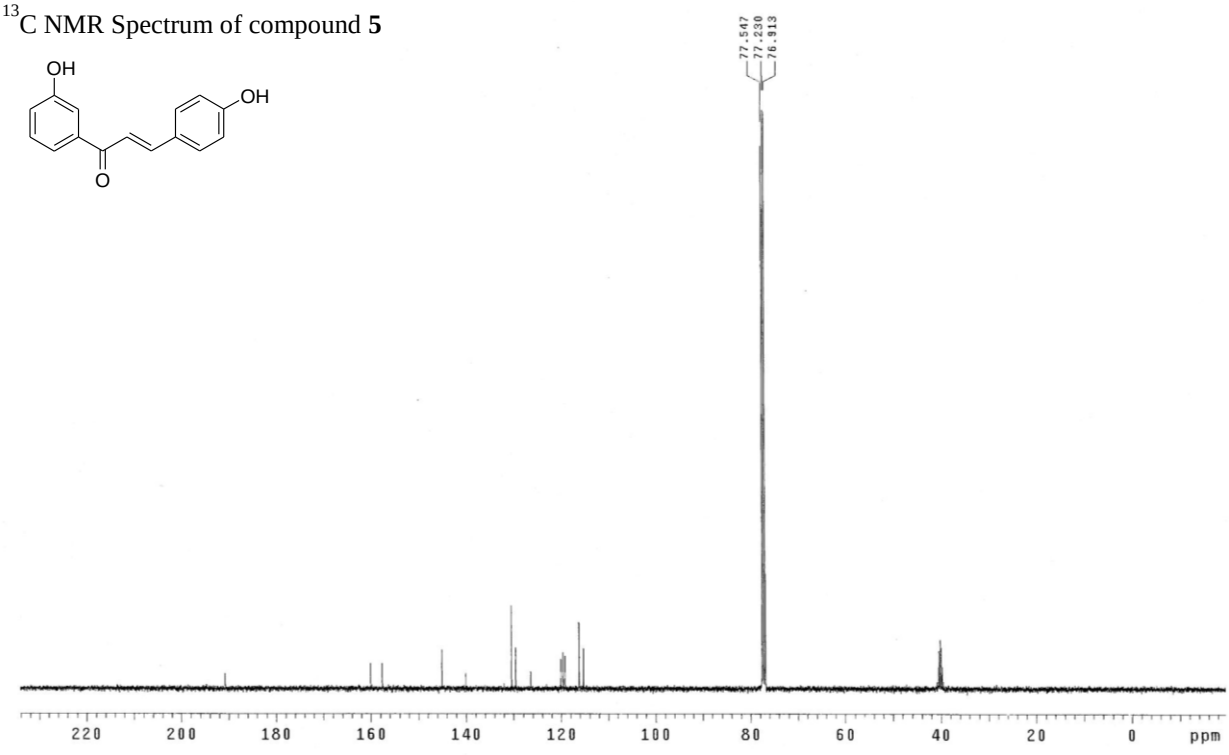
^{13}C NMR Spectrum of compound **4**



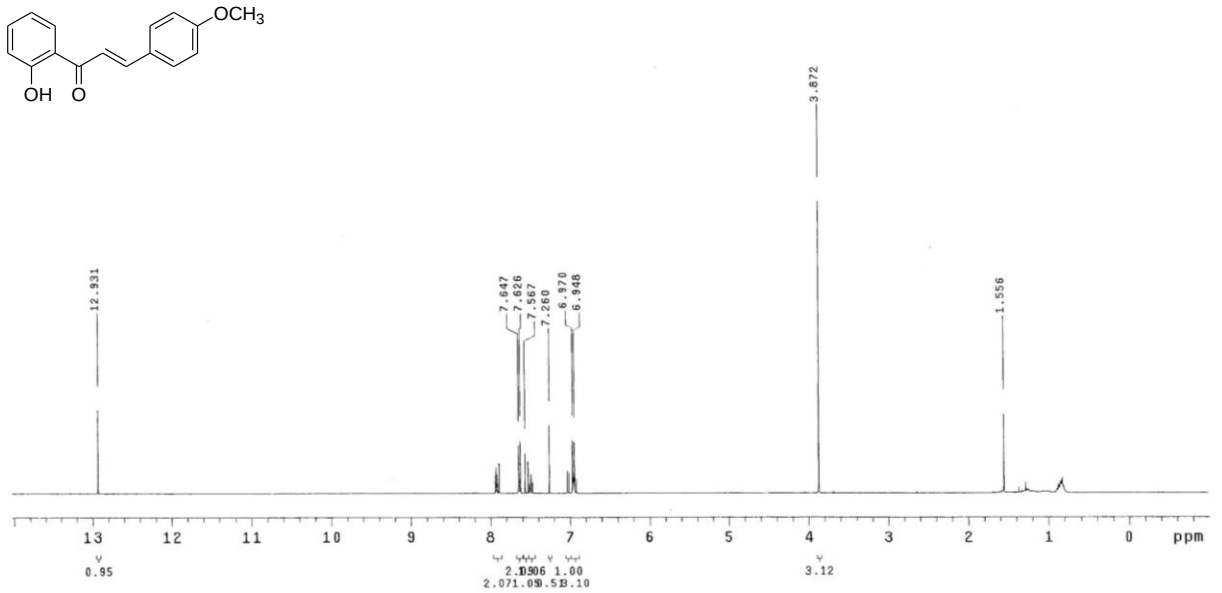
^{1}H NMR Spectrum of compound **5**



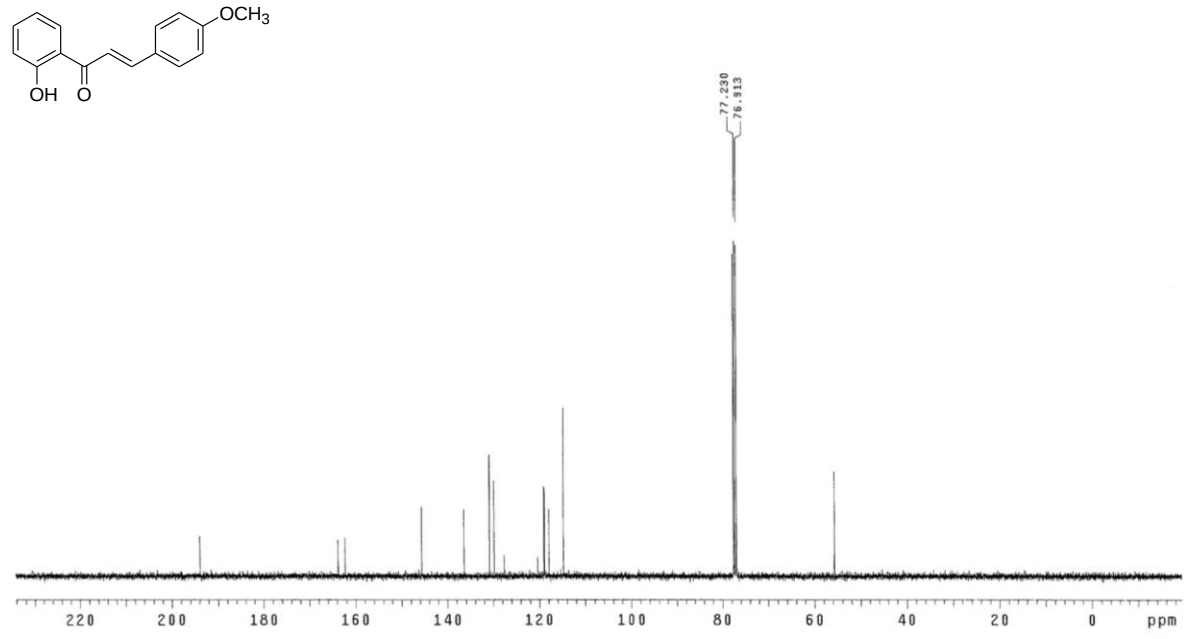
^{13}C NMR Spectrum of compound **5**



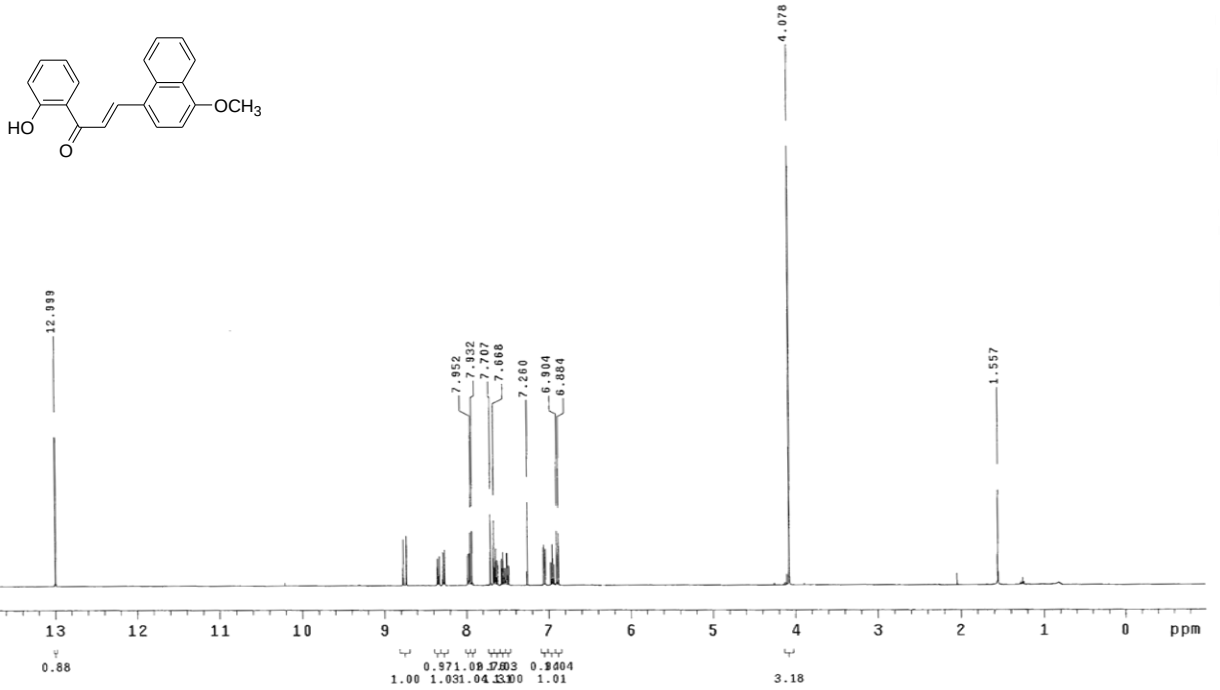
^{1}H NMR Spectrum of compound **6**



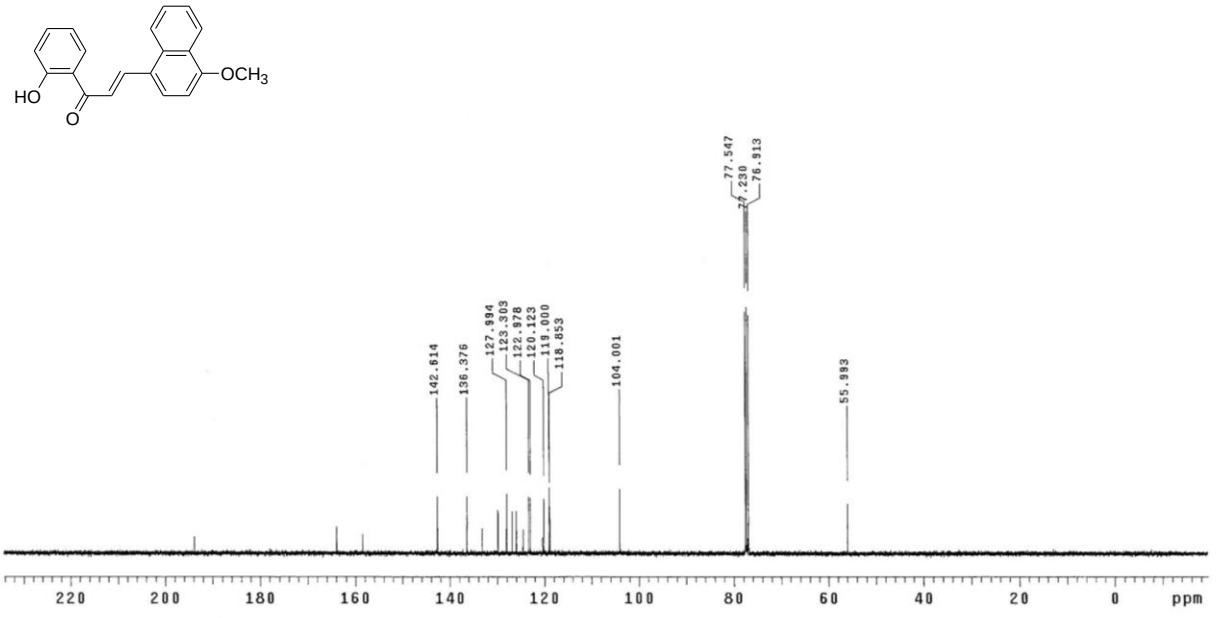
^{13}C NMR Spectrum of compound **6**



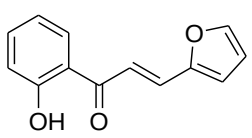
^{1}H NMR Spectrum of compound **7**



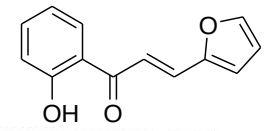
^{13}C NMR Spectrum of compound **7**

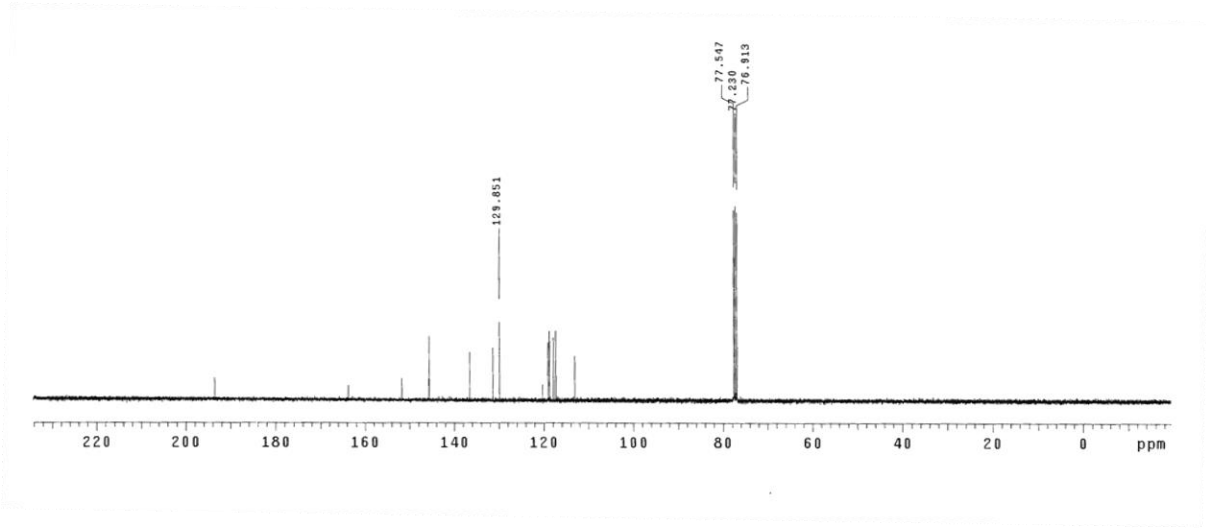


^{1}H NMR Spectrum of compound **8**

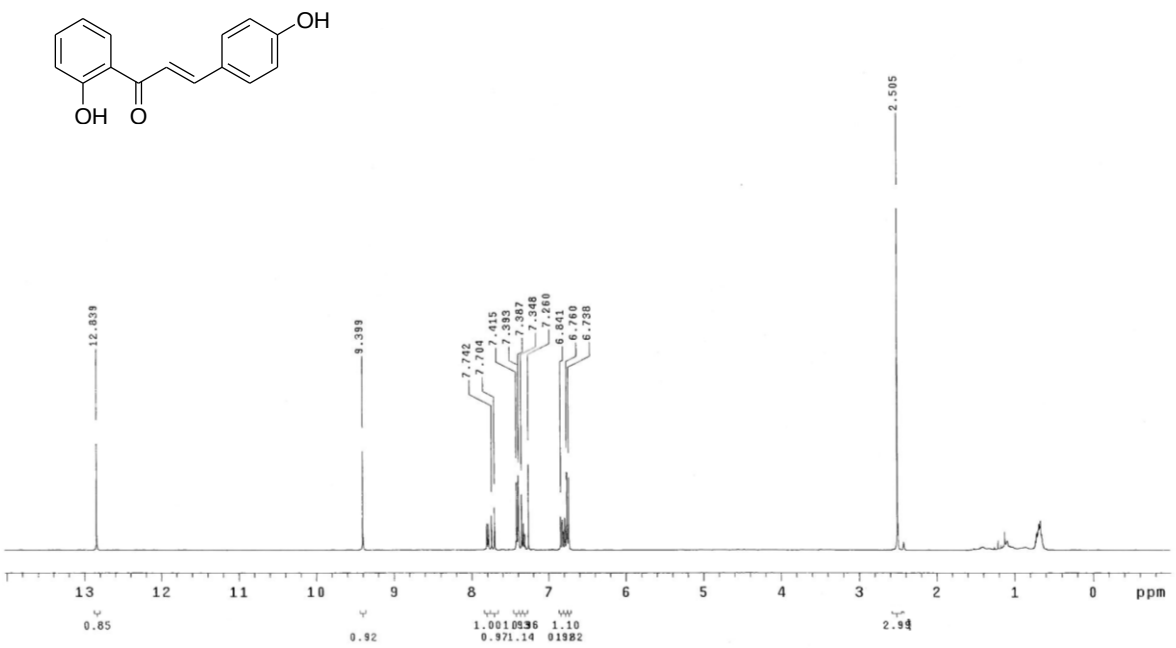


^{13}C NMR Spectrum of compound **8**

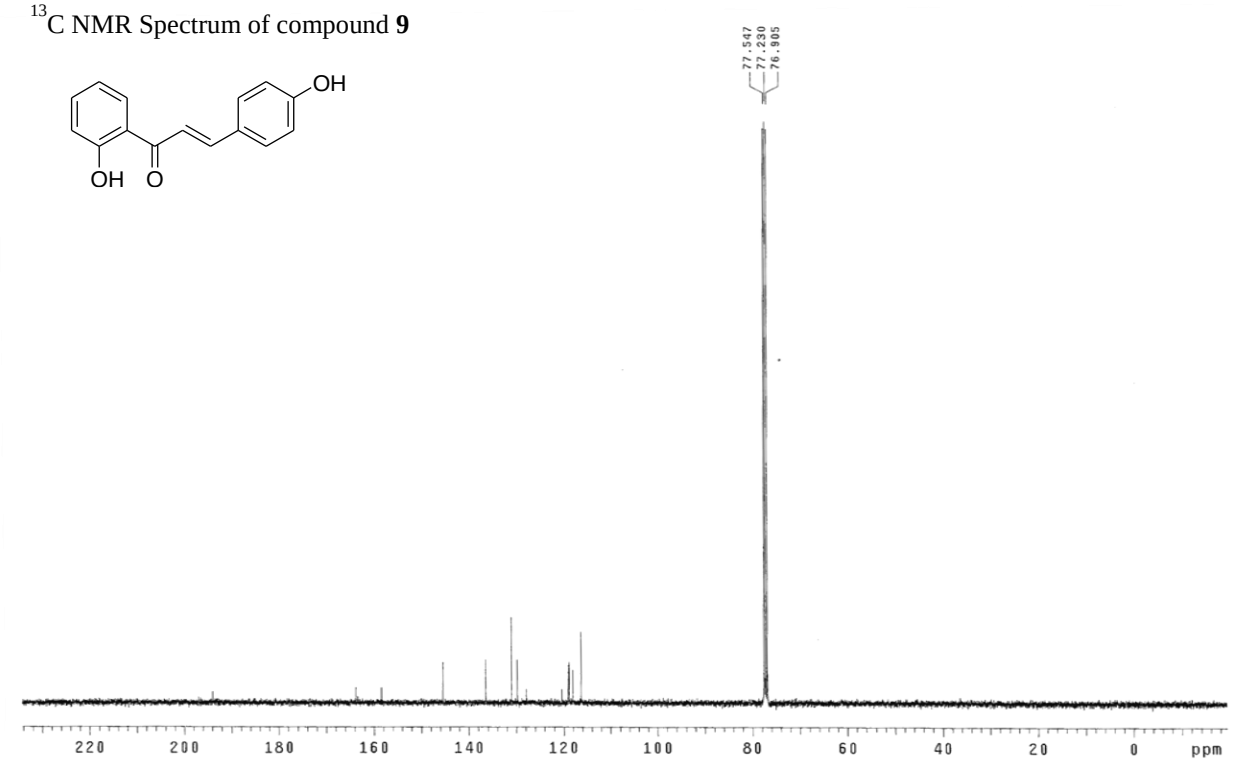




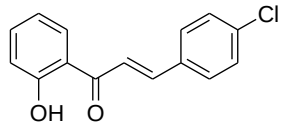
^{1}H NMR Spectrum of compound **9**

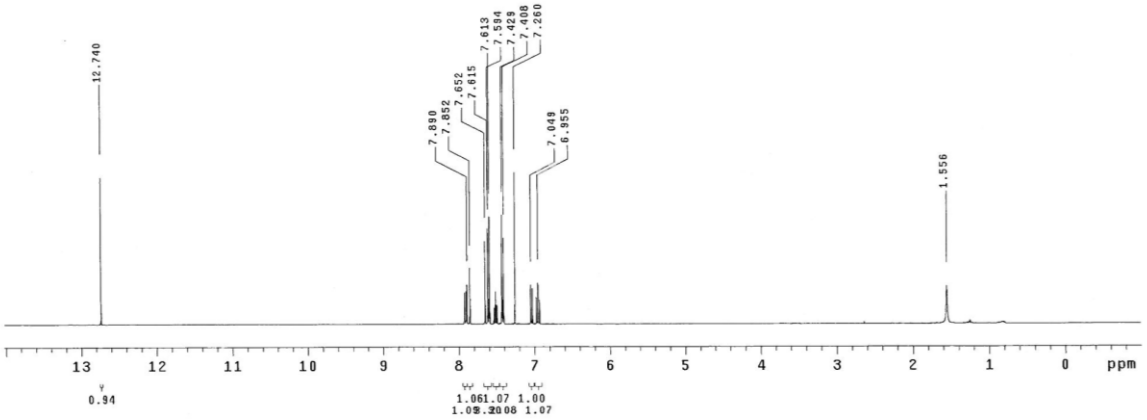


^{13}C NMR Spectrum of compound **9**

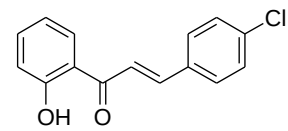


[1]H NMR Spectrum of compound **10**

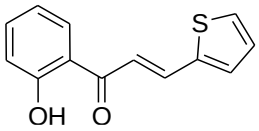


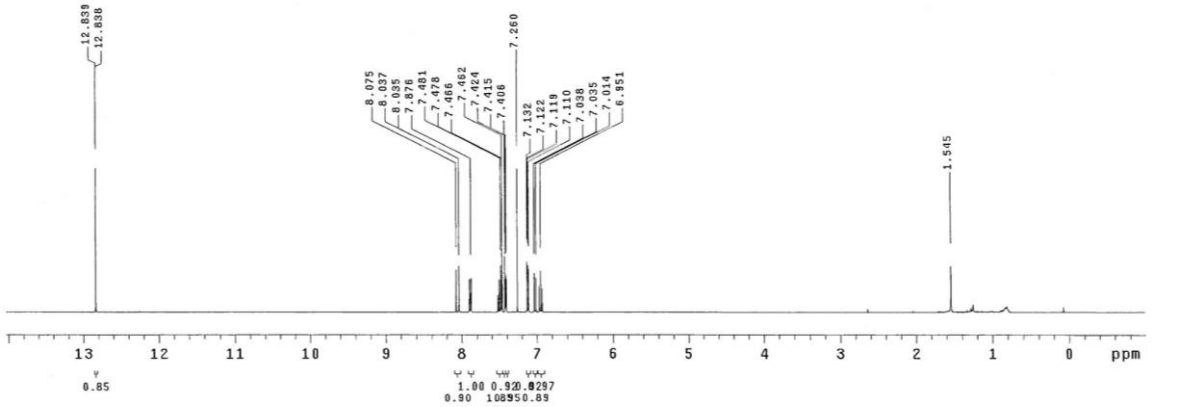


[13]C NMR Spectrum of compound **10**

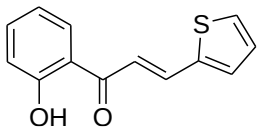


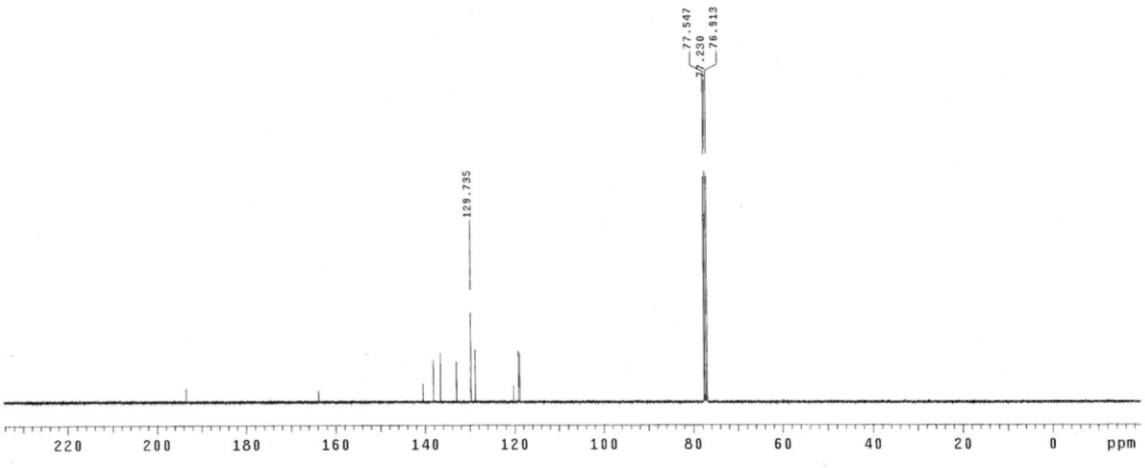
^{1}H NMR Spectrum of compound **11**



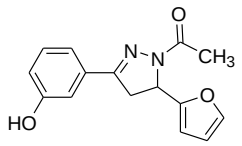


^{13}C NMR Spectrum of compound **11**

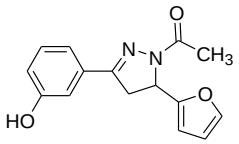




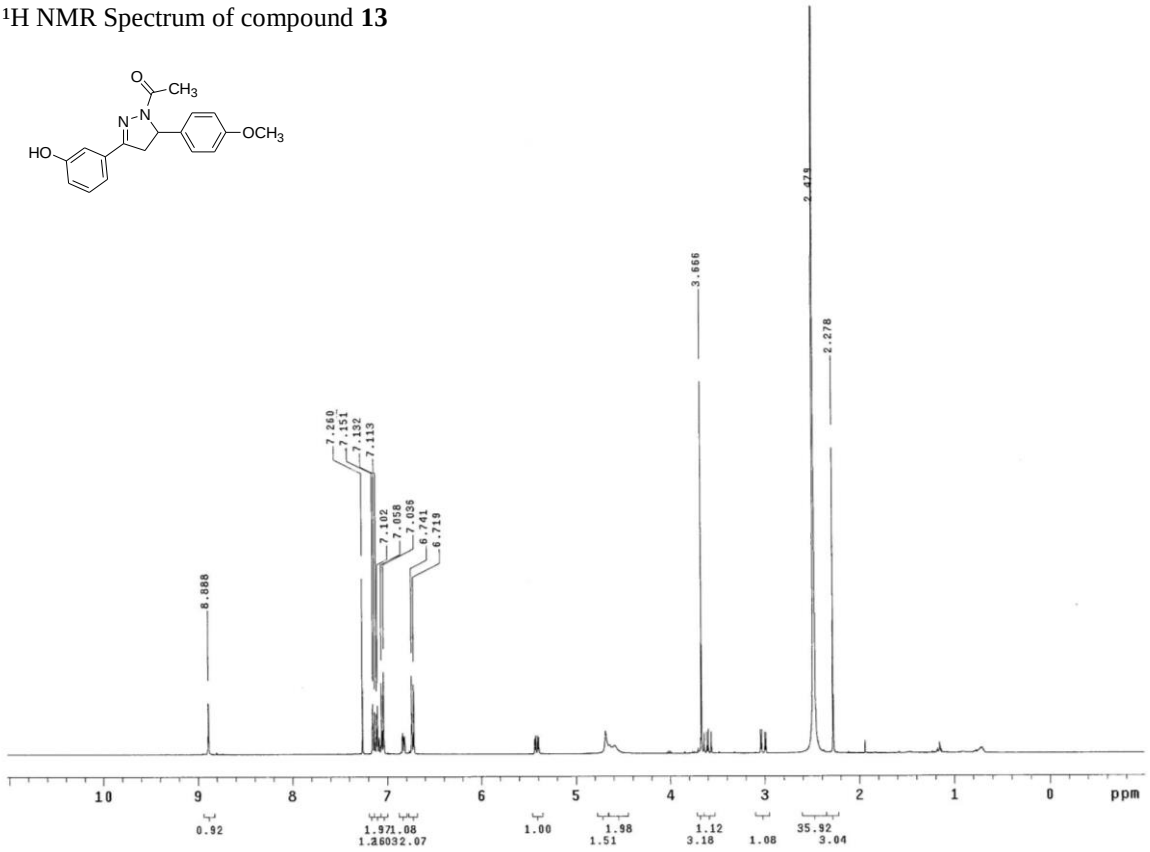
^{1}H NMR Spectrum of compound **12**



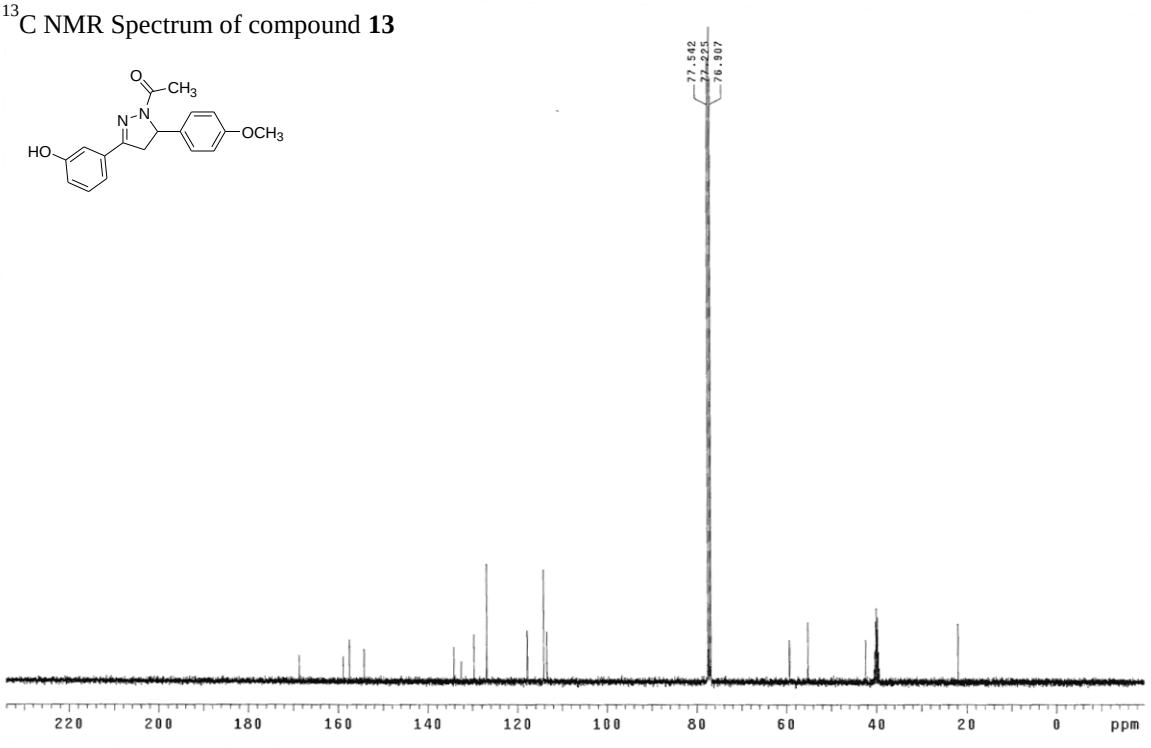
^{13}C NMR Spectrum of compound **12**



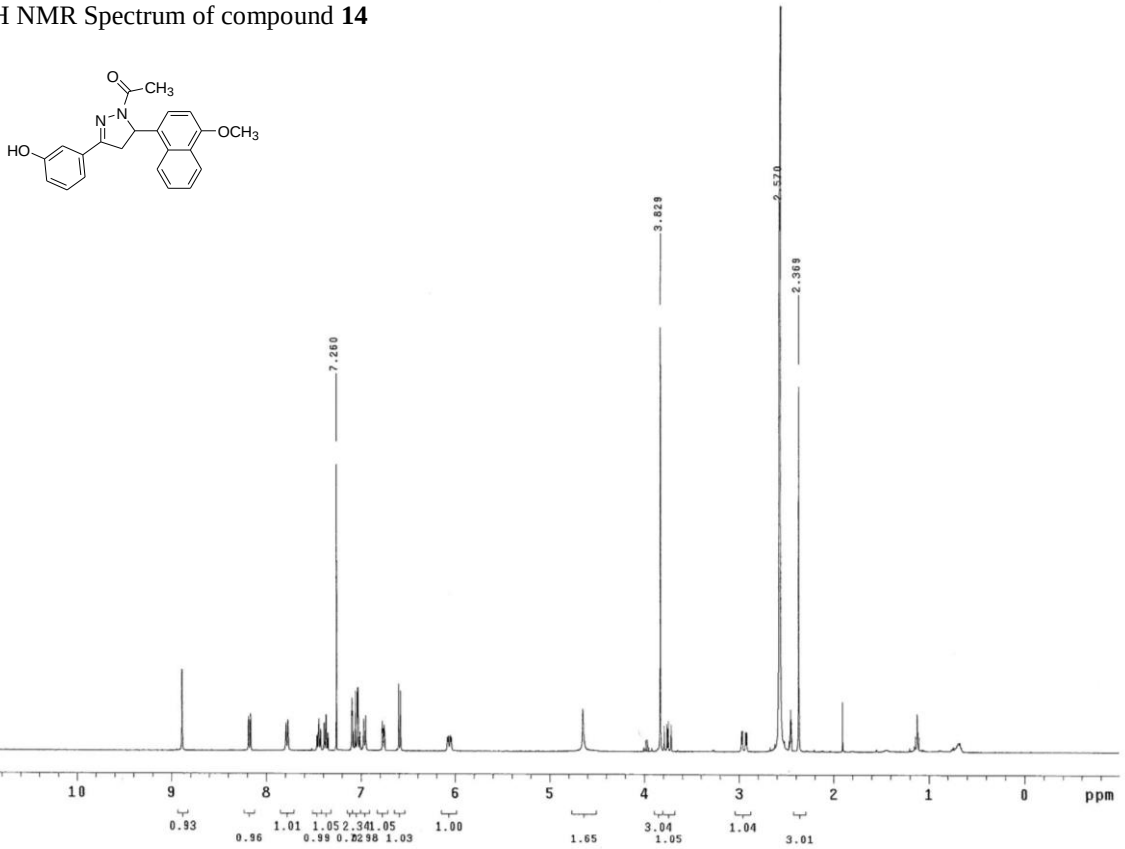
^{1}H NMR Spectrum of compound **13**



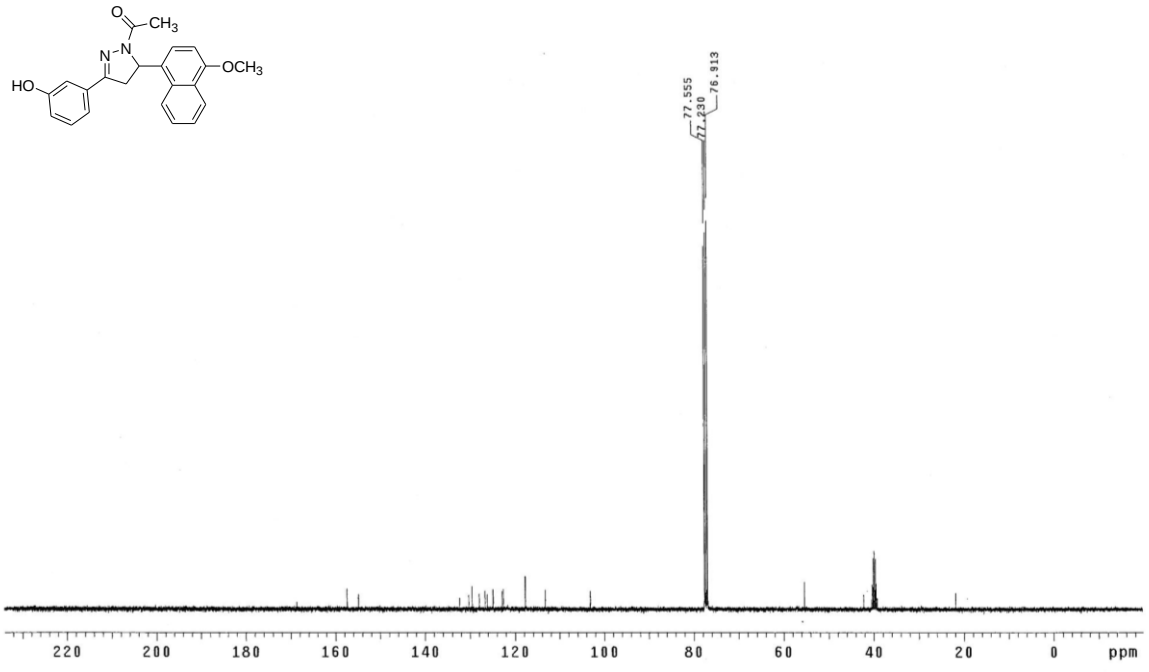
^{13}C NMR Spectrum of compound **13**



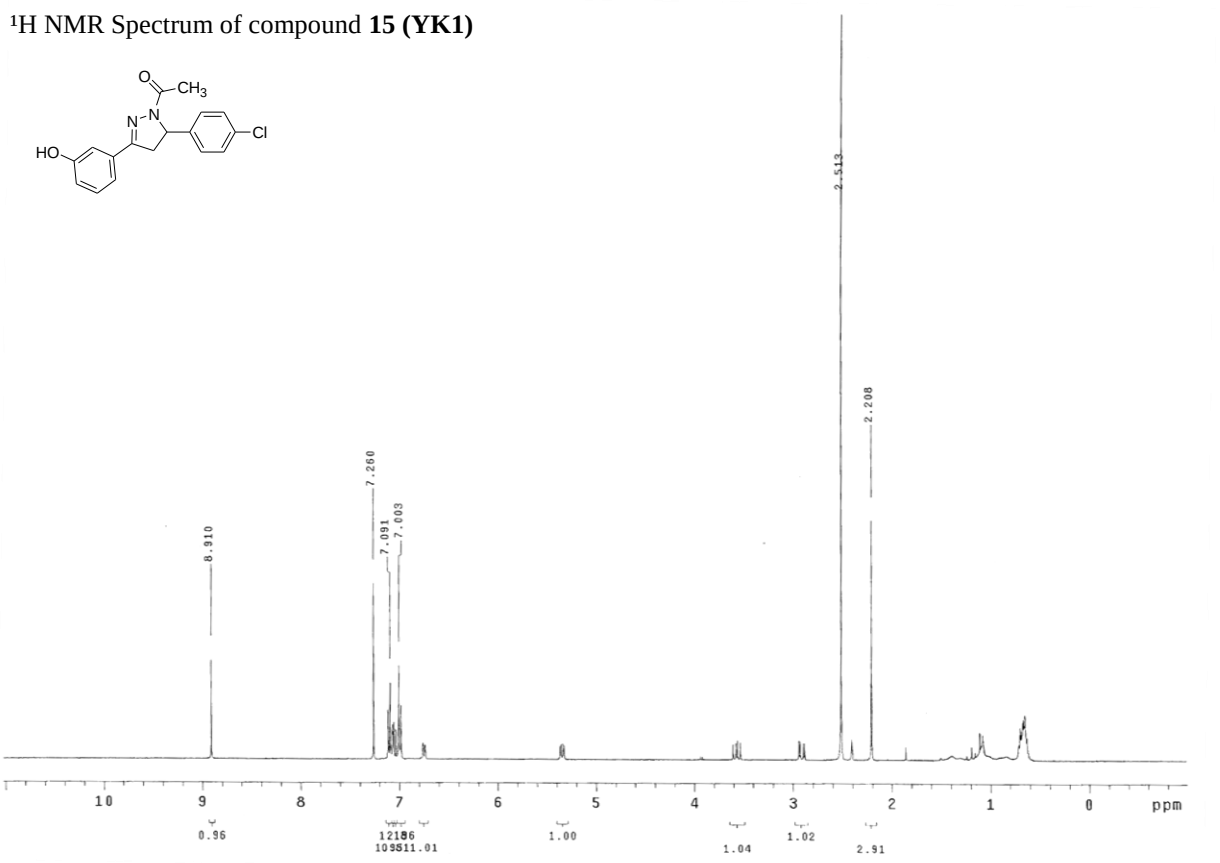
[1]H NMR Spectrum of compound **14**



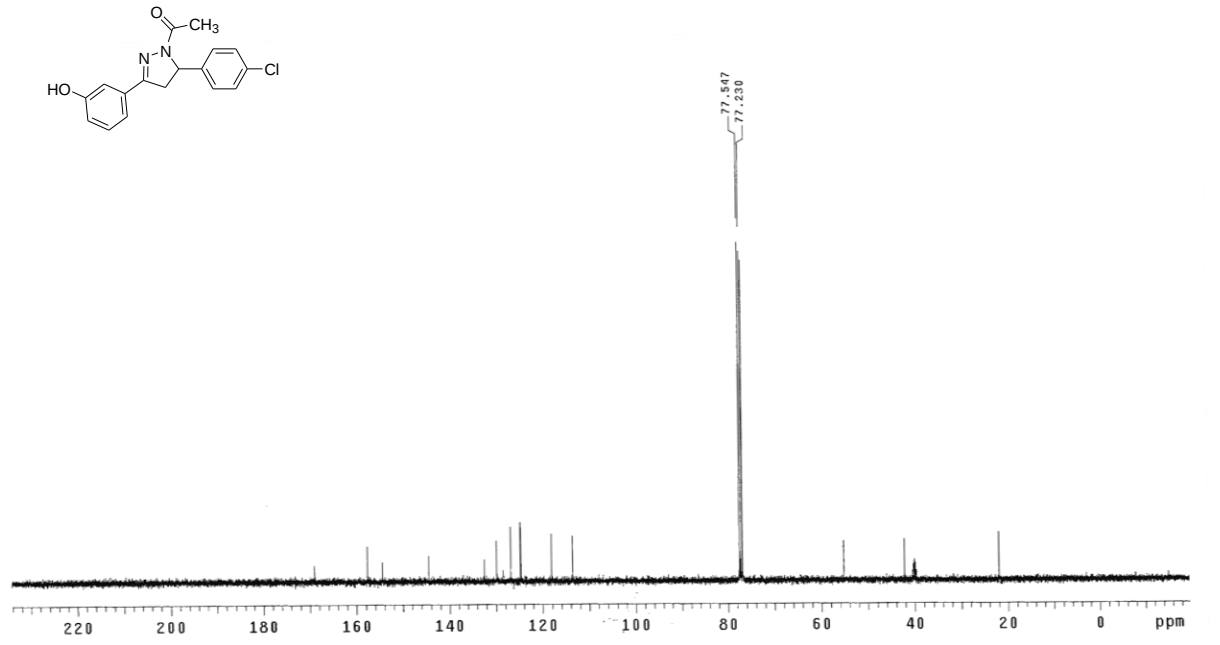
[13]C NMR Spectrum of compound **14**



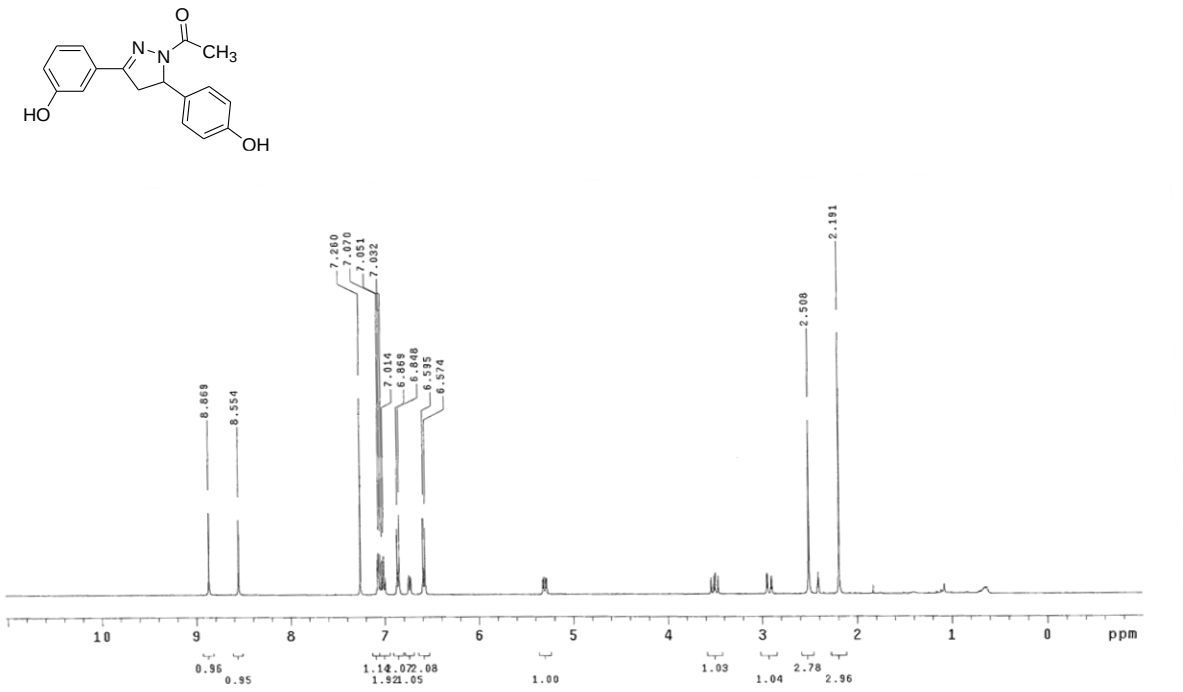
^{1}H NMR Spectrum of compound **15 (YK1)**



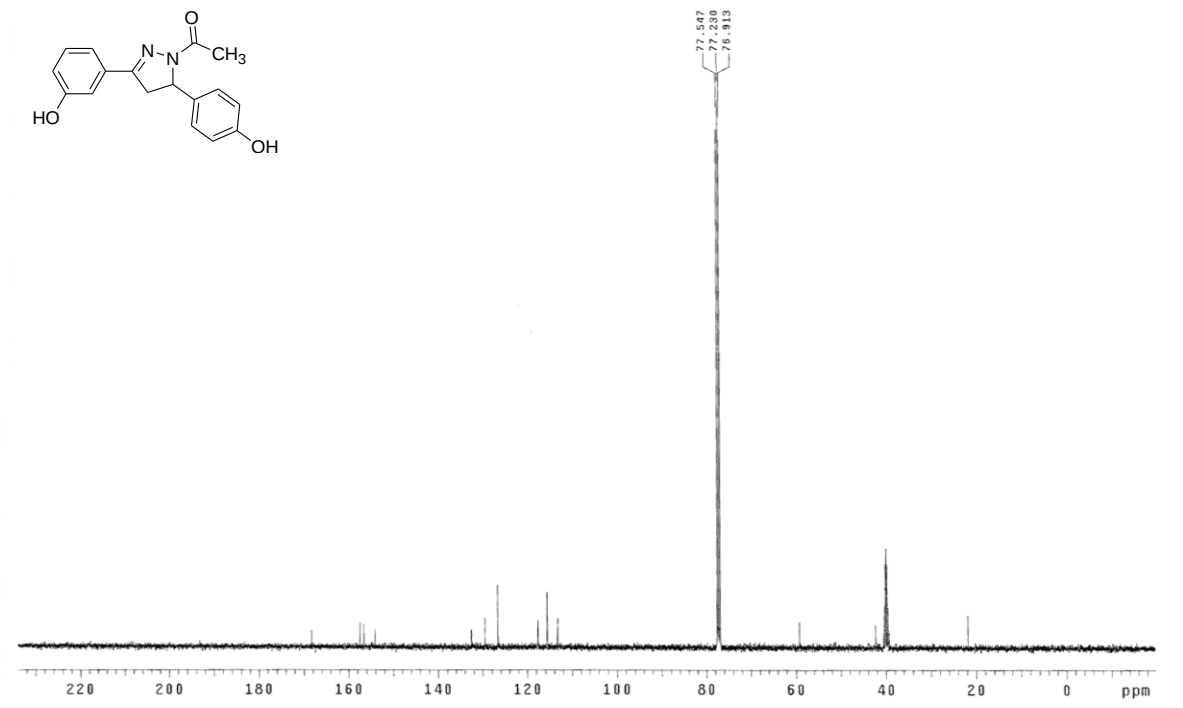
^{13}C NMR Spectrum of compound **15 (YK1)**



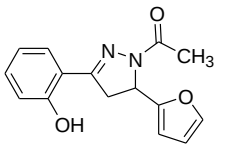
^{1}H NMR Spectrum of compound **16**



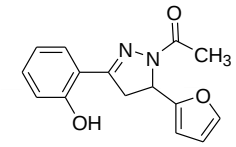
^{13}C NMR Spectrum of compound **16**

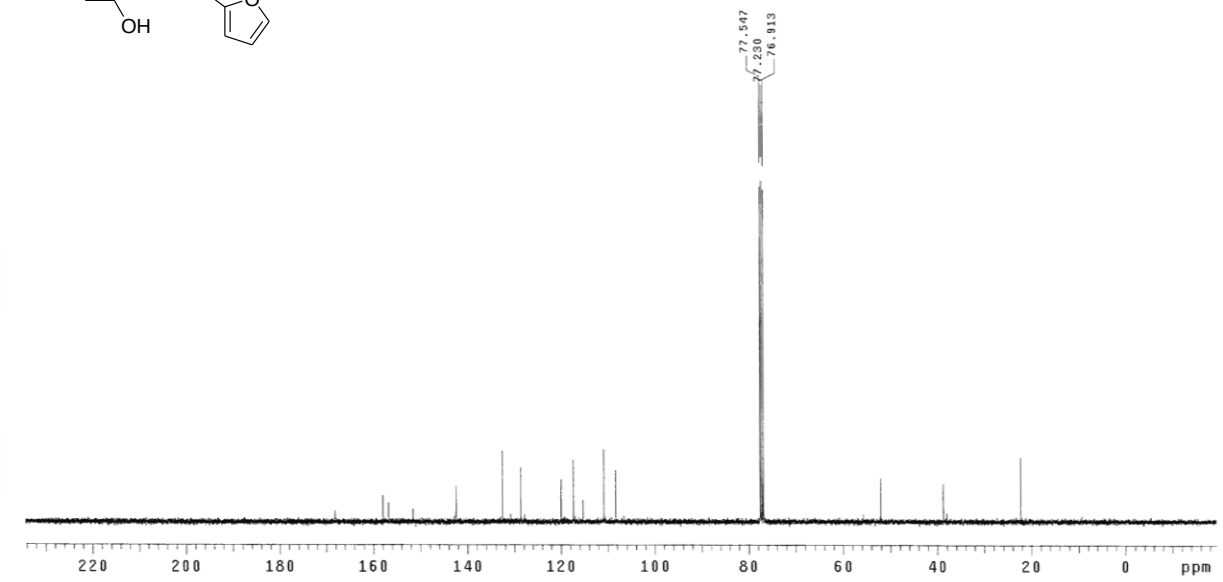


^{1}H NMR Spectrum of compound **17**

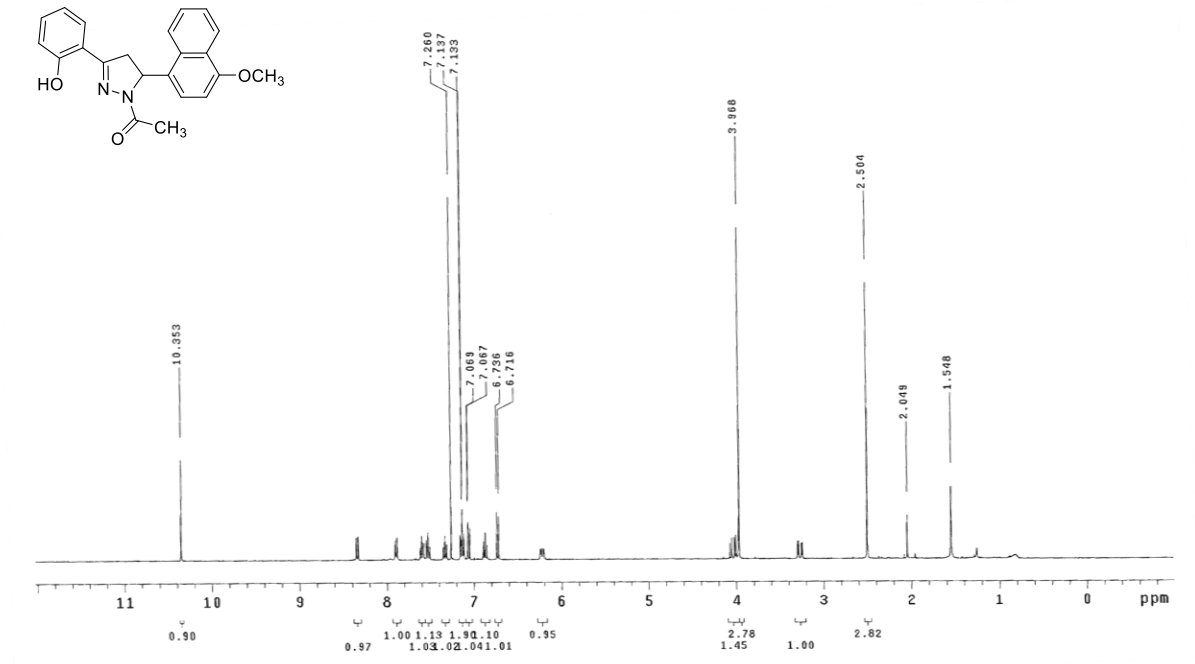


^{13}C NMR Spectrum of compound **17**

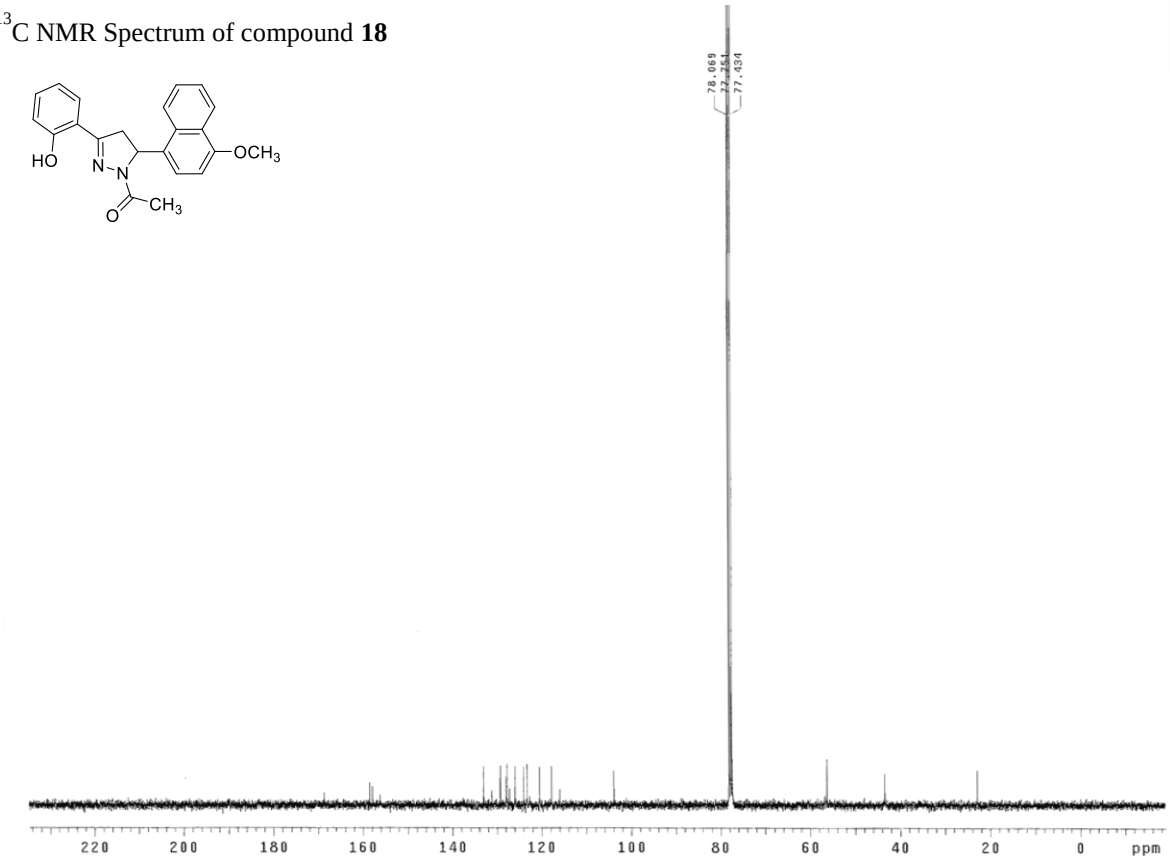




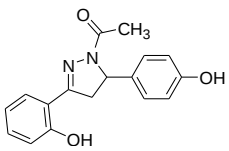
^{1}H NMR Spectrum of compound **18**



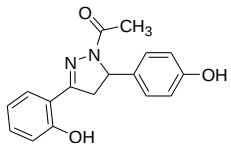
^{13}C NMR Spectrum of compound **18**



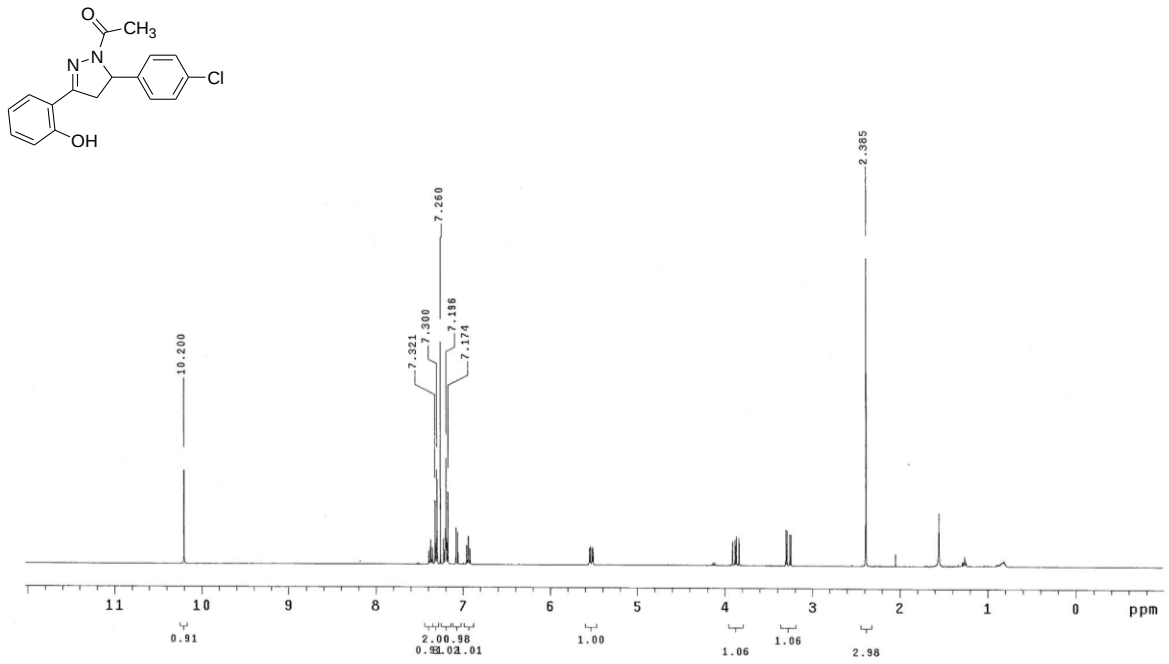
^{1}H NMR Spectrum of compound **19**



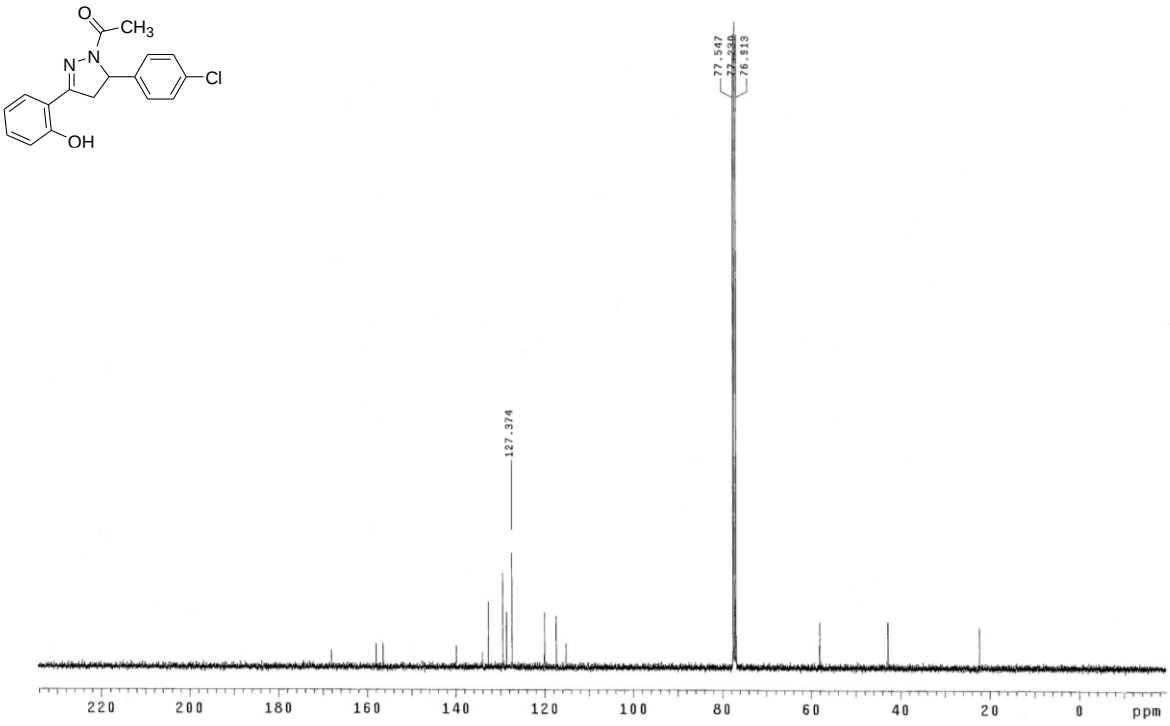
^{13}C NMR Spectrum of compound **19**



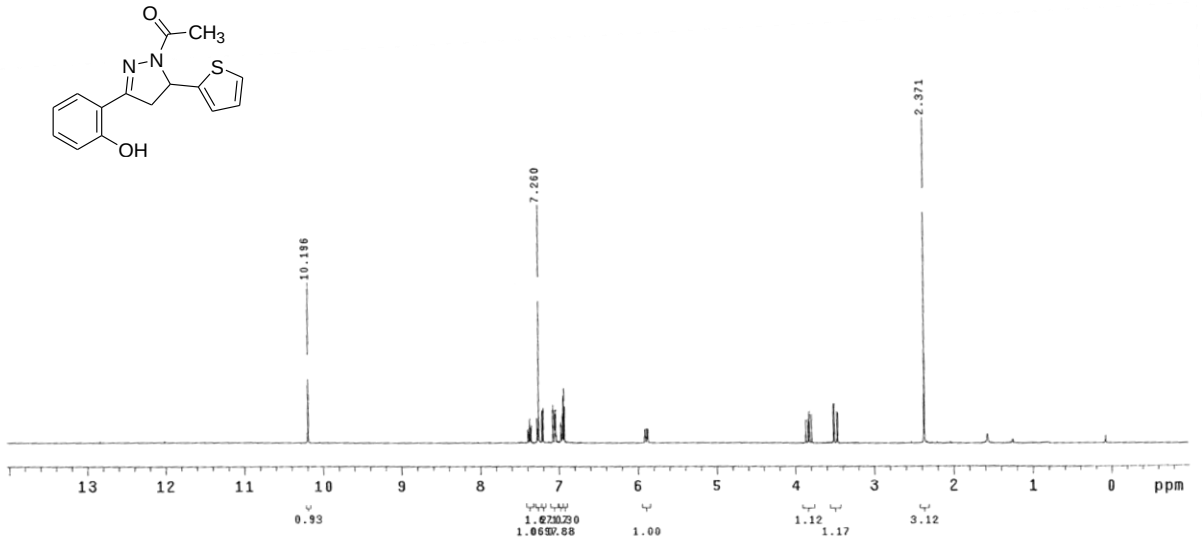
^{1}H NMR Spectrum of compound **20**



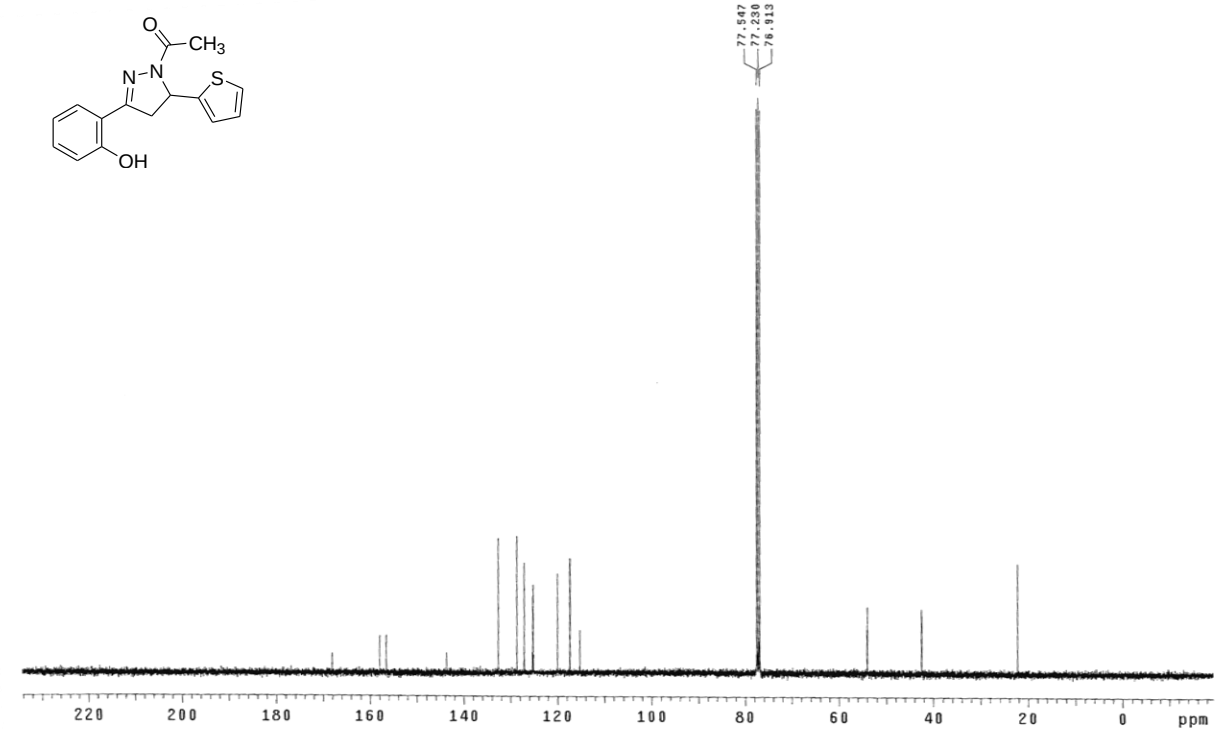
^{13}C NMR Spectrum of compound **20**



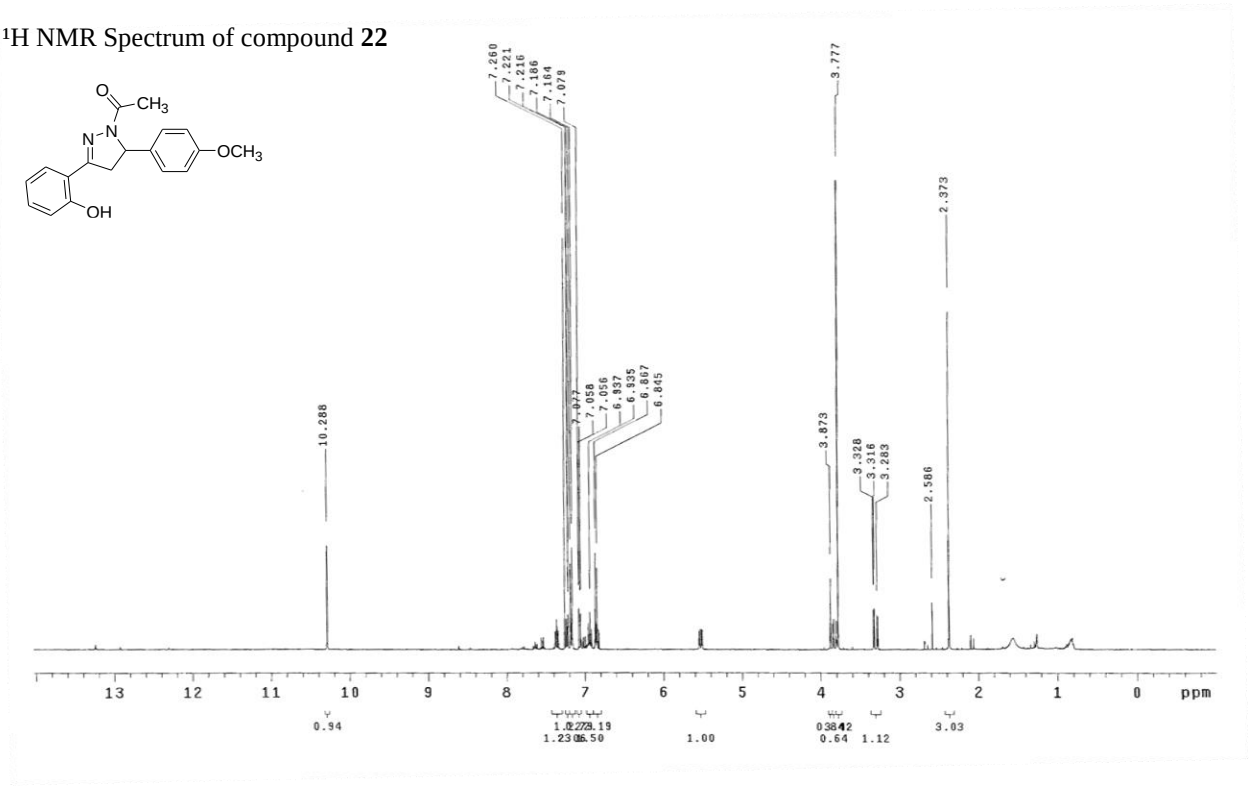
^{1}H NMR Spectrum of compound **21**



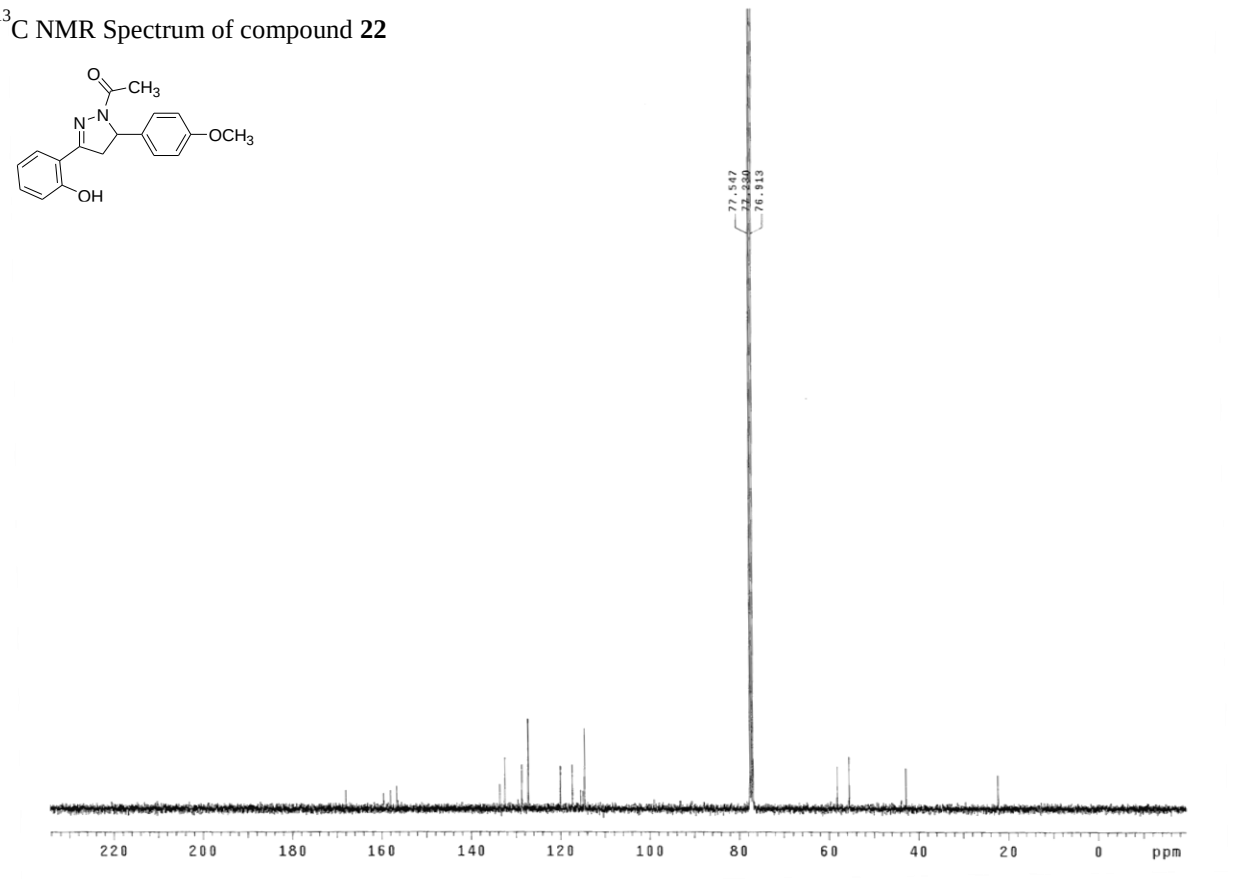
^{13}C NMR Spectrum of compound **21**



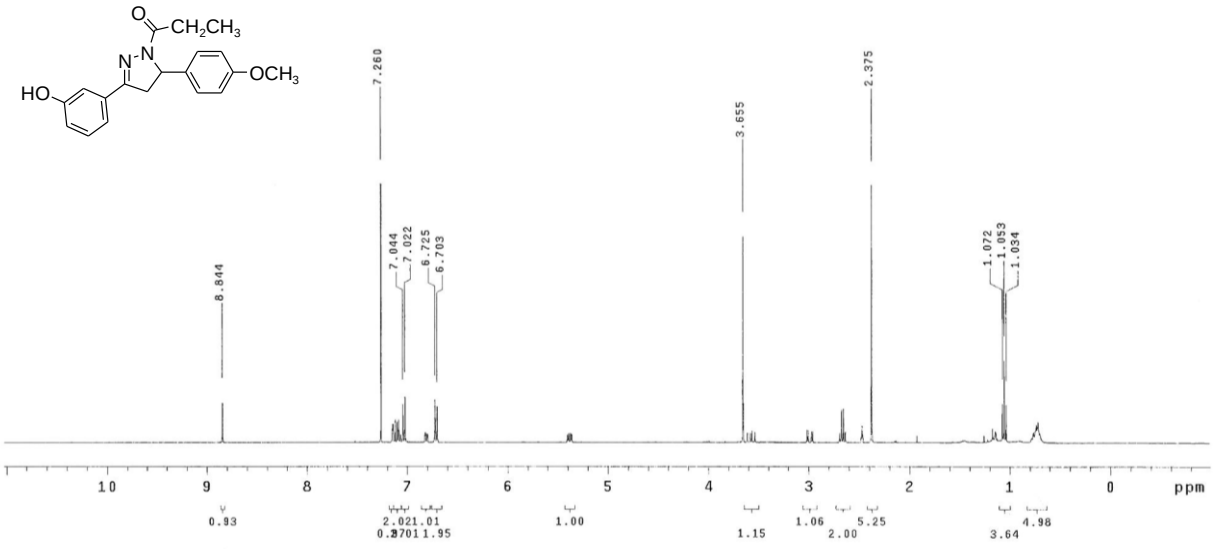
^{1}H NMR Spectrum of compound **22**



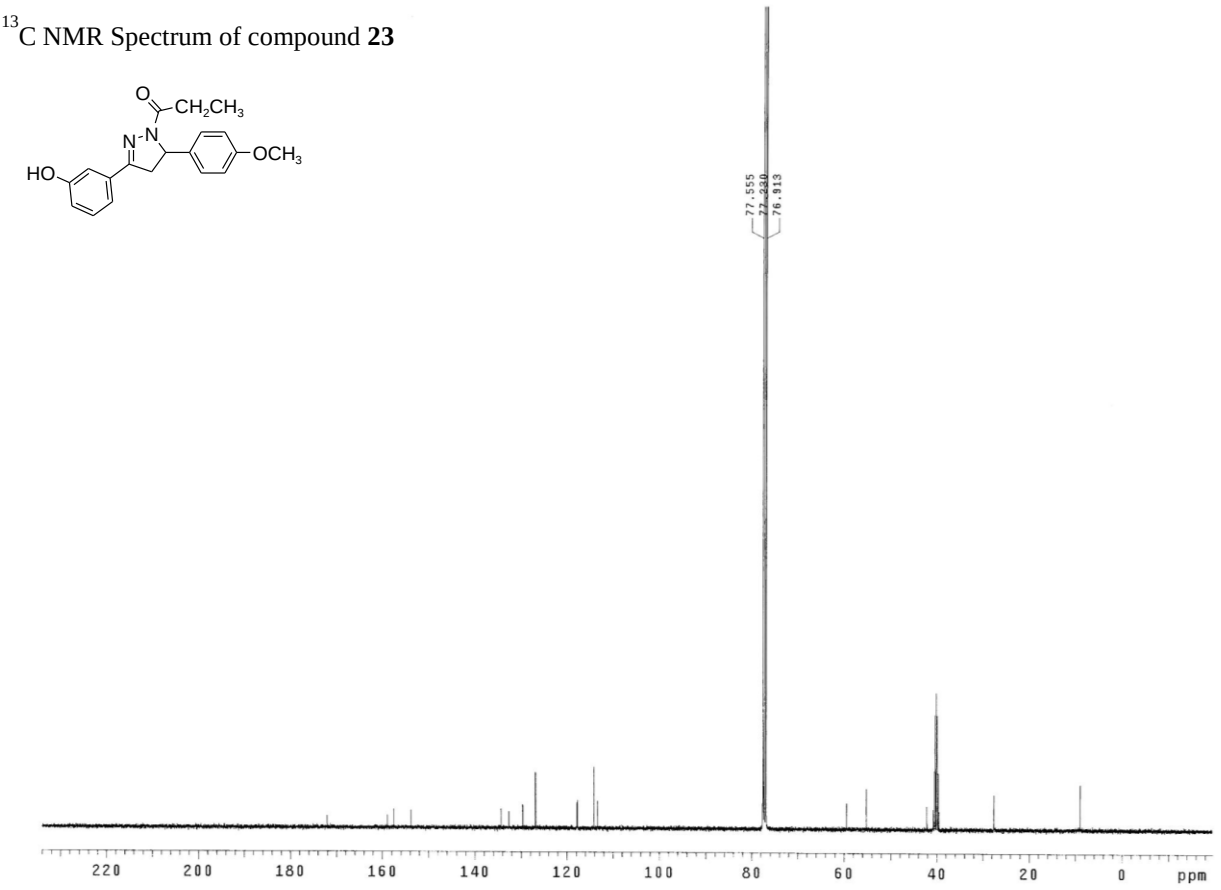
^{13}C NMR Spectrum of compound **22**



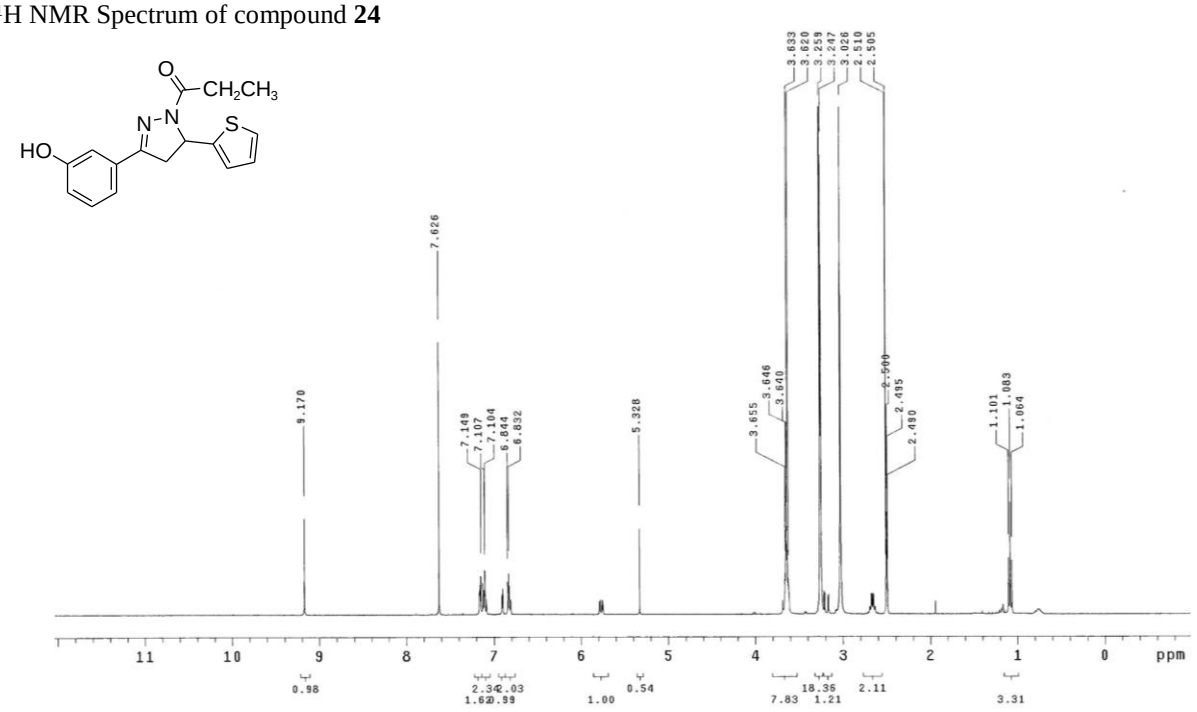
^{1}H NMR Spectrum of compound **23**



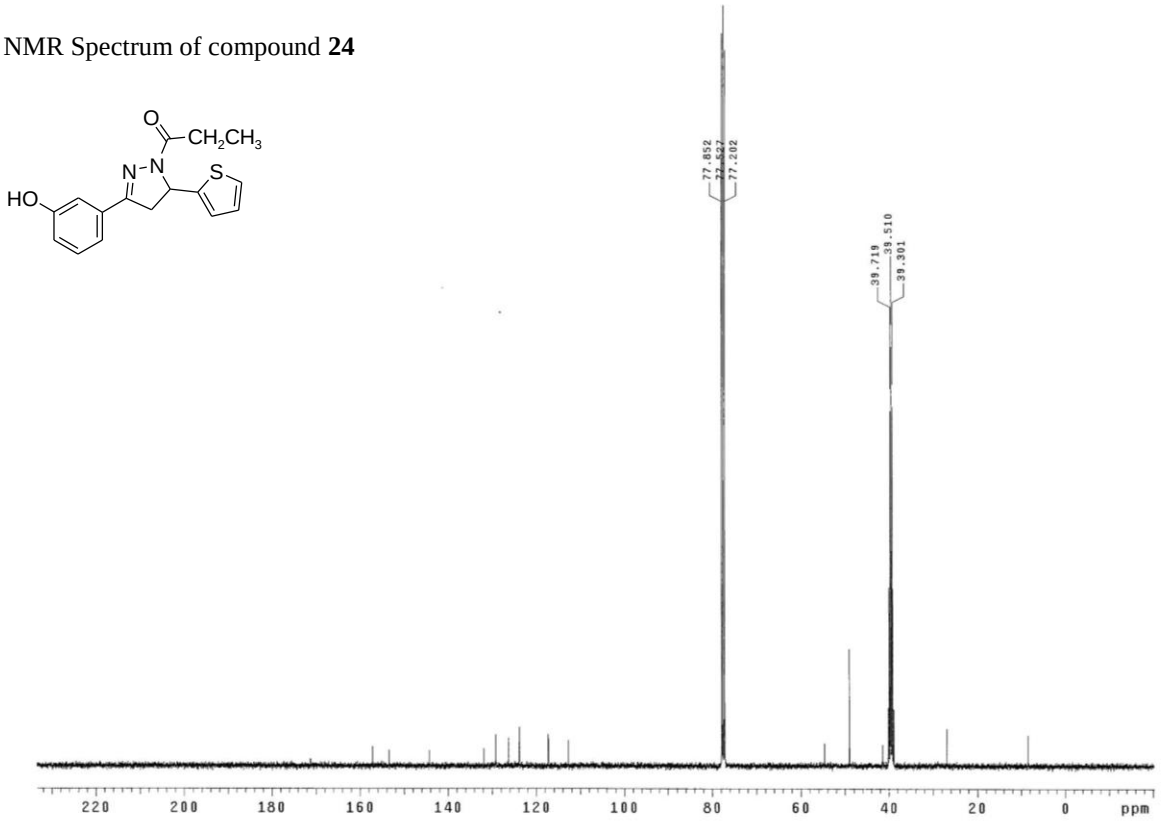
^{13}C NMR Spectrum of compound **23**



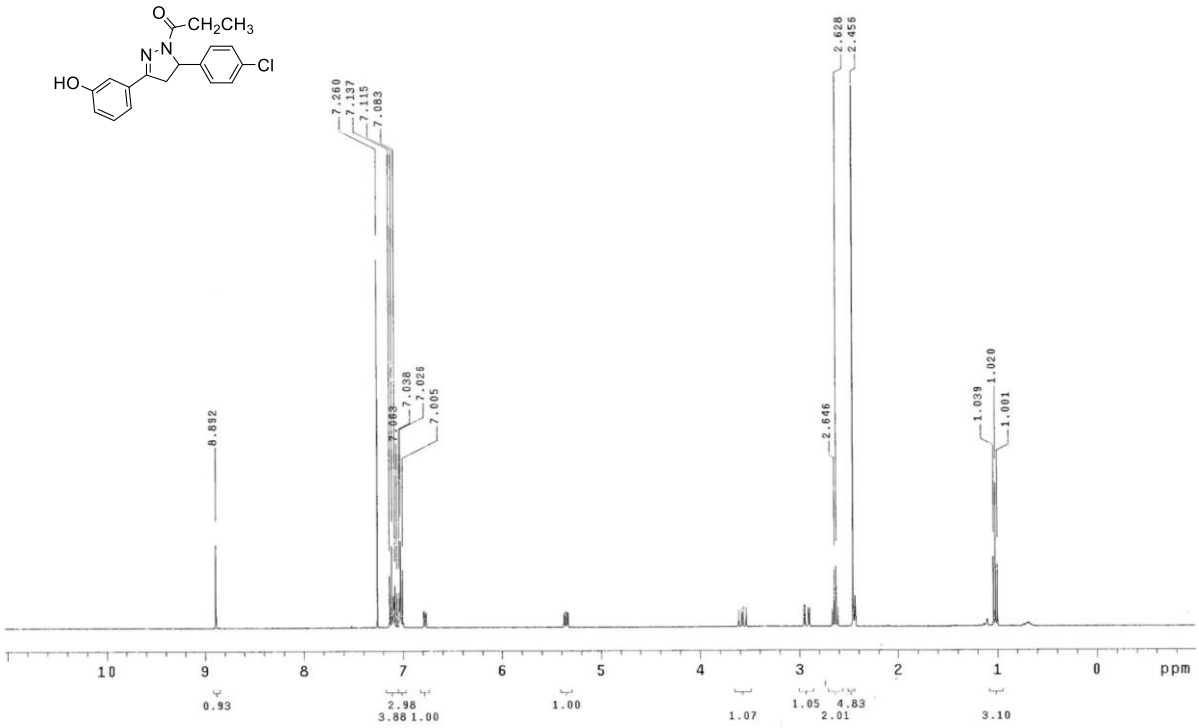
[1]H NMR Spectrum of compound **24**



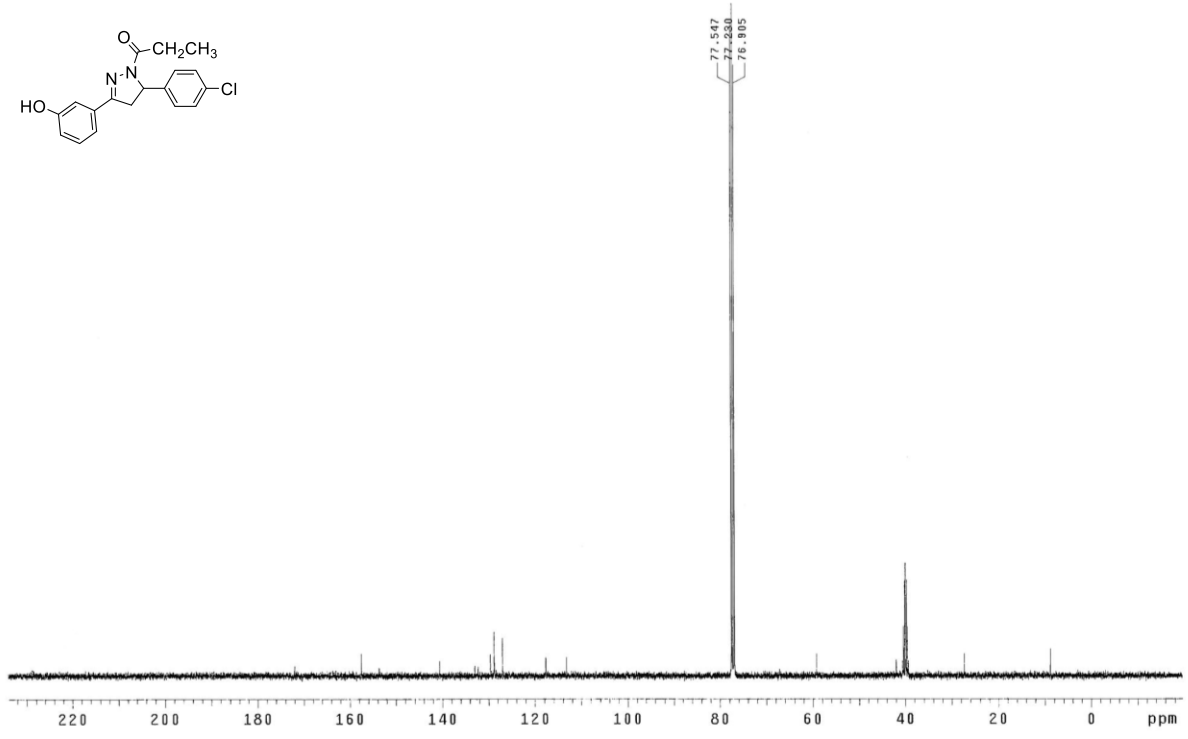
[13]C NMR Spectrum of compound **24**



[1]H NMR Spectrum of compound **25**



[13]C NMR Spectrum of compound **25**



Supplementary Tables

Table S1. Summary of the antibody lists used in this study.

Antibody	Vendor	Catalogue number	Application	Dilution
AKT	Cell signaling	#9272	WB	1:2000
c-PARP	Cell signaling	#9541	WB	1:2000
ELF3	Thermo	PA5-21293	WB	1:2000
GAPDH	MBL	M171-3	WB	1:10000
HER2	Thermo	MA5-13105	WB	1:1000
			IHC	1:100
Ki67	Dako	M7240	IHC	1:200
MAPK	Cell signaling	#9102	WB	1:2000
MED23	Novus	NB200-338	WB	1:2000
p-AKT (S473)	Santa cruz	sc-7985	WB	1:2000
p-MAPK (T202/Y204)	Cell signaling	#9101	WB	1:2000
α-tubulin	Cell signaling	#2144	WB	1:2000
β-actin	Cell signaling	#4967	WB	1:2000
$P27^{kip1}$	Santa cruz	sc-528	WB	1:1000
Cyclin D1	Cell signaling	#2922	WB	1:2000
Caspase7	Cell signaling	#9492	WB	1:1000
Bcl-2	Santa cruz	sc-7382	WB	1:1000

Table S2. Summary of the primer sequences used in this study. All sequences are provided from 5' to 3'. "F" indicates forward, and "R" indicates reverse primers.

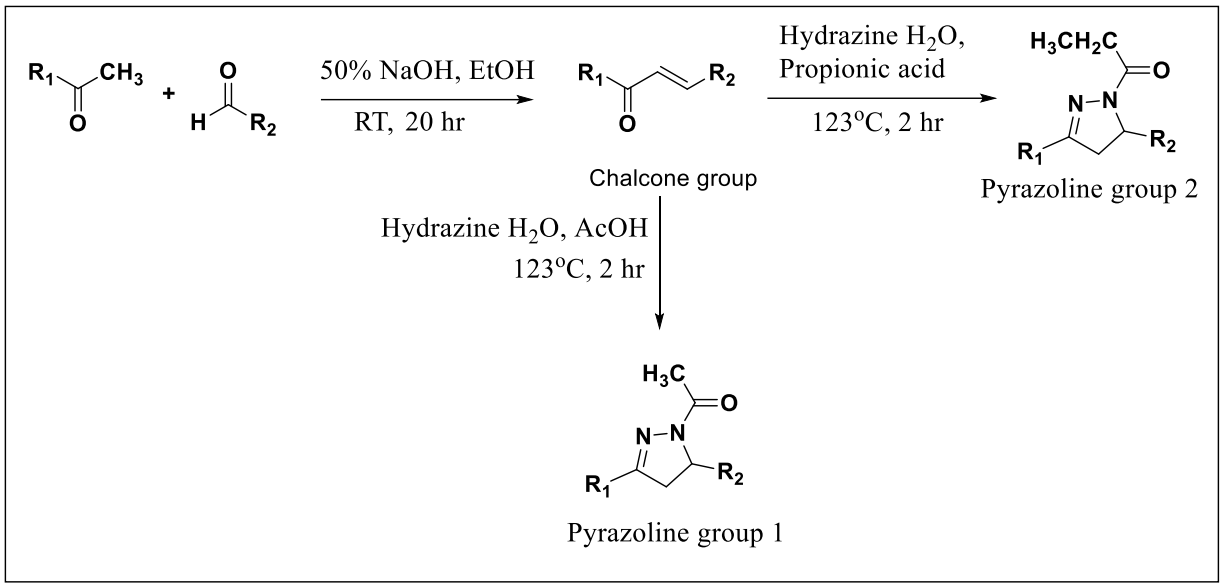
primer	sequence
Nluc-F	TGACGATGACAAGCTATGGAAGACGCCAAAAACATAAAGA
Nluc-R	GCCTCCAGCTCCTCCTCCATCCTTGTCAATCAAGGCGT
ELF3-F	GGAGGAGCTGGAGGCATGGCTGCAACCTGTGAGATTAG
ELF3-R	CTCTAGAGTCGACTGTCAGTTCCGACTCTGGAGAACC
MED23_full-length-F	GATGACAAGCTTGCGGCCGCGATGGAGACGCAACTGCAG
MED23_full-length-R	ACTACCTCCTCCTCCACTACCCTGATTAGACTGAGGTGC
Nluc-empty-mut F	TAAGGTACCAGTCGACTCTAGAGAACA
Nluc-empty-mut R	GCCTCCAGCTCCTCCTCCATCCTTGTC
Cluc-empty-mut-F	GATGCCTATGATTATGTCCGGTTATGT
Cluc-empty-mut-R	AAGCTTGTCATCGTCATCCTTGTAATC
MED23_391-582-F	GATGACAAGCTTGCGGCCGCGATGCAGAAAAATGCACTA
MED23_391-582-R	ACTACCTCCTCCTCCACTACCTATTTCCATATAGACCAA
MED23_391-462-F	GATGACAAGCTTGCGGCCGCGATGCAGAAAAATGCACTA
MED23_391-462-R	ACTACCTCCTCCTCCACTACCCTCATGGTGAAGTCTTAG
MED23_D400A-F	GCTCTTCGCTTTGCTATACCCAGAA
MED23_D400A-R	TTCATCACAGGGAGAAAATCAGCTA
MED23_H449G-F	AATACCTGGTTCCCTAAGACTTCAC
MED23_H449G-R	GGAATCTGTAGCTTGGAGTTGTCAT
MED23_K397A-F	TGTGATGGCTCTCTTCGACTTGCTA
MED23_K397A-R	GGGAGAAAATCAGCTAGTGCATTTT

Table S3. Kinase inhibitory activity of YK1.

Kinase	*In vitro* kinase inhibitory activity (%)[a] at the concentration of	
	10 μM	25 μM
c-RAF	3	10
EGFR	0	0
ErbB2	2	0
ErbB4	0	0
PI3 Kinase (p110a/p85a)	3	17

[a]The results of kinase inhibitory activity (%) of YK1 represent the mean value from individual duplicate experiments provided by KinaseProfiler Service Assay (Millipore, USA).

Supplementary Scheme and Figures



Scheme S1. General synthetic methods for the target compounds.

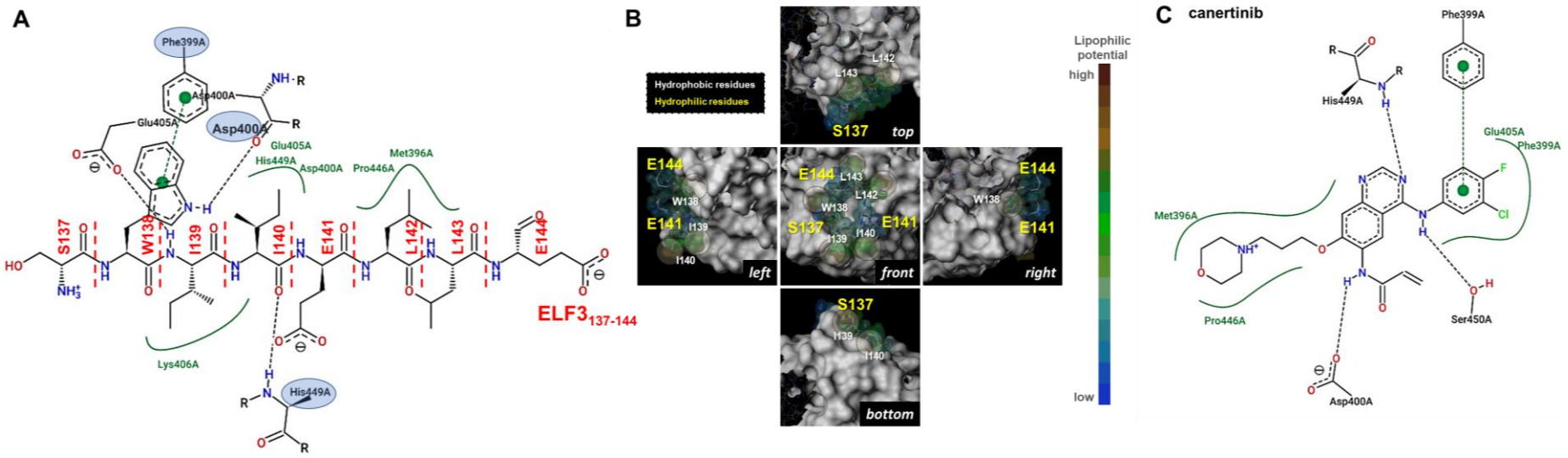


Figure S1. Detailed 2D interaction diagrams between ELF3$_{137\text{-}144}$ or canertinib and MED23 in the docking model from Figure 1B-C generated through PoseView (https://proteins.plus). (A) Strong π-π interactions with F399 along with specific H-bonding to the D400 and H449 residues of MED23 were commonly observed in both ELF3$_{137\text{-}144}$ and gefitinib (Figure S2). Indirect hydrophobic contacts via P446 and E405 were also found in common. (B) Docking pose of the ELF3$_{137\text{-}144}$ peptide on MED23 protein (PDB: 6H02) demonstrated from different viewpoints. Named in white are hydrophobic residues. Named in yellow are hydrophilic residues positioning to protrude outward without contacting MED23. (C) 2D diagrams of canertinib. ELF3$_{137\text{-}144}$ and canertinib created additional π-contacts with F399 of MED23

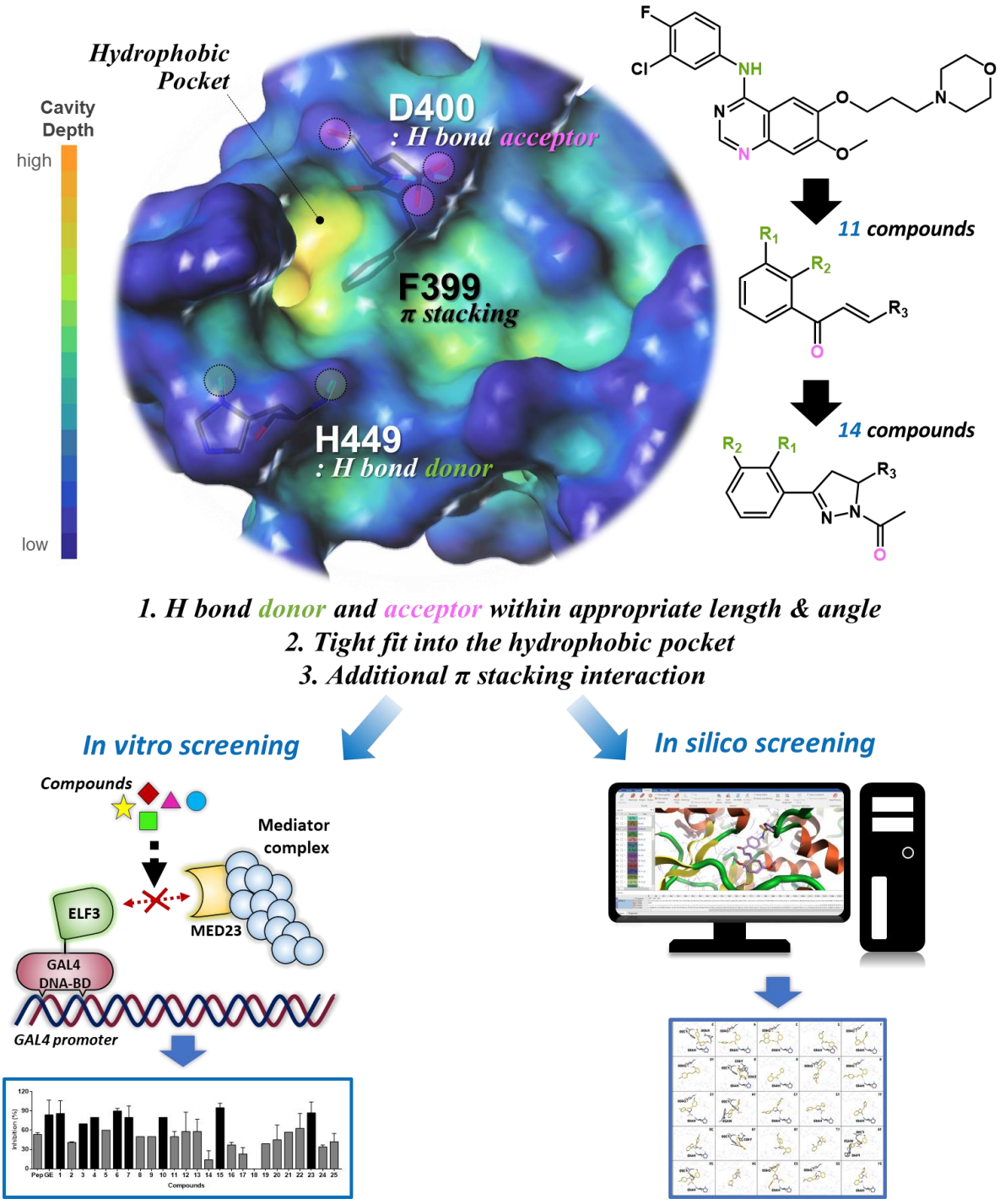


Figure S2. Design strategy of small molecules with minimal structural elements required to determine the hot-spot of ELF3-MED23 PPI. Focused series of 25 molecules were rationally designed using gefitinib as starting point. Three important emphasis points are mentioned above.

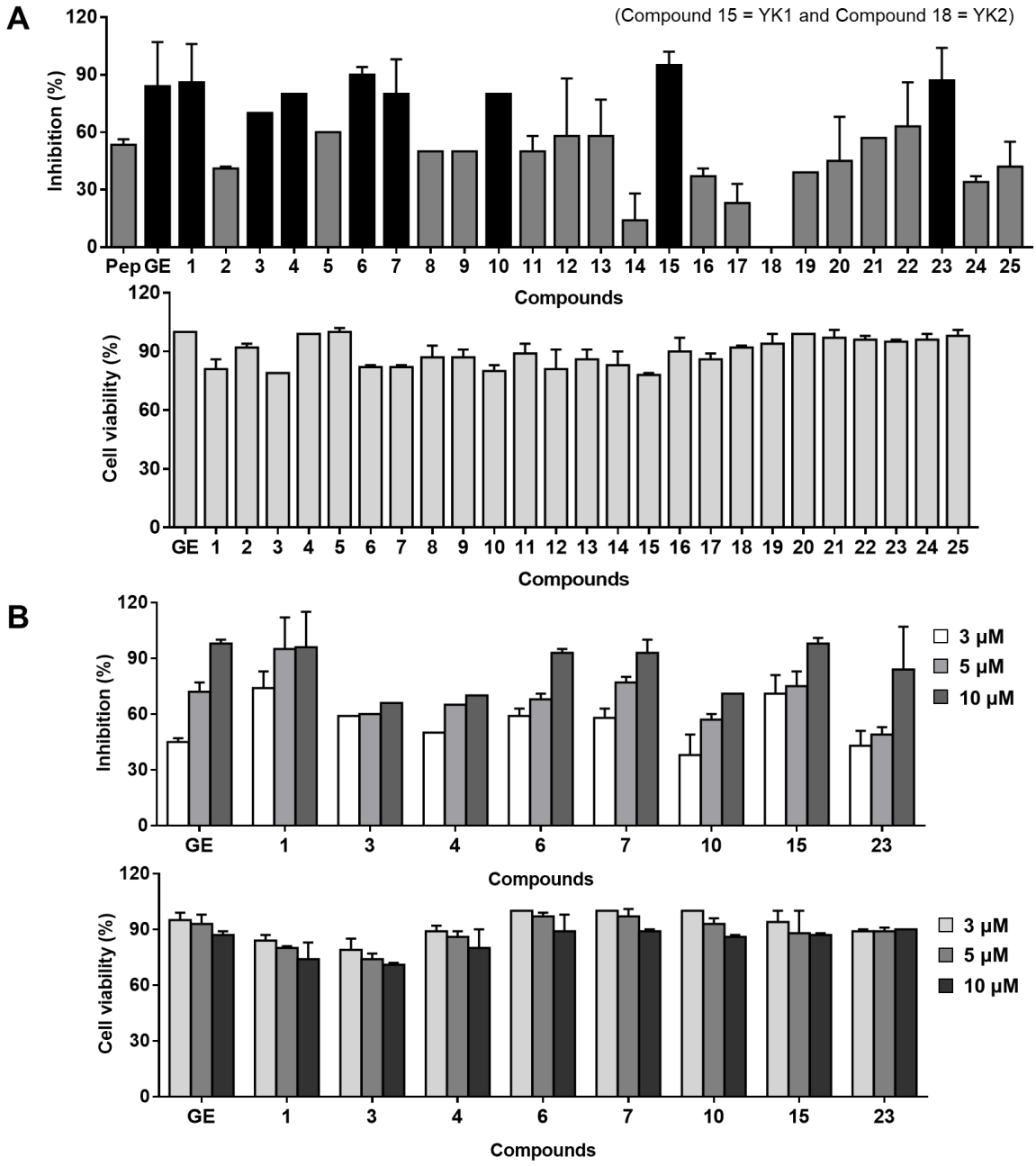


Figure S3. Biochemical evaluation of the prepared compounds to regulate HER2 gene transcription. (A) The synthesized compounds were screened to evaluate their inhibitory activity against ELF3–MED23 PPI. All the compounds were used at 10 μM for 12 h (SEAP reporter gene assay; n=3, mean ± S.D.). Cell viability was also measured in parallel using the same conditions as for the reporter gene assay (n=3, mean ± S.D.). (B) The compounds showing over 65% inhibition of ELF3-MED23 PPI (highlighted in black color in (A), were further tested at lower concentrations of 3, 5 and 10 μM for 12 h (n =3, mean ± S.D.). Cell viability was also measured under same experimental condition (n=3, mean ± S.D.).

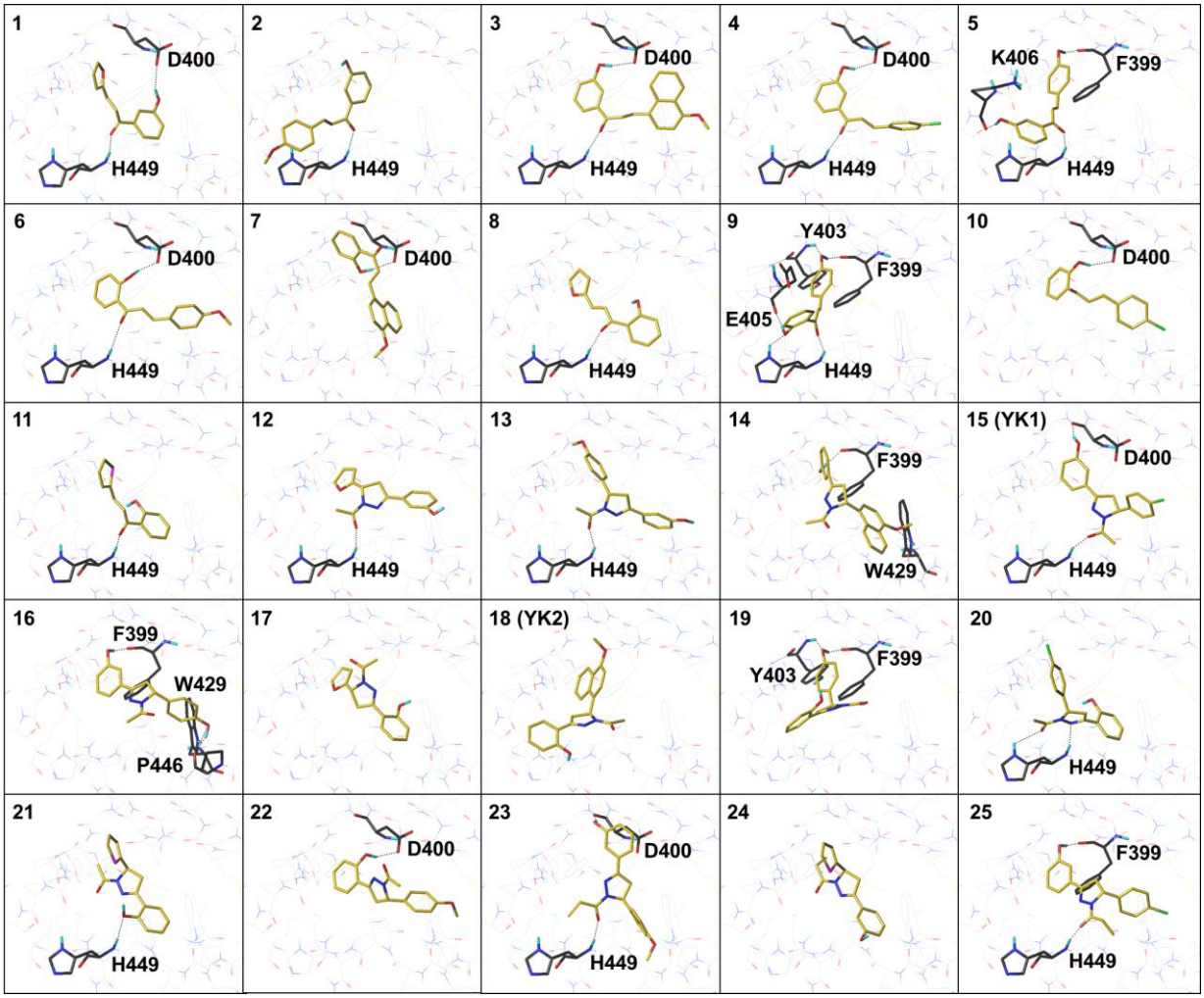


Figure S4. Docking pose of the total 25 compounds with chalcone and pyrazoline moieties. All the compounds were docked into the identified core binding interface of ELF3-MED23 using Sybyl-X 2.3 software.

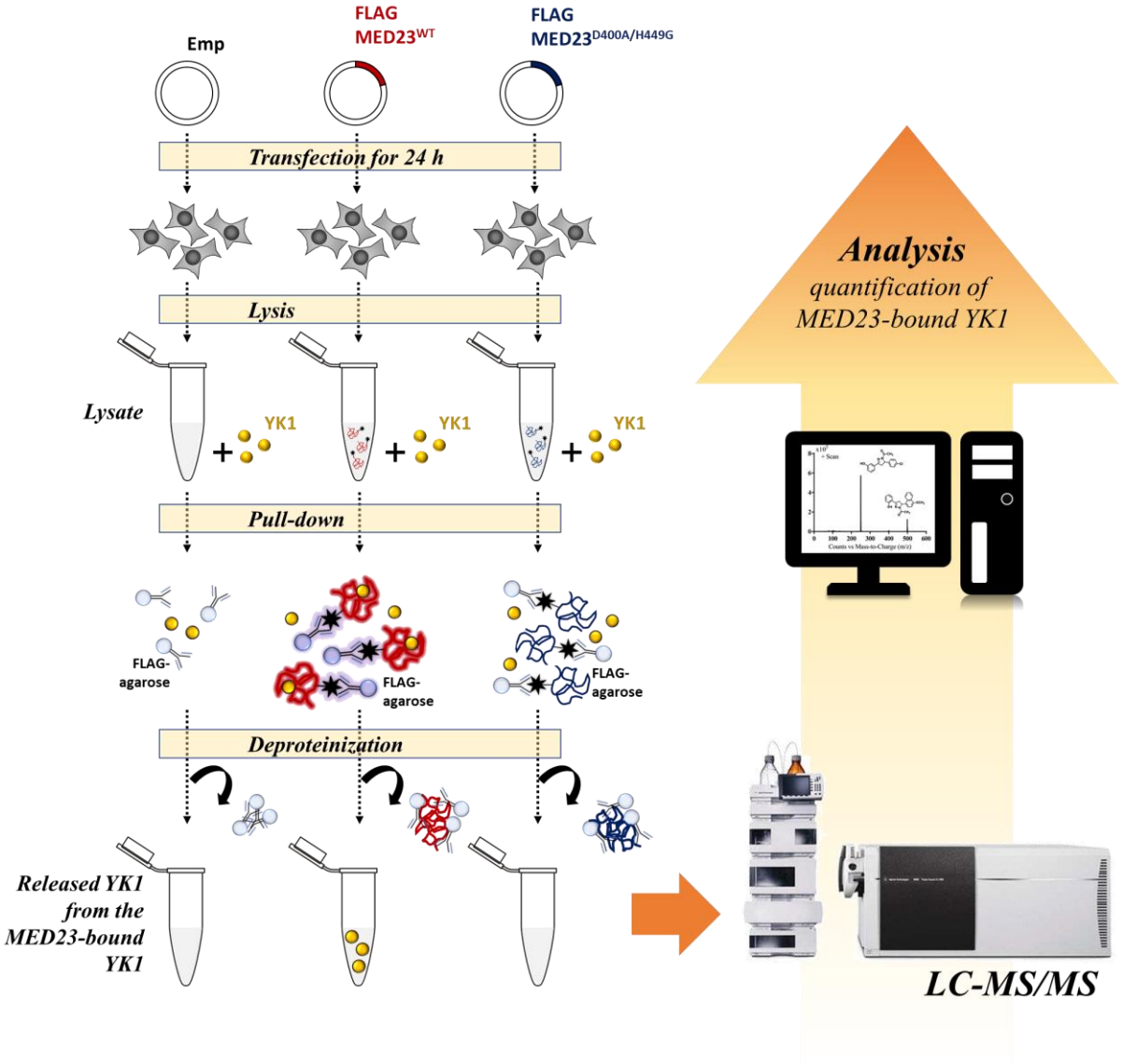


Figure S5. Graphical scheme of the LC-MS/MS method utilized in the study. Cells were transfected with FLAG-tagged MED23^{WT} and MED23$^{D400A/H449G}$ plasmids for 24 h followed by lysis. The lysate was incubated with YK1 for 4 h and finally pulled down using FLAG-agarose beads. The YK1 was extracted from pulled down MED23-bound YK1 with acetonitrile and centrifugation. See the detailed explanation of the process in the method of quantifying protein-small molecule interaction using LC-MS/MS.

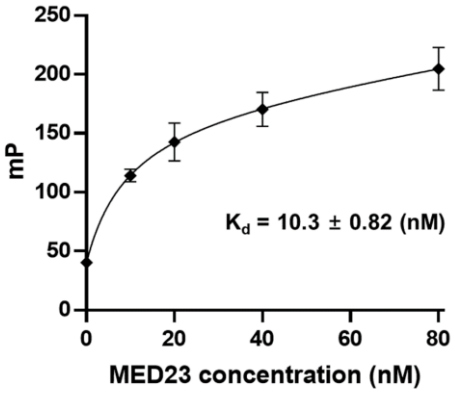
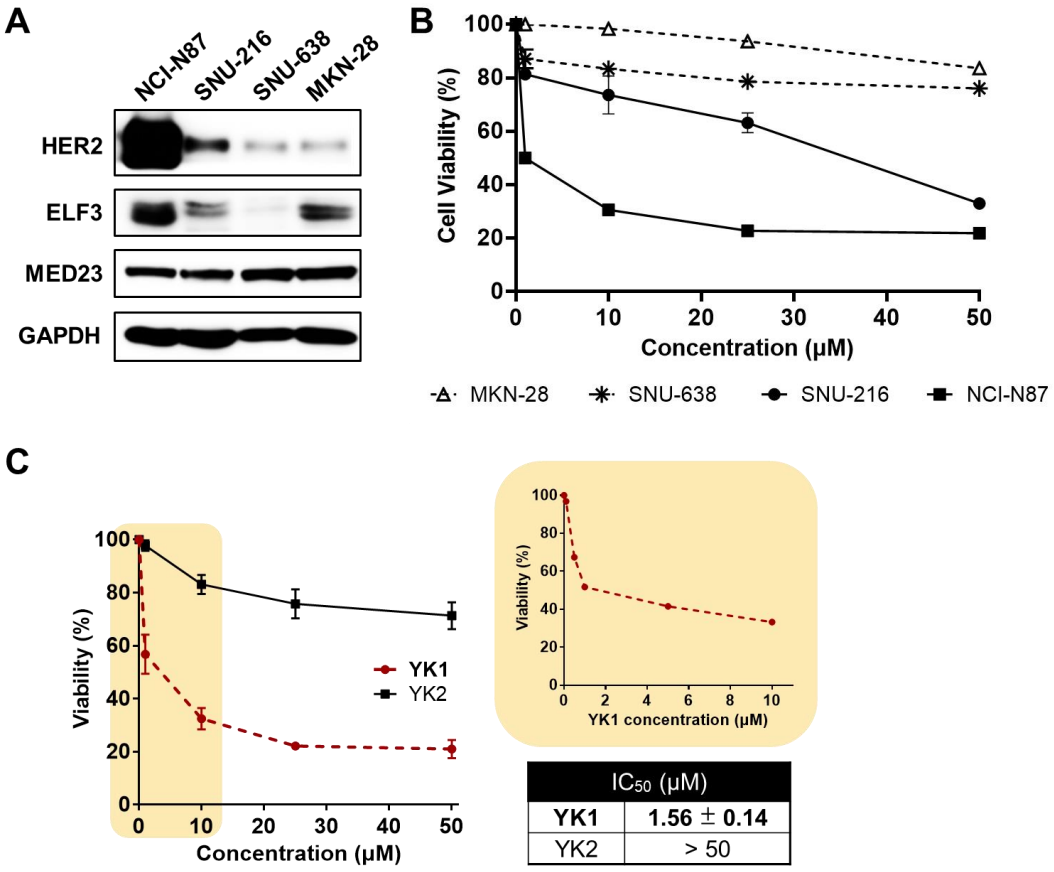


Figure S6. Titration curve of MED23$_{391–582}$ protein and FITC-labeled ELF3$_{129–145}$ peptide. Interaction of the MED23$_{391-582}$ protein and ELF3-FITC peptide (17 a.a.) was verified through a cell-free FP assay. K_d values were measured as 10.3±0.82 (nM) using the least squares non-linear fit method (n = 3, mean ± S.D).



IC_{50} (μM)	
YK1	**1.56 ± 0.14**
YK2	> 50

D

IC_{50} (μM) of YK1 in various normal mammalian cells[a]				
VERO	HFL-1	L929	NIH 3T3	CHO-K1
>100	69.0	>100	>100	91.4

[a]**VERO:** African green monkey kidney cell line; **HFL-1:** human embryonic lung cell line; **L929:** NCTC clone 929, mouse fibroblast cell line; **NIH 3T3:** mouse embryonic fibroblast cell line; **CHO-K1:** Chinese hamster ovary cell line

Figure S7. Biochemical evaluation of the target-specific anti-tumor activity of YK1. (A) The levels HER2, ELF3, and MED23 in diverse gastric cancer cell lines were evaluated to further identify the dependency of the compound's anticancer activity on the HER2 expression level. (B) YK1-mediated changes in cell viability of several gastric cancer cell lines possessing different HER2 level (72 h treatment at indicated doses; n = 3, mean ± S.D.). (C) The IC_{50} values of YK1 and YK2 against NCI-N87 cells were calculated at 72 h treatment (n = 3, mean ± S.D.). Full concentration-dependent cell viability curves are as indicated above. (D) The general cytotoxicity of YK1 was evaluated against various normal mammalian cells, VERO, HFL-1, L929, NIH 3T3 and CHO-K1 (24 h treatment at 0.01, 0.1, 1, 10, and 100 μM).

A

Solubility (pH 7.0)	Log P	Permeability Log P_{app} (cm/s)
327.8 ± 1.9 μM (103.2 ± 0.6 μg/ml)	2.98	-4.20 ± 0.097

B

Tester strain	Compound	Dose (μg/plate)	Revertant colonies /plate[a] (Mean ± S.D.) [Factor][#]	
			w/o S-9 mix	w/ S-9 mix
TA98	Vehicle	0	19±4	31±1
	YK1	40	28±1 [1.4]	33±5 [1.1]
TA100	Vehicle	0	86±5	117±14
	YK1	40	93±2 [1.1]	114±10 [0.9]

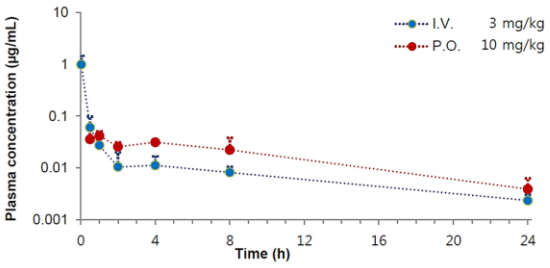
[a]**Ames test**: evaluating the number of revertant colonies of compound treated plate. The test was conducted with maximum concentration at which the compound was soluble and nontoxic to the S. typhimurium tester strains.
[#]No. of revertant colonies of treated plate/No. of revertant colonies of vehicle control plate.

C

Treatment	Concentration (μM)	% Inhibition of hERG ligand binding[a]	IC_{50} (μM)[b]
Positive control Astemizole	10	76.1±4.15	0.112
YK1	10	17.1±1.54	14.8

[a]**hERG channel binding assay**; measuring the inhibitory activity against hERG channel and its ligand using red fluorescent hERG channel ligand tracer. Final activity was assessed by decreased fluorescence polarization degree.
[b]**Patch clamp assay**; measuring the ionic currents flowing through hERG K^+ channel using HEK293 cell line stably expressing hERG gene. Automated Planar Patch Clamp (PatchXpress 7000A) was utilized.

D



Parameters	IV, 3 mg/kg	PO, 10 mg/kg
T_{max} (h)	NA	4.17 ± 3.75
C_{max} (μg/mL)	NA	0.04 ± 0.01
$T_{1/2}$ (h)	9.74 ± 2.76	10.9 ± 11.0
AUC_t (μg·h/mL)	0.51 ± 0.21	0.43 ± 0.16
AUC_∞ (μg·h/mL)	0.55 ± 0.22	0.50 ± 0.13
CL (L/h/kg)	6.17 ± 2.63	NA
V_{ss} (L/kg)	34.5 ± 26.4	NA
F_t (%)	NA	25.1

Figure S8. Pharmacokinetic evaluation of YK1 as a druggable candidate. (A) The physiochemical properties (e.g., solubility, log P, and permeability) of YK1 are summarized. Kinetic solubility (at pH 7) and log P were determined through nephelometry and the pH-metric method, respectively. Permeability was evaluated with a PAMPA assay using an artificially generated lipid-infused membrane. The measured values all generally meet the druglikeness criteria. (B), (C) Safety assessment of YK1 was conducted regarding genotoxicity (B), and cardiac toxicity (C). The IC_{50} value of the compound against the hERG K+ channel was measured using a patch clamp assay. Measured IC_{50} value was 14.8 μM, which was higher than 10 μM, supporting that YK1 is safe against cardiac liability, though caution is required for high-dosage uses. (D) The intravenous- and oral-route pharmacokinetic profiles of YK1 were evaluated after a single administration at each of the indicated doses. 8 wk-old male ICR mice were used for this experiment. YK1 was formulated in 5:55:40 of DMSO:DW:PEG400. All blood samples were taken from the retro-orbital vein at each time point (IV: 0.033, 0.5, 1, 2, 4, 8, 24 h; PO: 0.5, 1, 2, 4, 8, 24 h). The final extract of the plasma sample was analyzed using LC-MS/MS. All the calculated non-compartmental pharmacokinetic parameters were obtained from WinNonlin (Pharsight, USA).

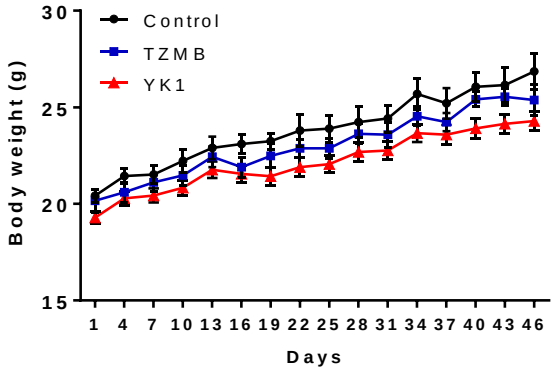


Figure S9. Changes in the body weight of JIMT-1 xenograft mice throughout the whole experiment. No significant differences were found between each experimental groups along with drug administration or tumor progression.