# Novel pyrano[2,3-c]pyrazolopyrimidines as promising anticancer agents: Design, synthesis, and cell cycle arrest of HepG2 cells at S phase

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Running title: **Novel pyrano[2,3-c]pyrazoles as anticancer agents**

## Experimental

### 1. Chemistry

Open capillary tubes on electrothermal melting point apparatus were used to measure the melting points which were uncorrected. The infrared spectra were recorded using the KBr wafer technique on Fourier Transform Infrared Thermo Electron Nicolet iS10 Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) at the Chemistry Department Laboratory, Faculty of Science, Ain Shams University. The $^{1}H$ and $^{13}C$ NMR spectra were measured on BRUKER 300; 400 and 75 *MHz* Spectrometer, with chemical shift (δ) expressed in ppm downfield with tetramethyl silane (TMS) as an internal standard, in DMSO-$d_6$ at the Microanalytical Centre (Faculty of Science, Cairo University). The mass spectra were recorded on a direct probe controller inlet part to single quadrupole mass analyzer (Thermo Scientific GCMS MODEL (ISQ LT)) using the Thermo X-CALIBUR software at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Elemental analyses were carried out at Ain Shams University's Faculty of Science using a PerkinElmer 2400 CHN elemental analyzer (PerkinElmer, Waltham, MA). Thin-layer chromatography (TLC) was run using TLC aluminum sheets silica gel F254 (Merck, Whitehouse Station, NJ).

***6-Amino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (1)*** [50]

A mixture of 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (0.98 g, 10 mmol), *p*-anisaldehyde (1.36 ml, 10 mmol), and malononitrile (0.66 g, 10 mmol) in absolute ethanol (30 mL) with piperidine (0.5 ml) was refluxed for 8 h. The precipitated solid while heating was separated off and recrystallized from ethanol/dioxane giving **1** as white crystals; Yield 88%; mp 216–218$^{o}$C; (Lit. [50] mp 210–212°C). IR (KBr, ν, cm$^{-1}$): 3304, 3248, 3127 (NH; $NH_2$), 2191 (C≡N), 1643 (C=N). $^{1}$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 1.82 (s, 3H, $CH_3$), 3.76 (s, 3H, $OCH_3$), 4.57 (s, 1H, H4-pyran), 6.82 (br.s, 2H, $NH_2$, exchangeable with $D_2O$), 6.90 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.12 (d, 2H, Ar-H, *J* = 8.4 Hz), 12.09 (br.s, 1H, NH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 8.95, 34.64, 54.17, 56.78, 97.07, 112.94, 120.04, 127.68, 134.74, 135.67, 153.94, 157.14, 159.87.

***Ethyl N-[5-cyano-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazol-6-yl]formimidate (2)*** [51]

A solution of enaminonitrile **1** (2.82 g, 10 mmol) and triethylorthoformate (10 mL) was heated at reflux for 6 h. The formed solid was filtered off, dried, and then crystallized from ethanol to give **2** as pale-yellow crystals; yield 80%; mp 295–297$^{o}$C (Lit. [51] mp 291–293°C). IR (KBr, ν, cm$^{-1}$): 3359 (NH), 2211 (C≡N), 1628 (C=N). $^{1}$H NMR (DMSO-$d_6$; 400 *MHz*) $\delta_{ppm}$: 1.32 (t, 3H, $CH_3$, *J* = 7.2 Hz), 1.80 (s, 3H, $CH_3$), 3.75 (s, 3H, $OCH_3$), 4.32 (q, 2H, $CH_2$, *J* = 7.2 Hz), 4.79 (s, 1H, H4-pyran), 6.91 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.16 (d, 2H, Ar-H, *J* = 8.4 Hz), 8.56 (s, 1H, N=CH), 12.25 (br.s, 1H, NH, exchangeable with $D_2O$). MS, m/z (%): 338 ($M^+$, 21.28), 283 (14.96), 268 (19.72), 254 (97.36), 238 (31.80), 227 (23.24), 212 (34.25), 166 (24.18), 143 (36.57), 125 (25.48), 108 (46.27), 96 (38.34), 49 (84.66), 81 (140.78), 56 (52.45), 55 (100).

***General procedure for synthesis of compounds 3a-c***

A solution of formimidate derivative **2** (1.69 g, 5 mmol) and aromatic amines namely, 3-amino pyridine, methyl 4-aminobenzoate, or 2-amino benzoic acid (10 mmol) in absolute ethanol (20 mL) was refluxed for 8-12 h. After cooling, the formed solid was filtered off, and then recrystallized from the appreciate solvent.

***(Syn, Anti)-N'-[5-Cyano-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazol-6-yl]-N-(pyridin-3-yl)formimidamide (3a):*** recrystallized from ethanol giving **3a** as white crystals; yield 80%; mp 250-252$^{o}$C; IR (KBr, ν, cm$^{-1}$): 3251, 3124 (NH), 2208 (C≡N), 1630 (C=N). $^{1}$H

NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 1.81 (s, 3H, $CH_3$), 3.74 (s, 3H, $OCH_3$), 4.77 (s, 1H, H4-pyran), 6.89-9.00 (m, 9H, Ar-H + CH=N), 10.49, 10.91 (two br.s, 1H, NH, *Syn*-, *Anti*- isomers, exchangeable with $D_2O$), 12.20 (br.s, 1H, NH pyrazole, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 10.58, 37.31, 55.80, 78.70, 79.66, 98.25, 114.68, 120.46, 124.38, 124.55, 127.36, 129.65, 136.06, 136.47, 136.54, 136.68, 136.74, 140.08, 142.11, 145.37, 149.70, 152.08, 155.92, 158.96, 160.34. MS, m/z (%): 387 (M+1, 14.82), 386 ($M^+$, missed), 385 (M-1, 5.60), 377 (14.40), 370 (16.89), 346 (12.94), 328 (22.91), 323 (28.01), 313 (17.93), 285 (44.37), 271 (28.88), 257 (17.28), 199 (11.34), 87 (30.49), 86 (100), 75 (30.63), 70 (74.41), 57 (68.01). Anal. Calcd. for $C_{21}H_{18}N_6O_2$ (386.42): C, 65.27; H, 4.70; N, 21.75. Found: C, 65.33; H, 4.63; N, 21.87.

***Methyl (Syn, Anti) -4-{N'-[5-cyano-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c] pyrazol-6-yl]formimidamido}benzoate (3b):*** recrystallized from dioxane giving **3b** as yellow crystals; yield 70%; mp 246-248$^o$C; IR (KBr, ν, $cm^{-1}$): 3296, 3206, 3108 (NH), 2198 (C≡N), 1710 (C=O ester), 1640 (C=N). $^1$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 1.85 (s, 3H, $CH_3$), 3.77 (s, 3H, $OCH_3$), 3.85 (s, 3H, COO$\underline{CH_3}$), 4.80 (s, 1H, H4-pyran), 6.92-9.01 (m, 9H, Ar-H + CH=N), 10.65, 11.16 (two br.s, 1H, NH, *Syn*-, *Anti*- isomers, exchangeable with $D_2O$), 12.24 (br.s, 1H, NH pyrazole, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 9.78, 36.65, 51.94, 55.02, 78.69, 79.51, 97.46, 113.93, 116.38, 119.63, 124.16, 128.91, 130.13, 130.90, 135.36, 135.93, 143.67, 148.63, 150.83, 155.18, 158.23, 159.52, 165.77. MS, m/z (%): 443 ($M^+$, 10.08), 425 (13.79), 396 (19.85), 364 (35.33), 350 (14.64), 282 (18.35), 256 (30.92), 353 (32.86), 221 (30.80), 179 (18.05), 158 (42.08), 130 (71.85), 105 (94.32), 94 (100), 80 (69.93), 71 (70.49), 65 (60.55), 59 (31.75), 45 (71.39). Anal. Calcd. for $C_{24}H_{21}N_5O_4$ (443.46): C, 65.00; H, 4.77; N, 15.79. Found: C, 65.18; H, 4.84; N, 15.68.

***(Anti)-2-{N'-[5-Cyano-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazol-6-yl]formimidamido}benzoic acid (3c):*** recrystallized from dioxane giving **3c** as white crystals; yield 70%; mp 266-268$^o$C; IR (KBr, ν, $cm^{-1}$): 3379 (OH), 3186, 3123 (NH), 2208 (C≡N), 1678 (C=O acid), 1629 (C=N). $^1$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 1.78 (s, 3H, $CH_3$), 3.69 (s, 3H, $OCH_3$), 4.73 (s, 1H, H4-pyran), 6.74-9.03 (m, 9H, Ar-H + CH=N), 11.15 (br.s, 1H, NH, exchangeable with $D_2O$), 12.20 (br.s, 1H, NH pyrazole, exchangeable with $D_2O$), 13.68 (br.s, 1H, OH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 11.73, 38.59, 56.96, 99.38, 115.85, 117.87, 121.46, 125.00, 130.83, 130.89, 137.17, 137.82, 142.64, 152.20, 156.99, 160.14, 171.23.

MS, m/z (%): 430 (M+1, 11.39), 429 ($M^+$, 28.80), 428 (M-1, 10.24), 412 (46.04), 384 (46.34), 365 (55.52), 346 (38.51), 323 (43.49), 316 (26.29), 298 (33.21), 262 (26.29), 247 (32.24), 233 (29.39), 213 (45.48), 187 (43.56), 171 (40.34), 154 (16.32), 125 (25.32), 115 (60.05), 104 (100), 69 (59.49), 59 (55.31), 48 (65.49). Anal. Calcd. for $C_{23}H_{19}N_5O_4$ (429.44): C, 64.33; H, 4.46; N, 16.31. Found: C, 64.27; H, 4.53; N, 16.28.

***4-[5-Imino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d] pyrimidin-6(5H)-yl]-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (4):*** A solution of compound **2** (1.69 g, 5 mmol) and 4-amino antipyrine (0.5 ml, 5 mmol) in absolute ethanol (20 ml) was refluxed for 10 h. After cooling, the deposited solid was filtered off, and recrystallized from ethanol giving **4** as white crystals; yield 55%; mp > 300$^o$C; IR (KBr, *v*, $cm^{-1}$): 3171, 3126 (NH), 1676 (C=O amide), 1641 (C=N). $^1$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 1.69 (s, 3H, $CH_3$ pyrazole), 2.02 (s, 3H, $CH_3$ pyrazolone), 3.03 (s, 3H, N-$CH_3$), 3.70 (s, 3H, $OCH_3$), 5.35 (s, 1H, H4-pyran), 6.84-7.54 (m, 9H, Ar-H), 7.63 (br.s, 1H, =NH, exchangeable with $D_2O$), 8.12 (s, 1H, =CH pyrimidine), 12.13 (br.s, 1H, NH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 9.39, 9.45, 31.72, 35.47, 54.66, 97.86, 99.27, 108.20, 113.36, 123.07, 125.81, 128.17, 128.69, 134.73, 134.98, 135.38, 152.89, 154.72, 155.72, 157.47, 161.16, 161.93, 162.45. MS, m/z (%): 495 ($M^+$, 7.88), 457 (6.08), 438 (6.29), 427 (11.06), 421 (10.09), 407 (5.90), 369 (46.98), 330 (23.54), 313 (21.35), 284 (39.43), 251 (12.77), 236 (24.26), 196 (19.51), 185 (18.31), 176 (16.66), 143 (29.06), 123 (40.48), 111 (100), 107 (46.82), 79 (36.60), 69 (70.66), 57 (37.58). Anal. Calcd. for $C_{27}H_{25}N_7O_3$ (495.54): C, 65.44; H, 5.09; N, 19.79. Found: C, 65.56; H, 5.22; N, 19.81.

***General procedure for compounds (7-9)***

A mixture of formimidate derivative **2** (1.69 g, 5 mmol) and acid hydrazide derivatives namely, semicarbazide hydrochloride, *p*-toluene sulfonohydrazide, or cyanoacetohydrazide (5 mmol) in absolute ethanol (20 mL) was refluxed for 8-10 h. After cooling, the formed solid was collected by filtration, and crystallized from the suitable solvent.

***11-(4-methoxyphenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano[3,2-e][1,2,4]triazolo-[1,5-c]pyrimidin-2(3H)-one (7):*** recrystallized from EtOH/dioxane producing **5** as white crystals; yield 61%; mp 242-244$^o$C; IR (KBr, *v*, $cm^{-1}$): 3324, 3189, 3118 (NH), 1673 (C=O amide), 1648 (C=N). $^1$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: for Lactam form: δ 1.95 (s, 3H, $CH_3$), 3.67 (s, 3H, $OCH_3$), 5.26 (s, 1H, H4-pyran), 6.81, 7.18 (dd, 4H, Ar-H), 7.85 (br.s, 1H, NH

triazole, exchangeable with $D_2O$), 8.27 (s, 1H, =CH pyrimidine), 12.26 (br.s, 1H, NH pyrazole, exchangeable with $D_2O$). For Lactim form: δ 8.41 (br.s, 1H, OH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 9.69, 34.29, 54.93, 97.61, 98.80, 99.20, 99.68, 113.48, 113.78, 128.59, 128.78, 135.72, 136.00, 136.22, 152.50, 153.62, 154.95, 156.08, 157.83, 157.93, 158.01, 158.09, 159.23, 162.58, 173.26. MS, m/z (%): 350 (M+1, 7.28), 349 ($M^+$, missed), 345 (27.96), 333 (25.22), 331 (46.36), 313 (64.75), 303 (48.40), 269 (45.19), 280 (27.44), 270 (20.22), 265 (19.68), 251 (13.23), 236 (21.72), 219 (17.25), 202 (25.21), 190 (27.06), 180 (100), 146 (25.54), 115 (47.67), 111 (53.05), 110 (38.83), 98 (18.77). Anal. Calcd. for $C_{17}H_{14}N_6O_3$ (349.35): C, 58.28; H, 4.03; N, 23.99. Found: C, 58.35; H, 3.87; N, 24.04.

***N-[5-Imino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d] pyrimidin-6(5H)-yl]-4-methylbenzenesulfonamide (8):*** recrystallized from dioxane producing **6** as white crystals; yield 60%; mp > 300$^o$C; IR (KBr, *v*, cm$^{-1}$): 3312, 3187 (NH), 1643 (C=N). $^1$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 2.01 (s, 3H, $CH_3$ pyrazole), 2.24 (s, 3H, $CH_3$), 3.79 (s, 3H, $OCH_3$), 5.18 (s, 1H, H4-pyran), 6.89-7.33 (m, 8H, Ar-H), 7.90 (br.s, 2H, =NH + N-NH, exchangeable with $D_2O$), 8.54 (s, 1H, =CH pyrimidine), 12.34 (br.s, 1H, NH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 9.62, 20.62, 32.53, 54.96, 98.00, 98.76, 113.82, 125.76, 128.32, 128.89, 133.93, 135.53, 140.06, 140.89, 152.28, 154.33, 155.36, 158.01, 159.60. MS, m/z (%): 478 ($M^+$, 49.60), 455 (54.04), 428 (54.39), 422 (81.15), 394 (29.48), 384 (52.18), 369 (52.56), 360 (58.87), 343 (34.77), 328 (36.50), 305 (49.84), 298 (56.22), 291 (80.09), 275 (49.39), 252 (100), 225 (95.14), 204 (60.17), 195 (70.46), 172 (60.29), 142 (40.67), 129 (64.56), 117 (44.36), 91 (25.22), 76 (76.72), 75 (35.07). Anal. Calcd. for $C_{23}H_{22}N_6O_4S$ (478.53): C, 57.73; H, 4.63; N, 17.56; S, 6.70. Found: C, 57.82; H, 4.59; N, 17.44; S, 6.78.

***2-[11-(4-Methoxyphenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl]acetonitrile (9):*** recrystallized from dioxane producing **7** as white crystals; yield 65%; mp 258-260$^o$C; IR (KBr, *v*, cm$^{-1}$): 3156, 3115 (NH), 2259 (C≡N), 1643 (C=N). $^1$H NMR (DMSO-$d_6$; 300 *MHz*) $\delta_{ppm}$: 1.92 (s, 3H, $CH_3$), 3.60 (s, 3H, $OCH_3$), 4.30 (s, 2H, $CH_2$), 5.46 (s, 1H, H4-pyran), 6.71-7.13 (dd, 4H, Ar-H), 8.40 (s, 1H, =CH pyrimidine), 12.23 (br.s, 1H, NH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 10.90, 19.03, 35.59, 56.06, 99.61, 103.81, 114.80, 115.15, 117.62, 118.90, 129.70, 130.12, 135.31, 136.85, 141.16, 152.08, 155.04, 155.10, 156.49, 159.32, 161.83. MS, m/z (%): 373 ($M^+$, 41.00), 362 (52.04), 353 (65.38), 335 (94.98), 318 (27.58), 285 (100), 274 (44.95), 252 (40.37), 220 (33.07), 210 (44.15),

195 (32.18), 137 (37.03), 122 (27.56), 100 (18.09), 78 (48.15), 74 (32.80), 50 (28.54), 43 (36.00). Anal. Calcd. for $C_{19}H_{15}N_7O_2$ (373.38): C, 61.12; H, 4.05; N, 26.26. Found: C, 61.12; H, 4.05; N, 26.26.

***3-(4-Chlorophenyl)-2-[11-(4-methoxyphenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl]acrylonitrile (10):*** A mixture of compound **7** (1.86 g, 5 mmol) and *p*-chlorobenzaldehyde (0.7 mL, 5 mmol) in absolute ethanol (30 mL) in presence of piperidine was refluxed for 6 h. The produced solid while refluxing was separated and recrystallized from dioxane yielding **8** as pale yellow crystals; yield 72%; mp > 300$^{o}$C; IR (KBr, *v*, cm$^{-1}$): 3185, 3122 (NH), 2224 (C≡N), 1631 (C=N). $^{1}$H NMR (DMSO-$d_6$; 300 *MHz*) δppm: 1.92 (s, 3H, $CH_3$), 3.59 (s, 3H, $OCH_3$), 5.51 (s, 1H, H4-pyran), 6.75 (d, 2H, Ar-H, *J*= 8.7 Hz), 7.20 (d, 2H, Ar-H, *J*= 8.4 Hz), 7.59 (d, 2H, Ar-H, *J*= 8.4 Hz), 7.97 (d, 2H, Ar-H, *J*= 8.7 Hz), 8.31 (s, 1H, =CH pyrimidine), 9.58 (s, 1H, CH=), 12.24 (br.s, 1H, NH, exchangeable with $D_2O$). $^{13}$C-NMR (DMSO-$d_6$) $\delta_{ppm}$: 11.76, 36.41, 56.90, 100.17, 103.76, 104.74, 115.59, 117.46, 131.24, 135.56, 137.40, 138.74, 142.14, 148.86, 155.54, 156.72, 157.45, 159.87, 163.74. MS, m/z (%): 498 (M+2, 36.44), 496 ($M^+$, 64.68), 483 (40.67), 478 (67.25), 451 (100), 408 (44.82), 400 (41.01), 393 (31.67), 365 (40.95), 354 (37.88), 341 (66.49), 330 (68.41), 312 (31.82), 285 (26.38), 270 (41.10), 266 (70.65), 251 (48.87), 238 (40.77), 215 (31.10), 205 (48.77), 186 (38.29), 180 (47.72). Anal. Calcd. for $C_{26}H_{18}ClN_7O_2$ (495.93): C, 62.97; H, 3.66; Cl, 7.15; N, 19.77. Found: C, 62.81; H, 3.58; Cl, 7.22; N, 19.69.

***Reaction of compound 2 with o-phenylene diamine: unexpected formation of compound (1) + benzimidazole***

A mixture of compound **2** (1.69 g, 5 mmol) and *o*-phenylene diamine (0.5 ml, 5 mmol) in ethanol (20 ml) was refluxed for 8 h. The solid left was separated and crystallized from ethanol/dioxane giving **1** as beige crystals; yield 50%. After leaving the mother liquor overnight at room temperature, it was crystallized from benzene-ethanol to give benzimidazole; white crystals, mp 170-172 $^{o}$C (Lit. [36] mp 170-171 $^{o}$C).

## 2. Biology

### 2.1.Cell lines and reagents

HepG2 cells were purchased from Nawah Scientific Company, Egypt. Cells were grown in DMEM medium (BioWhittaker™) containing 10% bovine serum albumin (Life Science Group L, UK, Cat No: S-001B-BR) and 100 IU/mL penicillin/ streptomycin (100 µg/mL)

(Lonza, 17-602E). The tested pyrazole derivatives were prepared in dimethyl sulfoxide (10 mM stock) (DMSO, Cat. No. 20385.02, Serva, Heidelberg, Germany) and stored at –20°C.

**2.2.Estimation of the antiproliferative activity of pyrazole derivatives by MTT assay** [37]

The MTT assay was used to measure the antiproliferative activity of pyrazole derivatives (0.5-100 µM) against the growth of human liver (HepG2) and normal fibroblasts (WI-38), and the results were compared with those of doxorubicin. The selectivity index (SI) was then calculated; SI = $IC_{50}$ against WI-38/ $IC_{50}$ against cancer cells. After 48 hours of incubation with the pyrazole derivatives, MTT was applied to the cells. The cells were further incubated for four hours. After adding DMSO, the absorbance at 570 nm was measured. The data are expressed as mean ± SEM for three independent experiments. The study was approved by the Research Ethics Committee at Ain shams University.

**2.3.Wound healing assay in HepG2 cells**

To investigate cell migration in vitro, the wound healing experiment was run. The details of the assay could be reviewed elsewhere [52-54]. The cells were treated with compound **4,** or compound **7** at their $IC_{50}$ concentration reported in Table 1. The images were taken using Optika B-159 (OPTIKA S.r.l., Italy). The size of the wound was measured using Image J 1.51 software from three independent experiments.

**2.4.Cell Cycle analysis by flow cytometry**

HepG2 cells were seeded ($1\times10^5$) in 6-well plate (2 mL/well) and incubated overnight at 37°C and 5% $CO_2$. Cell synchronization at G1 phase was performed by incubating the cells with serum-free medium for 24 hours. Then, cells were incubated with compound **4** or **7** at the $IC_{50}$ concentration (reported in Table 1) for 48 hours. The details could be reviewed elsewhere [55]. Propidium (PI)/RNase was obtained from BD Biosciences (BDB550825, NJ, USA). The experiment was repeated thrice for validity.

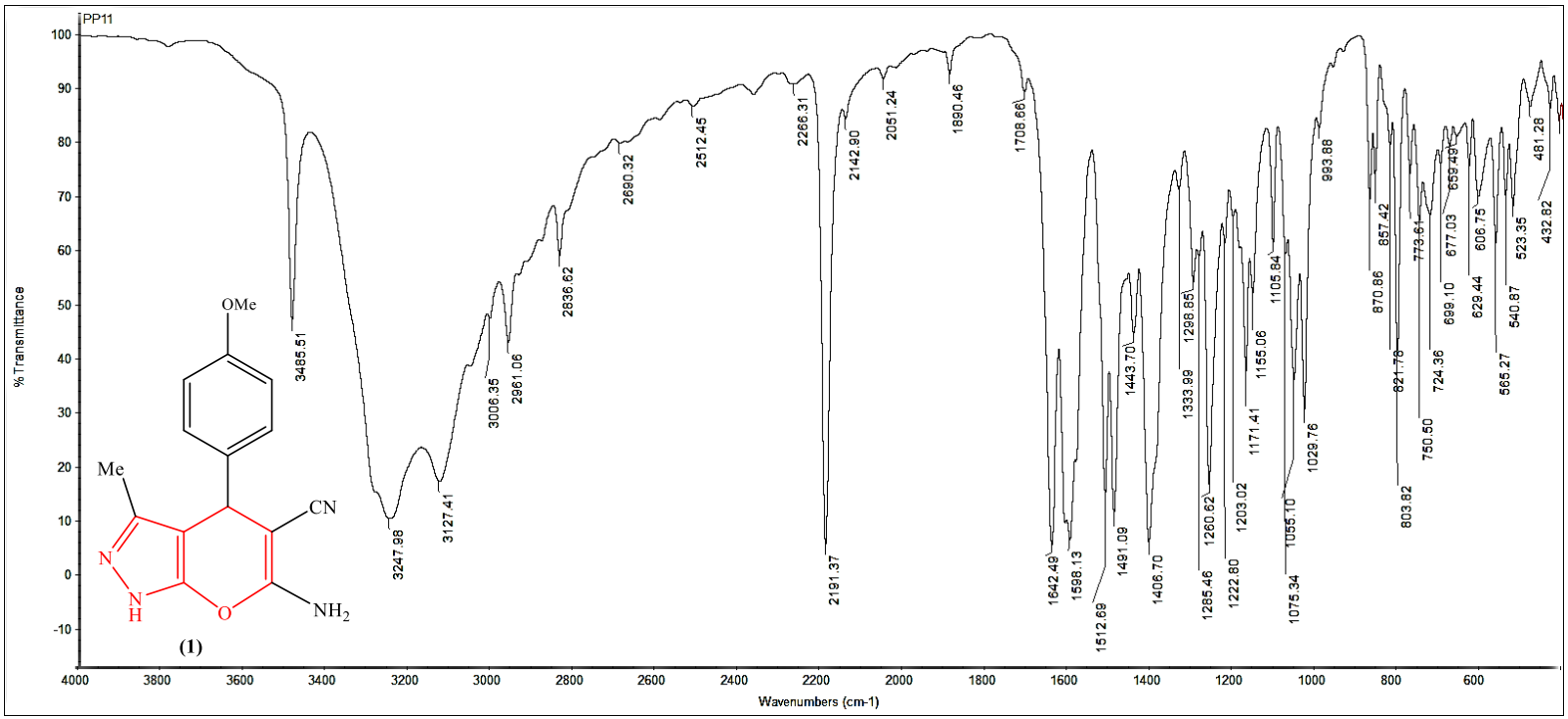
**2.5.Measurement of p21 by Western blotting**

HepG2 cells were seeded (1.5*10 cells/ml) in 25 cm flask (3 ml per flask) and incubated overnight. The cells were then treated either with DMSO (0.1%), compound **4**, or compound **7** at their $IC_{50}$ concentrations for 24 hours at 37°C and 5% $CO_2$. The mouse anti-human p21 and β-actin were obtained from Cell signaling technology (MA, USA). The details could be reviewed elsewhere [56]. The fold of change in p21 level was calculated using GraphPad Prism 8 Software after normalization to the level of β-actin.

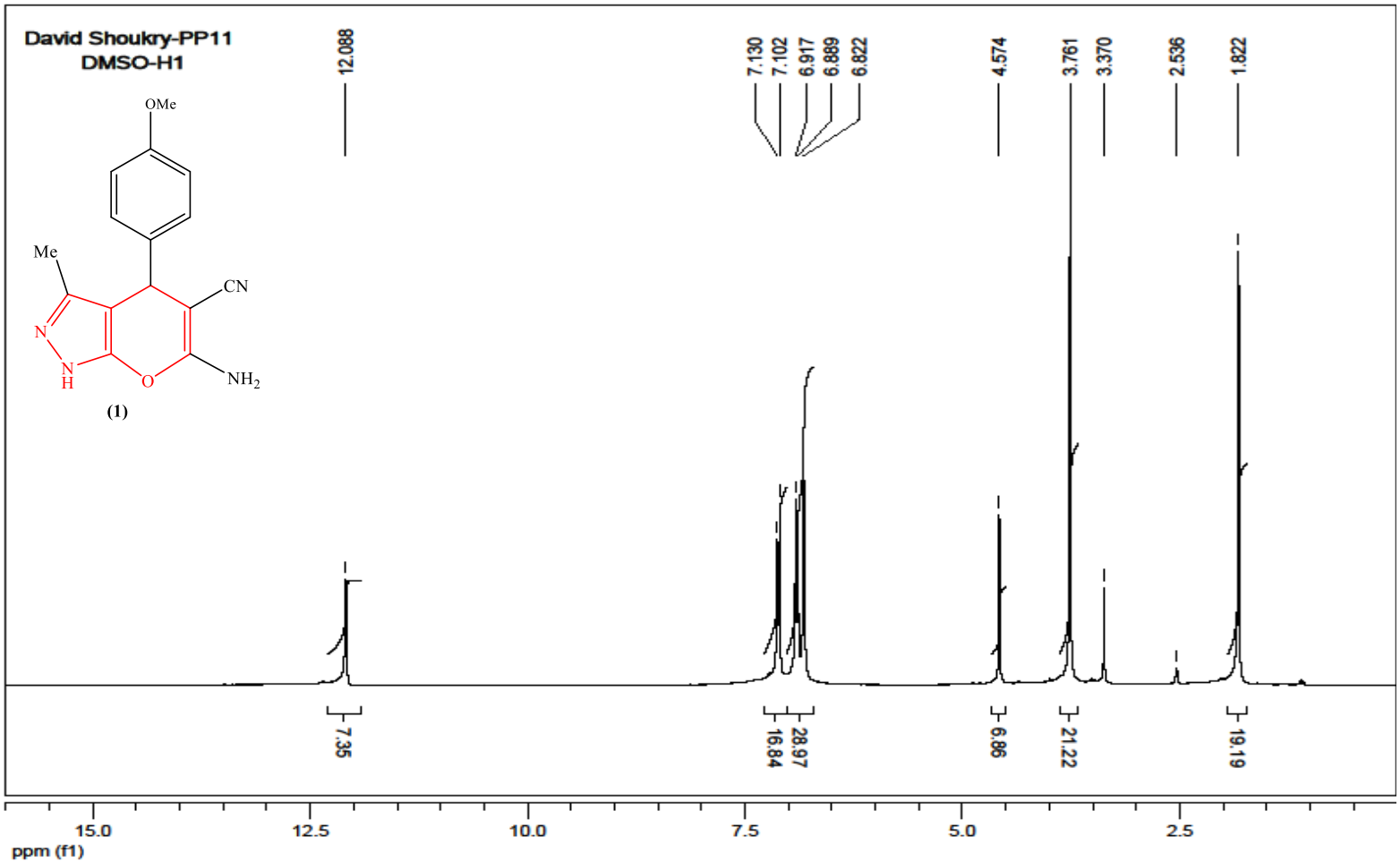
### 2.6.Statistical analysis of data

The Kolmogorov-Smirnov test was used to analyse the data distribution. The findings were presented as mean ± SEM. One Way ANOVA was used for the statistical analysis, and the Tukey-HSD test was used for multiple comparisons. When a difference was $P<0.05$, it was deemed significant.

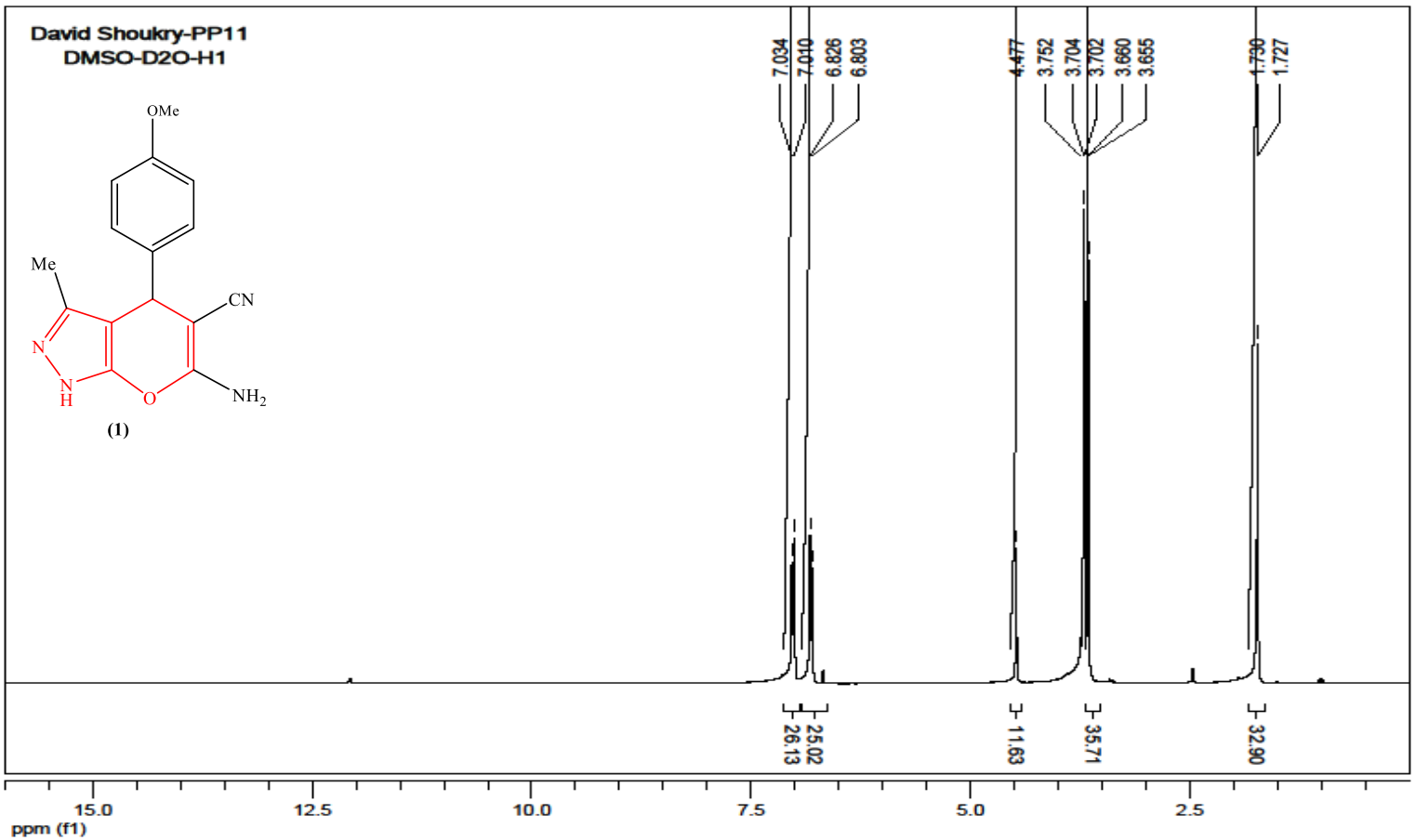
# Copies of the IR, NMR, and MS data for the synthesized compounds



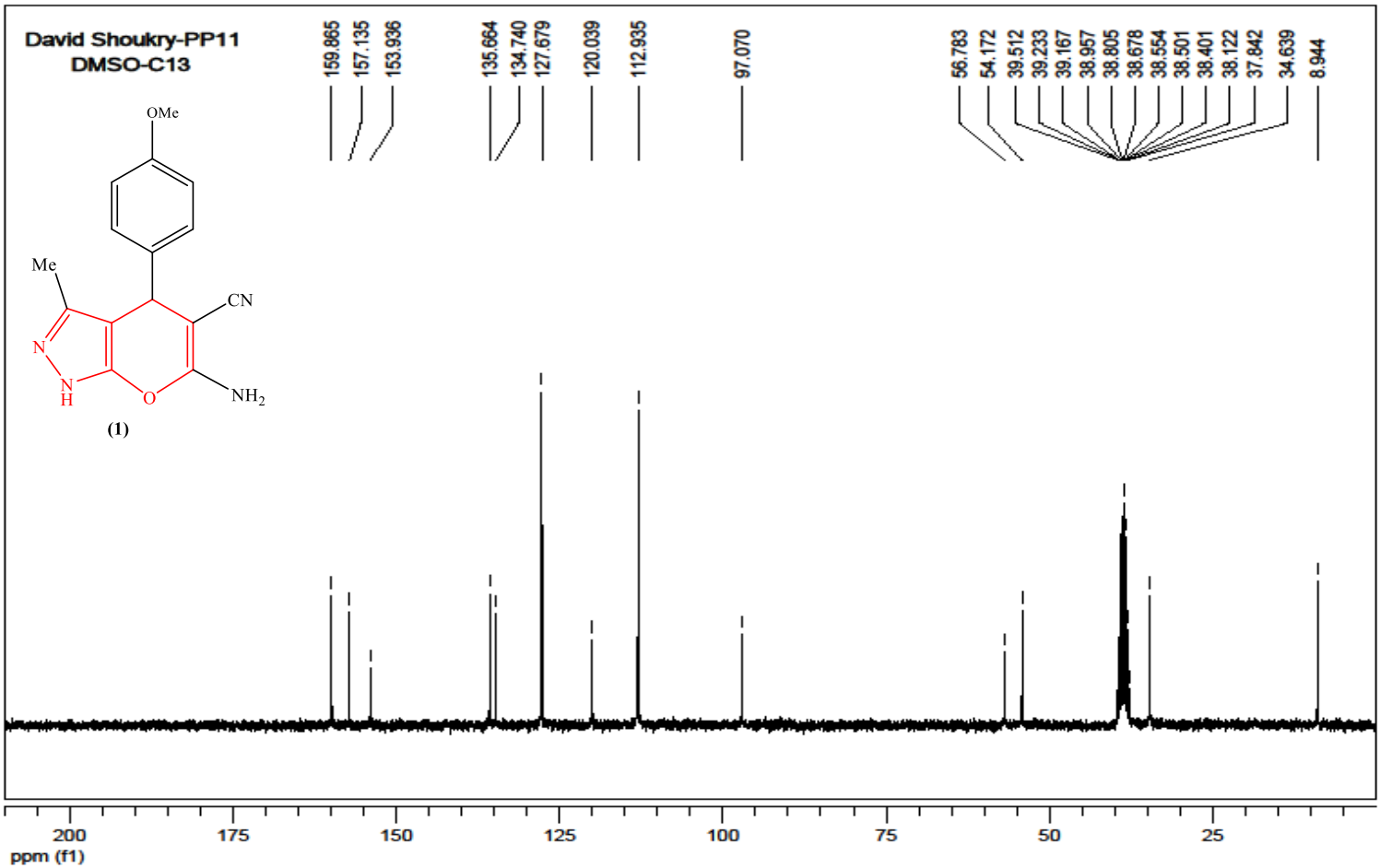

IR spectrum of compound **1**



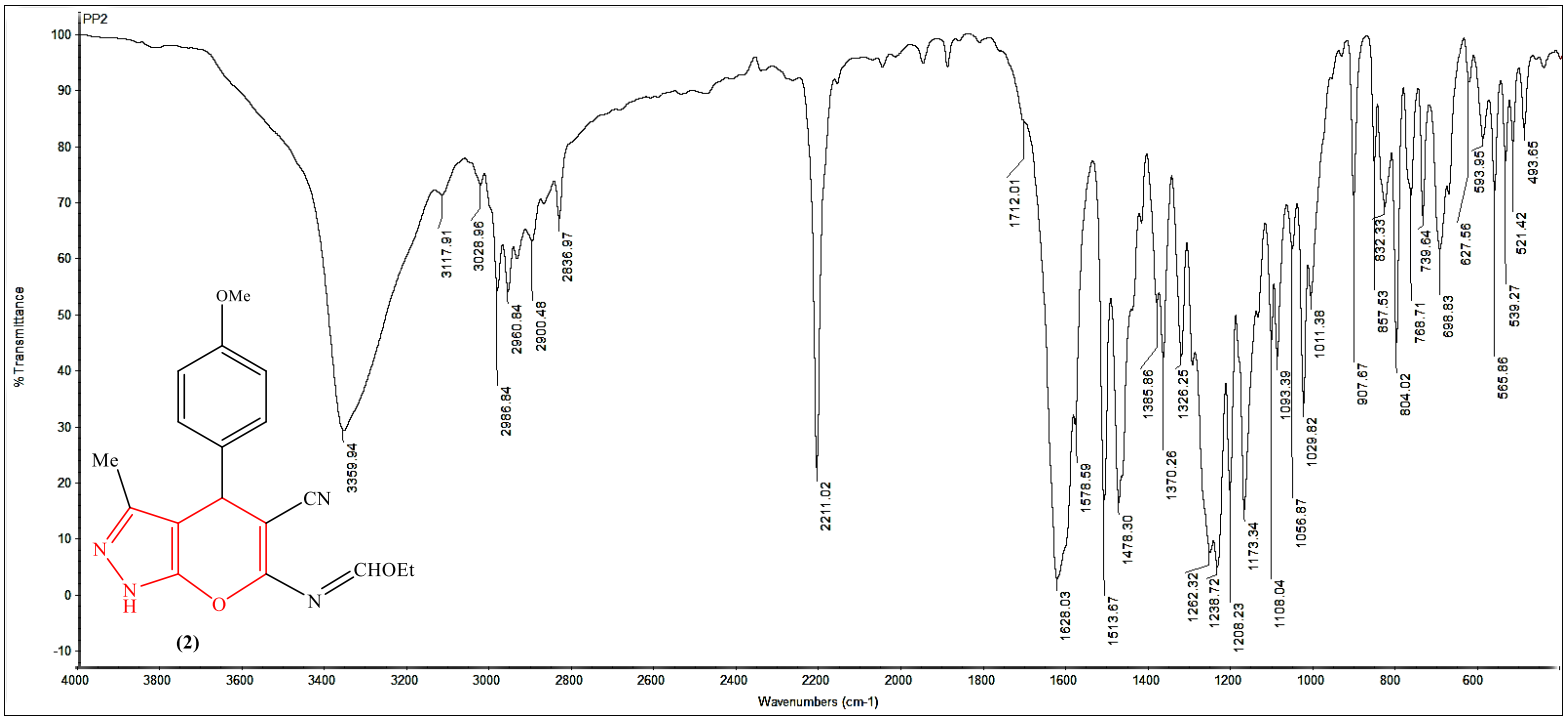

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **1**



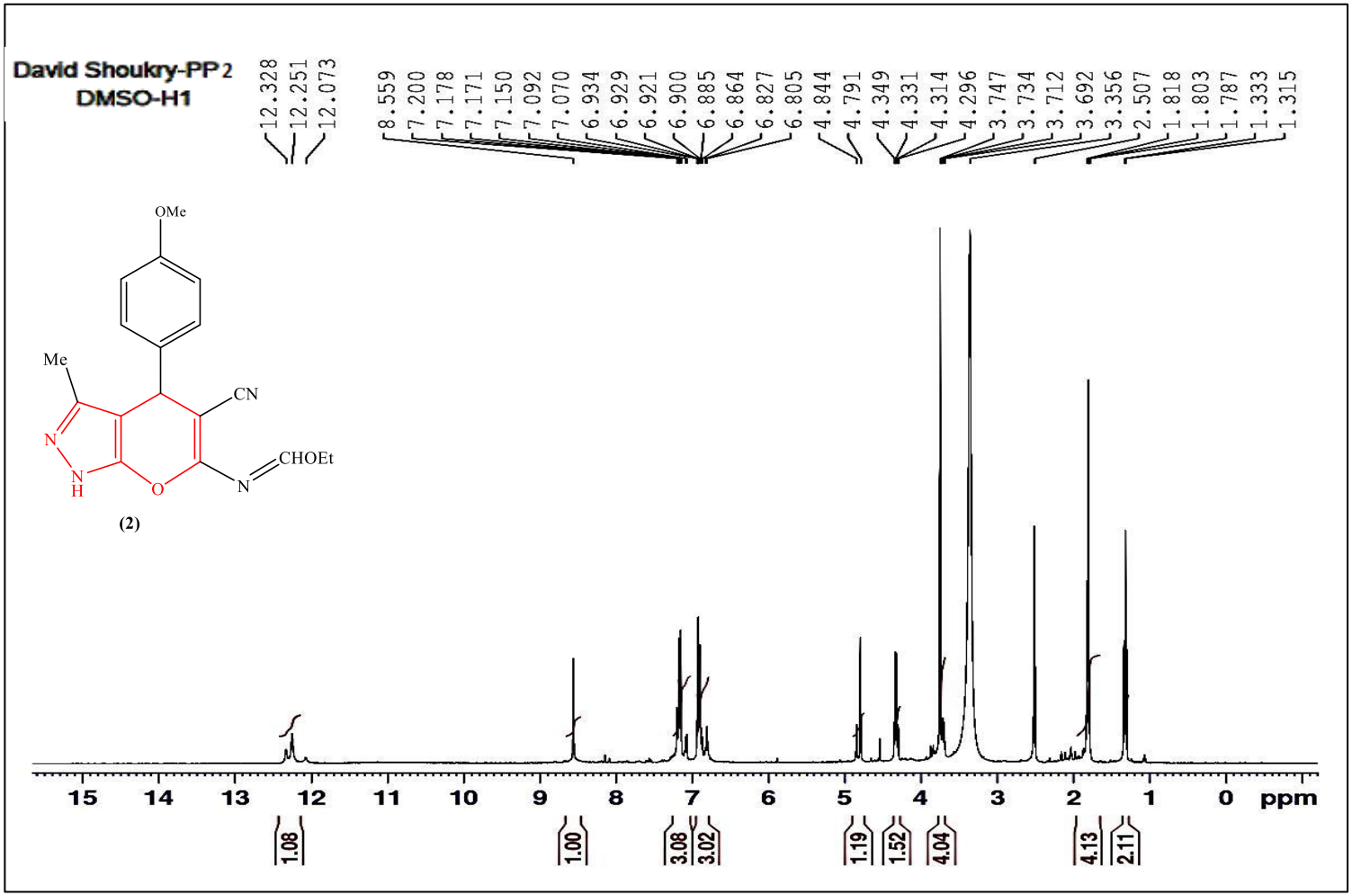

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **1**



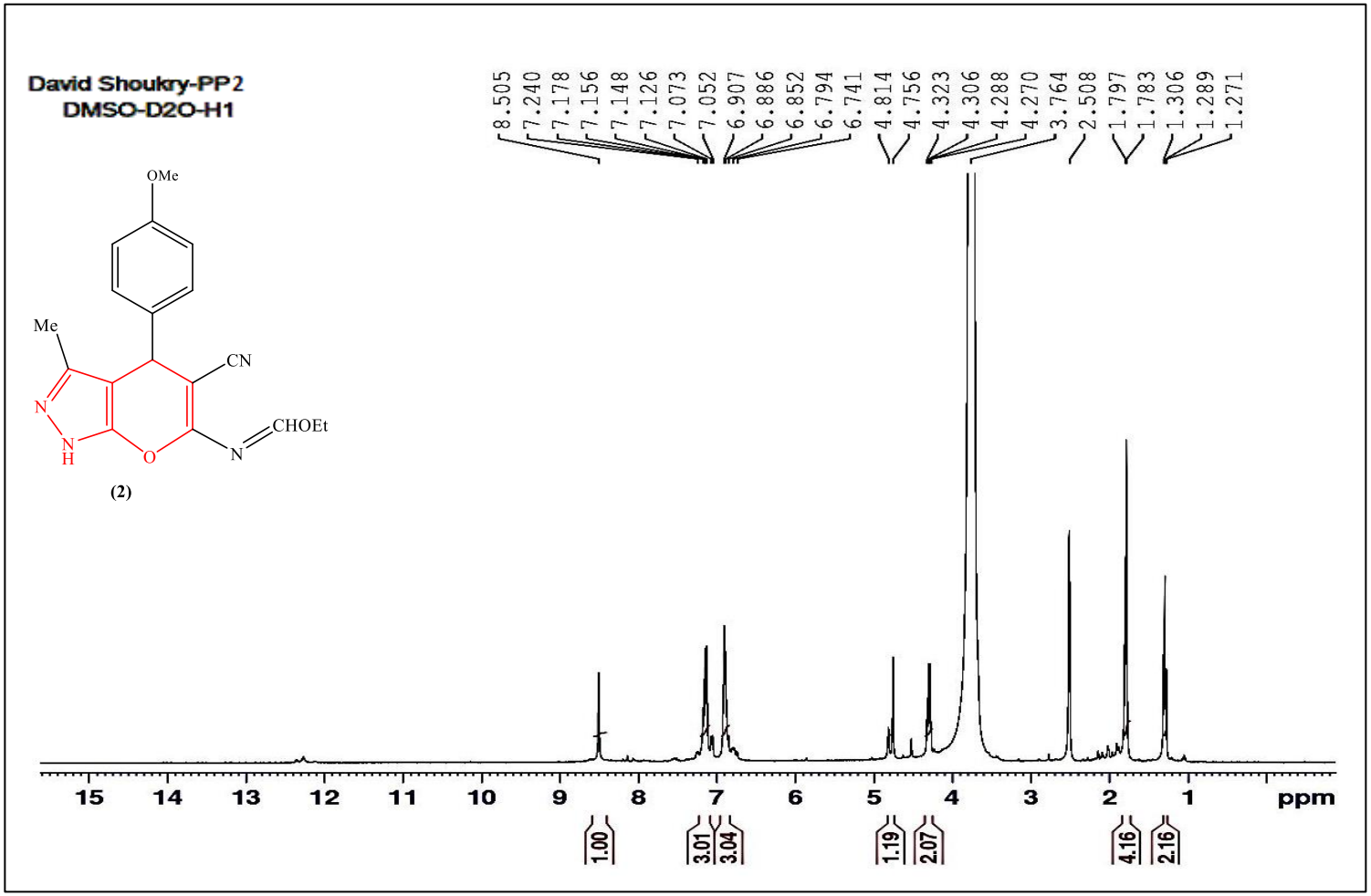

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **1**



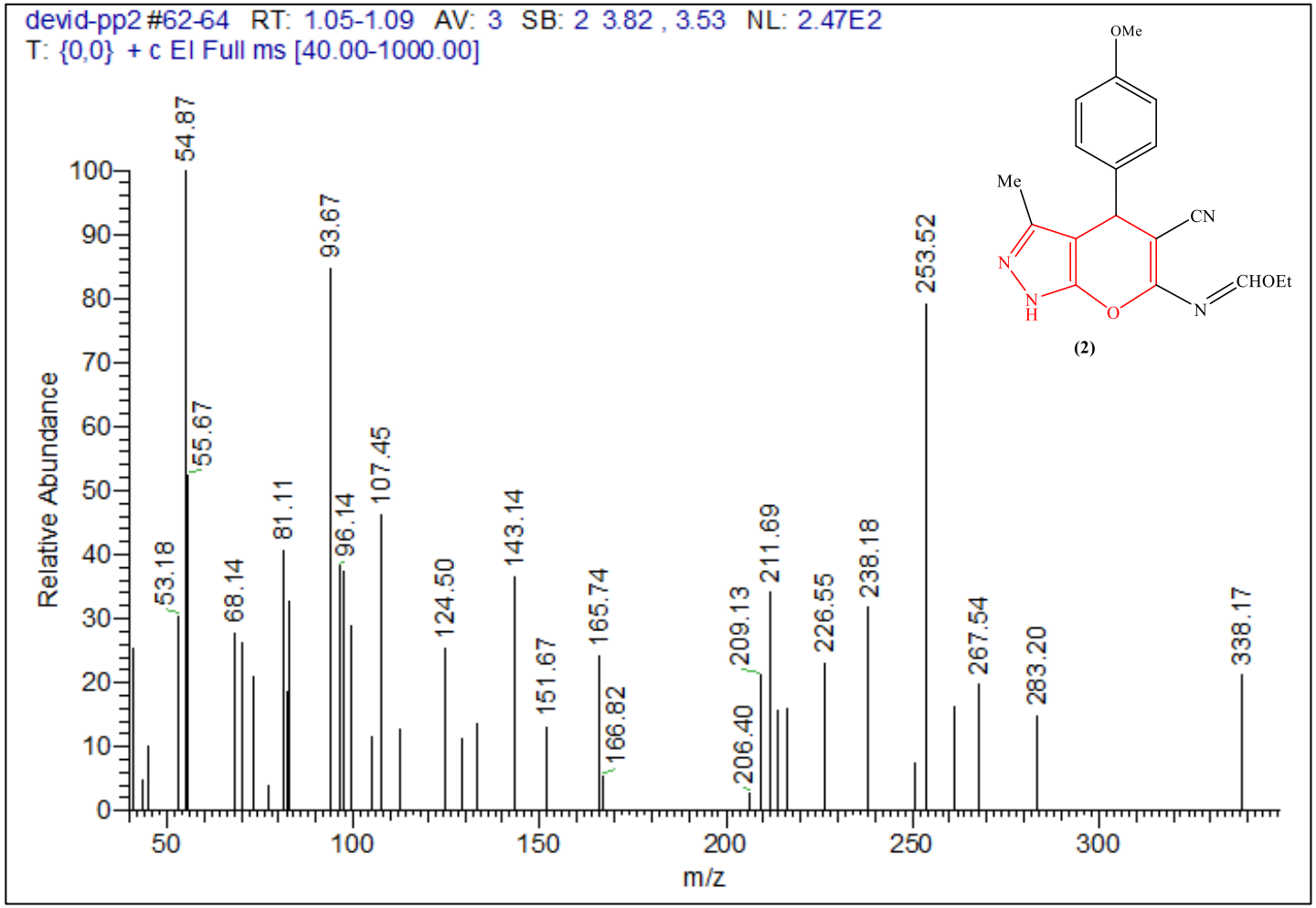

IR spectrum of compound **2**



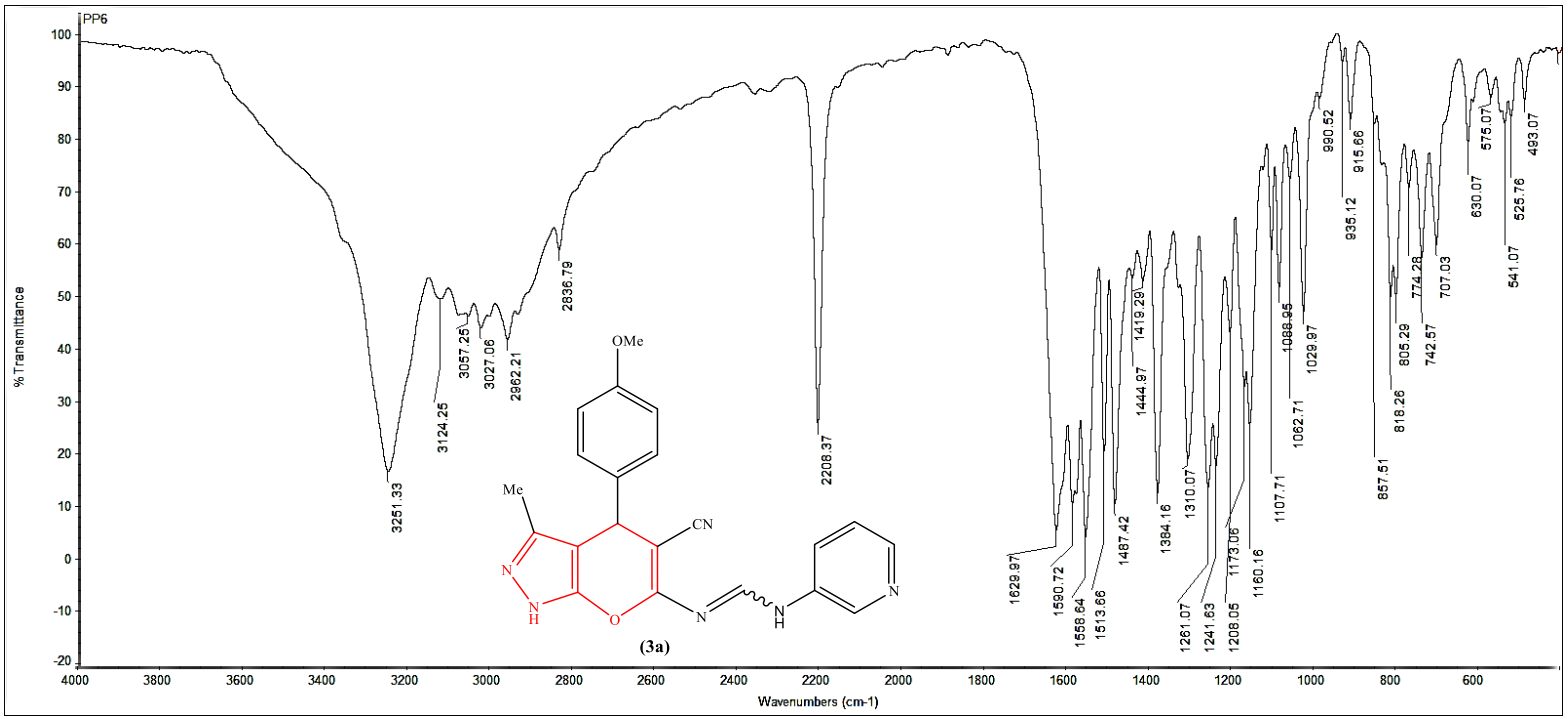

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **2**



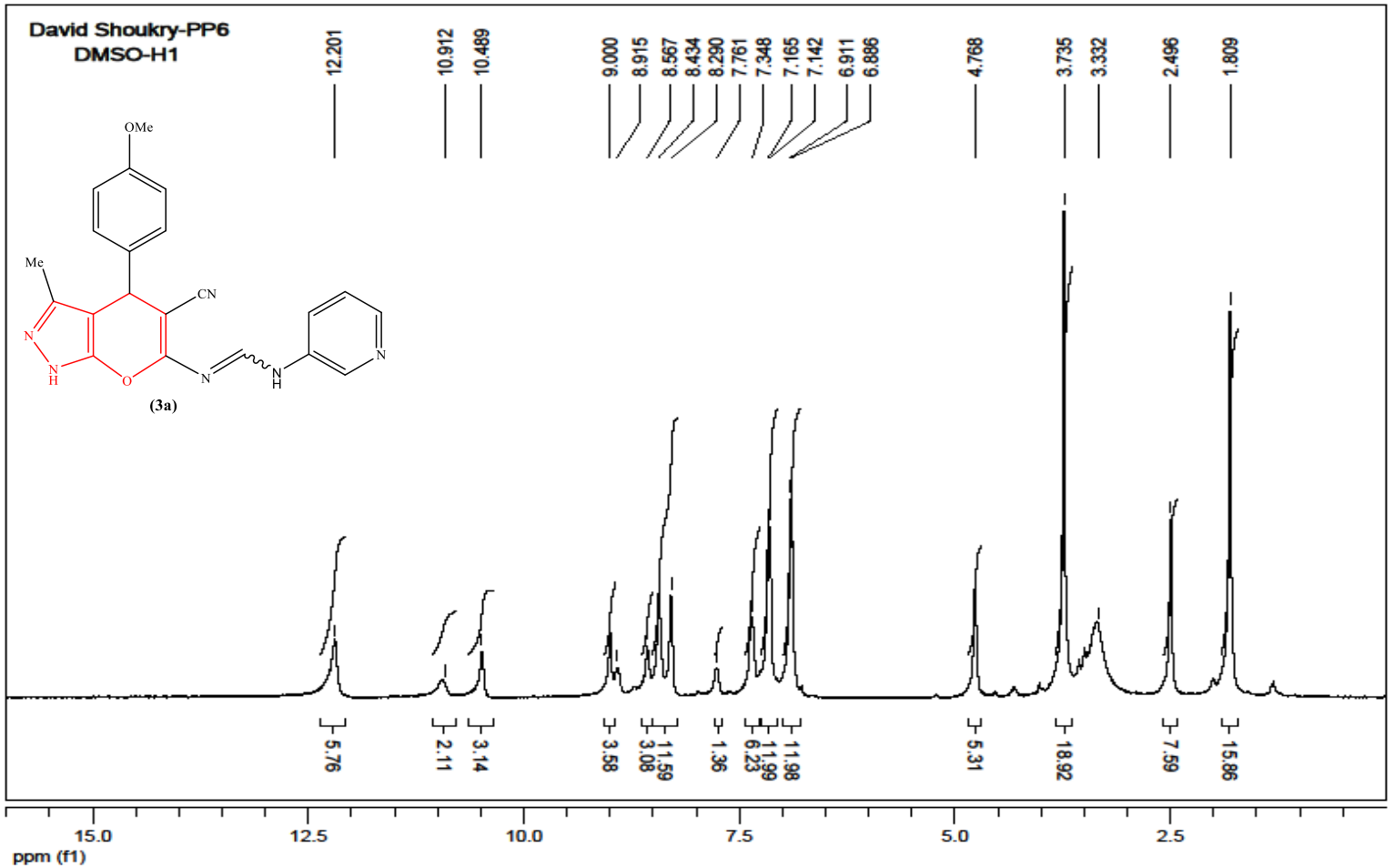

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **2**



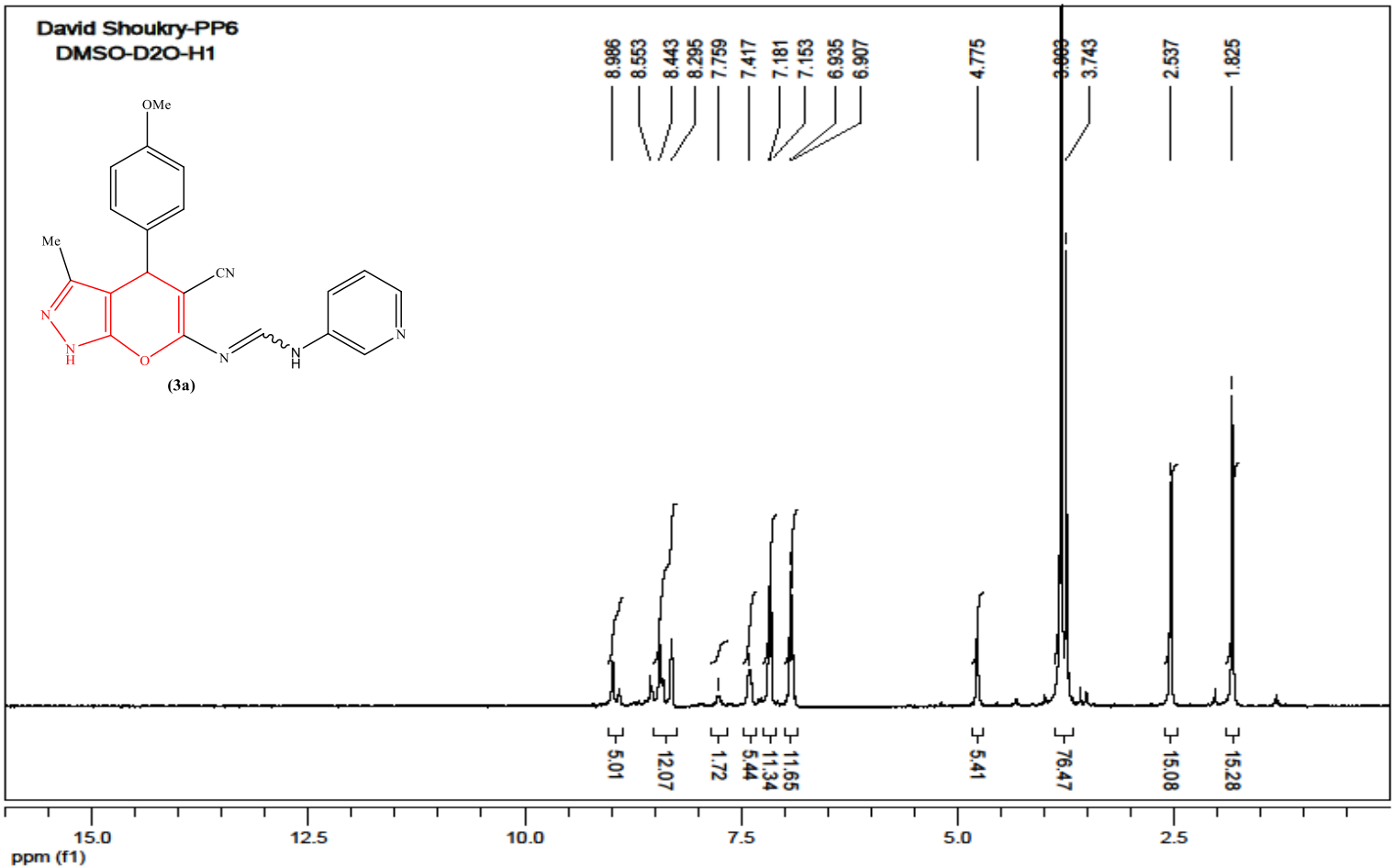

Mass spectrum of compound **2**



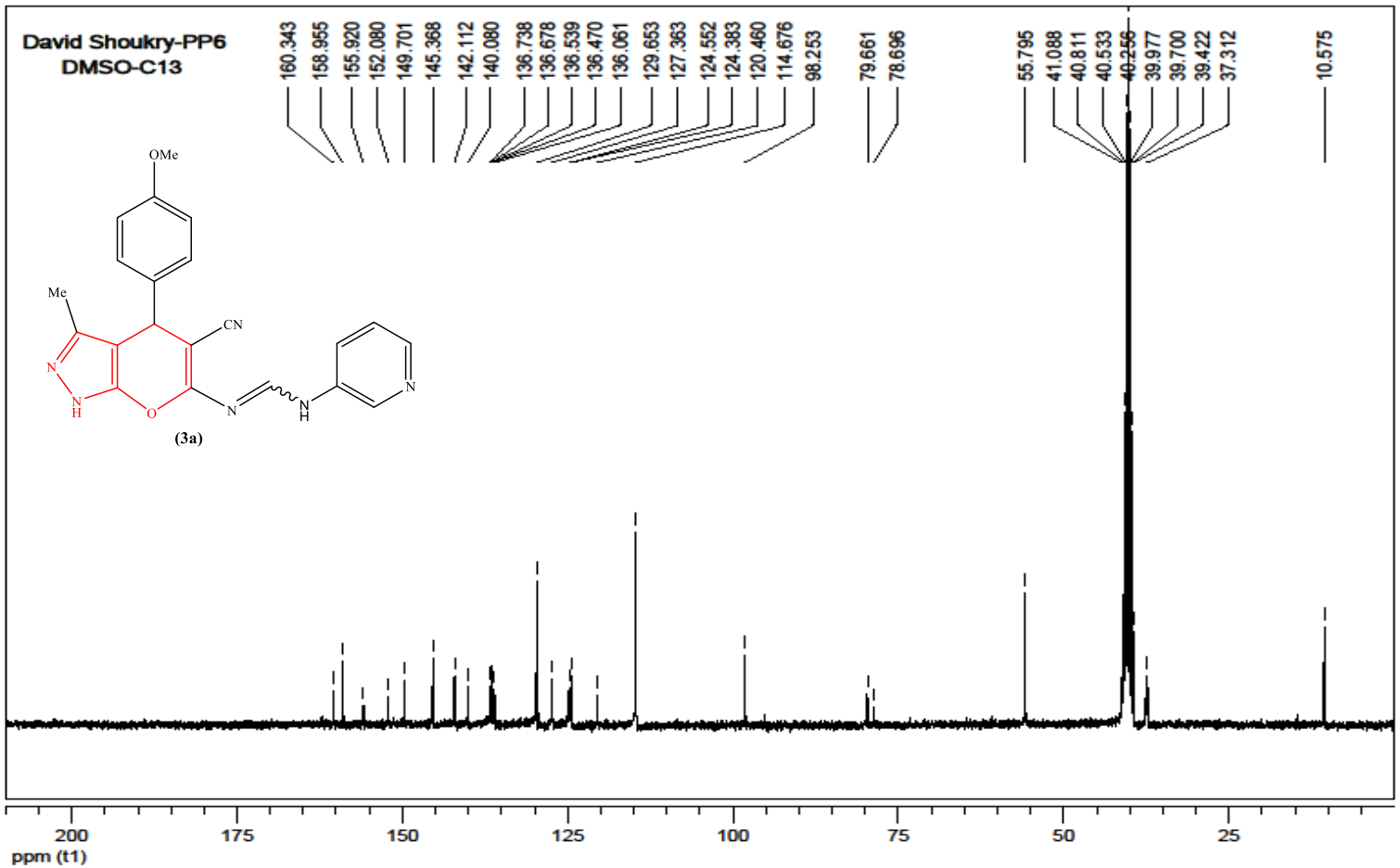

IR spectrum of compound **3a**



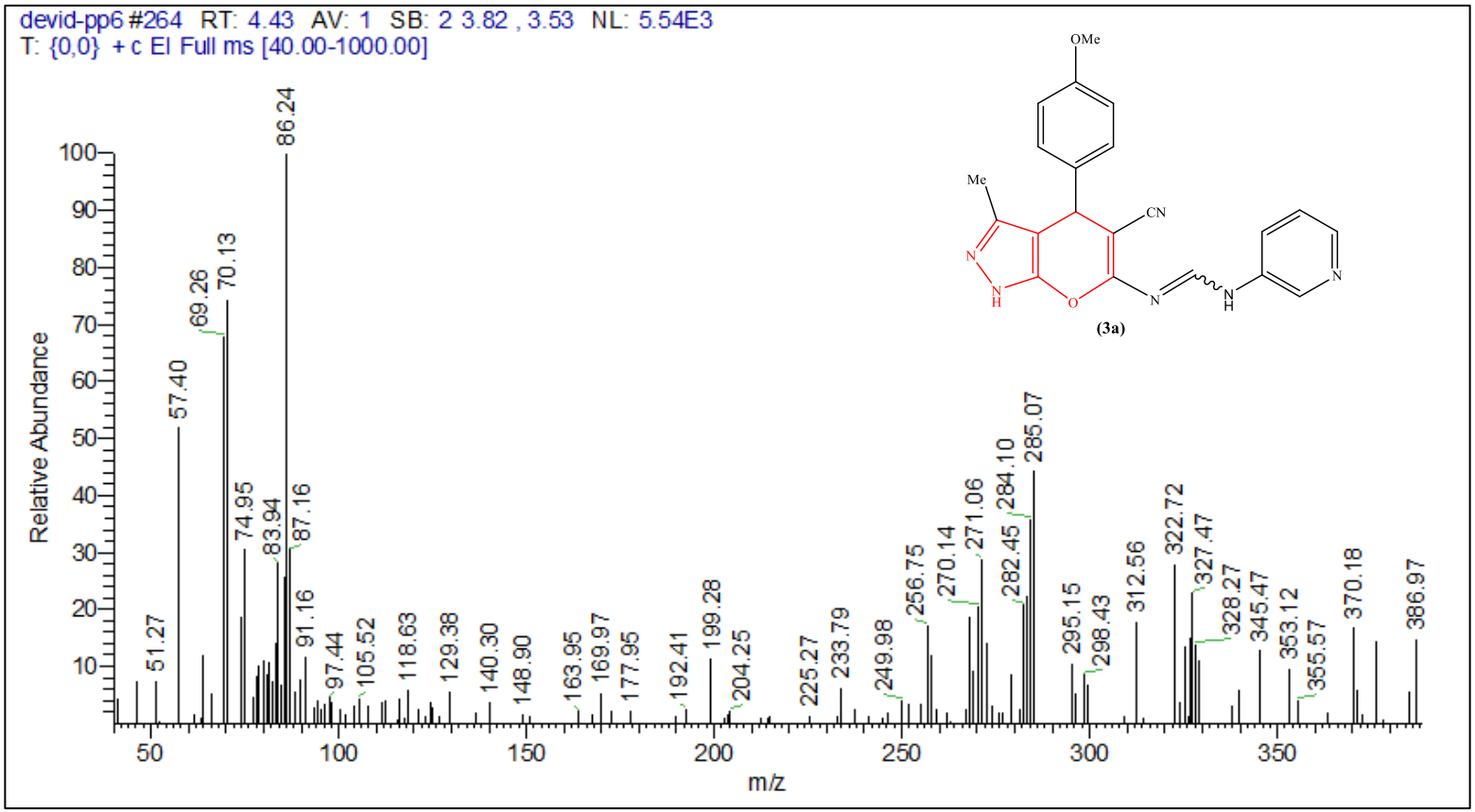

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **3a**



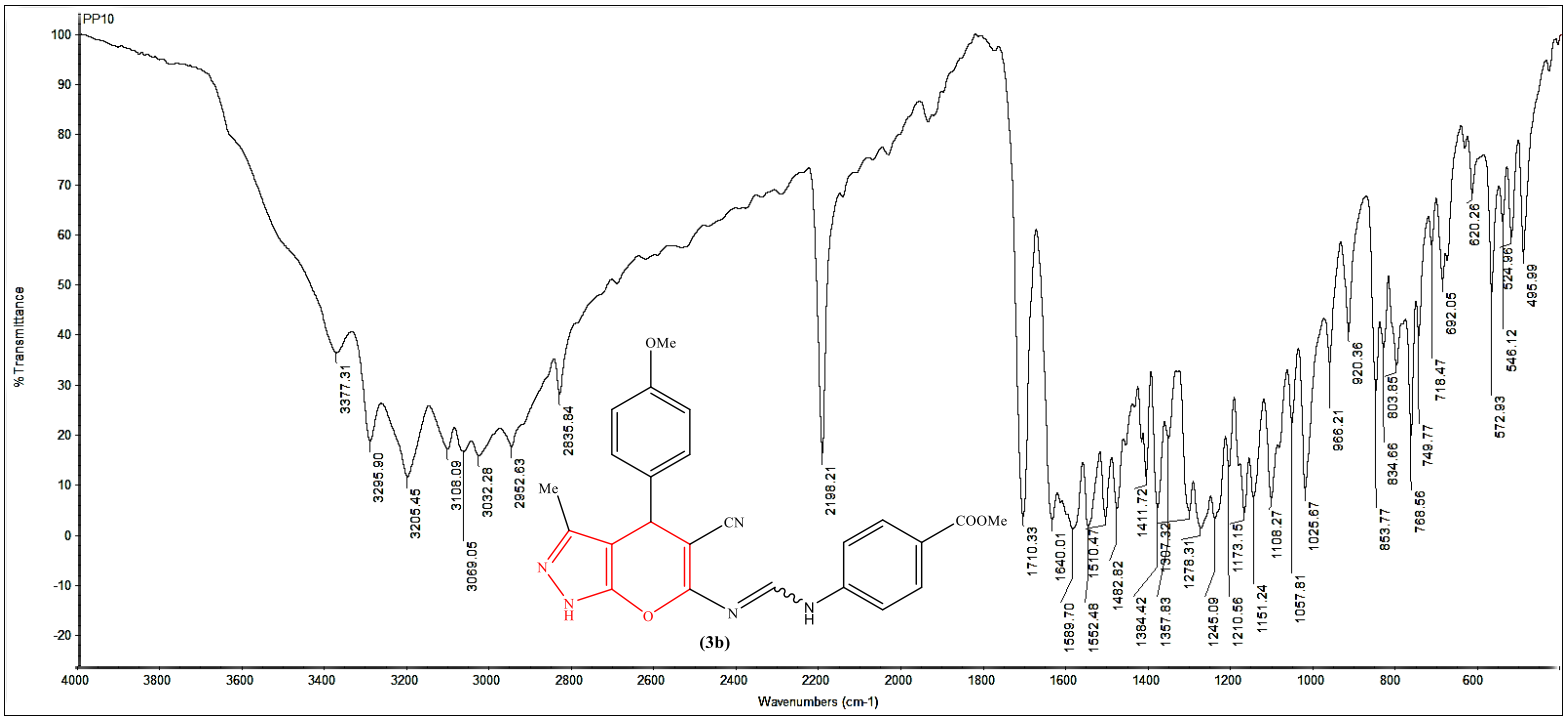

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **3a**



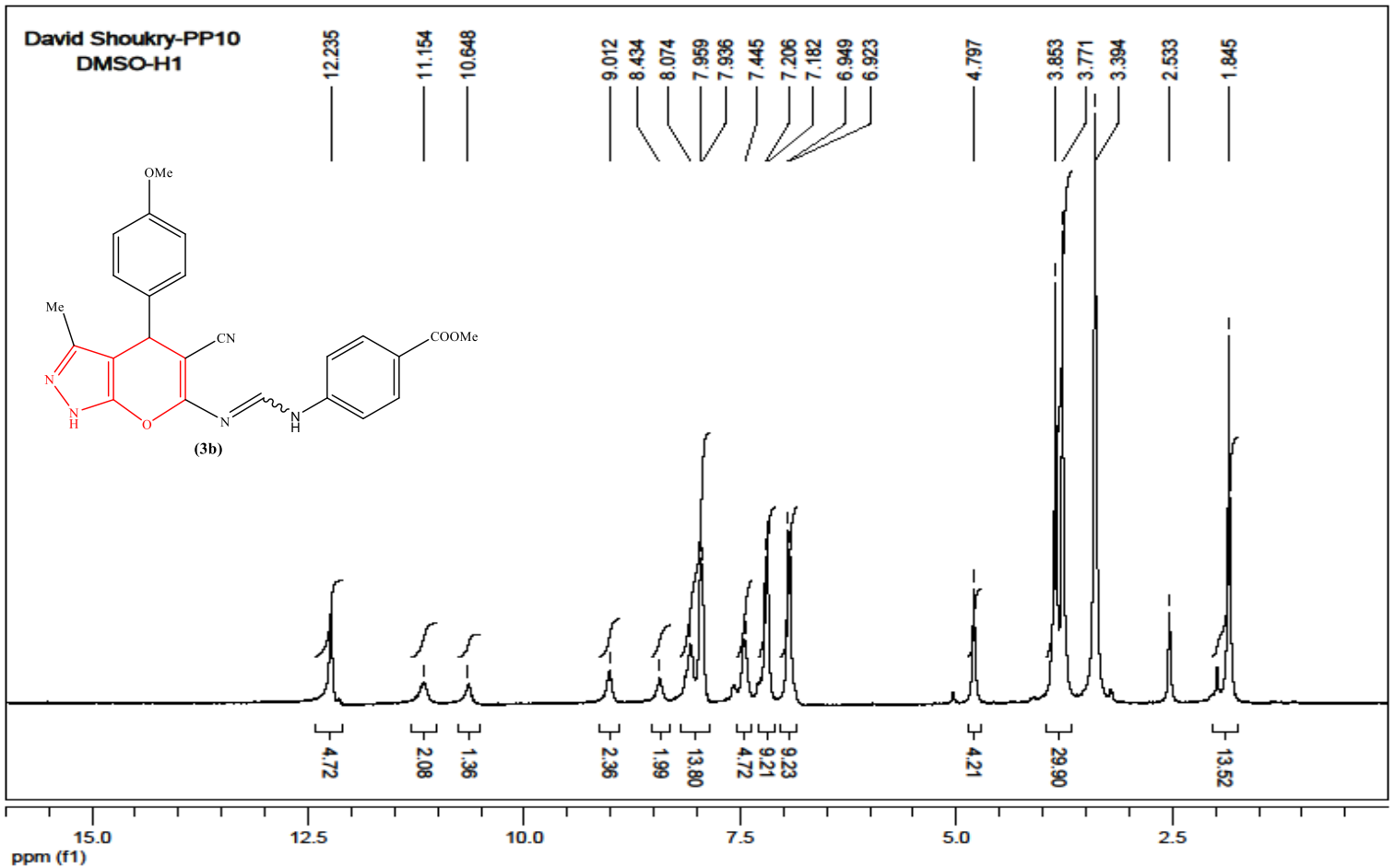

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **3a**



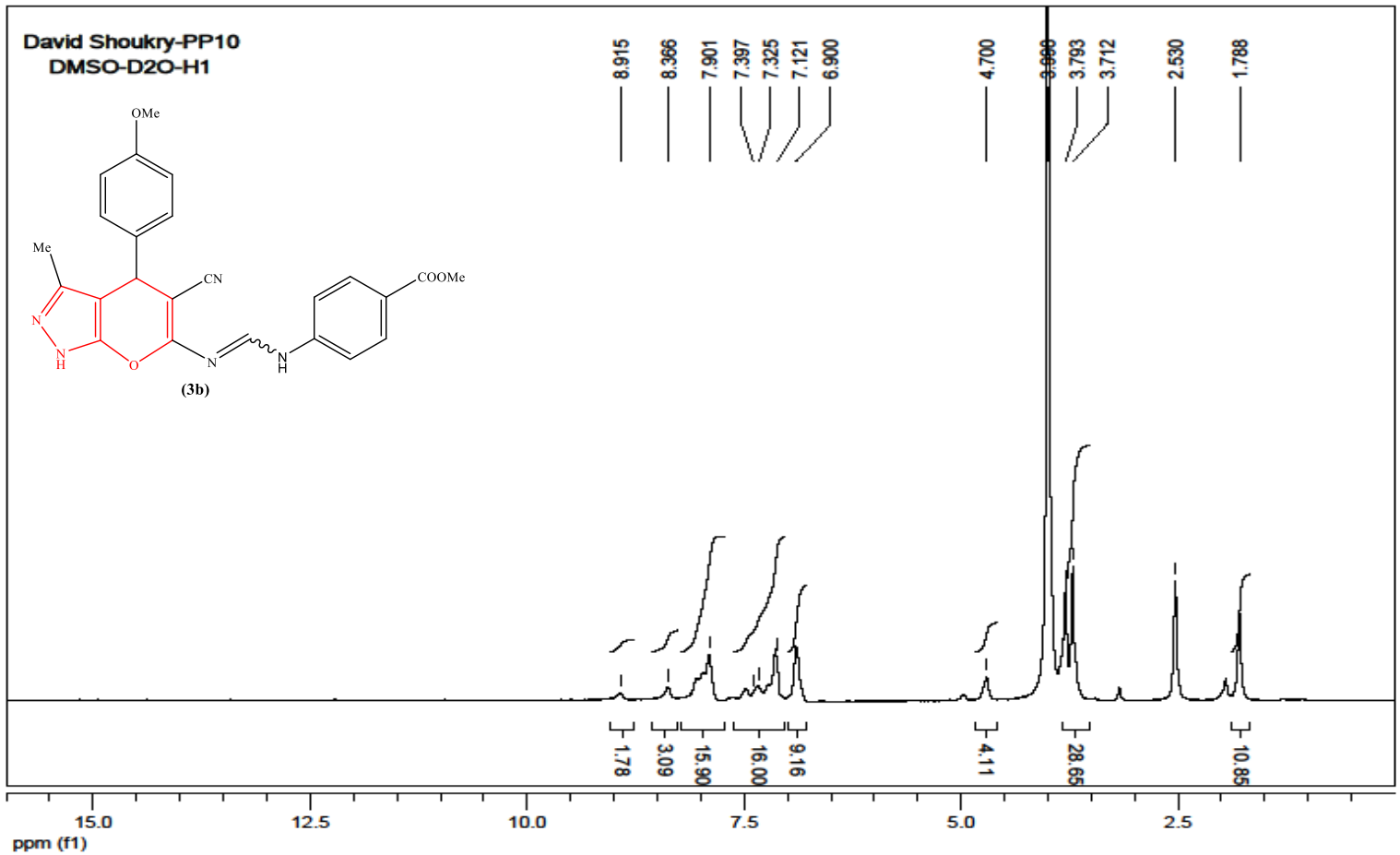

Mass spectrum of compound **3a**



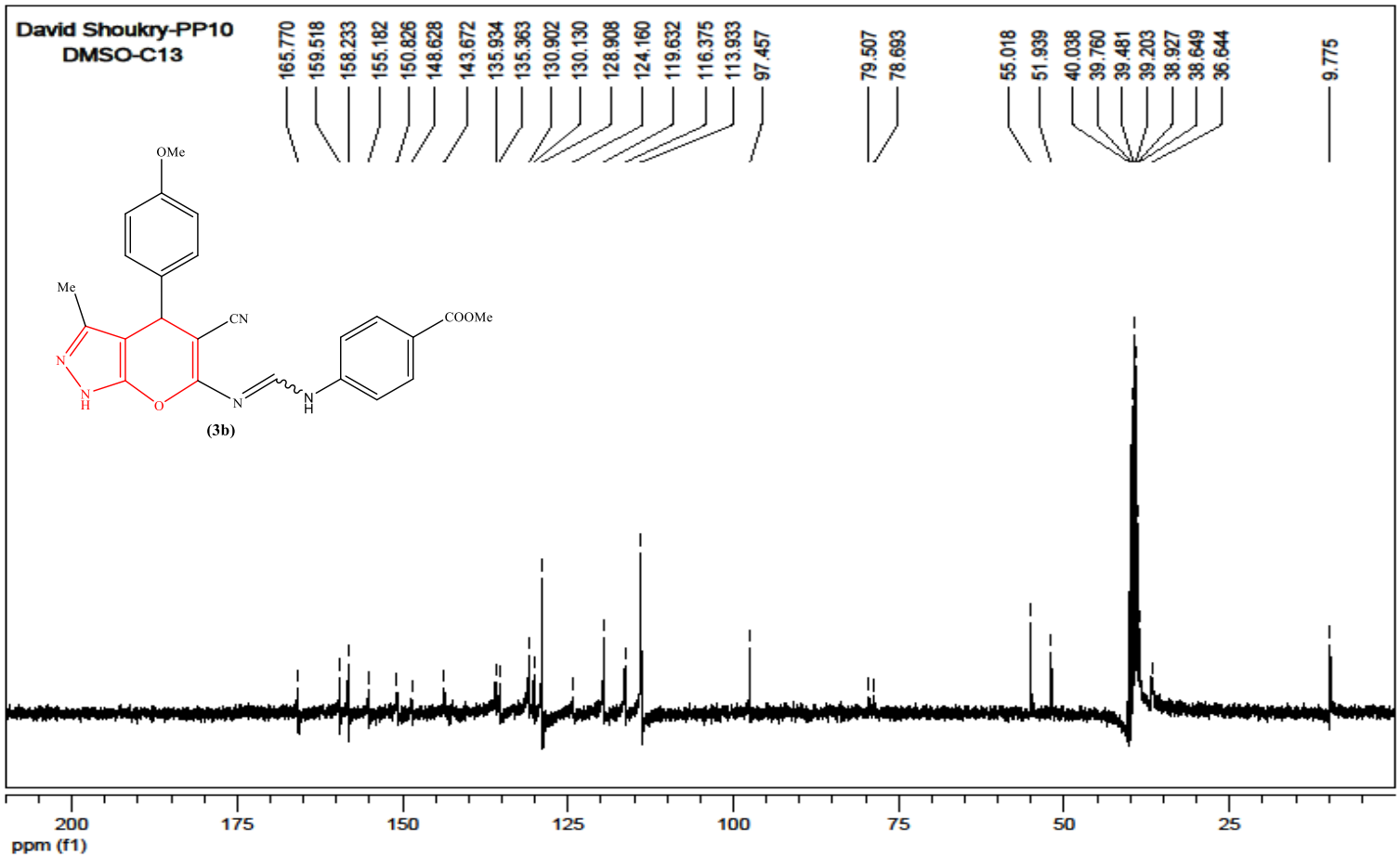

IR spectrum of compound **3b**



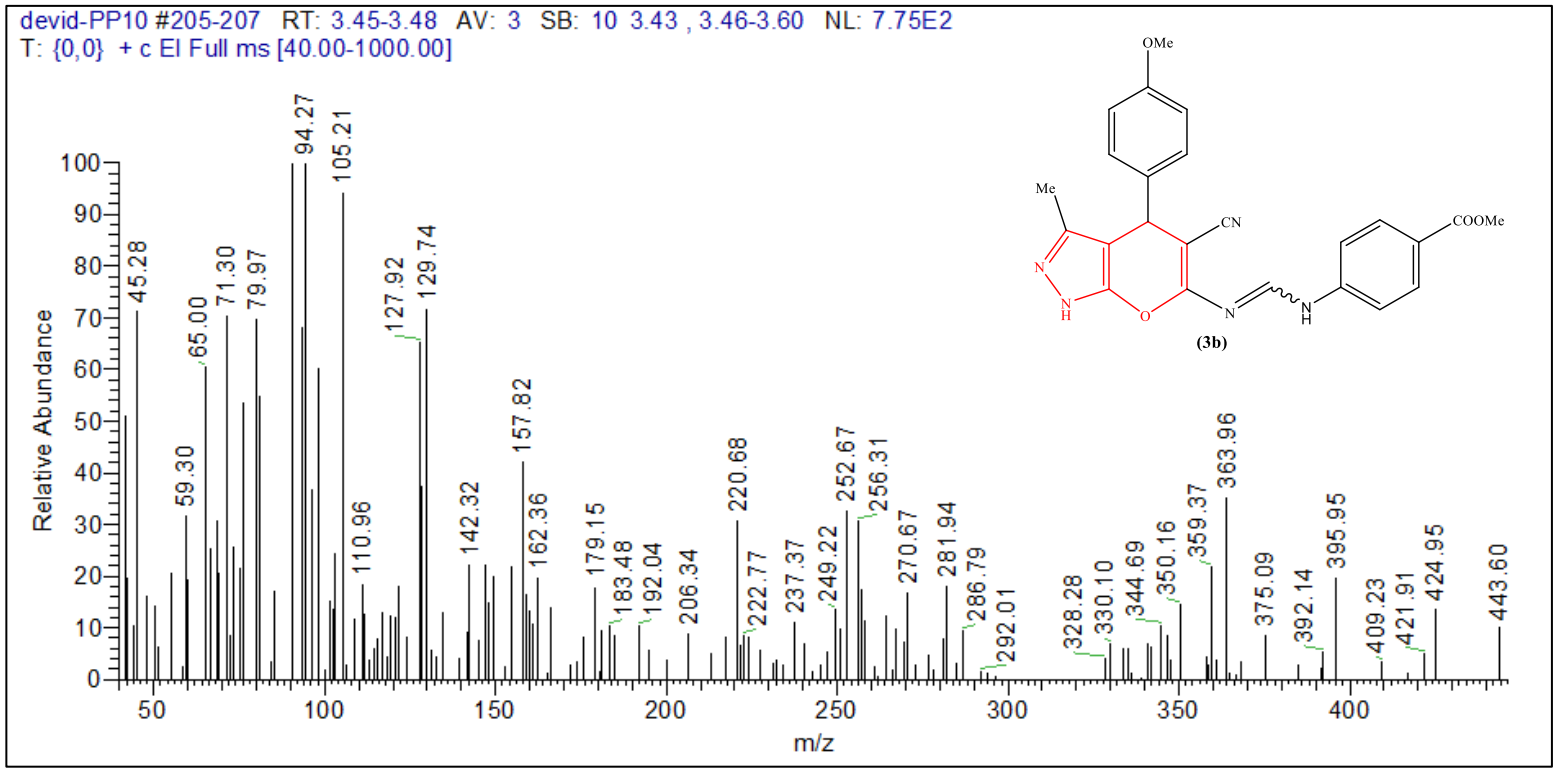

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **3b**



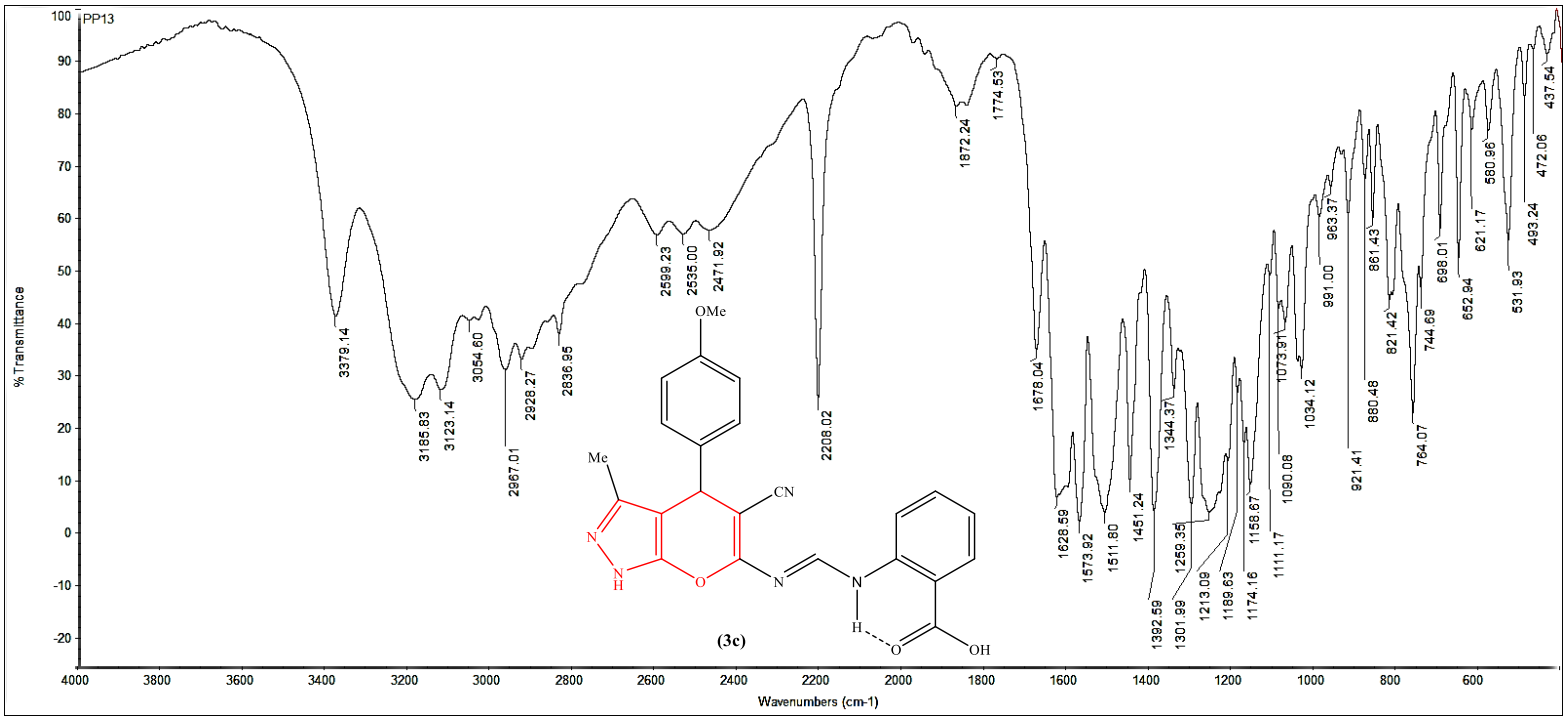

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **3b**



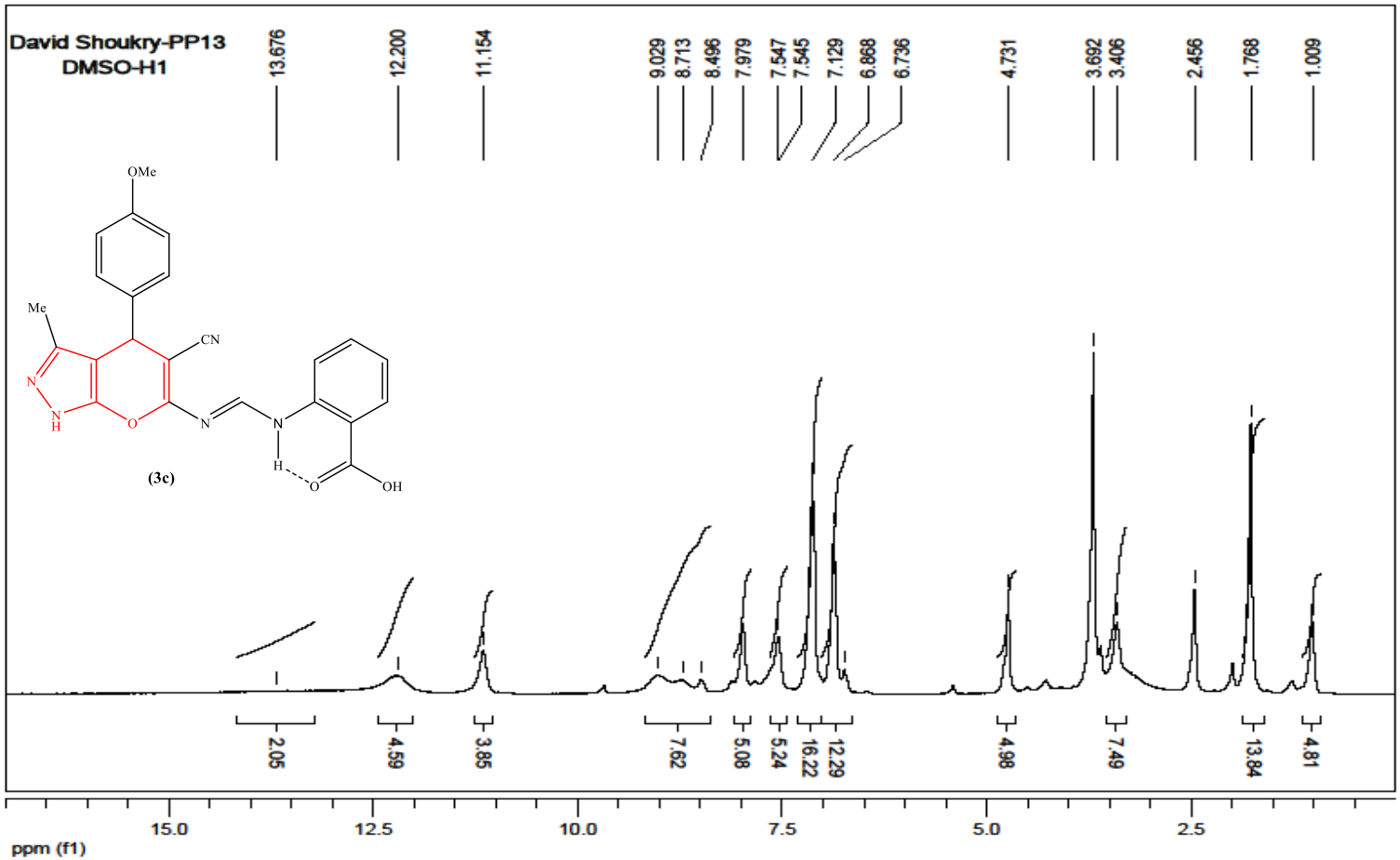

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **3b**



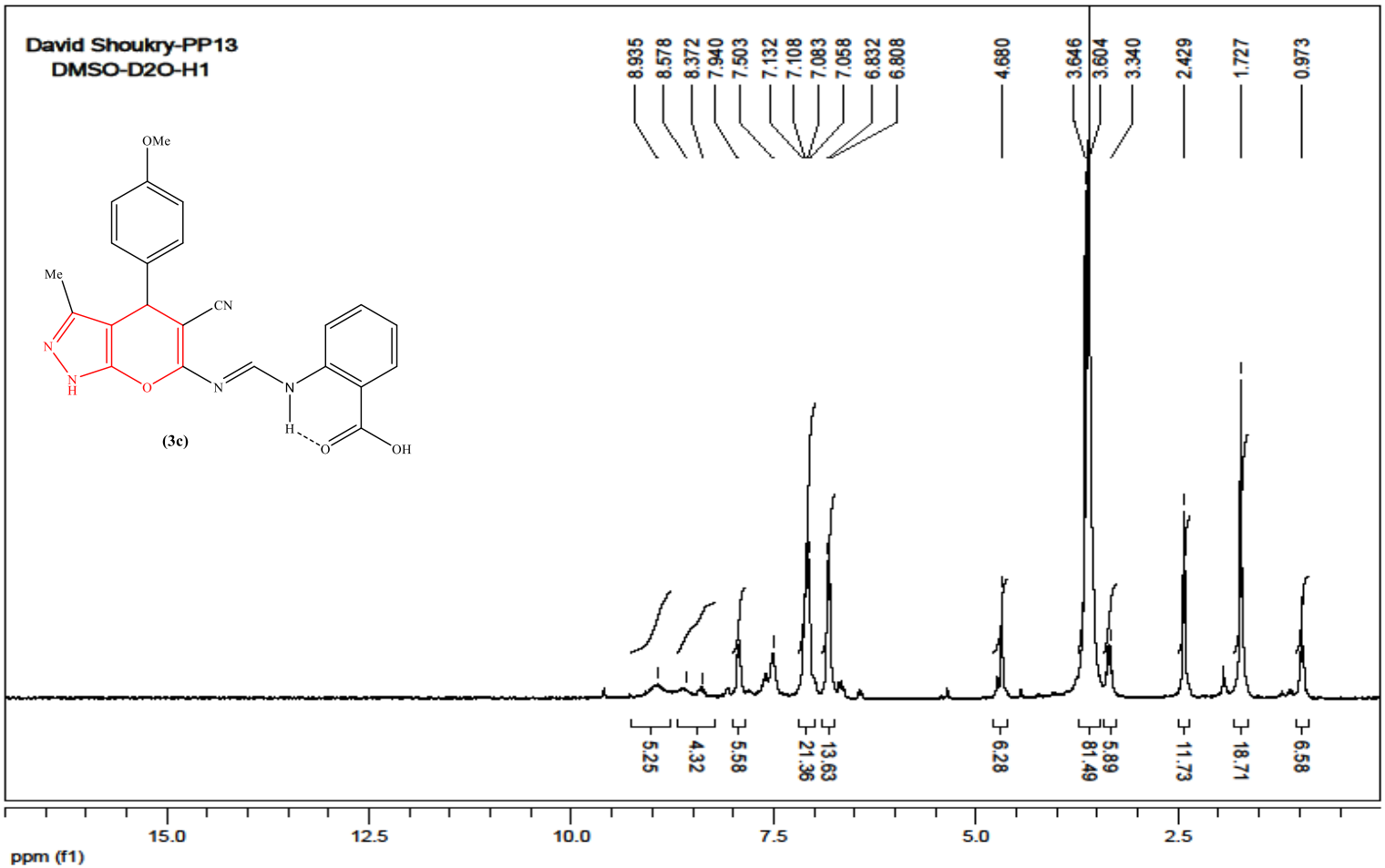

Mass spectrum of compound **3b**



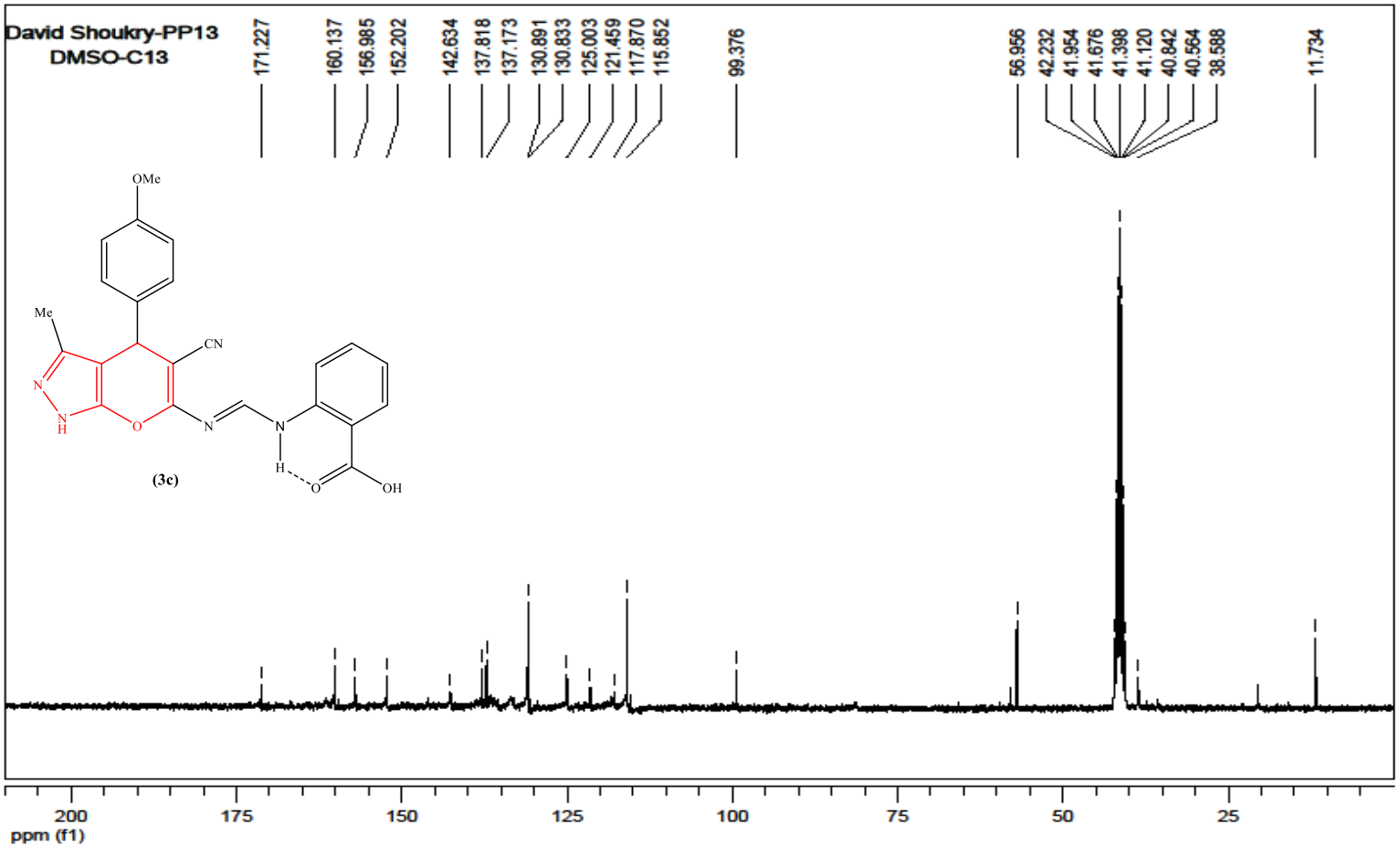

IR spectrum of compound **3c**



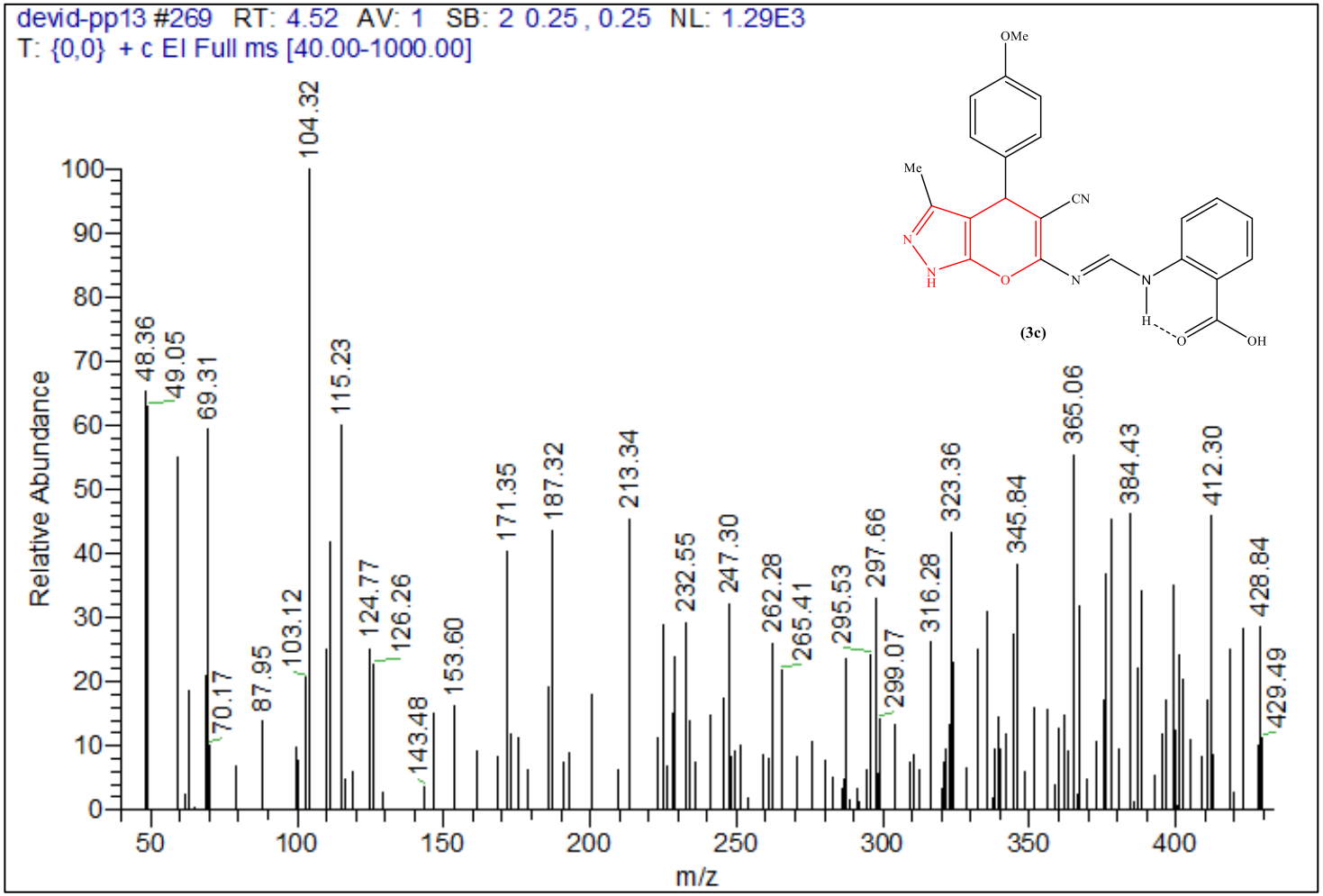

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **3c**



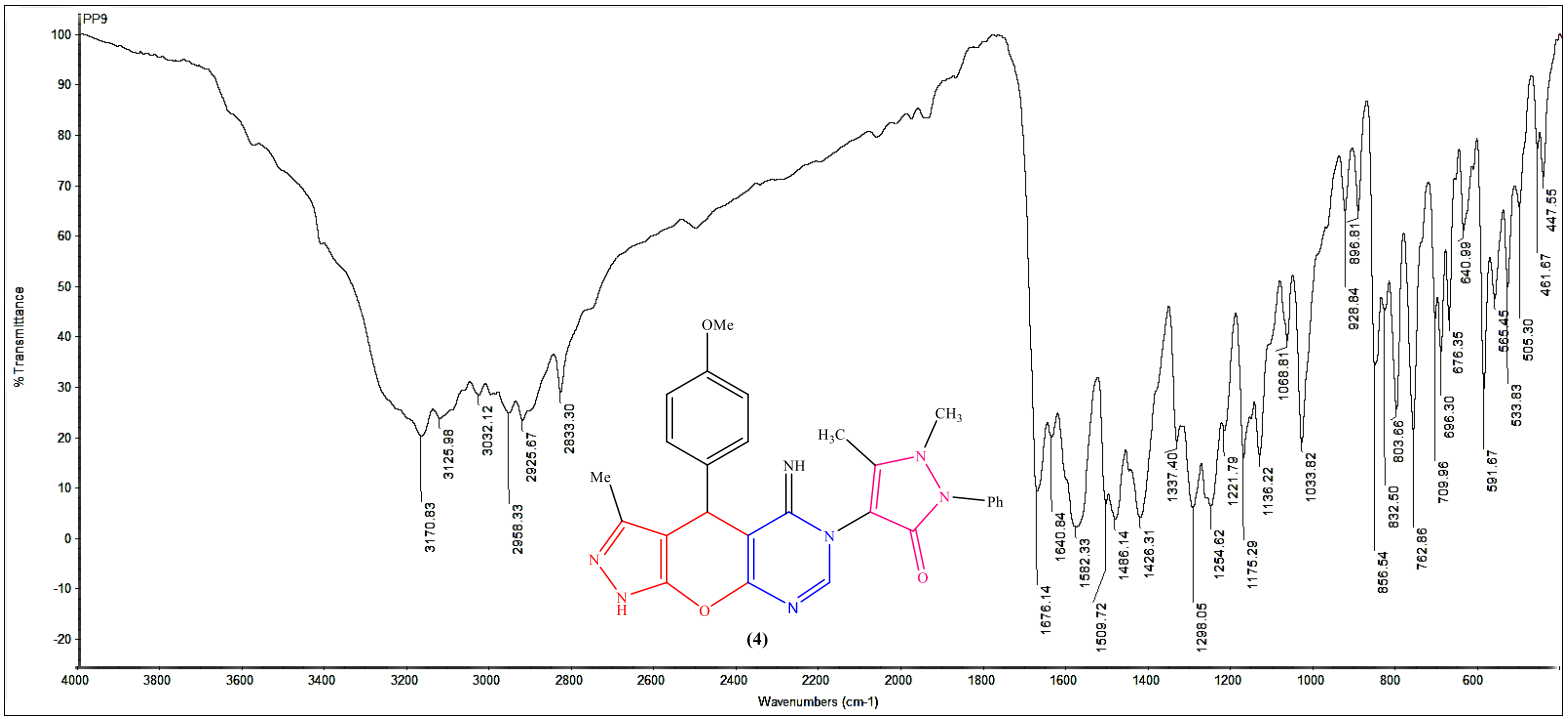

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **3c**




$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **3c**

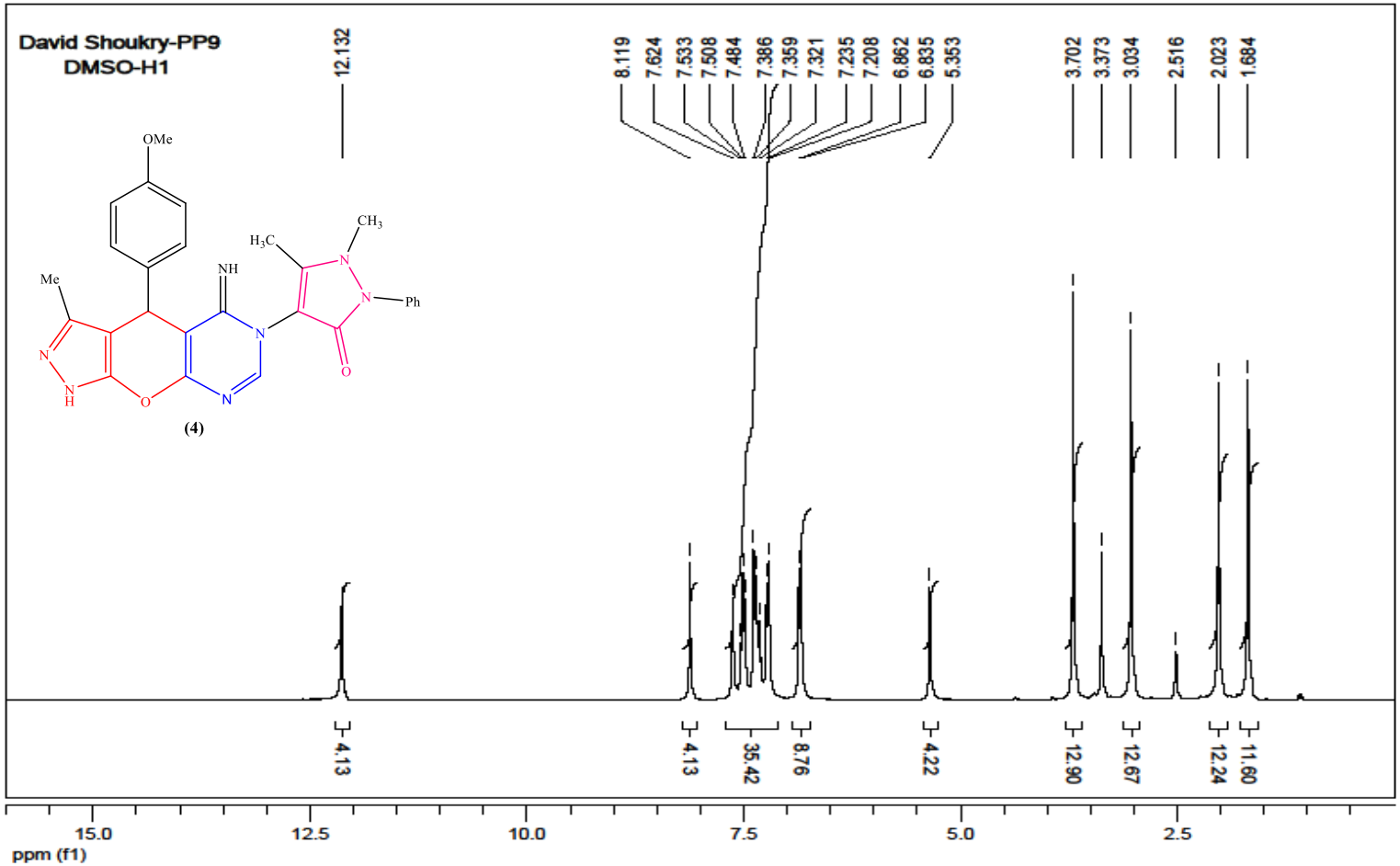



Mass spectrum of compound **3c**

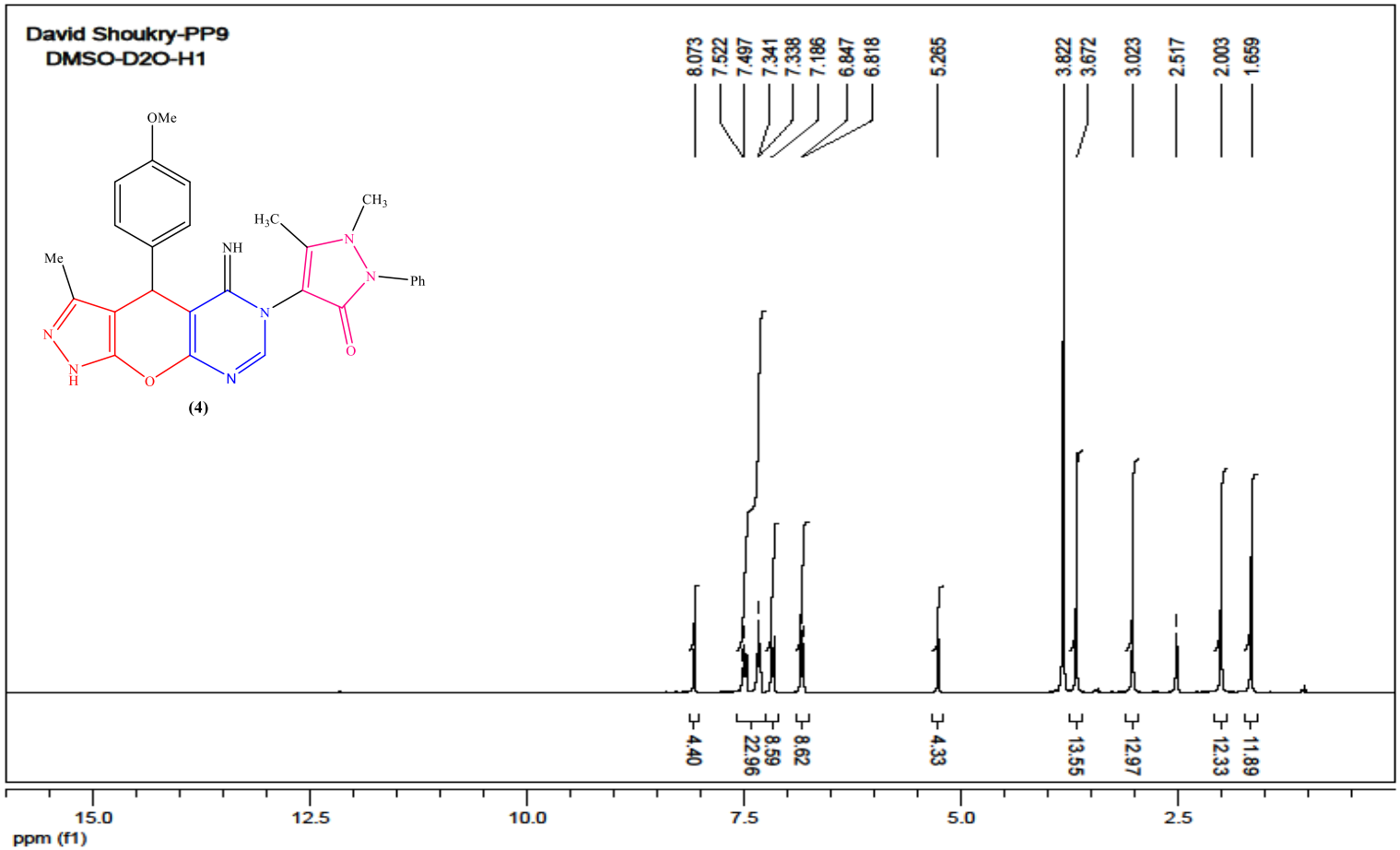



(4)

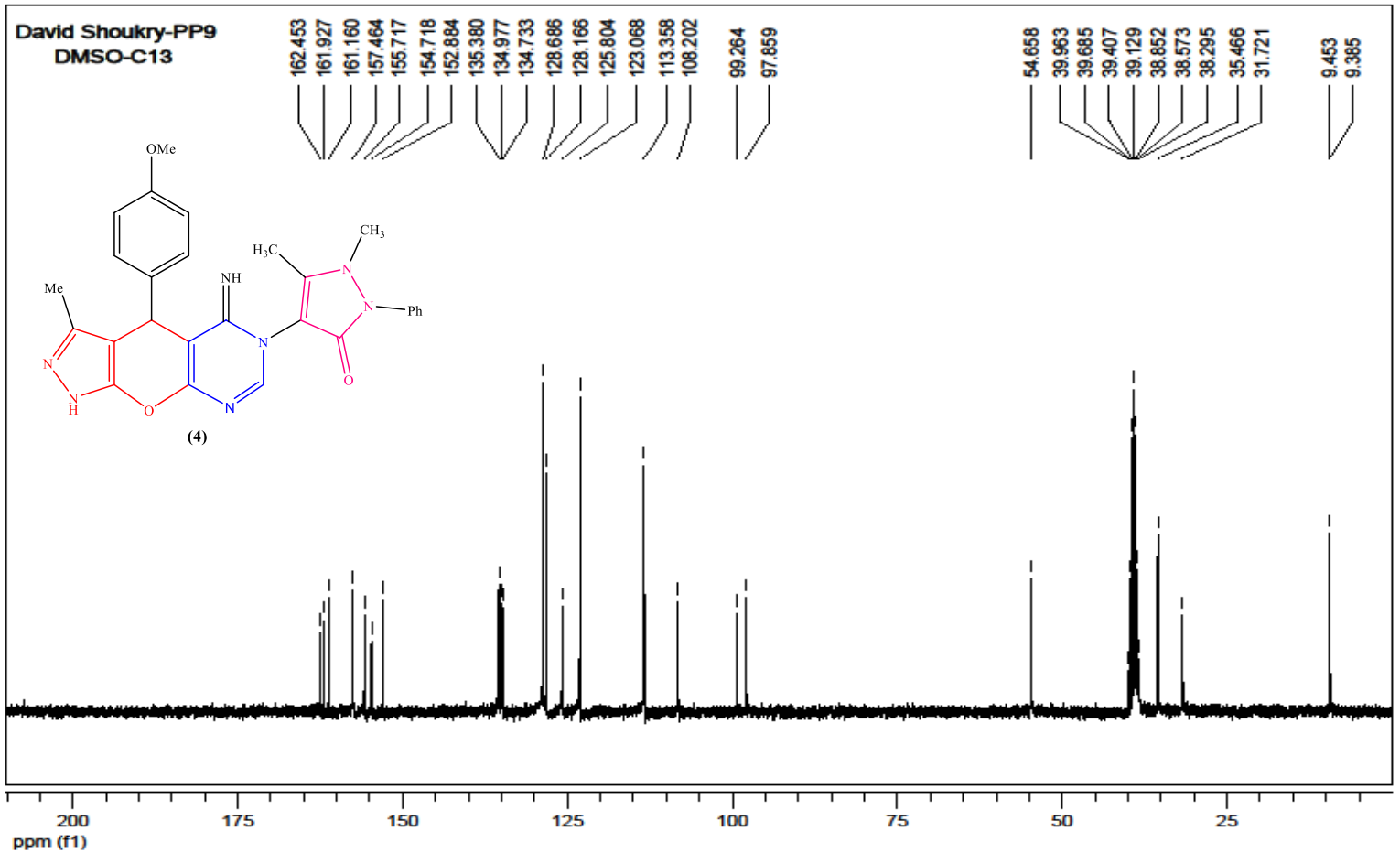
IR spectrum of compound **4**



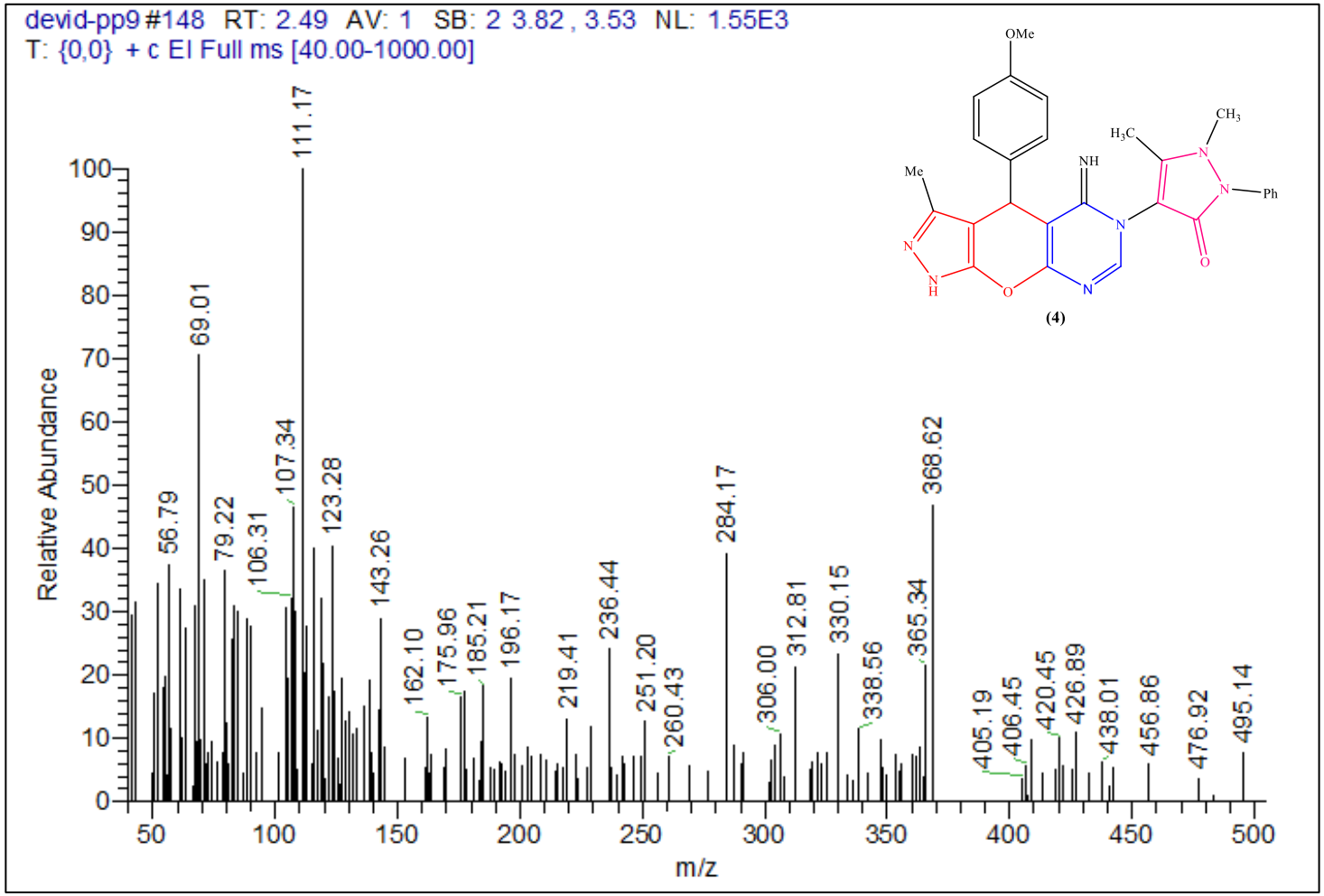

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **4**



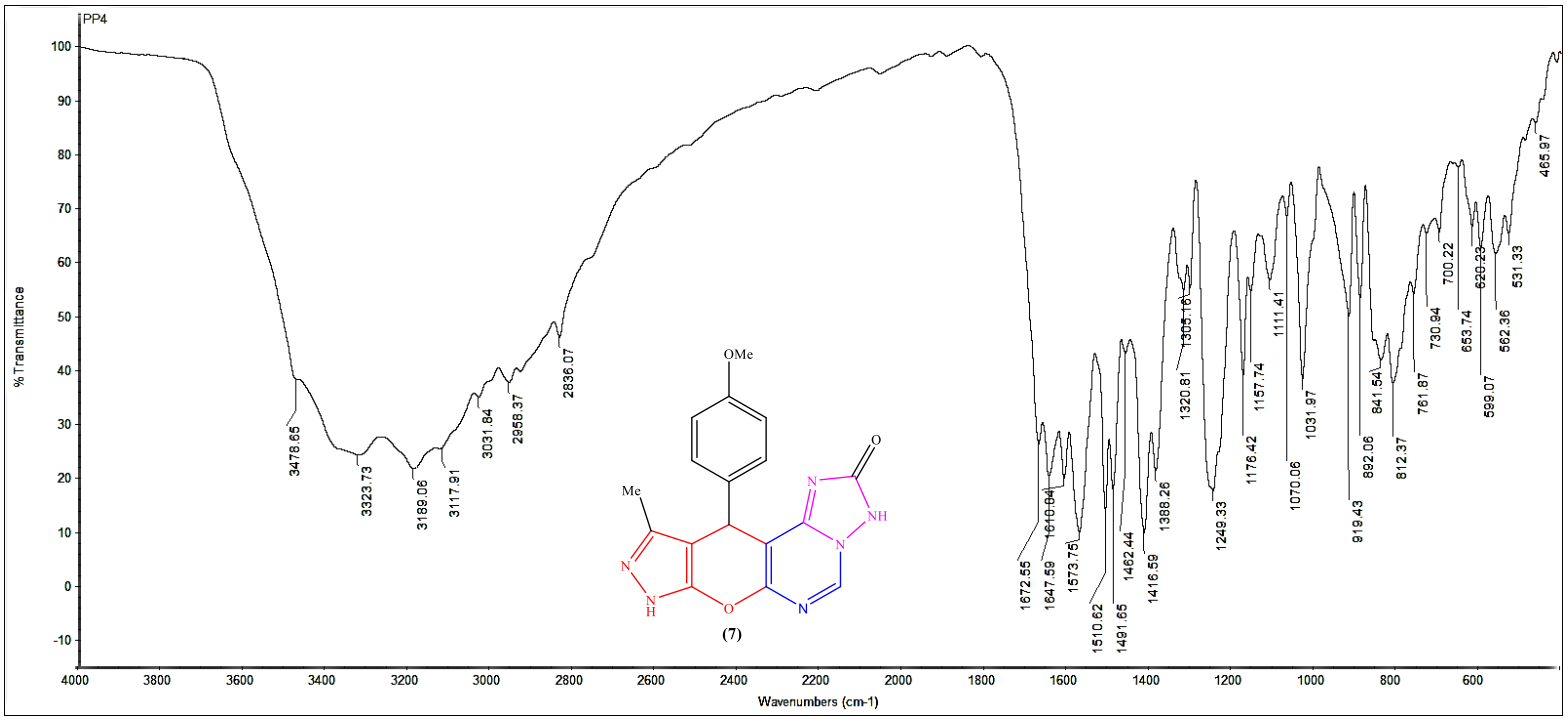

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **4**



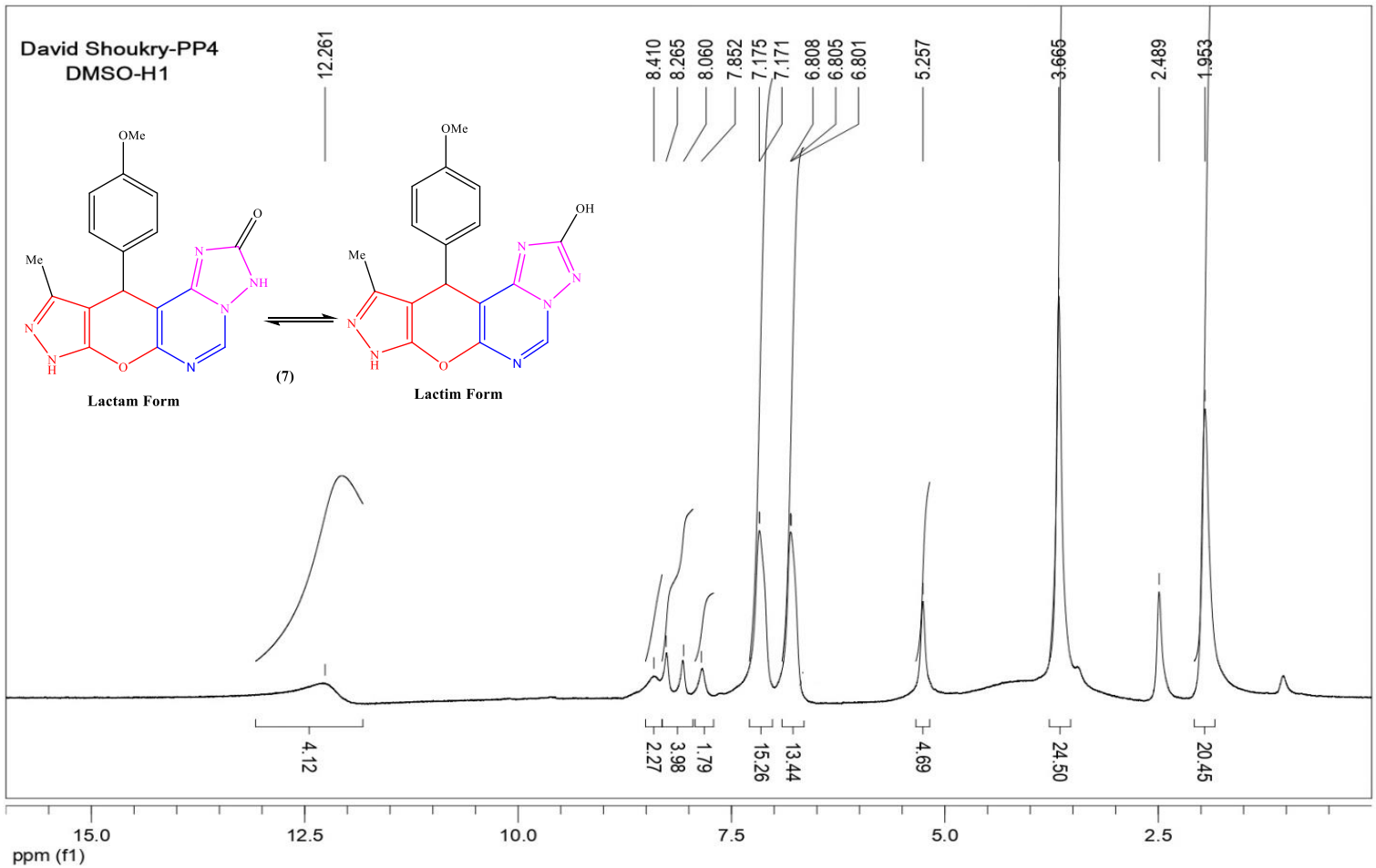

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **4**



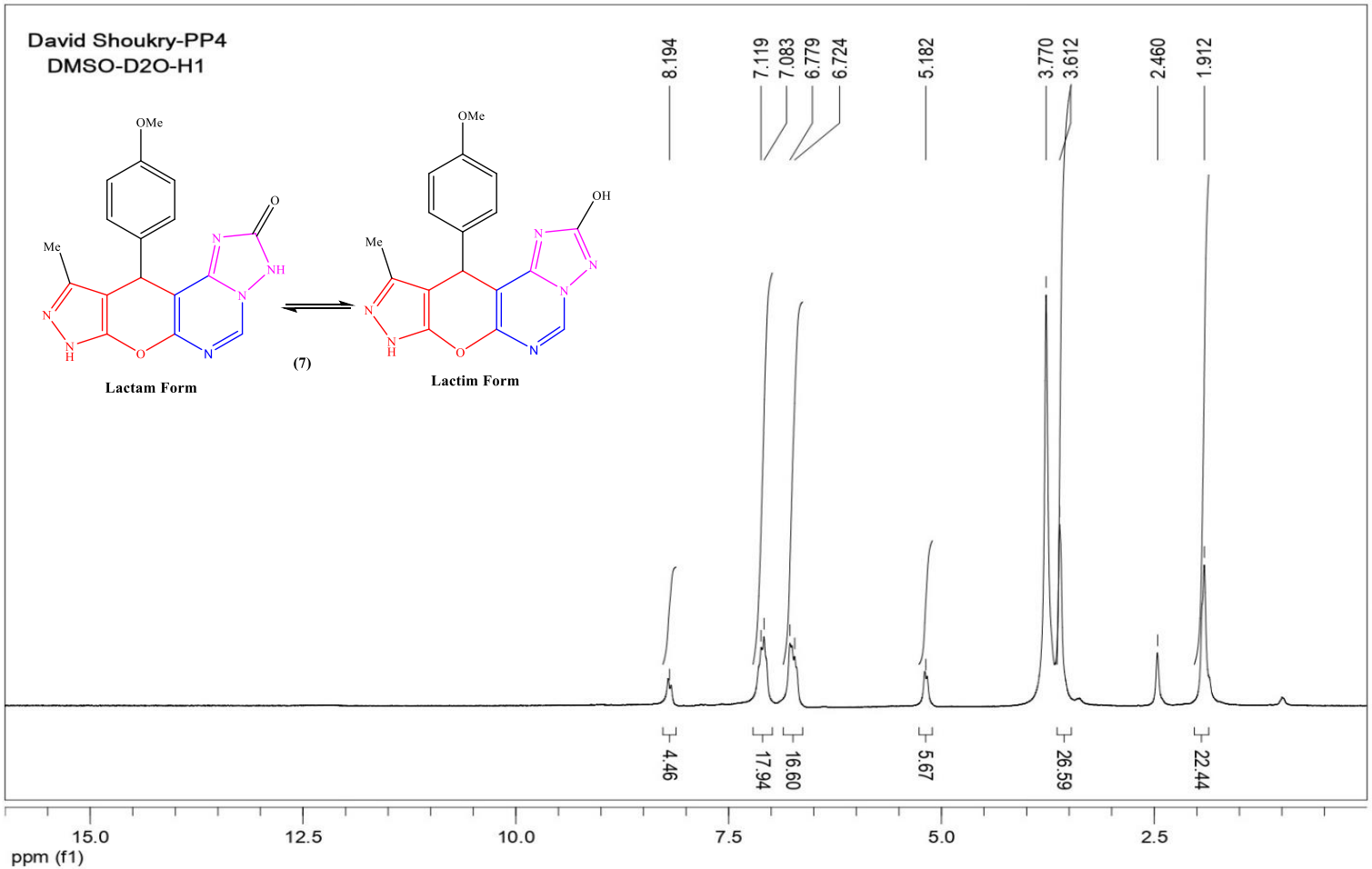

Mass spectrum of compound **4**



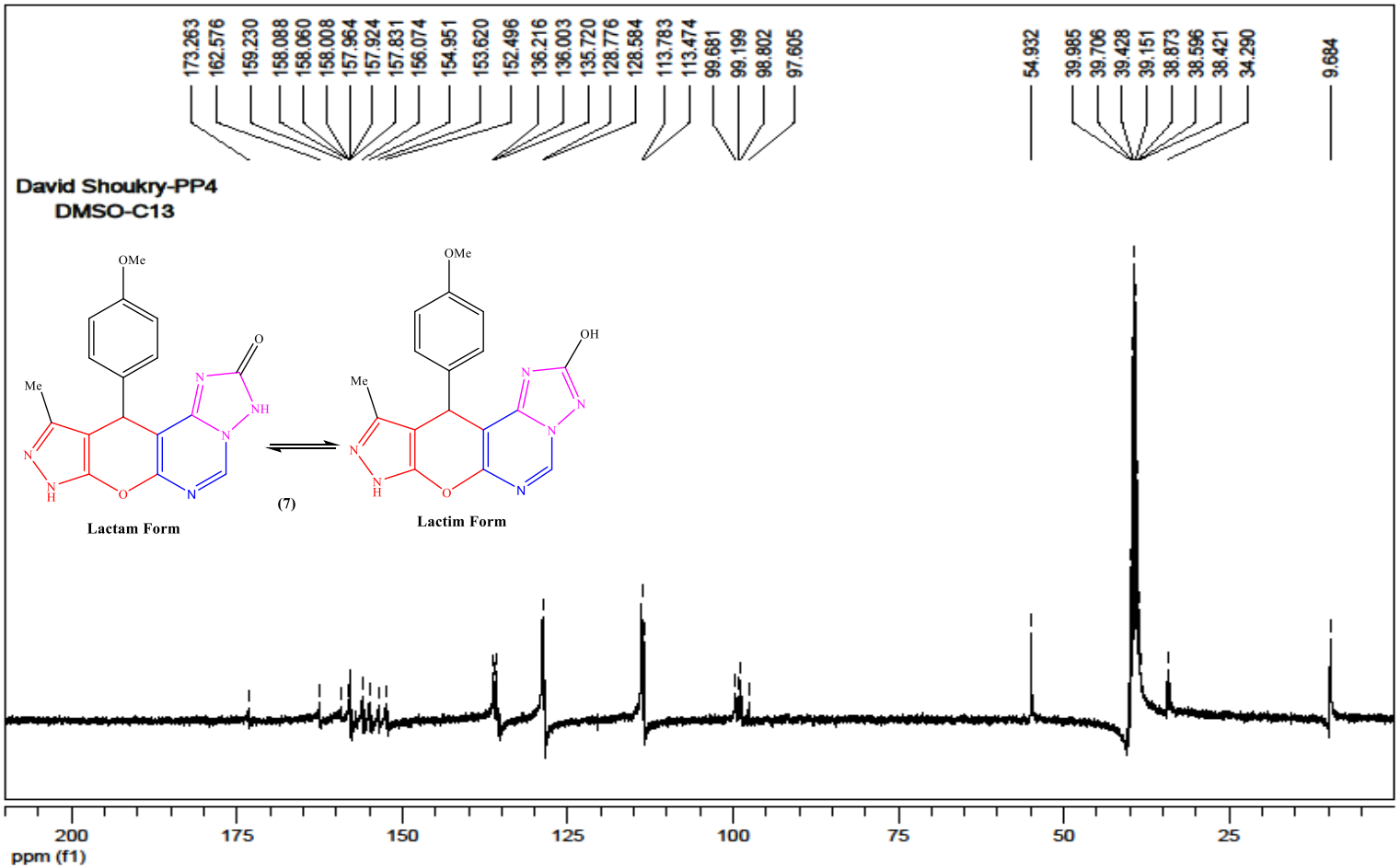

IR spectrum of compound **7**



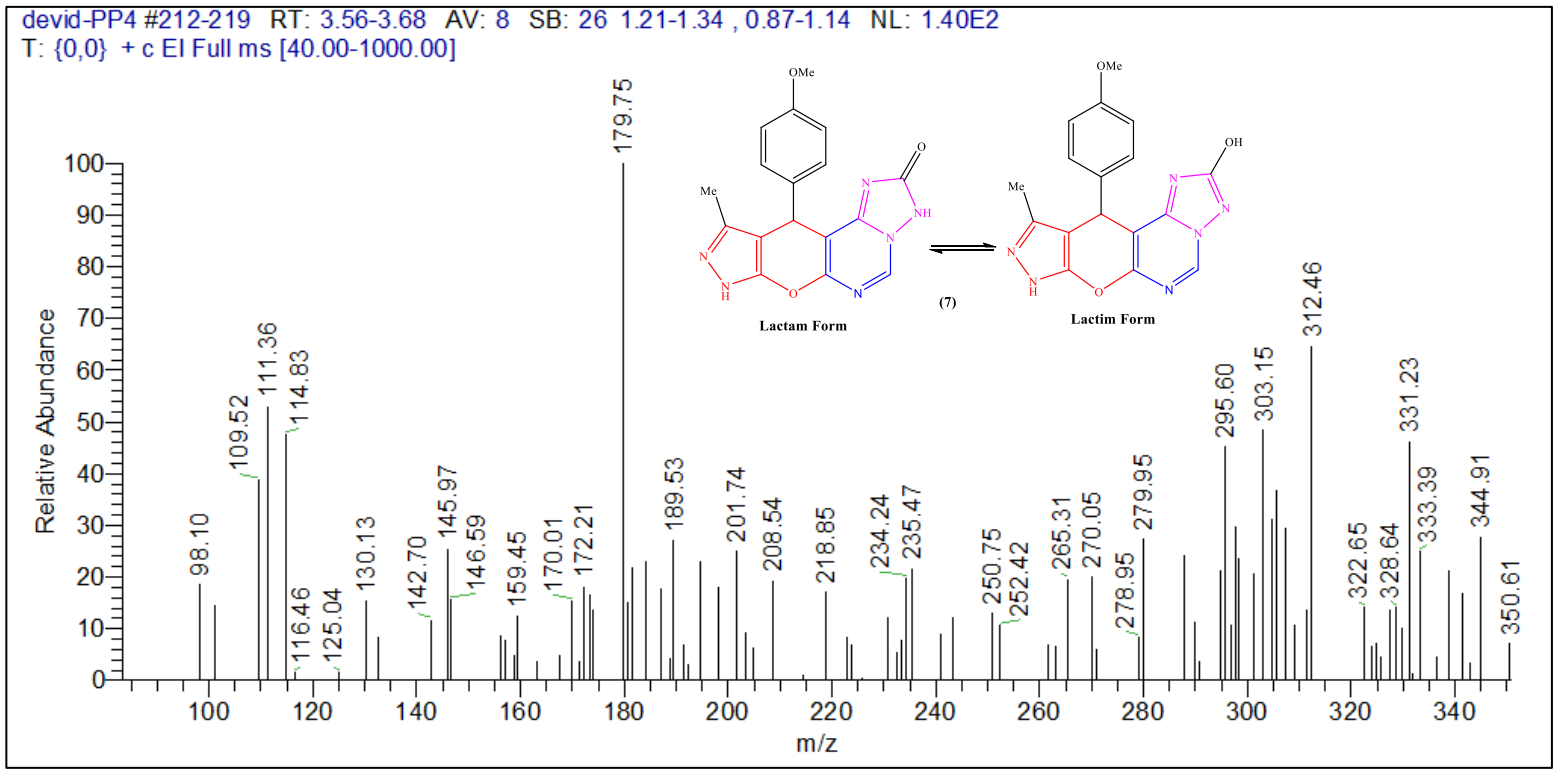

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **7**



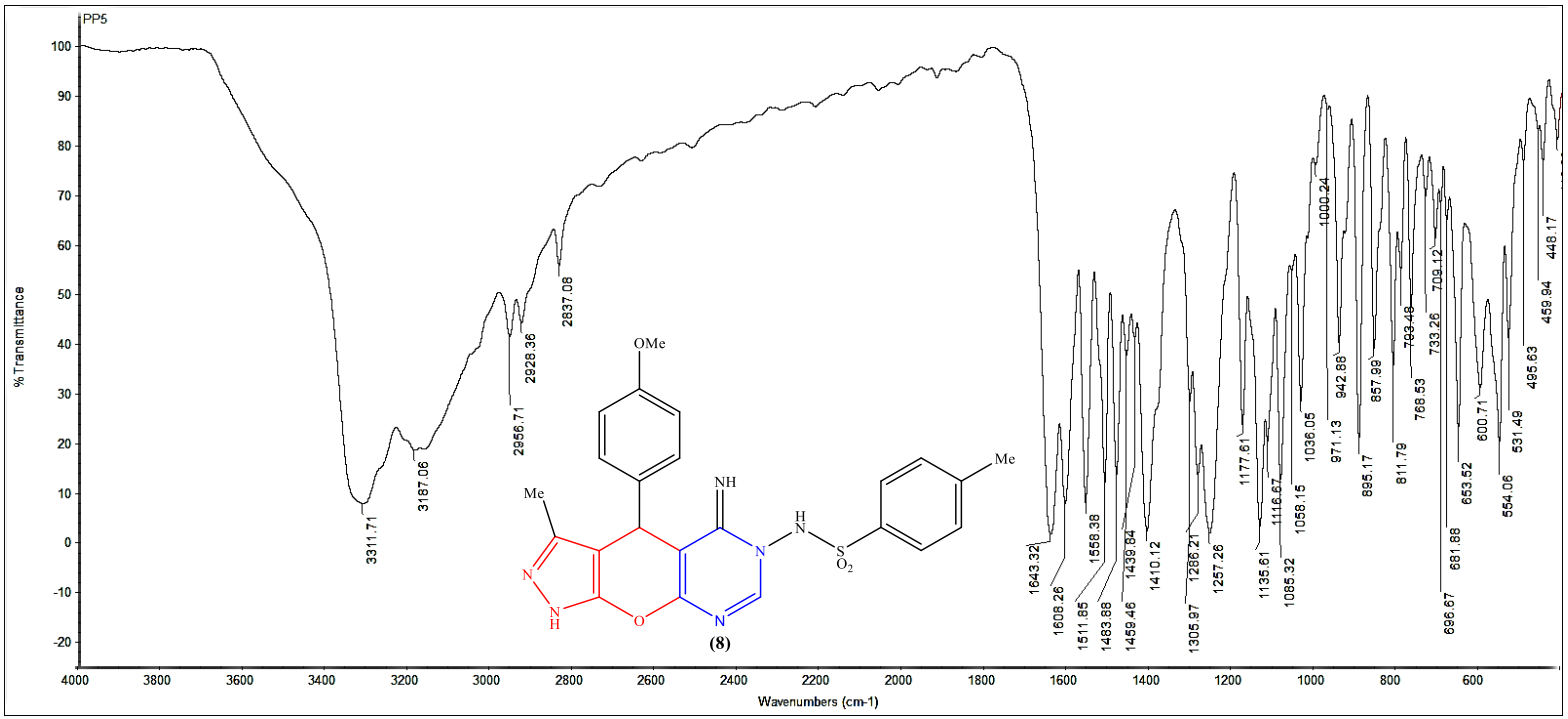

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **7**



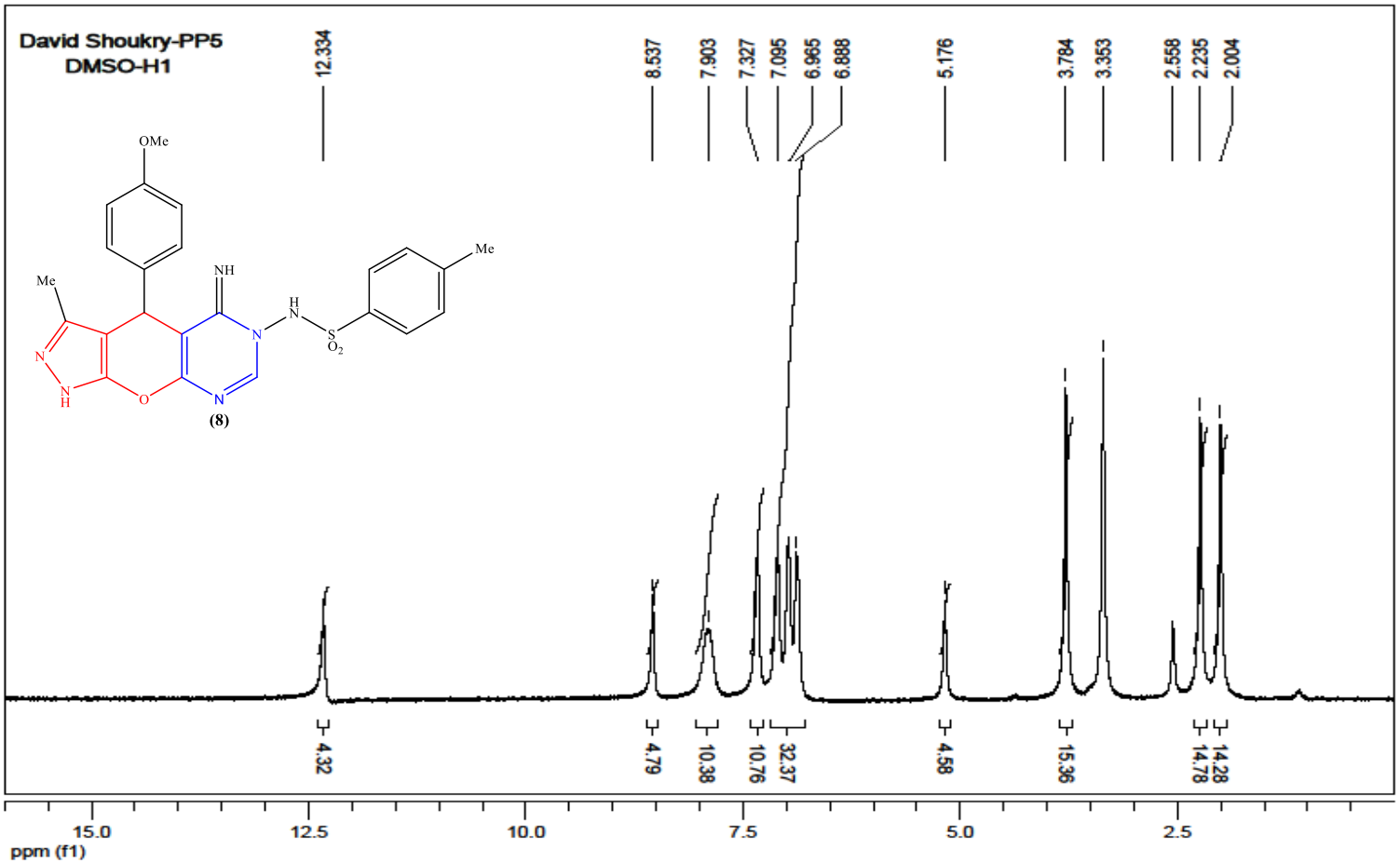

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **7**



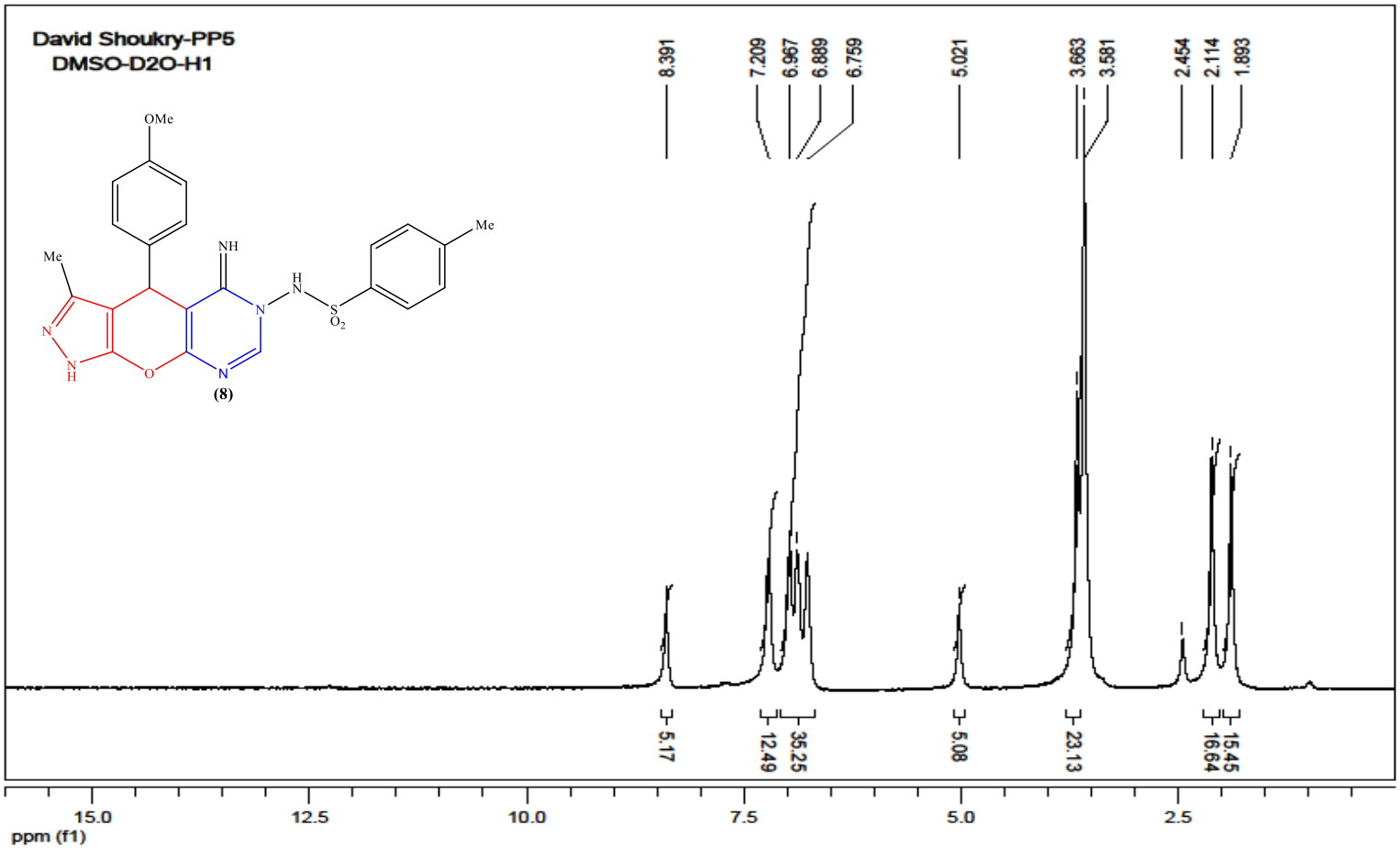

Mass spectrum of compound **7**



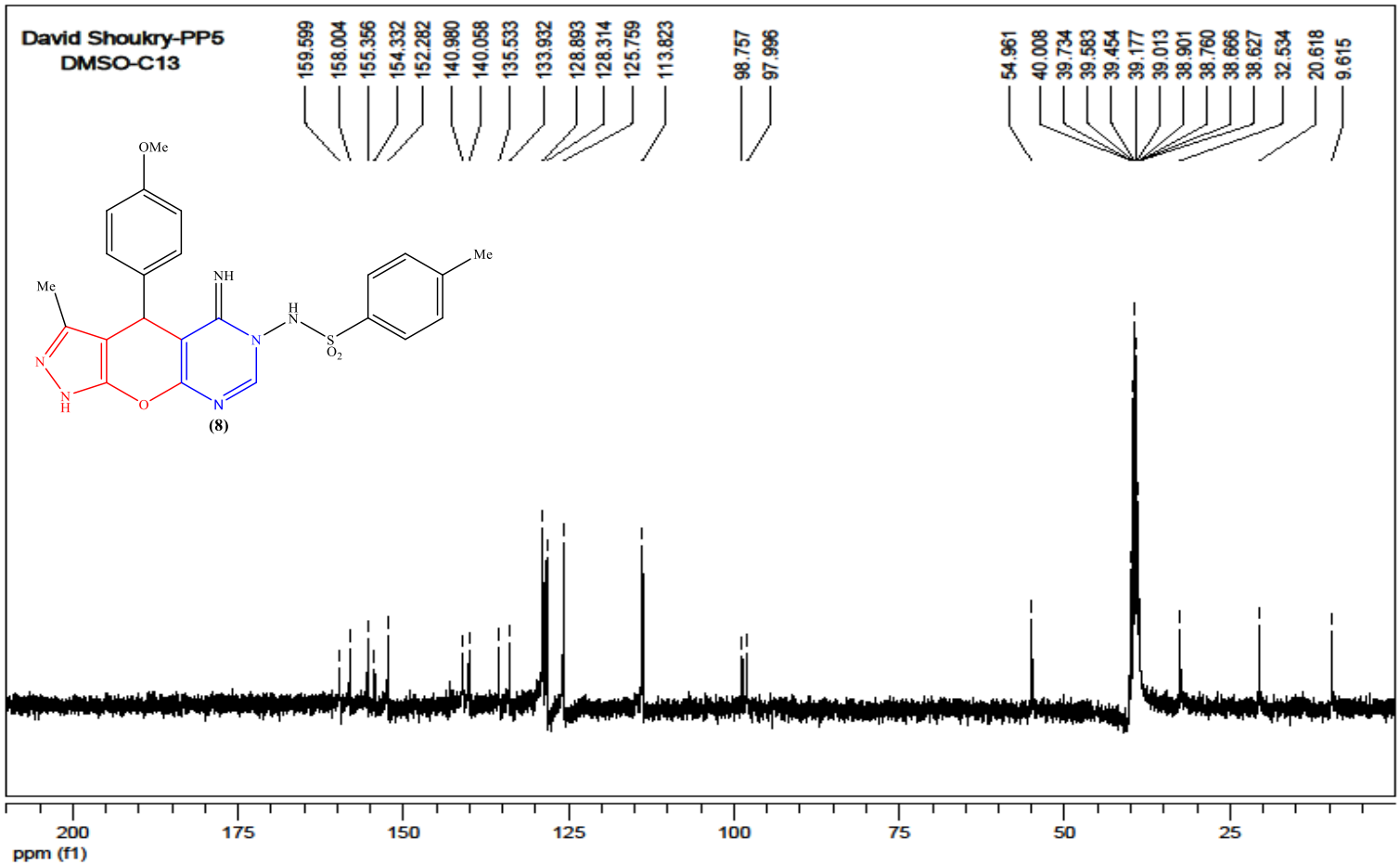

IR spectrum of compound **8**



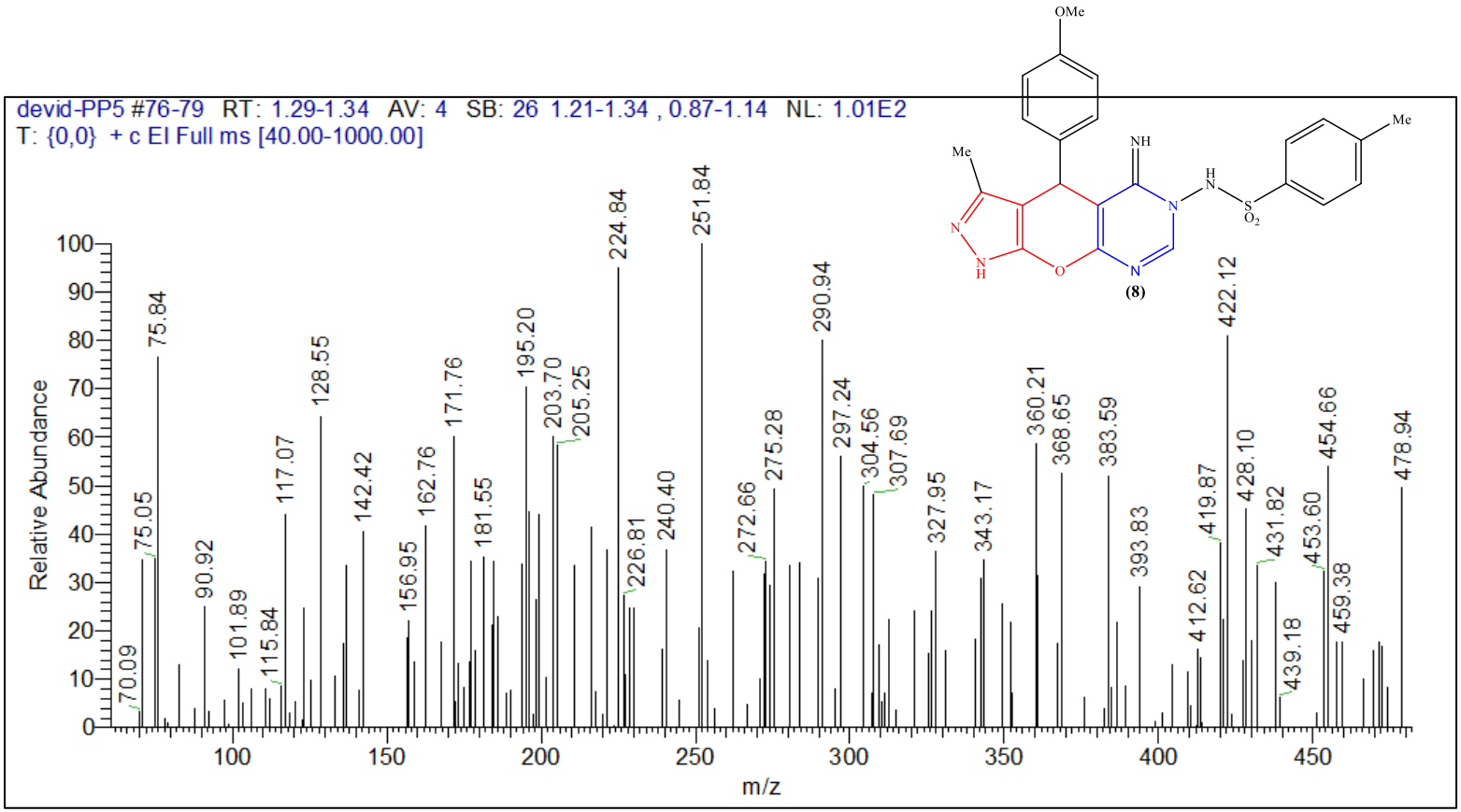

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **8**



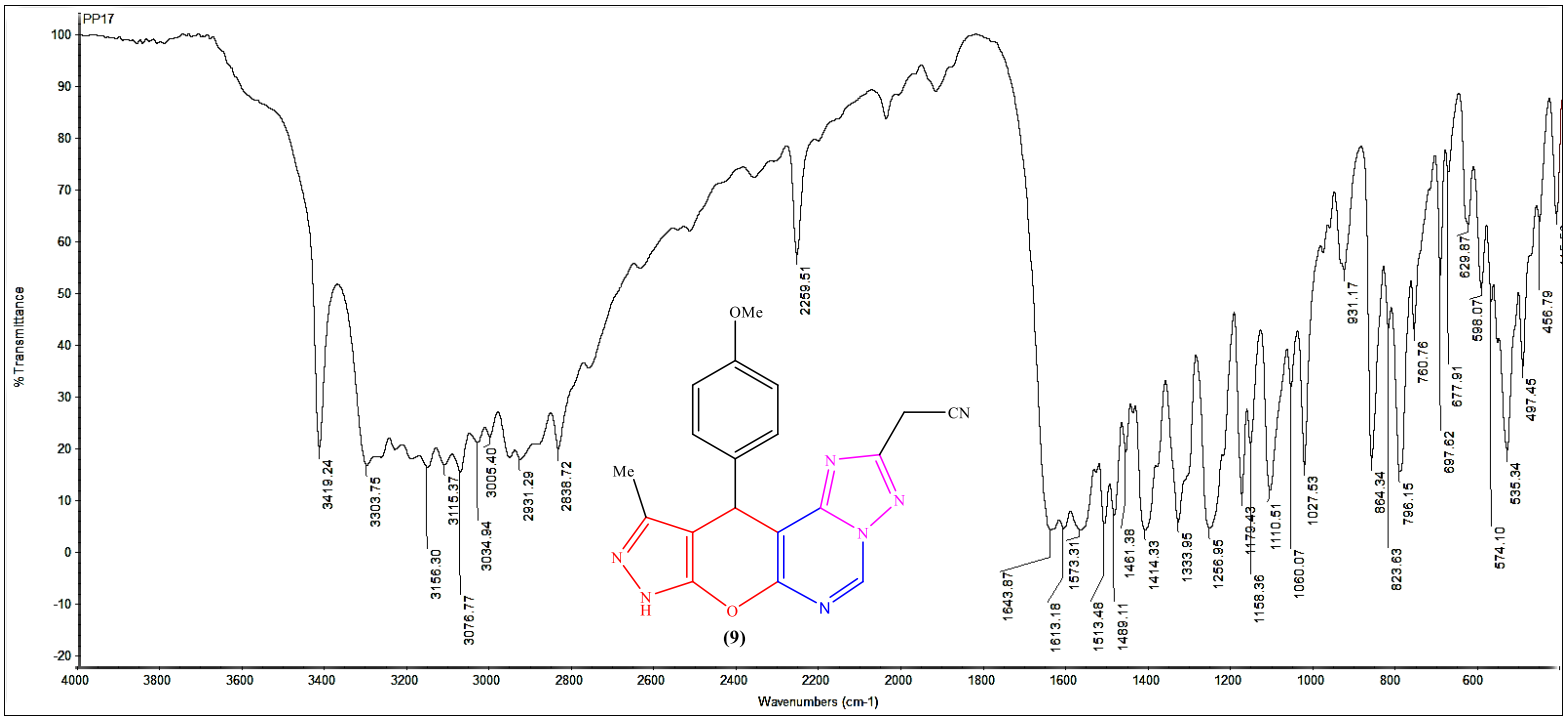

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **8**



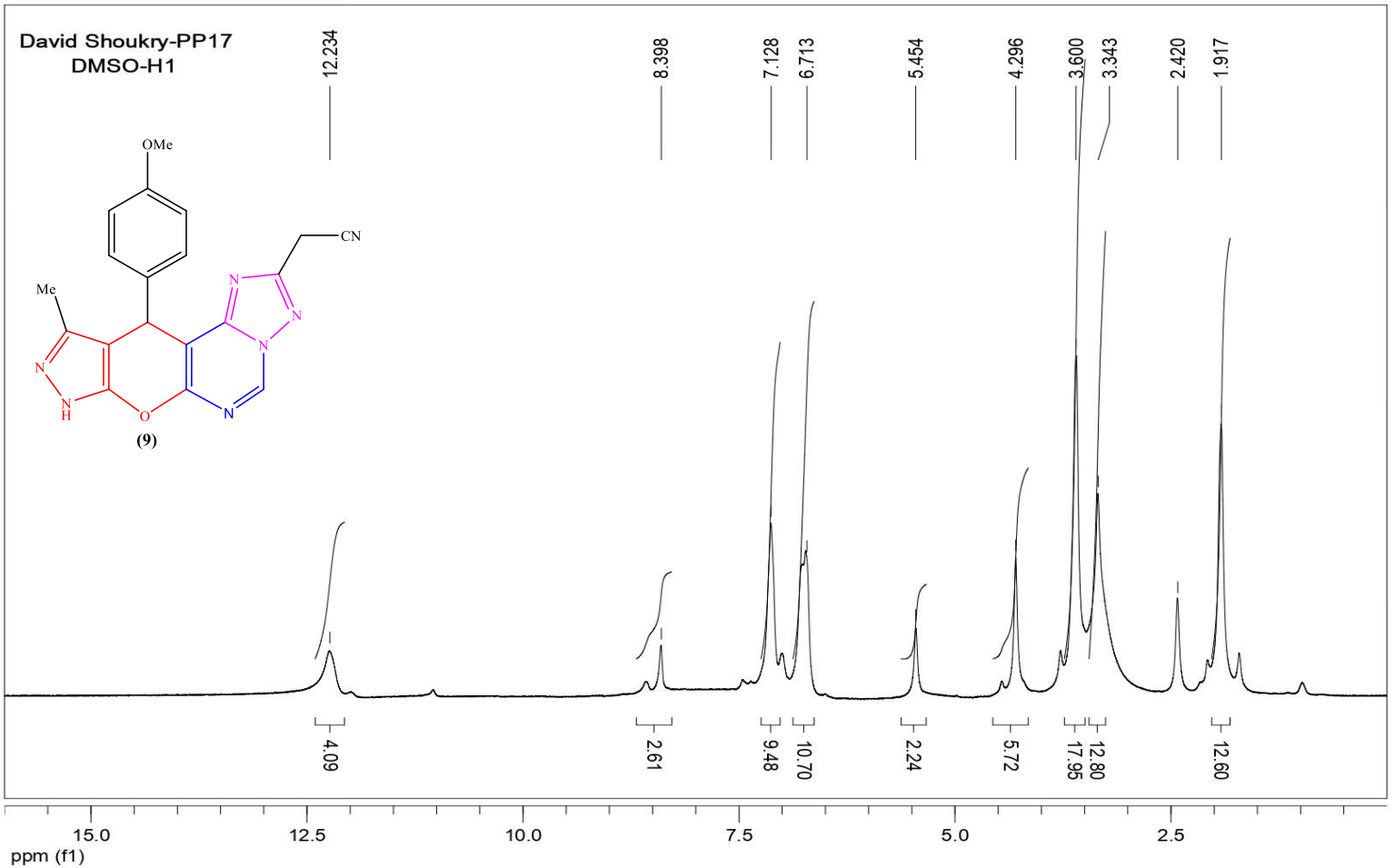

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **8**



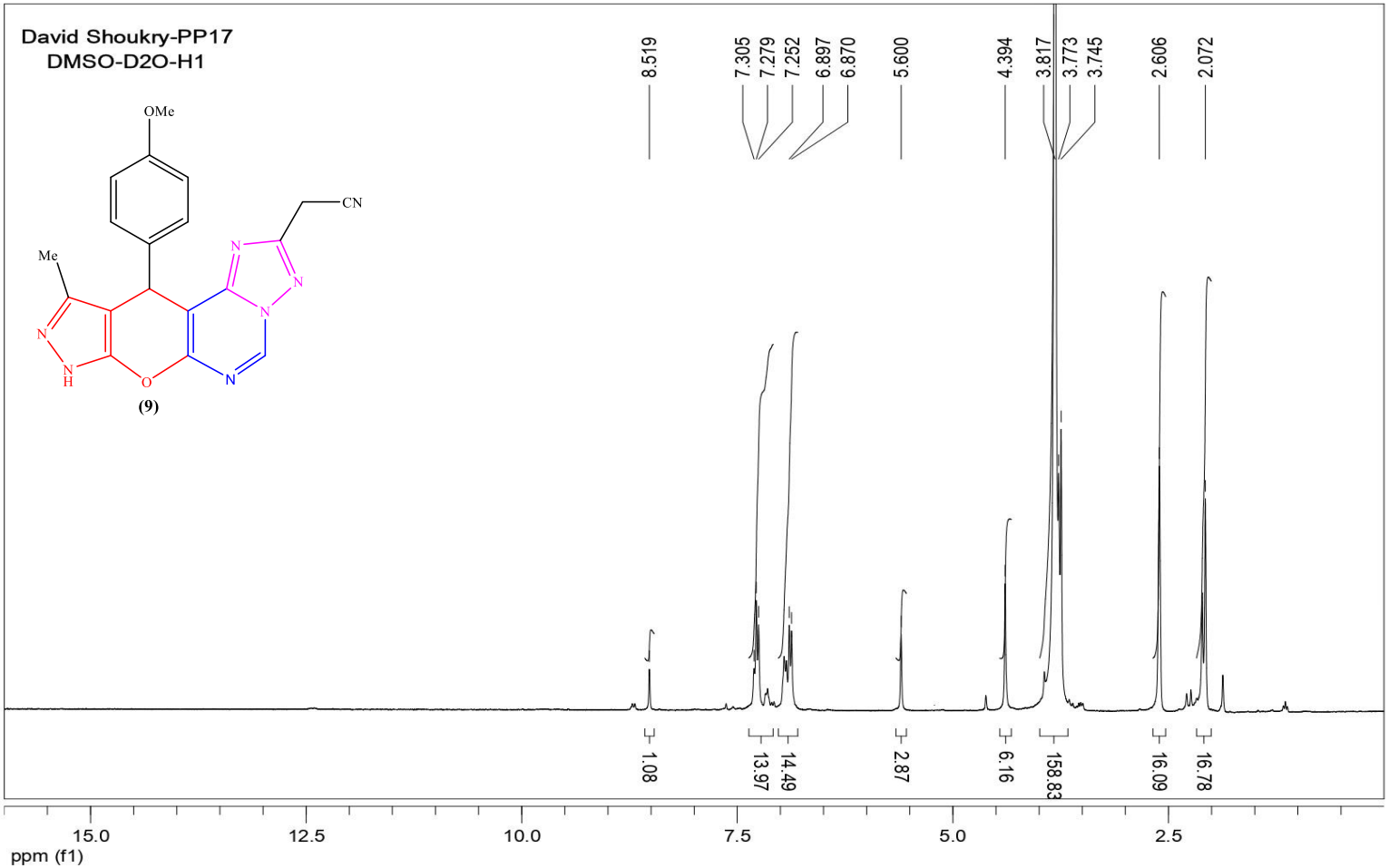

Mass spectrum of compound **8**



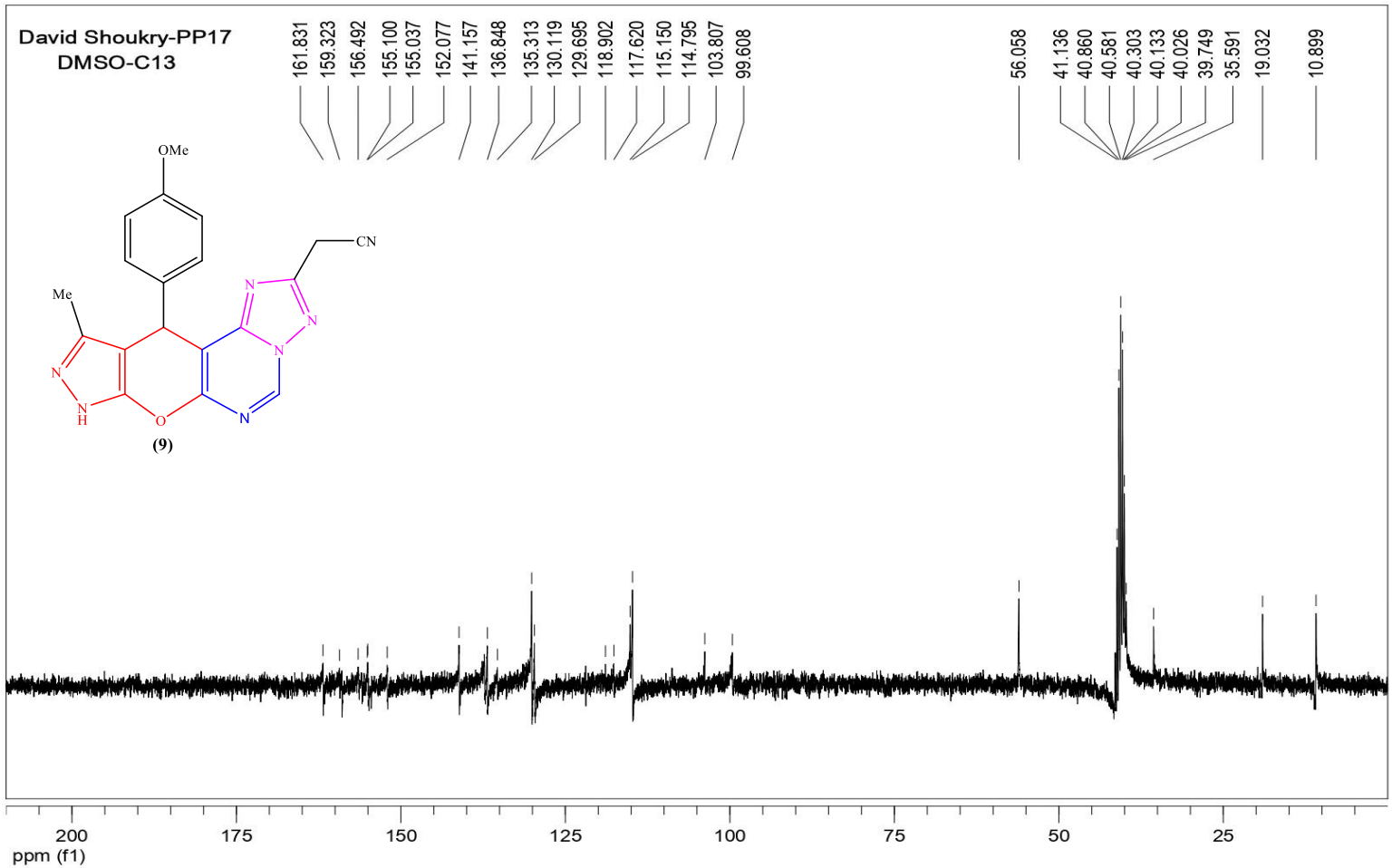

IR spectrum of compound **9**



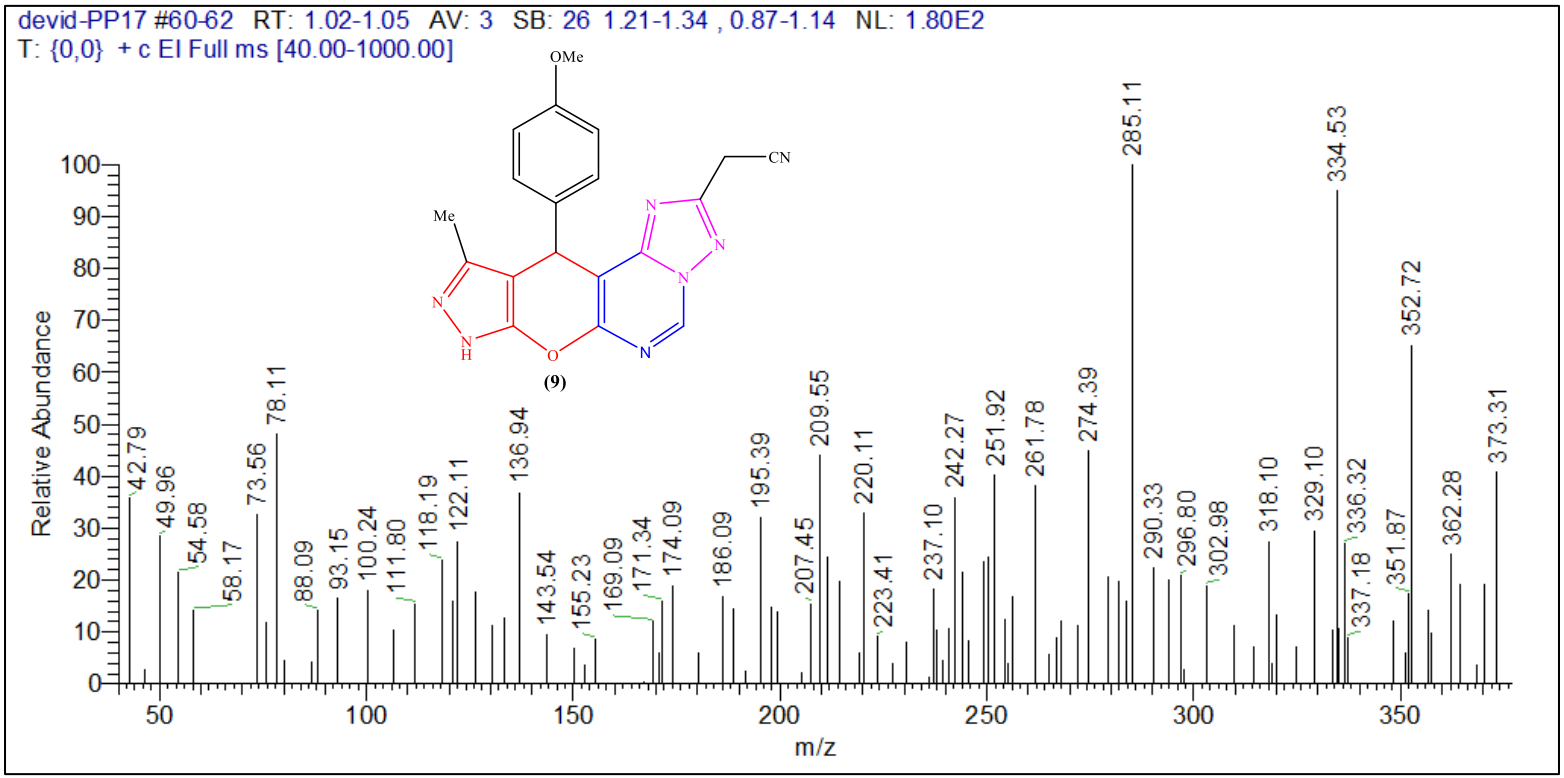

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **9**



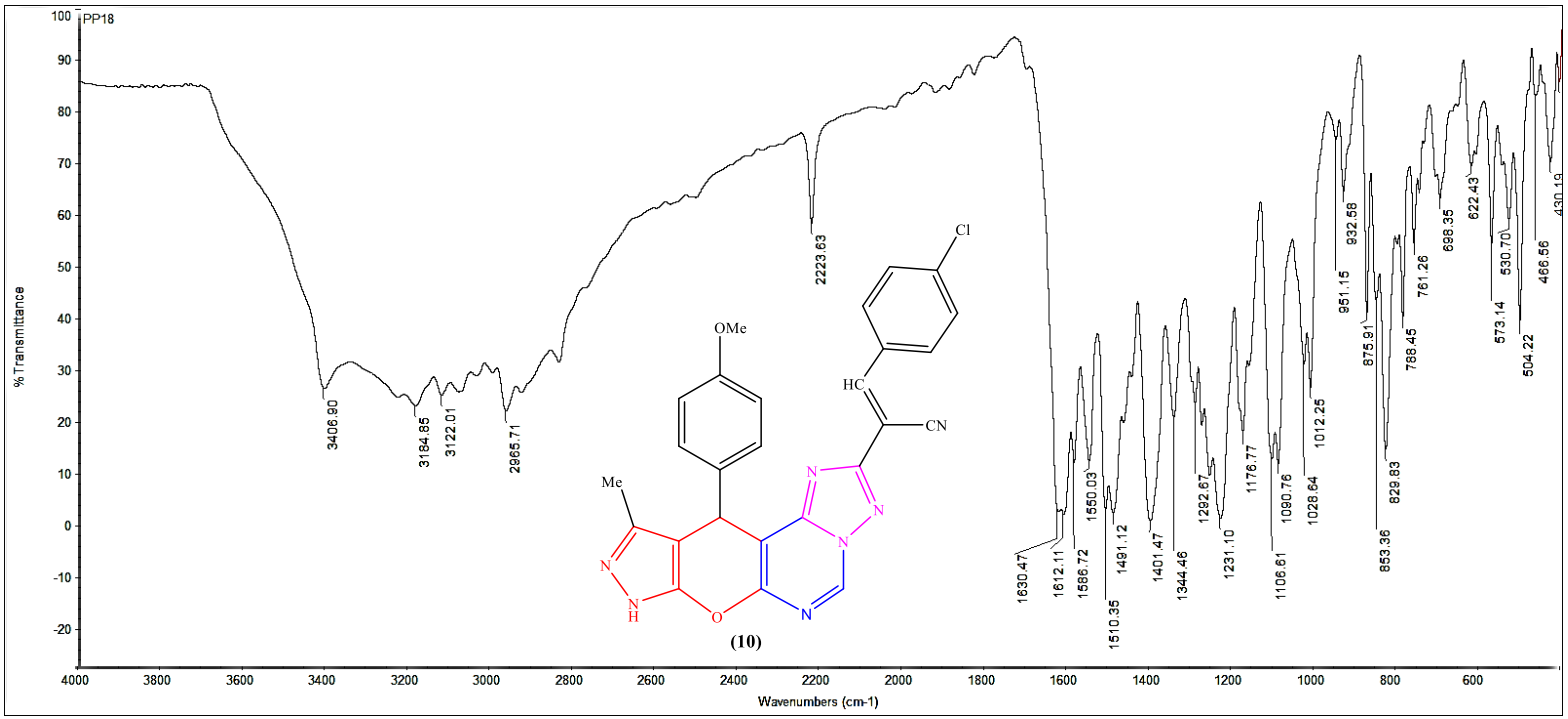

$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **9**



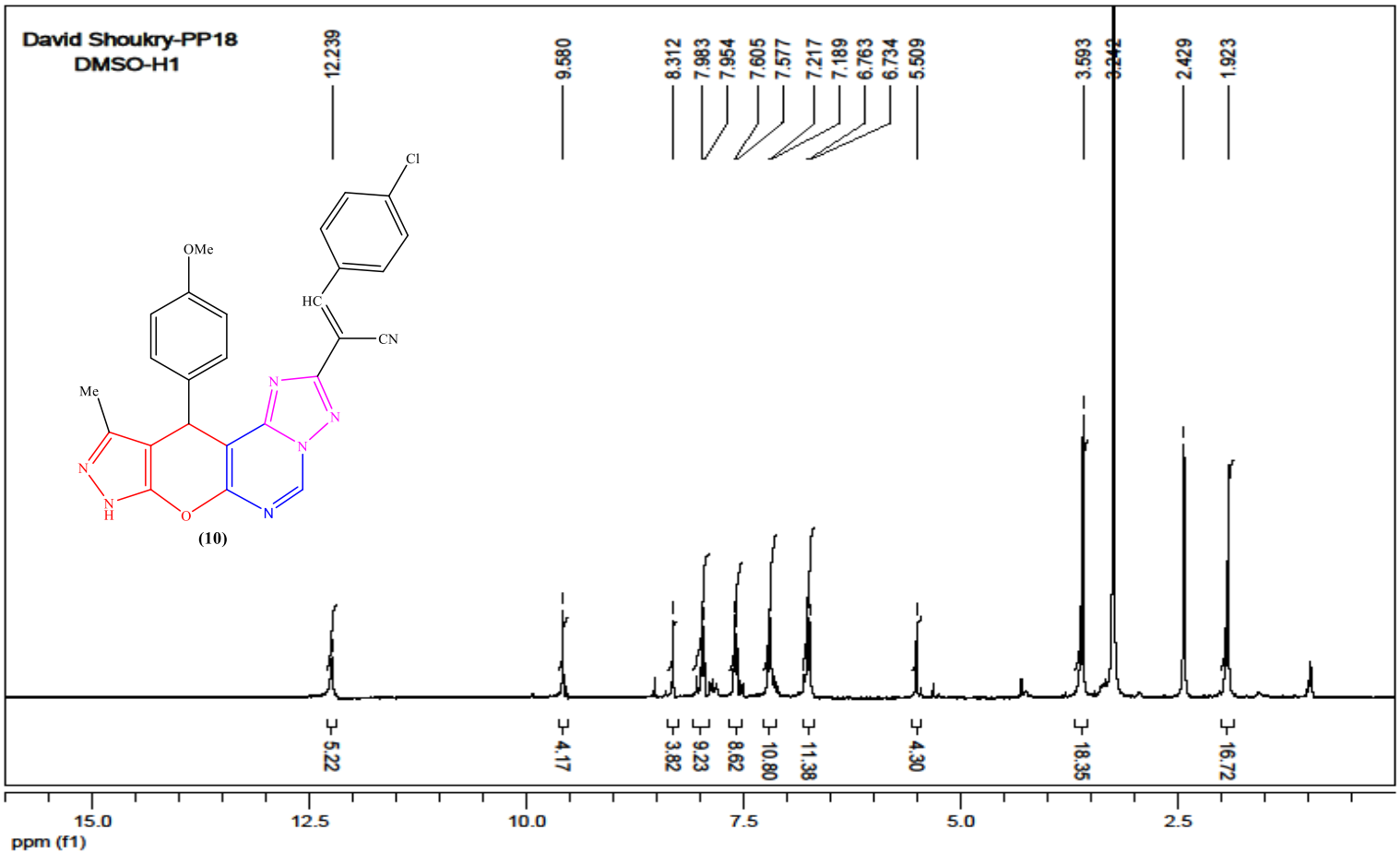

$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **9**



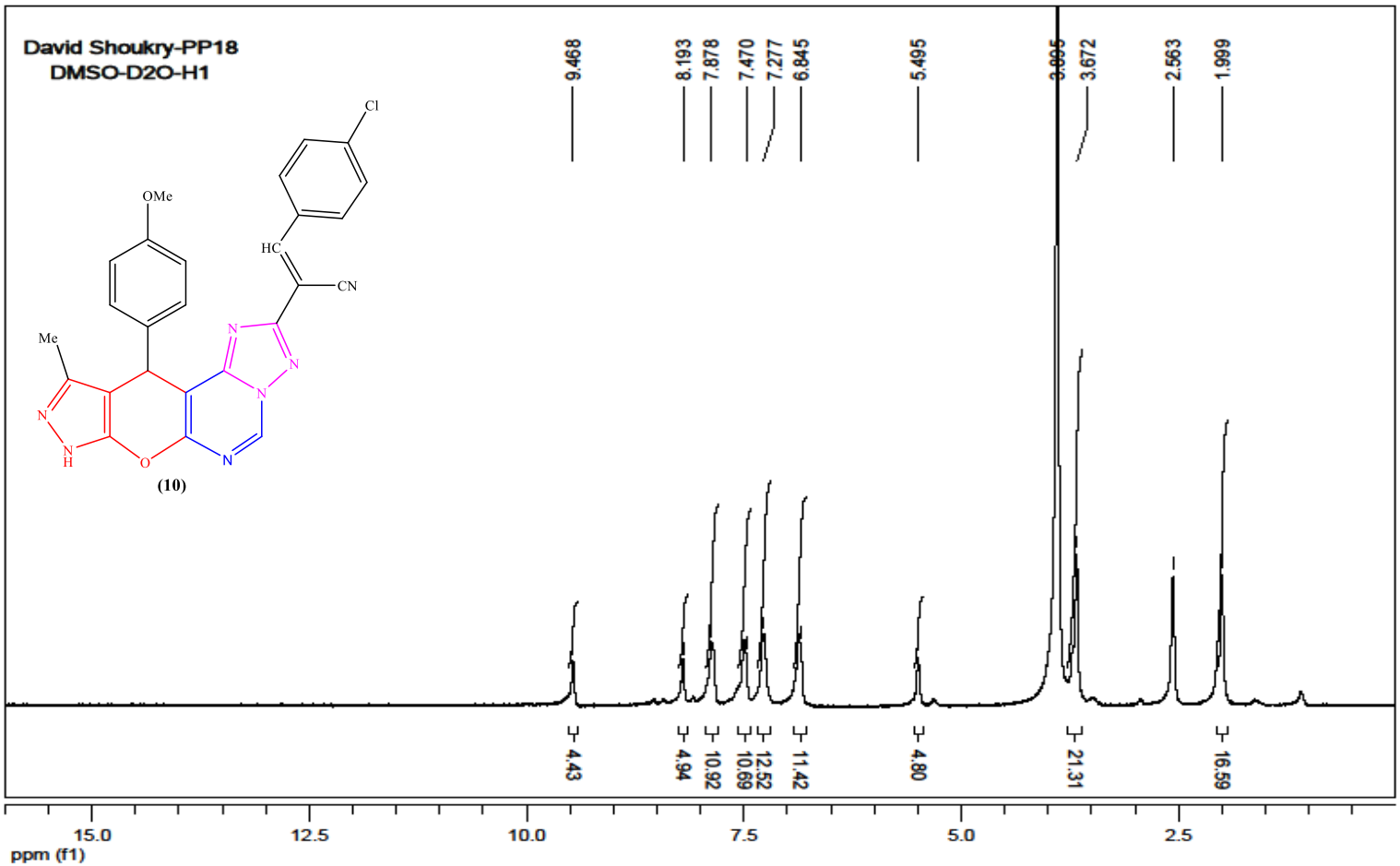

Mass spectrum of compound **9**



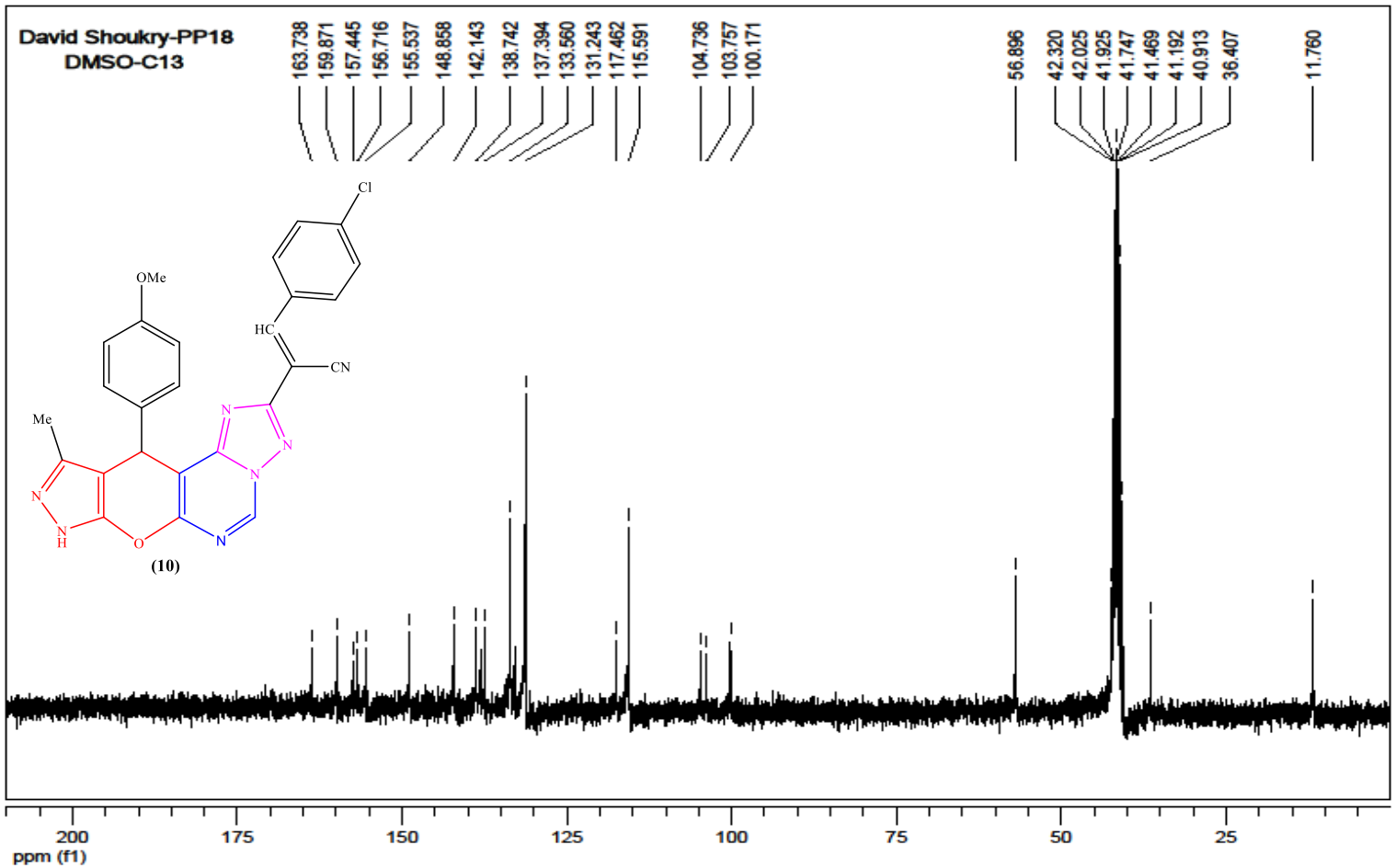

IR spectrum of compound **10**



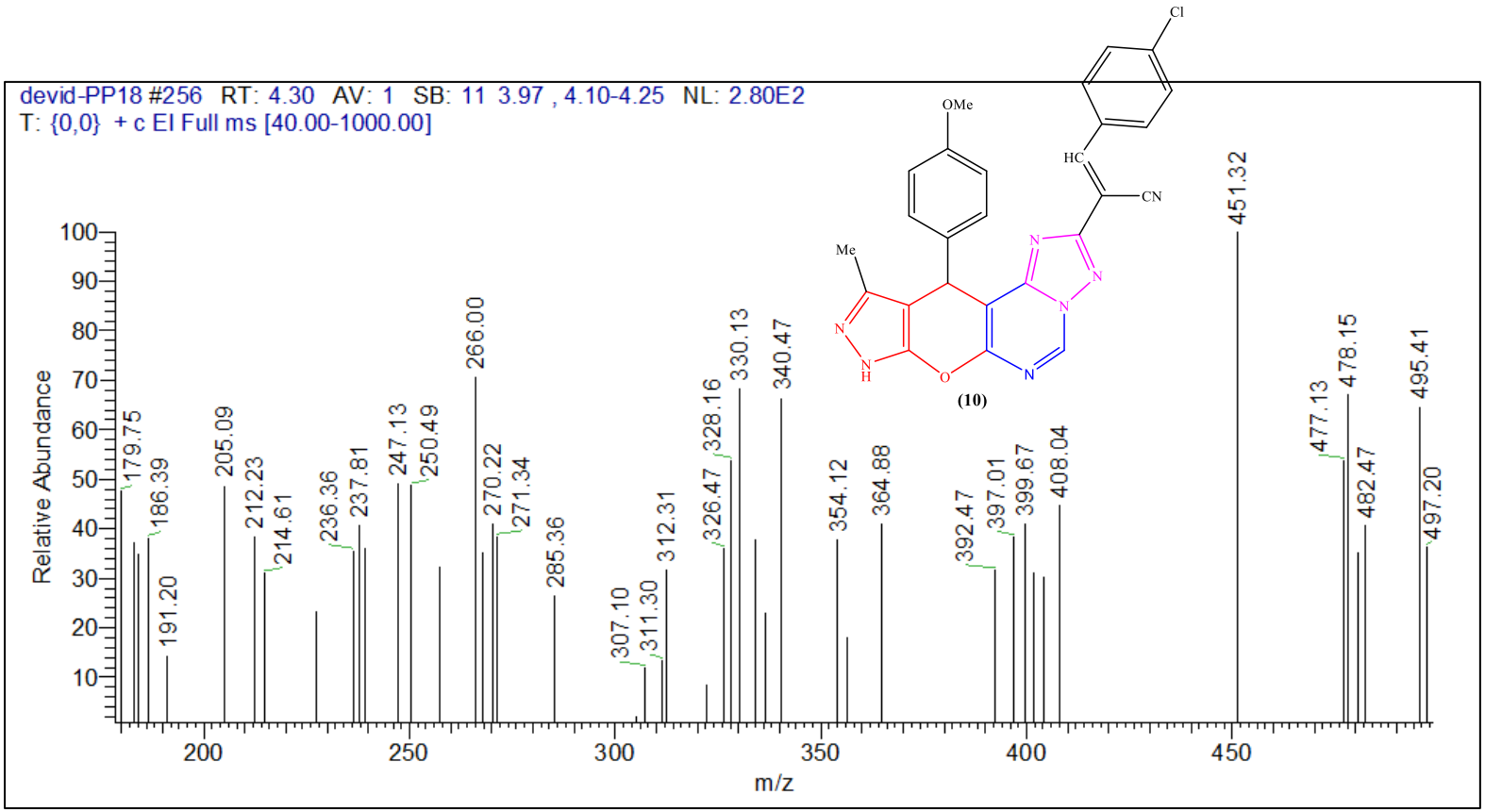

$^{1}$H-NMR spectrum (DMSO-$d_6$) of compound **10**




$^{1}$H-NMR spectrum (DMSO-$d_6$+ $D_2O$) of compound **10**




$^{13}$C-NMR spectrum (DMSO-$d_6$) of compound **10**




Mass spectrum of compound **10**

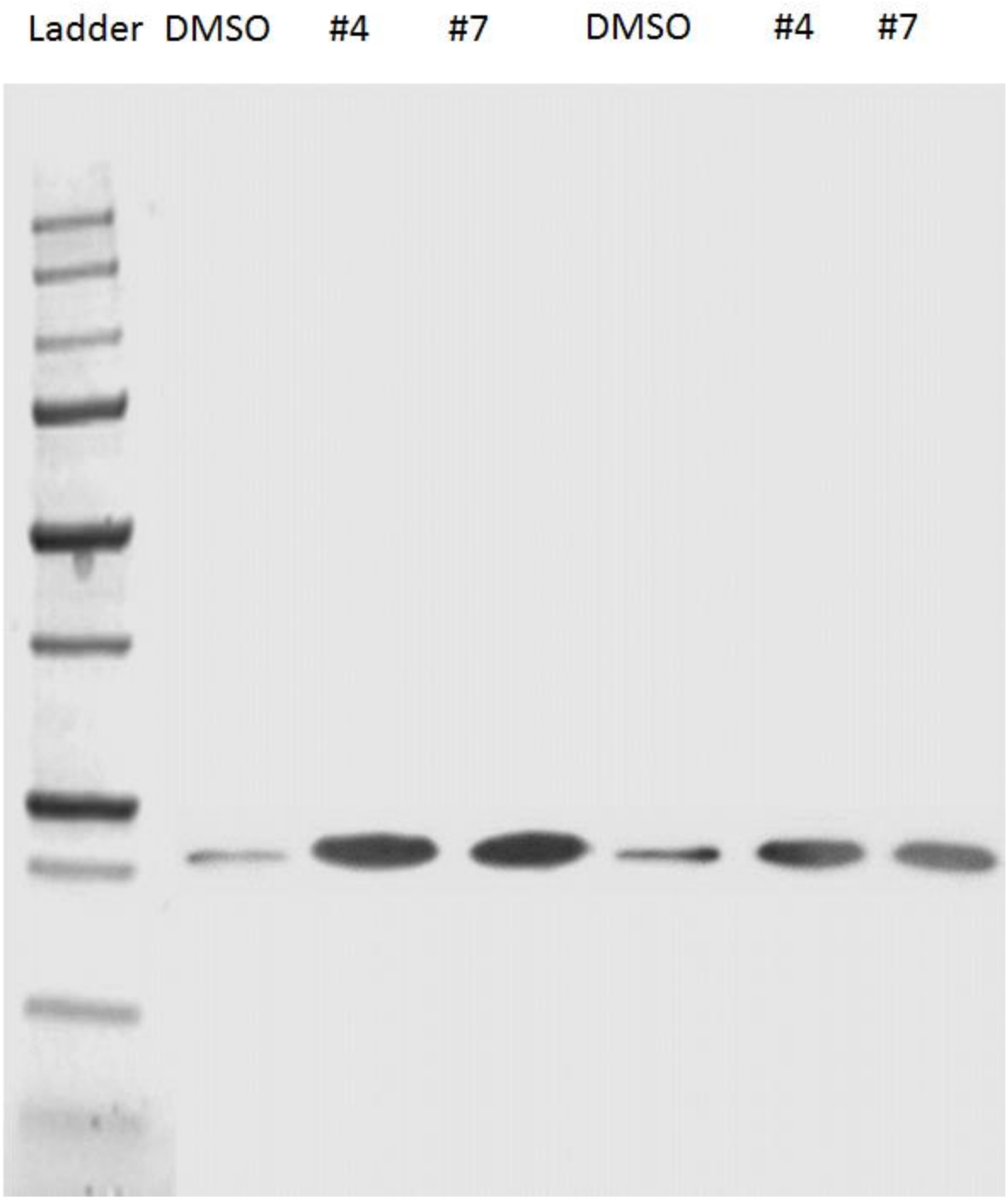



**Figure S1: Representative uncropped gel of p21 protein**