

Biochemical, structural analysis and docking studies of spiropyrazoline derivatives

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Diazomethane was prepared for the synthesis spiropyrazolines analogues as follows:

Briefly, 50% aqueous potassium hydroxide solution (30 mL) and 100 mL diethyl ether was placed in a 500 mL flat-bottomed flask. The solution was cooled in an ice-salt mixture to 5 °C and 0.2 M of *N*-nitrosomethylurea (10.3 g) was added in portions. The mixture was stirred. The solution turned yellow and was placed in a previously-cooled separatory funnel. The solution was separated and the ether layer of the diazomethane was placed in a flask covered with pellets of potassium hydroxide. The aqueous layer was placed into a flask with an aqueous solution of benzoic acid.

S1. Synthesis of 5'-(3-methylphenyl)-2-phenyl-4',5'-dihydro-4*H*-spiro[chromano-3,3'-pirazol]-4-one (compound I)

2.5 mmol (0.921 g) of (*E*)-3-(3-methylbenzylidene)-2-phenylchroman-4-one was dissolved in anhydrous acetone (5 mL) to give a light yellow solution. The flask containing the solution was placed in the ice bath and an ethereal solution of diazomethane (10 mmol) was added in excess. The mixture in the flask was left in the freezer for 48 h. The yellow precipitate was filtered off and purified by column chromatography and crystallized from methanol.

Yield: 51.3% **m.p.** 131–132.5 °C. **MS (ESI+):** *m/z* 369.4 C₂₄H₂₀N₂O₂ [M + H]⁺. **IR (KBr) ν (cm⁻¹):** 3027 (C-H arom.), 2975 (C-H aliph.), 1678 (C=O), 1602, 1579, 1472, 1461 (C=C, N=N), 1300 (C-N), 1211 (C-O), 1146 (C-O-C). **¹H-NMR (DMSO-*d*₆) δ (ppm):** 1.99 (3H, s, CH₃), 3.87 (1H, dd, *J*_{AB} = 3.1 Hz, *J*_{BX} = 6.9 Hz, CH), 4.96 (1H, dd, *J*_{AB} = 18 Hz, *J*_{BX} = 7.15 Hz CH₂), 6.19 (1H, s, C2-H), 6.34 (1H, dd, *J*_{AB} = 18 Hz, *J*_{BX} = 3 Hz CH₂), 6.85–7.86 (13H, m, C-H arom.). **¹³C-NMR (DMSO-*d*₆) δ (ppm):** 20.6 (CH), 39.8 (CH₃), 81.1 (C2-H), 118.6, 121.8, 126.8, 127.8, 128.2, 128.6, 138.0 (CH arom) 102.2, 119.8, 134.7, 134.9, 136.1, 159.4 (C arom), 185.5 (C=O). **Anal. Calc. For C₂₄H₂₀N₂O₂** (M = 368.44 g/mol) %C: 78.23; %H: 5.42; %N: 7.59. Found %C: 78.47; %H: 5.35; %N: 7.43.

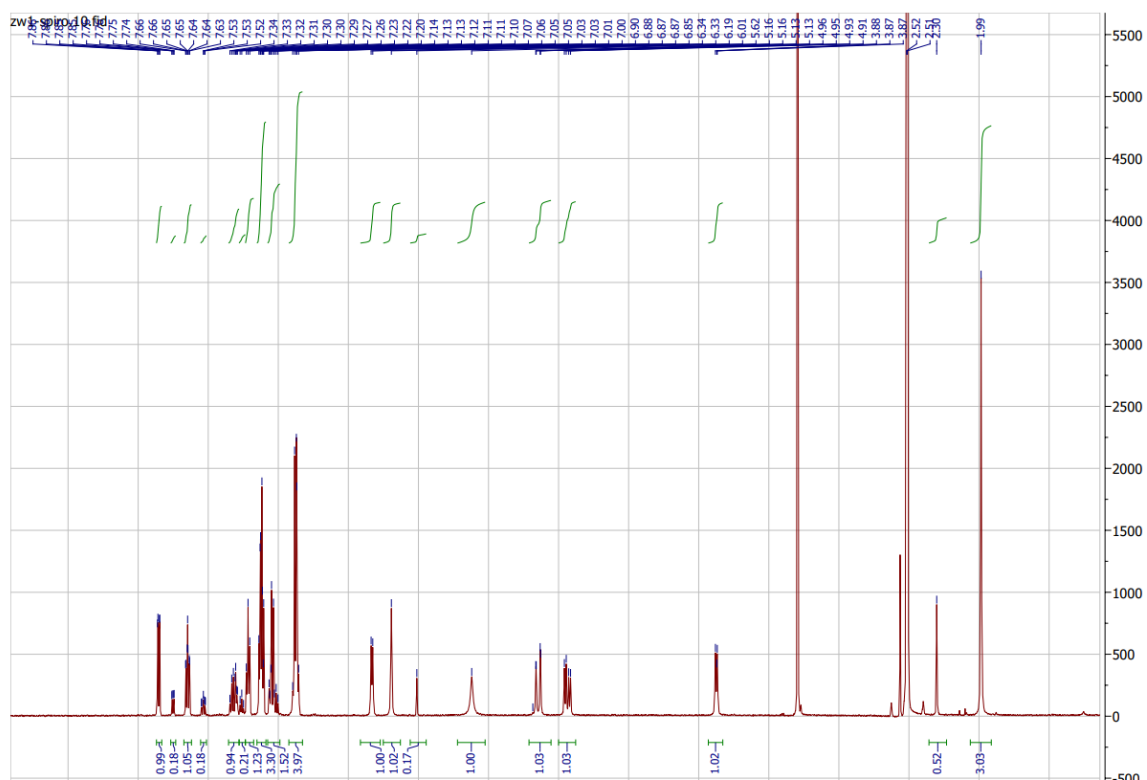


Figure S1a. ^1H NMR spectrum of compound I.

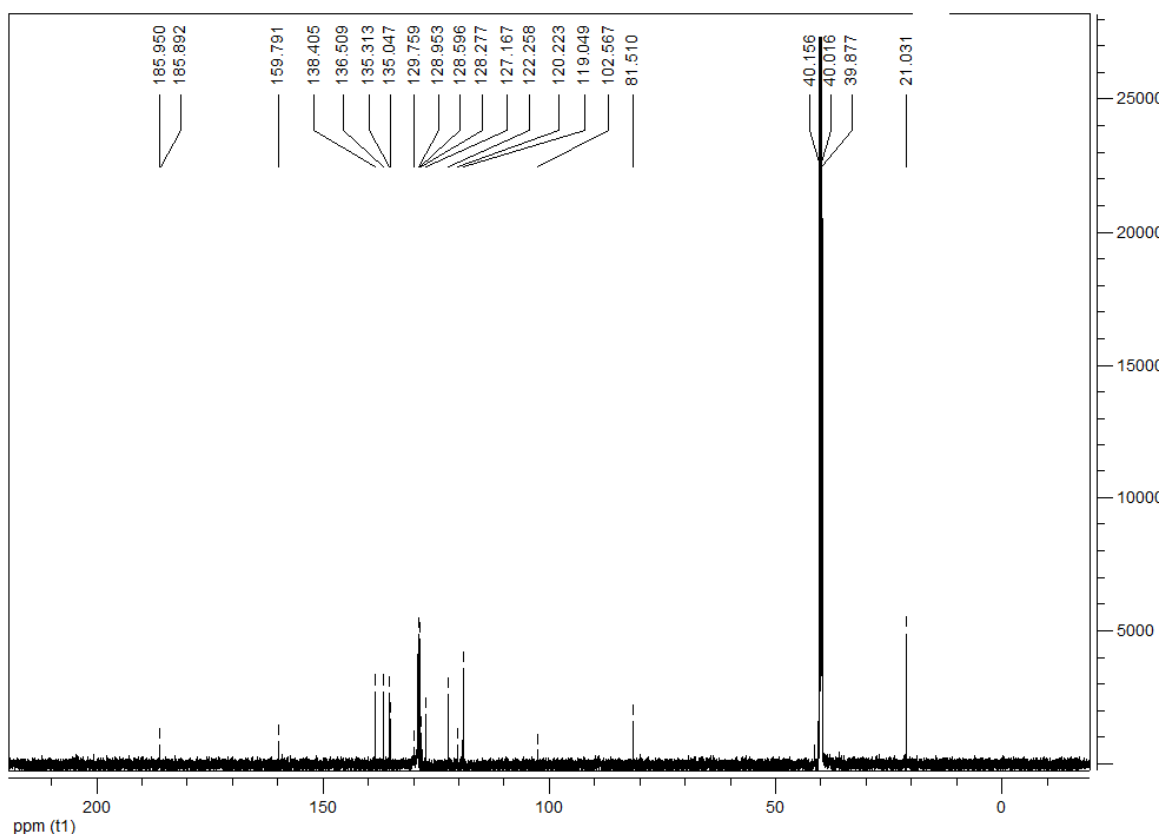


Figure S1b. ^{13}C NMR spectrum of compound I.

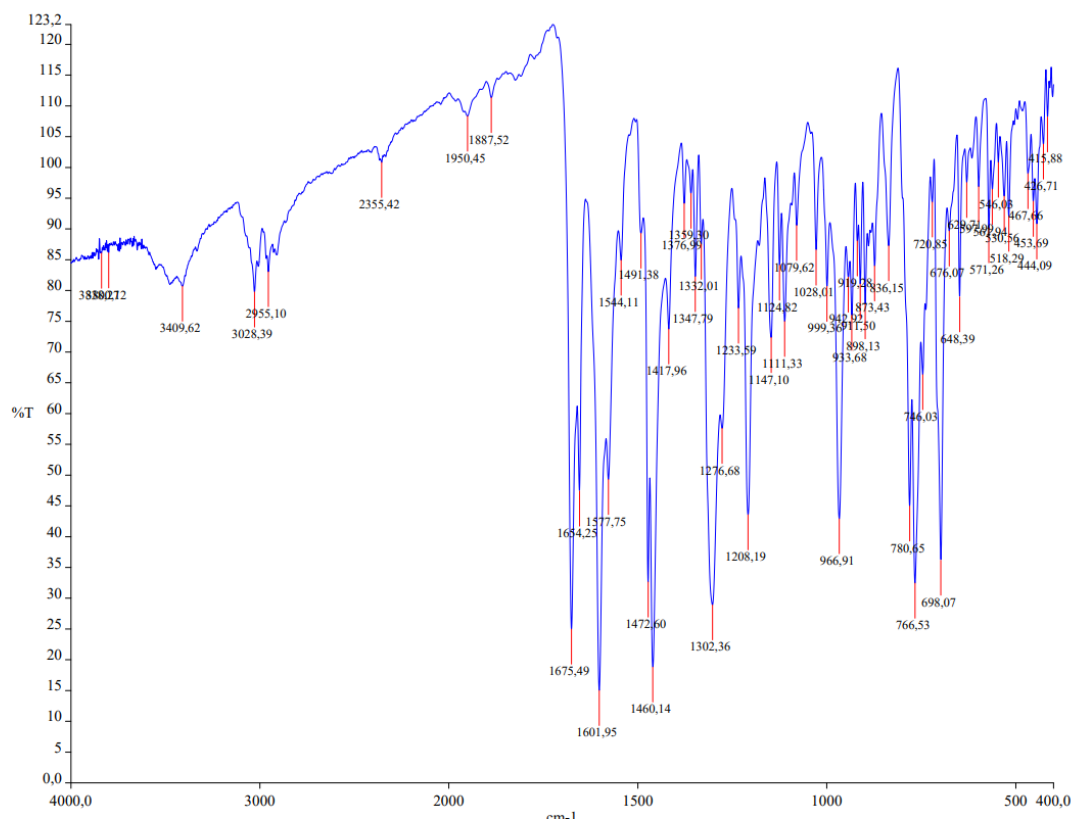


Figure S1c. IR spectrum of compound I.

S2. Synthesis of 5'-(4-methylphenyl)-2-phenyl-4',5'-dihydro-4*H*-spiro[chromano-3,3'-pirazol]-4-one (compound II)

2.5 mmol (0.921 g) of (*E*)-3-(4-methylbenzylidene)-2-phenylchroman-4-one was dissolved in anhydrous acetone (5 mL) to give a light yellow solution. The flask containing the solution was placed in the ice bath and an ethereal solution of diazomethane (10 mmol) was added in excess. The mixture in the flask was left in the freezer for 48 h. The yellow precipitate was filtered off and purified by column chromatography and crystallized from methanol.

Yield: 44% **m.p.** 138.2–140 °C. **MS (ESI+):** m/z 369.5 $C_{24}H_{20}N_2O_2$ $[M + H]^+$. **IR (KBr) ν (cm⁻¹):** 3027 (C-H arom.), 2975, 2928 (C-H aliph.), 1678 (C=O), 1602, 1579 1549, 1514, 1461 (C=C, N=N), 1300 (C-N), 1211 (C-O), 1146 (C-O-C). **¹H-NMR (DMSO-*d*₆) δ (ppm):** 1.98 (3H, s, CH₃), 3.88 (1H, dd, J_{AB} = 7.6 Hz, J_{BX} = 6.9 Hz, CH), 4.95 (1H, dd, J_{AB} = 12.27 Hz, J_{BX} = 1.96 Hz CH₂), 5.13 (1H, dd, J_{AB} = 12.27 Hz, J_{BX} = 7.15 Hz CH₂) 6,19 (1H, s, C2-H), 6.87–7.86 (13H, m, C-H arom.). **¹³C-NMR (CDCl₃) δ (ppm):** 23.6 (CH₃), 55,2 (CH), 77.3 (CH), 79.5 (C2-H), 112.3, 113.9, 118.2, 119.1, 123.4, 127.3, 128.5, 129.8, 138.9 (CH arom), 102.6, 121.3, 135.7, 142.6, 152.8, 158.7, 159.6 (C arom), 185.3 (C=O). **Anal. Calc. For $C_{24}H_{20}N_2O_2$** (M = 368.44 g/mol) %C: 78.23; %H: 5.42; %N: 7.59. Found %C: 78.37; %H: 5.21; %N: 7.49.

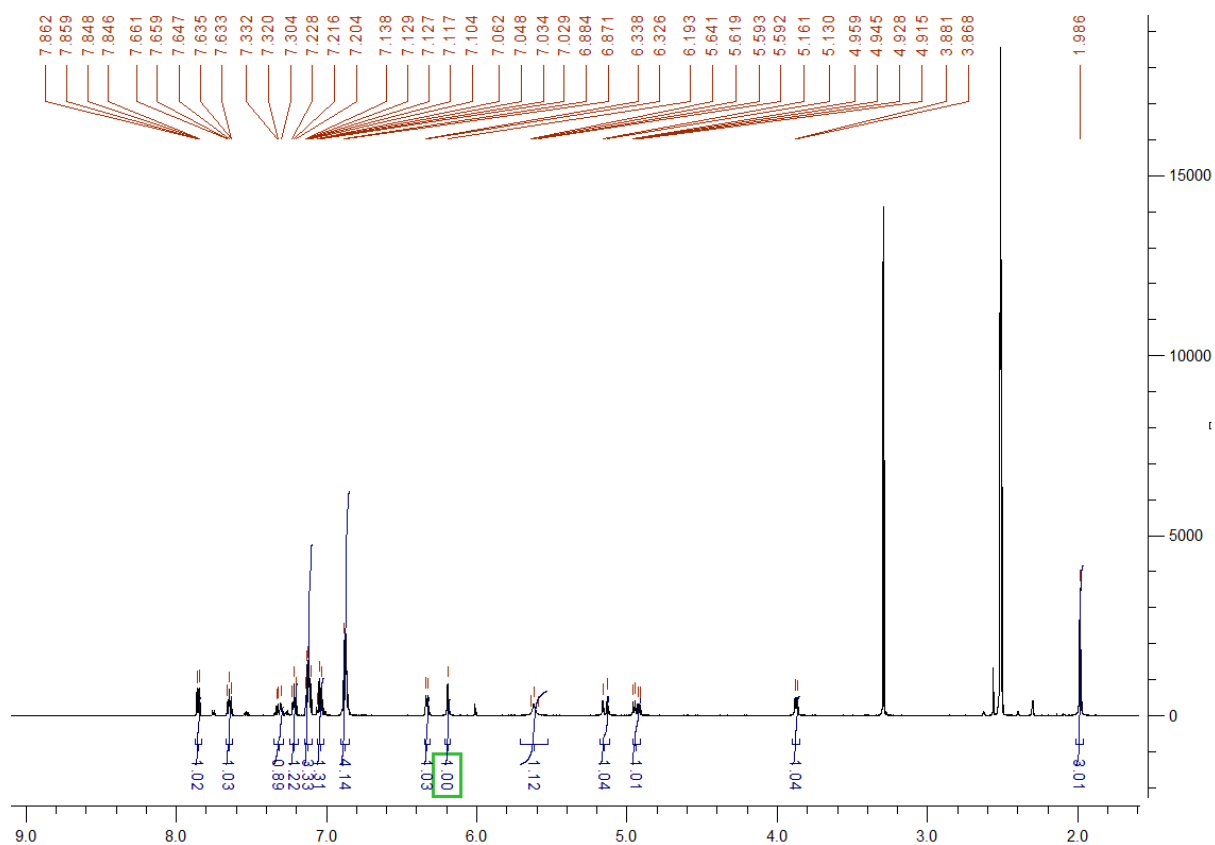


Figure S2a. ¹H NMR spectrum of compound II.

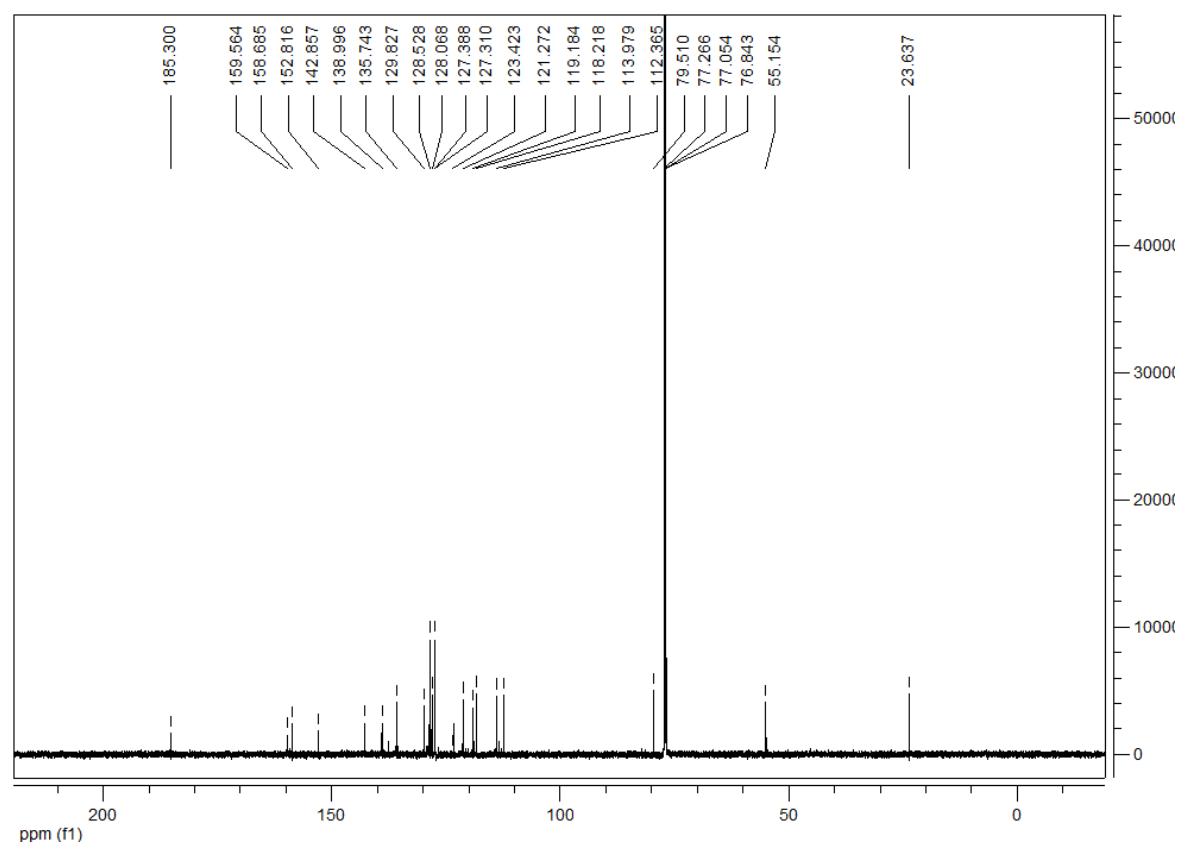


Figure S2b. ¹³C NMR spectrum of compound II.

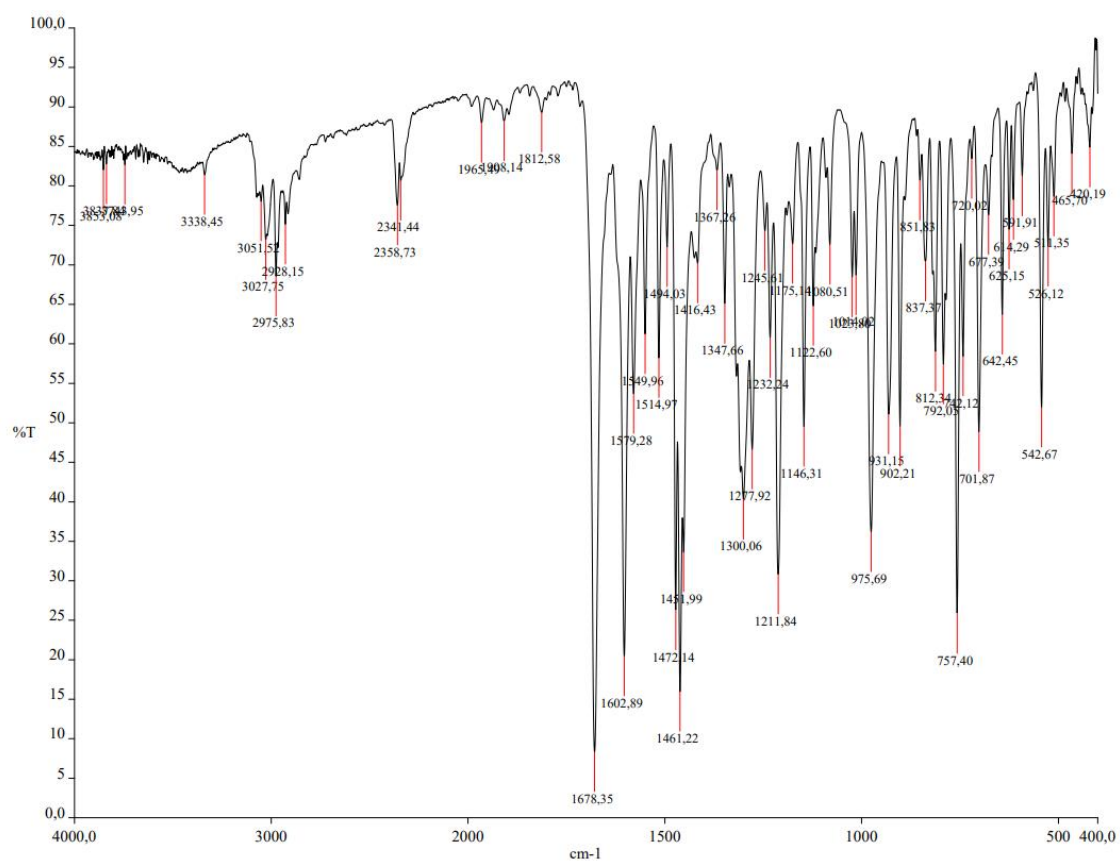


Figure S2c. IR spectrum of compound II.