

Phenylpyrazolone-1,2,3-triazole hybrids: A drug design approach towards the discovery of potent antiviral agents with promising anti-SARS-CoV-2 main protease inhibition potential

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1 Chemistry

1.1 General click procedure for the synthesis of 1,2,3-triazole-pyrazolehybrids **6a-q**

A solution of propargylated pyrazoles 4a-b (1 mmol) in DMSO was added with stirring to a solution of copper sulphate (0.10 g) and sodium ascorbate (0.15 g) in water (10 mL) (10 mL). The suitable azide **5a-o** (1 mmol) was then added to the reaction mixture, which was agitated at room temperature for 24-40 hours. The reaction was monitored by TLC (hexane-ethyl acetate). When the reaction was finished, the liquid was put into chilled water. The resulting precipitate was collected through filtering, washed with a saturated solution of ammonium chloride, and recrystallized from ethanol/DMF to get the desired 1,2,3-triazoles **6a-q**, all molecular characterizations are described in supplementary section.

1.1.1 (*Z*)-4-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid (6a)

It was obtained as orange pellets in 86 % yield, mp: 198-199 °C. IR (ν , cm⁻¹): 1550 (C=C), 1710 (C=O), 2930 (CH-Al), 3040 (CH-Ar). ¹H NMR (400 MHz, DMSO-*d*₆) δ _H = 2.36 (s, 3H, CH₃), 5.33 (s, 2H, OCH₂), 7.30 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.47 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.73 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.72-7.80 (m, 4H, Ar-H), 7.90-7.98 (m, 5H, H-C=C and Ar-H), 9.00 (s, 1H, CH-1,2,3-triazole), 12.76 (s, 1H, COOH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ _C = 13.60 (CH₃), 52.75 (OCH₂); 115.59, 117.89, 120.35, 124.31, 125.38,

126.48, 127.49, 128.68, 129.28, 136.67, 137.24, 137.67, 144.46, 147.76, 149.67, 152.75, 161.89, 162.89 (Ar-C, C=O), 168.87 (COOH). Calculated for C₂₇H₂₀ClN₅O₄: C, 63.10; H, 3.92; N, 13.63. Found: C, 63.29; H, 3.77; N, 13.49.

1.1.2 (Z)-4-((4-((3-Methyl-5-oxo-1-phenyl-1H-pyrazol-4(5H) ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid (6b)

It was obtained as orange pellets in 88 % yield, mp: 205-206 °C. IR (ν , cm⁻¹): 1560 (C=C), 1720 (C=O), 2960 (CH-Al), 3060 (CH-Ar). ¹H NMR (400 MHz, DMSO-d₆) δ_H = 2.34 (s, 3H, CH₃), 5.37 (s, 2H, OCH₂), 7.31 (d, 2H, J = 8.0 Hz, Ar-H), 7.49 (t, 1H, J = 8.0 Hz, Ar-H), 7.70 (d, 1H, J = 8.0 Hz, Ar-H), 7.76-7.83 (m, 4H, Ar-H), 7.91-8.00 (m, 6H, H-C=C and Ar-H), 9.02 (s, 1H, CH-1,2,3-triazole), 12.88 (s, 1H, COOH). ¹³C NMR (100 MHz, DMSO-d₆): δ_C = 13.64 (CH₃), 52.72 (OCH₂); 115.52, 117.80, 120.24, 124.38, 125.25, 126.39, 127.42, 128.64, 129.21, 136.52, 137.02, 137.58, 144.30, 147.84, 149.56, 152.86, 161.80, 162.92 (Ar-C, C=O), 168.46 (COOH). Calculated for C₂₇H₂₁N₅O₄: C, 67.63; H, 4.41; N, 14.61. Found: C, 67.88; H, 4.65; N, 14.89.

1.1.3 (Z)-4-((1-(4-Acetylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (6c)

It was obtained as orange pellets in 90 % yield, mp: 212-213 °C. IR (ν , cm⁻¹): 1565 (C=C), 1690 (C=O), 2945 (CH-Al), 3020 (CH-Ar). ¹H NMR (400 MHz, DMSO-d₆) δ_H = 2.33 (s, 3H, CH₃), 2.65 (s, 3H, COCH₃), 5.37 (s, 2H, OCH₂), 7.28 (d, 2H, J = 8.0 Hz, Ar-H), 7.46 (t, 1H, J = 8.0 Hz, Ar-H), 7.67 (d, 1H, J = 8.0 Hz, Ar-H), 7.73-7.80 (m, 4H, Ar-H), 7.89-7.96 (m, 5H, H-C=C and Ar-H), 9.01 (s, 1H, CH-1,2,3-triazole). ¹³C NMR (100 MHz, DMSO-d₆): δ_C = 13.60 (CH₃), 26.64 (CH₃); 52.72 (OCH₂); 115.50, 117.81, 120.04, 124.39, 125.32, 126.30, 127.39, 128.62, 129.18, 136.65, 137.23, 137.70, 144.06, 146.56,

149.15, 152.66, 161.77, 162.87 (Ar-C, C=O); 194.25 (CH₃C=O). Calculated for C₂₈H₂₂ClN₅O₃: C, 65.69; H, 4.33; N, 13.68. Found: C, 65.82; H, 4.56; N, 13.89.

1.1.4 (Z)-1-(4-Chlorophenyl)-4-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methyl-1H-pyrazol-5(4H)-one (6d)

It was obtained as orange pellets in 92 % yield, mp: 225-226 °C. IR (ν , cm⁻¹): 1550 (C=C), 1690 (C=O), 2910 (CH-Al), 3040 (CH-Ar). ¹H NMR (400 MHz, DMSO-d₆) δ_H = 2.31 (s, 3H, CH₃), 5.33 (s, 2H, OCH₂), 7.29 (d, 2H, J = 8.0 Hz, Ar-H), 7.54 (t, 1H, J = 8.0 Hz, Ar-H), 7.74 (d, 1H, J = 8.0 Hz, Ar-H), 7.79-7.85 (m, 4H, Ar-H), 7.90-7.97 (m, 5H, H-C=C and Ar-H), 9.04 (s, 1H, CH-1,2,3-triazole). ¹³C NMR (100 MHz, DMSO-d₆): δ_C = 13.63 (CH₃), 52.68 (OCH₂); 115.51, 117.86, 120.38, 124.78, 125.48, 126.51, 127.52, 128.81, 129.25, 136.60, 137.38, 137.46, 144.27, 146.78, 149.25, 152.58, 161.87, 162.84, 164.89 (Ar-C, C=O). Calculated for C₂₀H₁₅ClN₂O₂: C, 64.00; H, 3.93; N, 14.35. Found: C, 64.34; H, 3.69; N, 14.56.

1.1.5 (Z)-1-(4-Chlorophenyl)-3-methyl-4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)-1H-pyrazol-5(4H)-one (6e)

It was obtained as orange pellets in 88 % yield, mp: 232-234 °C. IR (ν , cm⁻¹): 1545 (C=C), 1695 (C=O), 2920 (CH-Al), 3040 (CH-Ar). ¹H NMR (400 MHz, DMSO-d₆) δ_H = 2.36 (s, 3H, CH₃), 5.34 (s, 2H, OCH₂), 7.29 (d, 2H, J = 8.0 Hz, Ar-H), 7.48 (t, 1H, J = 8.0 Hz, Ar-H), 7.72 (d, 1H, J = 8.0 Hz, Ar-H), 7.78-7.85 (m, 4H, Ar-H), 7.92-7.99 (m, 5H, H-C=C and Ar-H), 9.04 (s, 1H, CH-1,2,3-triazole). ¹³C NMR (100 MHz, DMSO-d₆): δ_C = 13.66 (CH₃), 52.78 (OCH₂); 115.57, 117.87, 120.15, 124.44, 125.30, 126.34, 127.48, 128.60, 129.26, 136.61, 137.09, 137.73, 144.25, 146.78, 149.24, 152.78, 161.85, 162.76 (Ar-C, C=O). Calculated for C₂₆H₁₉ClN₆O₄: C, 60.65; H, 3.72; N, 16.32. Found: C, 60.88; H, 3.93; N, 16.55.

(Z)-1-(4-Chlorophenyl)-4-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methyl-1H-pyrazol-5(4H)-one (6f)

It was obtained as orange pellets in 90 % yield, mp: 203-205 °C. IR (ν , cm⁻¹): 1570 (C=C), 1700 (C=O), 2970 (CH-Al), 3020 (CH-Ar). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.33 (s, 3H, CH₃), 5.39 (s, 2H, OCH₂), 7.28 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.51 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.75 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.81-7.87 (m, 4H, Ar-H), 7.93-7.98 (m, 5H, H-C=C and Ar-H), 9.00 (s, 1H, CH-1,2,3-triazole). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.61 (CH₃), 52.74 (OCH₂); 115.58, 117.79, 120.24, 124.49, 125.33, 126.39, 127.30, 128.68, 129.21, 136.68, 137.24, 137.58, 144.10, 146.90, 149.11, 152.70, 161.79, 162.90 (Ar-C, C=O). Calculated for C₂₆H₁₉Cl₂N₅O₂: C, 61.92; H, 3.80; N, 13.89. Found: C, 61.82; H, 3.99; N, 13.67.

(Z)-1-(4-Chlorophenyl)-4-(4-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methyl-1H-pyrazol-5(4H)-one (6g)

It was obtained as orange pellets in 89 % yield, mp: 244-245 °C. IR (ν , cm⁻¹): 1530 (C=C), 1680 (C=O), 2930 (CH-Al), 3070 (CH-Ar). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.30 (s, 3H, CH₃), 5.35 (s, 2H, OCH₂), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.53 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.72 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.82 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.87-7.92 (m, 6H, H-C=C and Ar-H), 9.01 (s, 1H, CH-1,2,3-triazole), 9.12 (s, 1H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.65 (CH₃), 52.73 (OCH₂); 115.56, 117.88, 120.14, 124.35, 126.36, 127.14, 128.60, 129.26, 136.80, 137.19, 137.68, 144.14, 146.97, 149.18, 152.75, 161.87, 162.76 (Ar-C, C=O). Calculated for C₂₆H₁₈Cl₃N₅O₂: C, 57.96; H, 3.37; N, 13.00. Found: C, 57.77; H, 3.54; N, 13.23.

(Z)-1-(4-Chlorophenyl)-4-(4-((1-(3-fluoro-4-methylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methyl-1H-pyrazol-5(4H)-one (6h)

It was obtained as orange pellets in 86 % yield, mp: 222-224 °C. IR (ν , cm⁻¹): 1540 (C=C), 1695 (C=O), 2910 (CH-Al), 3040 (CH-Ar). ¹H NMR (400 MHz, DMSO-*d*₆) δ _H= 2.32 (s, 3H, CH₃), 2.40 (s, 3H, CH₃), 5.32 (s, 2H, OCH₂), 7.27 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.51 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.88 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.91-7.98 (m, 6H, H-C=C and Ar-H), 9.05 (s, 1H, CH-1,2,3-triazole), 9.15 (s, 1H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ _C= 13.60 (CH₃), 14.89 (CH₃), 52.71 (OCH₂); 115.64, 117.80, 120.17, 124.21, 126.30, 127.19, 128.65, 129.18, 136.87, 137.23, 137.35, 144.32, 146.83, 149.13, 152.59, 161.75, 162.98 (Ar-C, C=O). Calculated for C₂₇H₂₁ClFN₅O₂: C, 64.61; H, 4.22; N, 13.95. Found: C, 64.87; H, 4.49; N, 13.66.

1.1.9 (Z)-4-(2-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1*H*-pyrazol-4(5*H*)-ylidene)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamido)benzoic acid (6i)

It was obtained as orange pellets in 90 % yield, mp: 178-179 °C. IR (ν , cm⁻¹): 1580 (C=C), 1730 (C=O), 2950 (CH-Al), 3050 (CH-Ar), 2600-3300 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ _H= 2.34 (s, 3H, CH₃), 5.30 (s, 2H, NCH₂), 5.46 (s, 2H, OCH₂), 7.29-7.49 (m, 4H, Ar-H), 7.80-7.98 (m, 5H, Ar-H and H-C=C), 8.20-8.38 (m, 3H, Ar-H and CH-1,2,3-triazole), 8.65-8.75 (m, 2H, Ar-H), 11.04 (s, 1H, NH), 12.72 (s, 1H, COOH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ _C= 13.60 (CH₃), 52.73 (NCH₂); 61.85 (OCH₂); 107.34, 115.89, 118.70, 121.45, 124.49, 126.09, 126.98, 128.56, 130.18, 135.48, 136.87, 144.29, 144.90, 146.78, 149.87, 151.97, 162.56, 163.09, 165.67 (Ar-C, C=O), 167.34 (COOH). Calculated for C₂₉H₂₃ClN₆O₅: C, 61.00; H, 4.06; N, 14.72. Found: C, 61.23; H, 4.29; N, 14.91.

1.1.10 (Z)-2-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1*H*-pyrazol-4(5*H*)-ylidene)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-(4-fluorophenyl)acetamide (6j)

It was obtained as orange pellets in 90 % yield, mp: 180-181 °C. IR (ν , cm⁻¹): 1545 (C=C), 1710 (C=O), 2940 (CH-Al), 3060 (CH-Ar), 3340 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.32 (s, 3H, CH₃), 5.35 (s, 2H, NCH₂), 5.39 (s, 2H, OCH₂), 7.17-7.27 (m, 5H, Ar-H), 7.48 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.63 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.78 (s, 1H, H-C=C), 7.97 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.34 (s, 1H, CH-1,2,3-triazole), 8.71 (d, 1H, *J* = 4.0 Hz, Ar-H), 10.77 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.60 (CH₃), 52.68 (NCH₂); 61.84 (OCH₂); 106.55, 115.44, 120.02, 124.01, 127.23, 129.19, 132.12, 137.61, 138.16, 142.35, 148.84, 152.69, 159.48, 161.94, 162.29, 162.91, 164.78 (Ar-C, C=O). Calculated for C₂₈H₂₂ClFN₆O₃: C, 61.71; H, 4.07; N, 15.42. Found: C, 61.52; H, 4.29; N, 15.62.

1.1.11 (Z)-*N*-(4-Fluorophenyl)-2-((4-((3-methyl-5-oxo-1-phenyl-1*H*-pyrazol-4(5*H*)-ylidene)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (6k)

It was obtained as orange pellets in 92 % yield, mp: 201-203 °C. IR (ν , cm⁻¹): 1555 (C=C), 1720 (C=O), 2960 (CH-Al), 3040 (CH-Ar), 3360 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.34 (s, 3H, CH₃), 5.33 (s, 2H, NCH₂), 5.37 (s, 2H, OCH₂), 7.17-7.29 (m, 6H, Ar-H), 7.47 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.76 (s, 1H, H-C=C), 7.96 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.37 (s, 1H, CH-1,2,3-triazole), 8.74 (d, 1H, *J* = 4.0 Hz, Ar-H), 10.81 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.63 (CH₃), 52.64 (NCH₂); 61.86 (OCH₂); 106.64, 115.51, 120.15, 122.22, 127.29, 129.34, 132.20, 137.69, 138.11, 142.34, 148.73, 152.46, 159.37, 161.87, 162.18, 162.85, 164.88 (Ar-C, C=O). Calculated for C₂₈H₂₃FN₆O₃: C, 65.87; H, 4.54; N, 16.46. Found: C, 65.63; H, 4.29; N, 16.23.

1.1.12 (Z)-2-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-nitrophenyl)acetamide (6l)

It was obtained as orange pellets in 92 % yield, mp: 199-200 °C. IR (ν , cm⁻¹): 1570 (C=C), 1710 (C=O), 2930 (CH-Al), 3080 (CH-Ar), 3320 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.31 (s, 3H, CH₃), 5.34 (s, 2H, NCH₂), 5.44 (s, 2H, OCH₂), 7.26-7.47 (m, 4H, Ar-H), 7.81-7.95 (m, 5H, Ar-H and H-C=C), 8.25-8.40 (m, 3H, Ar-H and CH-1,2,3-triazole), 8.71-8.79 (m, 2H, Ar-H), 11.11 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.63 (CH₃), 52.79 (NCH₂); 61.82 (OCH₂); 109.54, 115.70, 119.50, 120.10, 124.05, 125.64, 126.83, 128.62, 129.25, 135.79, 137.37, 143.07, 144.94, 146.34, 149.04, 152.80, 162.34, 162.96, 165.80 (Ar-C, C=O). Calculated for C₂₈H₂₂ClN₇O₅: C, 58.80; H, 3.88; N, 17.14. Found: C, 58.99; H, 4.09; N, 17.33.

1.1.13 (Z)-2-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-fluoro-4-methylphenyl)acetamide (6m)

It was obtained as orange pellets in 90 % yield, mp: 218-220 °C. IR (ν , cm⁻¹): 1580 (C=C), 1700 (C=O), 2950 (CH-Al), 3060 (CH-Ar), 3300 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.14 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 5.35 (bs, 2H, NCH₂ and OCH₂), 7.23-7.26 (m, 4H, Ar-H), 7.45-7.52 (m, 3H, Ar-H), 7.73-7.99 (m, 3H, Ar-H and H-C=C), 8.32 (s, 1H, CH-1,2,3-triazole), 8.68-8.75 (m, 2H, Ar-H), 10.62 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.61 (CH₃), 15.15 (CH₃), 52.66 (NCH₂); 61.89 (OCH₂); 110.32, 115.68, 119.52, 120.34, 121.78, 124.45, 125.60, 126.67, 128.90, 135.55, 139.50, 143.90, 145.18, 149.24, 152.45, 162.56, 163.28, 165.49 (Ar-C, C=O). Calculated for C₂₉H₂₄ClFN₆O₃: C, 62.31; H, 4.33; N, 15.03. Found: C, 62.52; H, 4.62; N, 15.34.

1.1.14 (Z)-N-(4-Chlorobenzyl)-2-(4-((4-((1-(4-chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)acetamide (6n)

It was obtained as orange pellets in 89 % yield, mp: 224-225 °C. IR (ν , cm⁻¹): 1560 (C=C), 1700 (C=O), 2930 (CH-Al), 3080 (CH-Ar), 3320 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.32 (s, 3H, CH₃), 4.32 (s, 2H, NHCH₂), 5.32 (s, 2H, NCH₂), 5.33 (s, 2H, OCH₂), 7.25-7.31 (m, 4H, Ar-H), 7.39-7.47 (m, 4H, Ar-H), 7.76 (s, 1H, H-C=C), 7.96 (s, 2H, Ar-H), 8.27 (s, 1H, CH-1,2,3-triazole), 8.69 (s, 2H, Ar-H), 8.88 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.63 (CH₃), 42.34 (NHCH₂); 52.54 (NCH₂); 61.81 (OCH₂); 106.87, 115.41, 120.24, 121.69, 127.35, 128.67, 132.03, 137.42, 137.89, 142.30, 148.25, 152.43, 156.87, 161.93, 162.43, 164.75 (Ar-C, C=O). Calculated for C₂₉H₂₄Cl₂N₆O₃: C, 60.53; H, 4.20; N, 14.60. Found: C, 60.38; H, 4.44; N, 14.87.

1.1.15 (Z)-2-(4-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-methylbenzyl)acetamide (6o)

It was obtained as orange pellets in 89 % yield, mp: 229-230 °C. IR (ν , cm⁻¹): 1550 (C=C), 1710 (C=O), 2900 (CH-Al), 3090 (CH-Ar), 3310 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.17 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 4.29 (s, 2H, NHCH₂), 5.35 (s, 2H, NCH₂), 5.38 (s, 2H, OCH₂), 7.23-7.28 (m, 3H, Ar-H), 7.33-7.44 (m, 5H, Ar-H), 7.79 (s, 1H, H-C=C), 7.92 (bs, 2H, Ar-H), 8.34 (s, 1H, CH-1,2,3-triazole), 8.73 (s, 2H, Ar-H), 8.93 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.60 (CH₃), 15.29 (CH₃), 42.41 (NHCH₂); 52.50 (NCH₂); 62.13 (OCH₂); 106.67, 115.62, 120.34, 121.74, 127.42, 128.54, 132.32, 137.23, 137.65, 142.12, 148.34, 152.13, 156.56, 162.08, 162.87, 164.90 (Ar-C,

C=O). Calculated for C₃₀H₂₇ClN₆O₃: C, 64.92; H, 4.90; N, 15.14. Found: C, 65.23; H, 4.63; N, 15.63.

1.1.16 (Z)-2-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-methoxybenzyl)acetamide (6p)

It was obtained as orange pellets in 92 % yield, mp: 208-209 °C. IR (ν , cm⁻¹): 1570 (C=C), 1690 (C=O), 2950 (CH-Al), 3060 (CH-Ar), 3290 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.34 (s, 3H, CH₃), 3.68 (s, 3H, CH₃), 4.35 (s, 2H, NHCH₂), 5.30 (s, 2H, NCH₂), 5.33 (s, 2H, OCH₂), 7.27-7.38 (m, 4H, Ar-H), 7.42-7.49 (m, 4H, Ar-H), 7.76 (s, 1H, H-C=C), 7.88 (bs, 2H, Ar-H), 8.31 (s, 1H, CH-1,2,3-triazole), 8.78 (s, 2H, Ar-H), 8.79 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.66 (CH₃), 42.53 (NHCH₂); 52.41 (NCH₂); 55.46 (OCH₃), 62.28 (OCH₂); 106.87, 115.89, 120.59, 121.79, 128.40, 129.82, 133.56, 138.76, 143.34, 147.78, 152.54, 156.78, 162.23, 162.57, 164.76 (Ar-C, C=O). Calculated for C₃₀H₂₇ClN₆O₄: C, 63.10; H, 4.77; N, 14.72. Found: C, 63.36; H, 4.59; N, 14.95.

1.1.17 (Z)-2-((4-((1-(4-Chlorophenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)methyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluorobenzyl)acetamide (6q)

It was obtained as orange pellets in 89 % yield, mp: 245-255 °C. IR (ν , cm⁻¹): 1570 (C=C), 1690 (C=O), 2910 (CH-Al), 3080 (CH-Ar), 3320 (N-H). ¹H NMR (400 MHz, DMSO-*d*₆) δ_H = 2.51 (bs, 3H, CH₃ overlap with DMSO-*d*₆), 4.31 (s, 2H, NHCH₂), 5.19 (s, 2H, NCH₂), 5.30 (s, 2H, OCH₂), 7.16-7.32 (m, 9H, Ar-H), 7.88 (bs, 3H, Ar-H and H-C=C), 8.28 (s, 1H, CH-1,2,3-triazole), 8.85 (s, 1H, Ar-H), 9.88 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ_C = 13.69 (CH₃), 42.26 (NHCH₂); 52.69 (NCH₂); 61.77 (OCH₂);

106.34, 115.72, 120.23, 122.45, 126.78, 129.54, 132.42, 137.55, 138.03, 142.19, 148.67, 152.87, 158.37, 162.06, 162.90, 165.31 (Ar-C, C=O). Calculated for C₂₉H₂₄ClFN₆O₃: C, 62.31; H, 4.33; N, 15.03. Found: C, 62.52; H, 4.56; N, 15.28.

2 Spectral Data of New Compounds

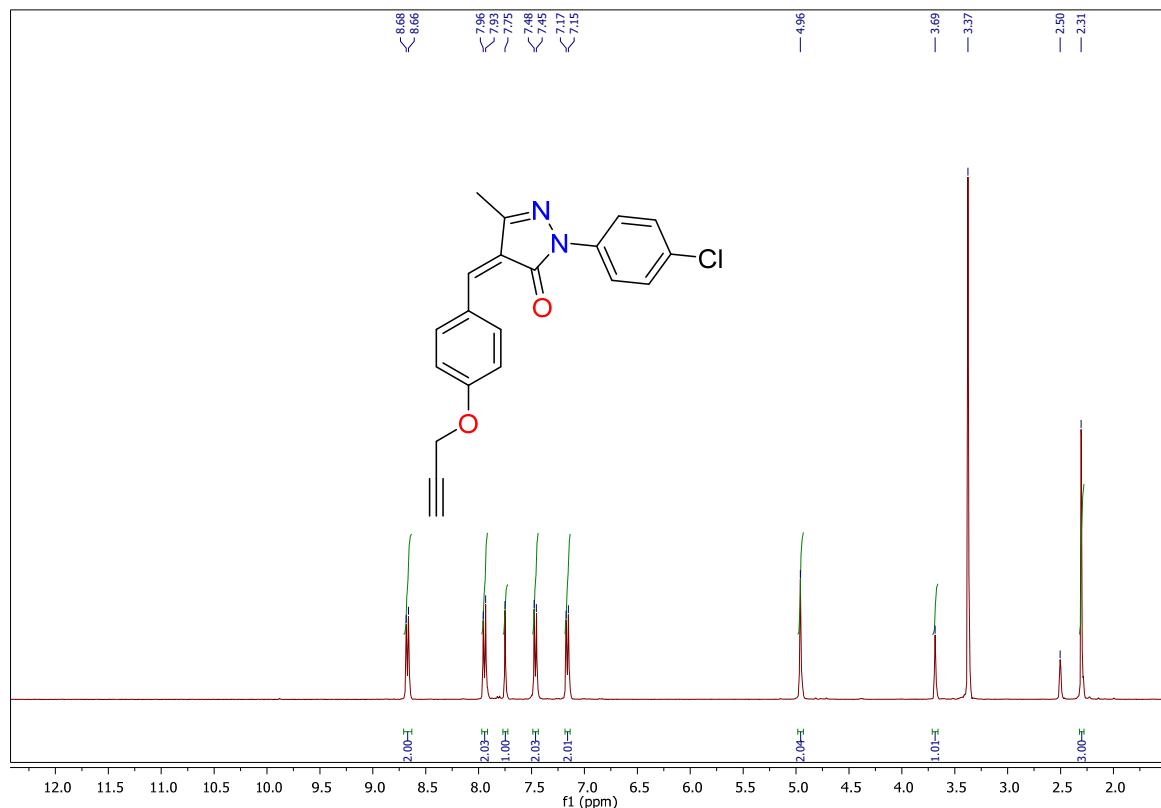


Figure S1: ¹H NMR spectrum of compound 4b.

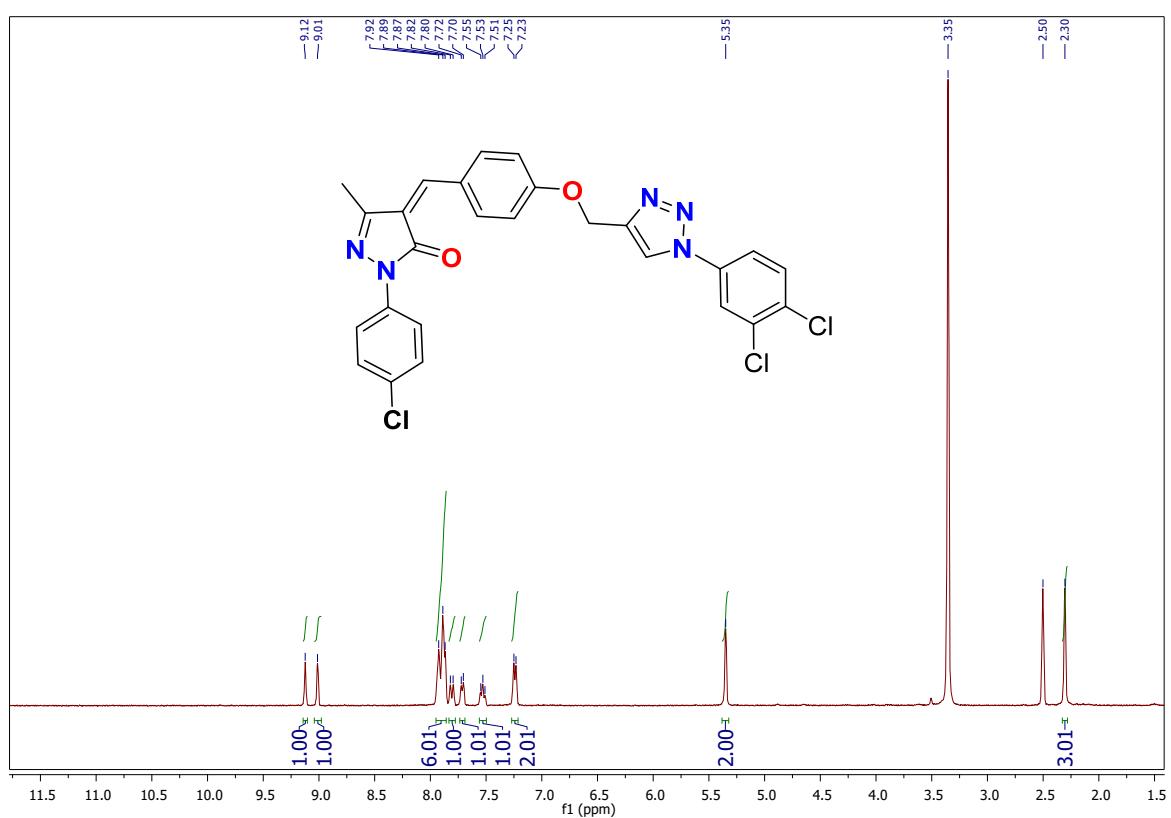


Figure S2: ¹H NMR spectrum of compound 6g.

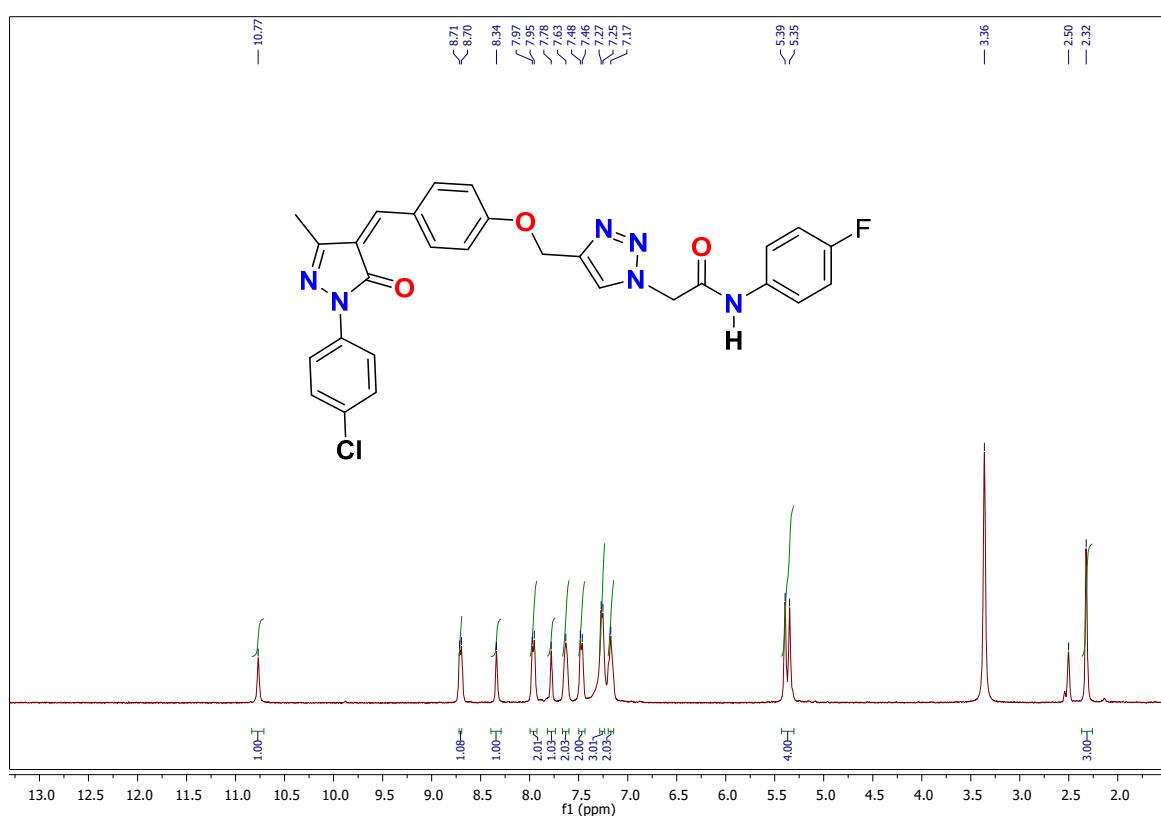


Figure S3: ¹H NMR spectrum of compound 6j.

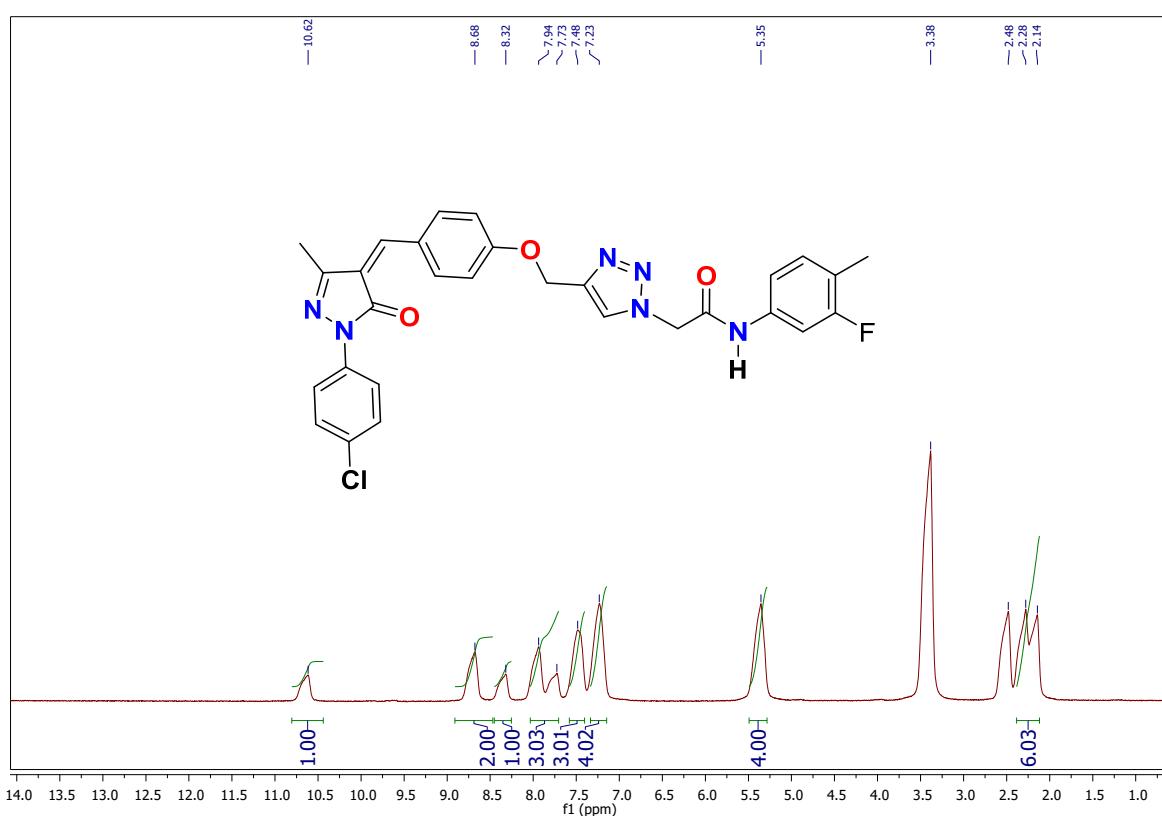


Figure S4: ¹H NMR spectrum of compound 6m.

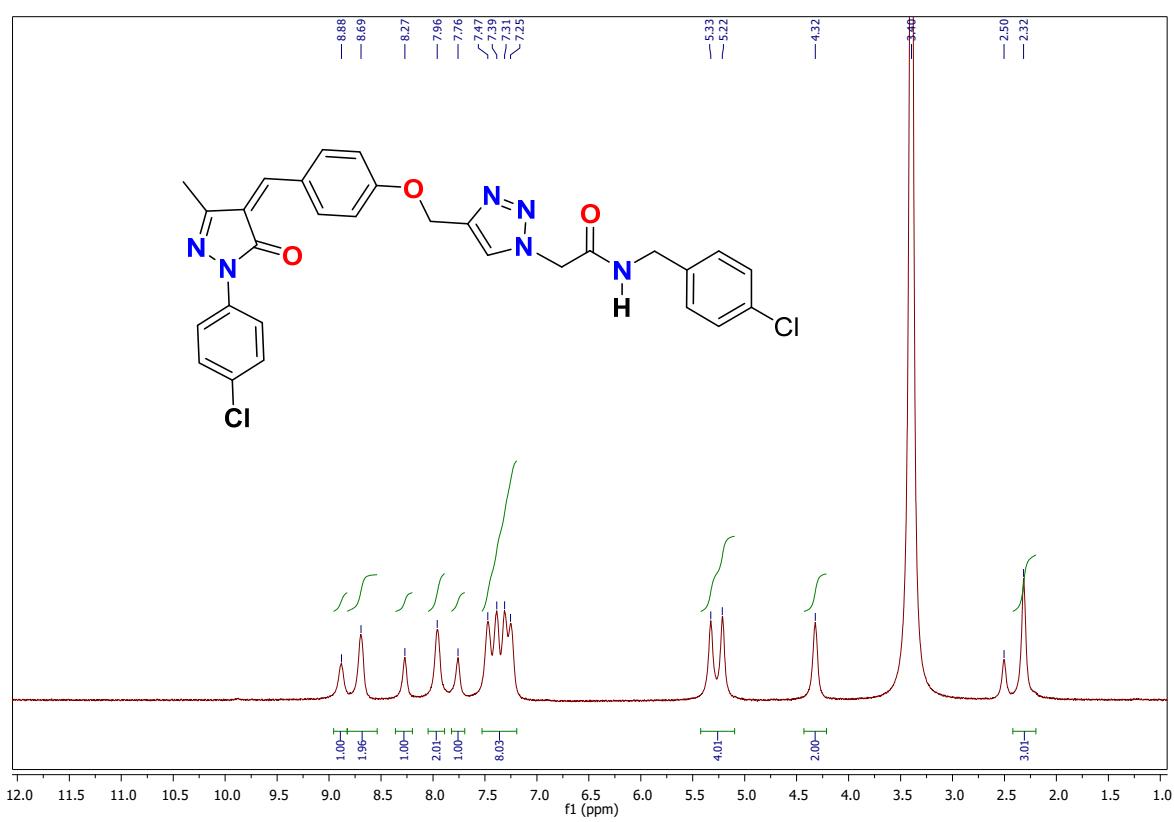


Figure S5: ¹H NMR spectrum of compound 6n.

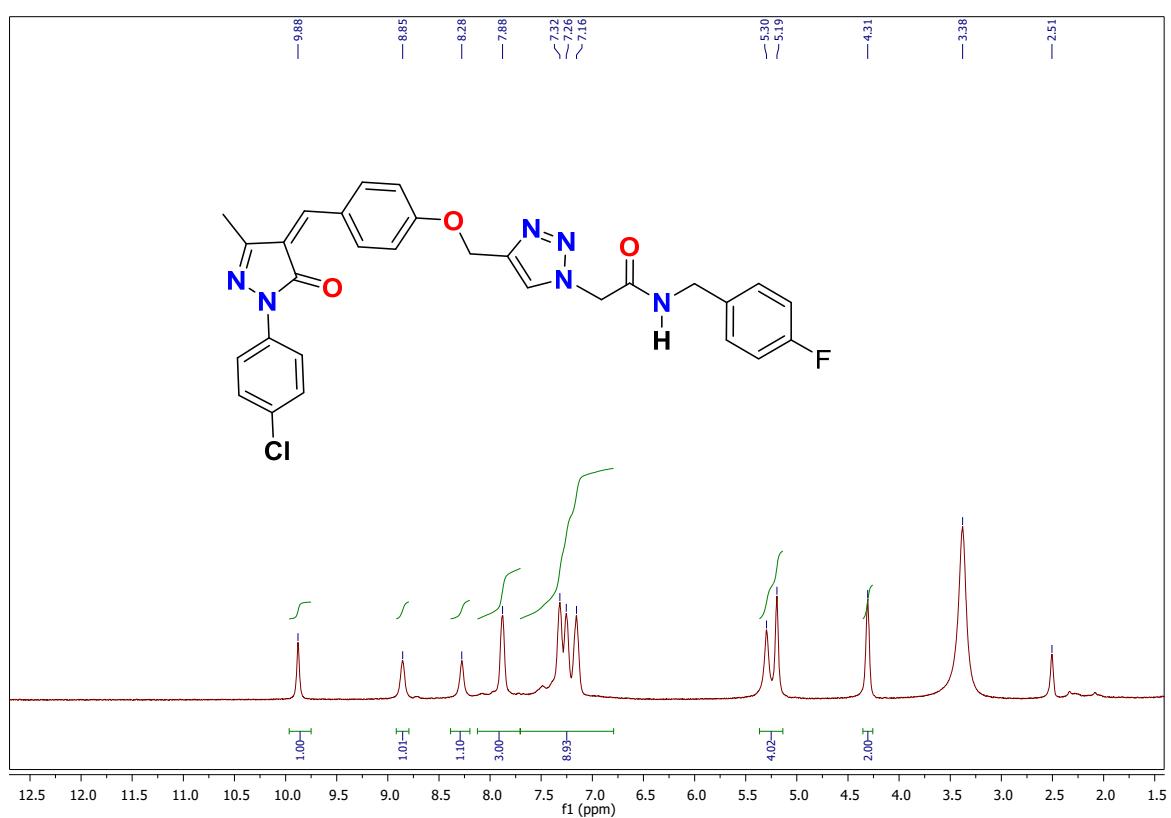


Figure S6: ¹H NMR spectrum of compound 6q.

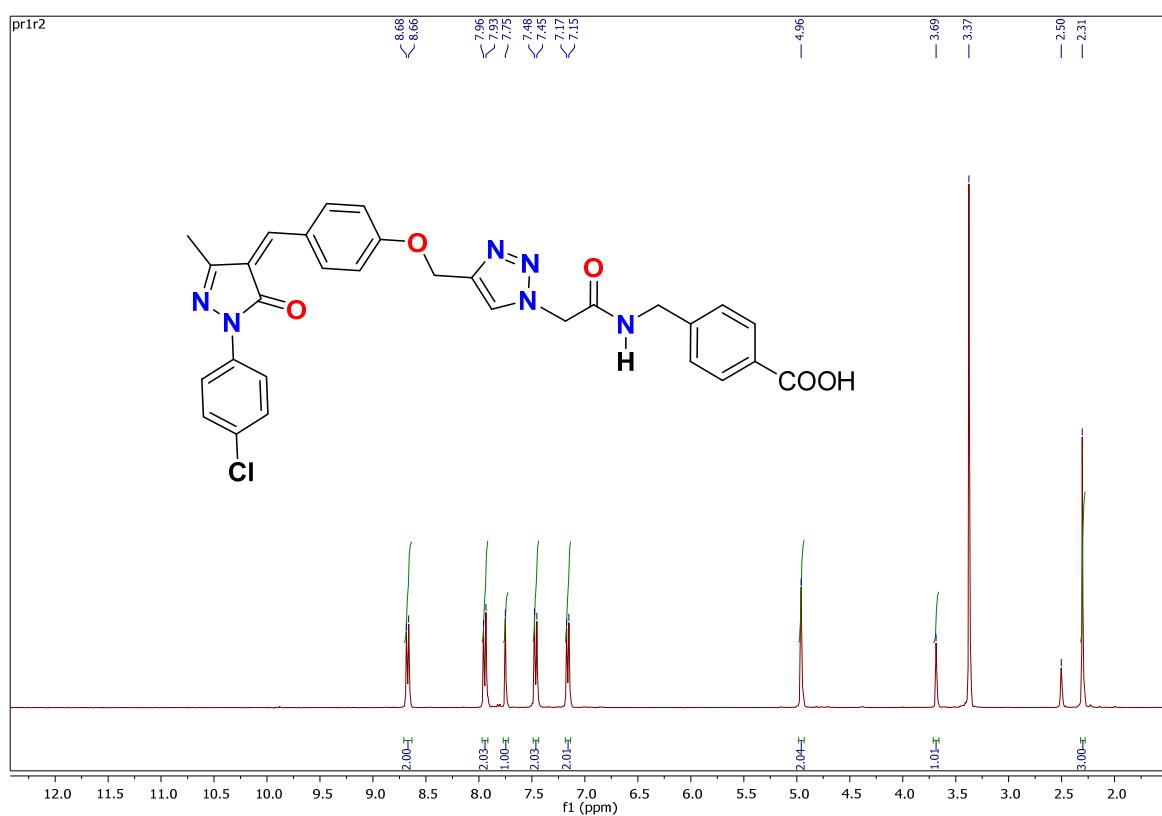


Figure S7: ¹H NMR spectrum of compound 6i.

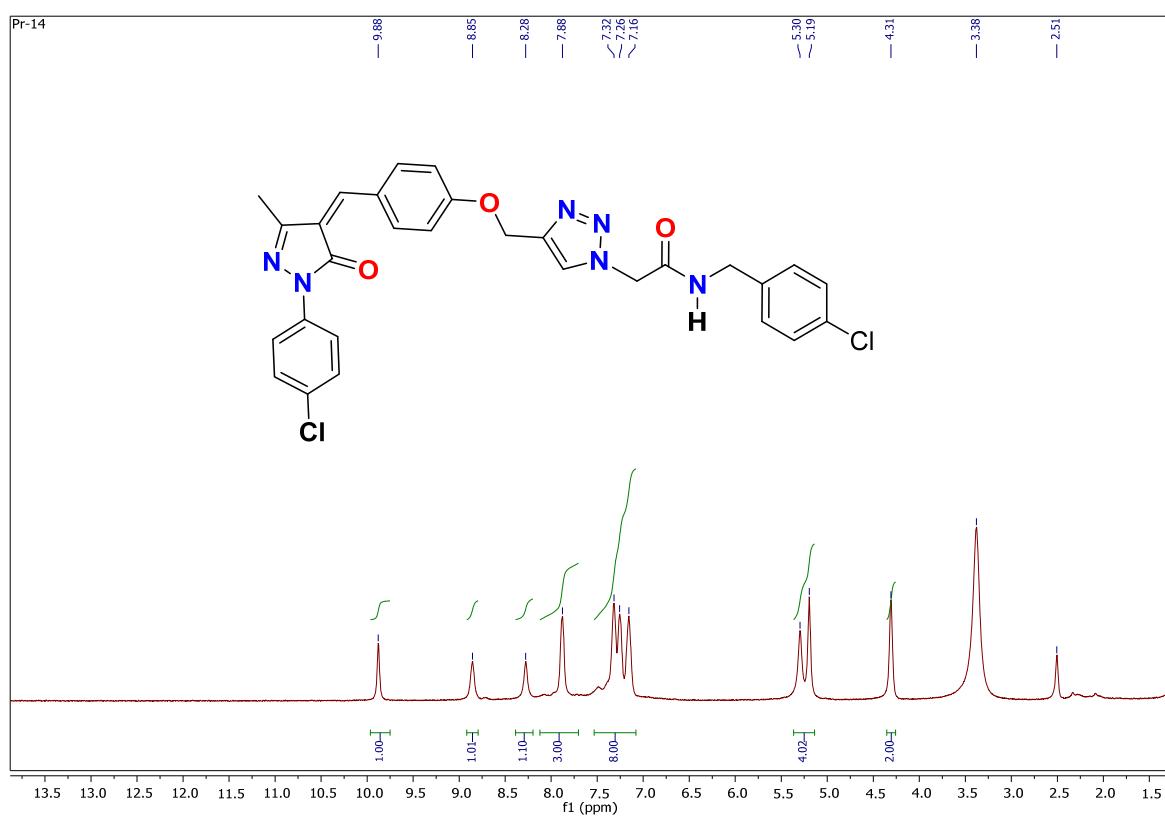


Figure S8: ¹H NMR spectrum of compound 6n.

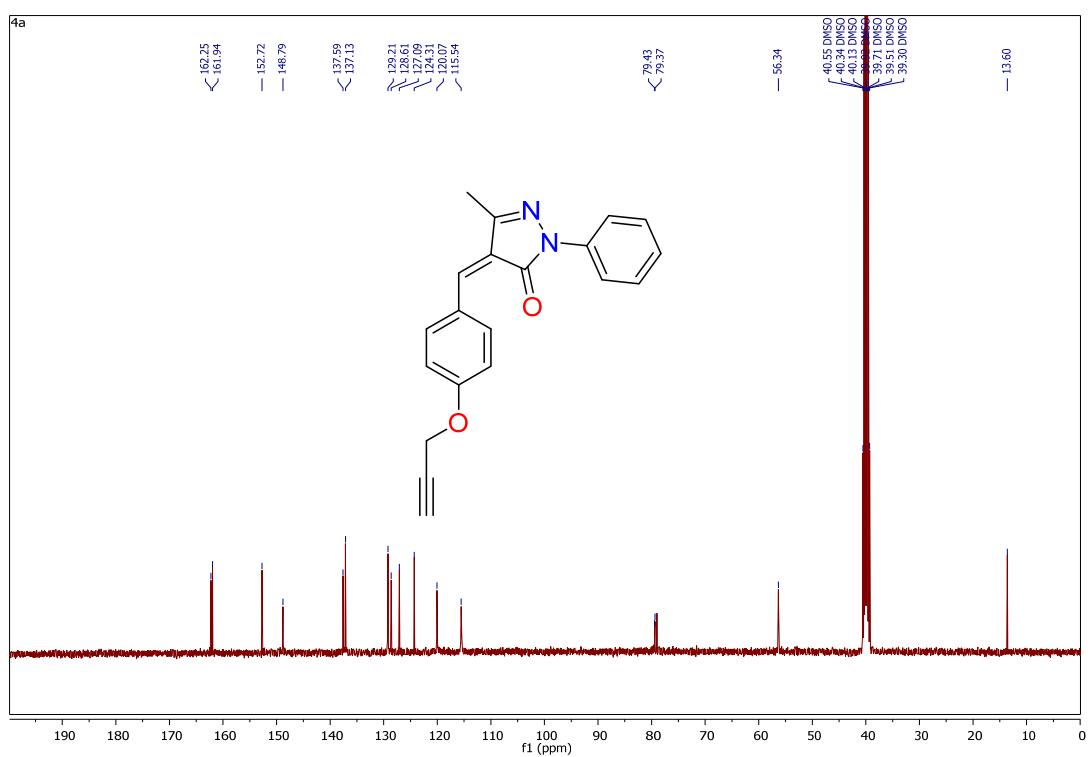


Figure S9: ^{13}C NMR spectrum of compound 4a.

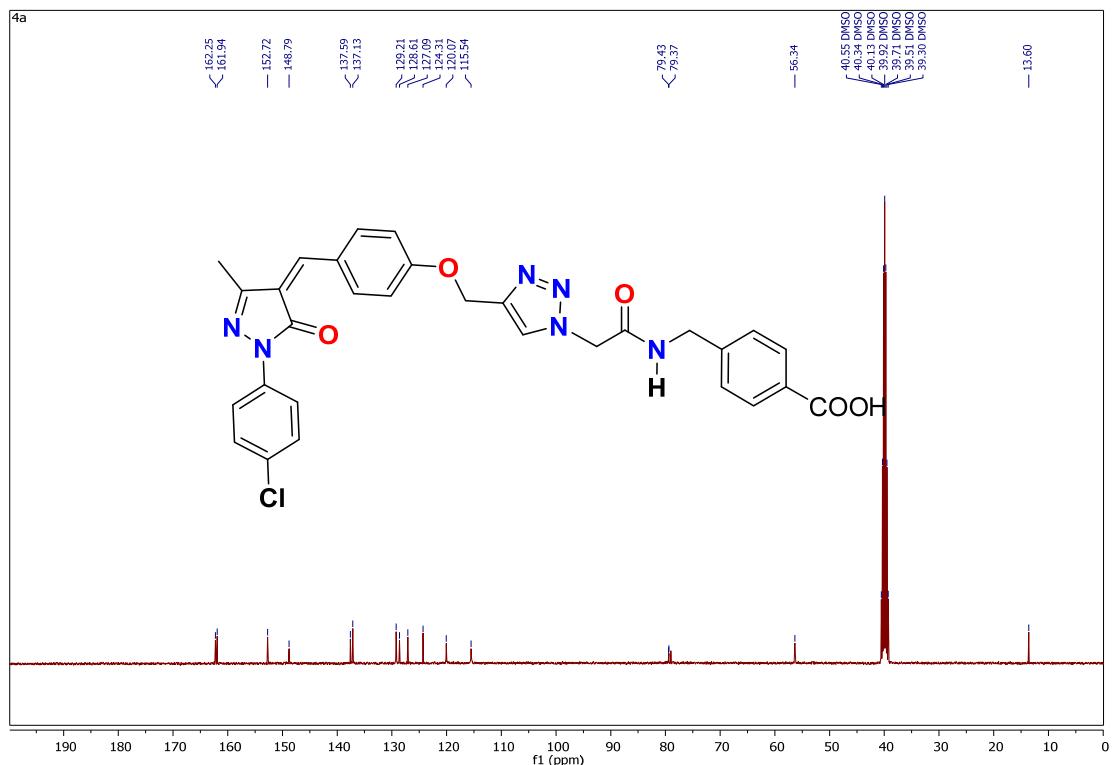


Figure S10: ^{13}C NMR spectrum of compound **6i**.

3 M^{pro} protease inhibition assay

The COV2-SARS-CoV-2 protease enzyme assay [52,53] was described in the manufacturing protocol (BPS Bioscience). In summary, it was performed by adding 30 μl of SARS-CoV-2 protease and 10 μl of compound diluted in 1 \times assay buffer. Then Incubated for 30 min at room temperature. The reaction began with the addition of 10 μl of the diluted substrate, which is a M^{pro} self-quenching 14-mer fluorogenic (FRET) peptide with a cleavage site indicated by an arrow ((Dabcyl-KTSAVLQ \downarrow SGFRKME-Edans) (BPS Bioscience) using a counter-screen assay for the quenching of inhibitors' fluorescence effect. Then Incubated at room temperature overnight and the plate was sealed with the plate sealer. The fluorescence intensity was measured in a microtiter plate-reading fluorimeter capable of excitation at a wavelength of 360 nm and detection of emission at a wavelength of 460 nm. The fluorescence intensity can also be measured kinetically. The "Blank" value is subtracted from all other values. The experiment was carried out three times to acquire the IC₅₀ value, and the error from the global fit with varied hill slope.

4 SARS-CoV-2 Antiviral assay

To assess the effect of different target chemical treatments on SARS-CoV-2 viral load (SARS-CoV-2 isolate EGY/WAT-2 VACCERA), a Real-Time PCR test was used to detect SARS-CoV-2 viral RNA [54,55]. Total RNA was extracted according to the manufacturer's instructions using the genesig® Coronavirus SARS-CoV-2 Real-Time PCR Assay kit (Primer design TM Ltd, Southampton, United Kingdom). This kit includes a master mix, primers, and probe for reverse transcription of extracted RNA

as well as Real-Time PCR for SARS-CoV-2 detection. The assay was carried out using the Rotor-Gene Q instrument (Qiagen, Germany) under the following amplification conditions: 10 minutes of reverse transcription at 55 °C, 2 minutes of initial activation at 95 °C, 45 cycles of 10 seconds denaturation at 95 °C and 60 seconds annealing and extension at 60 °C, and 45 cycles of annealing and extension at 60 °C.

5 Molecular dynamics simulation

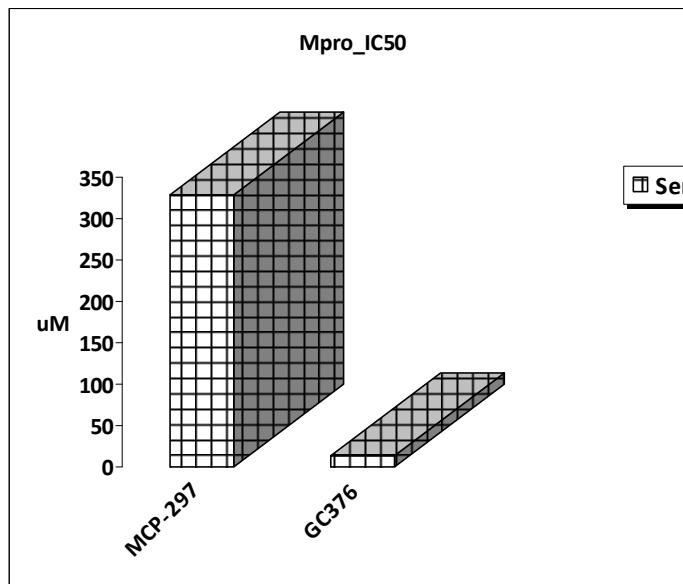
MD simulations were performed utilising templates from the 3D structure crystal of SARS-COV 19 Mpro protease (PDB ID: 5R80) complexed with the target active compound **6i** vs the reference bound drug. The simulation was run with the Desmond version 3.8 software and the OPLS2005 forcefield [61]. The initial docked model structure was refined using the protein preparation wizard in Maestro (Schrödinger, LLC) and placed into TIP3P water molecules solvated with 0.15 M NaCl. After minimization and relaxation of the model, the production molecular dynamics phase was performed for three independent 100 ns simulations in an isothermal-isobaric (NpT) ensemble at 300 K and 1 bar using Langevin dynamics. Long-range electrostatic interactions were computed using the Smooth Particle Mesh Ewald method. All system setups were performed using Maestro. Trajectory coordinates were recorded every 100 ps. The simulation trajectories were then analyzed for the sampled conformations in all structural models. The obtained trajectory was processed utilizing the AMBER11 tool ptraj for the calculations of RMSD and PMSF for protein RMSD. All molecular figures were generated using PyMOL (Schrödinger, LLC), see supplementary data.

6 The data of control drug GC-376

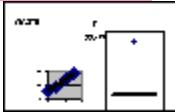
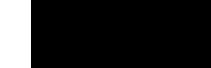
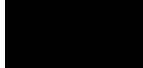
| | | | |
|------------|--------------------------|------------------------------|--------------------|
| Researcher | : Dr.Hany Emary | email: jan_25_misr@yahoo.com | mob. +966534391522 |
| Assay | : COV-3CL protease assay | | |
| Samples | : 01 compounds. | | |
| Cell line | : --- | | |
| Reference | : --- | | |

Lab Report I

| ser | Compound | Results | | | |
|-----|----------|---------|--------|------------------|------|
| | | code | MW | COV-3CL protease | SD |
| | | | g/mol | IC50 | |
| *** | GC-376 | | 507.53 | 12.85 | 0.74 |



Detailed results

| code | IC 50 | conc. uM | log | | | | | | | | | slop e | K.Activ ity |
|-------|---|-------------|---|---|---|---|--|---|----------|----------|----|-----------|----------------|
| | | | co nc | %in h | T 2 | T 1 | Δ T | RF U2 | RF U1 | ΔRF U | | | |
| GC376 |  | 1000 | 3 | 91 | 0 | 0 | 0 | 8.63 | 0 | 8.63 | 33 | 10.3561 | |
| | | 100 | 2 | 73 | 0 | 0 | 0 | 4 | 0 | 4 | 33 | 31.8483 | |
| | | 10 | 1 | 42 | 0 | 0 | 0 | 1 | 0 | 1 | 33 | 69.7327 | |
| | | 1 | 0 | 28 | 0 | 0 | 0 | 3 | 0 | 3 | 33 | 86.4369 | |
| | | | | | 3 | | 3 | | | | | 3.33 | |
| | | | | | 0 | 0 | 0 | 100 | 0 | 100 | 33 | 120 | |
| | | |  |  |  |  |  |  | | | | | |

