Supporting information

Development, anti-proliferative activity, multi-target kinase inhibition against CHK1, PIM1, and CDK-2, as well as computational insights of new thiazole-based hybrids

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2. Experimental

2.1. Chemistry

Melting points are uncorrected and were determined in open capillary tubes using electric melting point apparatus (G-K). Infrared spectra (KBr discs) were measured on a Shimadzu FTIR, 8300 PC IR spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}CNMR (101 MHz) was recorded with a Bruker model Ultra Shield NMR spectrometer with TMS as the internal standard and chemical shifts were reported on a

δ scale (ppm) using DMSO-d_6 as solvents. Micro analytical data were obtained from the Micro Analytical Research Centre, Faculty of Science, Cairo University, and the values found were within ± 0.3% of the theoretical. All reactions were monitored by TLC on Merck Silica Gel 60F254 and spots were detected using a UV lamp (254 nm) and different solvents as mobile phases.

2.1.2. 2-*Oxo-N'-(thiazol-2-yl)propanehydrazonoyl chloride (1)*

A solution of 2-aminothiazole (10 mmol) in concentrated HCl (5) mL was gently heated while stirring until completely dissolved, then cooled in an ice bath (0–5°C). Sodium nitrite (10 mmol) was dissolved in water (3 mL) and gradually added to the solution while maintaining the same temperature conditions to form the diazonium salt. The prepared diazonium salt was then added slowly to a solution mixture of 3-chloroacetyl acetone (10 mmol) and sodium acetate (2 g) in ethanol, ensuring the temperature remained consistent until the addition was complete. The reaction was stirred for an additional hour. The resulting precipitate was collected by filtration, thoroughly washed with cold water, and dried. The final product was recrystallized from ethanol to yield product **1** as a yellow powder.

Yield 89%; m.p. 223–225^{o}C; IR (ν_{max}/cm^{-1}): 3245 (NH), 3033 (CH-arom.), 1703 (CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 7.08 (d, 1H, CH-thiazole, J = 3.2 Hz), 7.13 (d,1H, CH-thiazole, J = 3.2 Hz), 7.32 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): 21.4 (CH_3), 120.8 (C_5-thiazole), 127.1 (C_4-thiazole), 149.7(C-hydrazonoyl), 156.6 (C_2-thiazole), 185.1(CO); Anal. Calcd. for $C_6H_6ClN_3OS$ (203.65): C, 35.39; H, 2.97; Cl, 17.41; N, 20.63; S, 15.75%; Found: C, 35.61; H, 2.75; Cl, 17.63; N, 20.84; S, 15.53%.

2.1.3. General Procedure for the Synthesis of Target Compounds 2-7

The suitable active nitriles, including malononitrile, ethyl 2-cyanoacetate, 2-cyanoacetamide, and 3-oxo-3-phenylpropanenitrile, as well as diketones such as pentane-2,4-dione and ethyl 3-oxobutanoate (10 mmol), were added to a sodium ethoxide solution prepared from sodium metal (10 mmol) in 15 mL of ethanol. After stirring the mixture for 30 minutes, compound **1** (5 mmol) was gradually introduced. The reaction was then stirred for an additional 8 hours. The resulting solid was filtered, washed with cold water, dried, and recrystallized from ethanol to yield compounds **2-7**, respectively.

2.1.3.1. 3-Acetyl-5-amino-1-(thiazol-2-yl)-1H-pyrazole-4-carbonitrile (2)

Yield 73%, orange powder; m.p. 177-179˚C; IR (ν_{max}/cm^{-1}): 3330, 3226 (NH_2), 2205 (CN), 1692 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.13 (s, 3H, CH_3), 7.44 (d, 1H, CH-thiazole, J = 3.6 Hz), 7.51 (d,1H, CH-thiazole, J = 3.6 Hz), 7.86 (s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): 25.1 ($CO\underline{C}H_3$), 109.6 (C_4-pyrazole), 114.9 (CN), 118.6 (C_5-thiazole), 132.2 (C_3-pyrazole), 138.5 (C_4-thiazole), 151.7 (C_2-thiazole), 156.4 (C_5-pyrazole), 186.3 (C=O); MS: m/z = 233 [M$^{\cdot+}$], 151 (100%). Anal. Calcd. for $C_9H_7N_5OS$ (233.25): C, 46.34; H, 3.02; N, 30.03; S, 13.75%; Found: C, 46.58; H, 3.25; N, 30.27; S, 13.54%.

2.1.3.2. Ethyl 3-acetyl-5-amino-1-(thiazol-2-yl)-1H-pyrazole-4-carboxylate (3)

Yield 70%, orange powder; m.p. 151-153˚C; IR (ν_{max}/cm^{-1}): 3415, 3377 (NH_2), 1693, 1720 (2C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.23 (t, 3H, -$OCH_2C\underline{H}_3$, J= 6.8 Hz,), 2.24 (s, 3H, CH_3), 4.24 (q, 2H, CH_2, $OC\underline{H}_2CH_3$, J= 6.8 Hz), 7.51 (d, 1H, CH-thiazole, J = 2.8 Hz), 7.70 (s, 2H, NH_2, D_2O exchangeable), 7.88 (d,1H, CH-thiazole, J= 2.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 14.5 ($CH_2\underline{C}H_3$), 28.3 ($CO\underline{C}H_3$), 61.40 ($\underline{C}H_2CH_3$), 103.6 (C_4-pyrazole), 110.2 (C_5-thiazole), 132.0 (C_4- thiazole), 141.8 (C_3--pyrazole), 153.2 (C_2-thiazole), 157.3 (C_5-pyrazole), 168.3 (C=O), 185.1 ($\underline{C}OCH_3$); MS: m/z = 280 [M$^{\cdot+}$]; Anal. Calcd. for $C_{11}H_{12}N_4O_3S$ (280.30): C, 47.13; H, 4.32; N, 19.99; S, 11.44%; Found: C, 47.35; H, 4.54; N, 19.77; S, 11.67%.

2.1.3.3. 3-Acetyl-5-amino-1-(thiazol-2-yl)-1H-pyrazole-4-carboxamide (4)

Yield 68%, orange powder; m.p. 166-168˚C; IR (ν_{max}/cm^{-1}): 3440-3125 ($2NH_2$), 1696, 1656 (2C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.11 (s, 3H, CH_3), 6.42 (s, 2H, NH_2, D_2O exchangeable), 7.02 (d, 1H, CH-thiazole, J = 4.1 Hz), 7.09 (d,1H, CH-thiazole, J= 4.1 Hz), 7.12 (s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): 26.4 ($CO\underline{C}H_3$), 105.0 (C_4-pyrazole), 111.0 (C_5-thiazole), 135.8 (C_4-thiazole), 148.6 (C_3-pyrazole), 156.5 (C_2-thiazole), 159.1 (C_5-pyrazole), 166.5 (CONH), 184.8 ($\underline{C}OCH_3$); MS: m/z = 251 [M$^{\cdot+}$]; Anal. Calcd. for $C_9H_9N_5O_2S$ (251.27): C, 43.02; H, 3.61; N, 27.87; S, 12.76%; Found: C, 43.02; H, 3.61; N, 27.87; S, 12.76%.

2.1.3.4. 3-Acetyl-5-phenyl-1-(thiazol-2-yl)-1H-pyrazole-4-carbonitrile (5)

Yield 78%, brown powder; m.p. 172-174˚C; IR (ν_{max}/cm^{-1}): 3079 (CH-arom.), 2215 (CN), 1695 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 7.07-7.88 (m, 5H, Ar-H + 2H, CH-thiazole); ^{13}C NMR (100 MHz, DMSO-d_6): 23.6 ($CO\underline{C}H_3$), 101.8 (C_4-pyrazole), 110.3 (C_5-thiazole), 115.3 (CN), 124.9, 128.6, 129.0, 136.2 (Ar-C), 136.3 (C_3-pyrazole), 139.9 (C_4-thiazole), 144.8 (C_5-pyrazole), 158.1 (C_2-thiazole),

187.2 (C=O); MS: m/z = 294 [$M^{\cdot+}$], 77 (100%). Anal. Calcd. for $C_{15}H_{10}N_4OS$ (294.33): C, 61.21; H, 3.42; N, 19.04; S, 10.89%; Found: C, 61.43; H, 3.64; N, 19.26; S, 10.68%.

2.1.3.5. 1,1'-(5-Methyl-1-(thiazol-2-yl)-1H-pyrazole-3,4-diyl)diethanone (6)

Yield 62%, Buff powder; m.p. 160-162˚C; IR (ν_{max}/cm^{-1}): 3068 (CH-arom.), 1706, 1693 (2C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 2.62 (s, 6H, $2CH_3CO$), 7.32 (d, 1H, CH-thiazole, J = 3.6 Hz), 7.35 (d,1H, CH-thiazole, J= 3.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 16.3 (CH_3), 25.1, 29.4 ($2CO\underline{C}H_3$), 111.2 (C_5-thiazole), 122.1 (C_4-pyrazole), 133.2 (C_4-thiazole), 141.8 (C_3-pyrazole), 149.5 (C_5-pyrazole), 155.6 (C_2-thiazole), 182.0 (CO), 183.3 (CO); MS: m/z = 249 [$M^{\cdot+}$], 235 (100%); Anal. Calcd. for $C_{11}H_{11}N_3O_2S$ (249.29): C, 53.00; H, 4.45; N, 16.86; S, 12.86%; Found: C, 53.21; H, 4.67; N, 16.75; S, 12.74%.

2.1.3.6. Ethyl 3-acetyl-5-methyl-1-(thiazol-2-yl)-1H-pyrazole-4-carboxylate (7)

Yield 71%, brown powder; m.p. 137-139˚C; IR (ν_{max}/cm^{-1}): 3045 (CH-arom.), 1710, 1688 (2C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.31 (t, J= 6.8 Hz, 3H, $-OCH_2\underline{CH_3}$), 2.12 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.09 (q, J= 6.8 Hz, 2H, CH_2, $OC\underline{H_2}CH_3$), 7.07 (d, 1H, CH-thiazole, J = 3.2 Hz), 7.33 (d,1H, CH-thiazole, J= 3.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 11.1 (CH_3), 14.4 ($CH_2\underline{C}H_3$), 23.6 ($CO\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$), 108.2 (C_5-thiazole), 110.9 (C_4-pyrazole), 130.7 (C_4-thiazole), 141.6 (C_3-pyrazole), 143.2 (C_5-pyrazole), 151.4 (C_2-thiazole), 168.5 ($\underline{C}OOC_2H_5$), 185.2 ($\underline{C}OCH_3$); MS: m/z = 279 [$M^{\cdot+}$]; Anal. Calcd. for $C_{12}H_{13}N_3O_3S$ (279.31): C, 51.60; H, 4.69; N, 15.04; S, 11.48%; Found: C, 51.81; H, 4.89; N, 15.27; S, 11.68%.

2.1.3.7. General Procedure for the Synthesis of 9a-c

Hydrazonoyl chloride **1** (10 mmol), along with the appropriate thiourea derivatives, such as thiourea, 1-methyl-2-thiourea, or *N*-phenyl-2-thiourea (10 mmol), and a catalytic amount of triethylamine (0.5 mL) were heated under reflux for nine hours in ethanol (10 mL). The reaction mixture was then cooled after the reaction's completion. The resulting residue was separated by filtration, washed with cold water, and recrystallized from dioxane to afford the corresponding products **9a–c**.

2.1.3.7.1. 4-Methyl-5-(thiazol-2-yldiazenyl) thiazol-2-amine (9a)

Yield 76%, reddish brown powder; m.p. 253-255˚C; IR (ν_{max}/cm^{-1}): 3354, 3275 (NH_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 6.70 (s, 2H, NH_2, D_2O exchangeable), 7.15 (d, 1H, CH-thiazole, J = 4.4 Hz), 7.48 (d,1H, CH-thiazole, J= 4.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 13.4 (CH_3), 112.1 (C_5-thiazol-2-yldiazenyl), 133.6 (C_4-thiazol-2-yldiazenyl), 142.8 (C_5-thiazol-2-amine), 148.7 (C_4-thiazol-2-

amine), 158.7 (C_2-thiazol-2-yldiazenyl), 162.5 (C_2-thiazol-2-amine); MS: m/z = 225 [$M^{\cdot+}$], 114 (100%); MS: m/z = 225 [$M^{\cdot+}$], 114 (100%); Anal. Calcd. for $C_7H_7N_5S_2$ (225.29): C, 37.32; H, 3.13; N, 31.09; S, 28.47%; Found: C, 37.53; H, 3.35; N, 31.31; S, 28.69%.

2.1.3.7.2. N,4-Dimethyl-5-(thiazol-2-yldiazenyl)thiazol-2-amine (9b)

Yield 60%, reddish brown powder; m.p. 266-268˚C; IR (ν_{max}/cm^{-1}): 3342 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.02 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 7.04 (d, 1H, CH-thiazole, J= 4.0 Hz), 7.12 (d,1H, CH-thiazole, J= 4.0 Hz), 7.60 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): 12.6 (CH_3), 31.4 (NH$\underline{C}H_3$), 111.3 (C_5-thiazol-2-yldiazenyl), 135.8 (C_4-thiazol-2-yldiazenyl), 141.6 (C_5-thiazol-2-amine), 144.1 (C_4-thiazol-2-amine), 155.4 (C_2-thiazol-2-yldiazenyl), 161.2 (C_2-thiazol-2-amine); MS: m/z = 239 [$M^{\cdot+}$]; 114 (100%); Anal. Calcd. for $C_8H_9N_5S_2$ (239.32): C, 40.15; H, 3.79; N, 29.26; S, 26.80%; Found: C, 40.37; H, 3.58; N, 29.46; S, 26.59%.

2.1.3.7.3. 4-Methyl-N-phenyl-5-(thiazol-2-yldiazenyl)thiazol-2-amine (9c)

Yield 74%, red powder; m.p. 261-263˚C; IR (ν_{max}/cm^{-1}): 3228 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.19 (s, 3H, CH_3), 7.06-7.27 (m, 7H, Ar-H+2CH-thiazole), 9.08 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO -d_6): 15.1 (CH_3), 112.9 (C_5-thiazol-2-yldiazenyl), 121.5, 129.0, 129.2, 131.1 (Ar-C), 140.0 (C_4-thiazol-2-yldiazenyl), 142.3 (C_5-thiazol-2-amine), 148.5 (C_4-thiazol-2-amine), 153.8 (C_2-thiazol-2-amine), 160.0 (C_2-thiazol-2-yldiazenyl); MS: m/z = 301 [$M^{\cdot+}$]; Anal. Calcd. for $C_{13}H_{11}N_5S_2$ (301.39): C, 51.81; H, 3.68; N, 23.24; S, 21.28%; Found: C, 51.60 ; H, 3.89; N, 23.46; S, 21.51%.

2.1.3.8. General Procedure for the Synthesis of 2-thioxo- pyrimidinones 11a-c

An equimolar amount of compound **1** (10 mmol) and different substituted 2-thioxo-pyrimidinones **10a–c** (10 mmol) in an ethanol solution having piperidine (0.5 mL) was refluxed for 6 hours. After cooling, the resulting solid was filtered, washed with water, dried, and recrystallized from ethanol/dioxane to yield targets **11a–c**.

2.1.3.8.1. 3-Acetyl-7-oxo-5-phenyl-1-(thiazol-2-yl)-1,7-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine -6-carbonitrile (11a)

Yield 80%, yellow powder; m.p. 282-284˚C; IR (ν_{max}/cm^{-1}): 2223 (CN), 1695, 1674 (2C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.13 (s, 3H, CH_3), 7.01 (d, 1H, CH-thiazole, J= 3.2 Hz), 7.03 (d,1H, CH-thiazole, J= 3.2 Hz), 7.30-7.83 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): 25.6 (CO$\underline{C}H_3$), 100.2 (C_6-triazolopyrimidine), 111.8 (C_5-thiazole), 115.2 (CN), 122.4, 127.1, 129.3, 134.9 (Ar-C), 139.9 (C_4-thiazole), 141.3

(C_3-triazolopyrimidine), 154.2 (C_9-triazolopyrimidine), 158.3 (C_2-thiazole), 162.2 (C_5-triazolopyrimidine), 169.5 (CO), 187.6 ($\underline{C}OCH_3$); MS: m/z = 362 [$M^{\cdot+}$]; Anal. Calcd. for $C_{17}H_{10}N_6O_2S$ (362.37): C, 56.35; H, 2.78; N, 23.19; S, 8.85%; Found: C, 56.57; H, 2.55; N, 23.41; S, 8.63%.

2.1.3.8.2. 3-Acetyl-5-(4-methoxyphenyl)-7-oxo-1-(thiazol-2-yl)-1,7-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (11b)

Yield 83%, yellow powder; m.p. 291-293˚C; IR (ν_{max}/cm^{-1}): 2216 (CN), 1702, 1672 (2C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.74 (d, 1H, CH-thiazole, *J*= 3.6 Hz), 7.09 (d, 2H, Ar-H, *J*= 8.8 Hz), 7.35 (d,1H, CH-thiazole, *J*= 3.6 Hz), 7.81 (d, 2H, Ar-H, *J*= 8.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 20.7 ($CO\underline{C}H_3$), 55.5 ($O\underline{C}H_3$), 104.6 (C_6-triazolopyrimidine), 110.4 (C_5-thiazole), 114.1 (CN), 121.8, 123.4, 128.7, 130.3 (Ar-C), 141.6 (C_4-thiazole), 153.9 (C_3-triazolopyrimidine), 158.3 (C_9-triazolopyrimidine), 161.2 (C_2-thiazole), 161.2 (C_5-triazolopyrimidine), 168.5 (CO), 185.1 ($COCH_3$); MS: m/z = 392 [$M^{\cdot+}$], 309 (100%); Anal. Calcd. for $C_{18}H_{12}N_6O_3S$ (392.39): C, 55.10; H, 3.08; N, 21.42; S, 8.17%; Found: C, 55.31; H, 3.29; N, 21.65; S, 8.39%.

2.1.3.8.3. 3-Acetyl-5-(4-chlorophenyl)-7-oxo-1-(thiazol-2-yl)-1,7-dihydro-[1,2,4]triazolo[4,3-a] pyrimidine-6-carbonitrile (11c)

Yield 83%, deep yellow powder; m.p. 291-293˚C; IR (ν_{max}/cm^{-1}): 2217 (CN), 1696, 1680 (2CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.14 (s, 3H, CH_3), 7.10 (d, 1H, CH-thiazole, *J*= 3.2 Hz), 7.33 (d,1H, CH-thiazole, *J*= 3.2 Hz), 7.92 (d, 2H, Ar-H, *J*= 8.4 Hz), 7.95 (d, 2H, Ar-H, *J*= 8.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 19.8 ($CO\underline{C}H_3$), 100.2 (C_6-triazolopyrimidine), 112.4 (C_5-thiazole), 115.1 (CN), 125.1, 125.5, 127.6, 131.5 (Ar-C), 131.7 (C_4-thiazole), 140.5 (C_3-triazolopyrimidine), 151.5 (C_9-triazolopyrimidine), 158.1 (C_2-thiazole), 160.4 (C_5-triazolopyrimidine), 168.8 (CO), 184.7 ($COCH_3$); MS: m/z = 396 [$M^{\cdot+}$]; Anal. Calcd. for $C_{17}H_9ClN_6O_2S$ (396.81): C, 51.46; H, 2.29; Cl, 8.93; N, 21.18; S, 8.08%; Found: C, 51.68; H, 2.51; Cl, 8.71; N, 21.39; S, 8.30%.

2.2. Biological evaluation

2.2.1. Antiproliferative activity

The cell lines were purchased from the American Type Culture collection as follows: non-small lung cancer (EKVX), breast carcinoma (MCF-7) and colorectal carcinoma (HCT-116) cell lines. Cytotoxic activity screening was performed using MTT assay [57], cells were placed in 10^4 cells/ well for 24 h, and then add fresh medium

which containing different concentration of the tested sample. Serial two-fold dilutions of the tested sample were added using a multichannel pipette. Moreover, all cells were cultivated at 37 °C, 5% CO_2 and 95% humidity. Also, incubation of control cells occurred at 37 °C. However, after incubation for 24 h different concentrations of samples (100, 50, 25 and 12.5 µM) were added and continued the incubation for 48 h, then, add the crystal violet solution 1% to each well for 0.5 h to examine viable cells. Rinse the wells using water until no stain. After that, add 30% glacial acetic acid to all wells with shaking plates on Microplate reader (TECAN, Inc.) to measure the absorbance, using a test wavelength of 490 nm. Besides, compare the treated samples with the control cell. The cytotoxicity was estimated by IC_{50} in (µM), the concentration that inhibits 50% of growth of cancer cell.

2.2.2. *In vitro* enzyme inhibitory assay against ALK2, PIM1, CK2*α*, CHK1, and CDK-2

ALK2 -TK inhibitory assay:

The promising derivative **11c** on the phosphorylation activity of ALK2 protein kinase was tested using the commercially available BPS Bioscience ALK2 luminescence kinase assay kit (BPS Bioscience. Inc. Corporate, 6405 Cornerstone Court W, Ste B San Diego, CA 92121, United States) [11]. At first, 12.5 µl of a master mixture composed of 3 µl 5x kinase buffer, 0.5 µl ATP (500 µM), 0.5 µl Casein (10mg/ml), and 8.5 µl water was dispensed onto each well. 2.5 µl of test compounds (100, 10, 1, 0.1, and 0.01 µM concentrations for the tested compound dissolved in 10% DMSO) were dispensed onto each well labeled as test, 2.5 µl of the same molar concentrations of Loratinib in 10% DMSO were dispensed onto each well labeled as standard; test and standard different concentrations were tested in triplicates. For the positive control and blank wells, 2.5 µL of 10% DMSO was dispensed into each well.

In the enzymatic step, 10 µl ALK2 solution in 1X kinase assay buffer (5 ng/µl) was added to each well designated as test, standard, or positive control; for blank wells, add 10 µl 1X kinase assay buffer. The plates were incubated for 45 min at 30°C.

25 µl kinase-glo reagent was added to each well, the plates were covered using aluminum foil and incubated for 45 min at room temperature. Then, 50 µl kinase detection reagent was added to each well; the plates were covered using aluminum foil and incubated for another 45 min at room temperature. Samples' chemiluminescence values were measured immediately, and the blank reading value was subtracted from

all readings. Spectrophotometry measures absorbance at a wavelength of 450 nm using Tecan –Spark Reader BIOLINE ELISA READER

PIM1-STK inhibitory assay:

The newly synthesized candidate **11c** was investigated for its inhibitory effect on PIM1-STK phosphorylation activity using Promega Corporation's PIM1 luminescence kinase assay kit (Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399 USA) [11].

First, dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer. Then add 1 µl of test compounds (100, 10, 1, 0.1, and 0.01 µM concentrations for the tested compound dissolved in 5% DMSO) was dispensed onto each well labeled as test; test different concentrations were tested in triplicate. Then add 2 µl of PIM enzyme (5 µg/µl) and 2 µl of substrate/ATP mixture to the wells of a 384-volume plate. The plates were incubated for 60 min at room temperature. Afterwards, 5 µl of ADP-Glo reagent was added to each well, the plates were incubated for 40 min at room temperature. Then, 10 µl of kinase detection reagent was added to each well; the plates were incubated for 30 min at room temperature. Samples' luminescence values were recorded (Integration time 0.5-1 second).

CK2α enzyme inhibition assay:

The derivative **11c** was investigated for its inhibitory activity against CK2 kinase inhibitor using the CycLex Corporation CK2 luminescence kinase assay kit (CycLex Corporation, 1063-103 Terasawaoka Ina, Nagano 396-0002 Japan). Silmitaserib was used as a reference standard [11].

At first, transfer the desired number of microtiter wells, provided coated with recombinant p53 N-terminus (1-99 a.a.) as a CK2 substrate, from the foil pouch to a well holder. Then 100 µl of a reaction mixture composed of test compounds or silmitaserib (100, 10, 1, 0.1, and 0.01 µM concentrations) dissolved in 95 µl kinase buffer and 5 µl ATP (2.5 mM) was added onto each well; test and standard different concentrations were tested in triplicate. For the positive control and blank wells, 95 µl kinase buffer and 5 µl ATP (2.5 mM) were dispensed onto each well.

10 µl partially purified CK2 solution (Cat # CY-E1170-1) (20 m units/well) was added to each well designated as test or standard on ice. 10 µl CK2 solution containing 20 m units was added to wells designated as positive control. 10 µl kinase reaction buffer was added to each well to initiate the kinase reaction. The plates were covered with sealer and incubated for 30 min at 30°C.

After that, the wells' contents were flicked out to stop the reaction. Wells were washed five times using wash buffer, and the residual wash buffer was removed by gentle tapping. 100 µl HRP conjugated Detection Antibody TK-4D4 was pipetted to each well, the plates were covered using sealer and incubated for 30 min at room temperature. Wash the wells five times as in the previous step.

N.B. a no enzyme control or blank well should be prepared using the same procedure but 10 µl buffer was added instead of CK2 enzyme fraction.

Then, 100 µl of substrate reagent was added to each well; the plates were incubated for another 10-15 min at room temperature. Thereafter, 100 µl of stop solution was added to each well. Samples' absobances were measured within 30 min using a spectrophotometric plate reader at 450 nm. Spectrophotometry measures absorbance at a wavelength of 450 nm using ROBONIK P2000 ELISA READER

CHK1 enzyme inhibition assay:

The derivative **11c** was investigated for its inhibitory effect on CHK1 phosphorylation activity using BPS Bioscience CHK1 luminescence kinase assay kit (BPS Bioscience. Inc. Corporate, 6405 Cornerstone Court W, Ste B San Diego, CA 92121, United States) [11]. At the beginning of the assay, 12.5 µl of a master mixture composed of 7 µl 1x kinase buffer, 0.5 µl ATP (500 µM) and 5 µl CHk1 substrate (1mg/ml) was dispensed onto each well. Then, 2.5 µl of test compounds (100, 10, 1, 0.1, and 0.01 µM concentrations for the tested compound dissolved in 10% DMSO) were dispensed onto each well labeled as test, 2.5 µl of the same molar concentrations of SCH900776 in 10% DMSO were dispensed onto each well labeled as standard; test and standard different concentrations were tested in triplicates. For the positive control and blank wells, 2.5 µL of 10% DMSO was dispensed into each well.

To initiate the enzymatic reaction, 10 µl of diluted CHK1 kinase solution in 1X kinase assay buffer (5 ng/µl) was added to each well designated as test, standard or positive control; for blank wells, add 10 µl 1X kinase assay buffer. The plates were incubated for 45 min at 30°C.

After that, 25 µl kinase-glo reagent was added to each well, the plates were covered using aluminum foil and incubated for 45 min at room temperature. Then, 50 µl kinase detection reagent was added to each well; the plates were covered using aluminum foil and incubated for another 45 min at room temperature. Samples' luminescence values were measured immediately and blank.

reading value was subtracted from all readings. Spectrophotometry measures absorbance at a wavelength of 450 nm using ROBONIK P2000 ELISA READER

CDK-2 Kinase assay:

The *In vitro* CDK-2 Kinase assay was carried out for the selected compound **11c** using Human Cyclin-dependent Kinase 2, CDK-2 ELISA Kit. This kit is an Enzyme-Linked Immunosorbent Assay (ELISA) [11]. The plate has been pre-coated with Human CDK-2 antibody. CDK-2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human CDK-2 Antibody is added and binds to CDK-2 in the sample. Then Streptavidin HRP is added and binds to the Biotinylated CDK-2 antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added, and color develops in proportion to the amount of Human CDK-2. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm. All reagents should be brought to room temperature before use. Standard: Reconstitute the 120ul of the standard (24ng/ml) with 120ul of standard diluent to generate a 12ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (12ng/ml) 1:2 with standard diluent to produce 6ng/ml, 3ng/ml, 1.5ng/ml and 0.75ng/ml solutions. Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows (12ng/ml, 6ng/ml, 3ng/ml, 1.5ng/ml, 0.75ng/ml. Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. The procedure runs as follow: Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C. Add 50ul standard to standard well. Add 40ul sample to sample wells and then add 10ul Human CDK-2 antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells. Mix well, cover the plate with a sealer. Incubate 60 minutes at 37°C. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50ul substrate solution A to each well and then add

50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50ul Stop Solution to each well, the blue color will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.2.3. Western blot analysis.

Western blot analysis was carried out to test the effect of compound **11c** on the expression level of the most sensitive kinases: PIM1, CDK-2 and CHK1 in a cellular environment using FLAG® Western Detection Kit (Agilent Technologies, Stratagene Products Division, 11011 North Torrey Pines Road, La Jolla, CA 92037) [58].

The experiment was terminated by lysing the cells in cold lysis buffer, 10mM Tris, 100mM NaCl, 25mM ethylenediaminetetra aceticacid (EDTA), 25mM Ethylene glycol bis(2-aminoethyl) tetraacetic acid (EGTA),0.1 Sodium dodecyl sulfate (SDS), % 1% (v/v) Triton X-100, 2% (v/v) NP-40 (pH 7.4), with 1:200 protease inhibitor cocktail (Sigma) and 1:300 phosphatase inhibitor cocktail Tablet (Roche). The cells were then immediately frozen at −20 °C for 1 h for further lysis, and collected by cell scraper and sonicated 2×10s, followed by centrifugation at 4000 rpm for 10 min under cooling. Total protein concentrations were determined colorimetrically in the supernatant using Bradford method before proceeding to the western blotting.

Western blotting: Equal amounts (20 μg) of protein samples were mixed and boiled with SDS Loading buffer for 10 min, allowed to cool on ice and then loaded into SDS-polyacrylamide gel and separated by Cleaver electrophoresis unit (Cleaver, UK), transferred onto polyvinylidene fluoride (PVDF) membranes (BioRad) for 30 min using a Semi-dry Electroblotter (Biorad, USA) at 2.5 A and 25 V for 30 min. The membrane was blocked with 5% nonfat dry milk in TBS-T for two hours at RT, in order to reduce non-specific protein interactions between the membrane and the antibody. The membrane was incubated overnight at 4°C with primary antibodies (Cell Signaling Technology) and β-actin (Sigma). The blots were then washed for three times (10 min each) with TBS-T. The membrane was then incubated with the corresponding horse radish peroxidase (HRP)- linked secondary antibodies (Dako) for another hour at room temperature, followed by washing for three times (10 min each) with TBS-T. The chemiluminescent Western ECL substrate (Perkin Elmer, Waltham, MA) was applied to the blot according to the manufacturer's recommendation. Briefly, the membranes were incubated for 1 min with a mixture of equal volumes from ECL solution A and

ECL solution B. The chemiluminescent signals were captured using a CCD camera-based imager (Chemi Doc imager, Biorad, USA), and the bands intensities were then measured by ImageLab (Biorad) Protein-sized markers were used in all gels to localize the gel transfer regions for specific proteins and determine the transfer efficiency.

2.2.4. Cell cycle arrest and apoptosis of compound 11c

Cell cycle analysis and apoptosis study were carried out using flow cytometry. MCF-7 cells were seeded at 8×10^4 and incubated at 37°C in 5% CO_2 overnight. After treatment with the tested compound **11c** for 24 h, cell pellets were collected and centrifuged (300 g, 5 min). For cell cycle analysis cell pellets were fixed with 70% ethanol on ice for 15 min and collected again. The pellets were incubated with propidium iodide (PI) staining solution at room temperature for 1 h and analyzed by a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). Apoptosis detection was carried out by FITC AnnexinV/PI commercial kit (Becton Dickenson, Franklin Lakes, NJ, USA) following the manufacturer protocol. The samples were analyzed by fluorescence-activated cell sorting (FACS) with a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) within 1 h after staining. Data were analyzed using Kaluza v 1.2 (Beckman Coulter) [64, 65].

2.2.5. Wound healing assay

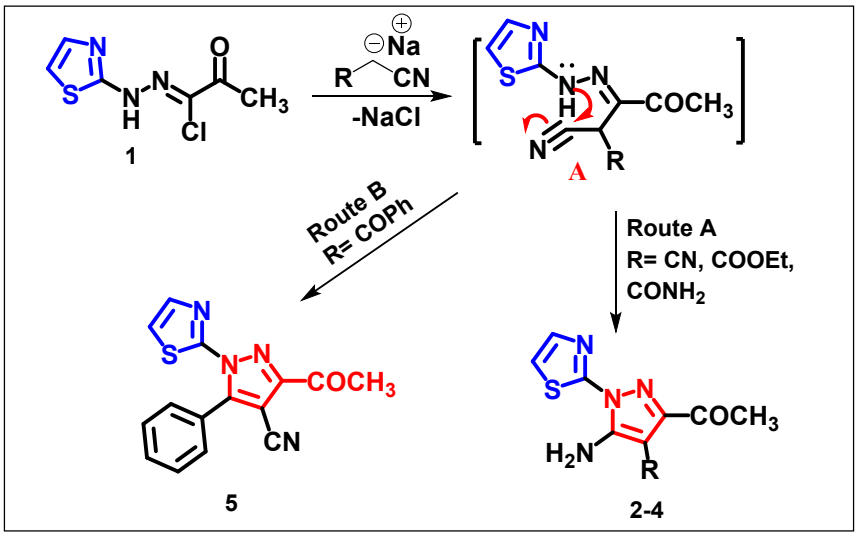
The anti-migratory effect of the compound was assessed using MCF-7 cells. After seeding 8×10^5 cells per well in a six-well plate and allowing them to adhere, a straight wound was created in the confluent cell monolayer using a 200 µL pipette tip. The wells were gently rinsed three times with sterile PBS to eliminate non-adherent cells, followed by the addition of 2 mL fresh medium. The initial wound width (0 h) was documented using an inverted microscope. The medium was then replaced with fresh medium containing varying concentrations of compound **11c**, and the cells were cultured for 24 h. After incubation for 72 h, the scratch area was re-examined under the microscope to evaluate migratory changes [66].

2.3. Molecular docking study

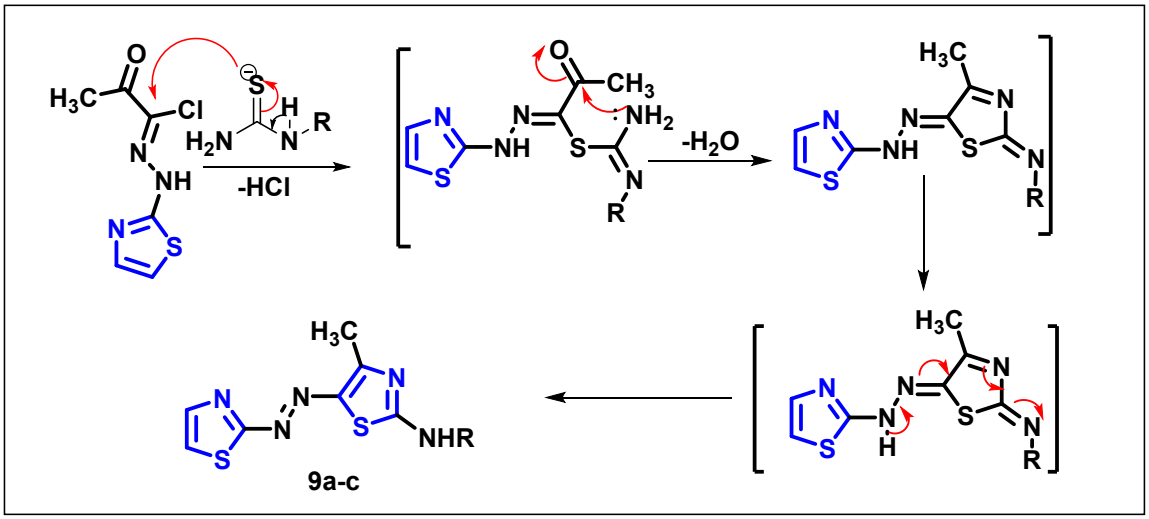
The 2D structure of triazolo[4,3-*a*]pyrimidine **11c** was drawn through Chem. Draw. The protonated 3D was employed using standard bond lengths and angles, using Molecular Operating Environment (MOE-Dock) software version 2024.0601 (Chemical Computing Group, Montreal, Canada) [67-70]). Then, the geometry optimization and energy minimization were applied to get the Conf Search module in

MOE, followed by saving of the moe file for upcoming docking process. The co-crystallized structures of PIM1, CHK1, and CDK-2 with their ligands (1R,2R)-N-[3-(naphthalen-2-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl]cyclohexane-1,2-diamine **26L**, 5-[(1R,3S)-3-aminocyclohexyl]-6-bromo-3-(1-methyl-1H-pyrazol-4-yl)pyrazolo[1,5-*a*]pyrimidin-7-amine **22K** and roscovitine were downloaded (PDB codes: 4MBL, 3OT3 and 3DDQ, respectively) from protein data bank. All minimizations were performed using MOE until an RMSD gradient of 0.05 kcal·mol^{-1}Å^{-1} with MMFF94x force field and the partial charges were automatically calculated. Preparation of the enzymes' structures were done for molecular docking using Protonate 3D protocol with the default options in MOE. London dG scoring function and Triangle Matcher placement method were used in the docking protocol. At first, validation of the docking processes were established by docking of the native ligands, followed by docking of the derivative **11c** within the ATP-binding sites after elimination of the co-crystallized ligands.

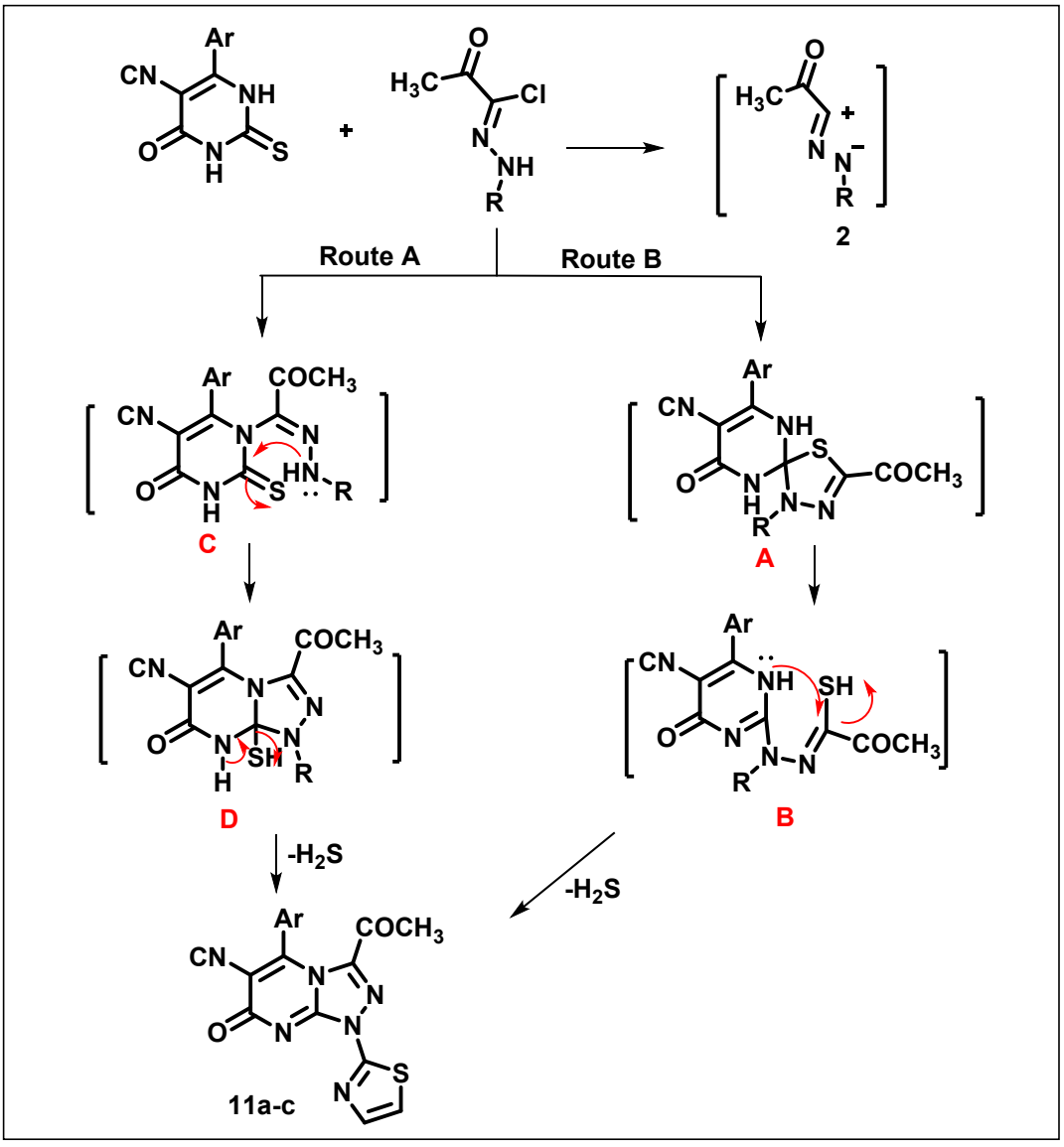
Schemes



Scheme S1. Proposed mechanistic pathway for preparing compounds **2-5**



Scheme S2. Proposed mechanistic pathway for preparing compounds **9a-c**



Scheme S3. Proposed mechanistic pathway for preparing compounds **11a-c**

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Tables

Table S1. The antitumor activities of the tested compounds expressed as IC_{50} values and compared with reference standard drugs evaluated on cancerous EKVX, MCF-7, and HCT-116 and normal FHC and MCF10A cell lines.

Compd. NO.	IC_{50} (μM) (mean±SEM)				
	EKVX	**MCF-7**	**HCT-116**	**FHC**	**MCF10A**
1	50.29±0.90	42.86±0.26	43.42±0.80	–	–
2	49.35±0.50	49.41±3.10	52.36±0.20	–	–
3	41.98±0.70	53.60±0.70	49.53±0.18	–	–
4	41.72±0.80	45.20±0.50	47.0±2.70	–	–
5	34.96±0.41	33.86±0.40	39.47±0.50	–	–
6	36.21±0.40	39.37±0.60	37.09±0.24	–	–
7	29.67±1.0	34.97±0.80	32.51±0.60	–	–
9a	23.47±0.09	26.98±0.70	24.12±0.30	–	–
9b	21.94±0.62	25.66±0.10	25.92±0.22	–	–
9c	21.01±0.30	28.85±0.28	30.96±0.50	–	–
11a	10.03±0.30	10.84±0.15	13.03±0.20	–	–
11b	13.20±0.29	10.37±0.20	12.73±0.30	–	–
11c	6.22±0.15	5.30±0.18	6.42±0.30	65.90±0.80	71.55±1.45
Doxorubicin	5.02 ± 0.01	6.12±0.10	6.63±0.25	33.78±0.15	45.80±1.22

Table S2. The percentage cytotoxicity of the active compounds on EKVX human tumor cell line at different concentrations

Compounds	100 µM	50 µM	25 µM	12.5 µM
1	76.65	54.22	31.95	21.83
2	74.38	49.55	23.93	18.57
3	84.33	68.7	48.98	38.72
4	82.8	65.05	30.2	24.27
5	80.66	62.57	38.82	22.51
6	87.23	65.49	40.86	19.51
7	90.68	68.86	39.86	13.51
9a	92.25	65.47	42.12	14.01
9b	93.17	72.87	46.04	17.9
9c	94.33	70.11	41.16	10.91
11a	96.81	75.03	46.08	8.9
11b	95.42	70.44	45.17	16.11
11c	97.31	79.44	52.17	5.41
Doxorubicin	98.88	75.44	55.17	5.01

Table S3. The percentage cytotoxicity of the active compounds on MCF-7 human tumor cell line at different concentrations

Compounds	100 µM	50 µM	25 µM	12.5 µM
1	84.87	64.05	37.31	25.52
2	74.38	49.55	23.93	18.57
3	84.33	70.33	56.98	48.72
4	82.80	65.05	30.2	29.27
5	89..77	73.18	50.81	33.12
6	86.01	72.85	46.5	39.01
7	87.12	68.45	43.98	25.01
9a	93.08	81.72	54.33	30.01
9b	94.76	75.08	43.37	12.28
9c	91.32	72.7	47.4	24.1
11a	95.7	74.36	47.01	10.87
11b	96.48	73.38	49.09	20.22
11c	98.82	78.38	55.09	5.22
Doxorubicin	98.01	78.38	52.09	1.22

Table S4. The percentage cytotoxicity of the active compounds on HCT-116 human tumor cell line at different concentrations

Compounds	100 µM	50 µM	25 µM	12.5 µM
1	79.46	65.25	39.02	36.08
2	72.17	55.08	39.25	33.83
3	75.27	51.14	26.3	22.13
4	82.62	61.14	35.2	30.36
5	86.46	61.14	36.2	11..36
6	89.12	62.91	36.6	10.63
7	90.17	75.91.	42.6	23.1
9a	92.46	80.65	58.32	35.21
9b	91.51	67.81	38.65	9.80
9c	91.01	71.26	35.82	8.81

11a	94.42	72.85	43.7	9.2
11b	94.72	73.85	43.7	6.2
11c	96.2	75.85	49.87	4.2
Doxorubicin	95.5	75.85	49.87	4.2

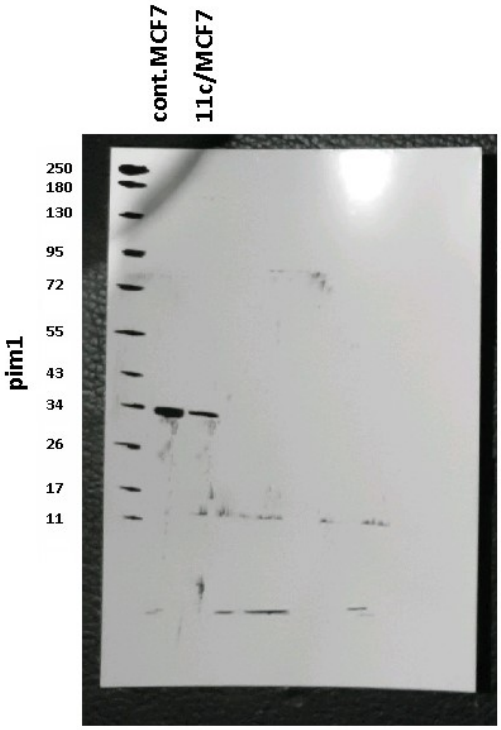
Table S5. The percentage inhibitory potency of the active triazolo[4,3-*a*]pyrimidine **11b** against ALK2, PIM1, CK2α, CHK1, and CDK-2 in comparison with lorlatinib, staurosporine, silmitasertib, SCH900776, and rescovitine, respectively at different concentrations

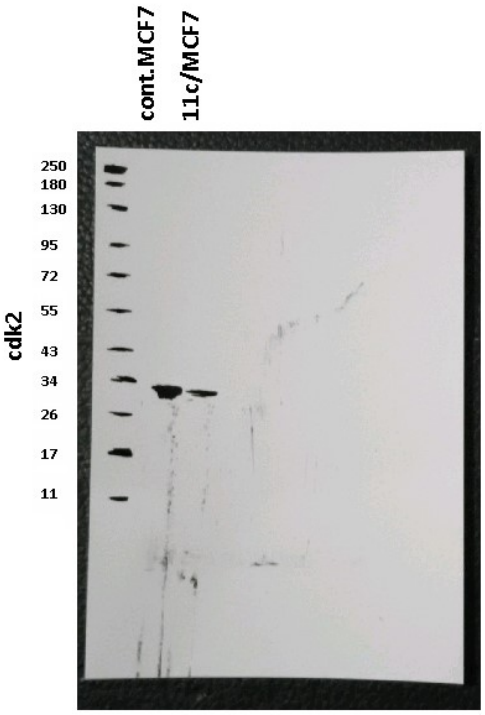
Compd./enzyme	**100 µM**	**10 µM**	**1 µM**	**0.1 µM**	**0.01 µM**
11c/ALK2	80.59	62.90	40.28	24.19	11.82
Lorlatinib/ALK2	97.44	88.36	71.55	48.25	29.45
11c/PIM1	92.15	83.55	62.11	40.6	23.9
Staurosporine/PIM1	96.25	87.40	73.21	42.10	25.80
11c/CK2α	77.23	53.12	35.14	9.24	5.22
Silmitasertib/CK2α	96.25	86.45	75.34	54.17	35
11c/CHK1	96.34	85.71	78.22	50.65	21.41
SCH900776 /CHK1	94.80	80.15	73.66	45.62	23.41
11c/CDK-2	95.25	88.23	69.17	52.13	41.72
Roscovitine/CDK-2	96.8	89.10	70.55	58.13	44.2

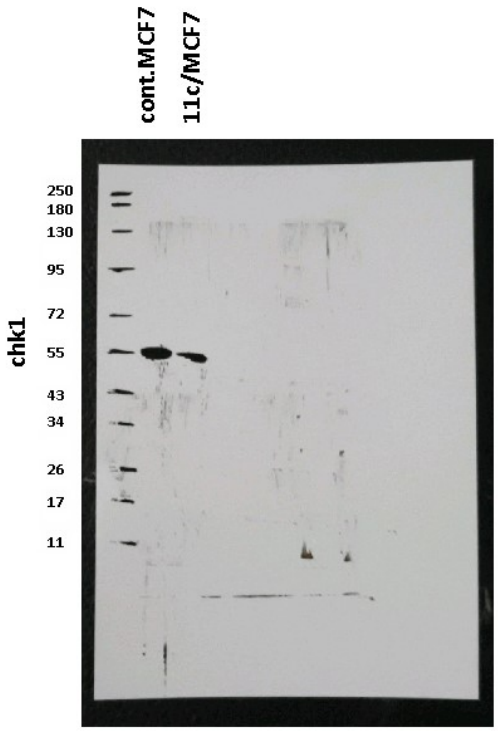
Western blotting results

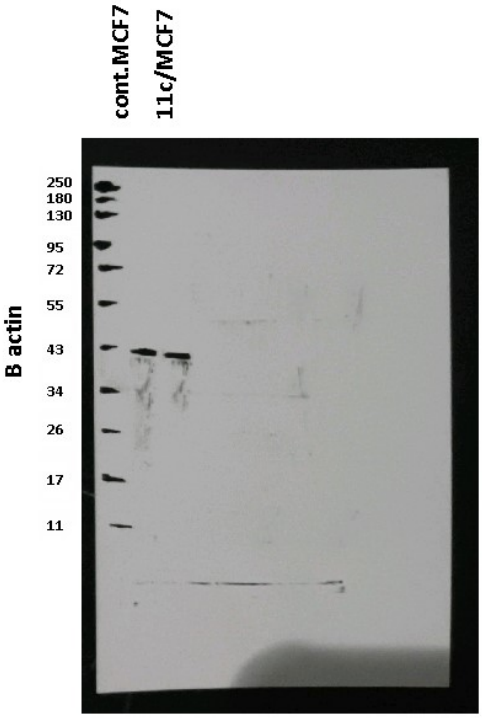
Compound			western blotting	β-actin
code	**cells**	**conc**	OD	

			MCF7			
			PIM1	CDK2	CHK1	
11c/MCF7	MCF7	---	2.461	2.661	2.912	√
cont.MCF7	---	---	6.093	6.219	5.714	√









Detailed Results:

PIM1	34 kda			
sample band	area	%	Rel.Density	adj. Density
Standared	1848	18.48	1	1
cont.MCF7	7038	70.38	3.808441558	6.093506494
11c/MCF7	2843	28.43	1.538419913	2.461471861

[redacted]

CDK2	34 kda			
sample band	area	%	Rel.Density	adj. Density
Standared	1917	19.17	1	1
cont.MCF7	7452	74.52	3.88732394	6.21971831
11c/MCF7	3188	31.88	1.66301513	2.6608242

[redacted]

CHK1	56 kda			
sample band	area	%	Rel.Density	adj. Density
Standared	2219	22.19	1	1
cont.MCF7	7925	79.25	3.571428571	5.714285714
11c/MCF7	4039	40.39	1.820189274	2.912302839

[redacted]

Table S6. Cell cycle analysis after 48 h incubation with compound **11c** compared with untreated MCF-7 cells.

Compound No.	%G0-G1	%S	%G2/M
11c /MCF-7	63.44	35.28	1.28
Cont./MCF-7	55.07	31.71	13.22

Table S7. Apoptosis induction analysis within MCF-7 cells treated with the active triazolo[4,3-*a*]pyrimidine **11c** compared with untreated MCF-7 cells.

	Apoptosis			Necrosis
	Total	Early	Late	
11c/MCF-7	33.21	6.49	19.57	7.15
Cont. /MCF-7	2.94	0.37	0.25	2.32

Wound healing results

Detailed results

**	% Closure	Total area	Migrated cells area	T	Length	L.of migration		L1	L2	L3
				72h	mm	ΔL				
11c/MCF7	67.407	0.81	0.546		0.9	0.3		0.29	0.31	0.31
cont.MCF7	94.815	0.81	0.768		0.9	0.43		0.43	0.42	0.43

Table S8. Anticipated ADMET profile of the active triazolo[4,3-*a*]pyrimidine **11c** using admetSAR 1.0.

Properties (Probability)	**11c**
Absorption	
BBB	
HIA	+ (1.0000)
P-glycoprotein Substrate	Non-substrate (0.7776)
P-glycoprotein Inhibitor	Non-inhibitor (0.5768)
Distribution	
Subcellular localization	Mitochondria (0.6087)
Metabolism	
CYP450 2C9 Substrate	Non-substrate (0.7617)
CYP450 2D6 Substrate	Non-substrate (0.8100)
CYP450 3A4 Substrate	Substrate (0.6140)
CYP450 1A2 Inhibitor	Non-inhibitor (0.7490)
CYP450 2C9 Inhibitor	Non-inhibitor (0.5089)
CYP450 2D6 Inhibitor	Non-inhibitor (0.9500)
CYP450 2C19 Inhibitor	Non-inhibitor (0.6907)
CYP450 3A4 Inhibitor	Non-inhibitor (0.9514)
Excretion & Toxicity	
hERG Inhibition T_hERG_I	Weak inhibitor (0.8797)
T_hERG_II	Non-inhibitor (0.6921)
AMES Toxicity	Non-AMES toxic (0.5000)
Carcinogens	Non-carcinogens (0.8143)
Acute Oral Toxicity (AO)	III (0.7282)
Carcinogenicity (Three-class)	non-required (0.5321)
Biodegradation	Not ready biodegradable (0.9941)

Figures

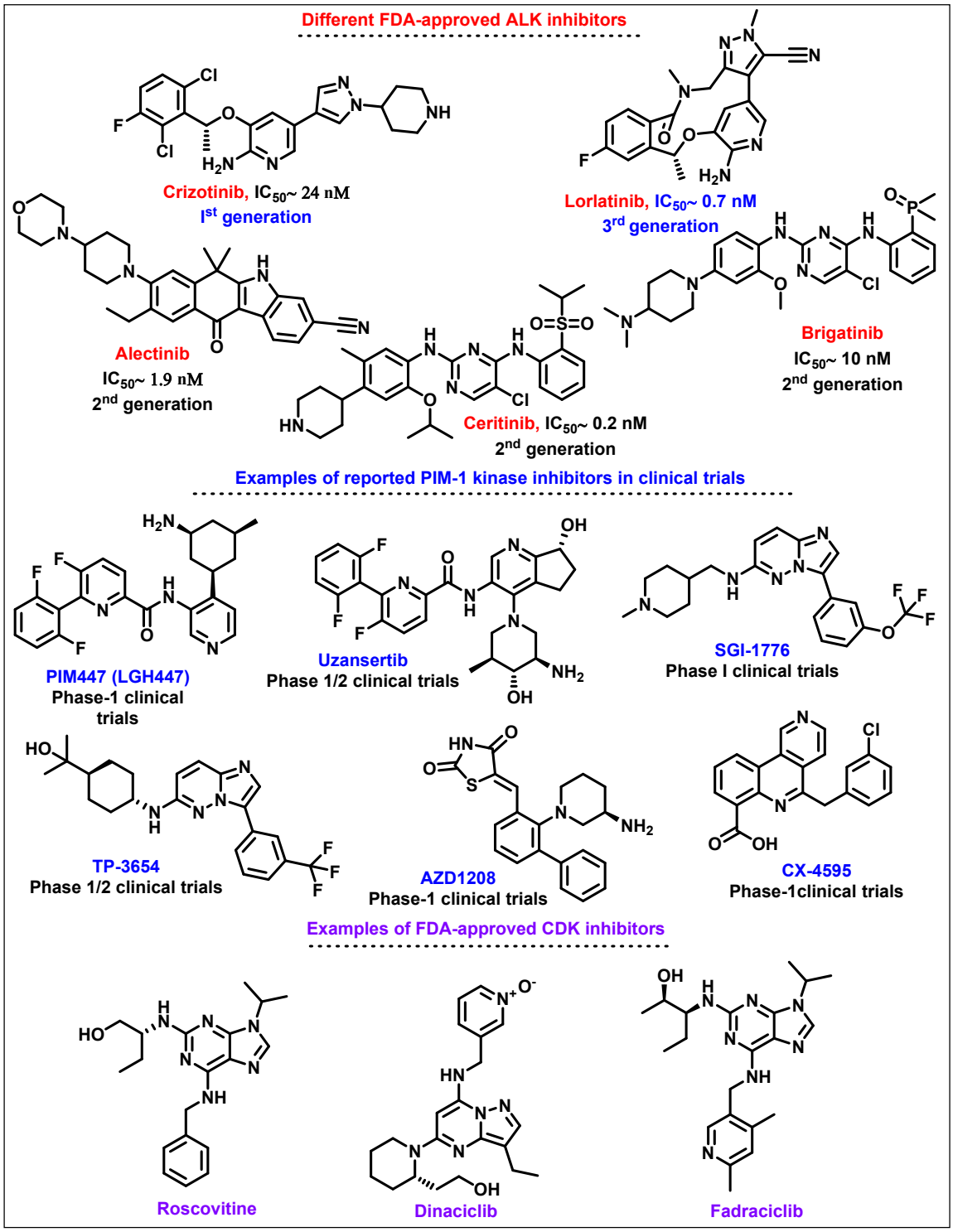


Fig. S1. Various antiproliferative drugs with ALK, PIM-1, and CDK-2 suppression activities

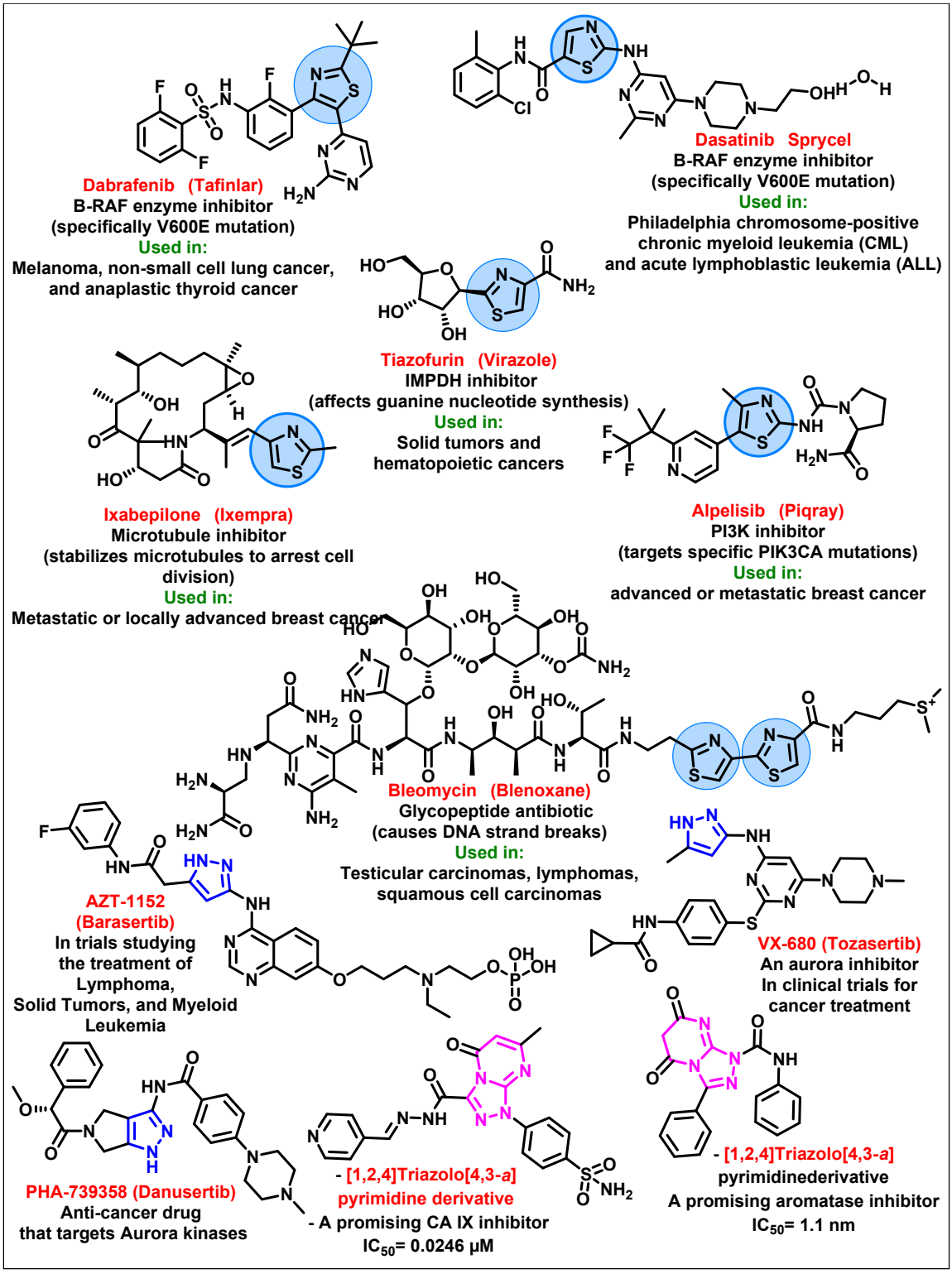


Fig. S2. Different examples of anticancer congeners based on the thiazole, pyrazole and [1,2,4]triazolo[4,3-*a*]pyrimidine ring systems

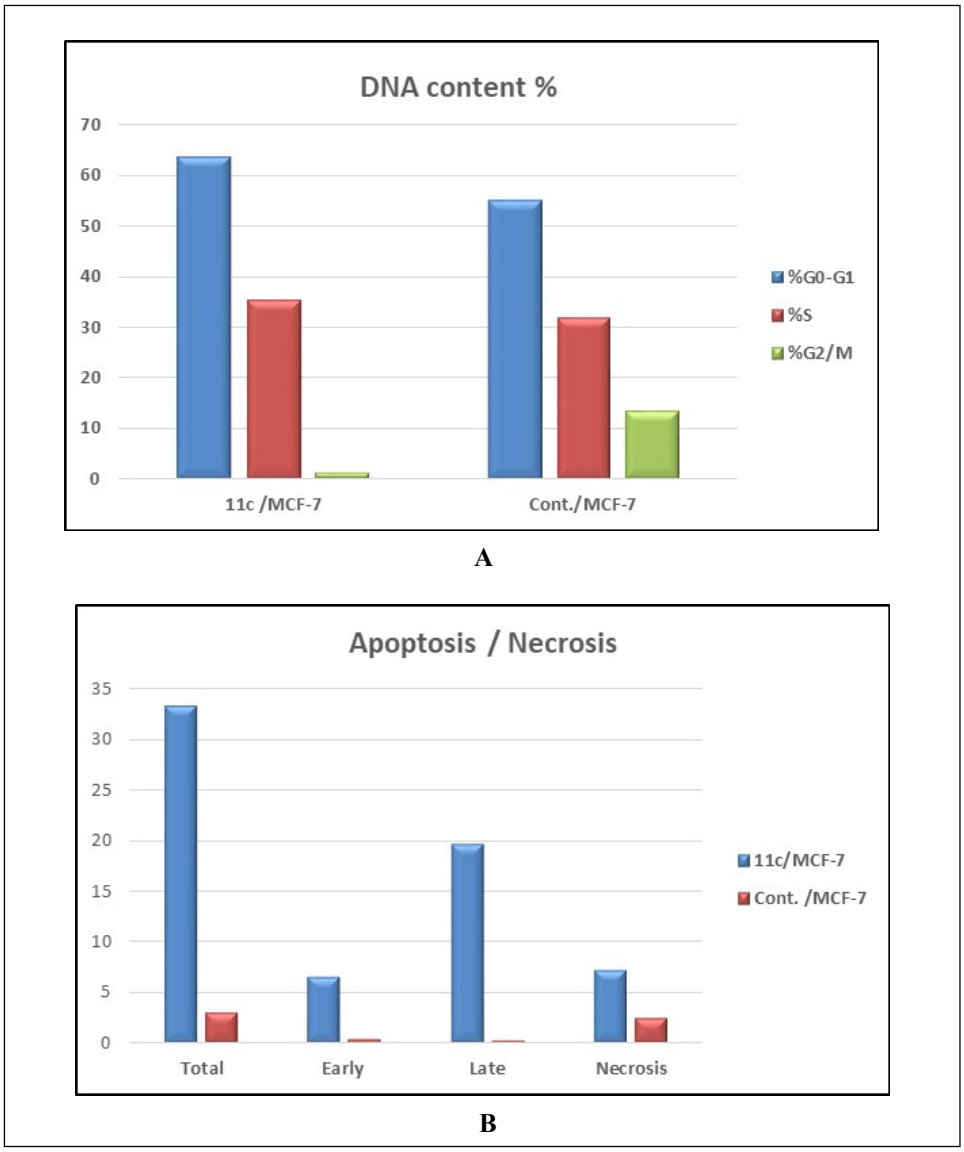
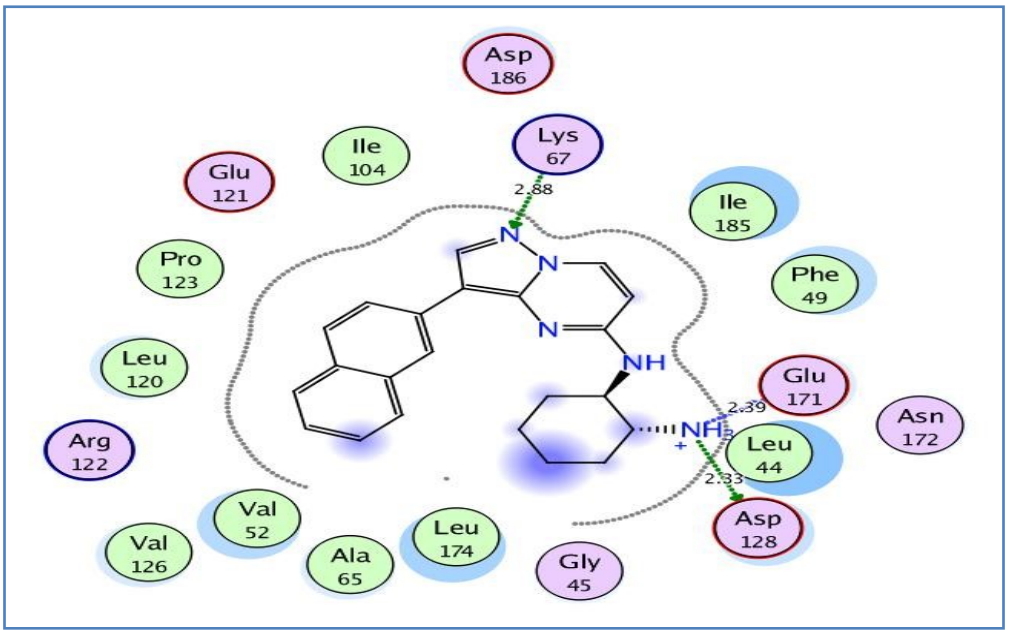


Fig. S3. A) Cell cycle analysis data, B) Apoptosis analysis for compound **11c** in MCF-7 following a 24-hour incubation period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Lys
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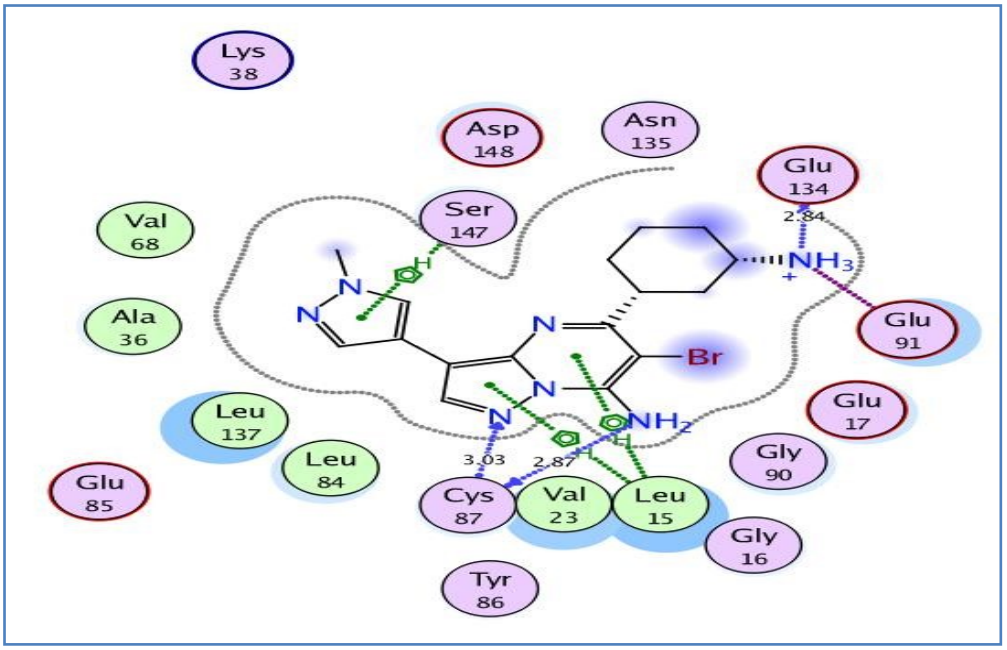
Ile
104
Glu
121
2.88
Ile
185
Pro
123
Phe
49
Leu
120
Glu
171
Asn
172
2.39
Arg
122
Leu
44
2.33
Asp
128
Val
52
Leu
174
Val
126
Ala
65
Gly
45

A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2.84
Ser
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Val
68
Glu
91
Ala
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Br
Leu
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Glu
17
Leu
84
3.03
2.87
Gly
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Glu
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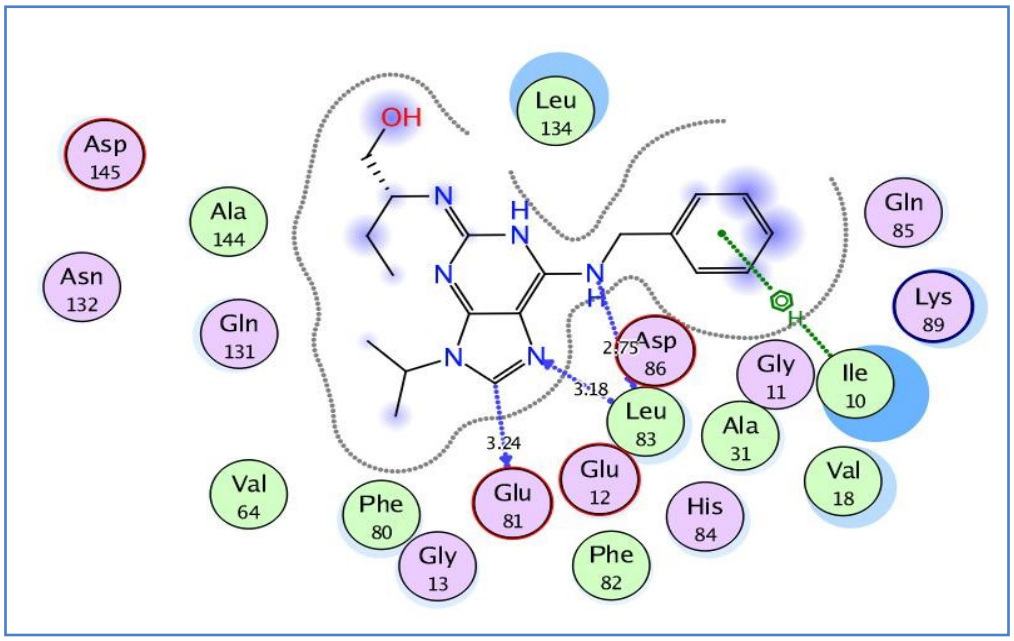
Cys
87
Val
23
Leu
15
Gly
16
Tyr
86

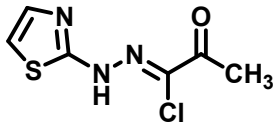
B



C

Fig. S4. A–C patterns illustrating the 2D binding interactions of the original ligands 26L, 22K, and roscovitine into active pockets of PIM1, CHK1, and CDK-2 (PDB codes: 4MBL, 3OT3, and 3DDQ, respectively)

Copies of IR, ^{1}H NMR and ^{13}C NMR spectra of the new compounds:



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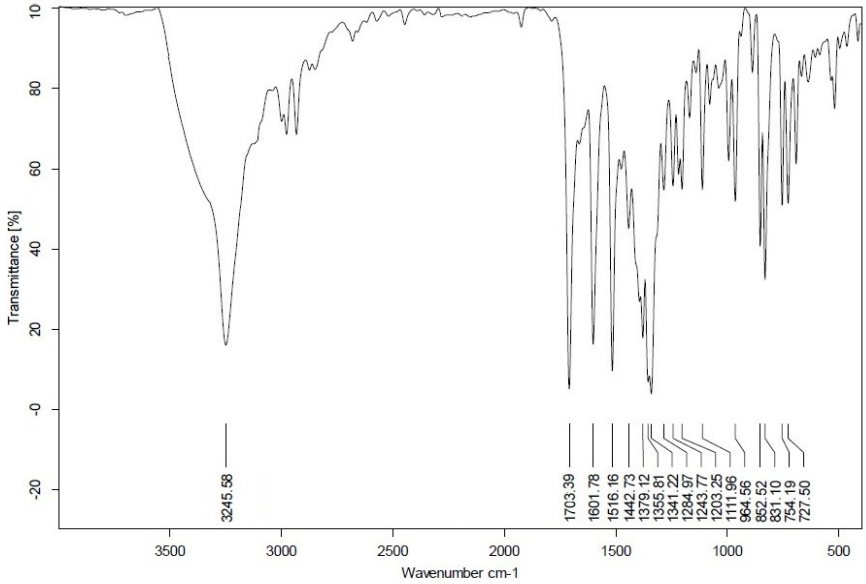


Fig. S5. IR spectrum of compound 1

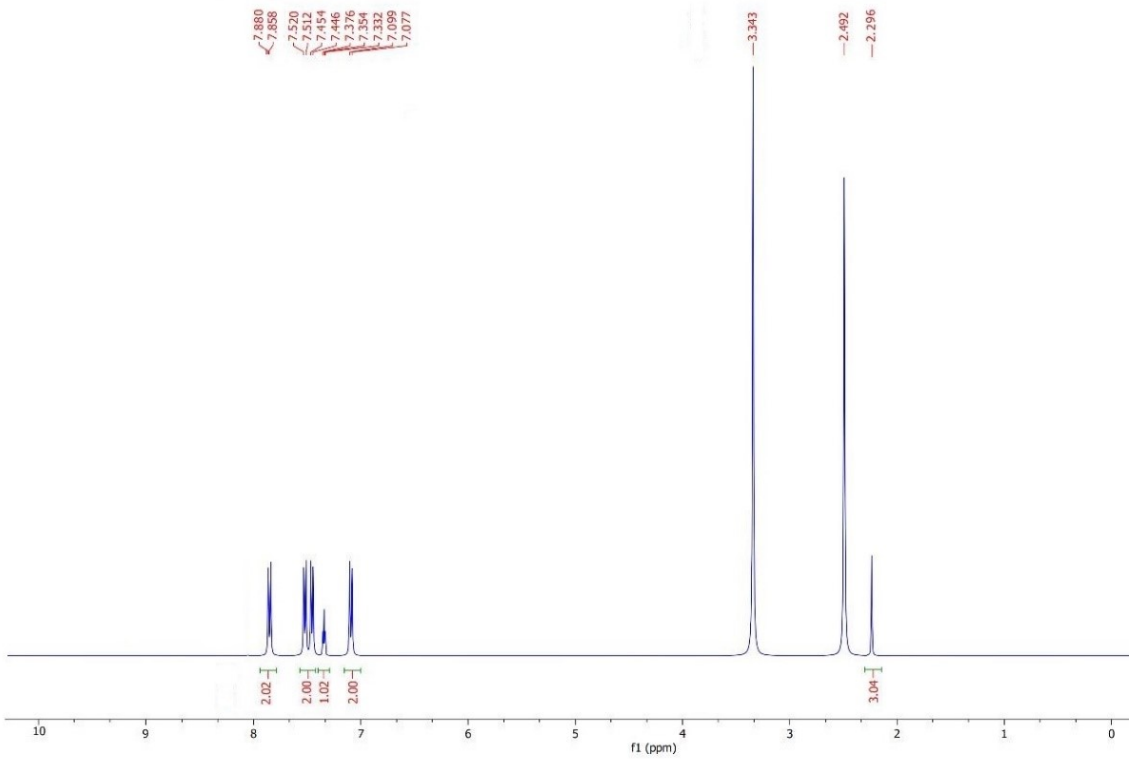


Fig. S6. ^{13}H-NMR of compound 1

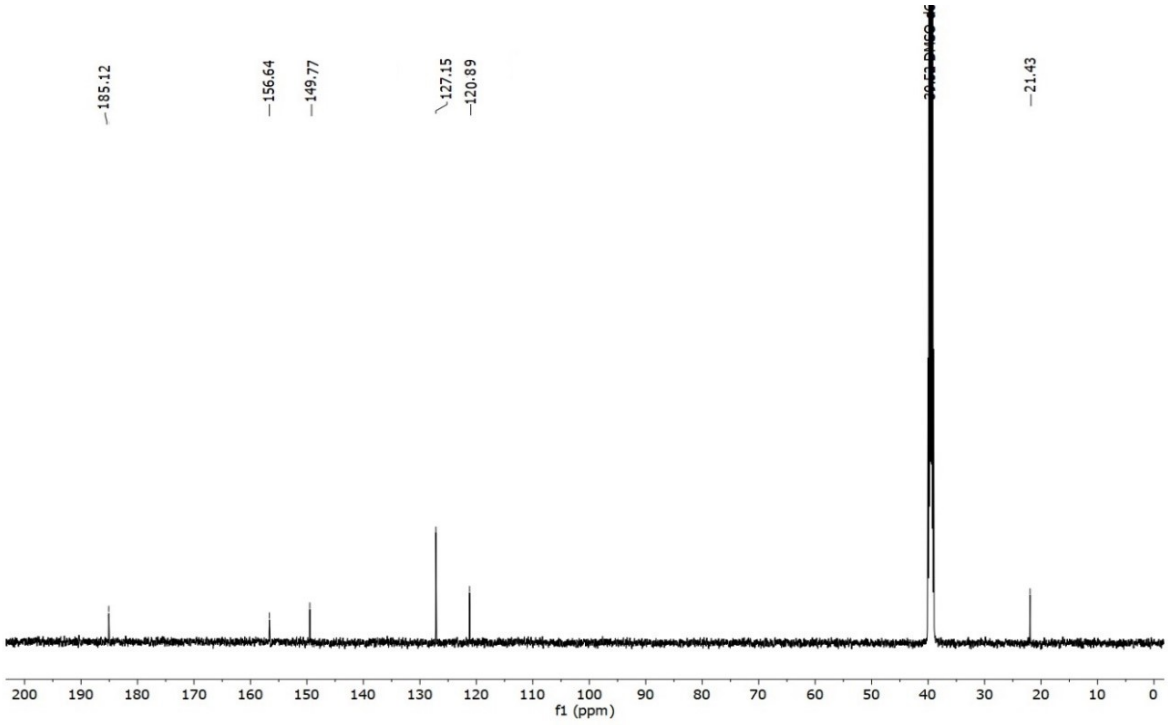
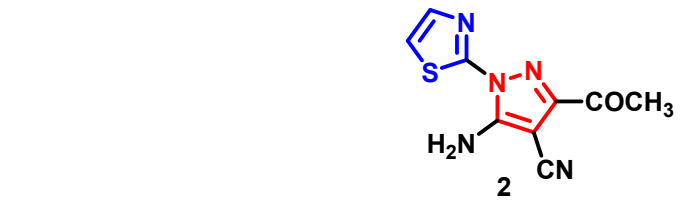


Fig. S7. ^{13}C-NMR of compound 1



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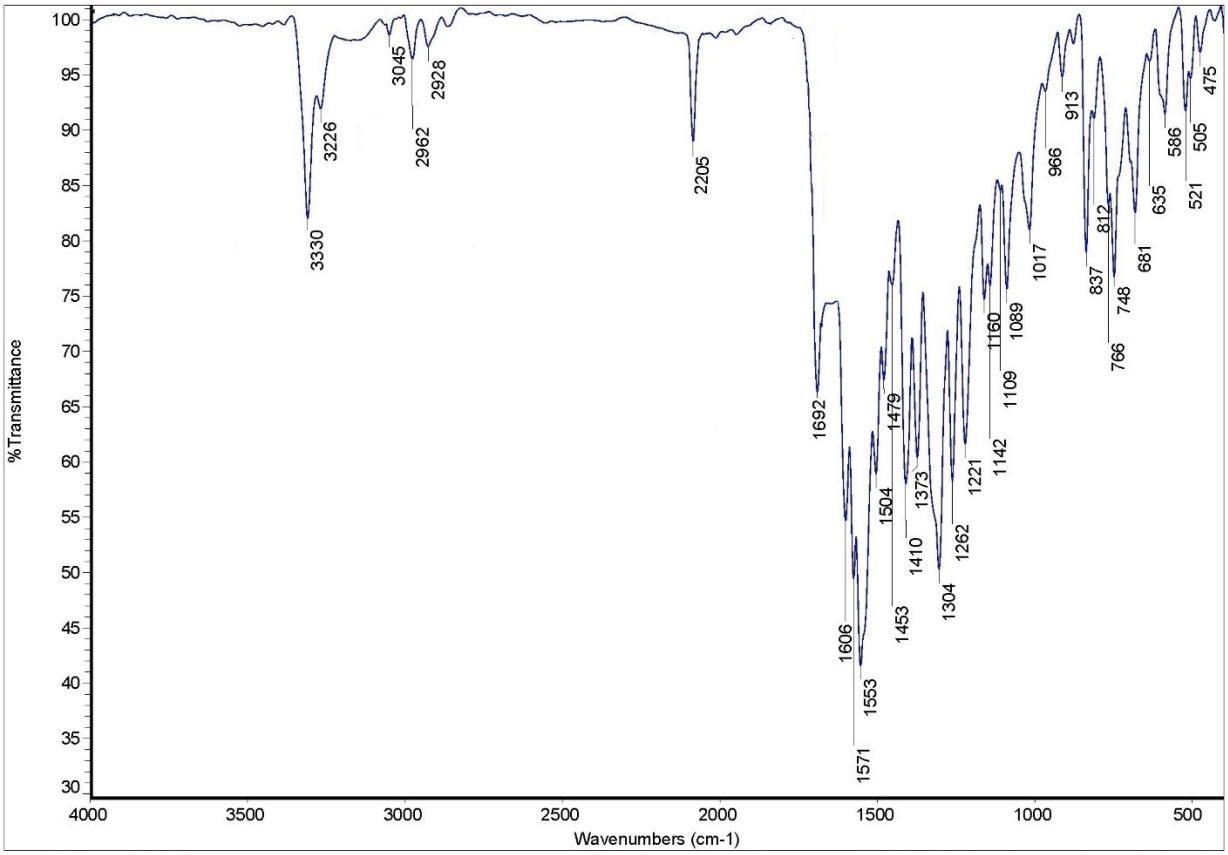


Fig. S8. IR spectrum of compound 2

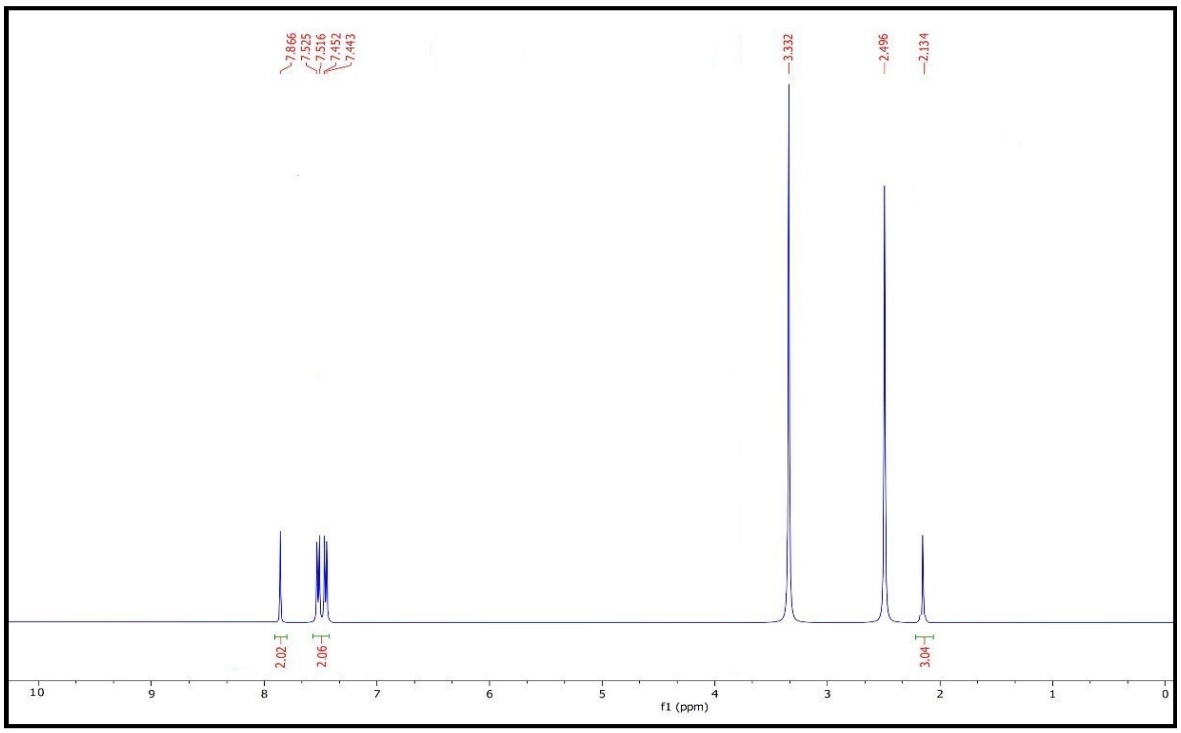


Fig. S9. ^{1}H-NMR of compound 2

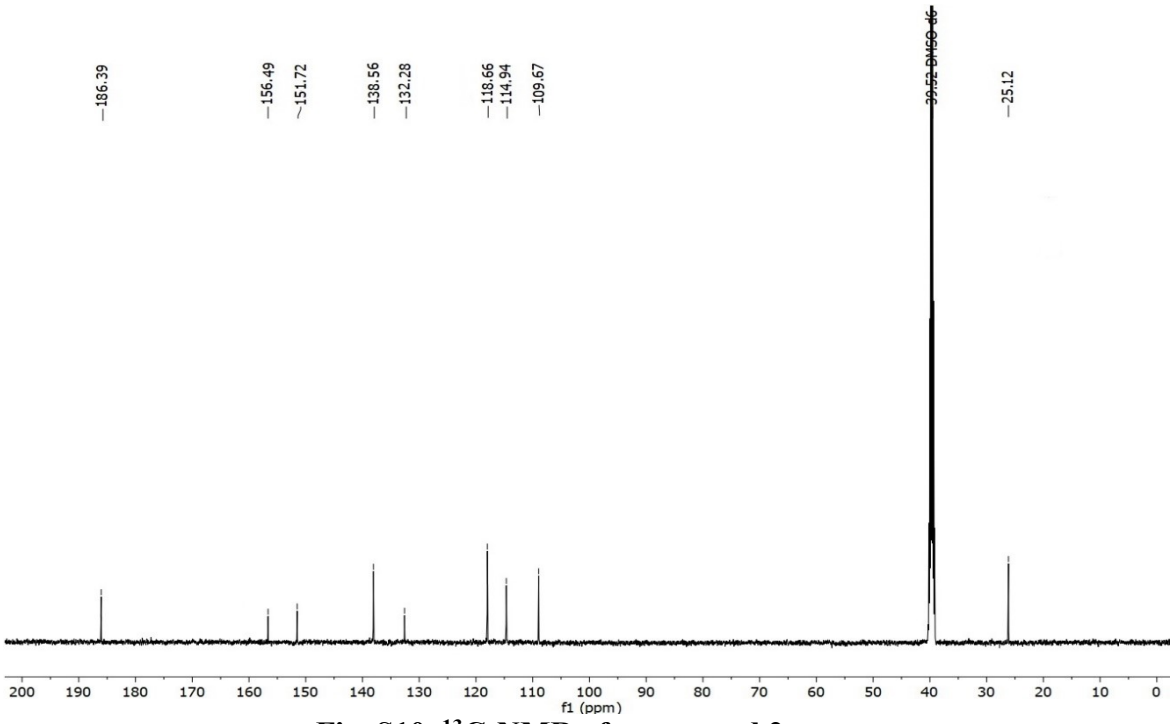


Fig. S10. ^{13}C-NMR of compound 2

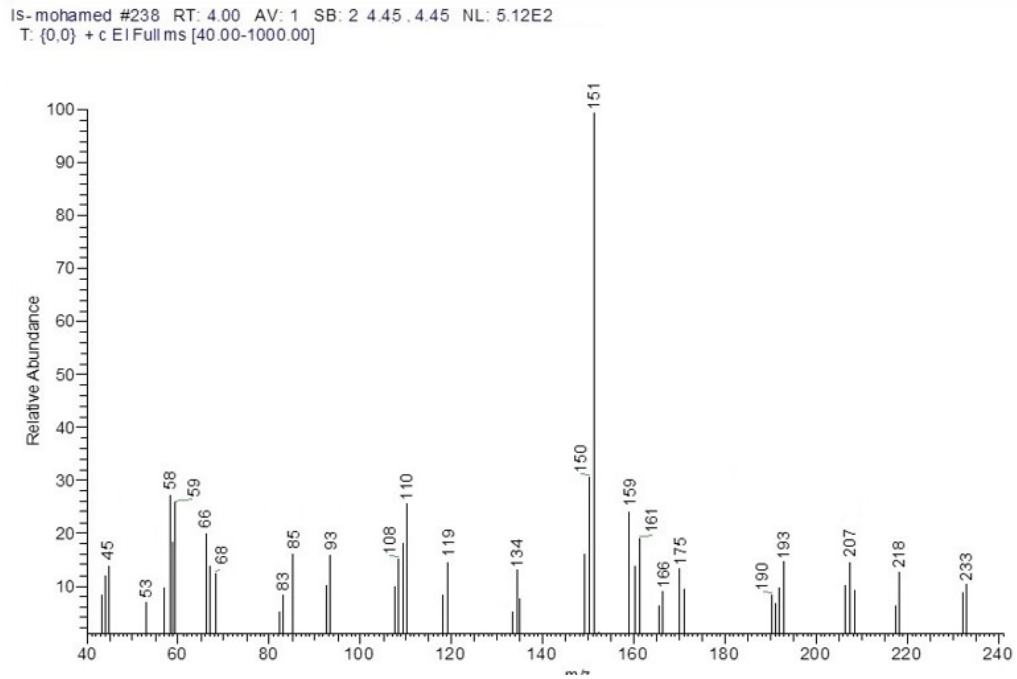
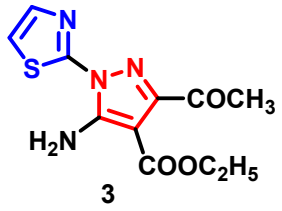


Fig. S11. Mass spectrum of compound 2



3

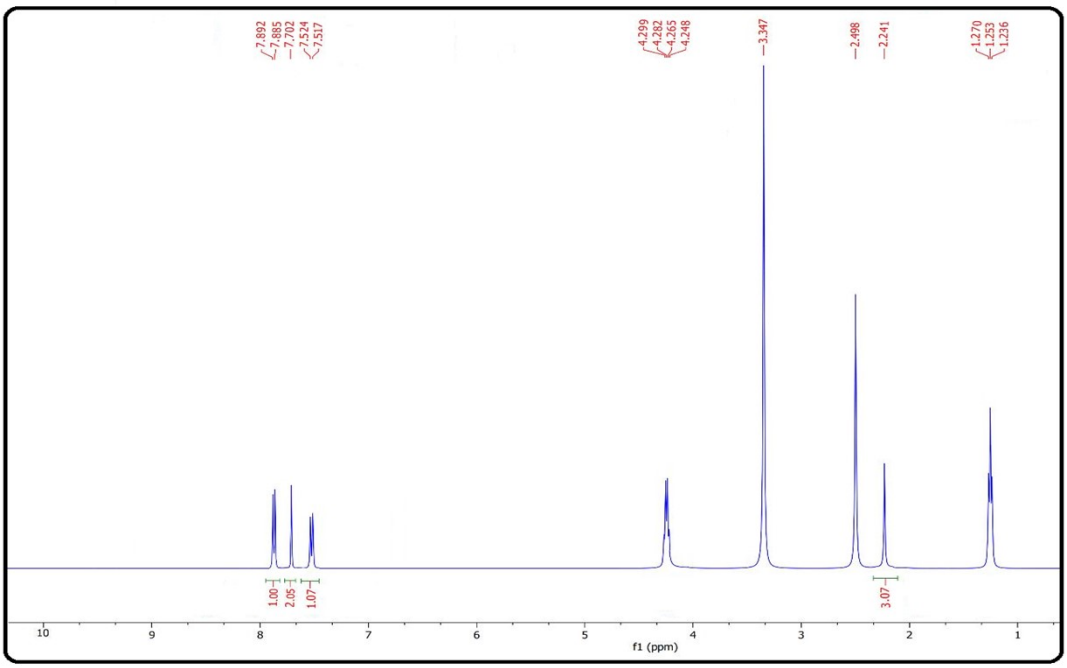


Fig. S12. ^{1}H NMR of compound 3

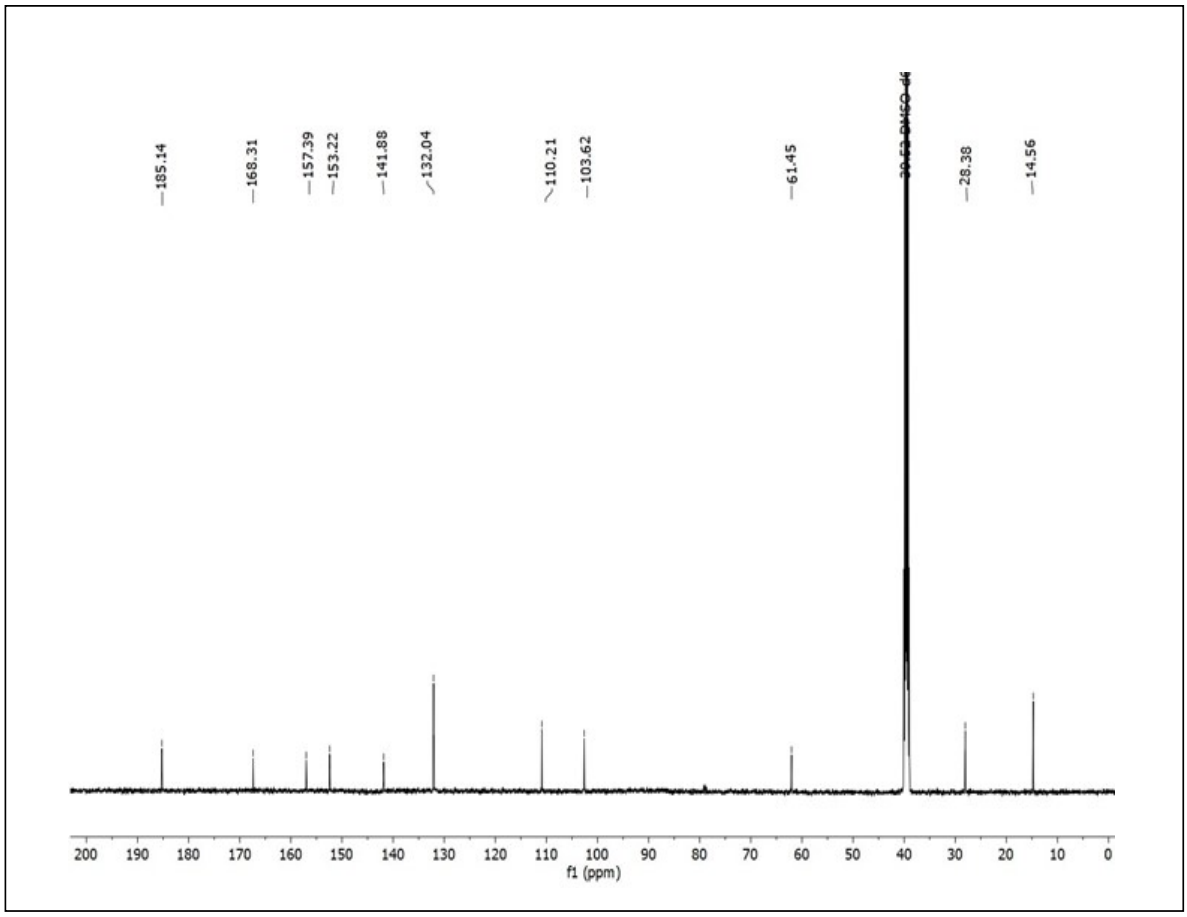
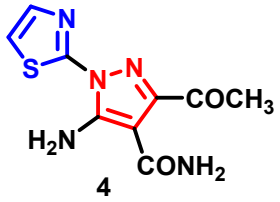


Fig. S13. ^{13}C-NMR of compound 3



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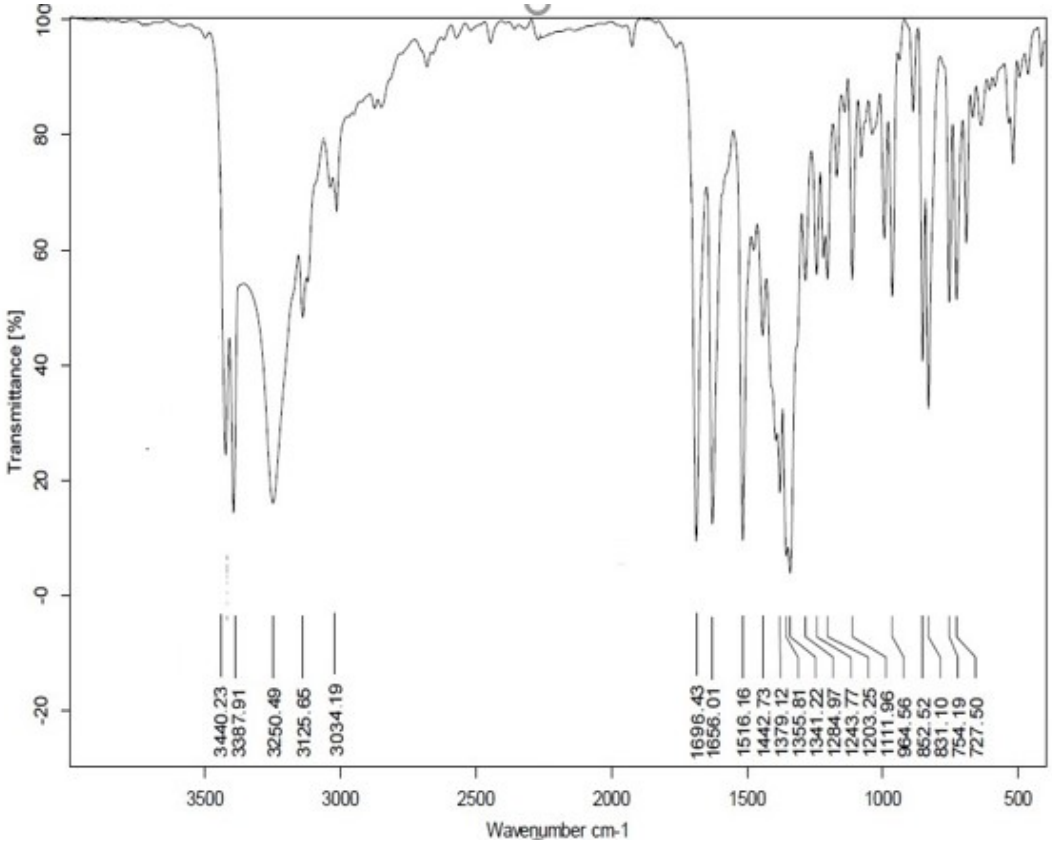


Fig. S14. IR of compound 4

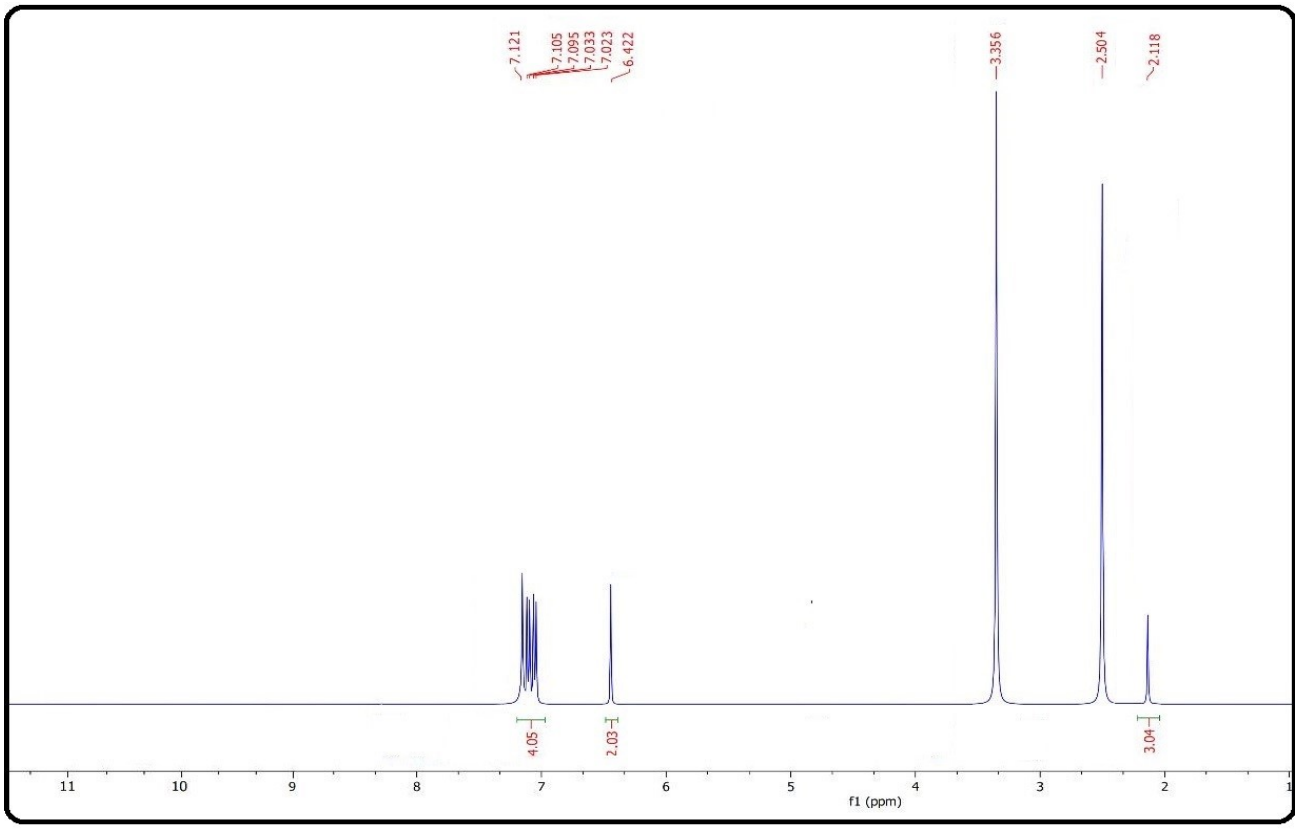


Fig. S15. ^{1}H-NMR of compound 4

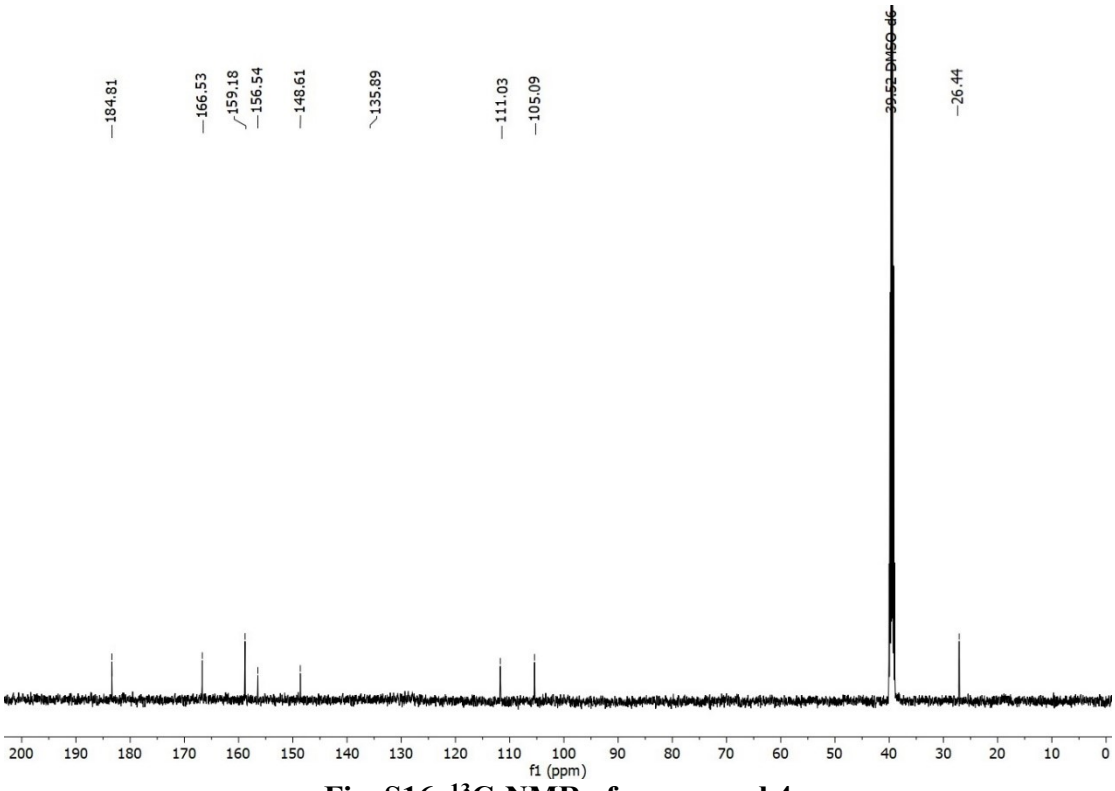
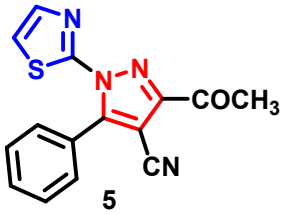


Fig. S16. ^{13}C-NMR of compound 4



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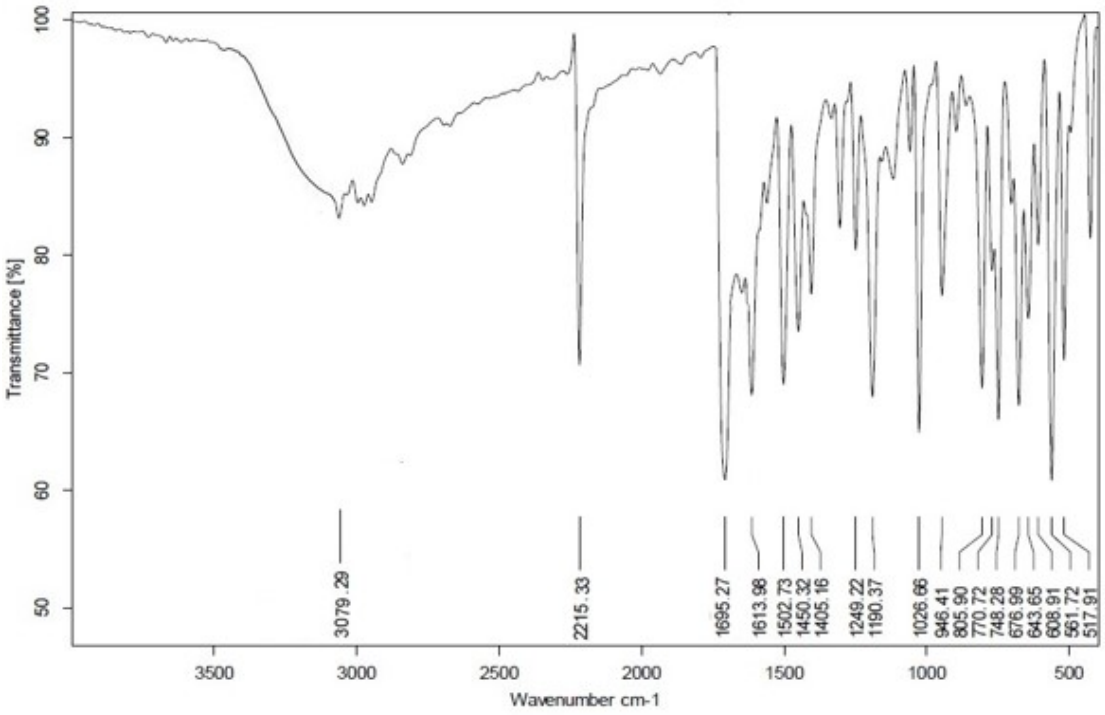


Fig. S17. IR spectrum of compound 5

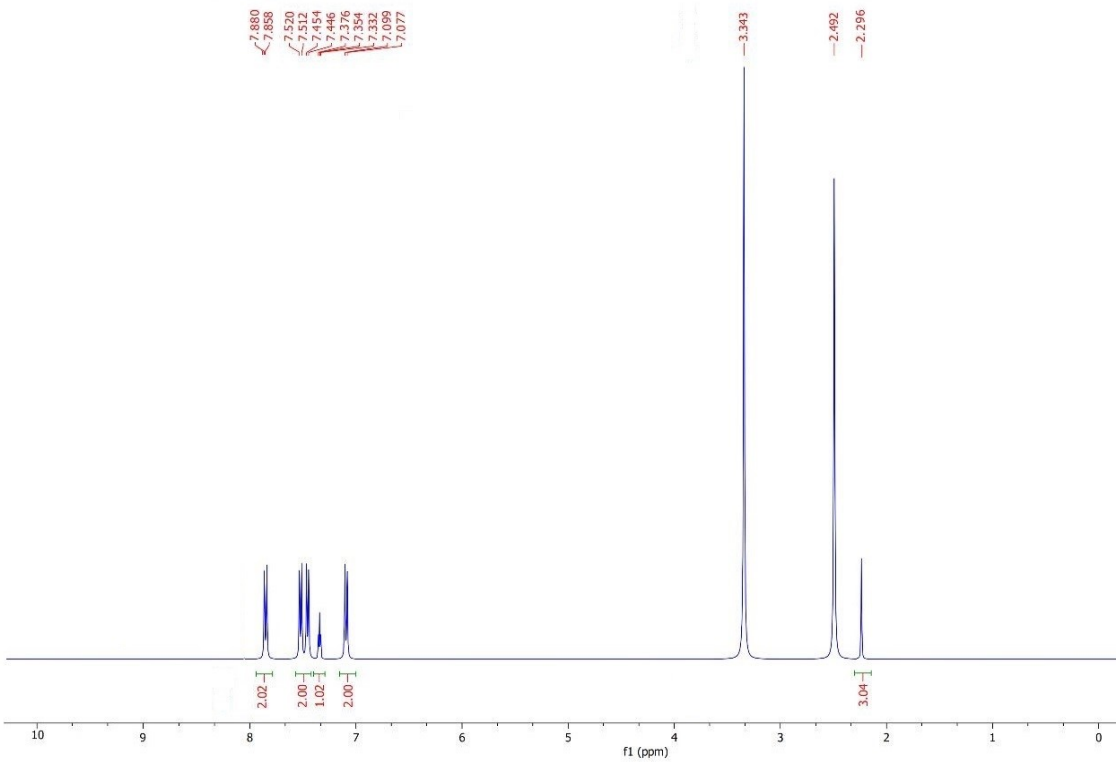


Fig. S18. ^{1}H NMR of compound 5

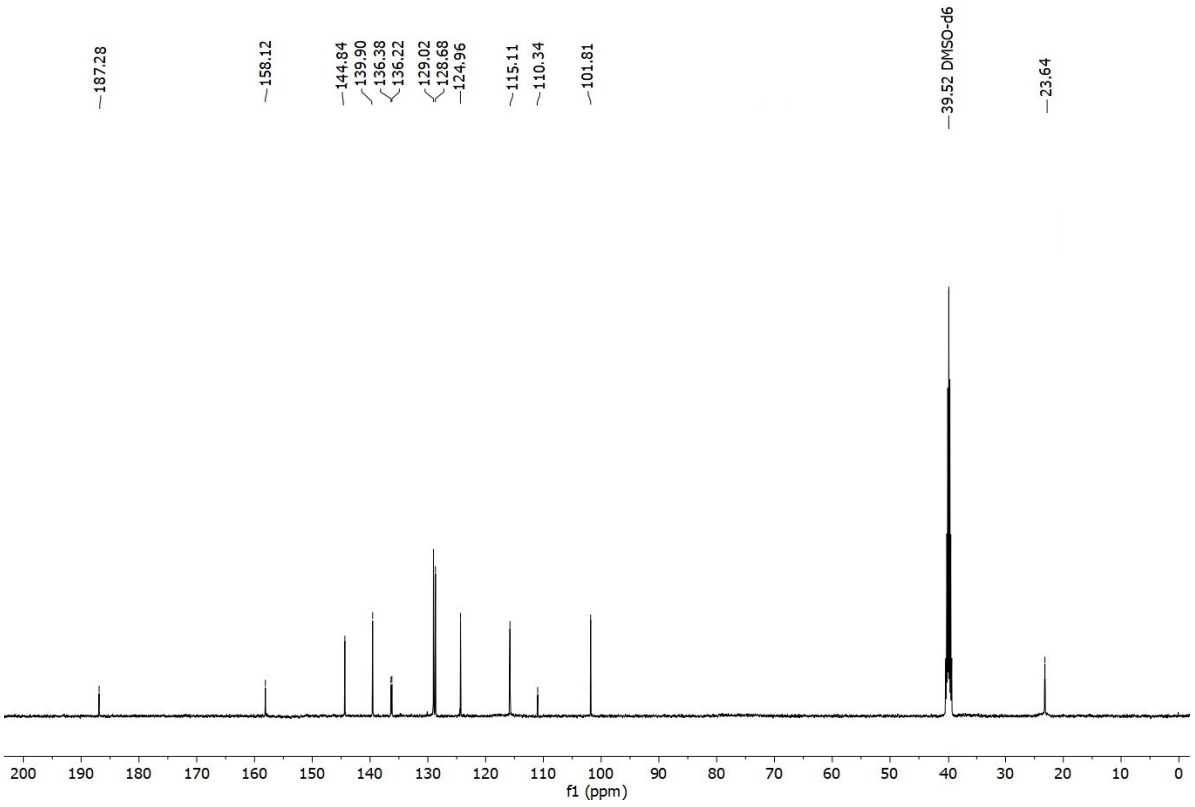


Fig. S19.^{13}C-NMR of compound 5

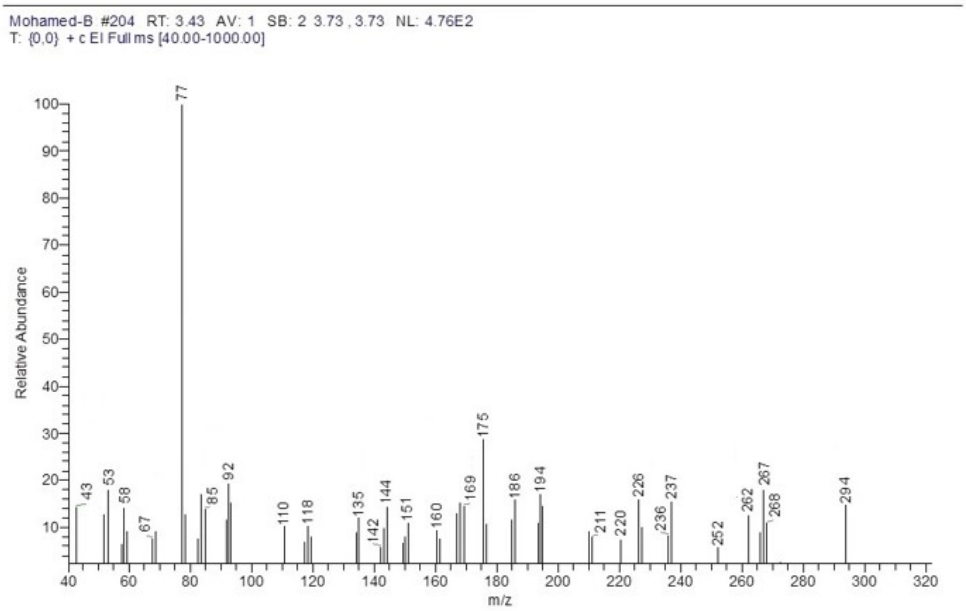
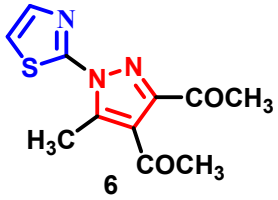


Fig. S20. Mass spectrum of compound 5



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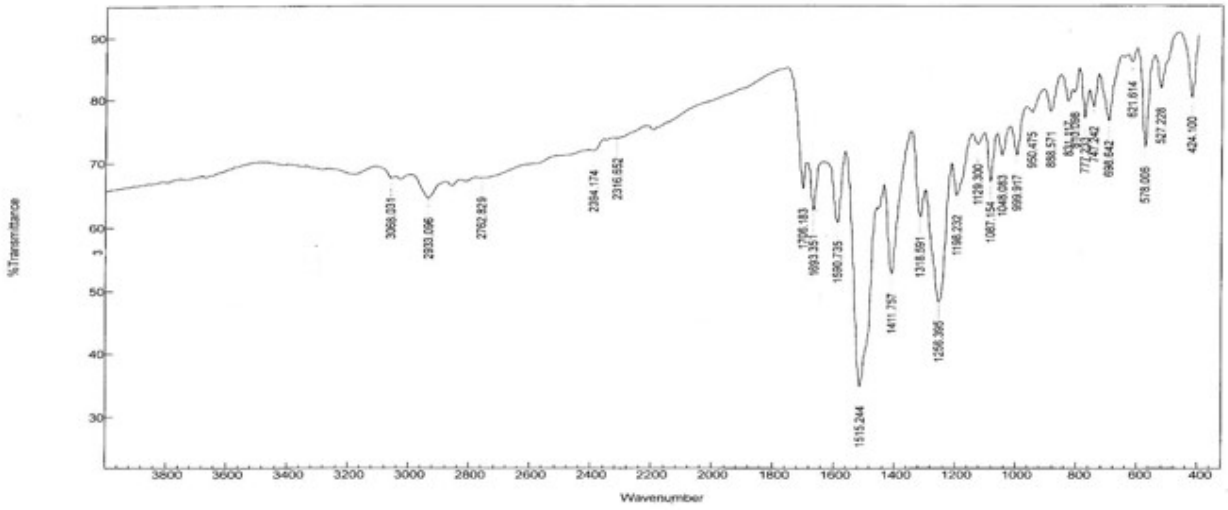


Fig. S21. IR spectrum of compound 6

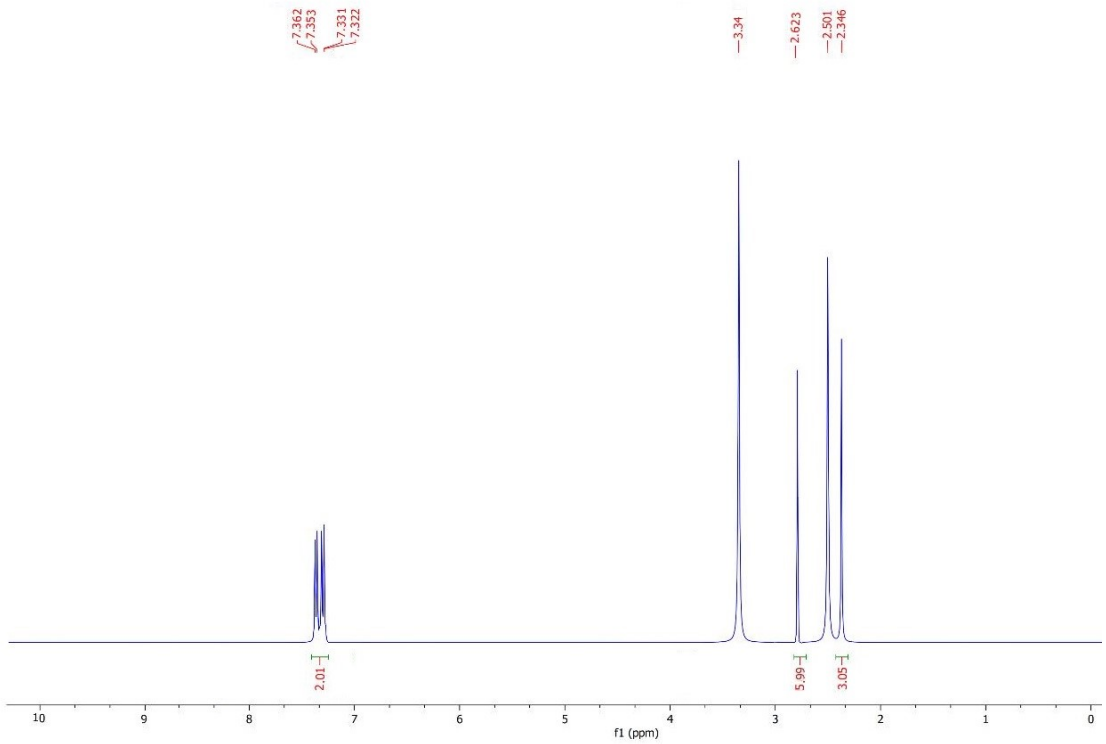


Fig. S22. [1] H-NMR of compound 6

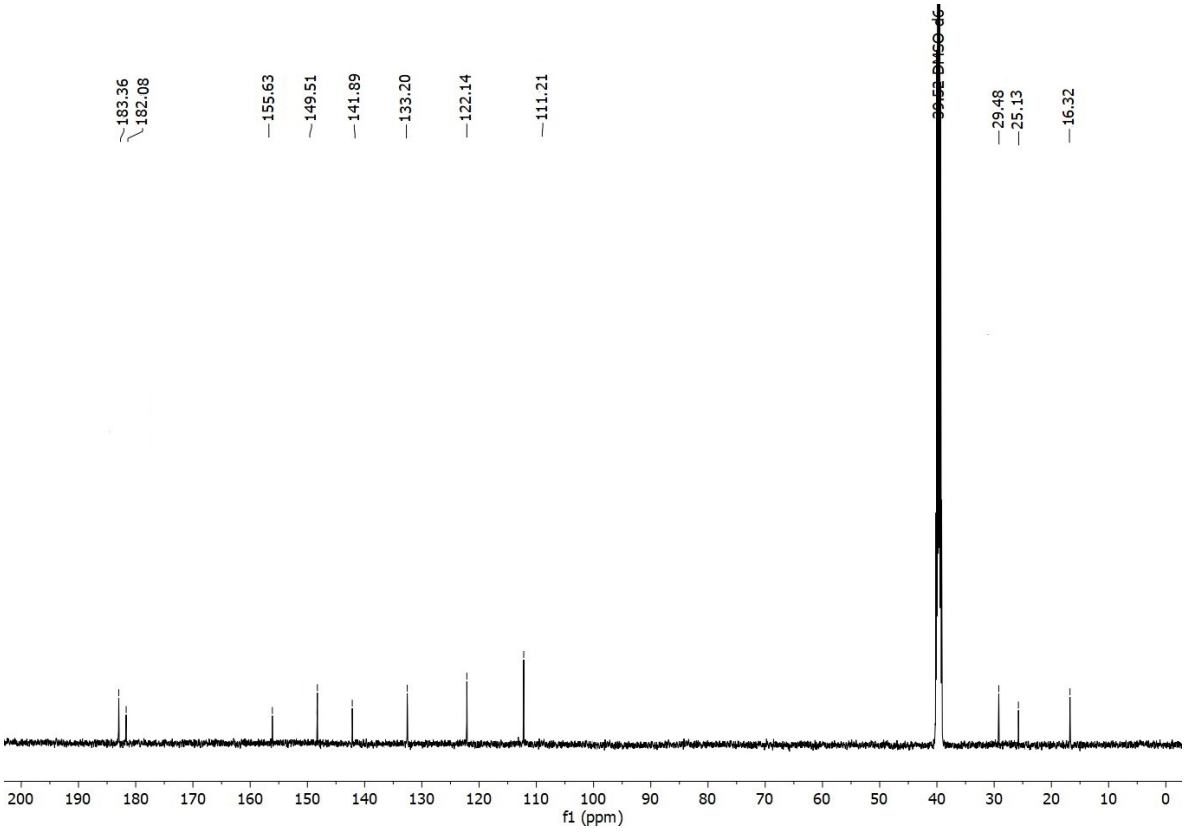


Fig. S23. ^{13}C-NMR of compound 6

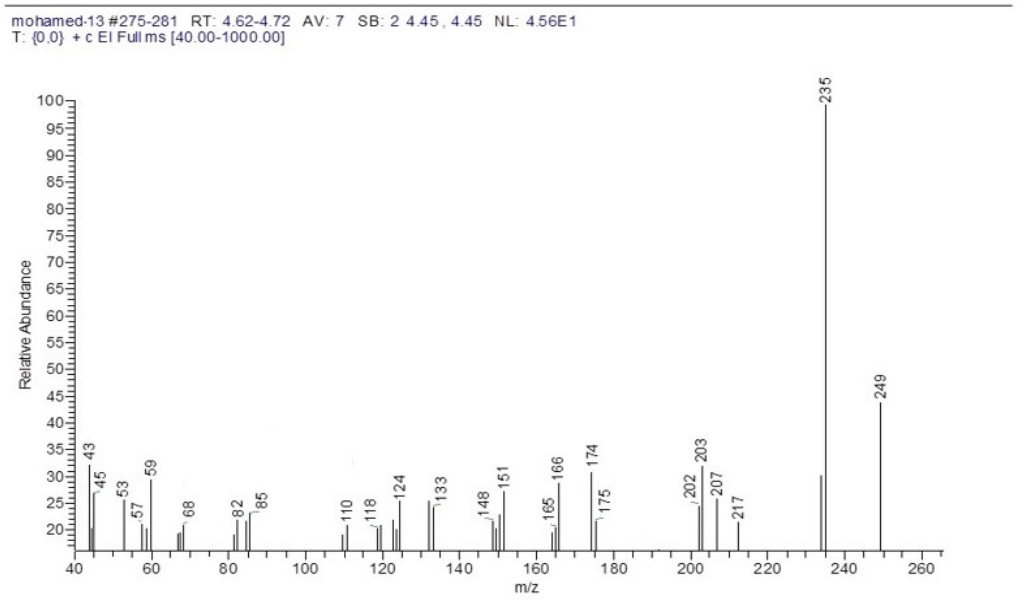
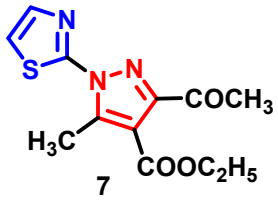


Fig. S24. Mass spectrum of compound 6



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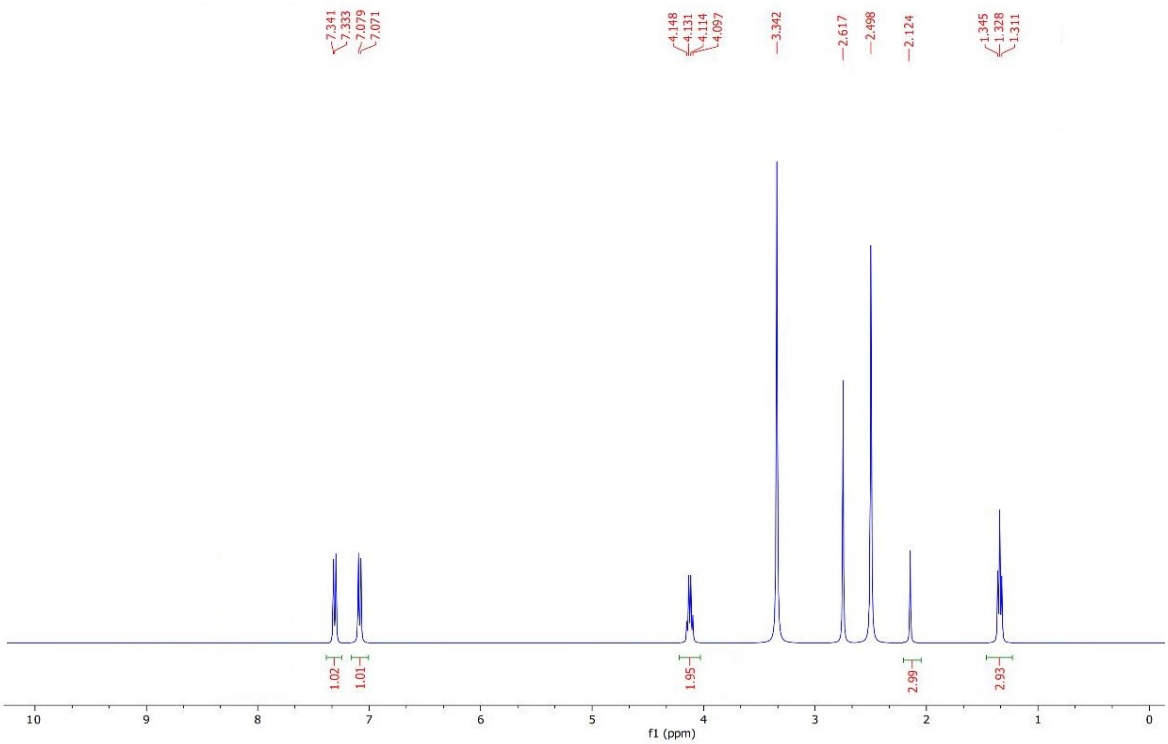


Fig. S25. [1] H-NMR of compound 7

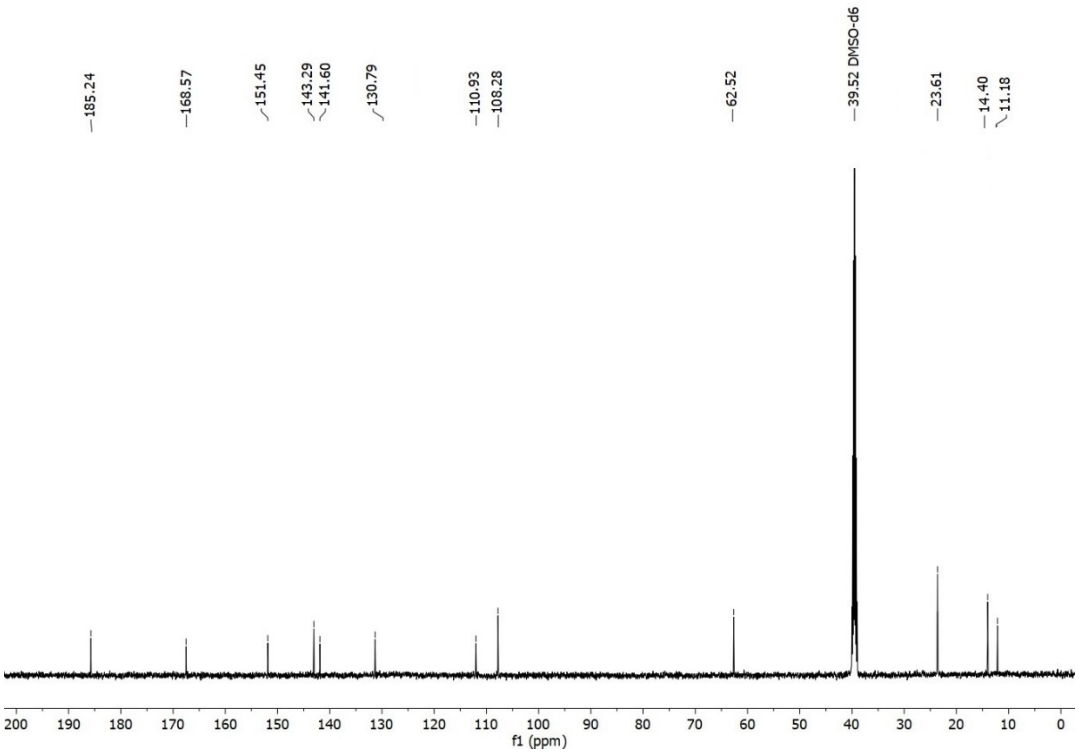
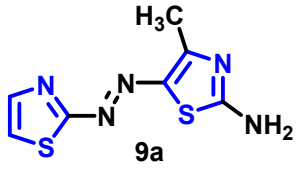


Fig. S26. ^{13}C-NMR of compound 7



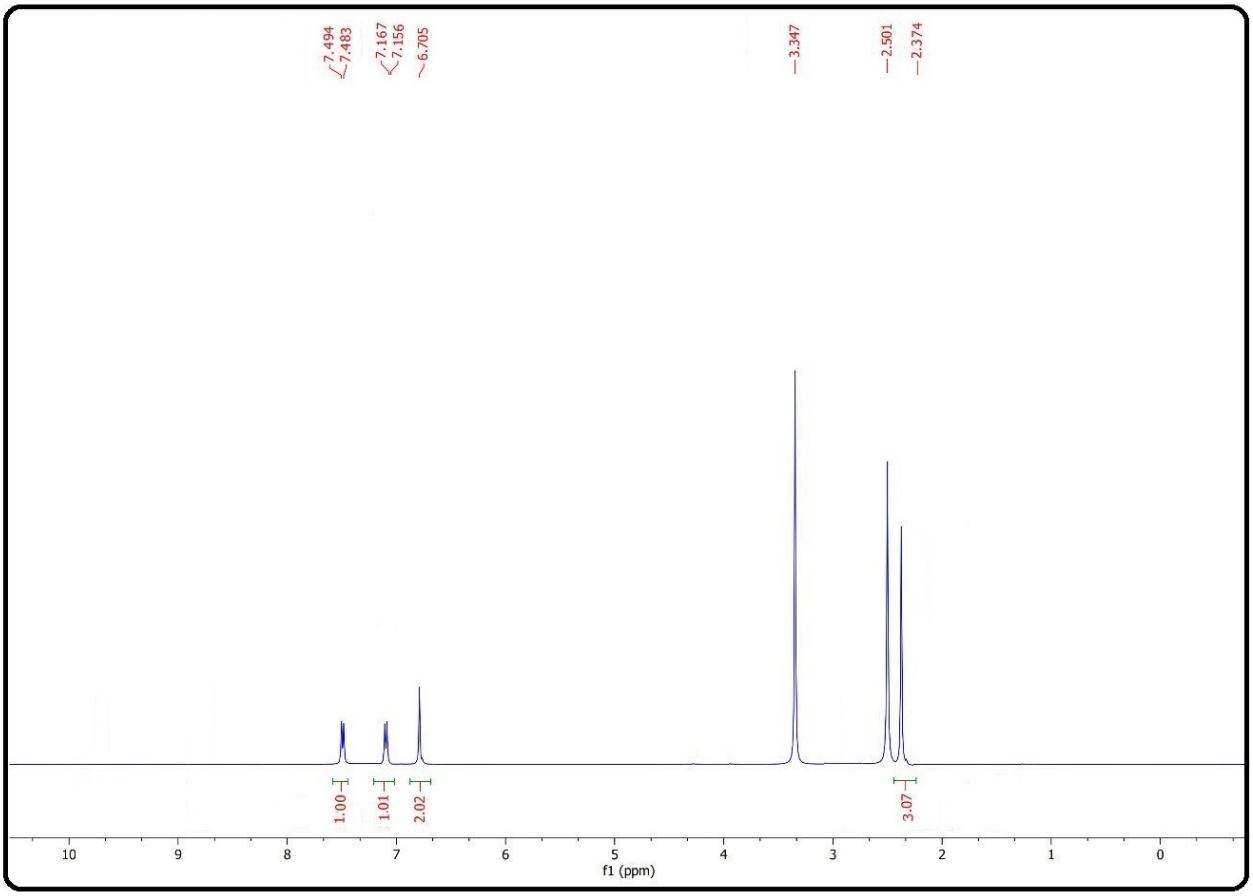


Fig. S27. [1] H-NMR of compound 9a

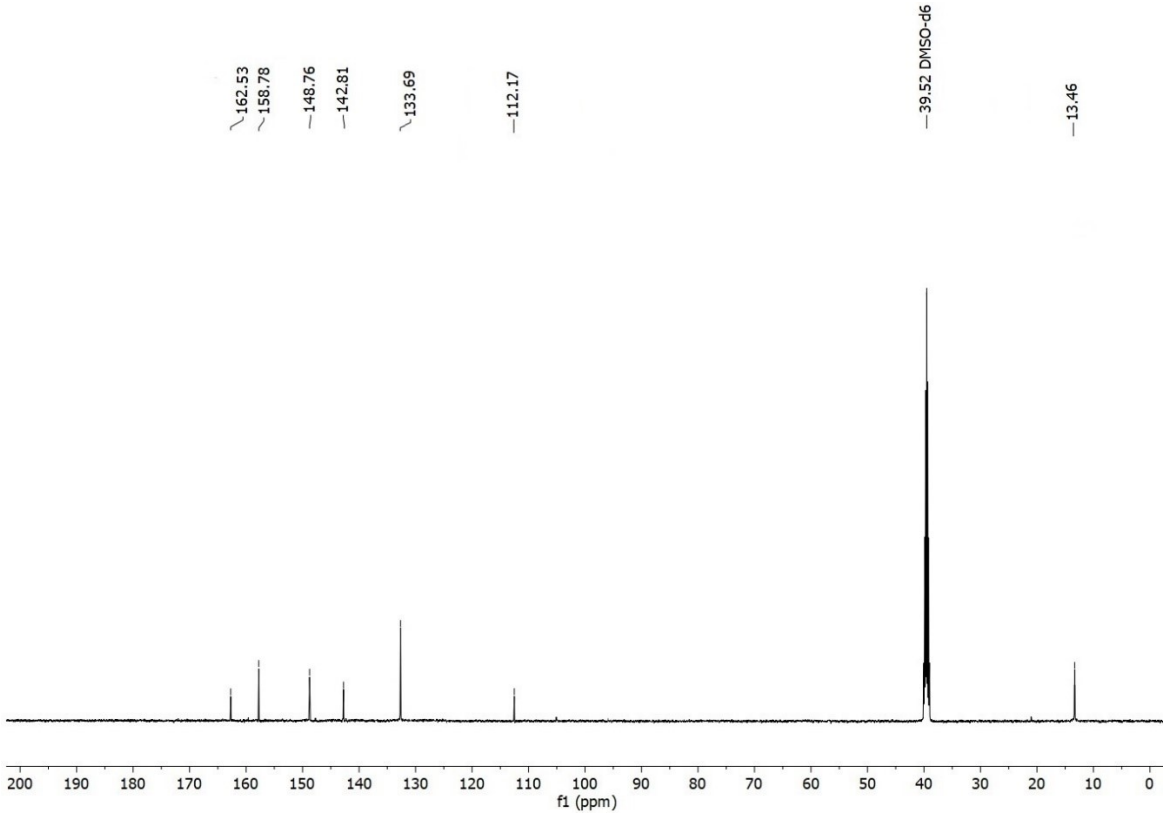


Fig. S28.^{13}C-NMR of compound 9a

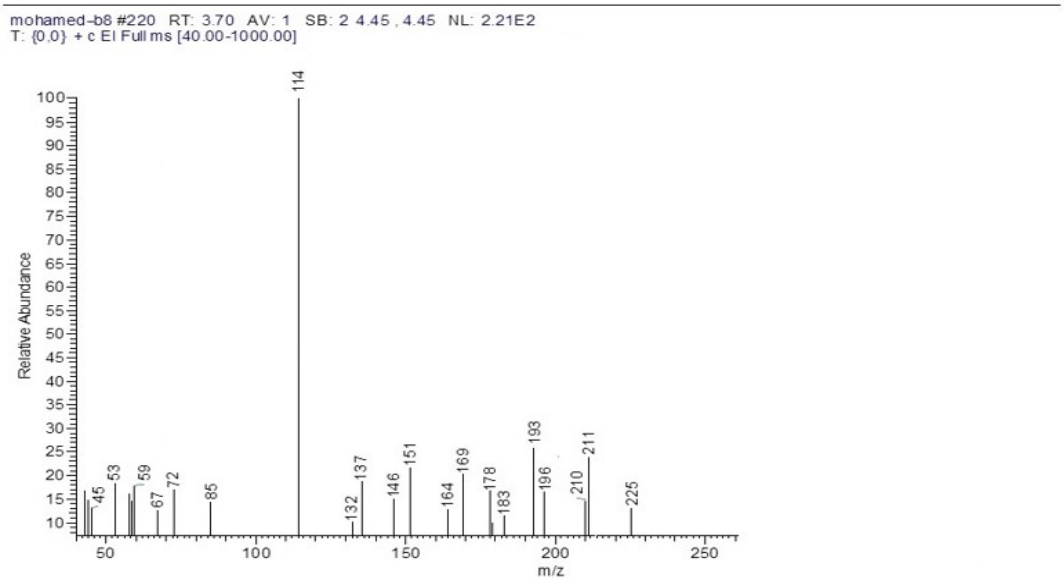


Fig. S29. Mass spectrum of compound 9a

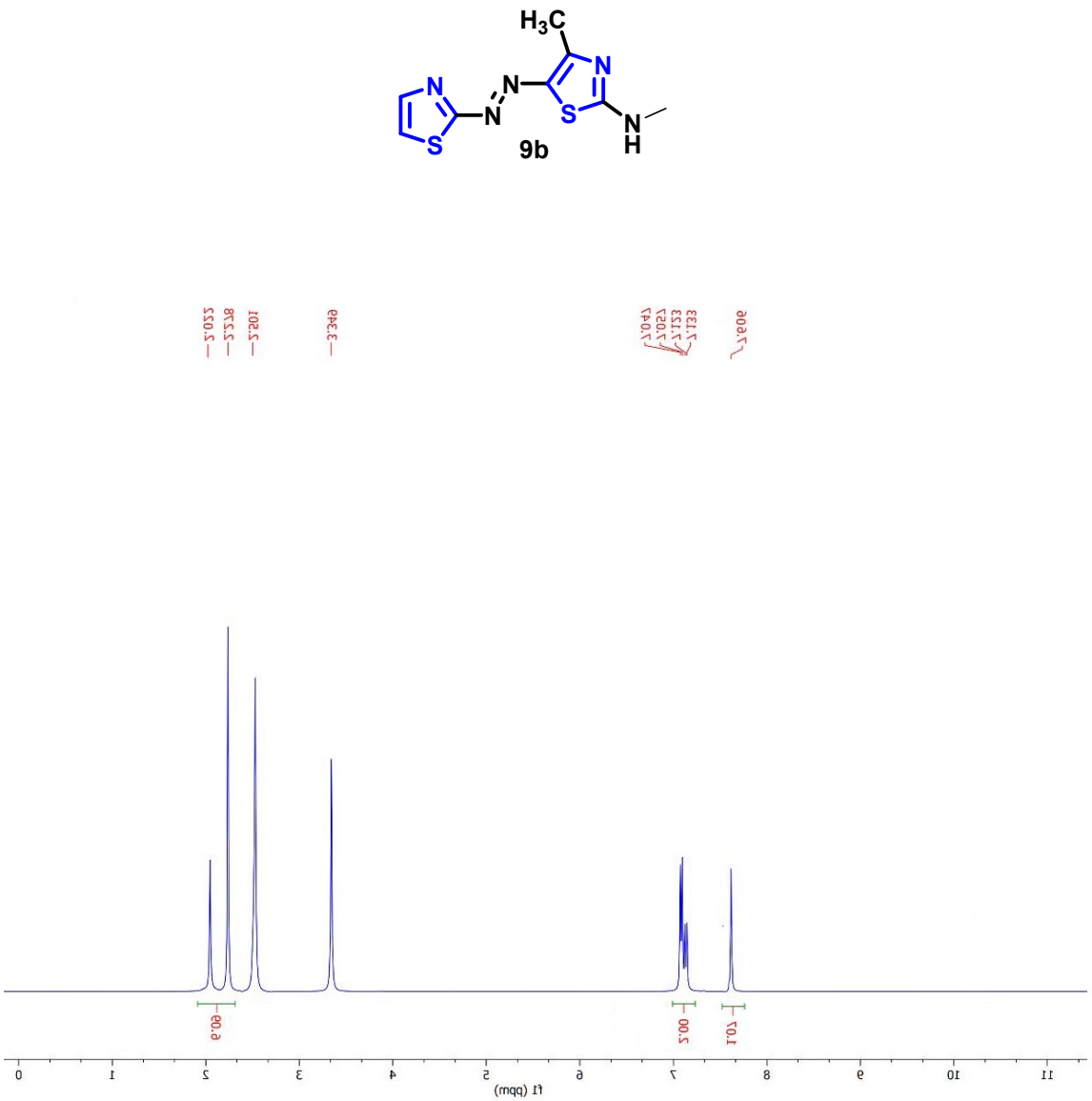


Fig. S30. [1] H-NMR of compound 9b

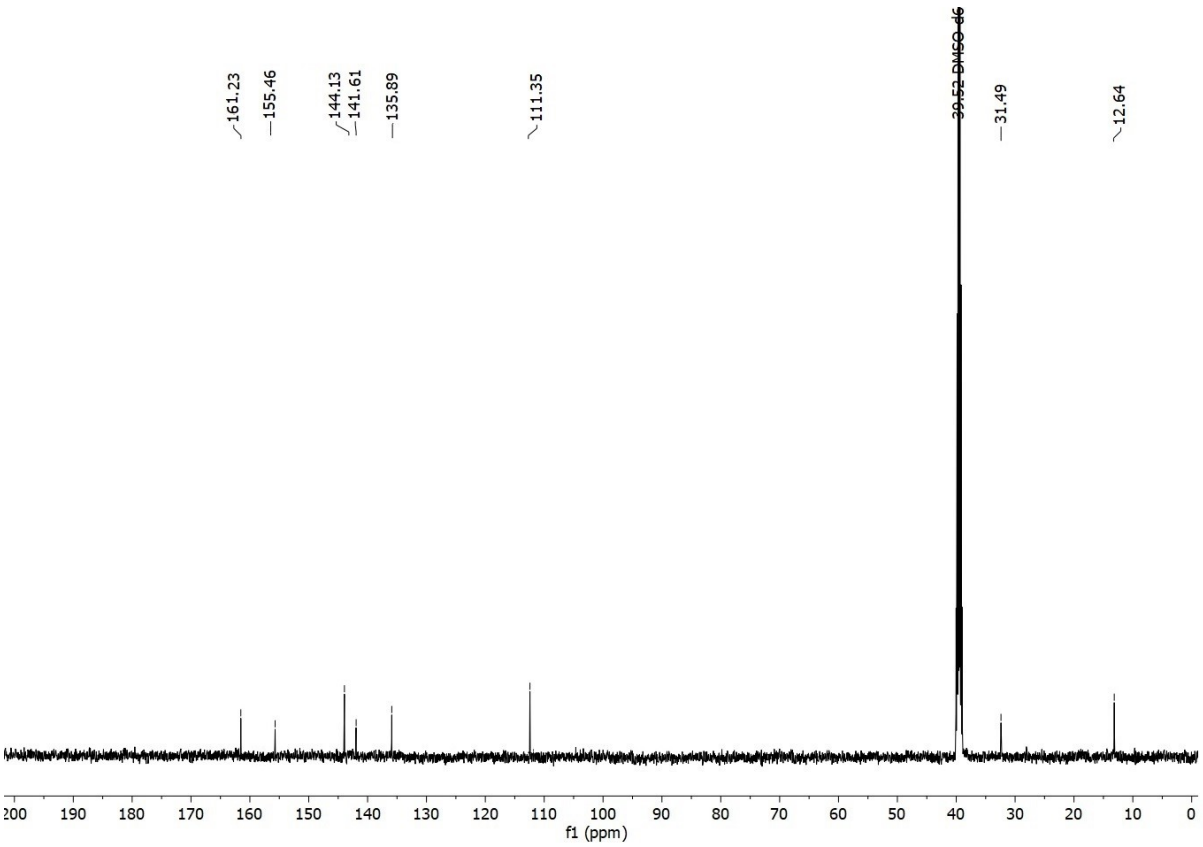
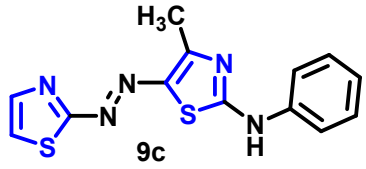


Fig. S31. ^{13}C-NMR of compound 9b



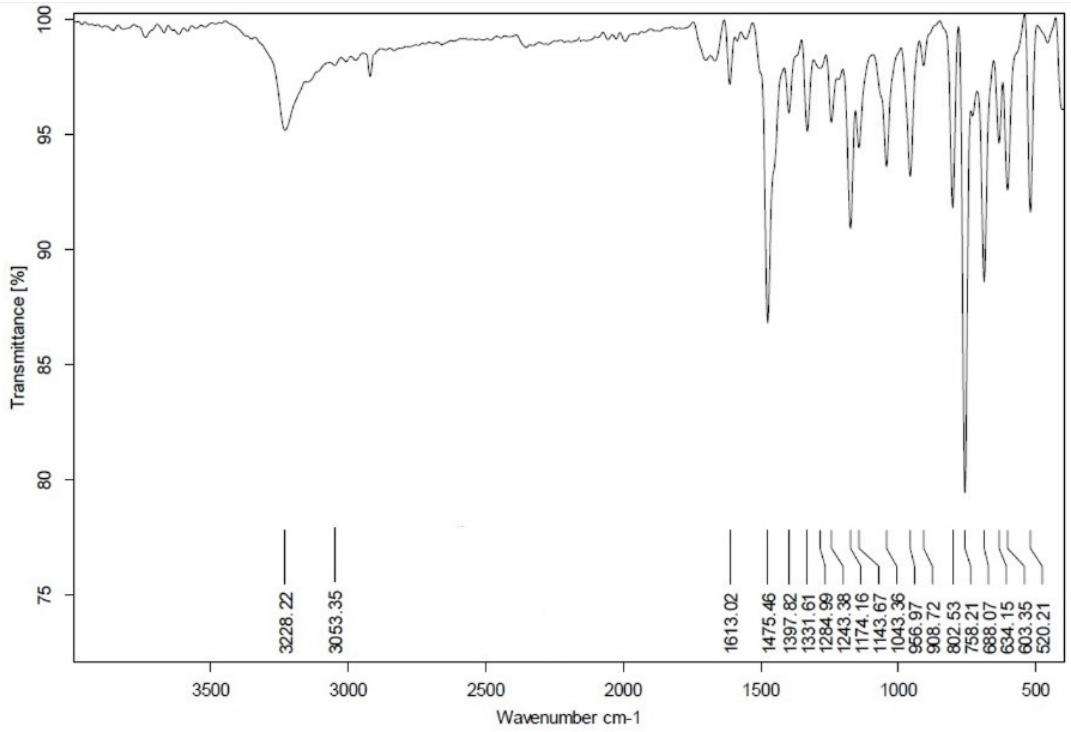


Fig. S32. IR spectrum of compound 9c

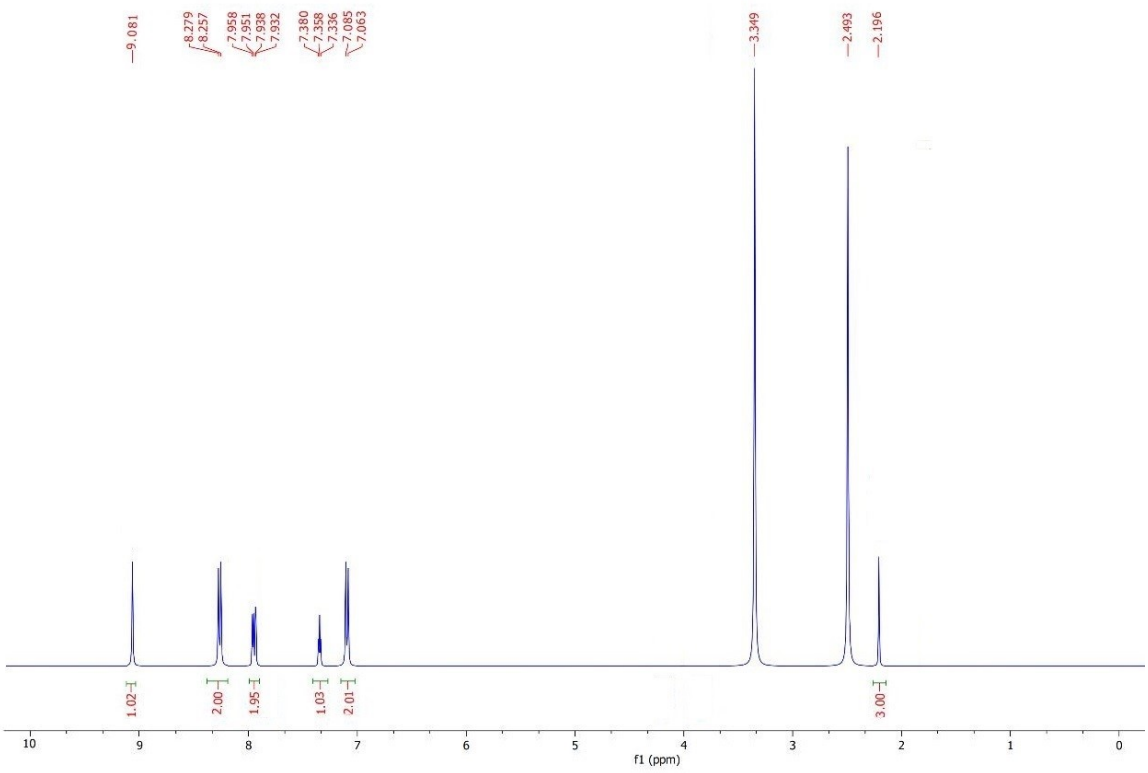


Fig. S33. [1] H-NMR of compound 9c

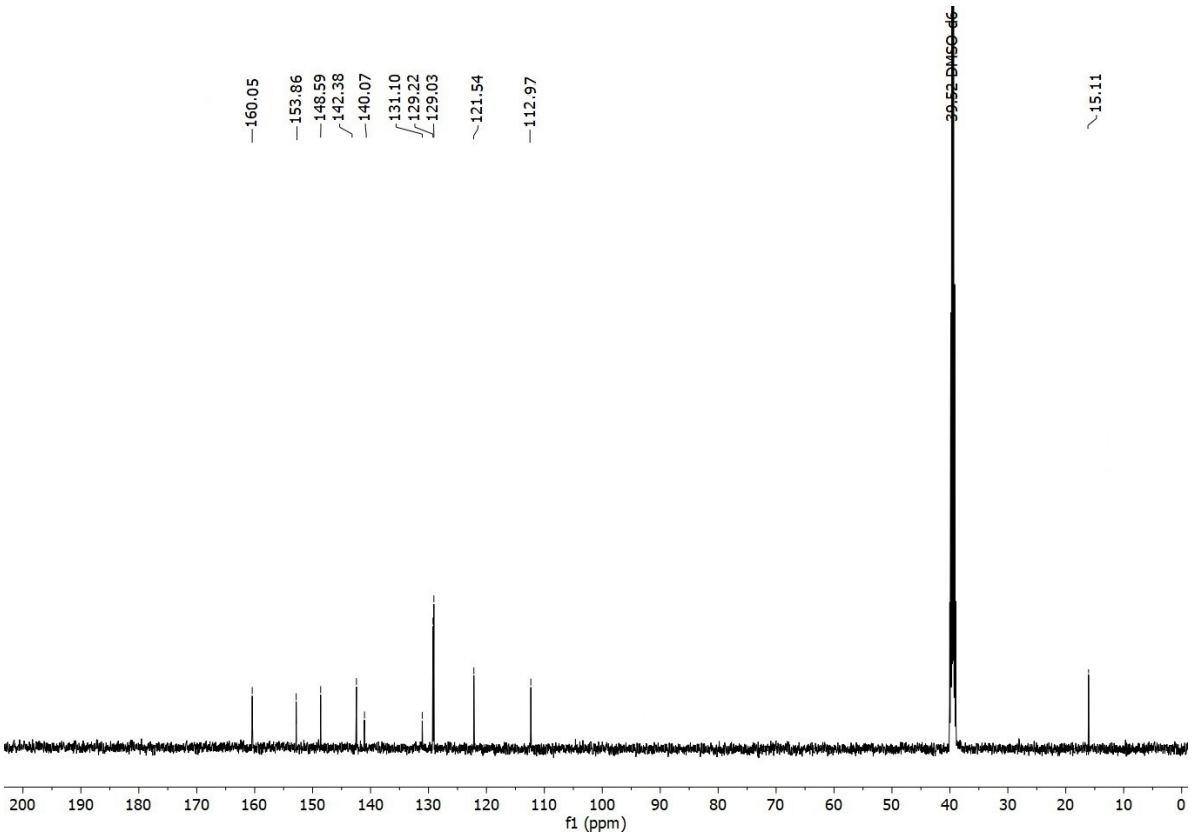


Fig. S34. ^{13}C-NMR of compound 9c

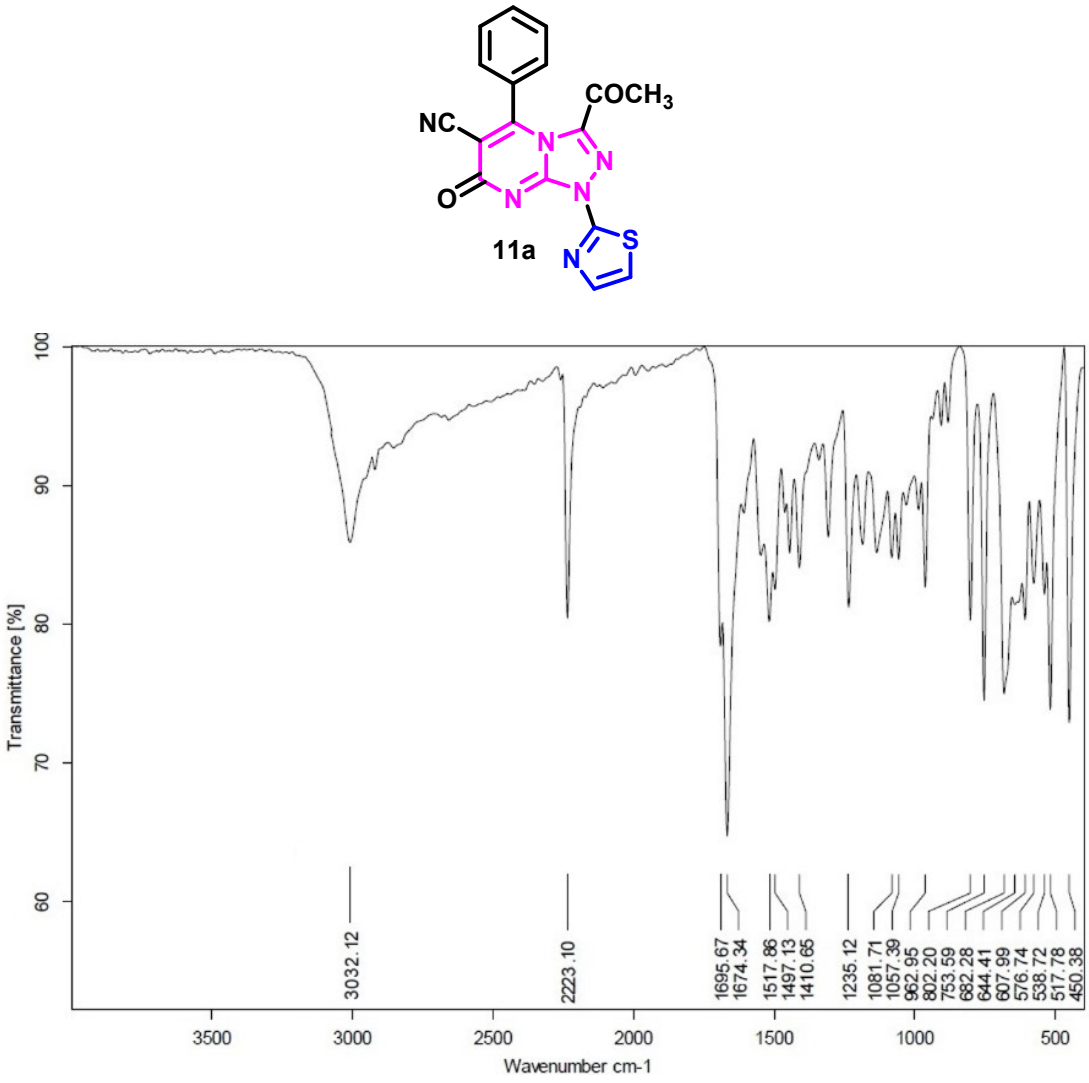


Fig. S35. IR spectrum of compound 11a

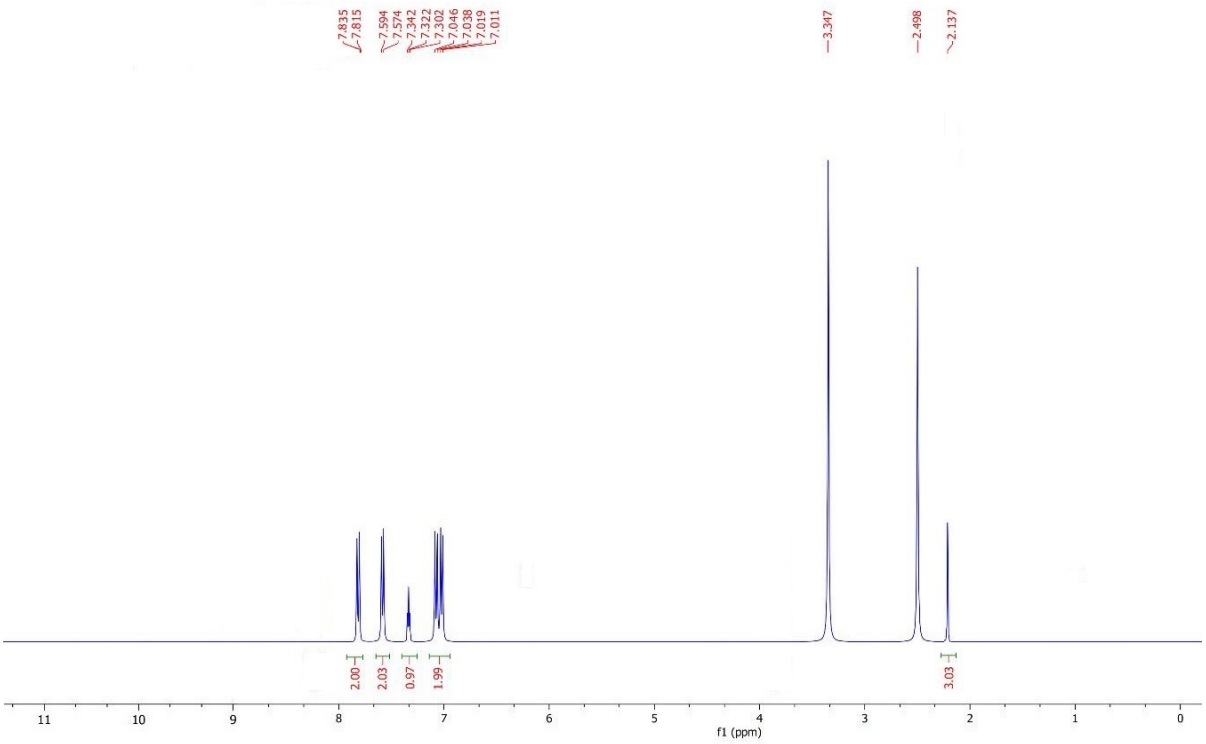


Fig. S36. [1] H-NMR of compound 11a

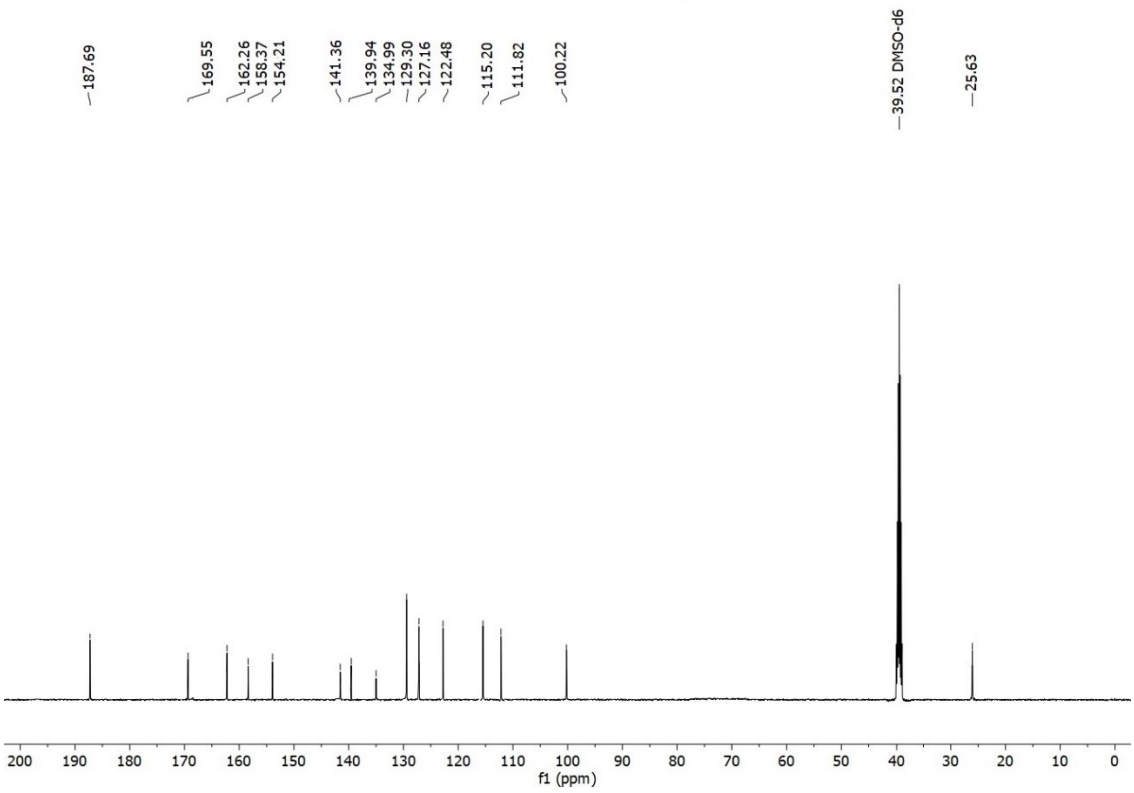


Fig. S37. ^{13}C-NMR of compound 11a

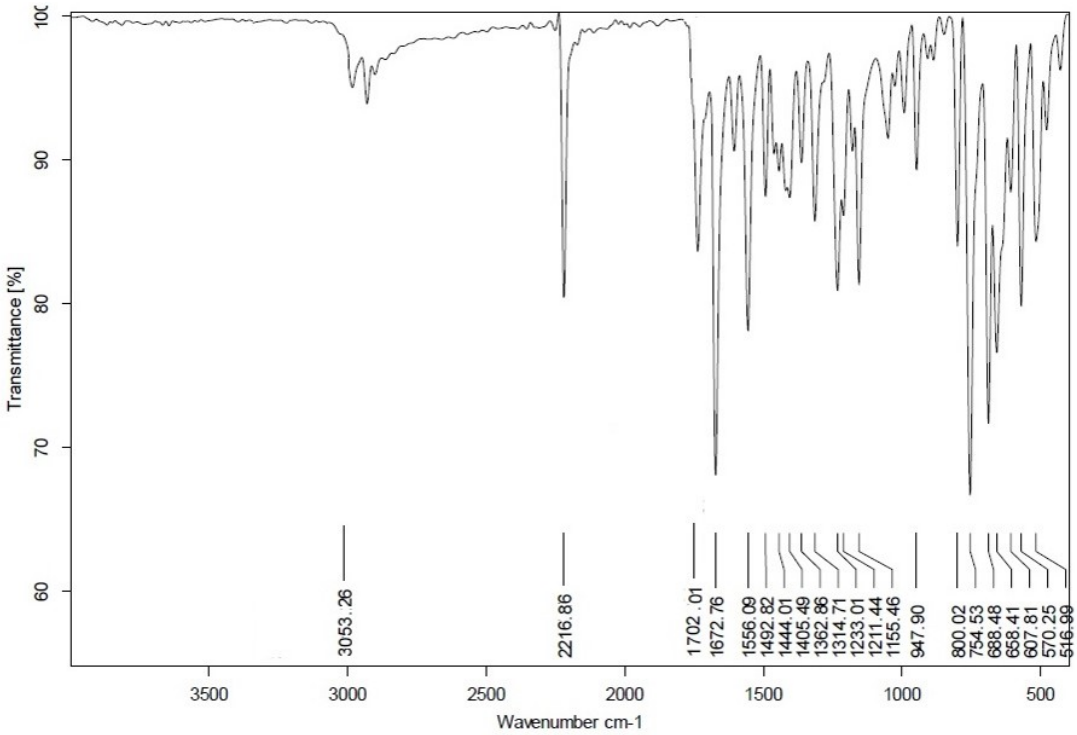


Fig. S38. IR spectrum of compound 11a

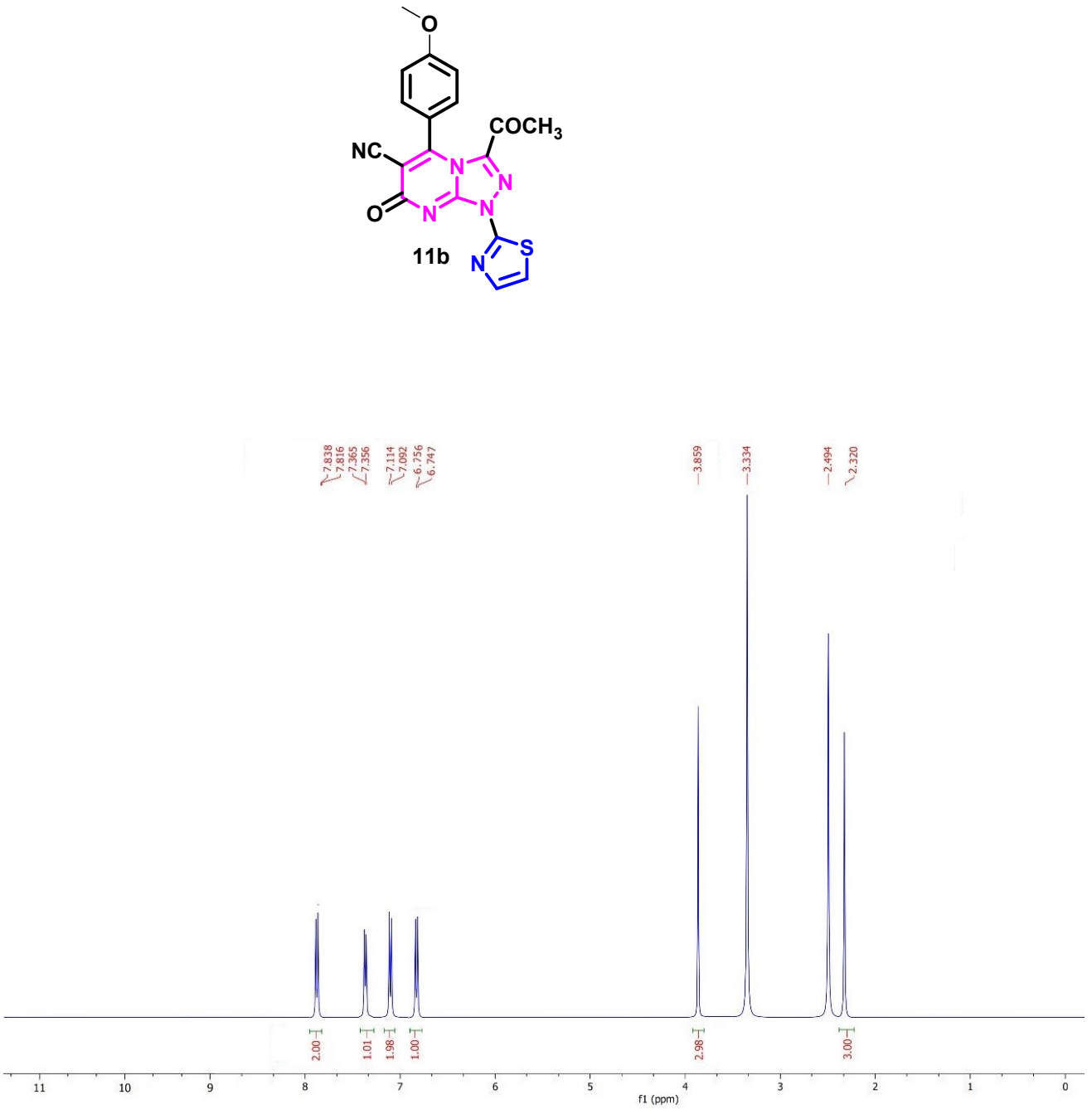


Fig. S39. [1] H-NMR of compound 11b

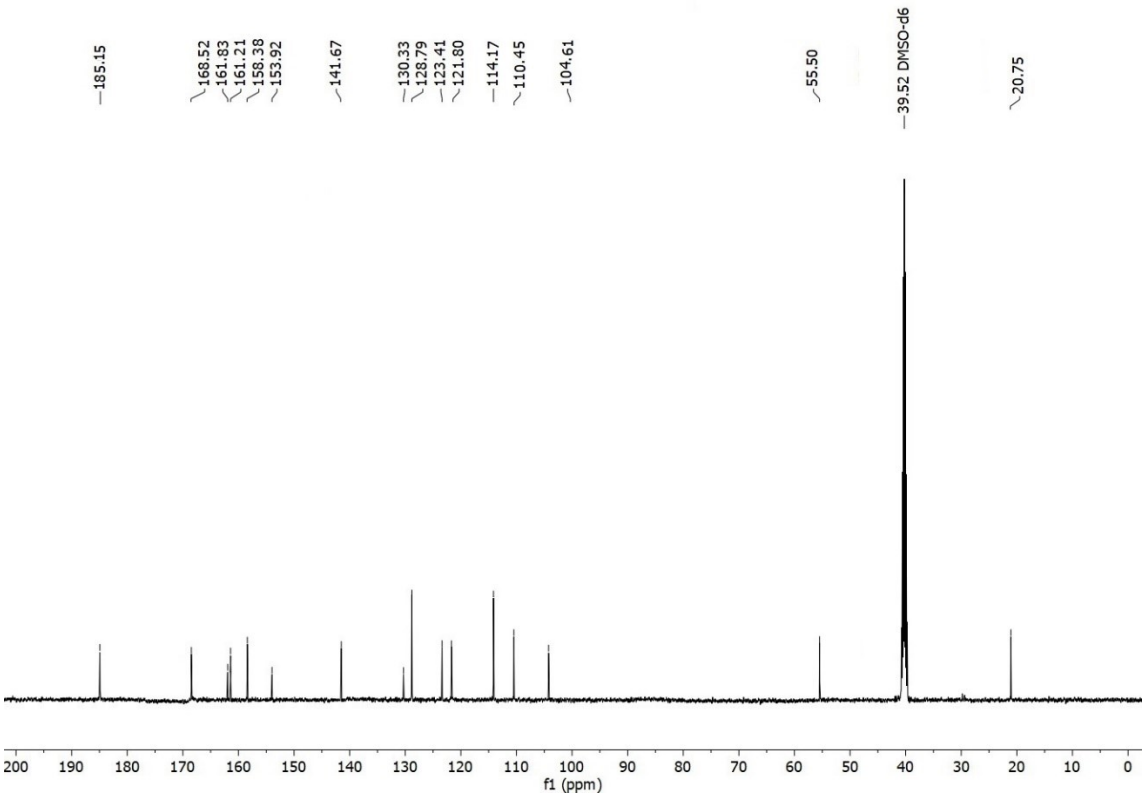


Fig. S40. ^{13}C-NMR of compound 11b

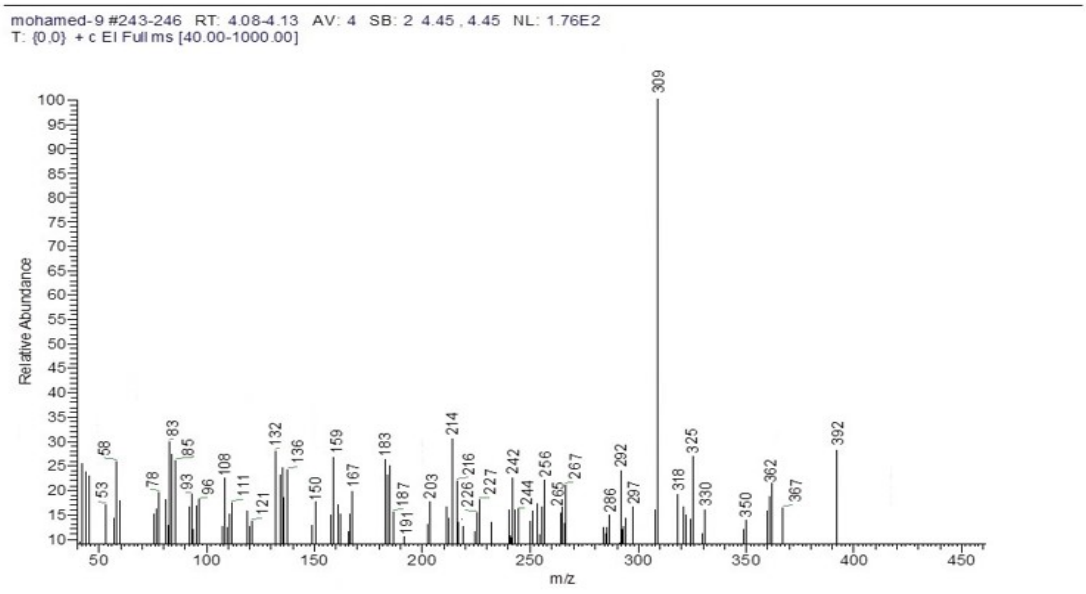


Fig. S41. Mass spectrum of compound 11b

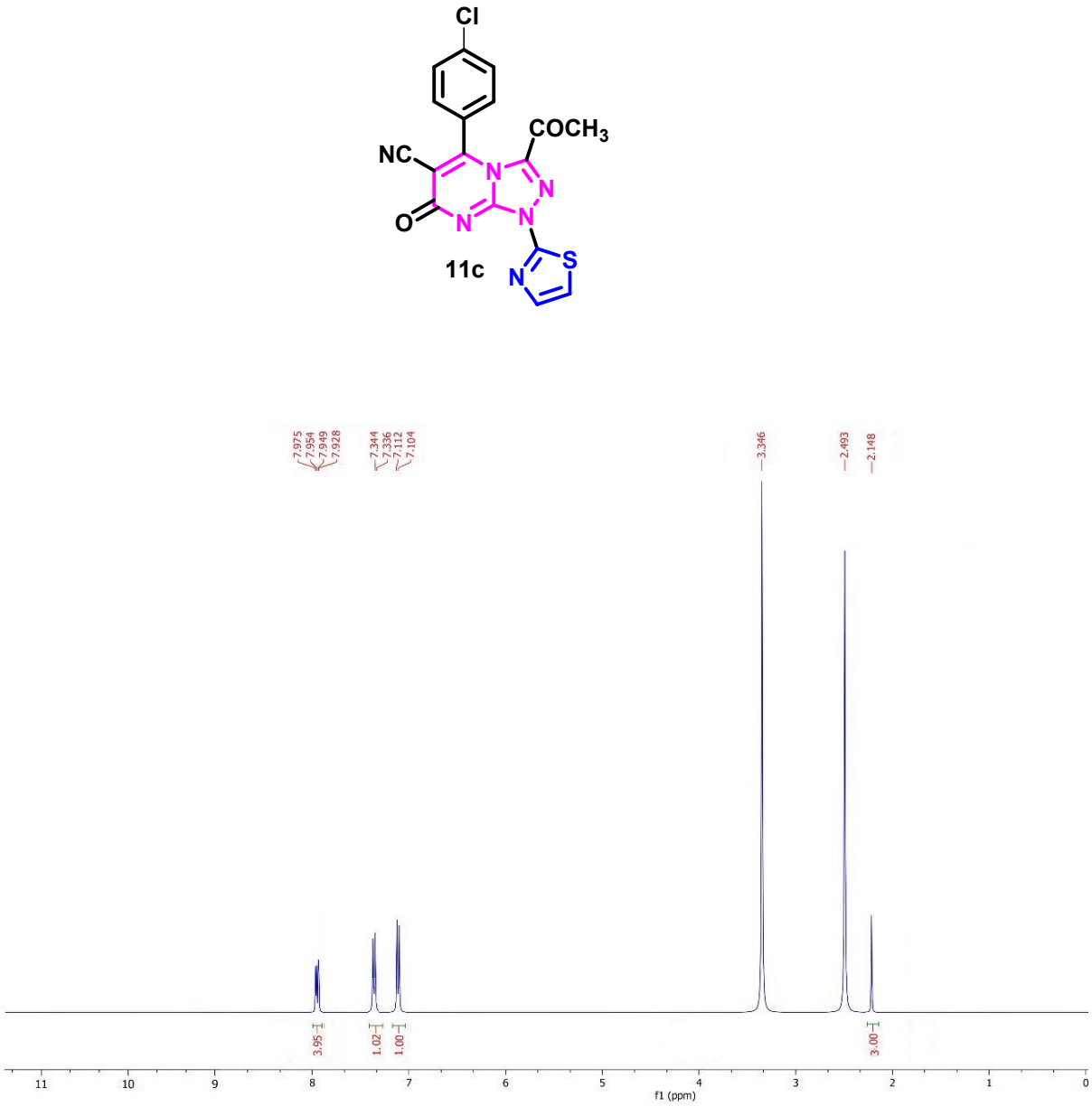


Fig. S42. [1] H-NMR of compound 11c

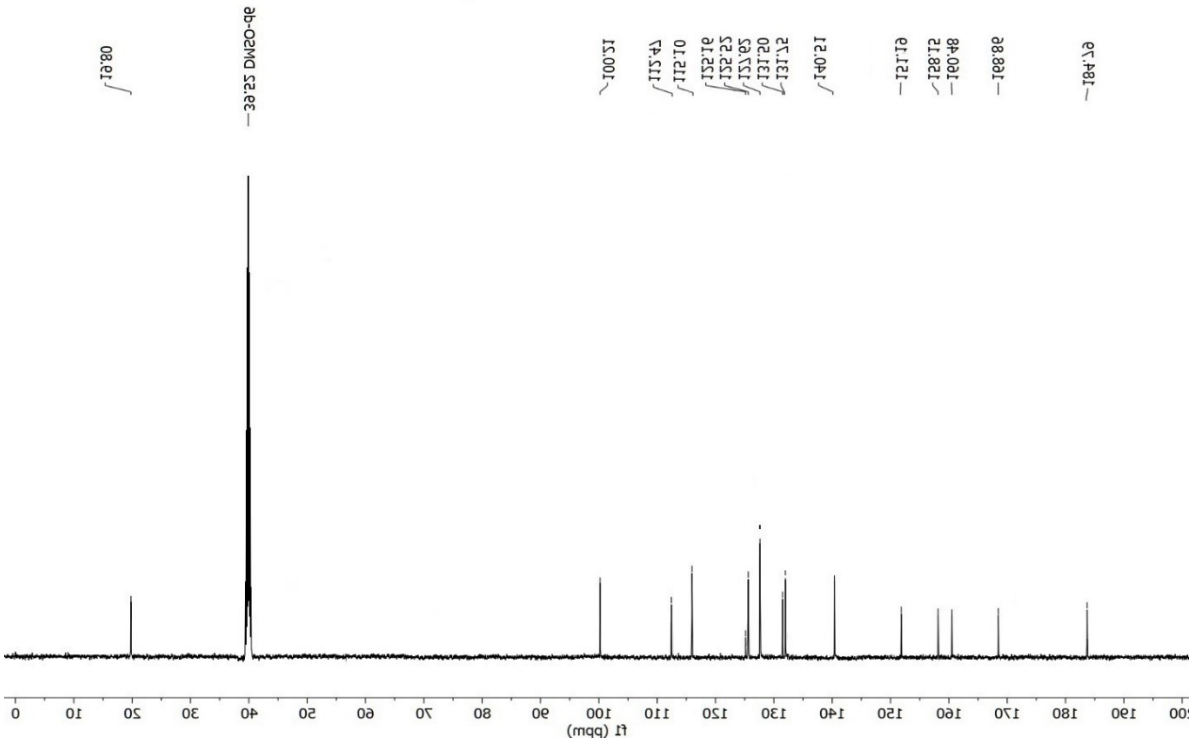


Fig. S43. ^{13}C-NMR of compound 11c