Synthesis and Biological Evaluation of *N/O*-Propargylated *di*-Arylpyrimidines as Dual Inhibitors of Acetylcholinesterase and Monoamine Oxidase.

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Chemistry

General procedure for the synthesis of diaryl substituted pyrimidinones

Urea (3 mmol) and iodine (0.3 mmol) was added to an ethanolic (5 ml) mixture of substituted acetophenone (1 mmol) and benzaldehyde (1 mmol). The mixture was heated to 80 ºC for 3-4 hours. The progress of the reaction was monitored by thin layer chromatography using 50 % ethyl acetate and pet. ether mixture. On completion of the reaction, excess solvent was evaporated on rotary evaporator. Iodine was quenched with 10 % aqueous sodium thiosulphate solution. Solid precipitates formed were filtered under vacuum and used further for next step.

General procedure for the synthesis of diaryl substituted chloro-pyrimidines

The synthesized pyrimidinones were reacted with $POCl_3$ and DIPEA in the presence of toluene at 80 ºC for 6-8 hours in a round bottom flask, evacuated, and purged with nitrogen. The progress of the reaction was monitored by thin layer chromatography using 10 % ethyl acetate and pet. ether mixture. After completion of the reaction, the reaction mixture was neutralised with aqueous $NaHCO_3$ solution, extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2CO_3. The solvent was evaporated under vacuum using rotary evaporator and the crude product was purified through column chromatography using pet ether, ethyl acetate mixture.

General procedure for the synthesis of diaryl substituted propargylated pyrimidines

The respective chloro-pyrimidines (1 mmol) were reacted with propargyl amine/alcohol (1 mmol) in the presence of K_2CO_3 (1 mmol) as base and DMF as solvent at 80 ºC for 3-4 hours. The completion of the reaction was monitored by thin layer chromatography using 30 % ethyl acetate and pet. ether mixture. After completion, the reaction mixture was extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4. Solvent was evaporated under vacuum on rotary evaporator and the product obtained was purified through column chromatography using petroleum ether and ethyl acetate mixture (3:1). The final products were characterized by NMR and HRMS spectral analysis.

Characteristic spectral data of the products

4-phenyl-N-(prop-2-yn-1-yl)-6-(p-tolyl)pyrimidin-2-amine (NV-1)

Brown solid, 78 % Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (t, 2.8 Hz, 1H), 2.42 (s, 3H), 4.41 (dd, 3.2 Hz, 2.4 Hz, 2H), 5.38 (s, 1H), 7.29 (d, 8.0 Hz, 2H), 7.46 (s, 1H), 7.49 (m, 3H), 8.01 (d, 8.0 Hz, 2H), 8.09 (dd, 3.6 Hz, 1.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.45, 31.47, 70.66, 81.20, 103.52, 127.06, 127.14, 128.72, 129.46, 130.43, 134.90, 137.84, 140.83, 162.09, 165.60, 165.67. HRMS: m/z $[M+Na]^+$ for $C_{20}H_{17}N_3Na^+$, calculated 322.1315; observed: 322.1361; HPLC percentage purity: 97.71 %

4-(3-bromophenyl)-6-phenyl-N-(prop-2-yn-1-yl)pyrimidin-2-amine (NV-2)

White crystal, 73 % Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (t, 1.6 Hz, 1H), 4.40 (dd, 2.0 Hz, 1.6 Hz, 2H), 5.41 (t, 3.6 Hz, 1H), 7.36 (t, 5.2 Hz, 1H), 7.36 (t, 5.2 Hz, 1H), 7.43 (s, 1H), 7.49 (t, 2.0 Hz, 3H), 7.60 (dq, 3.2 Hz, 0.8 Hz, 1H), 8.01 (d, 5.2 Hz, 1H), 8.09 (d, 2.4 Hz, 2H), 8.25 (t, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.50, 70.82, 80.97, 103.70, 123.01, 125.68, 127.16, 128.77, 130.22, 130.24, 130.67, 133.33, 137.51, 139.85, 162.13, 164.15, 166.09. HRMS: m/z $[M+Na]^+$ for $C_{19}H_{14}BrN_3Na^+$, calculated 386.0263; observed: 388.0258 (M and M+2 peak due to Br).

4-(4-bromophenyl)-6-(4-chlorophenyl)-N-(prop-2-yn-1-yl)pyrimidin-2-amine (NV-3)

Light brown solid, 70% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (t, 2.8 Hz, 1H), 4.39 (dd, 3.2 Hz, 2.8 Hz, 2H), 5.41 (s, 1H), 7.41 (s, 1H), 7.46 (dd, 4.4 Hz, 2.0 Hz, 2H), 7.62 (dd, 4.4 Hz, 2.0 Hz, 2H), 7.97 (d, 8.4 Hz, 2H), 8.04 (d, 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.47, 70.81, 80.90, 103.08, 125.19, 128.44, 128.68, 128.99, 131.96, 135.98, 136.46, 136.79, 162.12. HRMS: $[M+H]^+$ for $C_{19}H_{14}BrClN_3^+$, calculated 398.0054; observed: 398.0180.

4-(4-chlorophenyl)-6-phenyl-N-(prop-2-yn-1-yl)pyrimidin-2-amine (NV-4)

Light yellow solid, 77% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (t, 2.8 Hz, 1H), 4.40 (dd, 1.6 Hz, 1.6 Hz, 2H), 5.42 (s, 1H), 7.45 (d, 2.4 Hz, 2H), 7.47 (s, 1H), 7.50 (t, 3.6 Hz, 3H), 8.06 (d, 8.4 Hz, 2H), 8.09 (dd, 3.6 Hz, 2.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.47, 70.74, 81.04, 103.47, 127.14, 128.44, 128.77, 128.95, 130.61, 136.18, 136.62, 137.61, 162.14. HRMS: m/z $[M+Na]^+$ for $C_{19}H_{14}ClN_3Na^+$, calculated 342.0768; observed: 342.0982.

4-(4-bromophenyl)-6-(4-methoxyphenyl)-N-(prop-2-yn-1-yl)pyrimidin-2-amine (NV-5)

Brown solid, 73% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (t, 2.4 Hz, 1H), 3.88 (s, 3H), 4.40 (dd, 3.2 Hz, 2.4 Hz, 2H), 5.35 (t, 5.6 Hz, 1H), 7.01 (d, 9.2 Hz, 2H), 7.39 (s, 1H), 7.62 (d, 8.8 Hz, 2H), 7.97 (d, 8.4 Hz, 2H), 8.08 (d, 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.62, 55.57, 70.84,

81.27, 102.84, 114.26, 128.55, 128.80, 129.06, 130.17, 136.52, 136.63, 161.96, 162.24, 164.34, 165.58. HRMS: m/z $[M+H]^+$ for $C_{20}H_{17}BrN_3O^+$, calculated 394.0550; observed: 394.0498.

4-(4-methoxyphenyl)-6-phenyl-N-(prop-2-yn-1-yl)pyrimidin-2-amine (NV-6)

Light brown powder, 75% Yield; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.22 (t, 2.4 Hz, 1H), 3.87 (s, 3H), 4.40 (dd, 3.0 Hz, 3.0 Hz, 2H), 5.37 (t, 6.6 Hz, 1H), 7.00 (d, 9.6 Hz, 2H), 7.43 (s, 1H), 7.48 (m, 3H), 8.08 (d, 9.0 Hz, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 31.47, 55.41, 70.62, 81.26, 103.04, 114.07, 127.11, 128.64, 128.70, 130.18, 130.36, 137.95, 161.70, 162.10, 165.18, 165.49. HRMS: m/z $[M+Na]^+$ for $C_{20}H_{17}N_3NaO^+$, calculated 338.1264; observed: 338.1273.

4-(4-methoxyphenyl)-N-(prop-2-yn-1-yl)-6-(p-tolyl)pyrimidin-2-amine (NV-7)

Light yellow powder, 76% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (t, 2.8Hz, 1H), 2.42 (s, 3H), 3.87 (s, 3H), 4.40 (dd, 3.2 Hz, 2.4 Hz, 2H), 5.33 (t, 5.2 Hz, 1H), 7.00 (d, 8.8 Hz, 2H), 7.29 (d, 8.0 Hz, 2H), 7.41 (s, 1H), 7.99 (d, 8.0 Hz, 2H), 8.08 (d, 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.45, 31.44, 55.41, 70.60, 81.26, 102.73, 114.03, 127.01, 128.62, 129.42, 130.19, 135.00, 140.68, 161.62, 16197, 164.99, 165.37. HRMS: m/z $[M+H]^+$ for $C_{21}H_{20}N_3O^+$, calculated 330.1600; observed: 330.1592.

4-(4-chlorophenyl)-6-(4-methoxyphenyl)-N-(prop-2-yn-1-yl)pyrimidin-2-amine (NV-8)

Yellow solid, 72% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (t, 7.2 Hz, 1H), 3.88 (s, 3H), 4.40 (d, 4.4 Hz, 2H), 5.32 (s, 1H), 7.00 (d, 6.0 Hz, 2H), 7.39 (s, 1H), 7.45 (d, 5.6 Hz, 2H), 8.04 (d, 6.4 Hz, 2H), 8.07 (d, 6.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.48, 55.43, 70.70, 81.13, 102.70, 114.12, 128.41, 128.66, 128.91, 130.02, 136.38, 136.49, 161.82, 162.09, 164.20, 165.43. HRMS: m/z $[M+Na]^+$ for $C_{20}H_{16}ClN_3NaO^+$, calculated 372.0874; observed: 372.0874.

4-(3-nitrophenyl)-N-(prop-2-yn-1-yl)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine (NV-9)

Brown solid, 78% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (t, 2.4 Hz, 1H), 3.93 (s, 3H), 4.00 (s, 6H), 4.42 (dd, 3.2 Hz, 2.4 Hz, 2H), 5.47 (t, 5.2 Hz, 1H), 7.36 (s, 2H), 7.44 (s, 1H), 7.70 (t, 8.0 Hz, 1H), 8.35 (dq, 4.8 Hz, 1.2 Hz, 1H), 8.47 (d, 7.6 Hz, 1H), 8.95 (t, 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.57, 56.42, 61.01, 70.88, 80.79, 103.40, 104.65, 122.15, 124.98, 129.76, 132.70, 133.00, 139.58, 140.78, 148.75, 153.56, 162.08. HRMS: m/z $[M+Na]^+$ for $C_{22}H_{20}N_4NaO_5^+$, calculated 443.1326; observed: 443.1316; HPLC percentage purity: 95.53 %

4-(3-bromophenyl)-N-(prop-2-yn-1-yl)-6-(p-tolyl)pyrimidin-2-amine (NV-10)

White solid, 75% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (t, 3.2 Hz, 1H), 2.42 (s, 3H), 4.39 (dq, 2.4 Hz, 0.8 Hz, 2H), 5.40 (d, 4.0 Hz, 1H), 7.29 (d, 8.0 Hz, 2H), 7.35 (t, 8.0 Hz, 1H), 7.41 (d, 0.4 Hz, 1H), 7.60 (dq, 4.8 Hz, 0.8 Hz, 1H), 8.00 (d, 8.4 Hz, 3H), 8.24 (t, 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.61, 31.60, 70.91, 81.17, 103.51, 123.10, 125.79, 127.19, 129.62, 130.31, 130.35, 133.38, 134.74, 140.04, 141.16, 162.18. HRMS: m/z $[M+H]^+$ for $C_{20}H_{17}BrN_3^+$, calculated 378.0600; observed: 378.0572

4-(3-bromophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine (NV-11)

Off-white solid, 68% Yield; ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.50 (t, 2.4 Hz, 1H), 5.18 (d, 2.4 Hz, 2H), 7.39 (t, 7.8 Hz, 1H), 7.53 (dd, 3.6 Hz, 1.8 Hz, 3H), 7.64 (dq, 5.4 Hz, 1.2 Hz, 1H), 7.78 (s, 1H), 8.09 (dt, 4.8 Hz, 1.8 Hz, 1H), 8.17 (m, 2H), 8.32 (t, 1.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 55.10, 74.65, 78.62, 107.09, 123.18, 125.90, 127.40, 128.94, 130.39, 130.41, 131.33, 133.97, 136.43,138.76, 164.78, 165.67, 167.56. HRMS: m/z $[M+Na]^+$ for $C_{19}H_{13}BrN_2NaO^+$, calculated 387.0103; observed: 387.0075.

4,6-bis(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine (NV-12)

Brown solid, 77% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 1H), 3.88 (s, 6H), 5.16 (d, 2 Hz, 2H), 7.01 (d, 8.8 Hz, 4H), 7.69 (s, 1H), 8.14 (d, 8.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.74, 55.43, 74.32, 78.97, 105.27, 114.14, 128.86, 129.24, 162.03, 164.58, 166.30. HRMS: m/z $[2M+Na]^+$ for $C_{42}H_{36}N_4NaO_6^+$, calculated 715.2527; observed: 715.2463.

4-(4-chlorophenyl)-6-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine (NV-13)

White solid, 76% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (t, 2.0 Hz, 1H), 3.90 (s, 3H), 5.17 (d, 2.4 Hz, 2H), 7.03 (d, 9.2 Hz, 2H), 7.49 (d, 8.8 Hz, 2H), 7.73 (s, 1H), 8.12 (d, 8.8 Hz, 2H), 8.16 (d, 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.92, 55.47, 74.50, 78.53, 105.93, 114.25, 128.60, 128.90, 128.98, 129.10,135.28, 137.17, 162.28, 164.68, 165.59, 166.91. HRMS: m/z $[M+H]^+$ for $C_{20}H_{16}ClN_2O_2^+$, calculated 351.0895; observed: 351.0929

4-(4-methoxyphenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine (NV-14)

Off-white solid, 81% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 1H), 3.89 (s, 1H), 5.17 (d, 2.0 Hz, 2H), 7.02 (d, 8.8 Hz, 2H), 7.52 (d, 2.4 Hz, 3H), 7.76 (s, 1H), 8.16 (d, 8.4 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.84, 55.44, 74.40, 78.88, 106.17, 114.19, 127.29, 128.83, 128.93,129.06, 130.96,136.84, 162.15, 164.66, 166.66, 166.80. HRMS: m/z $[M+Na]^+$ for $C_{20}H_{16}N_2NaO_2^+$, calculated 339.1104; observed: 339.1085.

4-(2,4-dichlorophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine (NV-15)

White solid, 72% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 1H), 5.16 (d, 2.0 Hz, 2H), 7.40 (dd, 5.2 Hz, 1.6 Hz, 1H), 7.52 (d, 4.4 Hz, 3H), 7.54 (d, 1.2 Hz, 1H), 7.75 (d, 6.8 Hz, 1H), 7.84 (s, 1H), 8.15 (t, 2.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.11, 74.65, 78.62, 111.83, 127.49, 127.65, 128.97, 130.32, 131.39, 132.56, 133.09, 135.23, 136.30, 136.36, 164.62, 165.69, 166.66. HRMS: m/z $[M+H]^+$ for $C_{19}H_{13}C_{l2}N_2O^+$, calculated 355.0399; observed: 355.0446

4-(4-bromophenyl)-6-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine (NV-16)

White solid, 70% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (t, 2.4 Hz, 1H), 3.89 (s, 3H), 5.17 (d, 2.4 Hz, 2H), 7.02 (d, 9.2 Hz, 2H), 7.65 (d, 8.8 Hz, 2H), 7.73 (s, 1H), 8.04 (d, 8.4 Hz, 2H), 8.16 (d, 9.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.92, 55.47, 74.50, 78.75, 105.89, 114.25, 125.63, 128.82, 128.89, 128.99, 132.07, 135.75, 162.30, 164.70, 165.67, 166.95. HRMS: m/z $[M+H]^+$ for $C_{20}H_{16}BrN_2O_2^+$, calculated 395.0390; observed: 395.0403

4-(3-bromophenyl)-2-(prop-2-yn-1-yloxy)-6-(p-tolyl)pyrimidine (NV-17)

Brownish powder, 74% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 2.50 (t, 2.4 Hz, 1H), 5.17 (d, 2.4 Hz, 2H), 7.32 (d, 8.0 Hz, 2H), 7.38 (8.4 Hz, 1H), 7.64 (dq, 5.2 Hz, 0.8 Hz, 1H), 7.76 (s, 1H), 8.08 (d, 8.0 Hz, 3H), 8.31 (t, 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.65, 55.17, 74.74, 78.79, 106.85, 123.26, 126.00, 127.44, 129.80, 130.49, 130.50, 133.70, 134.00, 138.95, 142.00, 164.83, 165.57, 167.60. HRMS: m/z $[M+H]^+$ for $C_{20}H_{16}BrN_2O^+$, calculated 379.0441; observed: 379.0439

4-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)-6-(p-tolyl)pyrimidine (NV-18)

White solid, 71% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 2.48 (t, 2.4 Hz, 1H), 3.88 (s, 3H), 5.16 (d, 2.4 Hz, 2H), 7.01 (d, 8.8 Hz, 2H), 7.31 (d, 8.0 Hz, 2H), 7.73 (s, 1H), 8.06 (d, 8.0 Hz, 2H), 8.15 (d, 9.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.61, 54.91, 55.56, 74.48, 79.07, 105.92, 114.29, 127.34, 129.03, 129.30, 129.69, 134.14, 141.53, 162.21, 164.76, 166.59, 166.88. HRMS: m/z $[M+H]^+$ for $C_{21}H_{19}N_2O_2^+$, calculated 331.1441; observed: 331.1440

4-(4-chlorophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine (NV-19)

Light brown solid, 80% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 1H), 5.18 (d, 2H), 7.49 (d, 6.4 Hz, 2H), 7.53 (s, 3H), 7.79 (s, 1H), 8.12 (d, 6.4 Hz, 2H), 8.17 (d, 3.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.16, 74.72, 78.81, 106.95, 127.50, 128.78, 129.06, 129.29, 131.40, 135.25,

136.66, 137.49, 164.91, 166.11, 167.59. HRMS: m/z $[M+Na]^+$ for $C_{19}H_{13}ClN_2NaO^+$, calculated 443.0609; observed: 443.0617.

4-(3-nitrophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine (NV-20)

Light brown solid, 79% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (t, 2.4 Hz, 1H), 5.21 (d, 2.4 Hz, 2H), 7.56 (dd, 3.2 Hz, 2.0 Hz, 3H), 7.73 (t, 8.0 Hz, 1H), 7.90 (s, 1H), 8.21 (dd, 3.2 Hz, 2.0 Hz, 2H), 8.39 (dq, 4.8 Hz, 1.2 Hz, 1H), 8.56 (dt, 4.8 Hz, 1.2 Hz, 1H), 9.00 (t, 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.41, 74.97, 78.57, 107.31, 122.40, 125.70, 127.61, 129.18, 130.18, 131.75, 133.33, 136.28, 138.63, 148.91, 164.76, 165.03, 168.26. HRMS: m/z $[M+H]^+$ for $C_{19}H_{14}N_3O_3^+$, calculated 332.1030; observed: 332.1030

4-(3-bromophenyl)-6-(3,4-dimethoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine (NV-21)

Yellow solid, 75% Yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (t, 2.0 Hz, 1H), 3.97 (s, 3H), 4.01 (s, 3H), 5.17 (d, 2.0 Hz, 2H), 6.98 (d, 6.8 Hz, 1H), 7.39 (t, 6.4 Hz, 1H), 7.64 (d, 6.4 Hz, 1H), 7.74 (dd, 5.2 Hz, 1.6 Hz, 2H), 7.83 (d, 1.6 Hz, 1H), 8.08 (d, 6.4 Hz, 1H), 8.31 (t, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.18, 56.21, 56.28, 74.68, 78.89, 106.55, 110.34, 111.07, 120.77, 123.27, 126.01, 129.33, 130.50, 133.98, 139.07, 149.54, 152.12, 164.81, 165.36, 167.29. HRMS: m/z $[M+H]^+$ for $C_{21}H_{18}BrN_2O_3^+$, calculated 425.0495; observed: 425.0528

Biological Studies

Determination of acetylcholinesterase and MAO inhibition activities

Elleman's assay kit for acetylcholinesterase activity was purchased from the Molecular Probes, Inc./Invitrogen. Briefly, an adequate volume of acetylcholinesterase (0.5 U/ml) was incubated with three different concentrations of the test compounds in a CO_2 incubator for 30 min. at 37 °C in flat-bottom 96-well plates (Tarsons). After incubation period of 30 minutes, appropriate volumes of dye (DTNB) and substrate solution was added to above 96-well plates. The inhibition assays for the calculation of IC_{50} values were performed with a final substrate concentration of 2 mM. Donepezil was used as a positive control. A multiplate reader was used to record the absorbance spectra at 417 nm. The experiments were performed in triplicates (n = 3). All the synthesized compounds were then evaluated for their inhibitory potential against both the isoforms of MAO (A and B) using Amplex Red assay kit. Fluorometric method was performed as described by our previous experiments.[35] In a similar way to AChE, an adequate amount of enzyme (hMAO-

A/B) was incubated with three different concentrations of tested compounds for 30 min. at 37 °C in a flat-bottom 96 well plate. After 30 min. of incubation period, a 50 µl working solution containing Amplex red, horseradish peroxidase and substrate tyramine was added to start the reaction with uninhibited enzyme. After a suitable incubation of the mixture, H_2O_2 production was quantified using microplate fluorescence reader (TECAN instrument) at an excitation wavelength of 545 nm and emission wavelength of 590 nm. Clorgyline and pargyline were used as standards for MAO-A and MAO-B respectively. The final fluorescence calculations were done after subtracting the blank from the readings. The protocols were performed in triplicates (n= 3). IC_{50} values for the compounds were calculated and reported in micromolar range. The detailed procedure for these assays is reported in our previous reports.[35]

Reversibility inhibition and kinetics of AChE inhibition studies

Dilution methodology was adopted to study the reversibility inhibition of the leads. Briefly, the leads were incubated with the enzymes at concentrations of 10 x IC_{50} and 100 x IC_{50} for 30 min at 37 ºC. The samples were subsequently diluted with buffer and substrate to attain a final inhibitor concentration of 0.1 x IC_{50} and 1 x IC_{50} value, respectively. Finally, the experiments were performed in triplicate and enzyme activities were expressed as mean ± SD. Kinetic studies were performed to know the mechanism of AChE inhibition. The most potent lead (NV-9) was investigated. A double reciprocal Lineweaver-Burk graph was plotted at five different concentrations of substrate (0.25 – 6 mM) and three different final inhibitor concentrations (2 µM, 4 µM, 6 µM). 40 µL of recombinant enzyme (AChE, 0.5 U/mL) was added to each well during the experimentation along with substrate, inhibitors, and dye. Other details of the experiment are given in our previous reports.[35]

Neuroprotection and cytotoxicity studies

Neuroprotection potential and cytotoxicity evaluation of the leads was performed using neuronal cell lines, SH-SY5Y. The neuroprotective potential of the leads was accessed through the treatment with neurotoxin, 6-hydroxy dopamine (6-OHDA). The cells were cultured using a humidified atmosphere containing 5 % CO_2 at 37 ºC in a 96-well plate. Formation of the formazan byproduct was measured at a wavelength of 594 nm.[36] The experiments were performed in triplicate and repeated for validation. Cytotoxicity studies were also performed using the similar cell lines, SH-SY5Y. The cells were seeded at a density of approximately 10000 cells per well in 96 well plate.

The wells without compound treatment were treated as control. As an output of this assay, cellular reductases catalyse the conversion of MTT to blue formazan which is soluble in DMSO. The absorbance was recorded at 595 nm using a multiplate reader and finally the values were plotted as graphs. Further details of neuroprotection and cytotoxicity studies can be found in our previous reports.[35]

Molecular Docking Studies

Docking studies give critical insights into the ligand's spatial orientation and its interactions within the receptor binding sites. To elucidate the interaction mechanisms of the synthesized ligands at the active sites of hMAO-B and AChE enzymes, molecular docking[37] was performed using Maestro 12.8 (Schrödinger LLC). X-ray crystal structures of hMAO-B (PDB ID-2BYB) and AChE (PDB ID-1EVE) were imported from Protein Data Bank (PDB). The protein structure was prepared using the "Protein Preparation Wizard" tool. Energy minimization was performed using the OPLS2005 force field. Ligands were designed in ChemBioDraw Ultra-12 and subsequently prepared using the ligand preparation tool within the Schrödinger Suite 2017. The docking procedure was validated by redocking the co-crystallized ligand. The QikProp application was utilized to assess the drug-like properties and to calculate the ADME characteristics of the leads.

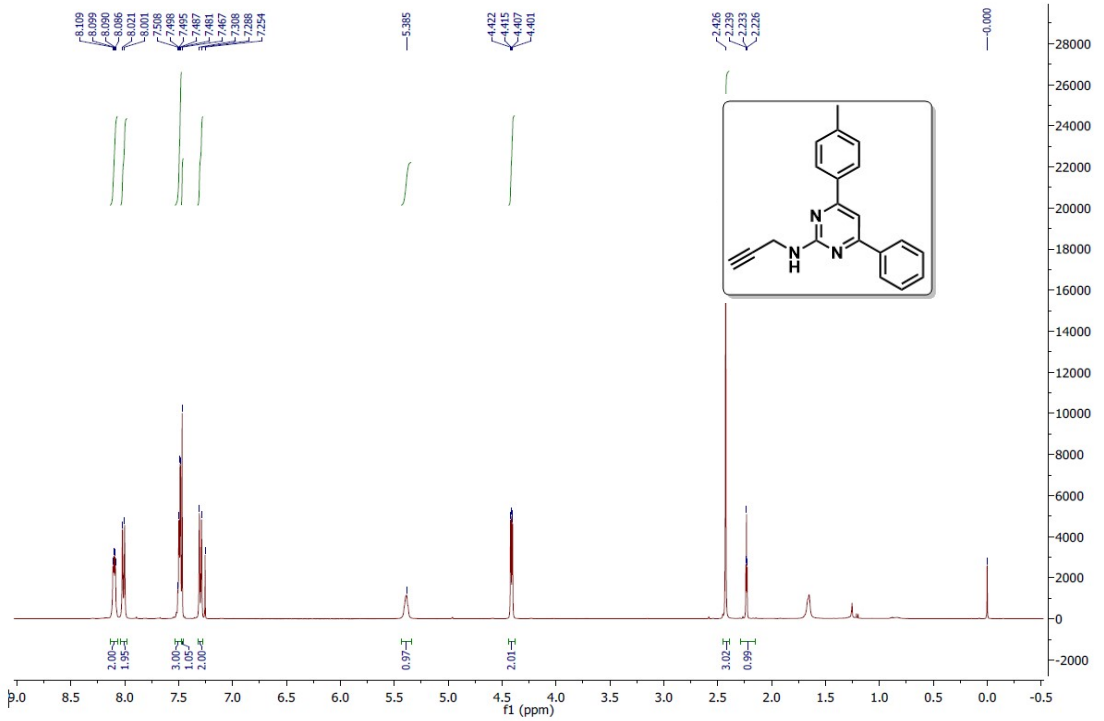
Molecular Dynamics Simulation Studies

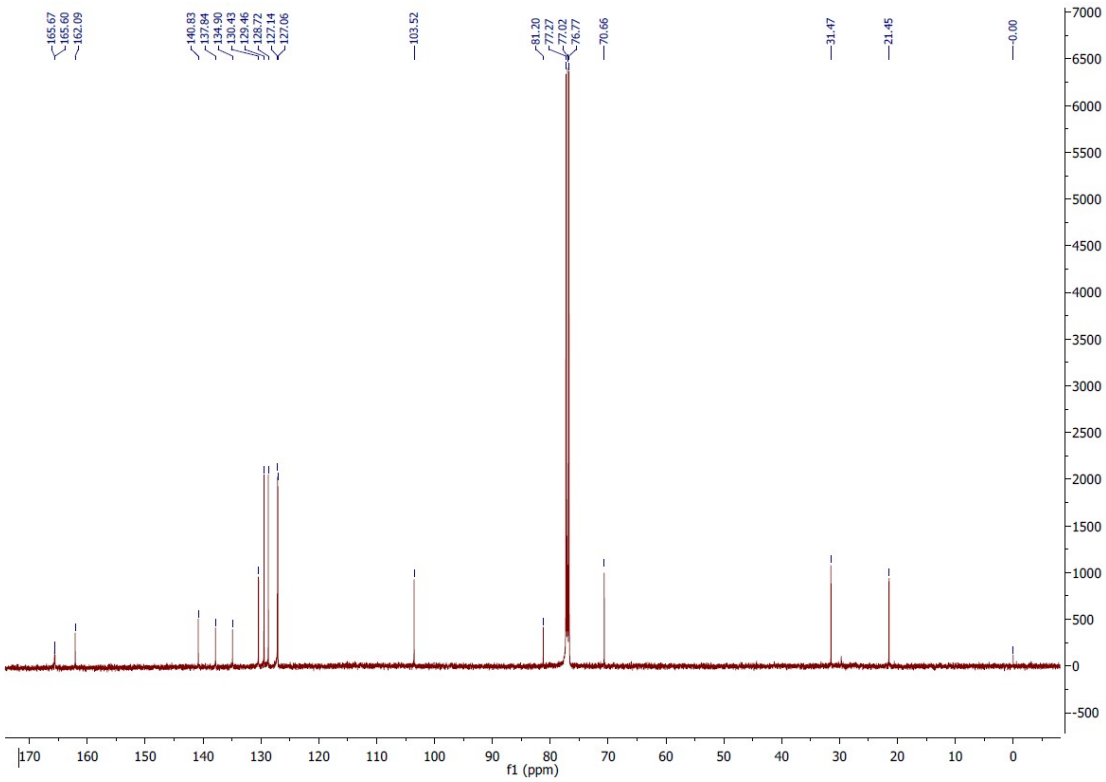
The potent leads of the series were investigated to check their stability with the active cavity for up to 100 ns. CHARMM36-Mar2019 force field[38] was utilized for the generation of complex topology with TIP3P model to solvate water box possessing dimension of at least 1.5 nm away from the surface of protein. Counter ions (Na^+ and Cl^-) were added in an appropriate amount to neutralize the system (protein + ligand). Three consecutive steps viz. energy minimization, NVT, and NPT equilibration were taken to minimize the system. Initially, the energy minimization was done using the steepest descent algorithm followed by NVT equilibration for 500 ps, where the protein was restrained with a fix temperature of 310 K using the velocity-rescaling algorithm having a coupling constant of 0.1 ps. The Berendsen barostat algorithm was applied for the NPT equilibration of the system for 500 ps at 1 bar of pressure and a coupling constant of 1 ps. Finally, the production run for 100 ns was performed. Meanwhile, the time step was set at 2 fs throughout the simulation process. The LINCS (LINear Constraint Solver) algorithm was used for bond’s

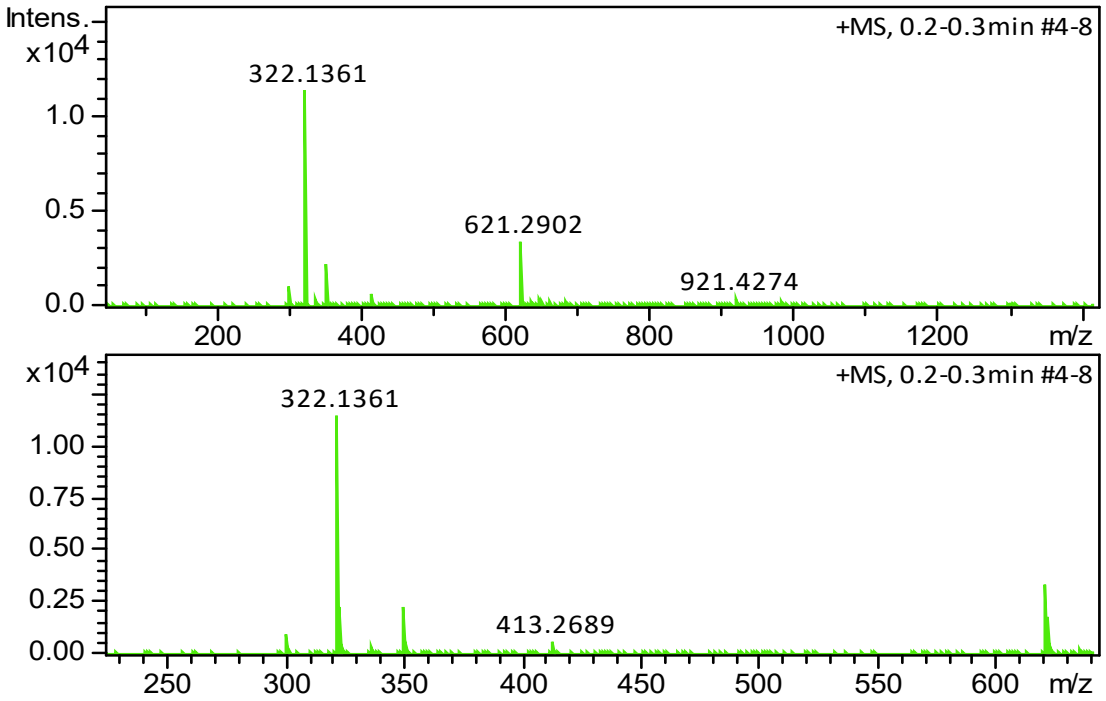
length constraints. The Particle mesh Ewald (PME) strategy was adopted with a cut-off radius of 1.2 nm and 0.16 nm grid spacing. The Van der Waals cut-off distance was set to 1 nm. Using the GROMACS analytic tools like rms, rmsf, and gyration; root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (rGy) were computed to analyze the trajectory.

Spectral data:

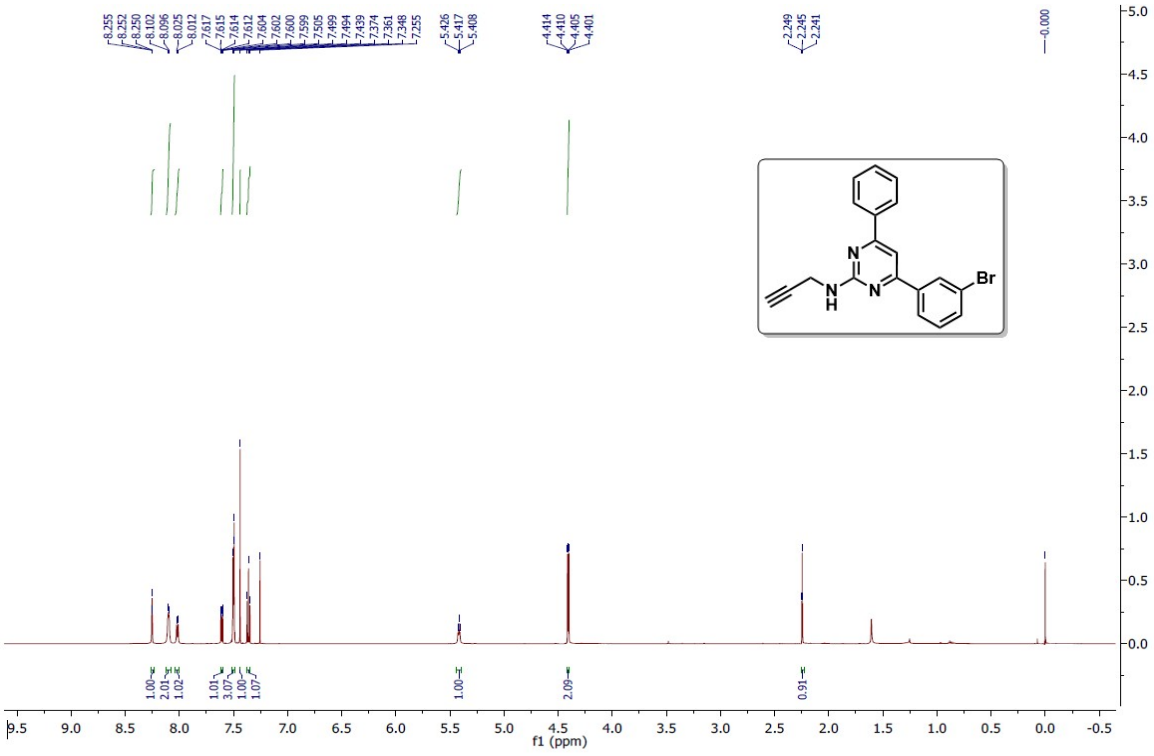
4-phenyl-N-(prop-2-yn-1-yl)-6-(p-tolyl)pyrimidin-2-amine **(NV-1)**

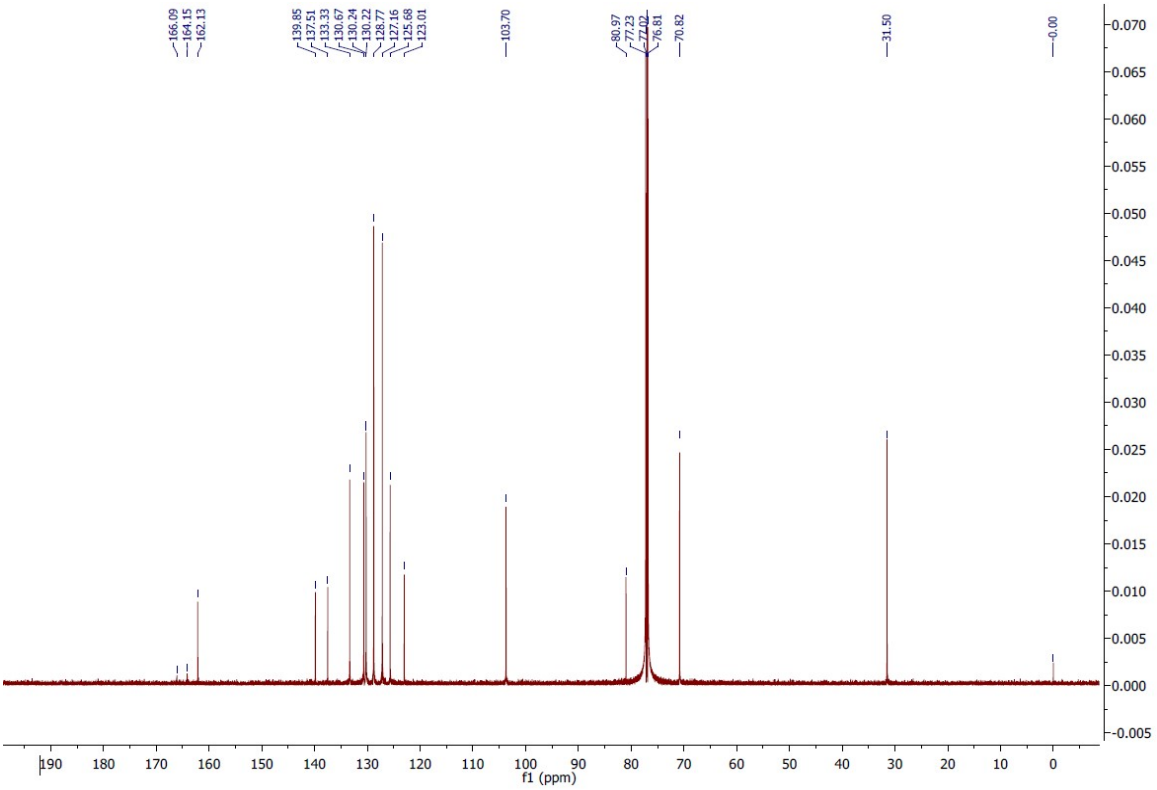






4-(3-bromophenyl)-6-phenyl-N-(prop-2-yn-1-yl)pyrimidin-2-amine **(NV-2)**

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164.15
162.13
139.85
137.51
133.33
130.67
130.24
128.77
127.16
125.68
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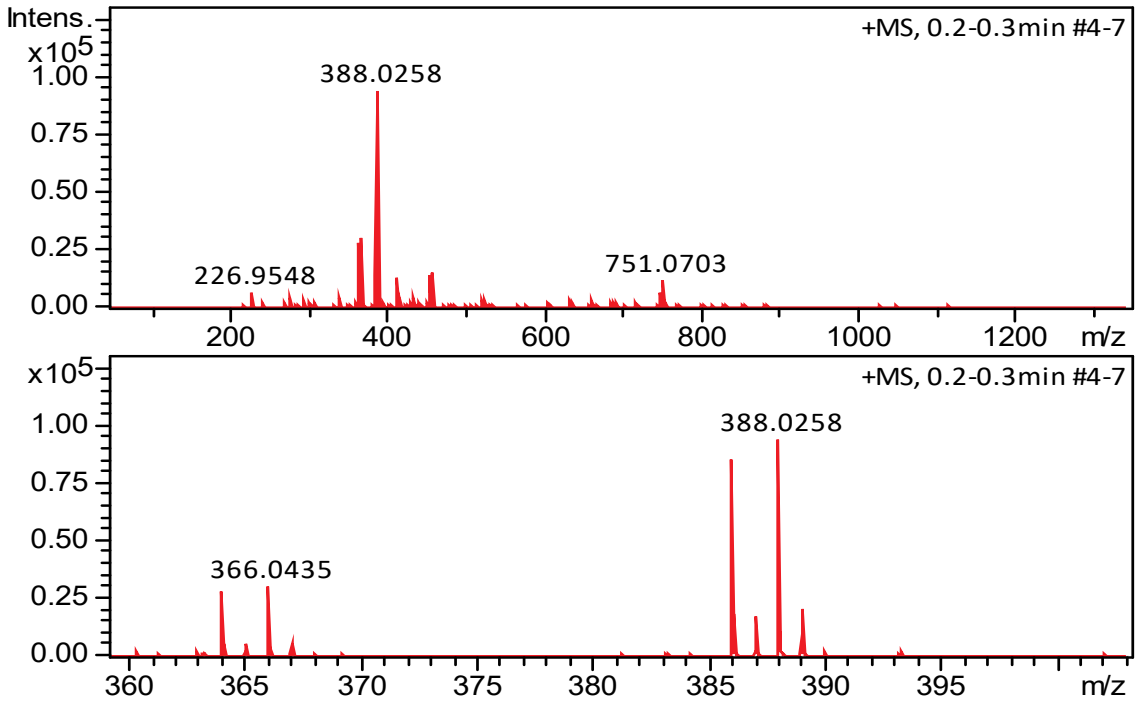
103.70
80.97
77.23
77.02
76.81
70.82
31.50
0.00
f1 (ppm)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x10^5
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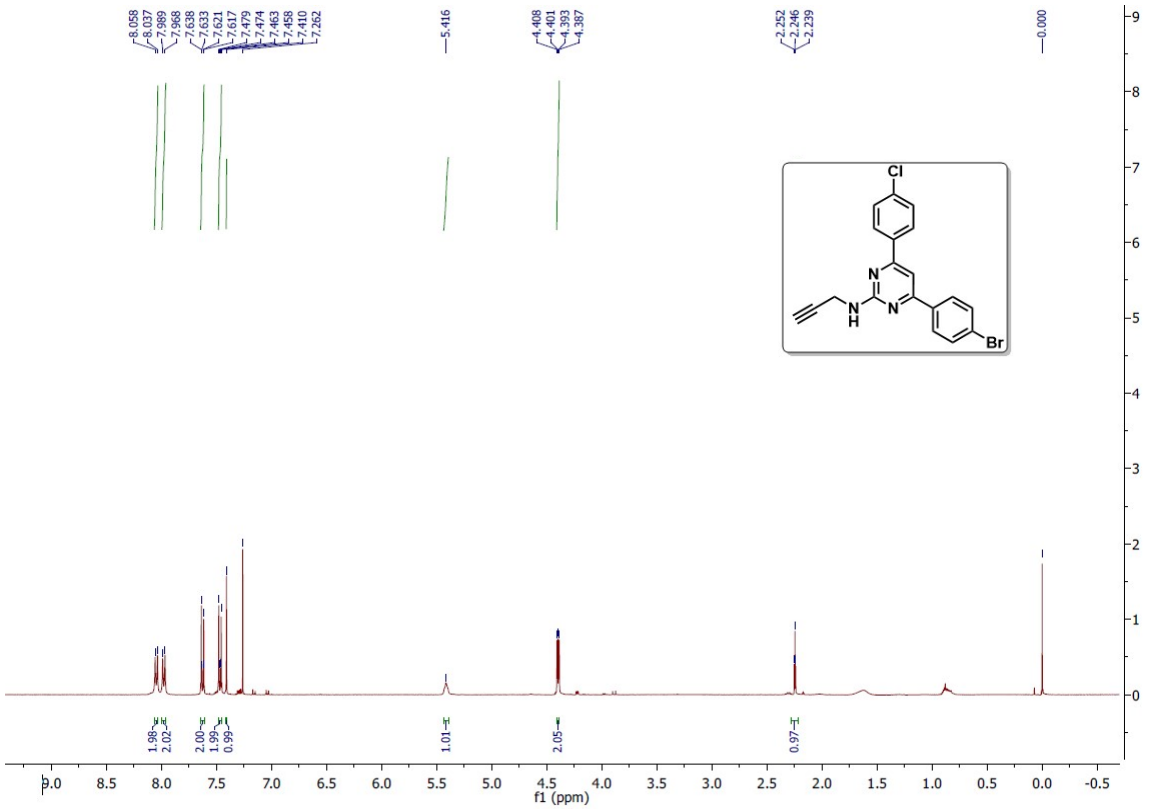
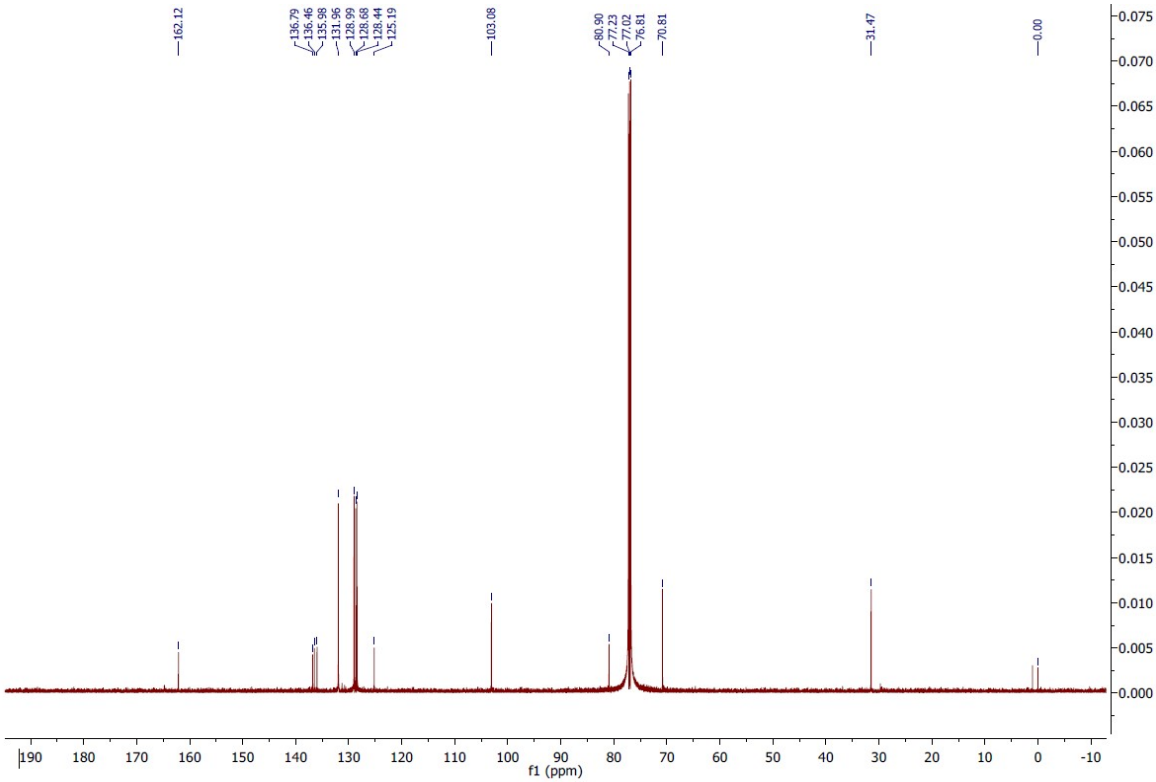
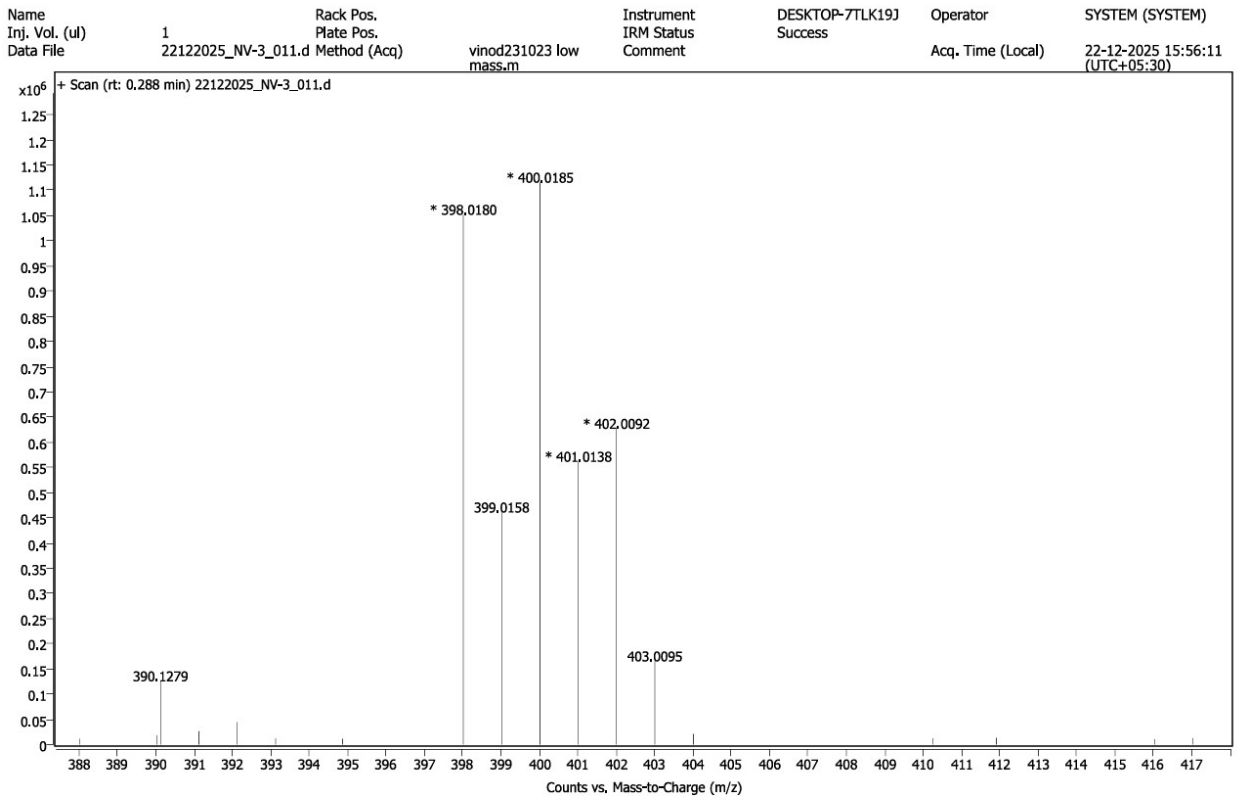
751.0703
m/z
+MS, 0.2-0.3min #4-7
388.0258
366.0435
m/z

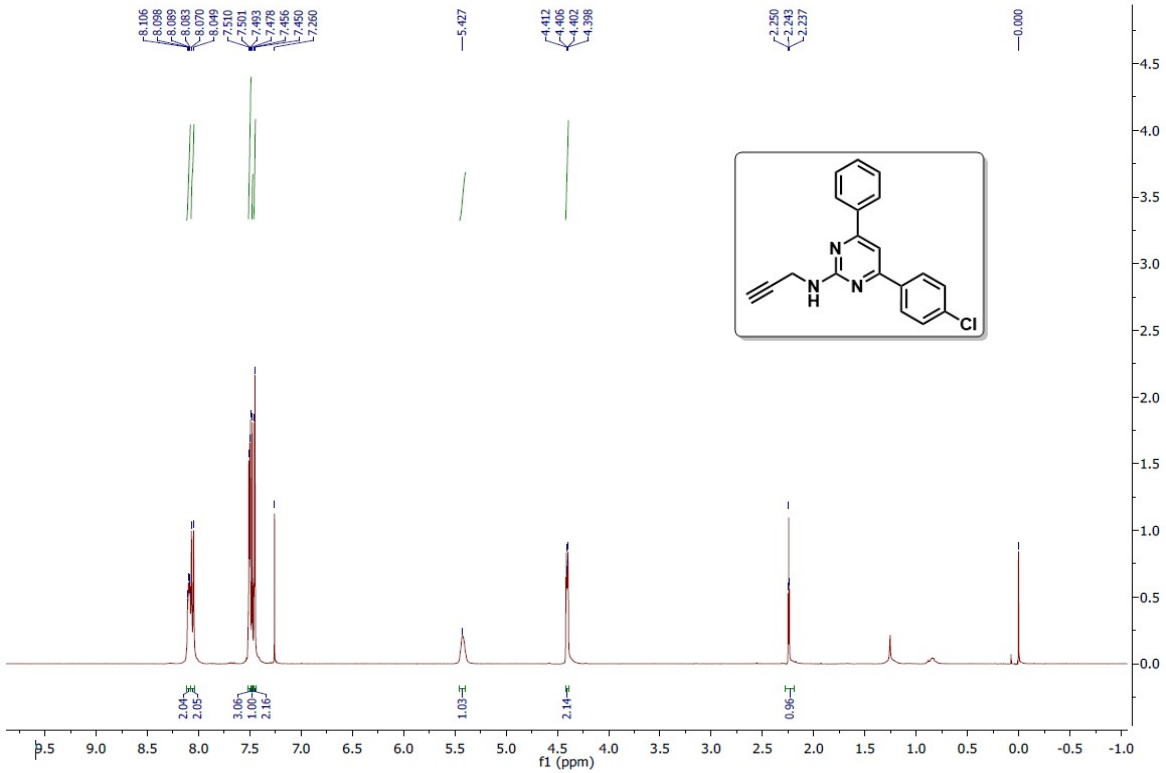
4-(4-bromophenyl)-6-(4-chlorophenyl)-N-(prop-2-yn-1-yl)pyrimidin-2-amine **(NV-3)**

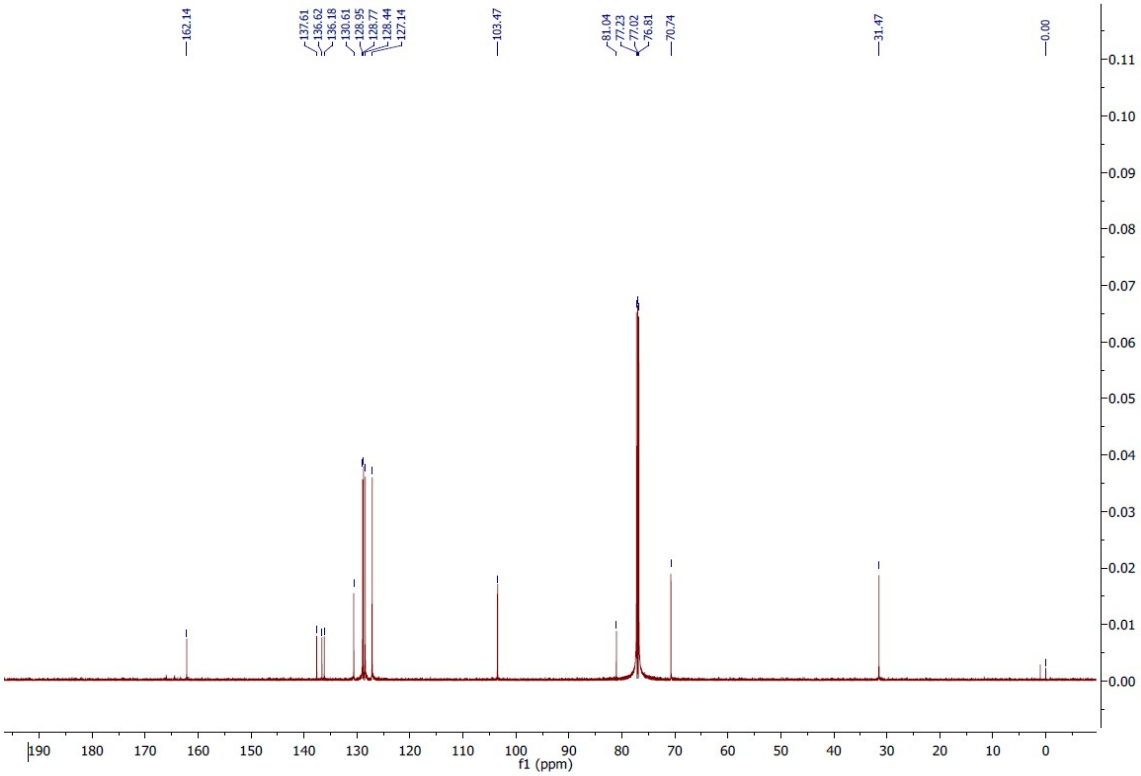






4-(4-chlorophenyl)-6-phenyl-N-(prop-2-yn-1-yl)pyrimidin-2-amine **(NV-4)**




162.14
137.61
136.62
136.18
130.61
128.95
128.77
128.44
127.14
103.47
81.04
77.23
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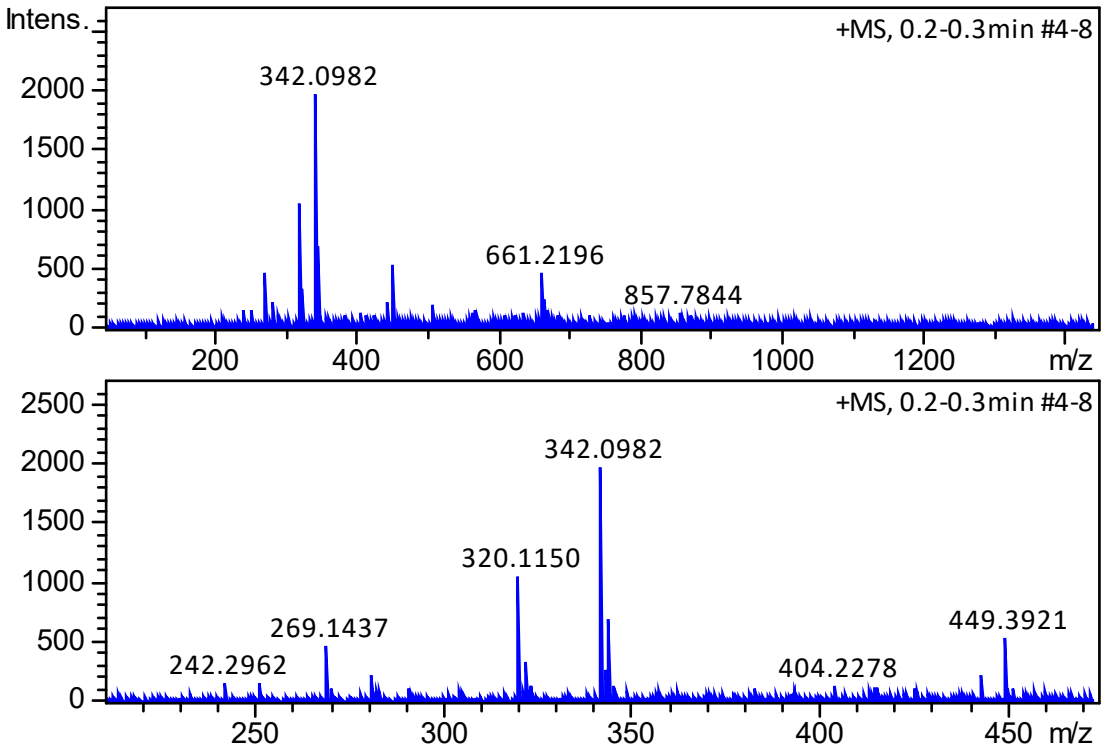
76.81
70.74
31.47
0.00
f1 (ppm)


Intens.
+MS, 0.2-0.3min #4-8
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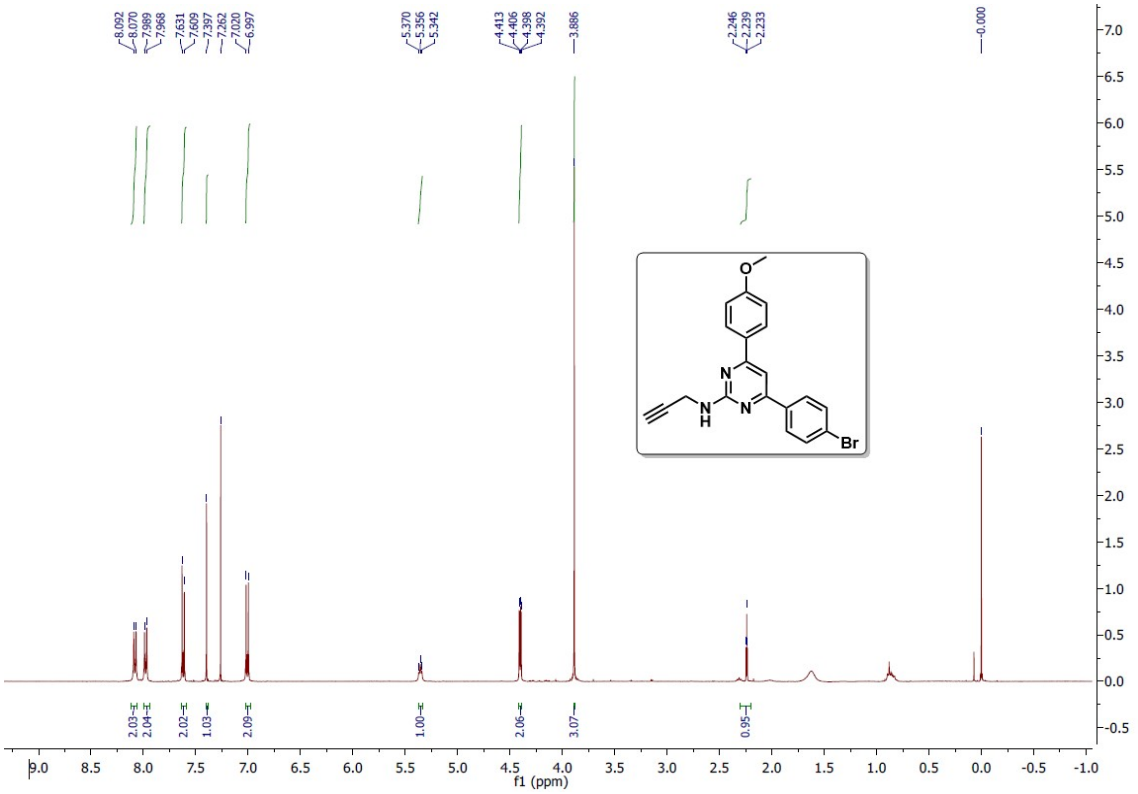
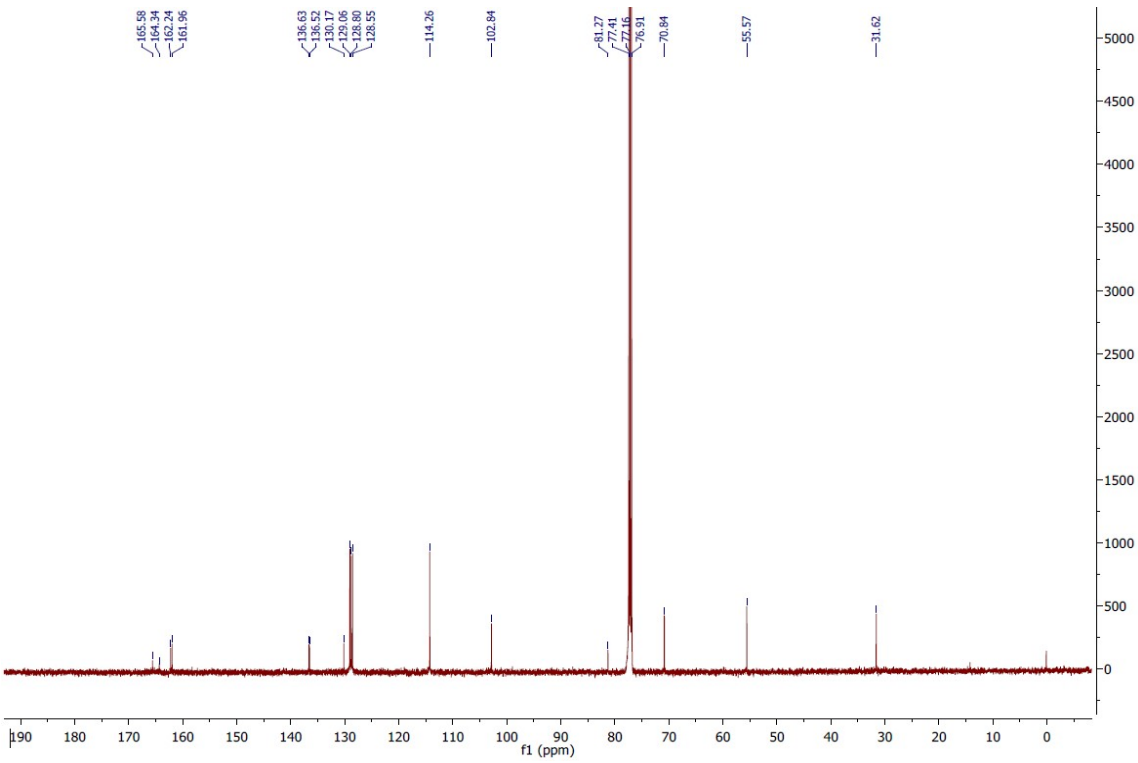
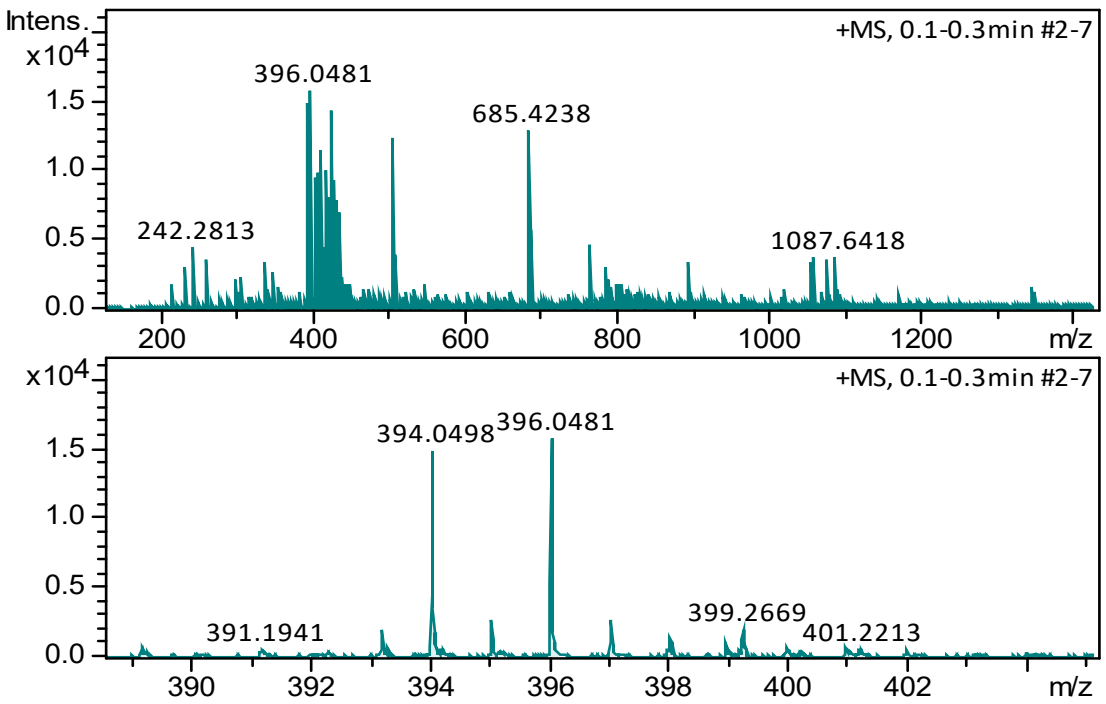
661.2196
857.7844
m/z
+MS, 0.2-0.3min #4-8
342.0982
320.1150
269.1437
242.2962
404.2278
449.3921
m/z

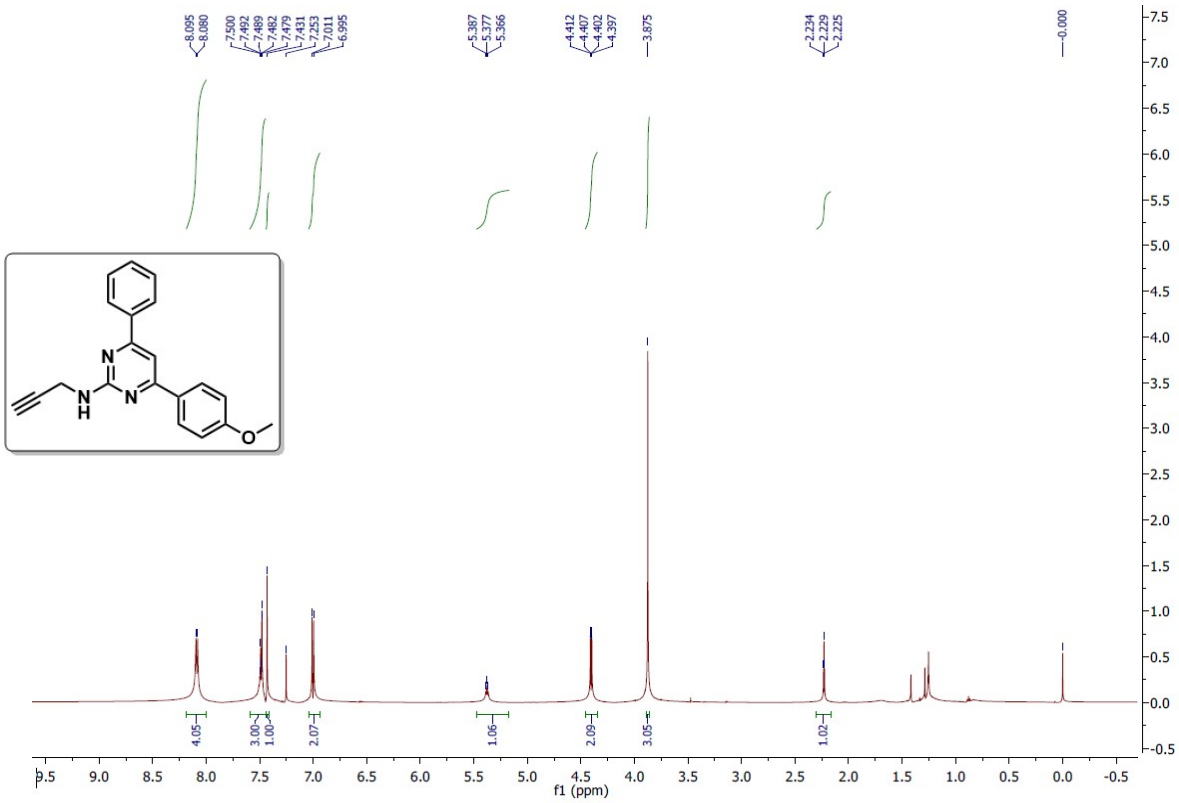
4-(4-bromophenyl)-6-(4-methoxyphenyl)-N-(prop-2-yn-1-yl)pyrimidin-2-amine **(NV-5)**

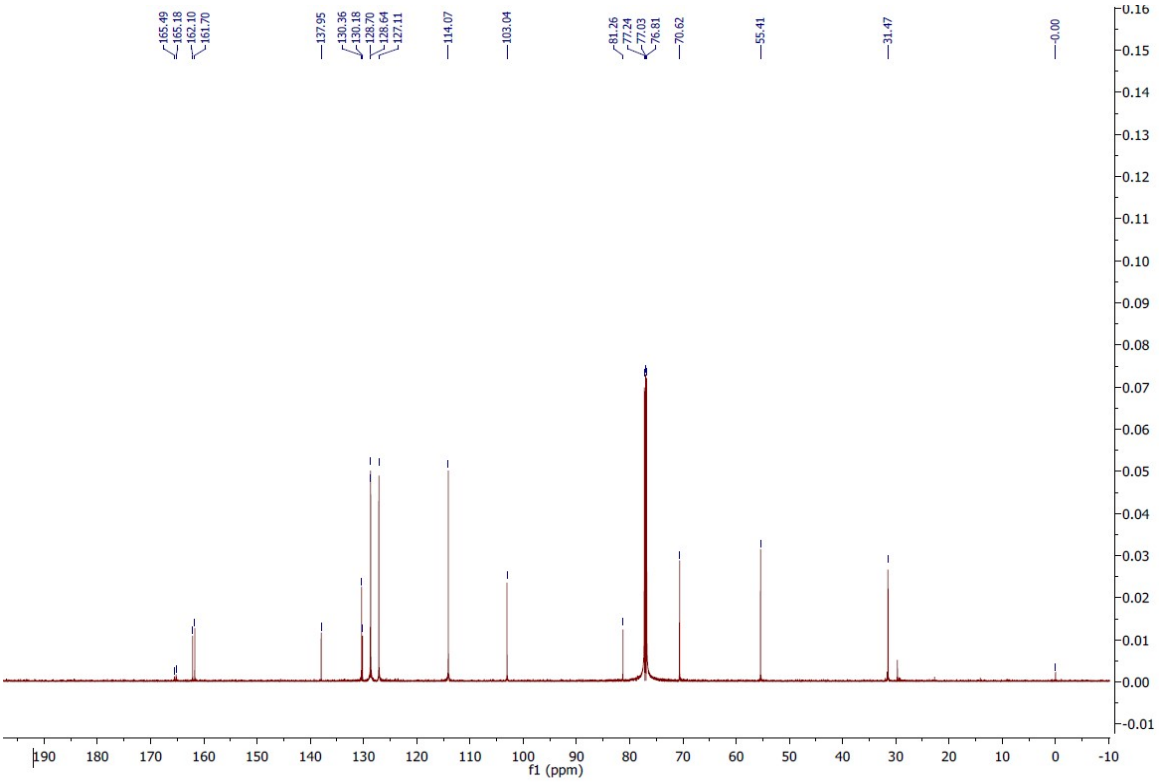






4-(4-methoxyphenyl)-6-phenyl-N-(prop-2-yn-1-yl)pyrimidin-2-amine **(NV-6)**

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165.18
162.10
161.70
137.95
130.36
130.18
128.70
128.64
127.11
114.07
103.04
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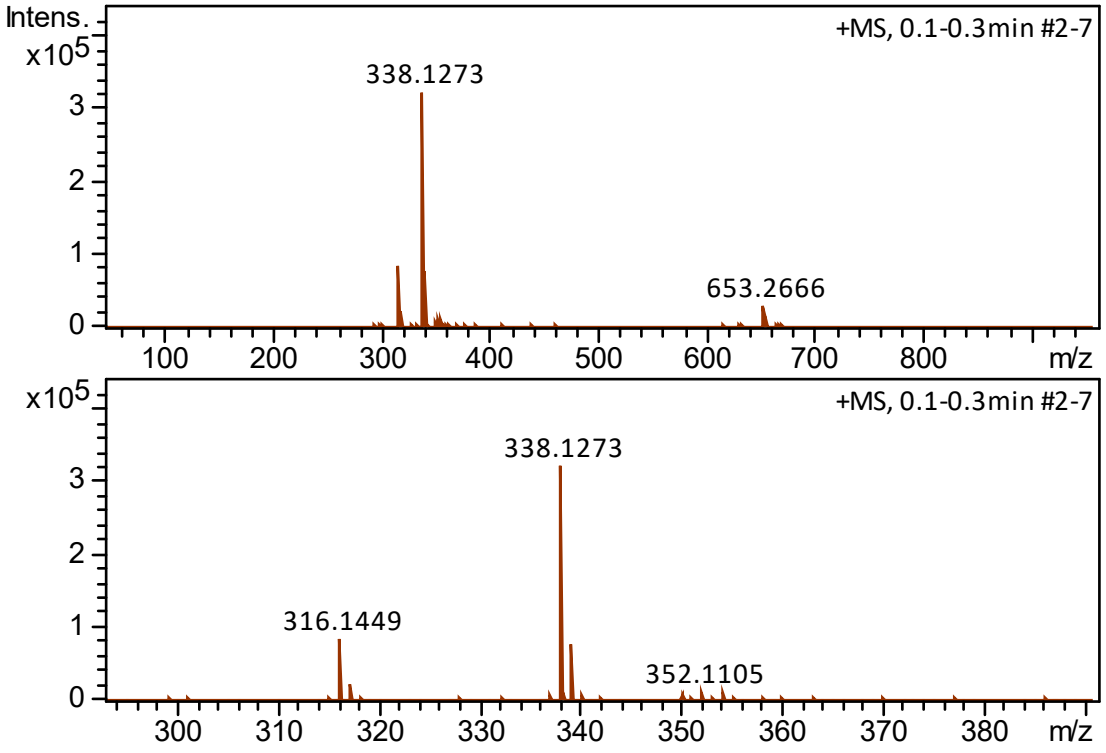
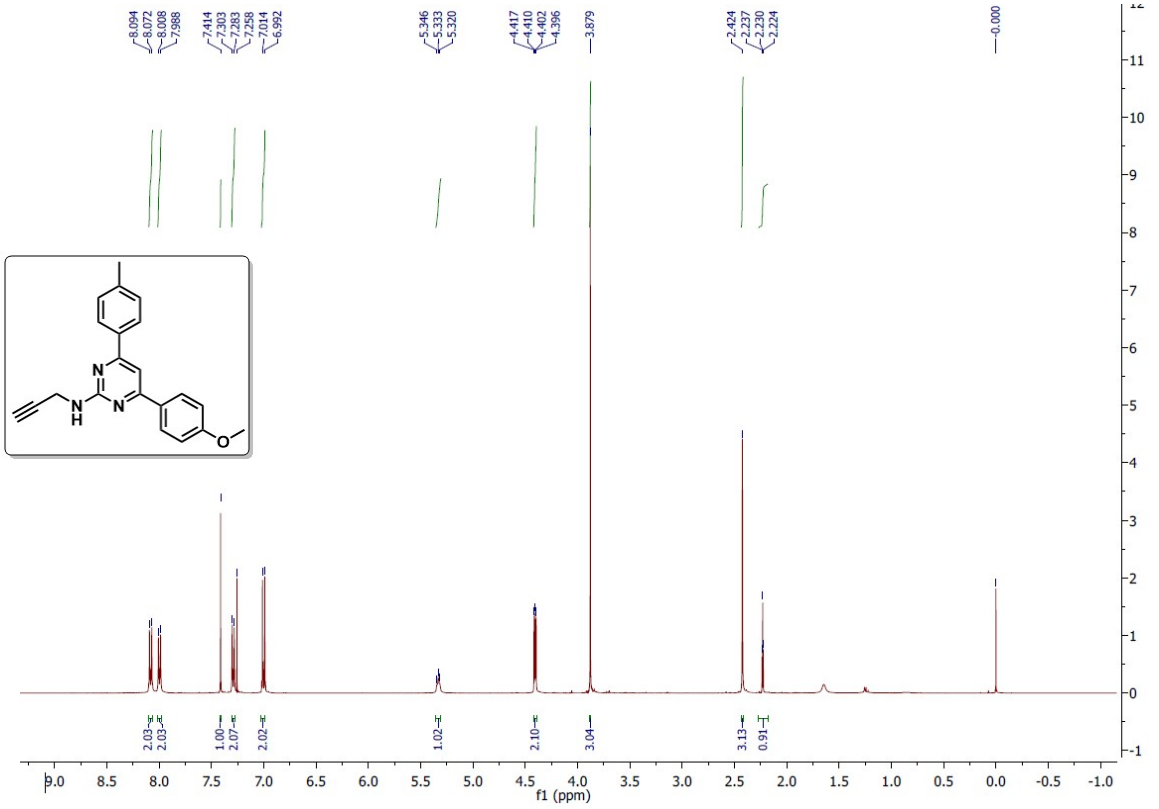
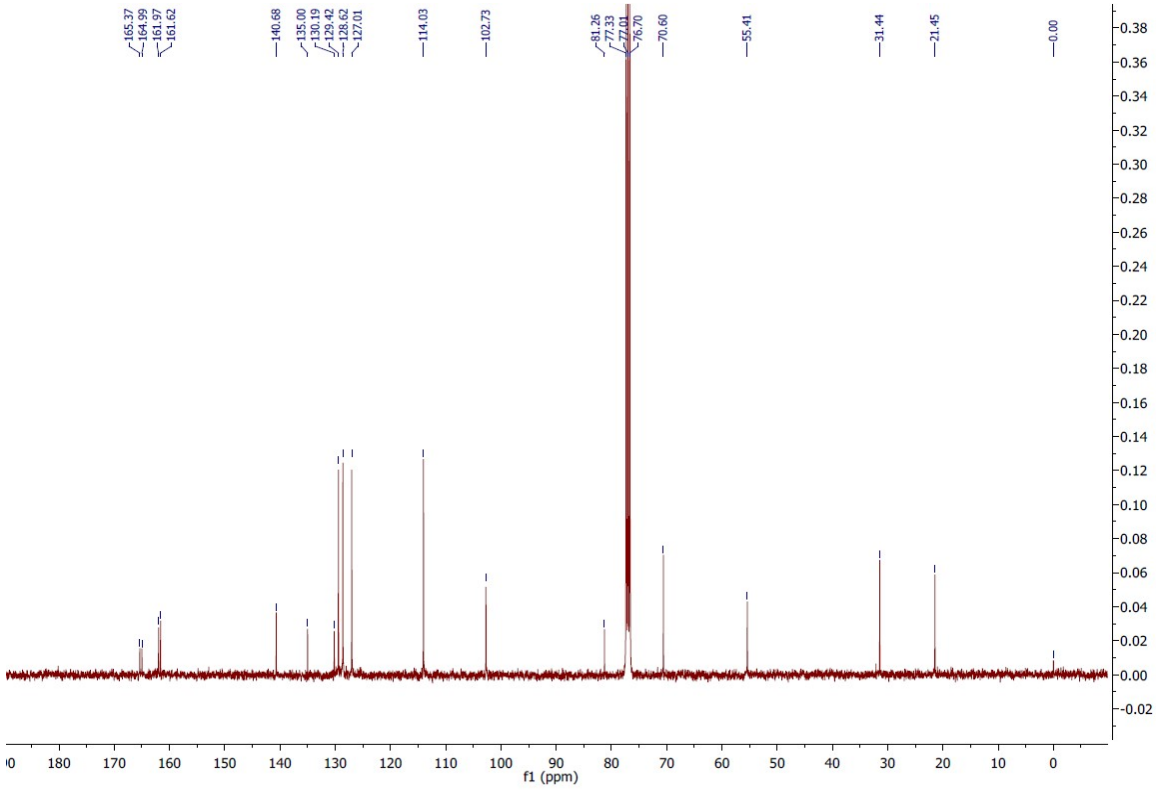
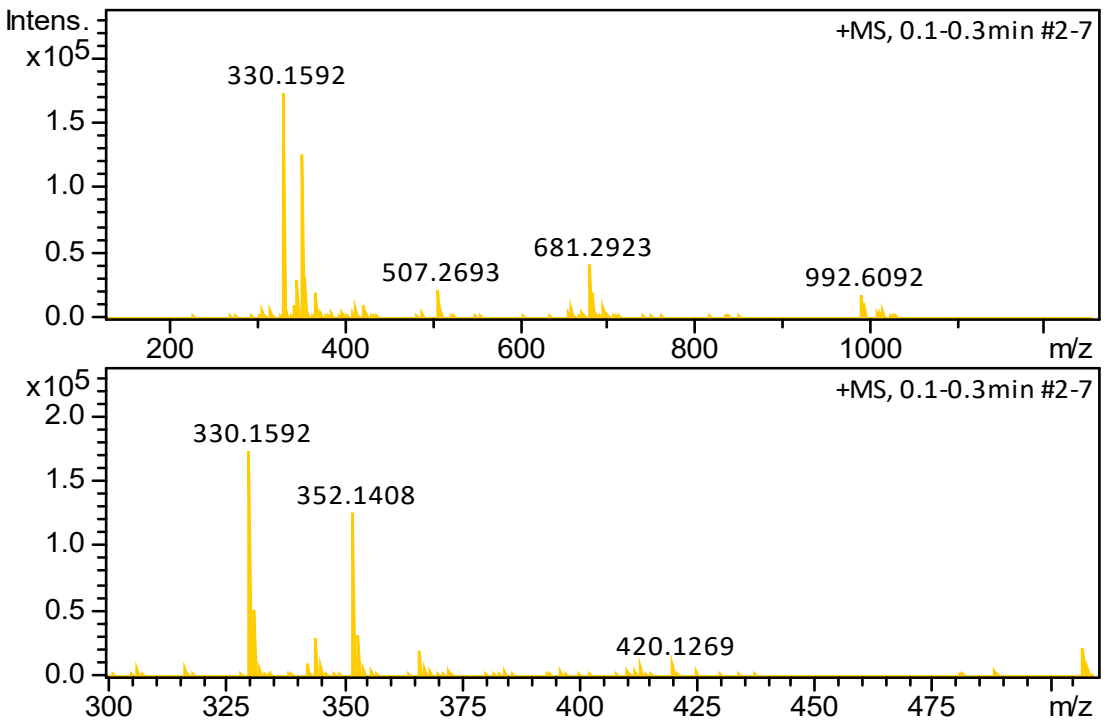
70.62
55.41
31.47
0.00
f1 (ppm)


Intens.
x10^5
+MS, 0.1-0.3min #2-7
338.1273
653.2666
m/z
x10^5
+MS, 0.1-0.3min #2-7
338.1273
316.1449
352.1105
m/z

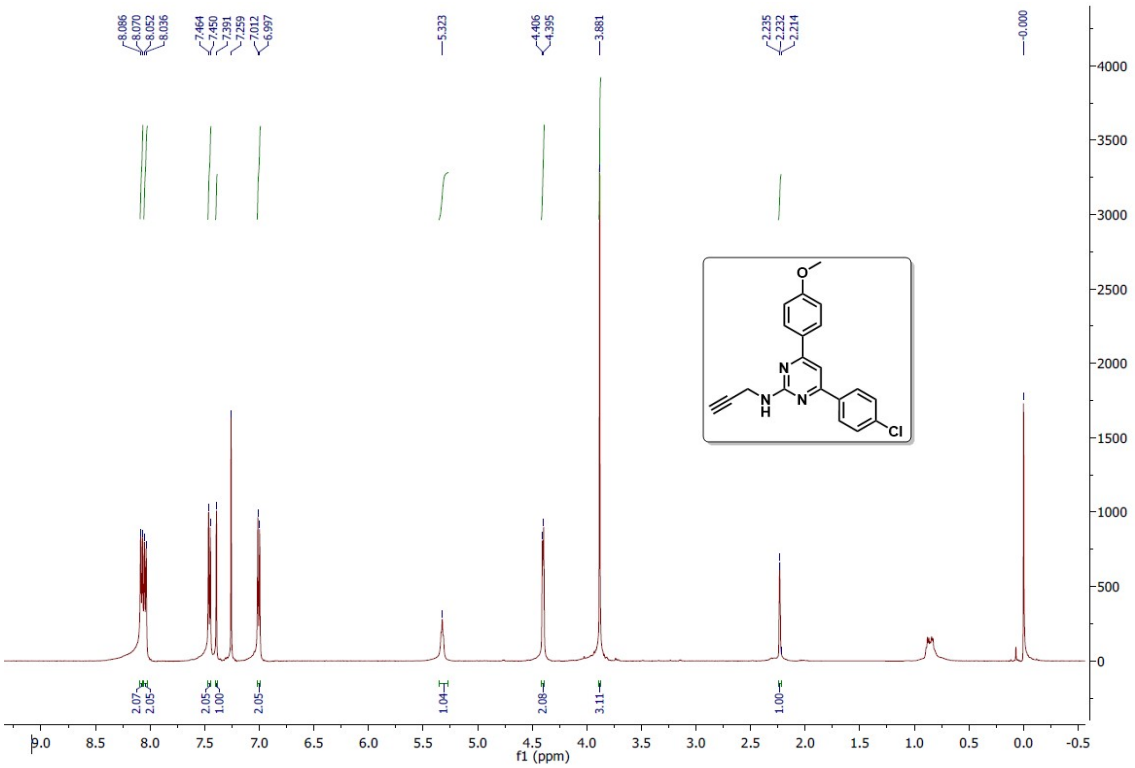
4-(4-methoxyphenyl)-N-(prop-2-yn-1-yl)-6-(p-tolyl)pyrimidin-2-amine **(NV-7)**

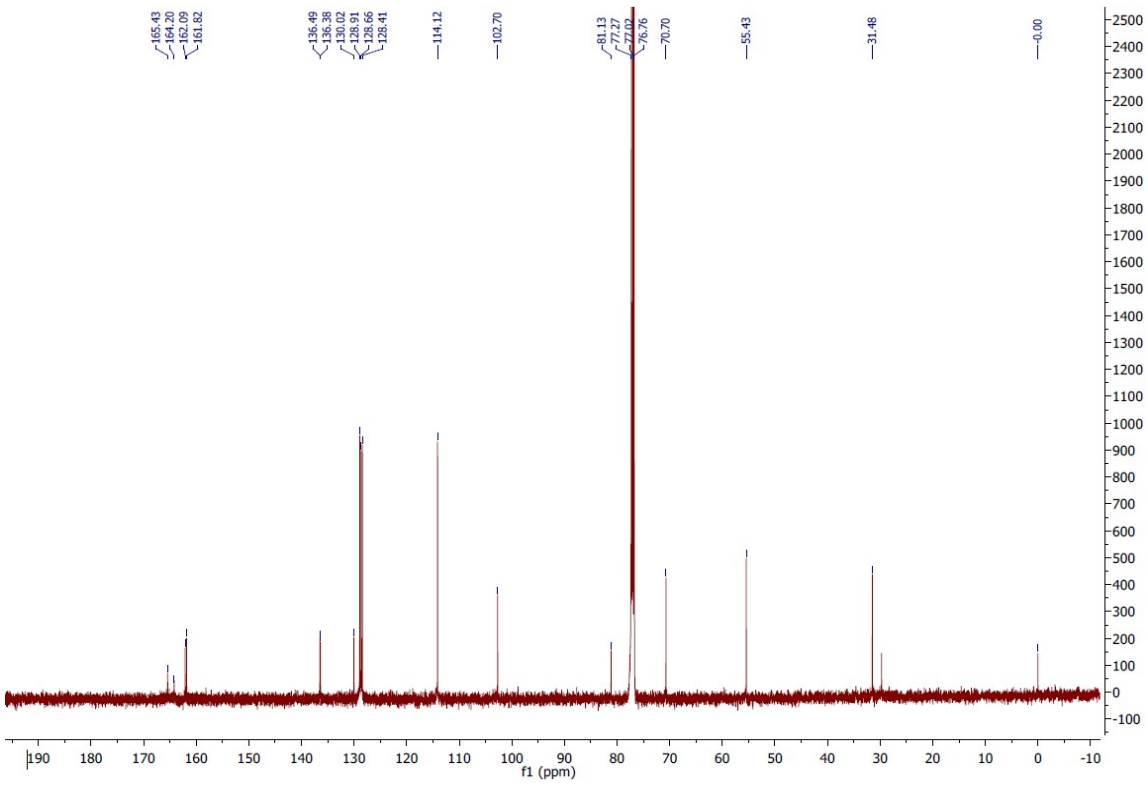


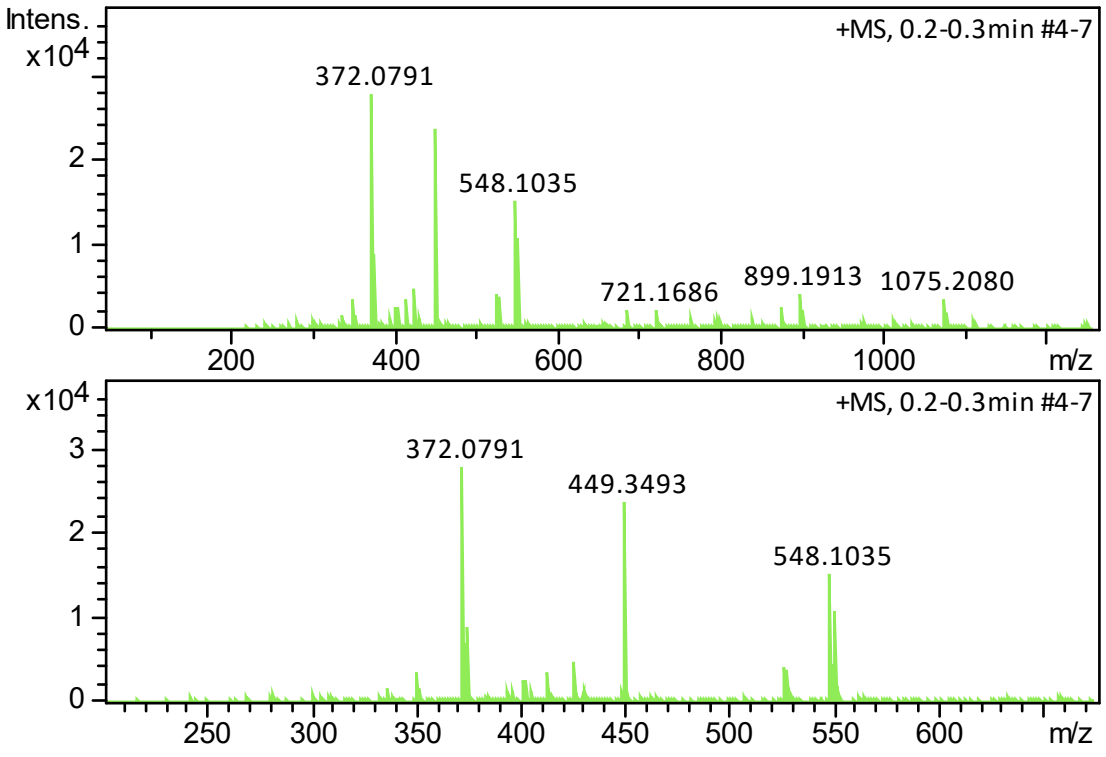




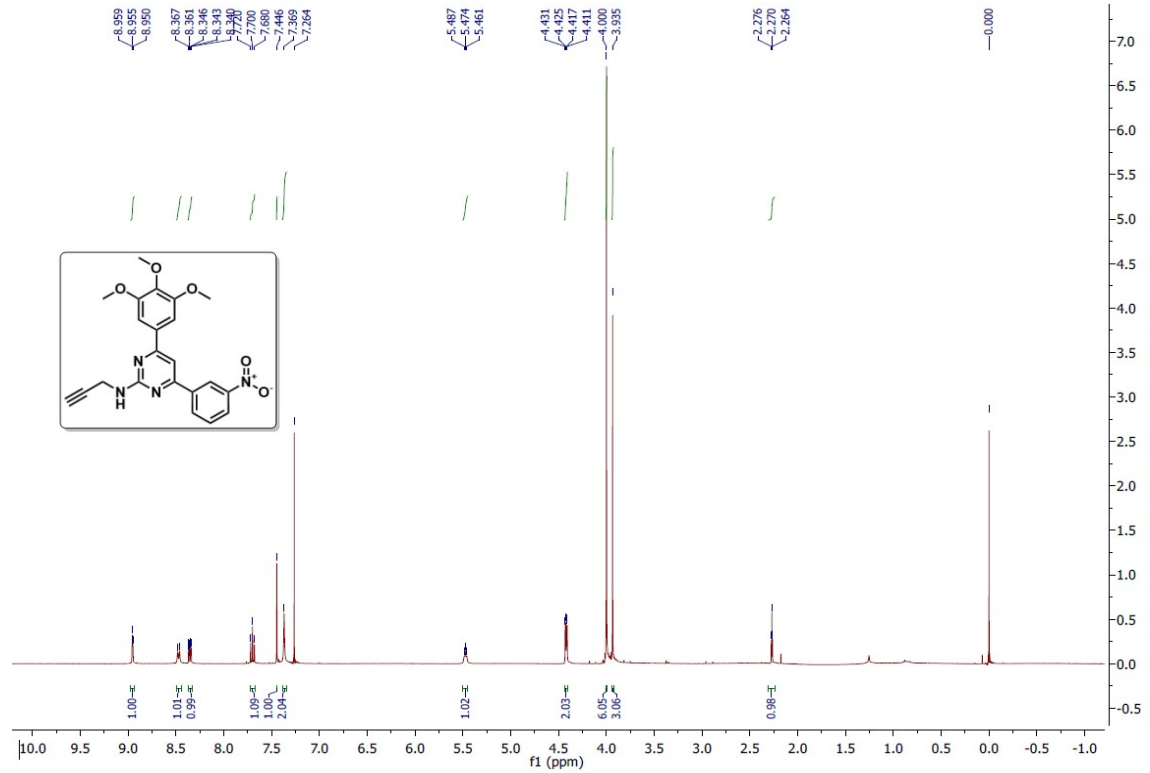
4-(4-chlorophenyl)-6-(4-methoxyphenyl)-N-(prop-2-yn-1-yl)pyrimidin-2-amine **(NV-8)**

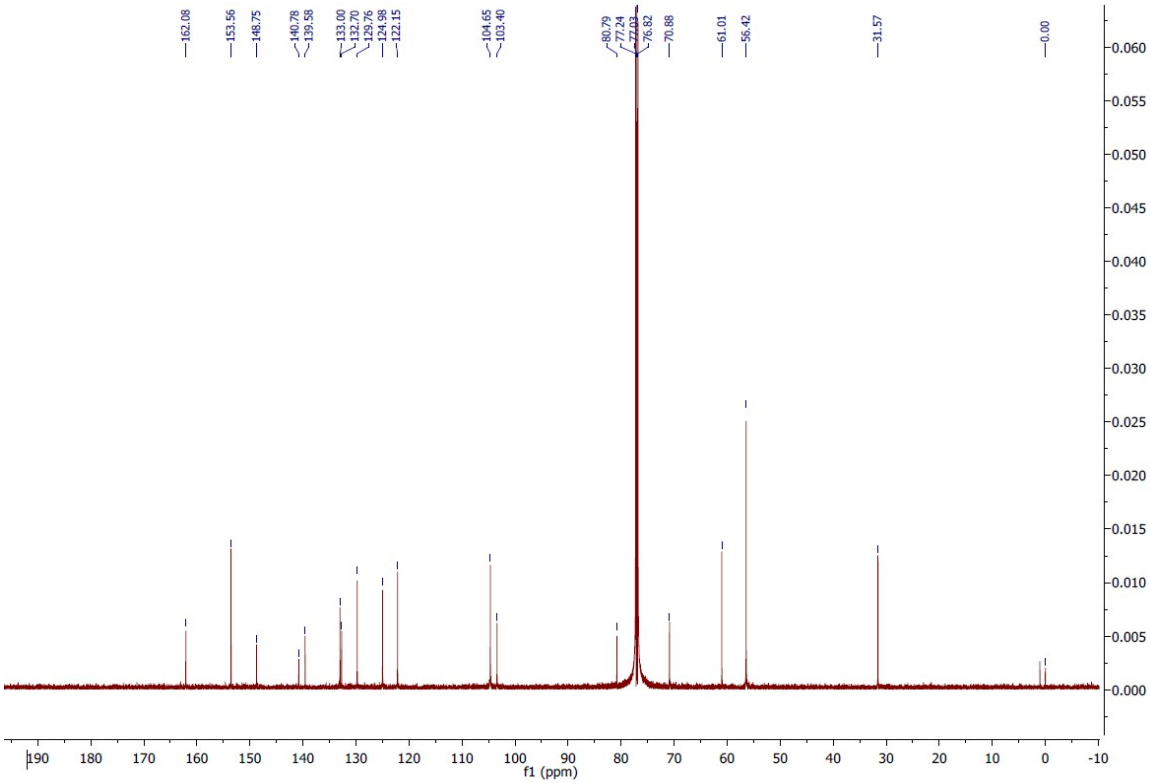


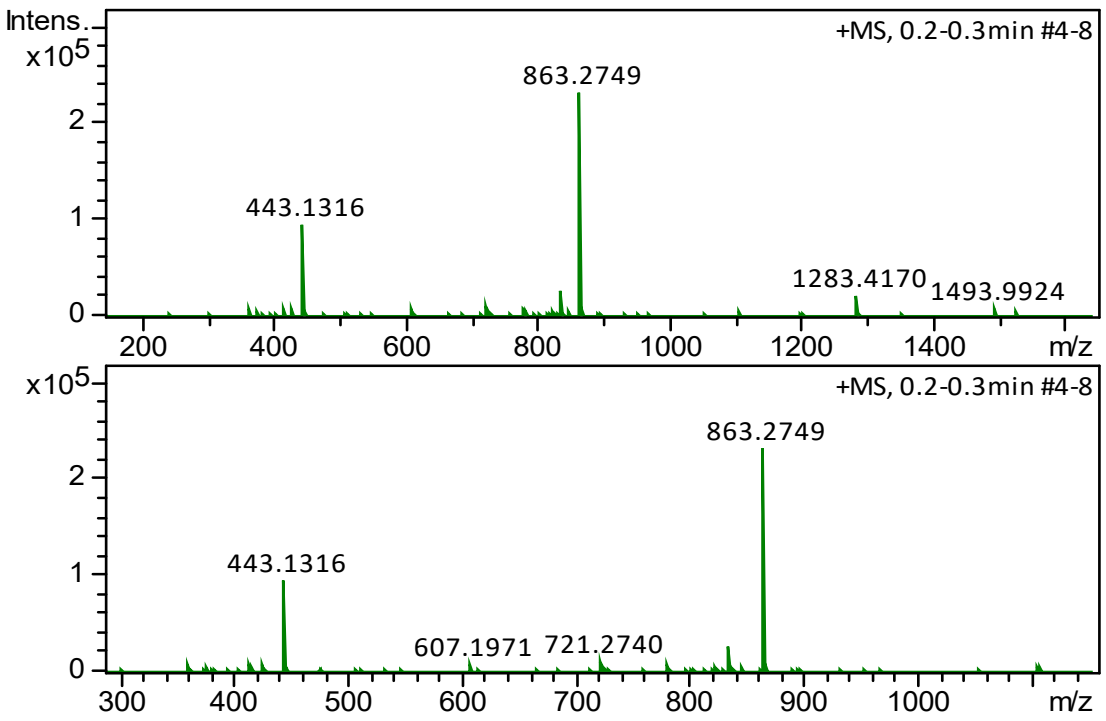

165.43
164.20
162.09
161.82
136.49
136.38
130.02
128.91
128.66
128.41
114.12
102.70
81.13
77.27
77.02
76.76
70.70
55.43
31.48
0.00
f1 (ppm)


Intens.
x10^4
+MS, 0.2-0.3min #4-7
372.0791
548.1035
721.1686
899.1913
1075.2080
m/z
x10^4
+MS, 0.2-0.3min #4-7
372.0791
449.3493
548.1035
m/z

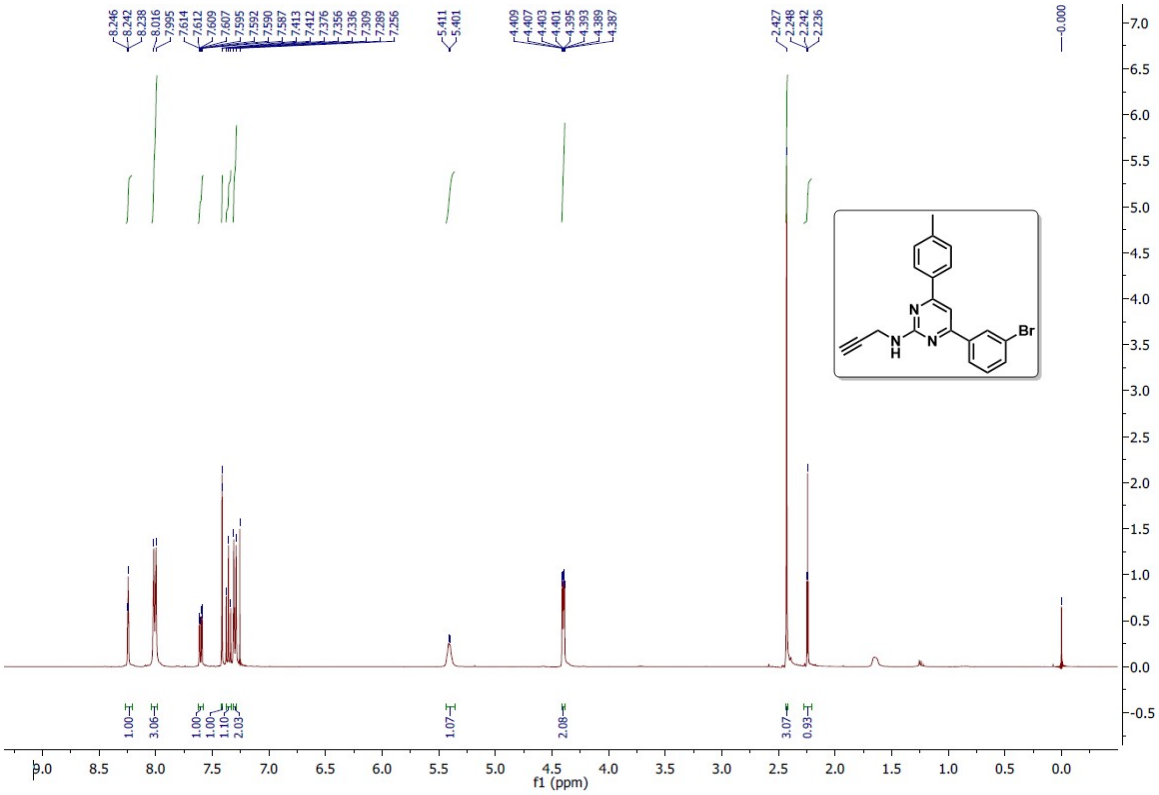
4-(3-nitrophenyl)-N-(prop-2-yn-1-yl)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine **(NV-9)**

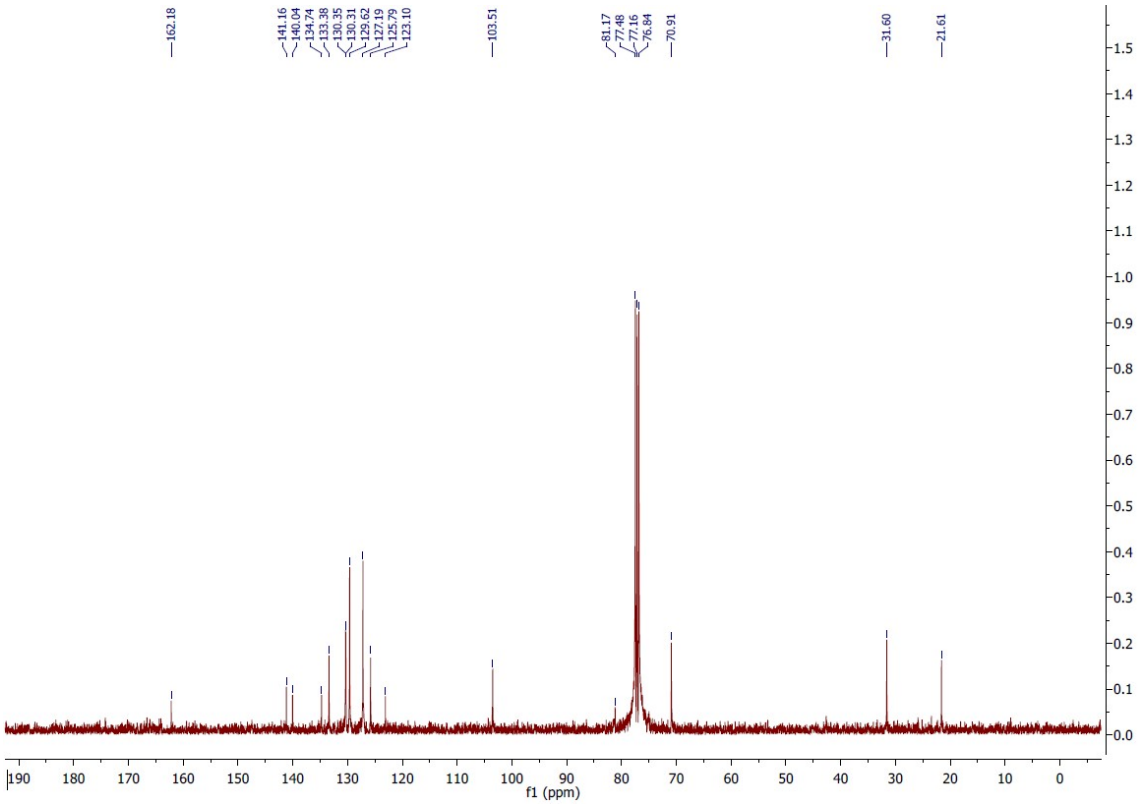






4-(3-bromophenyl)-N-(prop-2-yn-1-yl)-6-(p-tolyl)pyrimidin-2-amine **(NV-10)**

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134.74
133.38
130.35
130.31
129.62
127.19
125.79
123.10
103.51
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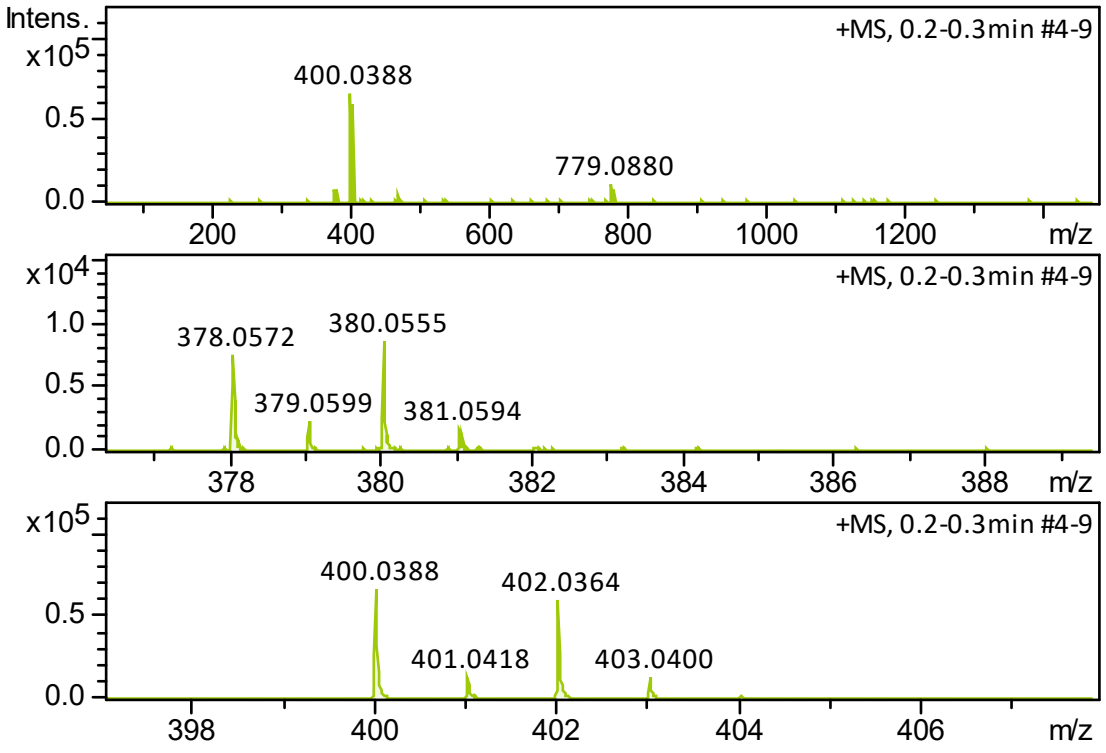
76.84
70.91
31.60
21.61
f1 (ppm)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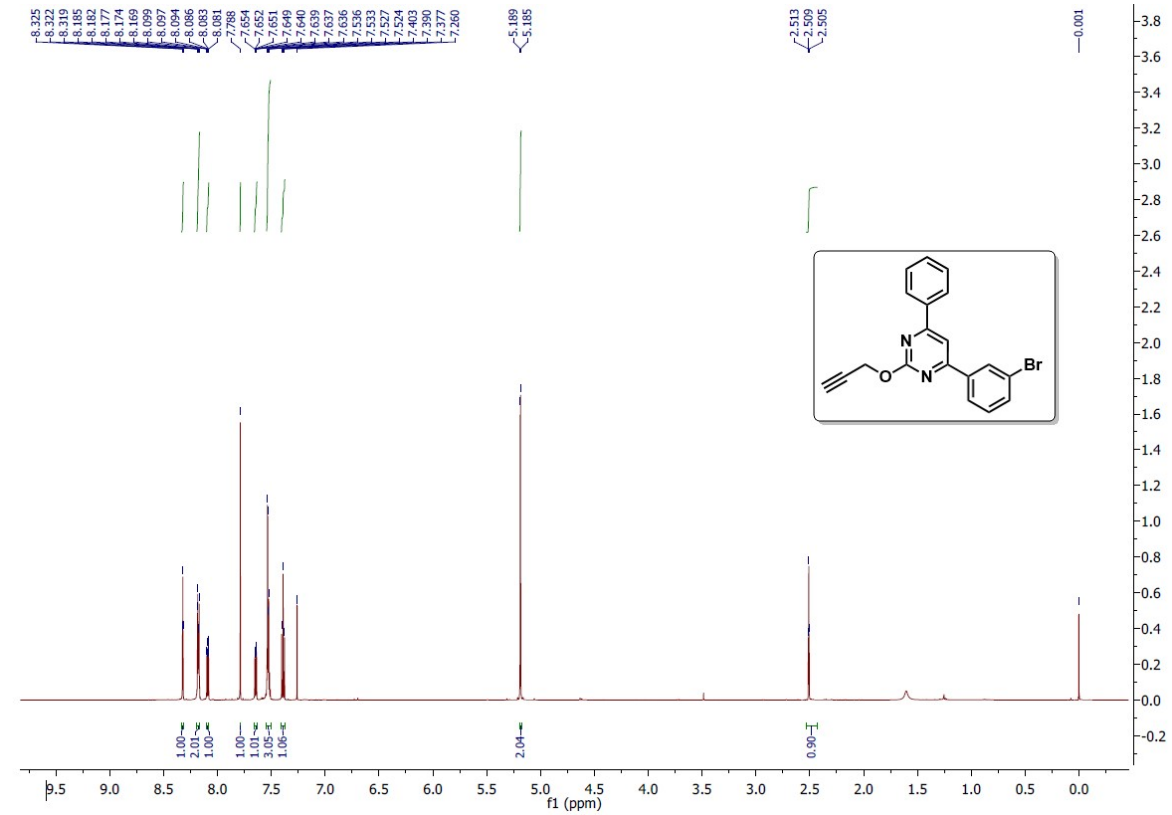
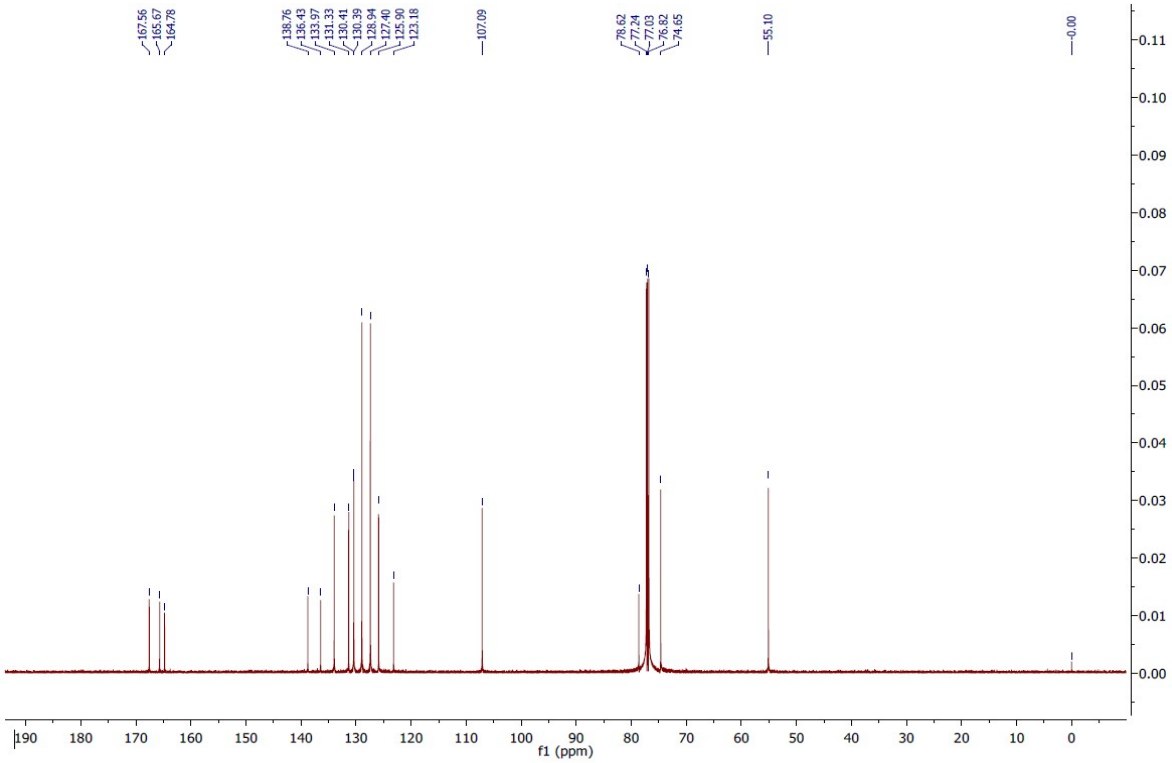
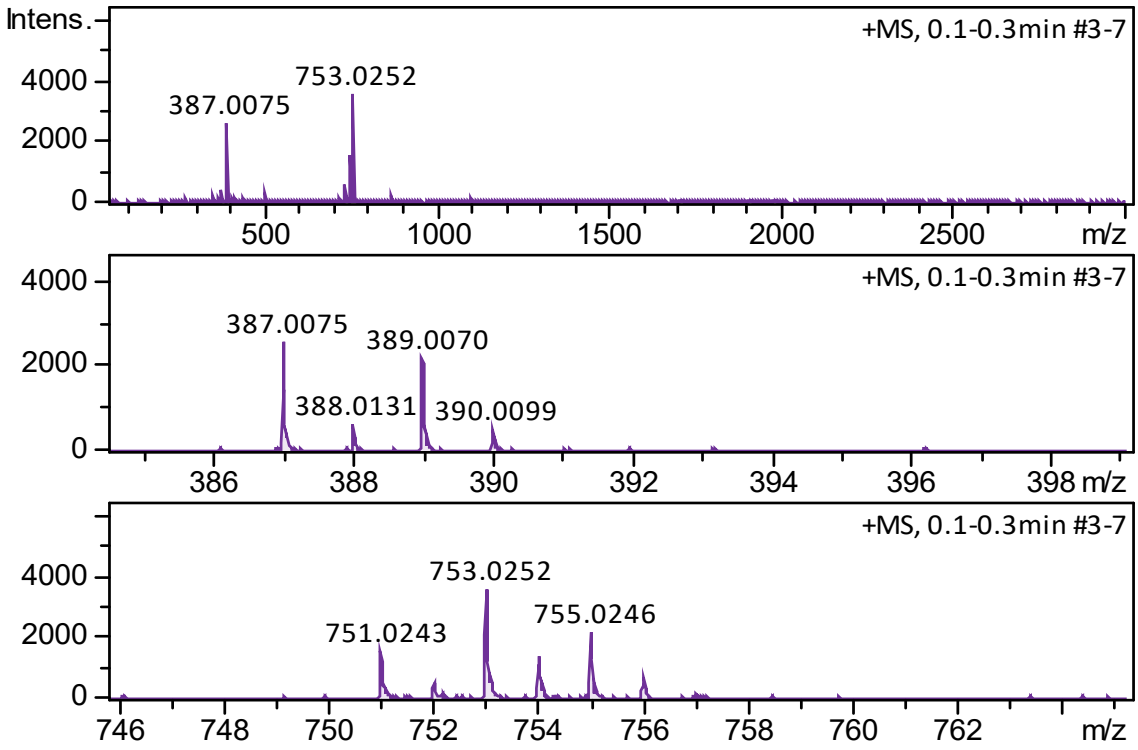
779.0880
m/z
x10^4
+MS, 0.2-0.3min #4-9
378.0572
380.0555
379.0599
381.0594
m/z
x10^5
+MS, 0.2-0.3min #4-9
400.0388
402.0364
401.0418
403.0400
m/z

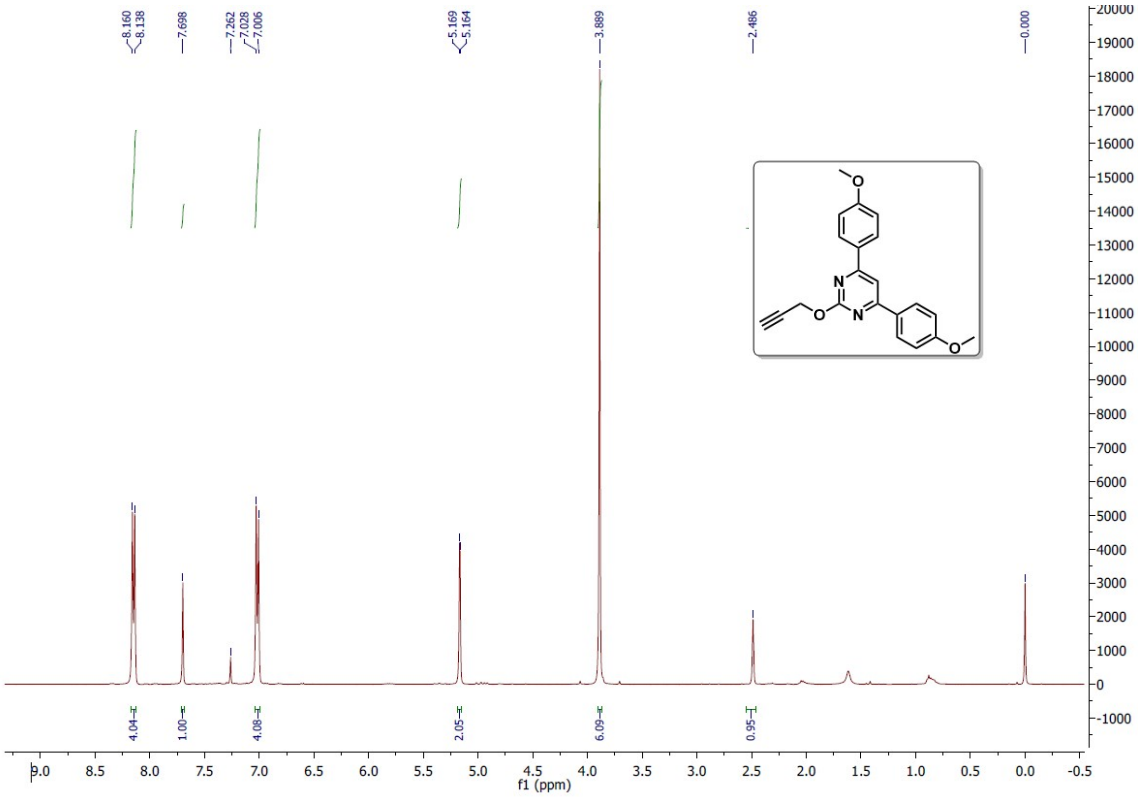
4-(3-bromophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-11)**

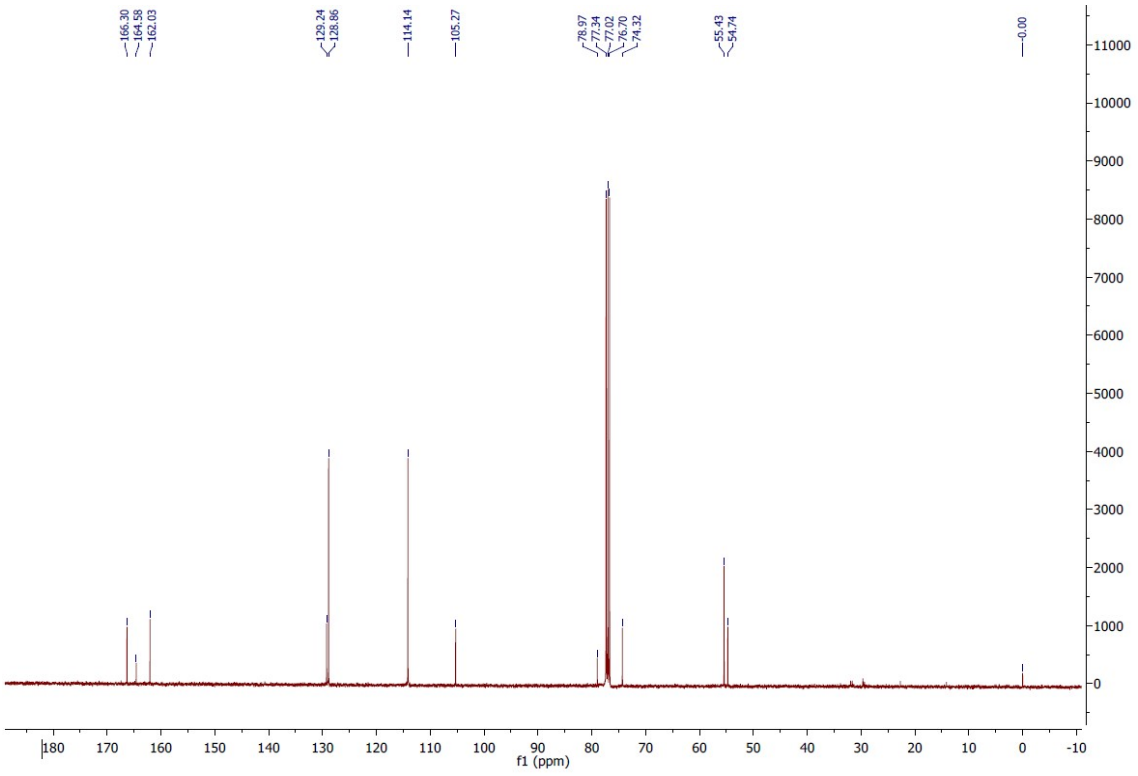


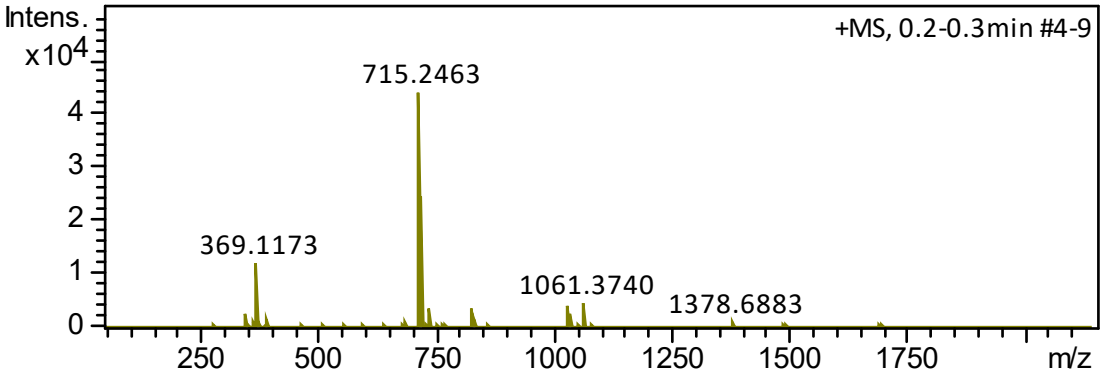


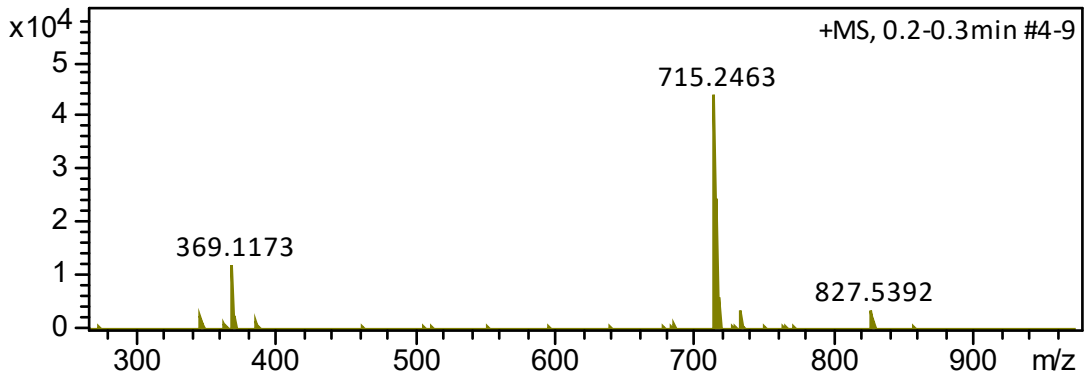


4,6-bis(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-12)**

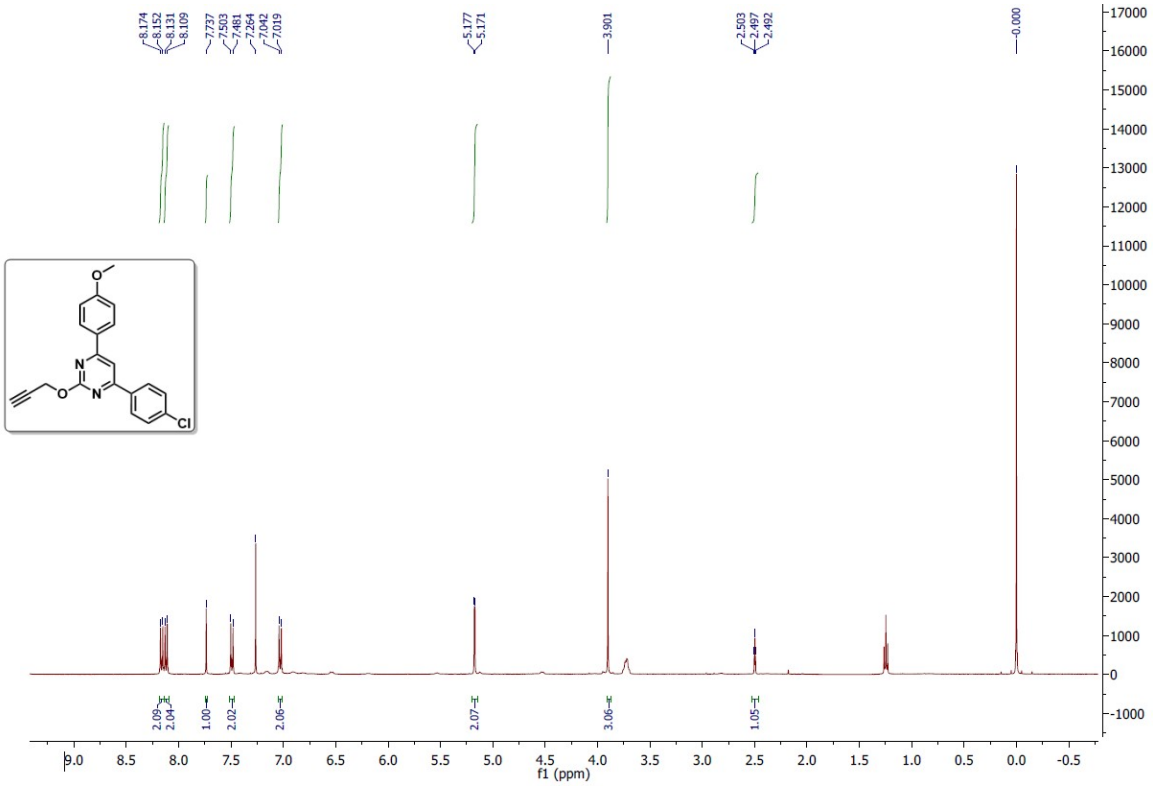


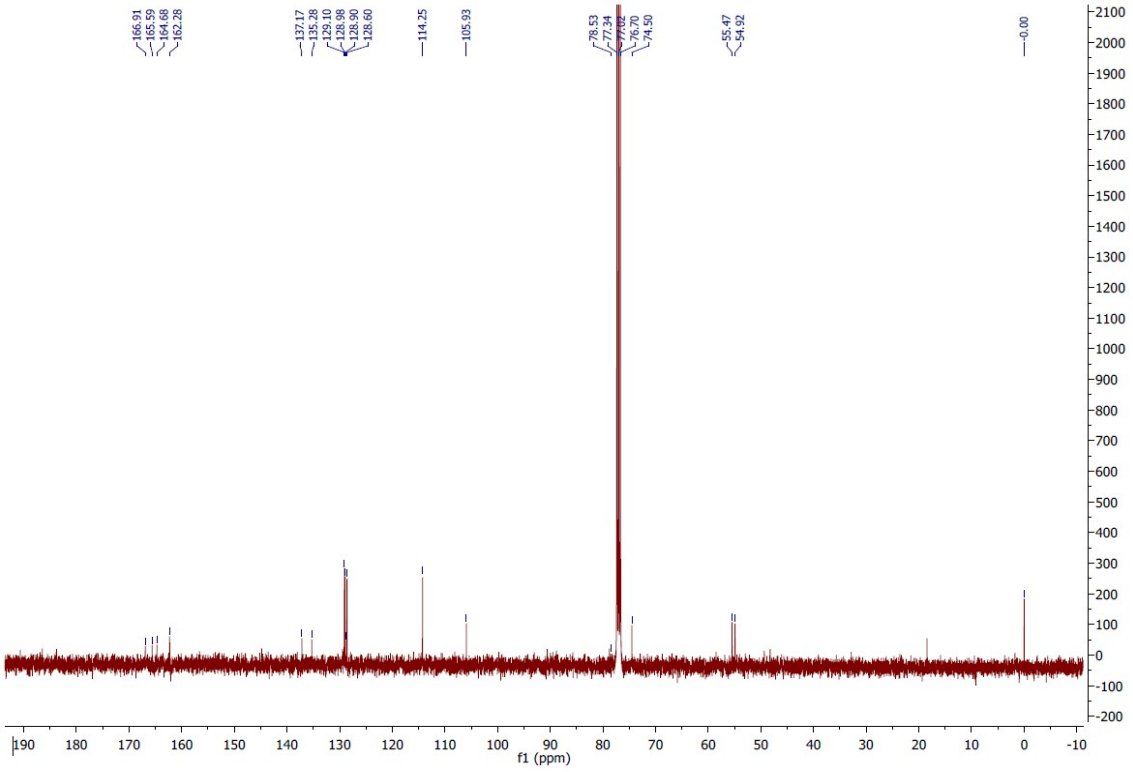

166.30
164.58
162.03
129.24
128.86
114.14
105.27
78.97
77.34
77.02
76.70
74.32
55.43
54.74
0.00
f1 (ppm)

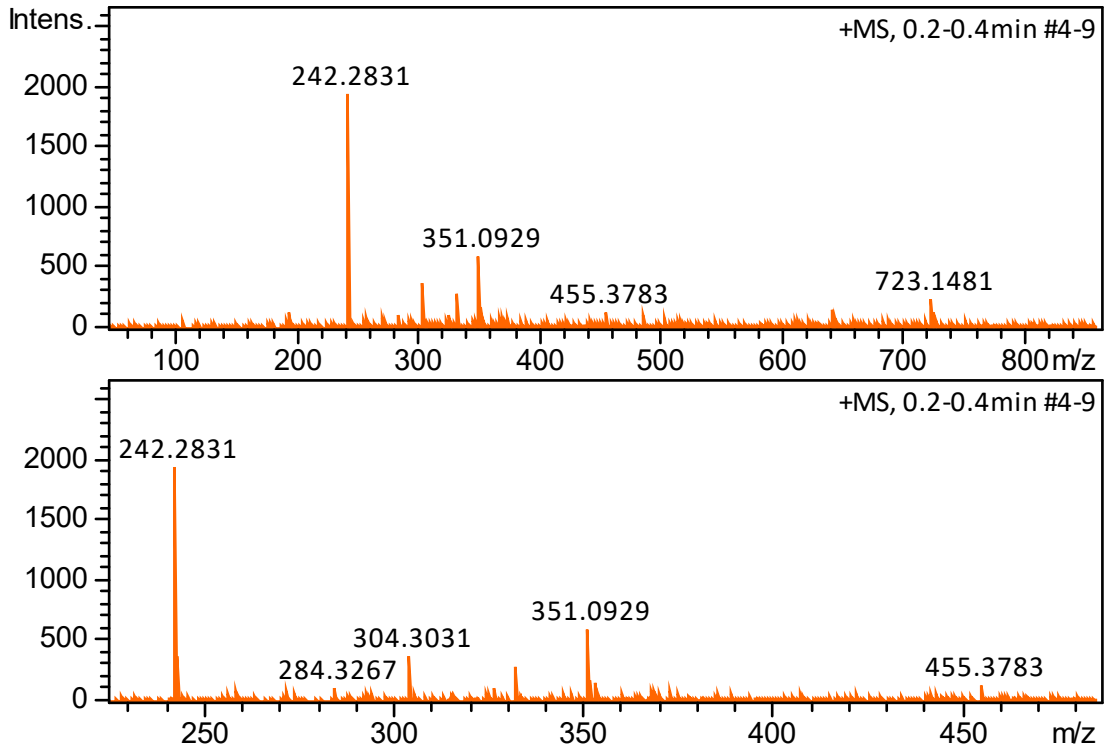

Intens.
x10^4
+MS, 0.2-0.3min #4-9
715.2463
369.1173
1061.3740
1378.6883
m/z


x10^4
+MS, 0.2-0.3min #4-9
715.2463
369.1173
827.5392
m/z

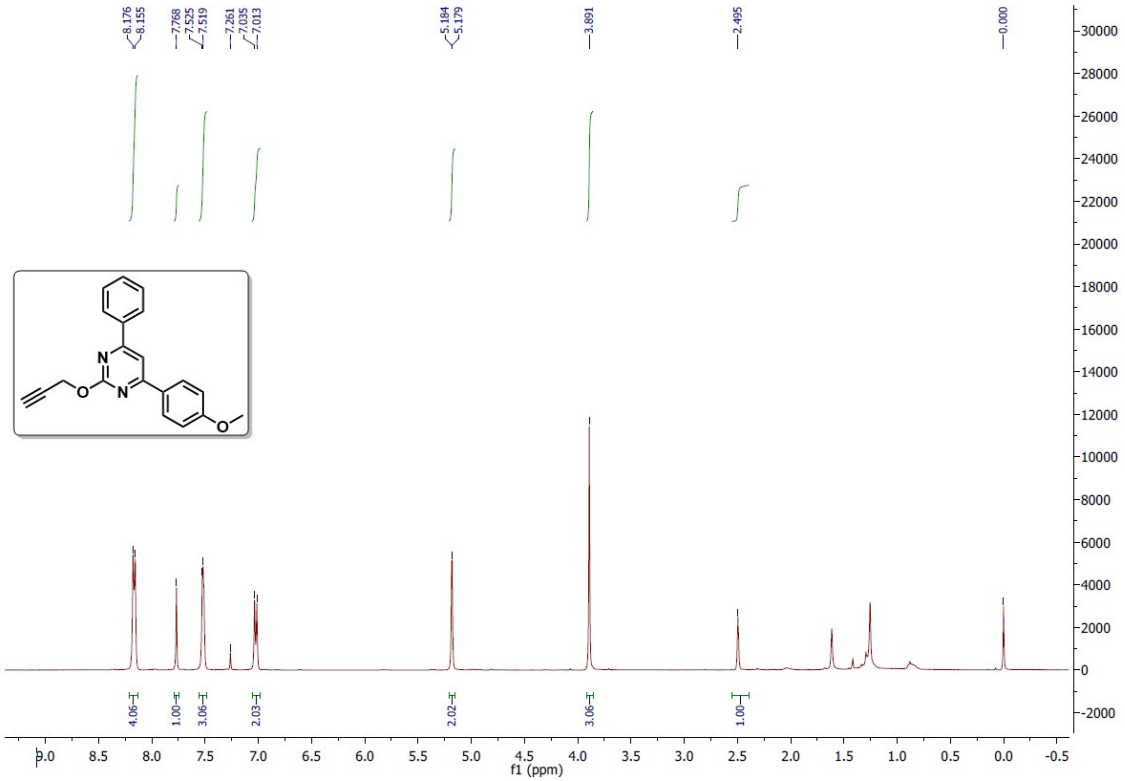
4-(4-chlorophenyl)-6-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-13)**

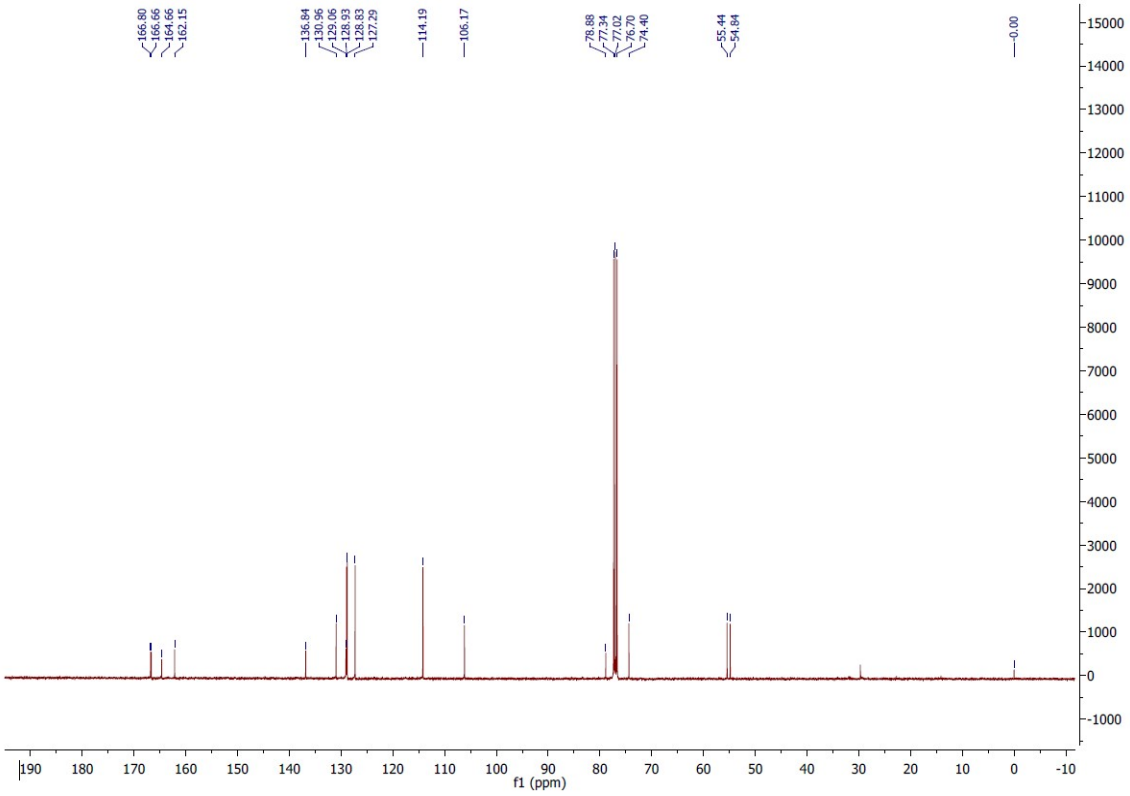


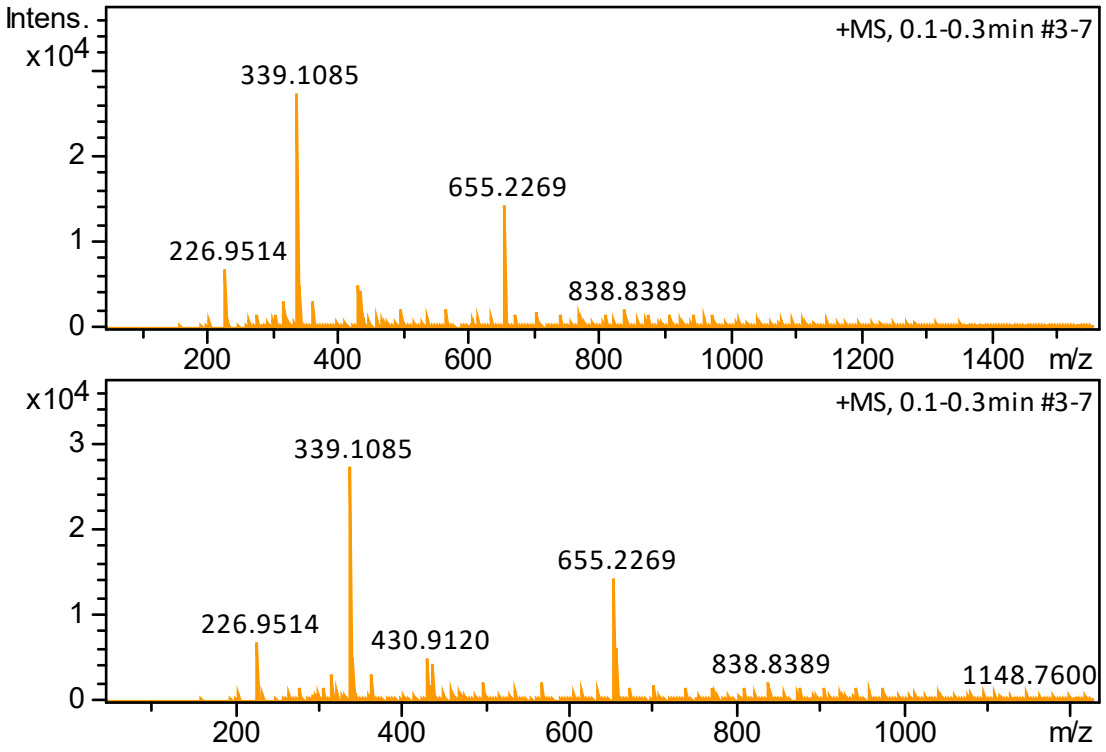




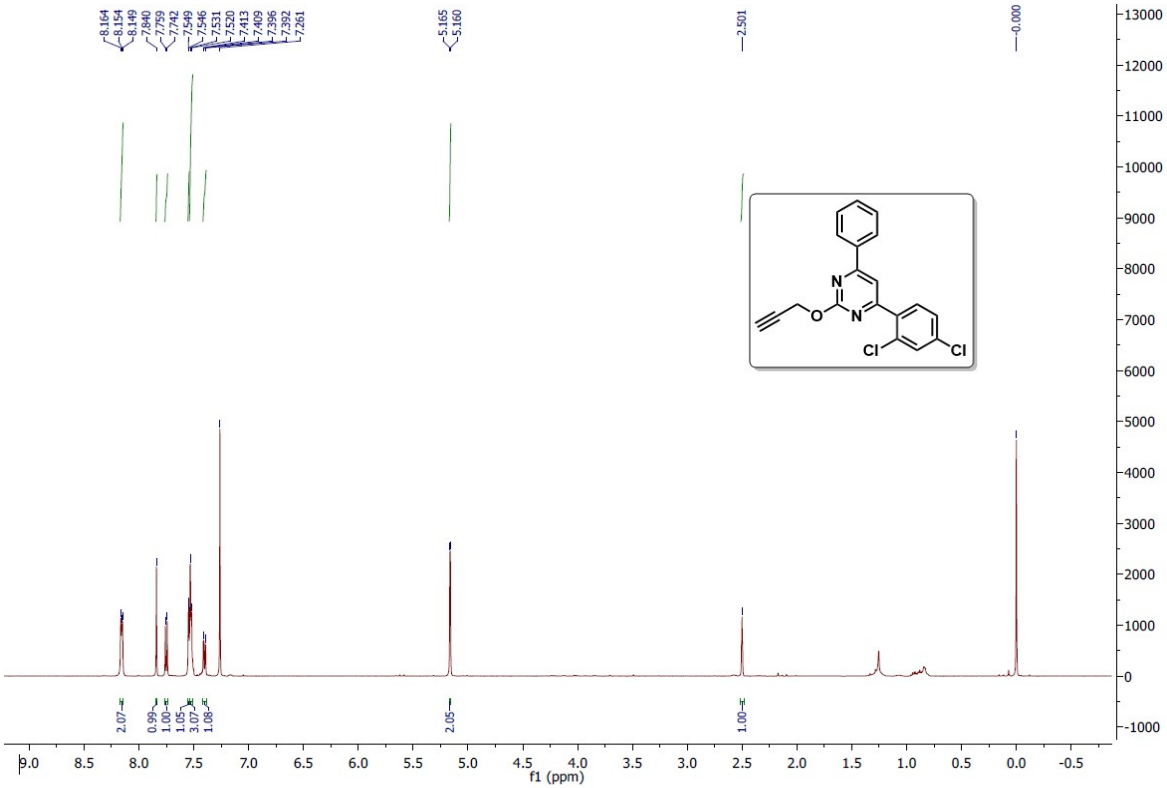
4-(4-methoxyphenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-14)**

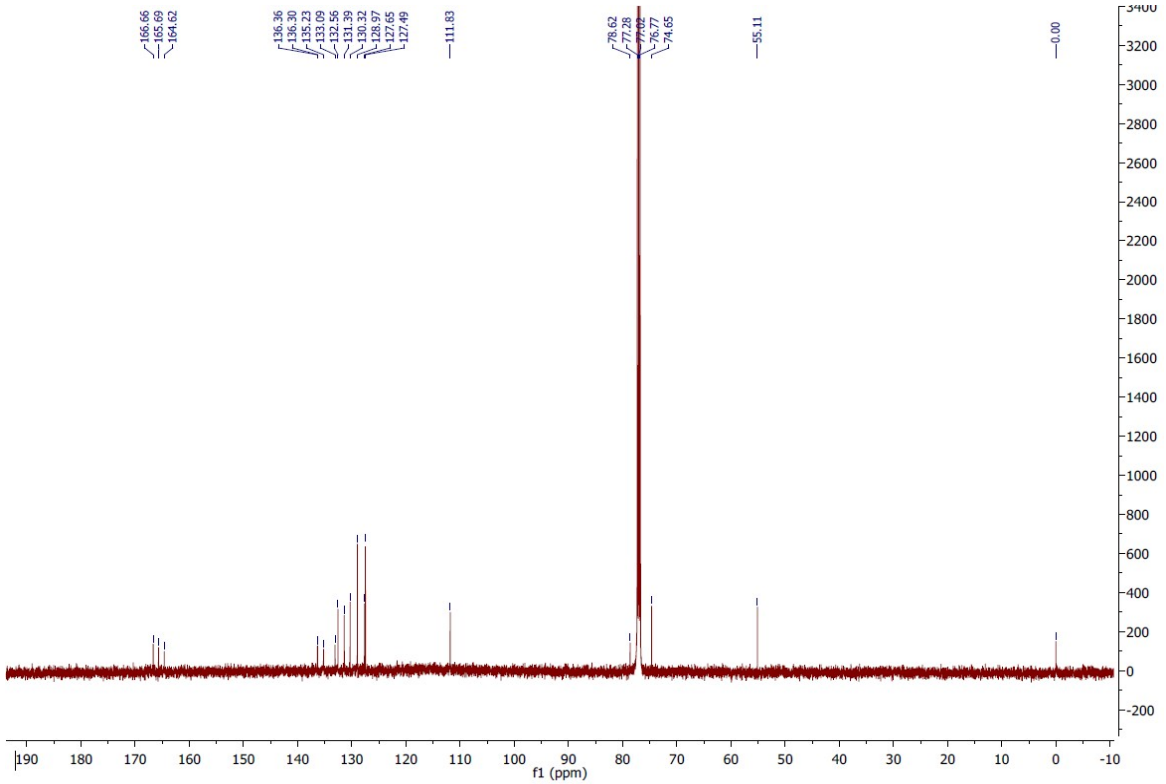


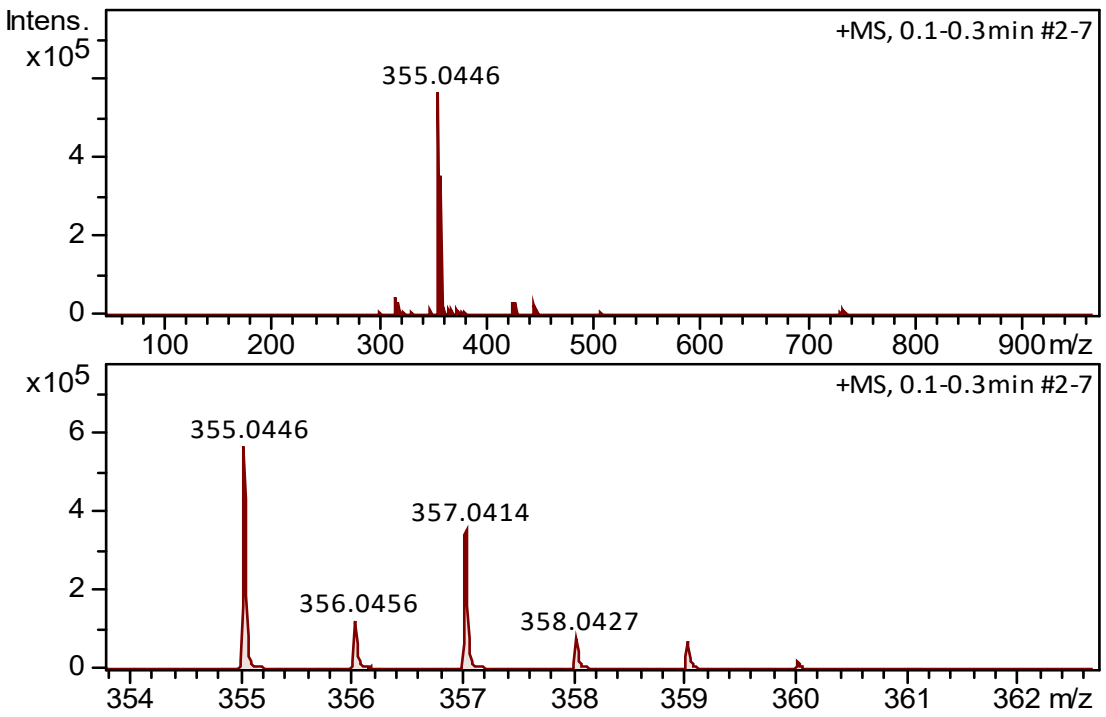

166.80
166.66
164.66
162.15
136.84
130.96
129.06
128.93
128.83
127.29
114.19
106.17
78.88
77.34
77.02
76.70
74.40
55.44
54.84
0.00
f1 (ppm)


Intens.
x10^4
+MS, 0.1-0.3min #3-7
339.1085
655.2269
226.9514
838.8389
m/z
x10^4
+MS, 0.1-0.3min #3-7
339.1085
655.2269
226.9514
430.9120
838.8389
1148.7600
m/z

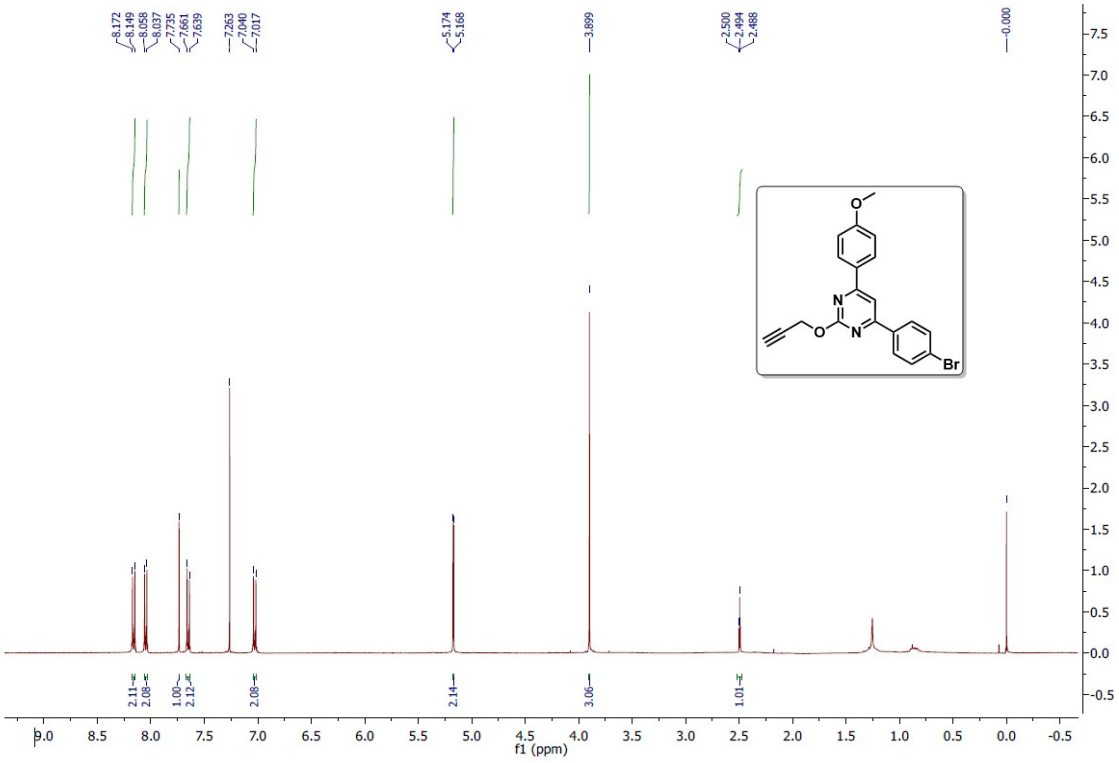
4-(2,4-dichlorophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-15)**

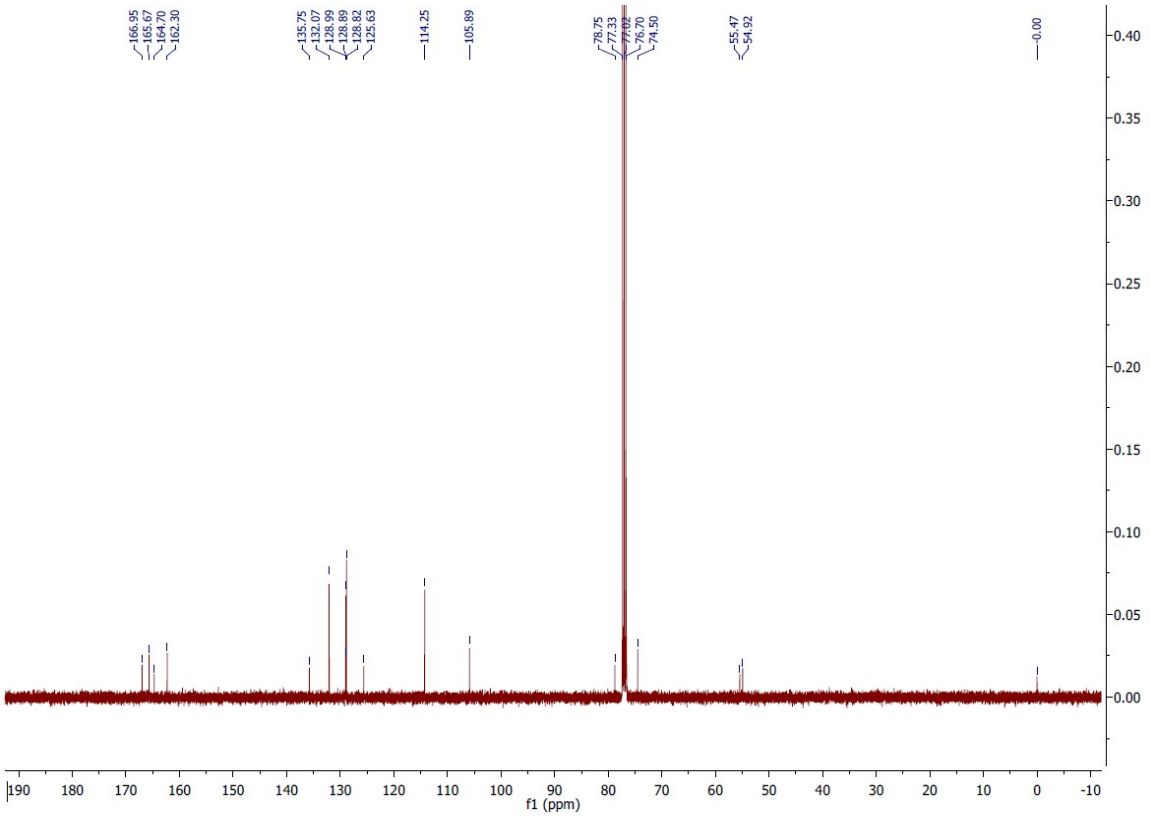






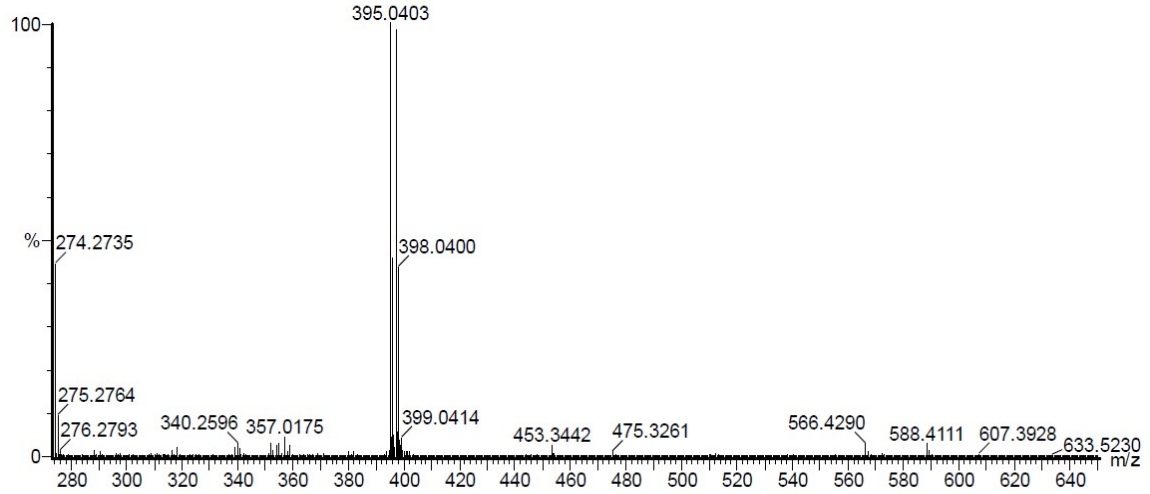
4-(4-bromophenyl)-6-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-16)**





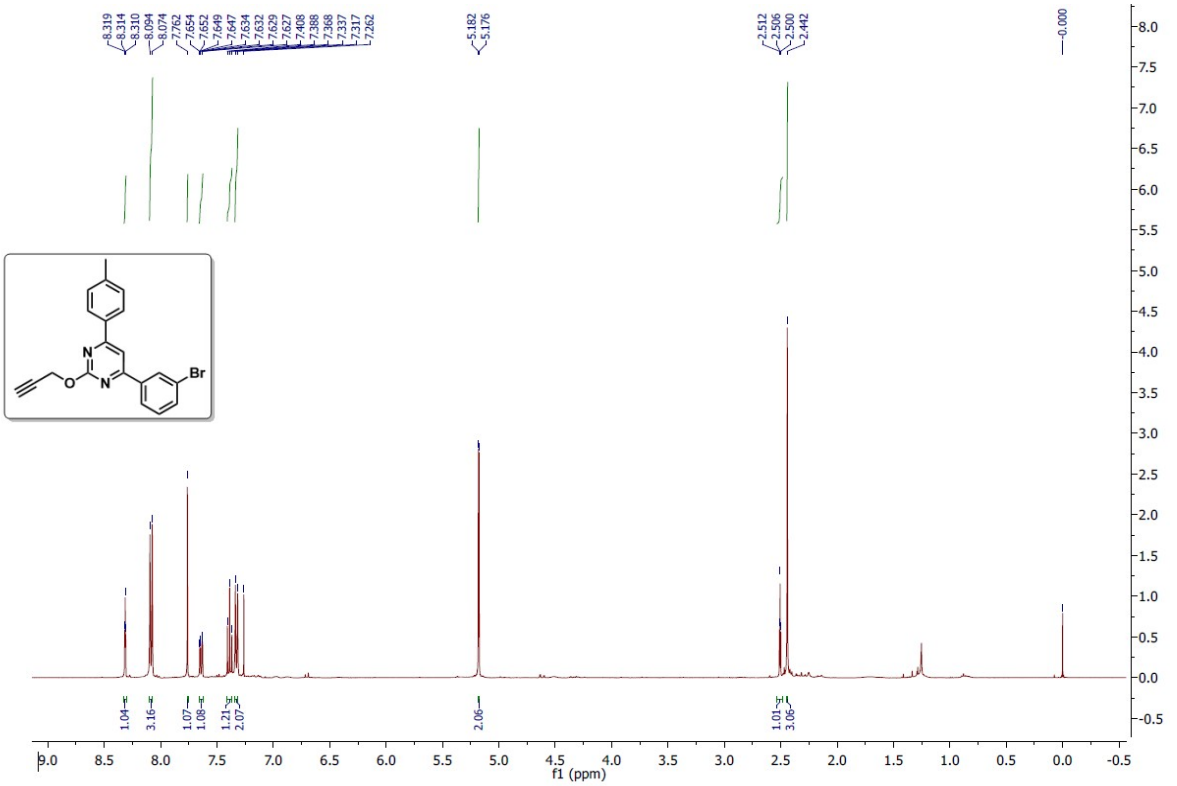
Test Name : HRMS-1
010221-B4P23 15 (0.157)

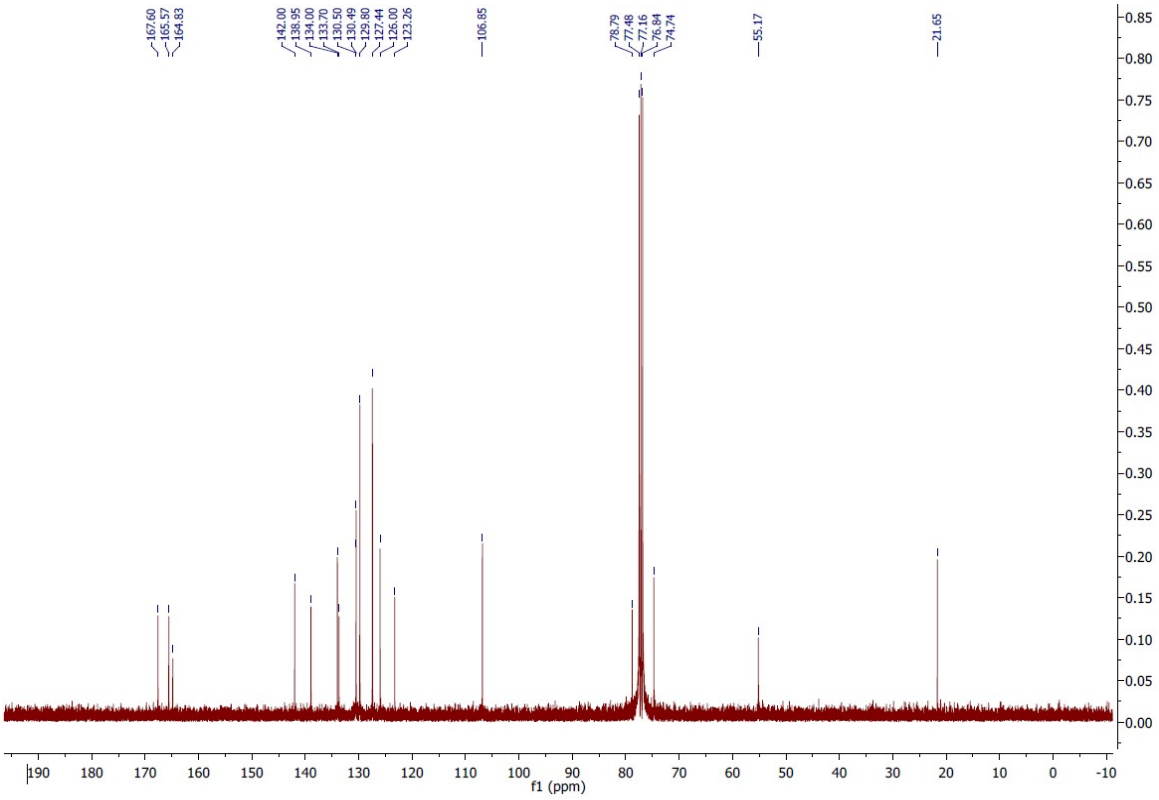
1: TOF MS ES+
9.03e+007



Minimum:				-1.5				
Maximum:		2.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
395.0403	395.0395	0.8	2.0	13.5	1702.8	n/a	n/a	C20 H16 N2 O2 Br

4-(3-bromophenyl)-2-(prop-2-yn-1-yloxy)-6-(p-tolyl)pyrimidine **(NV-17)**



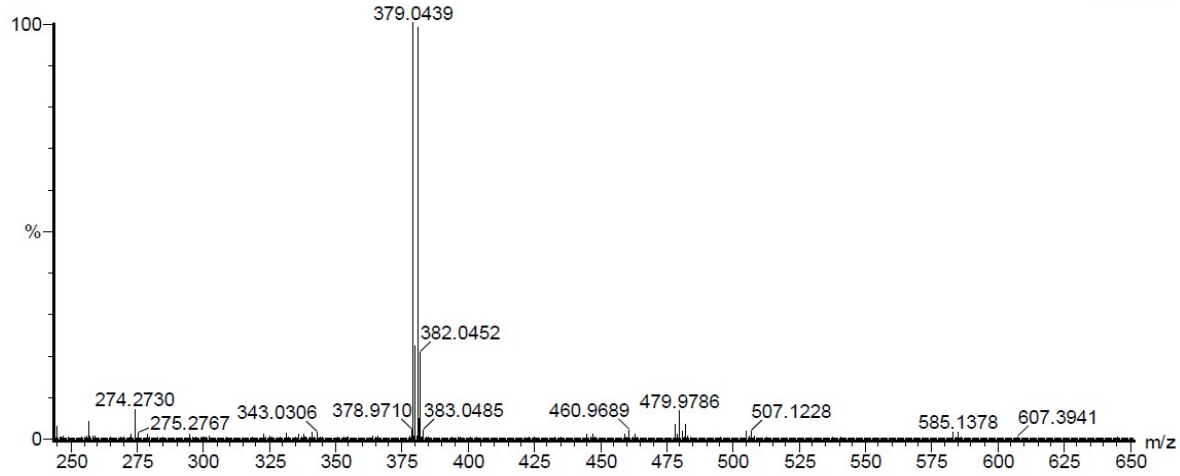


Sample Name : NKS-113
Test Name : HRMS-1
010221-NKS-113 19 (0.203)

IITRPR

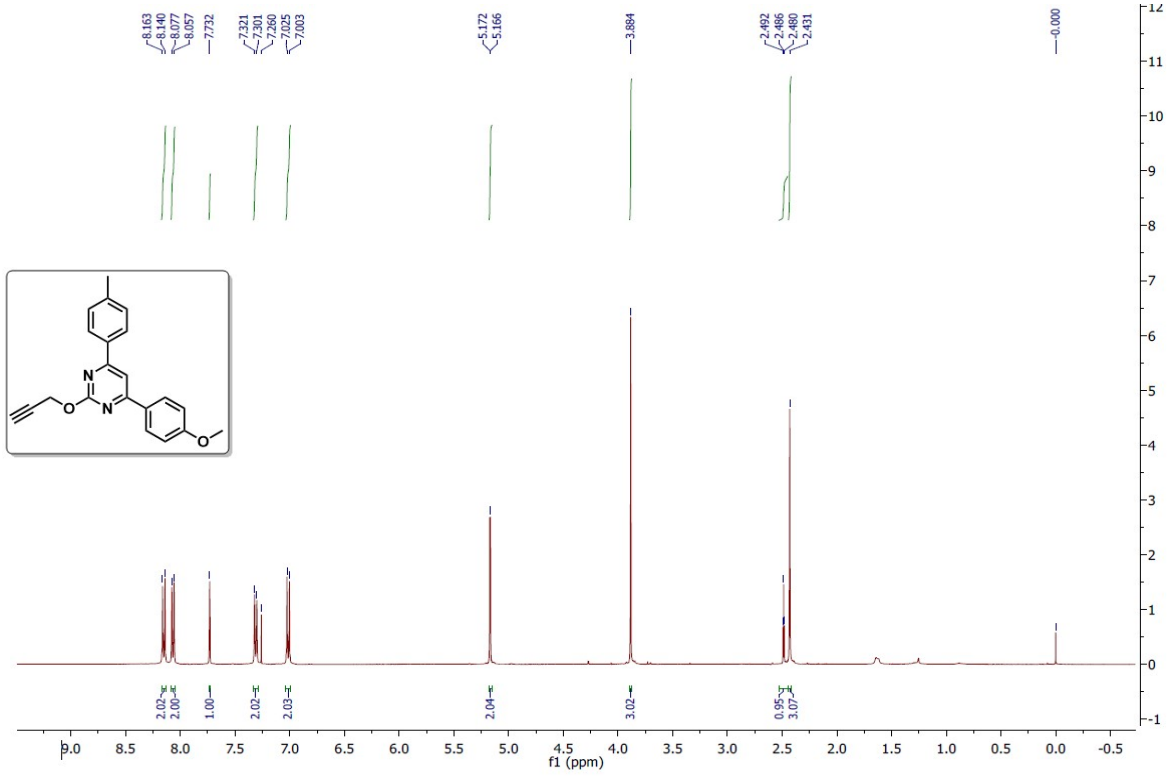
XEVO G2-XS QTOF

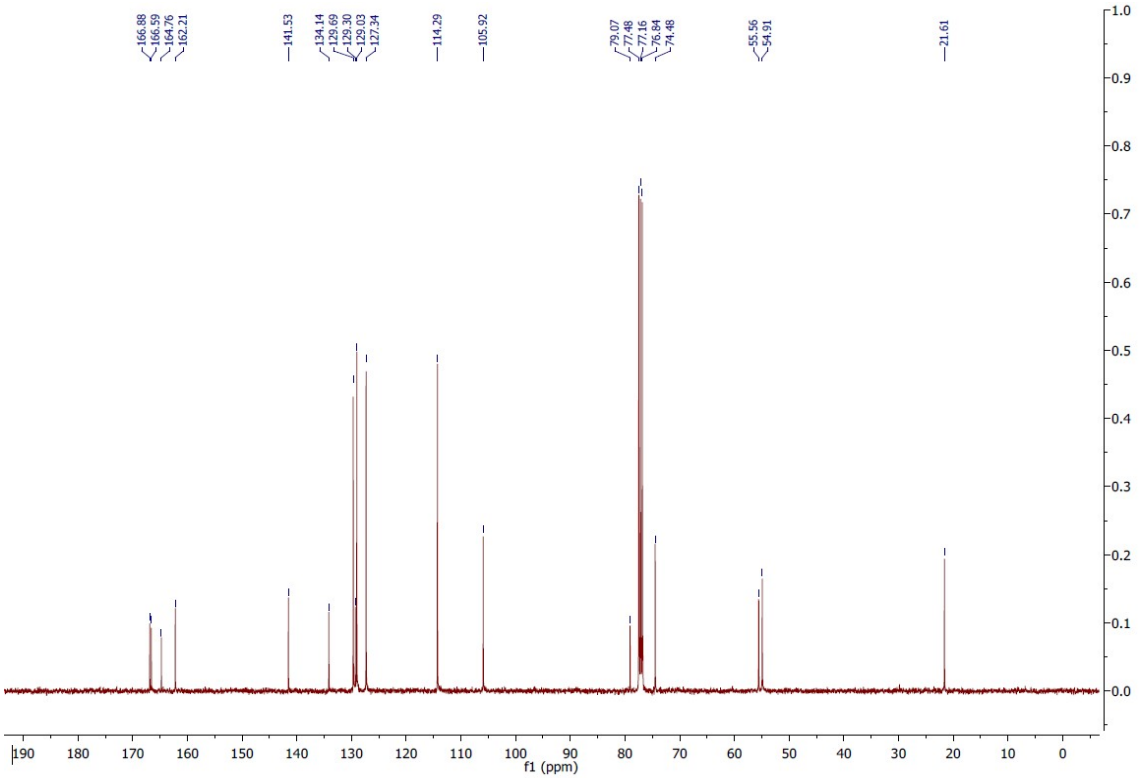
1: TOF MS ES+
3.78e+007



Minimum:				-1.5				
Maximum:		2.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
379.0439	379.0446	-0.7	-1.8	13.5	1567.5	n/a	n/a	C20 H16 N2 O Br

4-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy)-6-(p-tolyl)pyrimidine **(NV-18)**



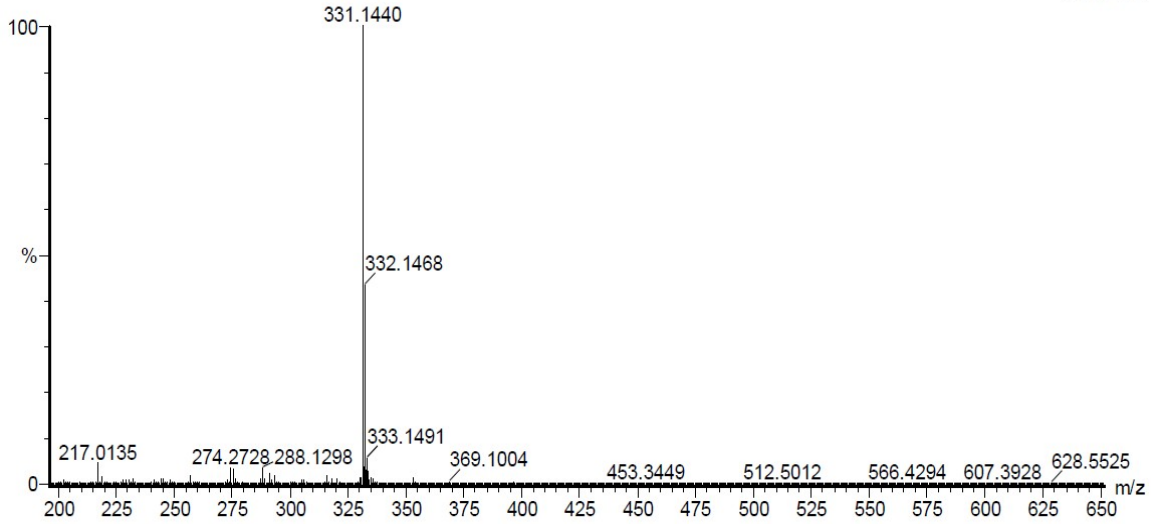


Sample Name : NKS-114
Test Name : HRMS-1
010221-NKS-114 18 (0.183)

IITRPR

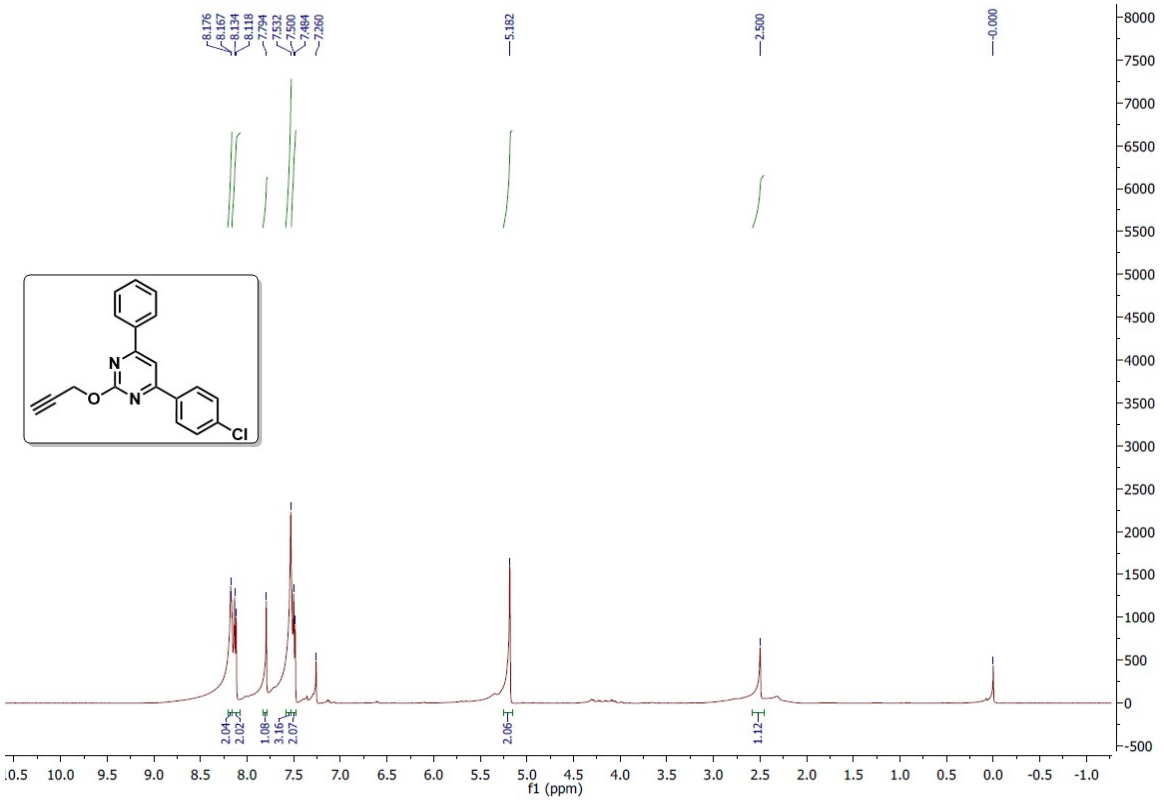
XEVO G2-XS QTOF

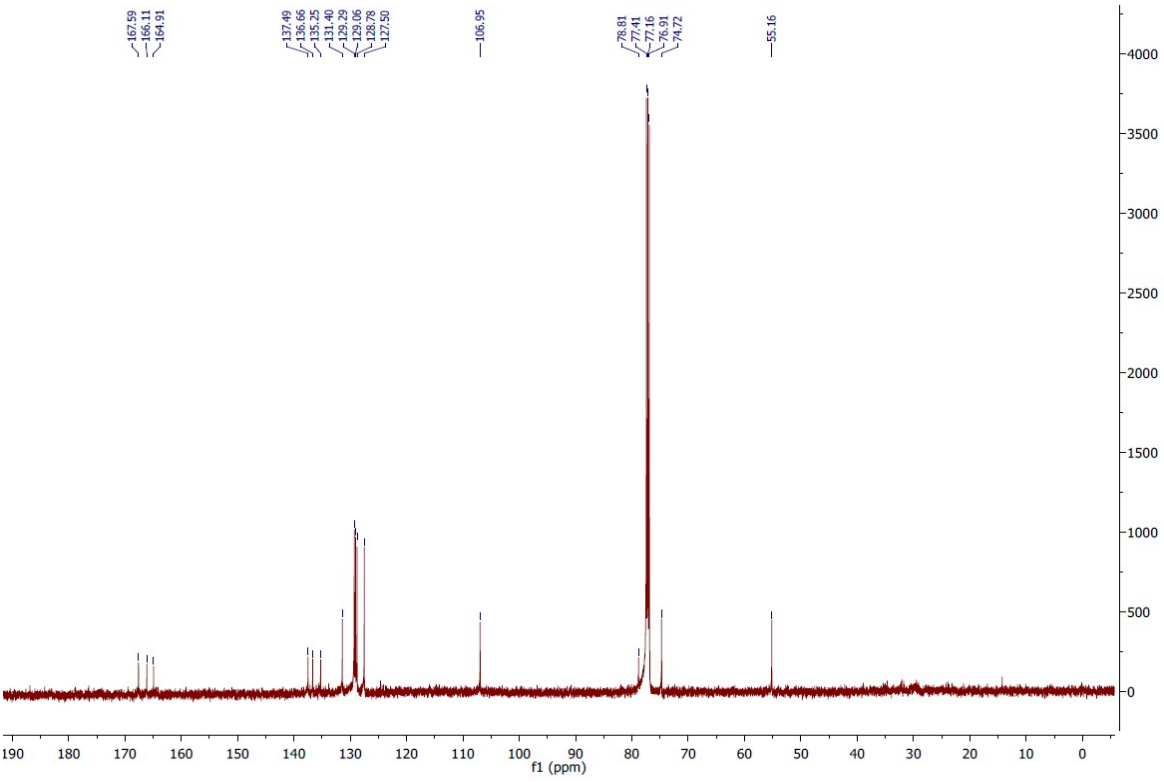
1: TOF MS ES+
7.90e+007

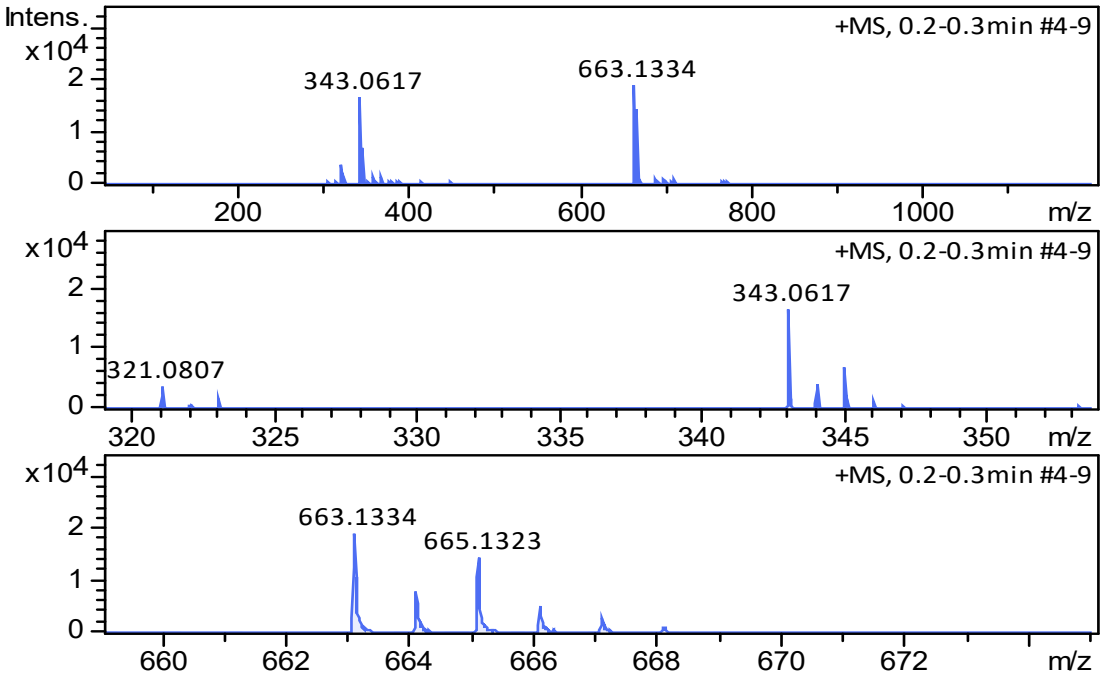


Minimum:				-1.5				
Maximum:		2.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
331.1440	331.1447	-0.7	-2.1	13.5	1798.8	n/a	n/a	C21 H19 N2 O2

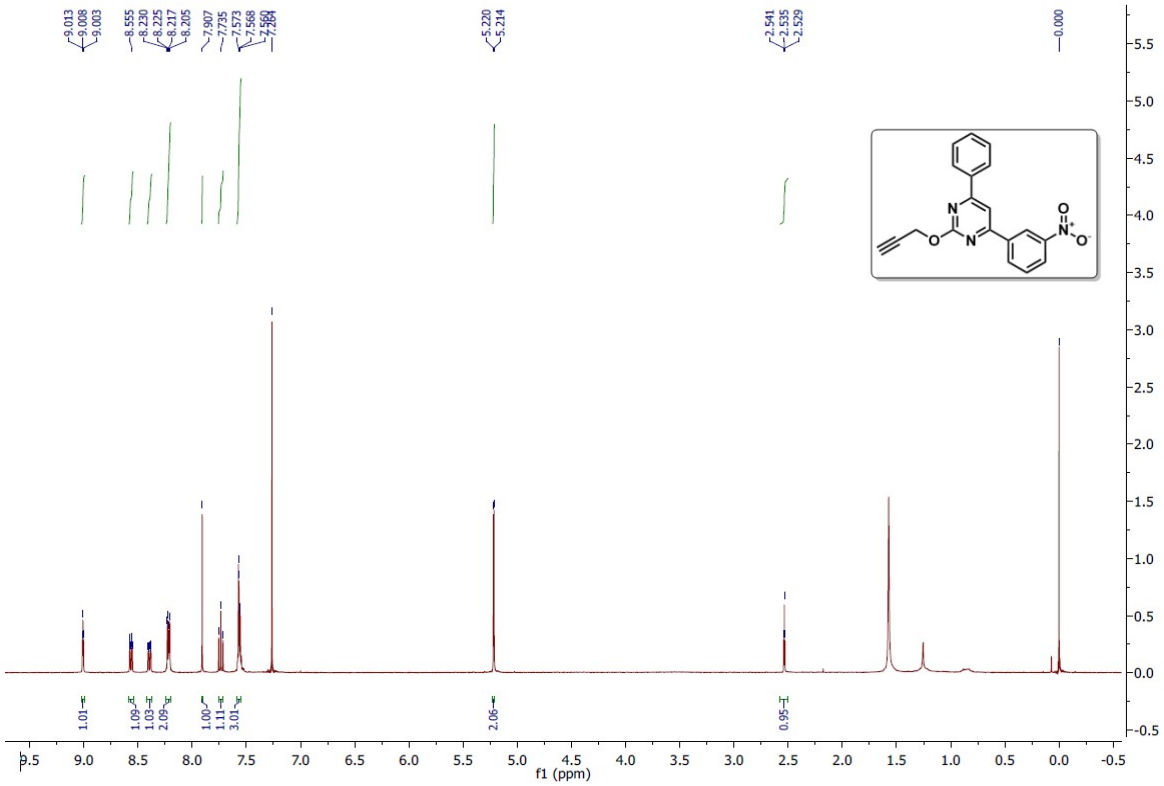
4-(4-chlorophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-19)**

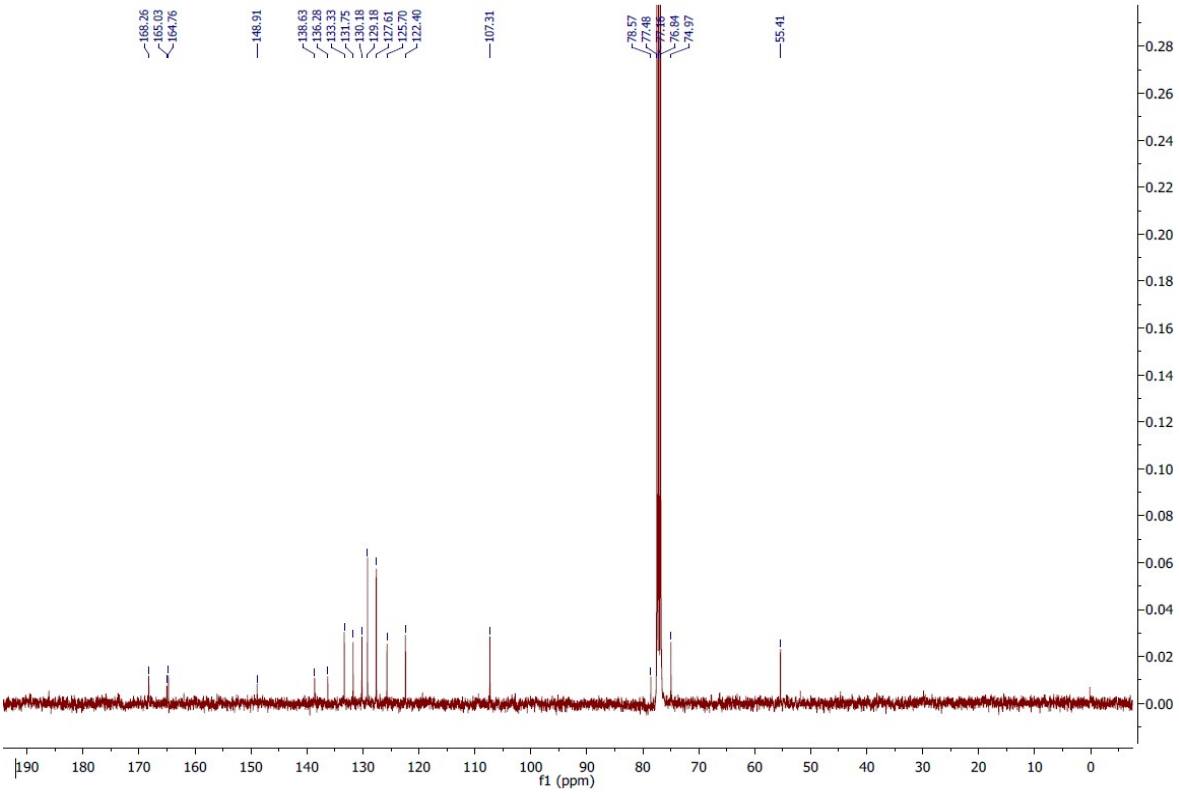






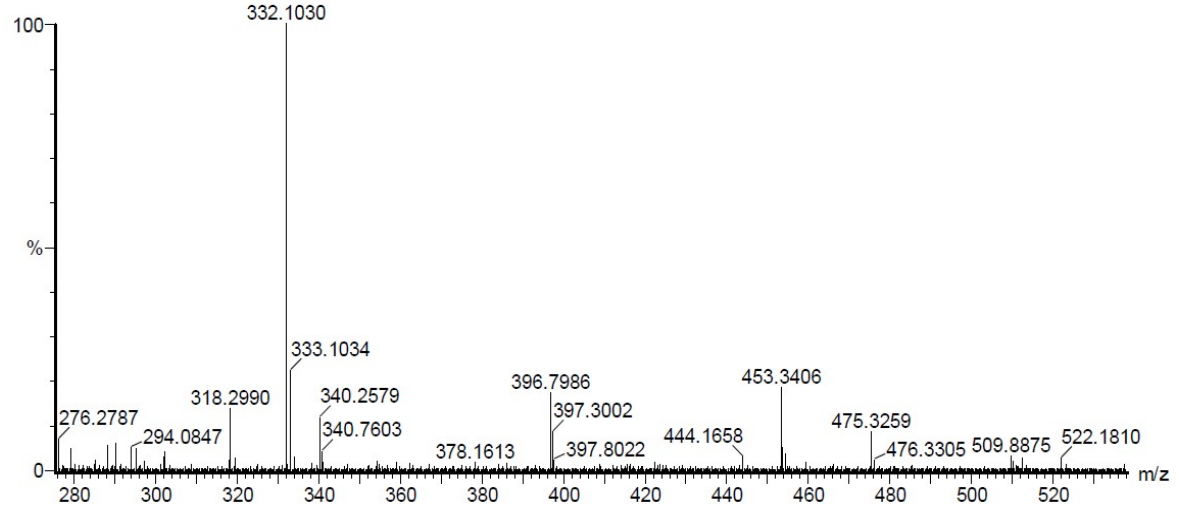
4-(3-nitrophenyl)-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-20)**





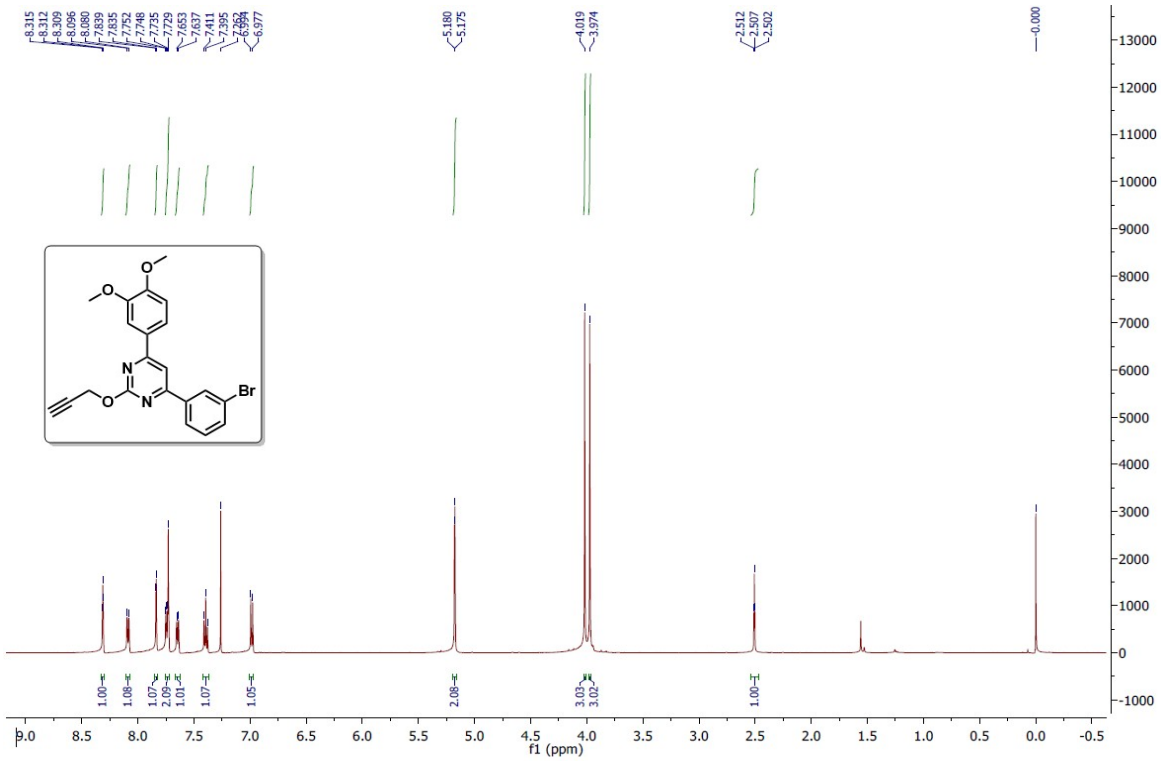
Test Name : HRMS-1
010221-NKS-118 17 (0.174)

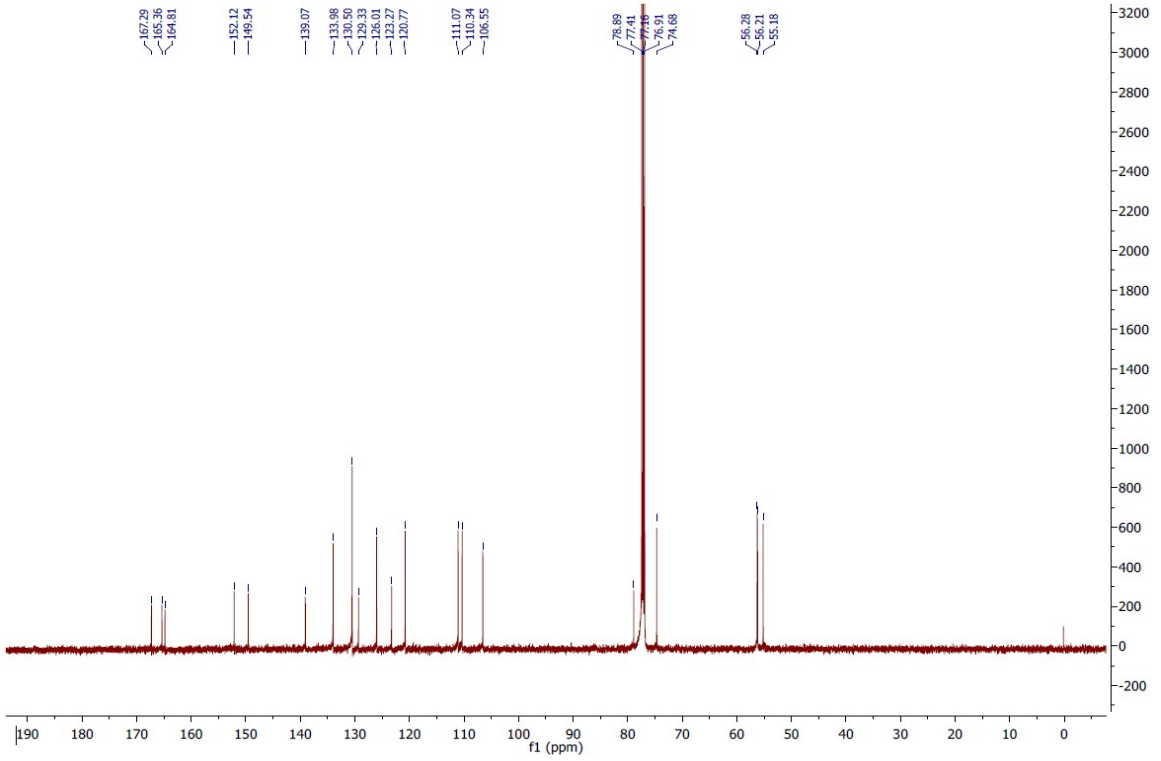
1: TOF MS ES+
3.09e+006

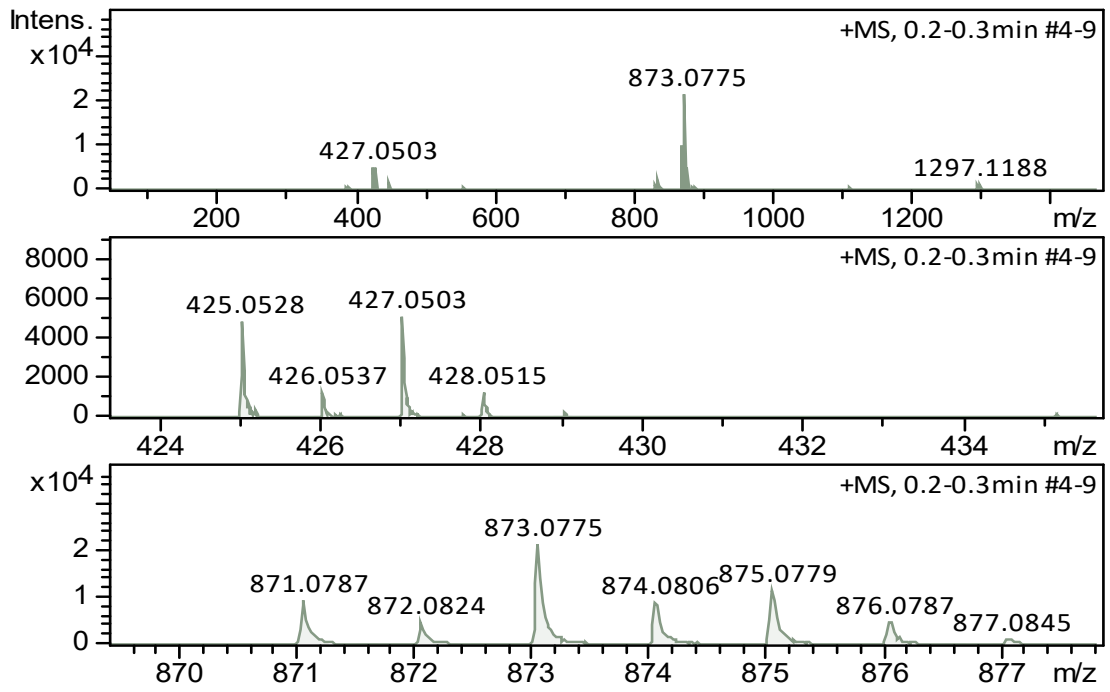


Minimum:				-1.5				
Maximum:		2.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
332.1030	332.1035	-0.5	-1.5	14.5	1291.2	n/a	n/a	C19 H14 N3 O3

4-(3-bromophenyl)-6-(3,4-dimethoxyphenyl)-2-(prop-2-yn-1-yloxy)pyrimidine **(NV-21)**

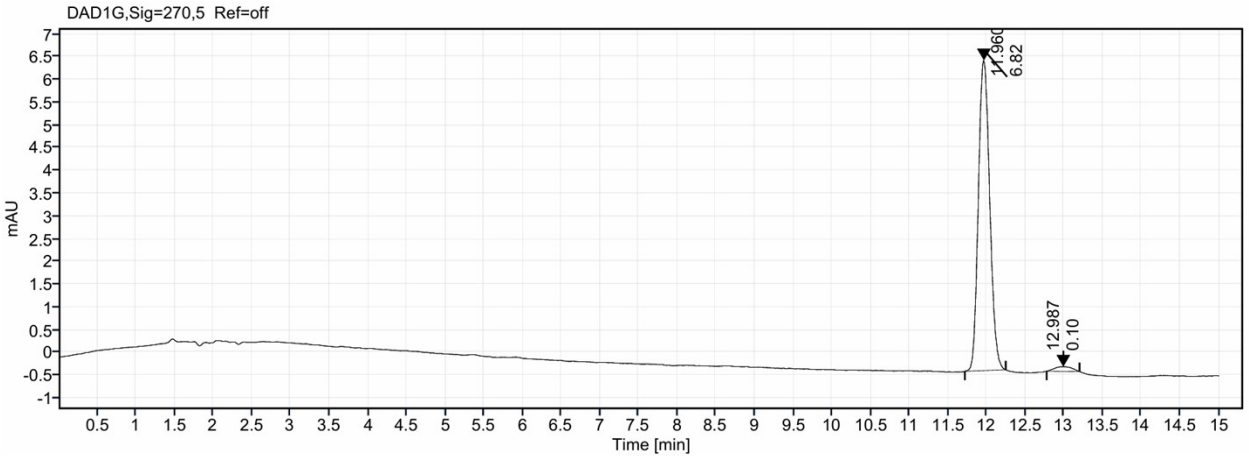






HPLC data:

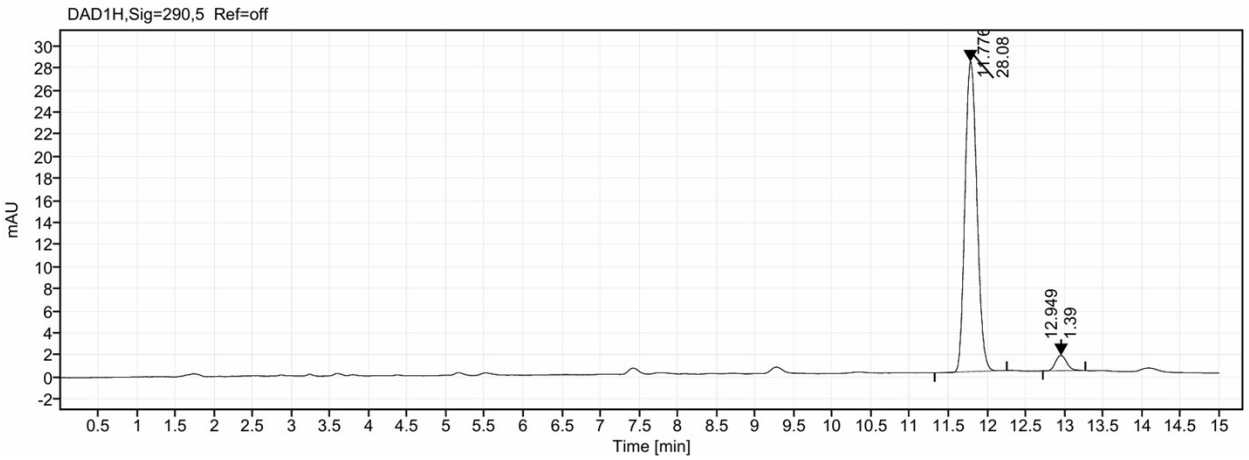
NV-1



Signal: DAD1G,Sig=270,5 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
NV 1 -1	11.960	69.528	97.71	6.815	98.53
NV 1-2	12.987	1.627	2.29	0.102	1.47

NV-9



Signal: DAD1H,Sig=290,5 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
nv-9 1	11.776	300.678	95.53	28.083	95.28
nv-9 2	12.949	14.069	4.47	1.390	4.72

Lipophilicity of the lead compound using an octanol/water partition coefficient study:

Sr. no.	Concentration (µg/mL)	AUC
1	500	18927.347
2	250	9571.976
3	125	4713.044
4	62.5	2352.627
5	31.25	1173.889

Table S1: Calibration curve data (concentration and AUC)

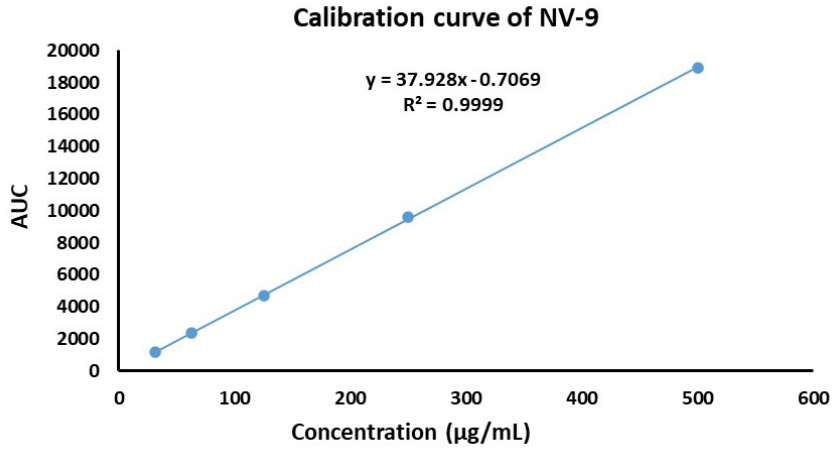
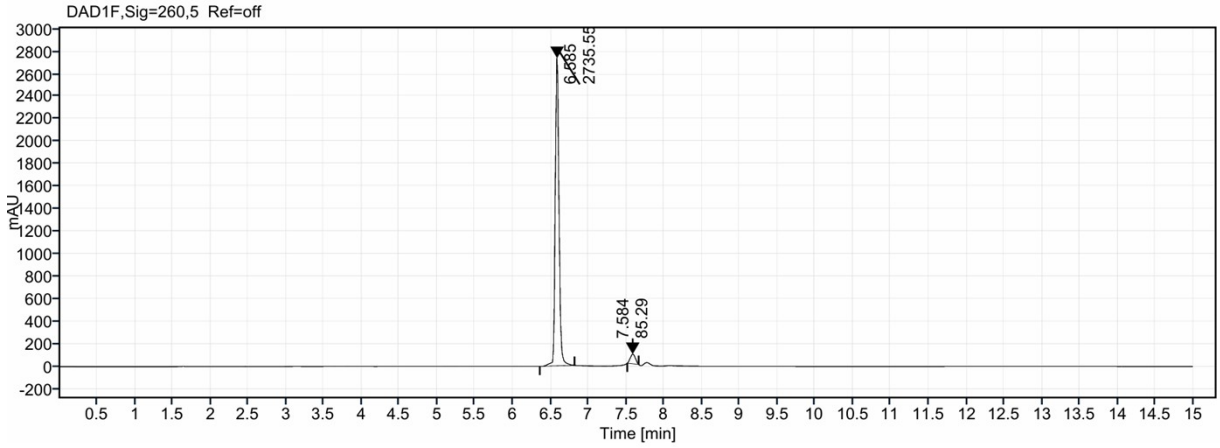


Fig. S1: HPLC calibration curve of NV-9



Signal: DAD1F,Sig=260,5 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
peak@6.585min	6.585	9529.379	95.95	2735.549	96.98
peak@7.584min	7.584	402.174	4.05	85.290	3.02

Fig. S2: HPLC data of NV-9 in octanol

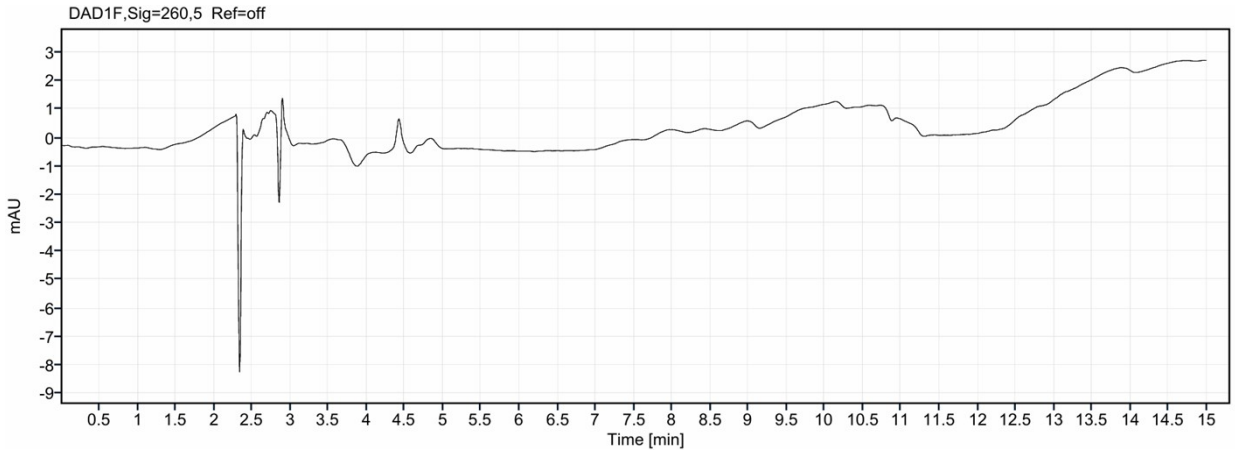


Fig. S3: HPLC data of NV-9 in water