Supporting Information for

Microwave-assisted synthesis, biological evaluation and molecular docking studies of new coumarin based 1,2,3-triazoles

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Supporting Information

1. General Methods

All the chemicals were purchased from Sigma Aldrich, SD-Fine, SRL and Fischer chemicals like commercial sources and used without further purification. Reactions were performed by using dry solvents, oven-dried RB Flasks in Nitrogen gas atmosphere. Transfer of solvents into the RB flask was done with glass syringes. All the intermediates and final products were synthesized through conventional heating, microwave irradiation method by using green solvents. Reaction progress was monitored by TLC on silica gel plates 60 F254 (Merck), the reaction mixture is poured onto crushed ice, filtered and purified with column chromatography by using 60-120 (SRL) mesh silica gel. Melting points of the compounds recorded with Cintex

apparatus. IR (KBr) spectra were recorded on a Shimadzu FT-IR-8400S spectrophotometer. Microwave reactions were carried out in a Multisynth series microwave system (Milestone). ^{1}H, and ^{13}C (proton decoupled) NMR recorded in $CDCl_3$, DMSO solvents on Bruker Avance II 400 and 100 MHz spectrometer (TMS internal standard). Chemical shifts (δ) were reported in parts per million (ppm) with respect to TMS as an internal standard. Coupling constants (J) are quoted in hertz (Hz). Mass spectra and HRMS were recorded on Mass spectrometry by Electron spray ionization (ESI) or Atmospheric pressure chemical ionization (APCI) technique.

2. Synthesis procedure

Synthesis of *3-(4-fluorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-one* 6(a-d):

i). **Conventional Method:** A mixture of *1-(2-hydroxy-4-(prop-2-yn-1-yloxy)phenyl)ethanone* **5**(1g, 5.26 mol), substituted *1-(1H-imidazol-1-yl)-2-phenylethanone* **3**(5.37 mol**)** and anhydrous K_2CO_3 were refluxed in acetone (20 mL) for 4-6h to get *3-(4-fluorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-ones* (6a-d). The progress of the reaction was monitored by TLC, after the completion of reaction crude was poured onto ice cold water and precipitate obtained was filtered and dried. The compound was purified by column chromatography using 60-120 mesh, with 6% Ethyl acetate – n hexane mixture as an eluent to get purification of desired compounds *3-(4-fluorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-ones* **6(a-d)** in high yields.

ii) **Microwave Irradiation Method:** *1-(2-hydroxy-4-(prop-2-yn-1-yloxy)phenyl)ethanone* ***5****(1g, 5.26 mol),* few substituted *1-(1H-imidazol-1-yl)-2-phenylethanone* **3**(5.37 mol**)**, anhydrous K_2CO_3 and to this mixture 4-azido-phenols (1.2 equival.) were suspended in a quartz tube and inserted into the Teflon vial with screw capped and subjected to microwave irradiation. The mixture is then irradiated 5-8 min at affixed temperature (40-100^{0}C). Microwave irradiation power set to 200 W maximum. After the completion of the reaction crude is poured on crushed ice. the precipitate is filtered off, washed with water and dried

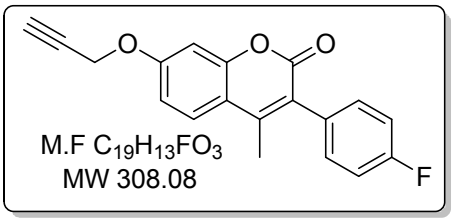
Synthesis of various substituted *4-methyl-7-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-3-(p-tolyl)-2H-chromen-2-ones 8(a-l)*

iii) Conventional Method: To the mixture of *3-(4-fluorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-ones* **6(a-d)** (200mg, 1 equival.), added CuI (5 mol %) in 10 mL of DMF-Water (1:1) solvent and 4-azido-phenols (1.2 equival.). The reaction mixture was stirred at room temperature for 5-6 hours and the progress of the reaction was monitored by TLC. The obtained crude was poured over crushed ice, the precipitate is filtered off, washed with water and dried under sunlight. The crude was purified by column chromatography using 60-120 mesh, with 50% Ethyl acetate – n hexane mixture as an eluent to get purification of desired 1,2,3-triazole derivatives **8(a-l).**

iv) Microwave Irradiation Method: To the mixture of *3-(4-fluorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-ones* **6(a-d)** (0.2g, 1 equival.), CuI (5 mol %) in 10 mL of DMF-Water (1:1) solvent and to this mixture 4-azido-phenols (1.2 equival.) were suspended in a were suspended in a quartz tube and inserted into the Teflon vial with screw capped and subjected to microwave irradiation. mixture is then irradiated 5-8 min at affixed temperature (40-100^{0}C). Microwave irradiation power set to 200 W maximum. After the completion of the reaction crude is poured on crushed ice. The precipitate is filtered off, washed with water and dried under sunlight. The crude was purified by column chromatography using 60-120 mesh, with 50% Ethyl acetate – n hexane mixture as an eluent to get purification of desired 1,2,3-triazole derivatives **8(a-l).**

3. Characterization data of products:

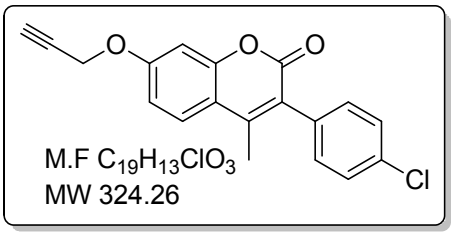
6a. 3-(4-fluorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-one



White solid; Yield: 86%; mp: 102-104 ^{o}C; IR (KBr, cm^{-1}): 3251, 2131, 1703, 1226; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 8.78 Hz, 1H), 7.29-7.27 (m, 2H), 7.16-7.14 (m, 2H), 6.99-6.95 (m, 2H), 4.78 (d, J = 2.51 Hz, 2H), 2.59-2.58 (t, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.7, 161.2, 160.1, 154.0, 148.2, 132.1, 130.4, 130.0, 126.3, 123.6, 115.6, 115.4, 114.6,

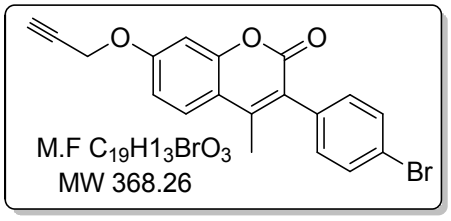
112.9, 101.9, 76.6, 56.2, 16.7; MS (m/z) ESI calcd for $C_{19}H_{13}FO_3$ $[M + 1]^+$ 308.08, found: 309.00.

6b. ***3-(4-chlorophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-one***



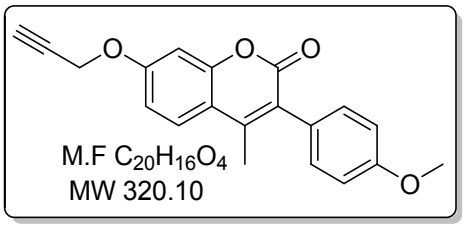
White solid; Yield: 89%; mp:110-112 ^oC; IR (KBr, cm^{-1}): 3267, 2137, 1705, 1143; ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 8.53 Hz, 1H), 7.43 (d, J = 8.53 Hz, 2H), 7.24 (d, J = 8.53 Hz, 2H), 6.99-6.95 (m, 2H), 4.78 (d, J = 2.51 Hz, 2H), 2.59-2.58 (t, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.2, 154.1, 148.2, 134.1, 132.9, 131.7, 130.4, 128.7, 126.3, 123.5, 114.6, 113.0, 101.9, 76.6, 56.2, 16.7; MS (m/z) ES^+ calcd for $C_{19}H_{13}ClO_3$ $[M + 1]^+$ 324.26, found: 325.00.

6c. ***3-(4-bromophenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-one***



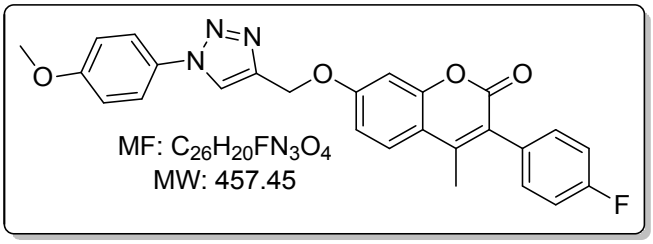
White solid; Yield: 90%; mp: 114-116 ^oC; IR (KBr, cm^{-1}): 3246, 2115, 1710, 1743; ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.58 (d, J = 8.53 Hz, 2H), 7.18 (d, J = 8.53 Hz, 2H), 6.98-6.95 (m, 2H), 4.78 (d, J = 2.51 Hz, 2H), 2.59-2.58 (t, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.2, 154.1, 148.2, 134.4, 132.0, 131.6, 131.2, 128.7, 126.3, 123.5, 122.4, 113.0, 102.0, 76.6, 56.2, 16.7; MS (m/z) ES+ calcd for $C_{19}H_{13}BrO_3$ $[M + 1]^+$ 368.26, found: 369.00.

6d. 3-(4-methoxyphenyl)-4-methyl-7-(prop-2-yn-1-yloxy)-2H-chromen-2-one



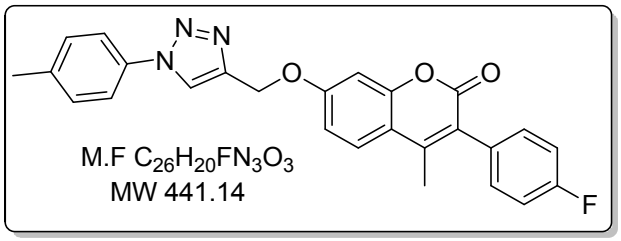
White solid; Yield: 86%; mp: 120-122 ^oC; IR (KBr, cm^{-1}): 3248, 2117, 1708, 1182; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.53 Hz, 1H), 7.23 (d, J = 8.78 Hz, 2H), 6.99-6.95 (m, 4H), 4.78 (d, J = 2.51 Hz, 2H), 3.85 (s, 3H), 2.59-2.58 (t, 1H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 159.9, 159.3, 154.0, 147.5, 126.6, 126.2, 124.4, 114.9, 113.9, 113.0, 101.9, 76.5, 56.2, 55.3, 16.7; MS (m/z) ESI calcd for $C_{20}H_{16}O_4$ $[M + 1]^+$ 320.10, found: 321.05.

8a. ***3-(4-fluorophenyl)-7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one***



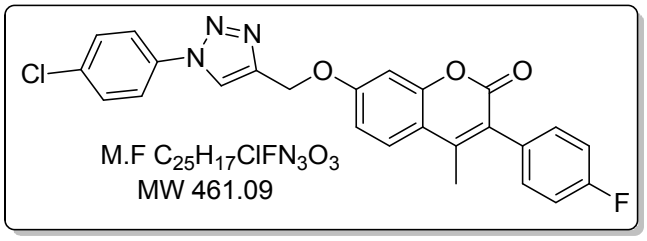
White solid; Yield: 85%; mp:198-200 ^oC; IR (KBr, cm^{-1}): 3153, 2250, 1714, 1189; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.58-7.52 (m, 3H), 7.23-7.19 (m, 2H), (6.97-6.94 (m, 4H), 5.29 (s, 2H), 3.81 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 162.6, 160.2, 159.8, 159.0, 153.1, 147.5, 142.5, 131.0, 131.0, 129.3, 129.2, 125.4, 122.6, 121.3, 120.4, 114.6, 114.3, 113.8, 113.5, 111,5, 100.9, 61.2, 54.6, 15.6; HRMS (m/z) calcd for M.F $C_{26}H_{20}FN_3O_4$ $[M + 1]^+$ 457.14, found: 458.5149.

8b. ***3-(4-fluorophenyl)-4-methyl-7-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one***



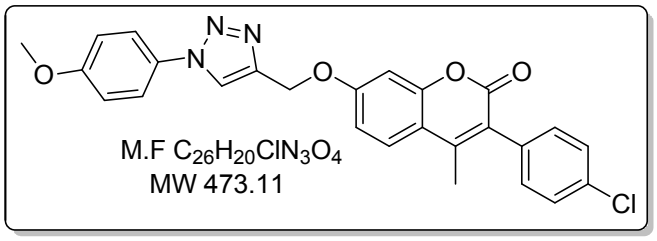
White solid; Yield: 82%; mp 182-184 ^{o}C; IR (KBr, cm^{-1}): 3125, 2210, 1712, 1145; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.63-7.59 (m, 3H), 7.34-7.27 (m, 4H), 7.16-7.12 (m, 2H), 7.04-7.00 (m, 2H), 5.36 (s, 2H), 2.43 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 160.8, 154.1, 148.1, 143.1, 139.2, 134.5, 132.2, 131.9, 130.3, 126.3, 123.6, 121.2, 120.5, 115.6, 115.3, 114.5, 112.5, 101.9, 62.2, 21.1, 16.6; HRMS (m/z) calcd for M.F $C_{26}H_{20}FN_3O_3$ [M + 1]$^{+}$ 441.14, found: 442.1676.

8c. ***7-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-(4-fluorophenyl)-4-methyl-2H-chromen-2-one***



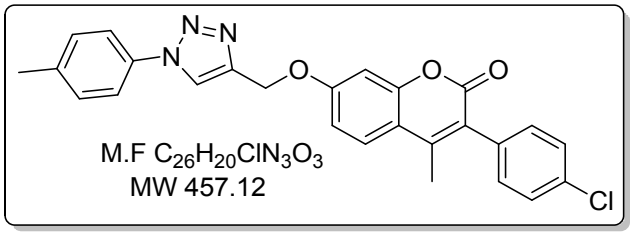
White solid; Yield: 84%; mp: 204-206 ^{o}C; IR (KBr, cm^{-1}): 3212, 2150, 1708, 1186; ^{1}H NMR (400 MHz, DMSO) δ 9.05 (s, 1H), 7.98-7.97 (m, 2H), 7.89-7.69 (m, 3H), 7.37-7.11 (m, 6H), 5.40 (s, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 161.2, 154.6, 148.5, 144.0, 139.6, 135.0, 132.5, 132.4, 130.7, 126.8, 124.1, 121.6, 121.0, 116.0, 115.7, 114.9, 113.0, 102.4, 62.7, 17.0; HRMS (m/z) calcd for $C_{25}H_{17}ClFN_3O_3$ [M + 1]$^{+}$ 461.19, found: 462.1033.

8d. ***3-(4-chlorophenyl)-7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one***



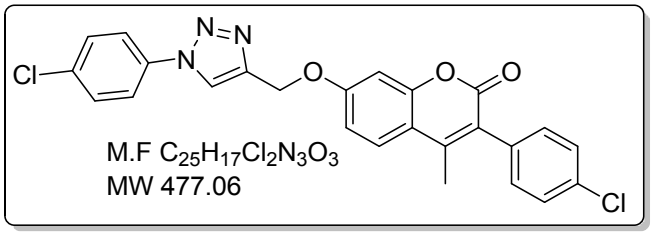
White solid; Yield: 85%; mp: 208-210 ^{o}C; IR (KBr, cm^{-1}): 3183, 2191, 1704, 1176; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.59-7.53 (m, 3H), 7.36 (d, J = 8.44 Hz, 2H), 7.17 (s, 2H), 6.99-6.94 (m, 4H), 5.29 (s, 2H), 3.81 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 159.9, 159.0, 153.2, 147.1, 146.7, 142.4, 133.1, 131.9, 130.6, 127.7, 125.4, 124.3, 121.3, 120.4, 113.8, 113.4, 111.6, 111.0, 61.3, 54.6, 15.6; HRMS (m/z) calcd for $C_{26}H_{20}ClN_3O_4$ [M + 1]$^{+}$ 473.11, found: 474.1223.

8e. ***3-(4-chlorophenyl)-4-methyl-7-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one***



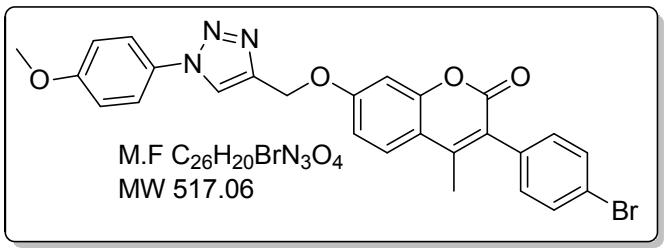
White solid; Yield: 85%; mp: 200-202 ^{o}C; IR (KBr, cm^{-1}): 3125, 2210, 1712, 1145; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.59-7.53 (m, 3H), 7.36 (d, J = 8.53 Hz, 2H), 7.19 (d, J = 8.78 Hz, 2H), 6.98-6.94 (m, 4H), 5.29 (s, 2H), 3.81 9s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 156.5, 154.1, 151.1, 148.1, 143.5, 139.2, 134.5, 134.1, 131.6, 130.3, 128.6, 126.4, 121.2, 120.5, 114.4, 112.6, 111.6, 106.5, 101.9, 62.2, 21.5, 16.0; HRMS (m/z) calcd for $C_{26}H_{20}ClN_3O_3$ [M + 1]$^{+}$ 473.11, found: 458.1275.

8f. ***3-(4-chlorophenyl)-7-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one***



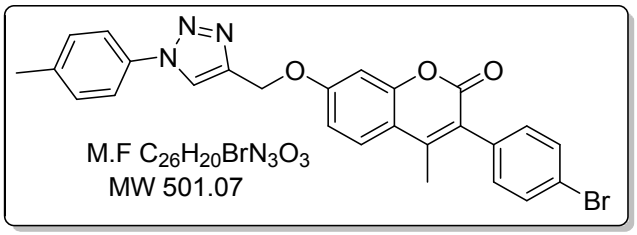
White solid; Yield: 88%; mp: 196-198 ^{o}C; IR (KBr, cm^{-1}): 3224, 2182, 1704, 1172; ^{1}H NMR (400 MHz, DMSO) δ 9.05 (s, 1H), 7.97 (d, J = 8.53 Hz, 2H), 7.80 (d, J = 8.78 Hz, 1H), 7.69 (d, J = 8.53 Hz, 2H), 7.51 (d, J = 853 Hz, 2H), 7.36-7.35 (m, 3H), 7.12-7.10 (m, 1H), 5.40 (s, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.8, 157.8, 154.2, 151.9, 148.1, 134.9, 134.2, 132.9, 131.7, 130.1, 128.7, 126.5, 123.6, 121.8, 121.1, 114.6, 112.6, 102.0, 62.2, 16.6; HRMS (m/z) calcd for $_{25}H_{17}Cl_2N_3O_3$ $[M + 1]^+$ 477.06, found: 478.0727.

8g. ***3-(4-bromophenyl)-7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one***



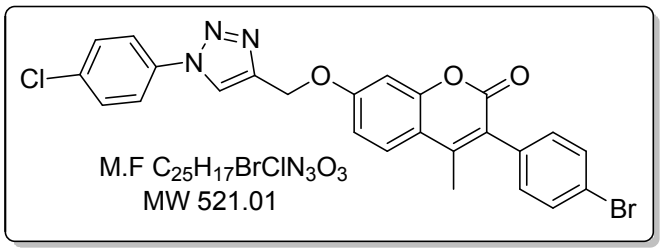
White solid; Yield: 82%; mp: 210-212 ^{o}C; IR (KBr, cm^{-1}): 3393, 2257, 1713, 1186; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.59-7.50 (m, 5H), 6.98 (d, J = 8.53 Hz, 2H), 6.96-6.94 (m, 4H), 5.30 (s, 2H), 3.81 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.0, 154.2, 148.1, 144.7, 143.4, 133.4, 132.0, 131.6, 130.27, 126.4, 123.5, 122.3, 121.4, 114.9, 114.5, 112.6, 102.0, 62.3, 55.9, 16.6; HRMS (m/z) calcd for $C_{26}H_{20}ClN_3O_4$ $[M + 1]^+$ 517.06, found: 518.0719.

8h. ***3-(4-bromophenyl)-4-methyl-7-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-one***



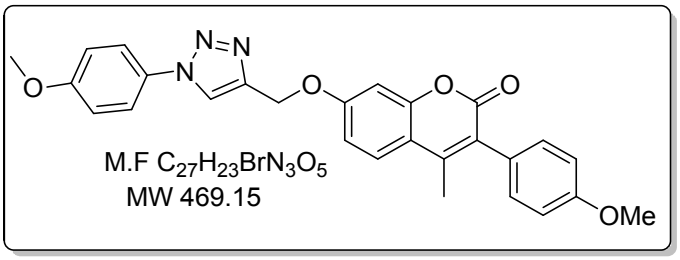
White solid; Yield: 80%; mp: 213-215 ^oC; IR (KBr, cm^{-1}): 3183, 2191, 1704, 1176; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.63-7.57 (m, 5H), 7.33 (d, J = 8.53 Hz, 2H), 7.18 (d, J = 8.53 Hz, 2H), 7.04-7.00 (m, 2H), 5.37 (s, 2H), 2.44 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 154.2, 148.1, 143.5, 139.3, 134.6, 133.4, 132.0, 131.6, 130.3, 126.4, 123.5, 122.4, 121.3, 120.6, 114.5, 112.6, 101.9, 62.3, 21.5, 16.7; HRMS (m/z) calcd for $C_{26}H_{20}BrN_3O_3$ $[M + 1]^+$ 501.07, found: 502.3170.

8i. ***3-(4-bromophenyl)-7-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one***



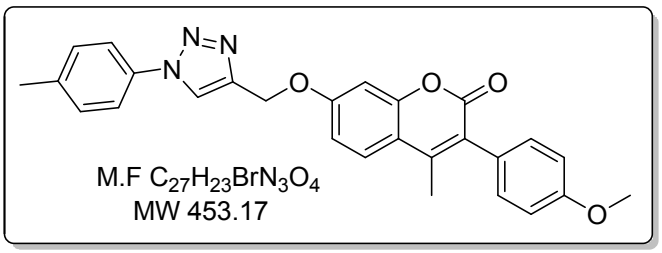
White solid; Yield: 85%; mp: 180-182 ^oC; IR (KBr, cm^{-1}): 3254, 2212, 1706, 1163; ^1H NMR (400 MHz, DMSO) δ 7.96 (s, 1H), 7.59-7.53 (m, 3H), 7.36 (d, J = 8.44 Hz, 2H), 7.17 (s, 2H), 6.99-6.94 (m, 4H), 5.29 (s, 2H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.8, 154.1, 148.1, 134.9, 134.1, 132.9, 131.6, 130.0, 129.8, 128.6, 128.2, 126.6, 126.4, 123.5, 121.7, 114.5, 112.6, 101.9, 62.2, 16.6; HRMS (m/z) calcd for $C_{25}H_{17}ClBrN_3O_3$ $[M + 1]^+$ 521.01, found: 522.1237.

8j. ***3-(4-methoxyphenyl)-7-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methyl-2H-chromen-2-one***



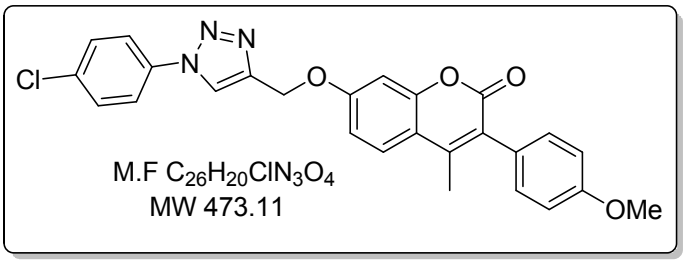
White solid; Yield: 80%; mp: 174-176 ^{o}C; IR (KBr, cm^{-1}): 3233, 2212, 1705, 1180; ^{1}H NMR (400 MHz, DMSO) δ 8.10 (s, 1H), 7.71 (d, J = 8.53 Hz, 2H), 7.60 (d, J = 8.53 Hz, 1H), 7.23 (d, J = 8.69 Hz, 2H), 7.02 (d, J = 8.53 Hz, 2H), 6.99-6.96 (m, 4H), 5.37 (s, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 159.9, 159.0, 153.2, 147.1, 146.7, 142.4, 133.1, 131.9, 130.6, 127.7, 125.4, 124.3, 121.3, 120.4, 113.8, 113.4, 111.6, 100.9, 61.2, 55.3, 54.6, 15.6; HRMS (m/z) calcd for $C_{27}H_{23}BrN_3O_5$ [M + 1]$^{+}$ 469.15 found: 470.1742.

8k. ***3-(4-methoxyphenyl)-4-methyl-7-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)-2H-chromen-2-on***



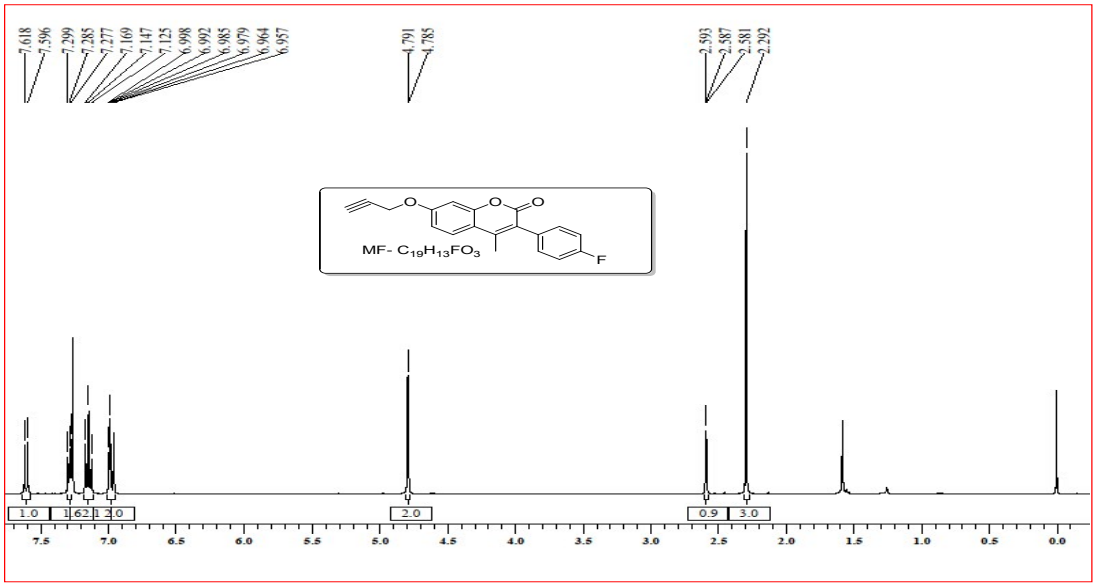
White solid; Yield: 87%; mp: 186-188 ^{o}C; IR (KBr, cm^{-1}): 3210, 2203, 1708, 1192; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.62-7.58 (m, 3H), 7.33 (d, J = 8.54 Hz, 2H), 7.22 (d, J = 8.69 Hz, 2H), 7.02-6.97 (m, 4H), 5.36 (s, 2H), 3.84 (s, 3H), 2.43 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 160.6, 159.3, 154.0, 147.5, 143.6, 139.2, 134.5, 131.4, 130.3, 126.3, 124.3, 121.2, 120.5, 114.8, 113.8, 112.4, 101.9, 62.2, 55.8, 21.1, 16.6; HRMS (m/z) calcd for $C_{26}H_{20}BrN_3O_3$ [M + 1]$^{+}$ 453.17, found: 454.1780

8l. ***7-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-(4-methoxyphenyl)-4-methyl-2H-chromen-2-one***

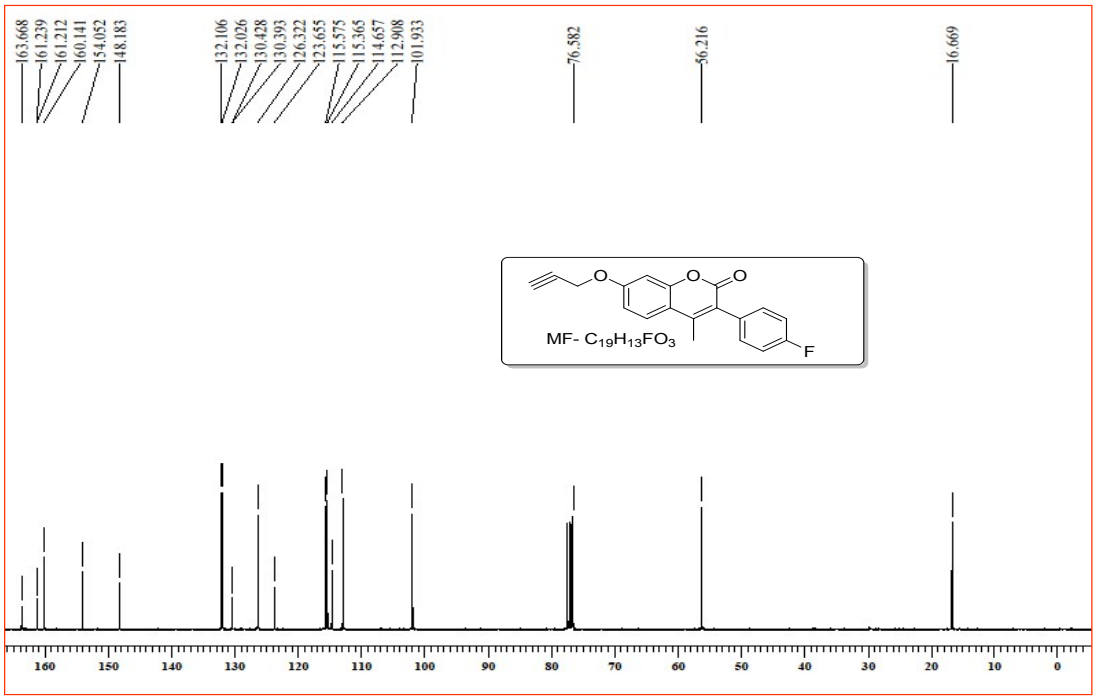


White solid; Yield: 80%; mp: 206-208 ^{o}C; IR (KBr, cm^{-1}): 3242, 2223, 1712, 1173; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.07 (d, J = 8.53 Hz, 2H), 7.59 (d, J = 8.53 Hz, 1H), 7.23 (d, J = 8.69 Hz, 2H), 7.02 (d, J = 8.53 Hz, 2H), 6.99-6.96 (m, 4H), 5.37 (s, 2H), 3.85 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 160.5, 159.3, 154.1, 147.5, 135.3, 134.9, 131.5, 130.0, 126.6, 126.3, 124.4, 121.8, 121.1, 114.9, 113.9, 112.4, 101.9, 62.2, 55.3, 16.7; HRMS (m/z) calcd for $C_{26}H_{20}ClN_3O_4$ $[M + 1]^+$ 473.11, found: 474.1223.

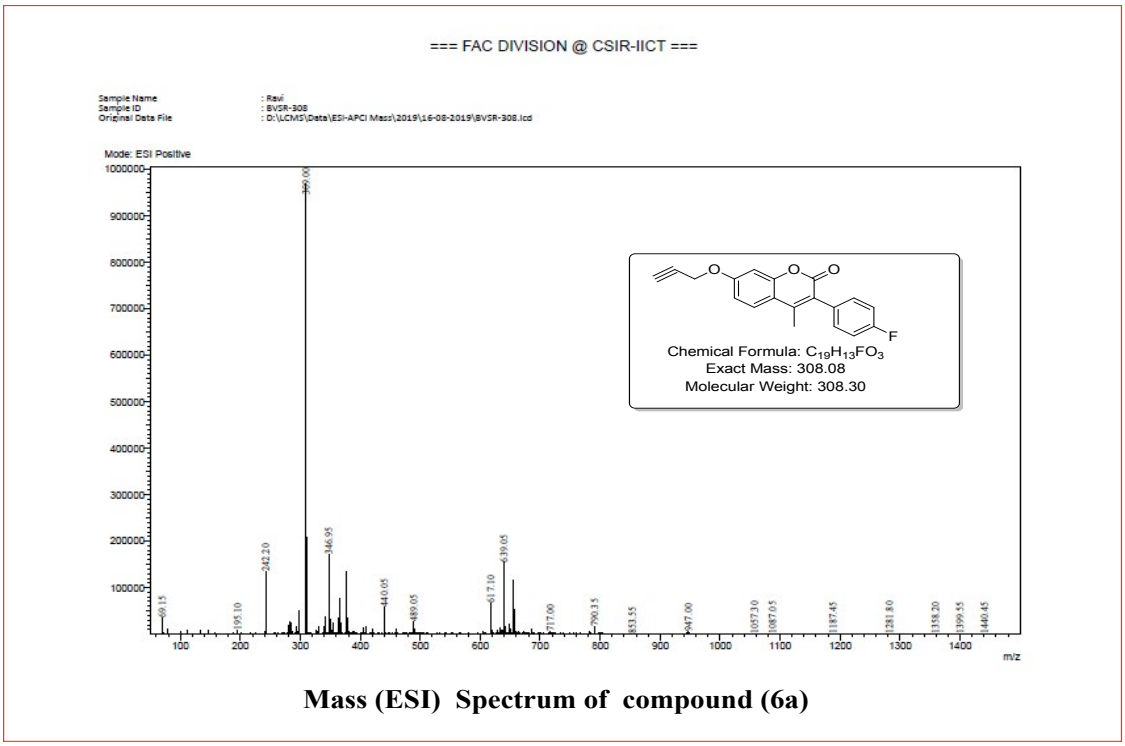
4. Copies of Spectral data



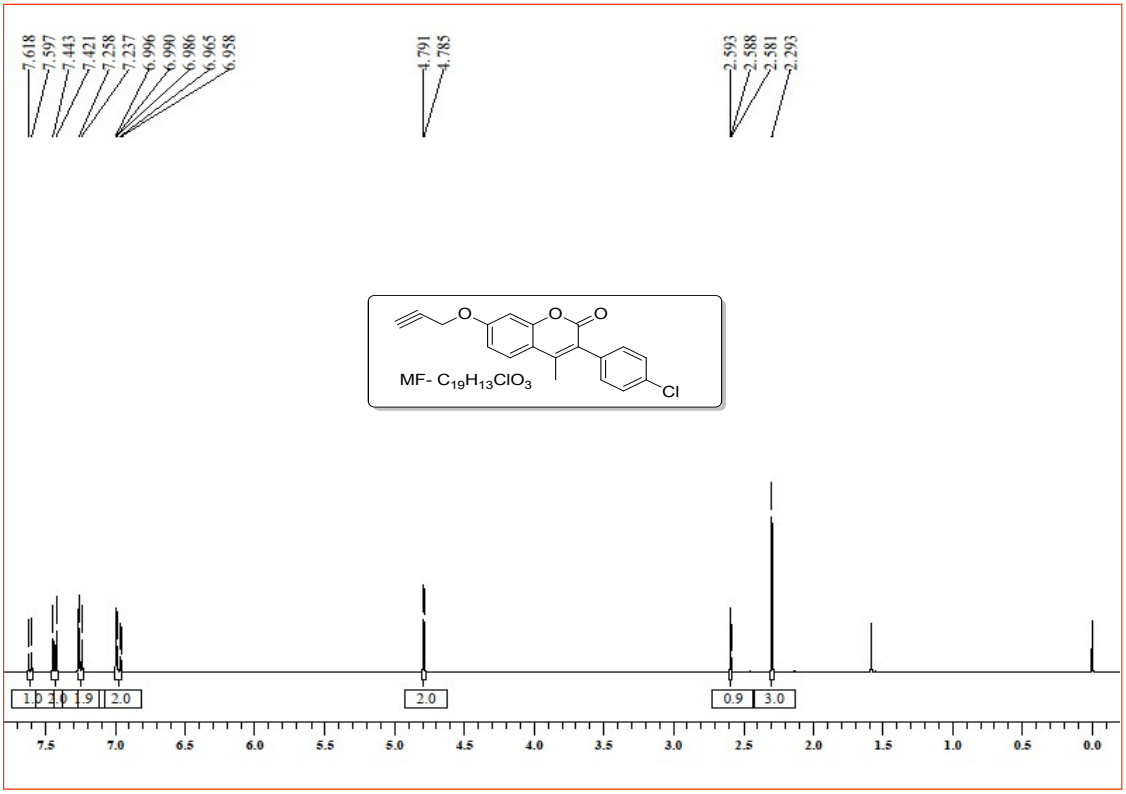
1H NMR (400 MHz, CDCl3) Spectrum of compound (6a)



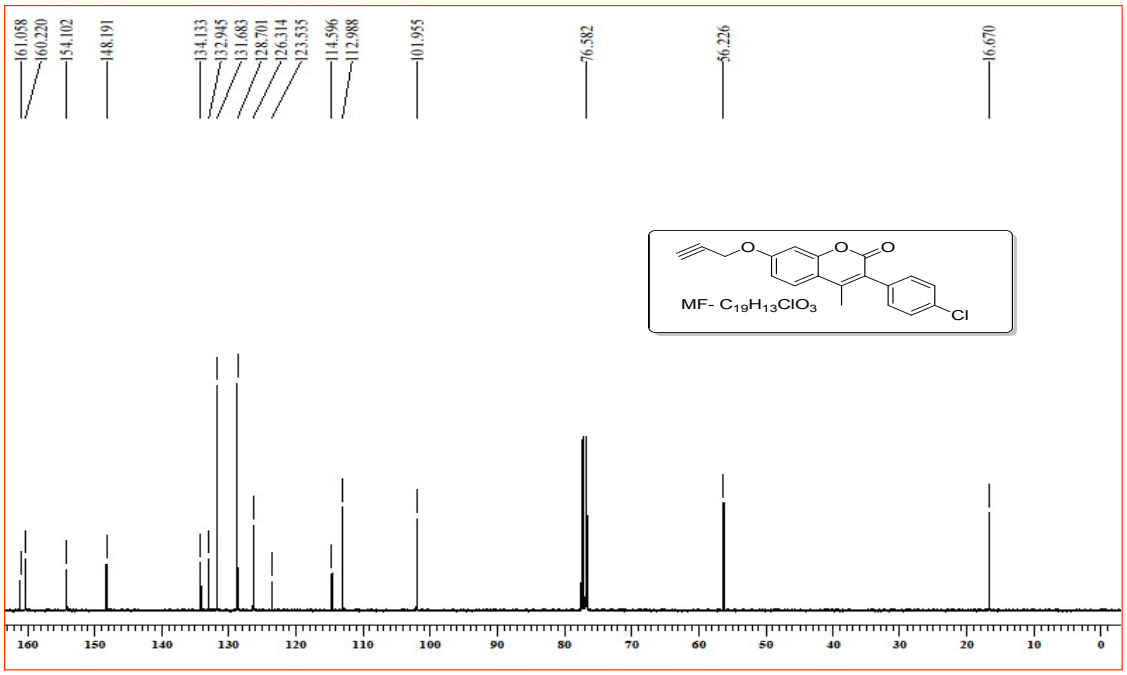
13 C NMR (400 MHz, CDCl3) Spectrum of compound (6a)



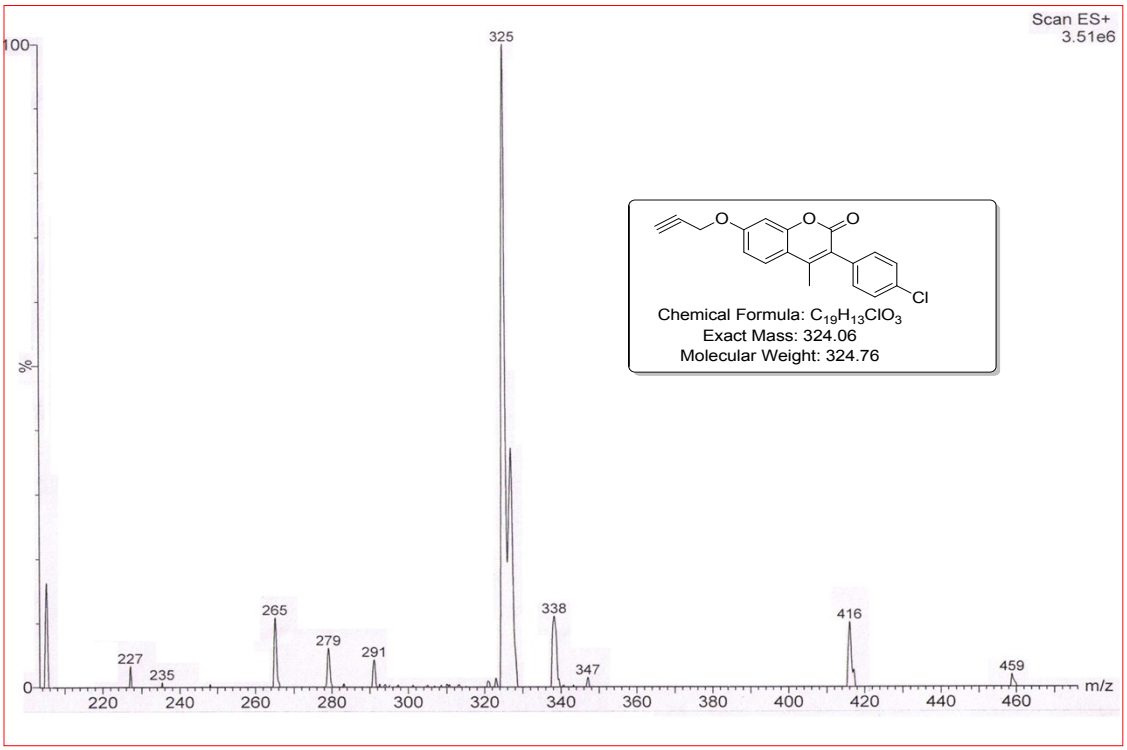
Mass (ESI) Spectrum of compound (6a)



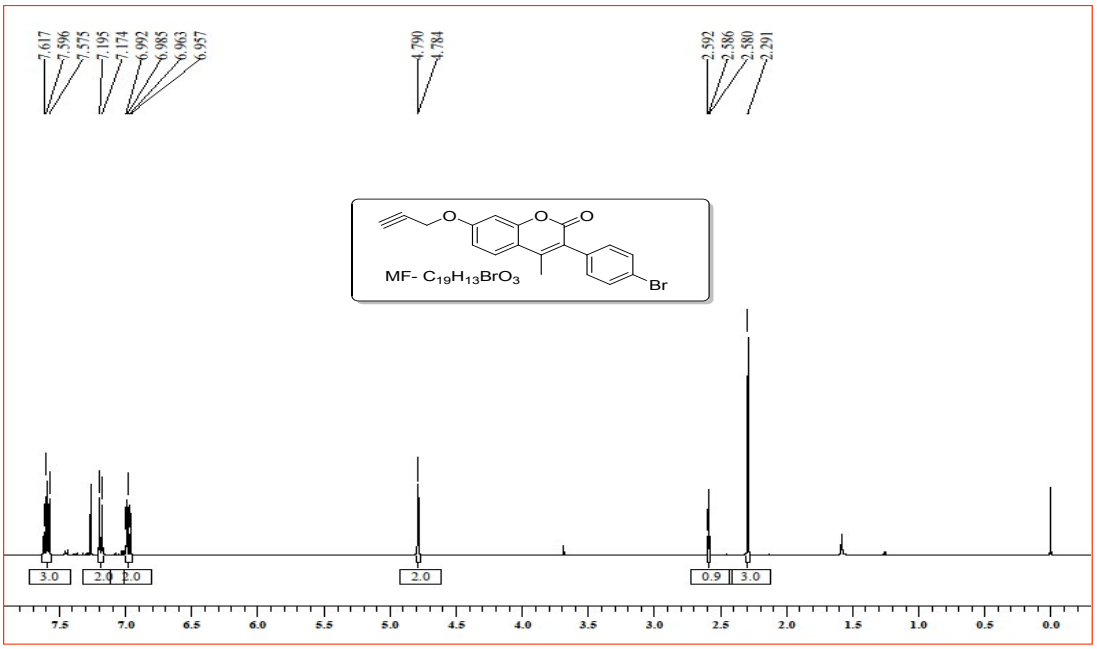
1H NMR (400 MHz, CDCl3) Spectrum of compound (6b)



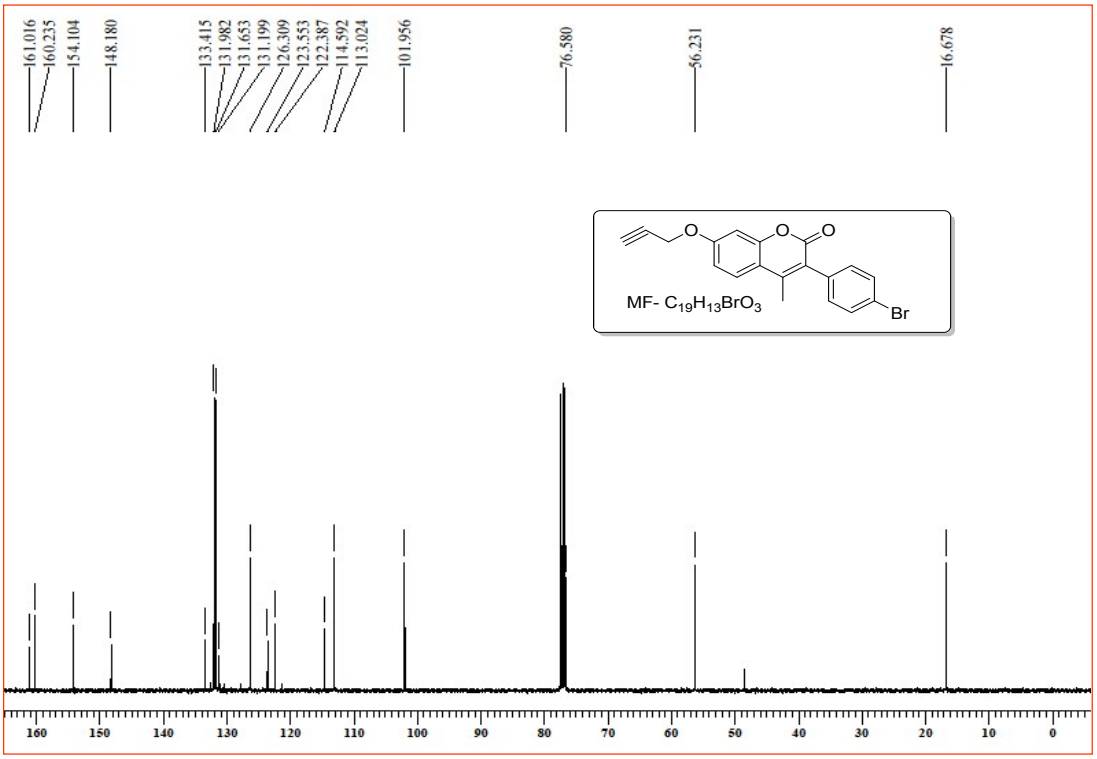
13 C NMR (400 MHz, CDCl3) Spectrum of compound (6b)



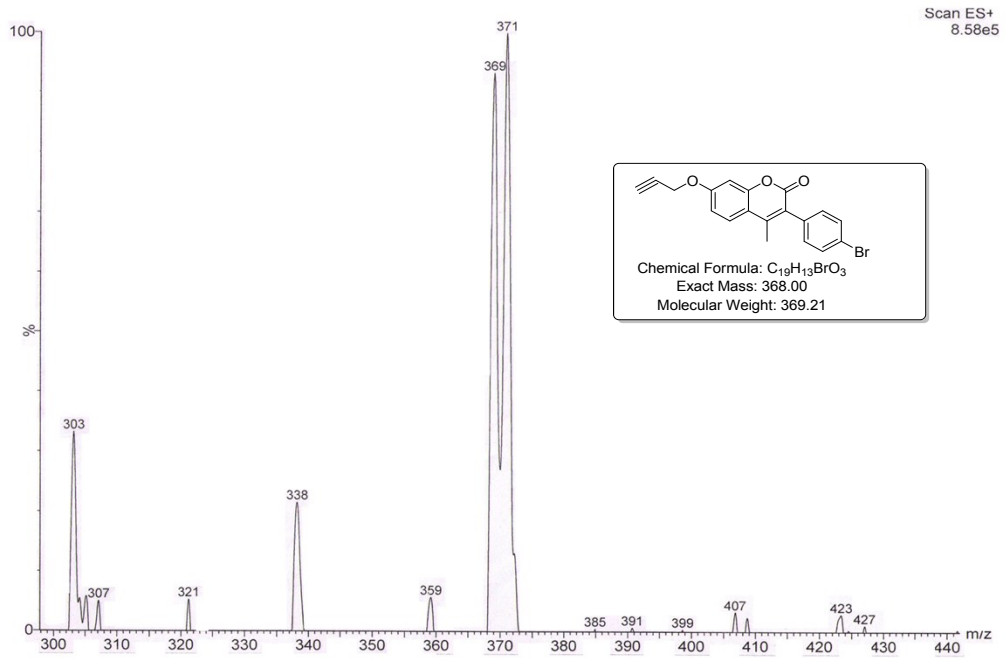
Mass (ESI) Spectrum of compound (6b)



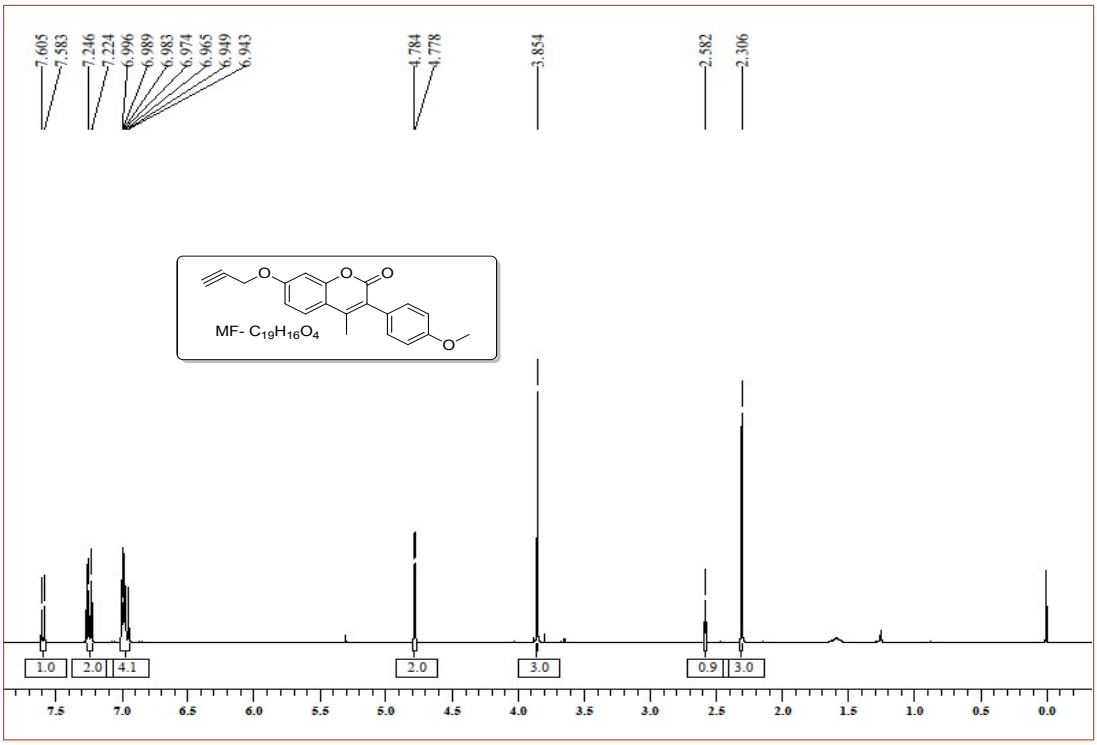
1H NMR (400 MHz, CDCl3) Spectrum of compound (6c)



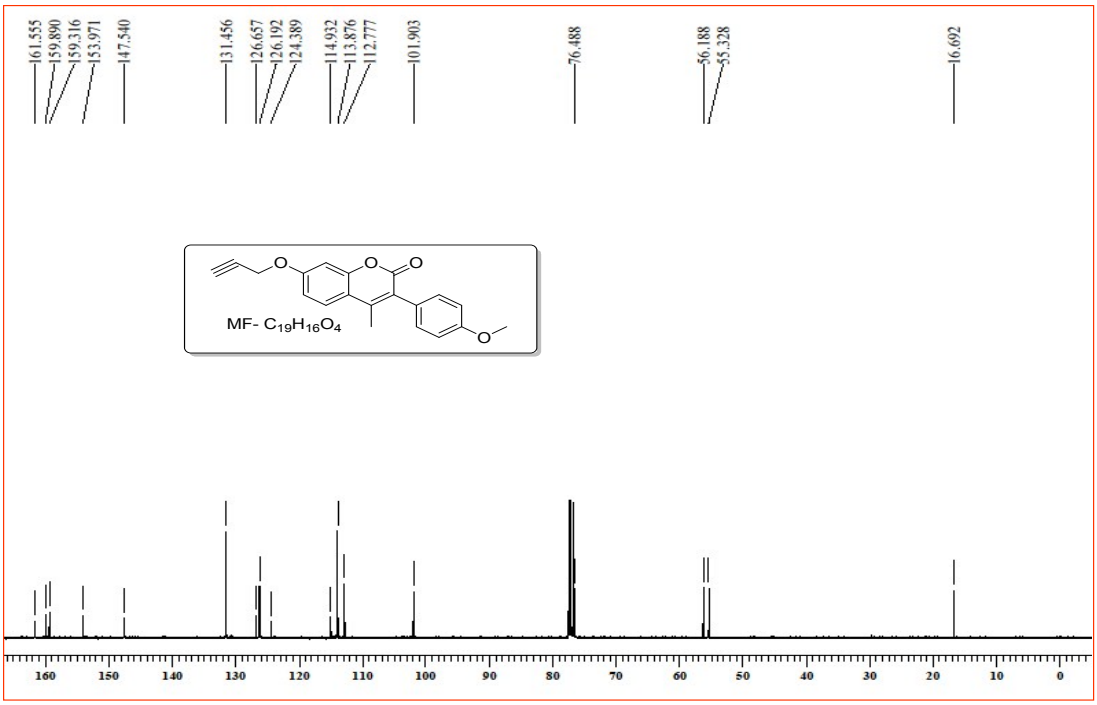
13 C NMR (400 MHz, CDCl3) Spectrum of compound (6c)



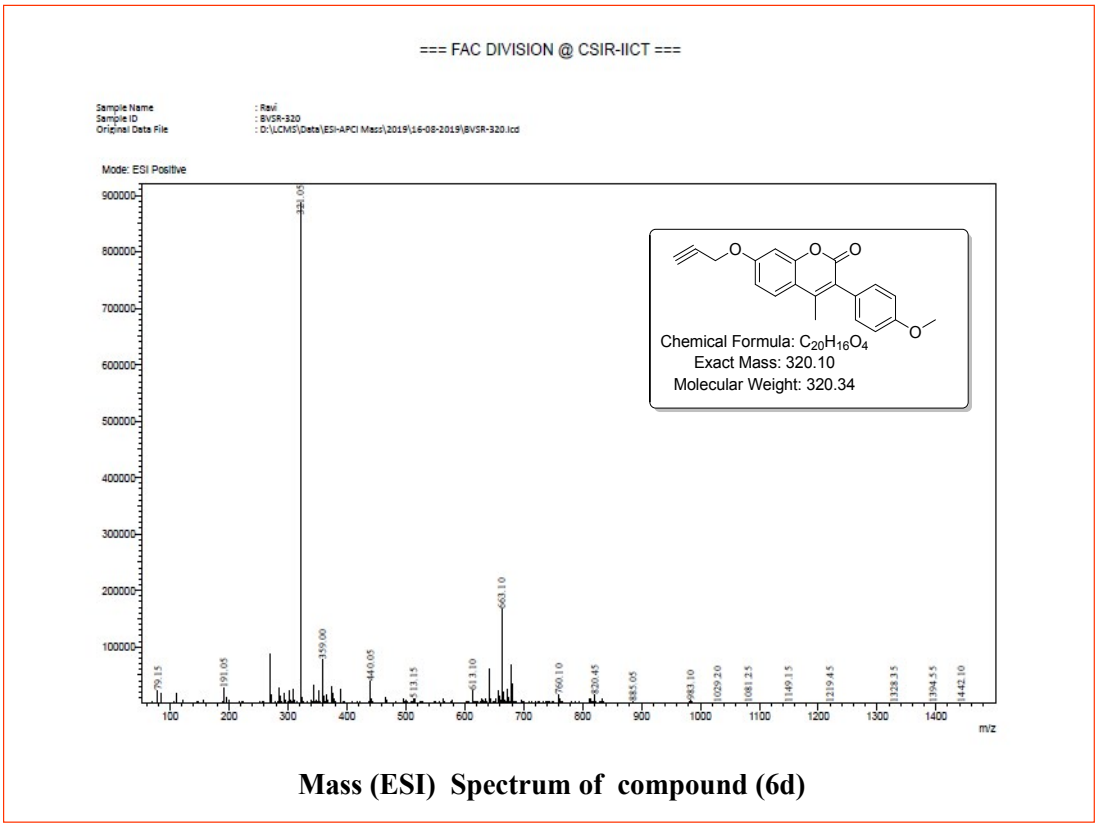
Mass (ESI) Spectrum of compound (6c)



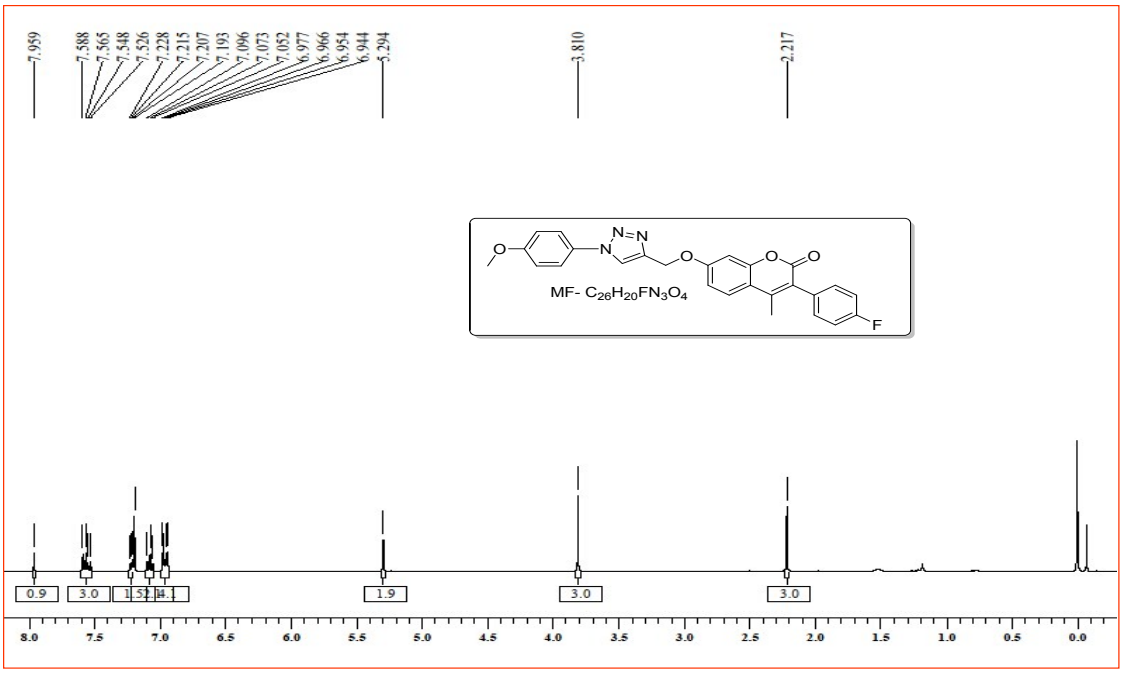
1H NMR (400 MHz, CDCl3) Spectrum of compound (6d)



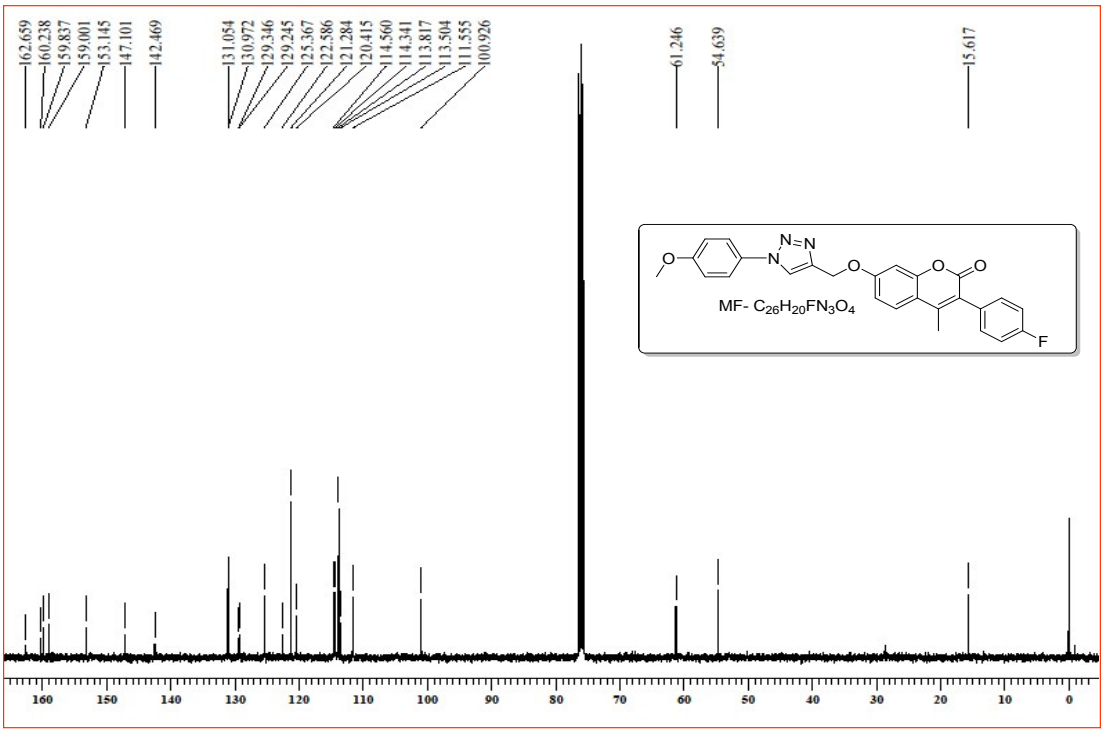
13 C NMR (400 MHz, CDCl3) Spectrum of compound



Mass (ESI) Spectrum of compound (6d)

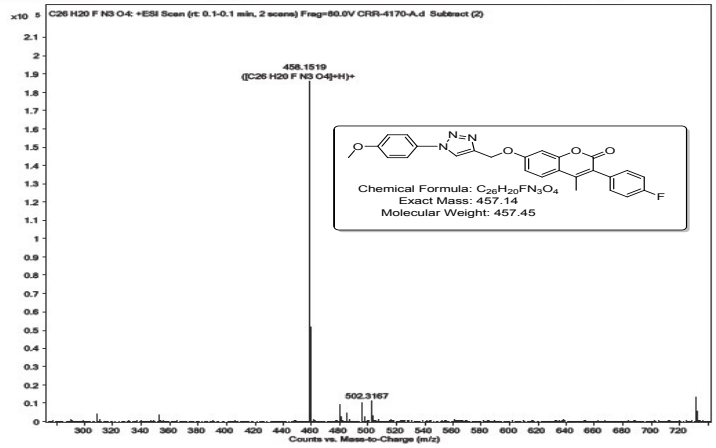


1H NMR (400 MHz, CDCl3) Spectrum of compound (8a)



13 C NMR (400 MHz, CDCl3) Spectrum of compound

Sample Name	CRR-4170-A	Position	P2-A7	Instrument Name	Instrument 1
User Name	CSIR-IICT\Analyst	Inj Vol	1	InjPosition	
Sample Type	Sample	IRM Calibration Status	Success	Data Filename	CRR-4170-A.d
ACQ Method	NME-AA-FS10-02-17.m	Comment		Acquired Time	25-06-2019 22:58:20



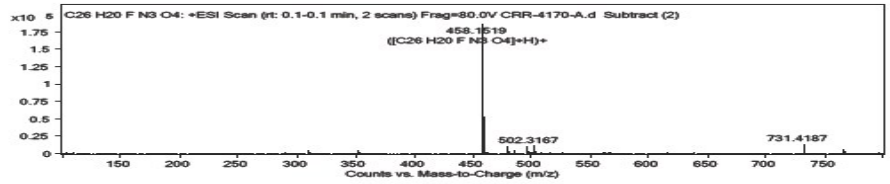
HRMS Spectrum of compound (8a)

Qualitative Analysis Report

Data File	CRR-4170-A.d	Sample Name	CRR-4170-A
Sample Type	Sample	Position	P2-A7
Instrument Name	Instrument 1	User Name	CSIR-IICT\Analyst
Acq Method	NME-AA-FS10-02-17.m	Acquired Time	25-06-2019 22:58:20
IRM Calibration Status	Success	DA Method	HRMS TEMPLATE.m
Comment			
Sample Group		Info.	
Stream Name	LC 1	Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B6172 SP1)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
80	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
458.1519	1	186209.44	C26 H20 F N3 O4	(M+H)+
915.295	1	86193.34		

Formula Calculator Element Limits

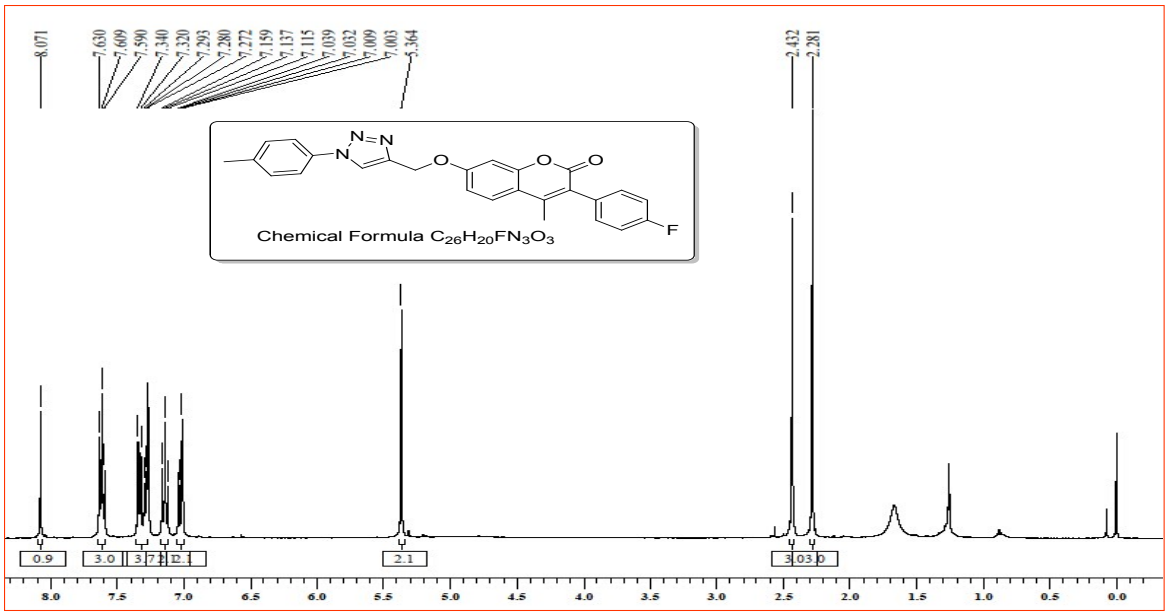
Element	Min	Max
C	0	26
H	0	21
O	1	4
N	0	3
Si	0	0
Cl	0	0
F	0	1

Formula Calculator Results

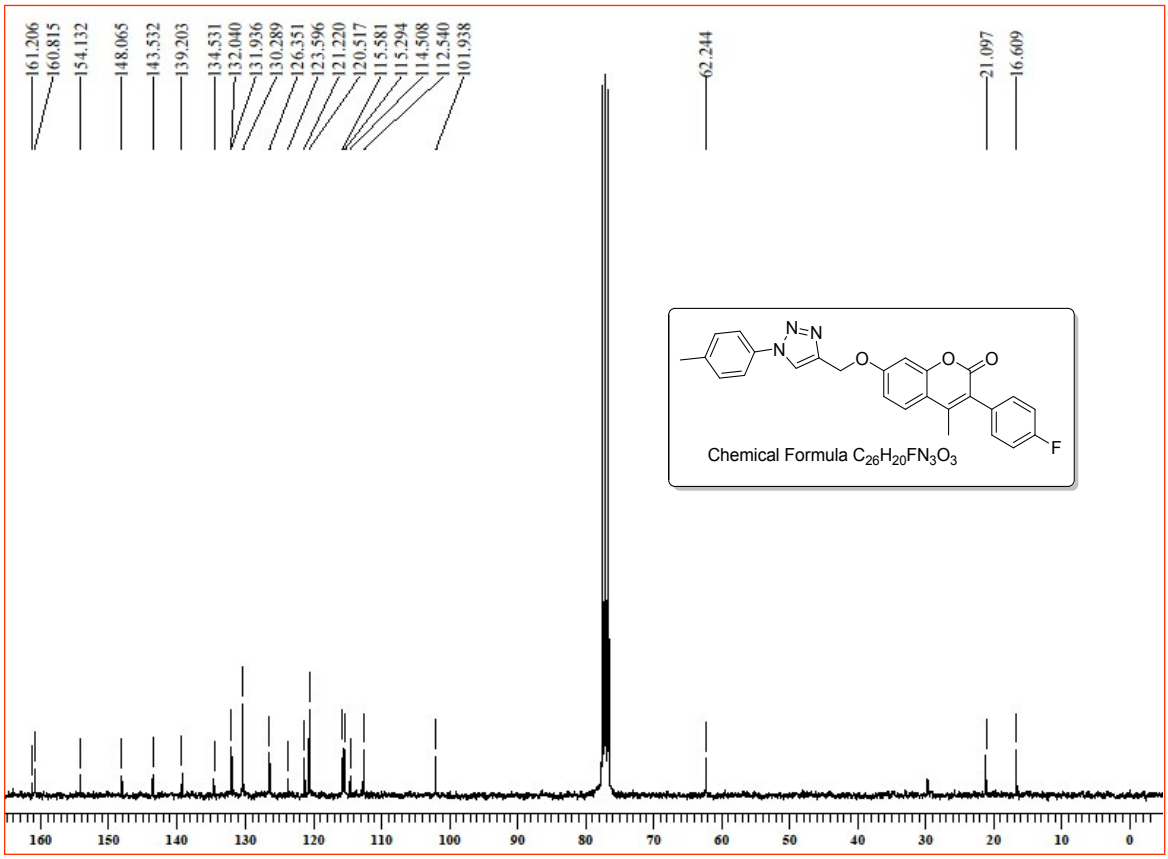
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C26 H21 F N3 O4	True	458.1524	458.1516	1.64	C26 H21 F N3 O4	97.88
C26 H20 F N3 O4	True	457.1445	457.1438	1.65	C26 H21 F N3 O4	97.88

Agilent Technologies

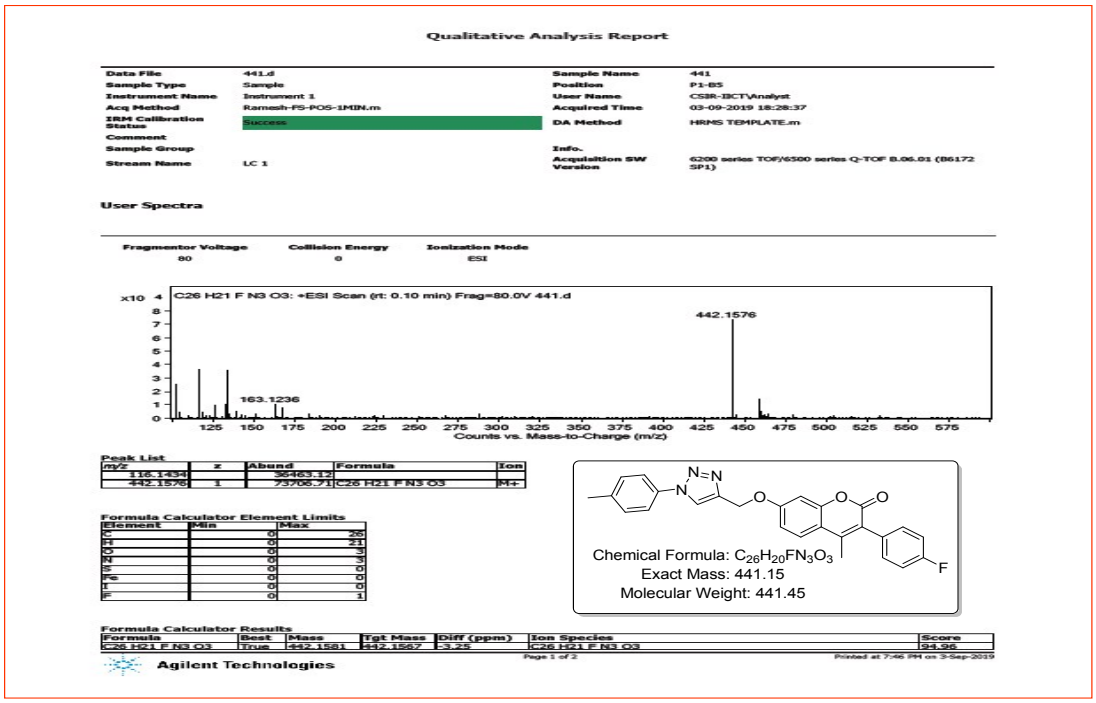
HRMS Spectrum of compound (8a)



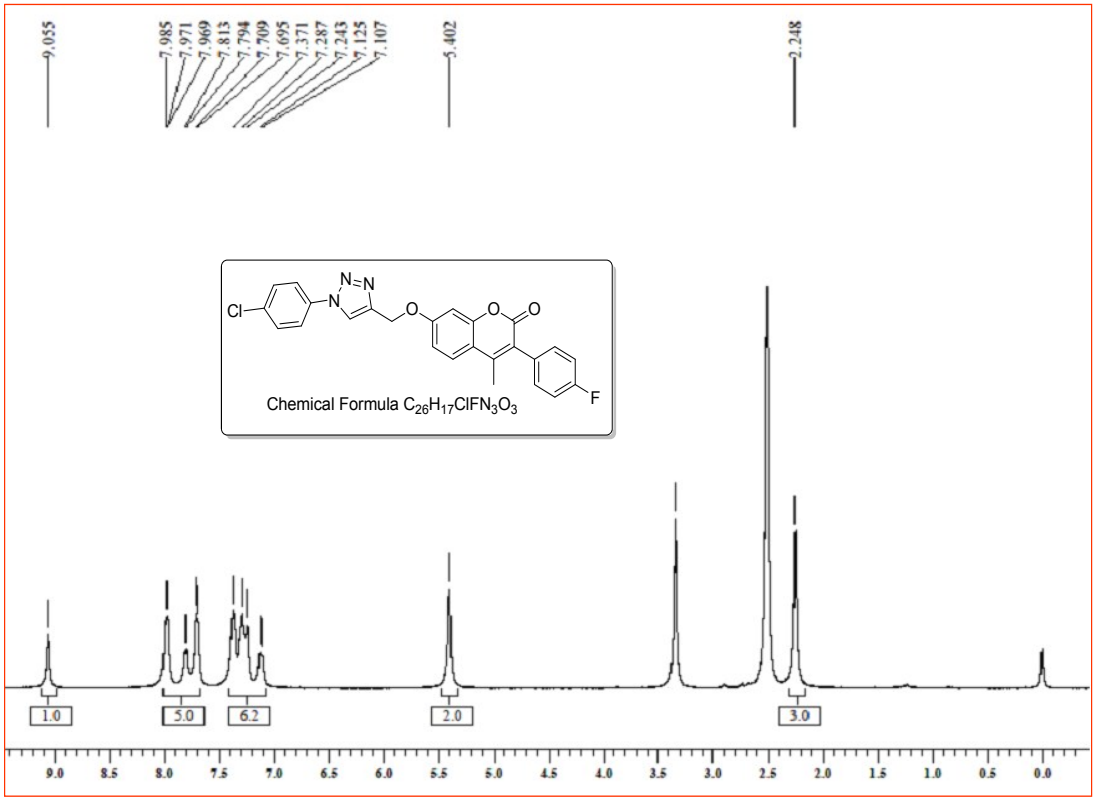
1H NMR (400 MHz, CDCl3) Spectrum of compound (8b)



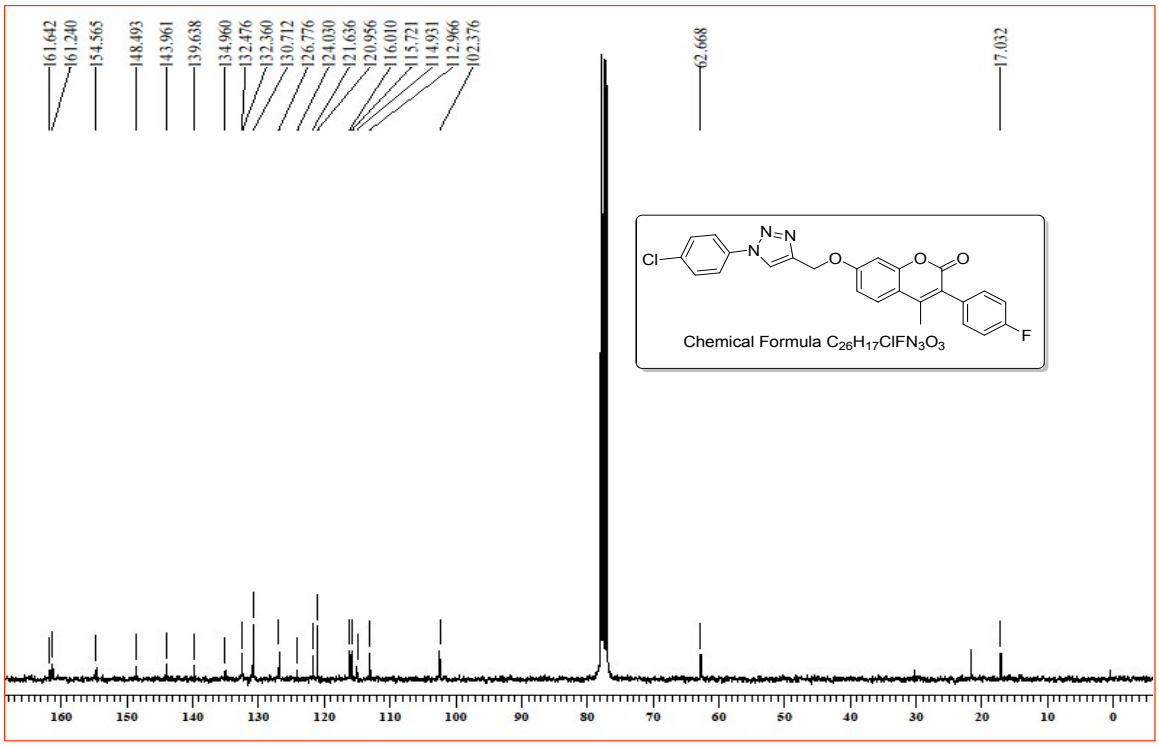
13 C NMR (400 MHz, CDCl3) Spectrum of compound



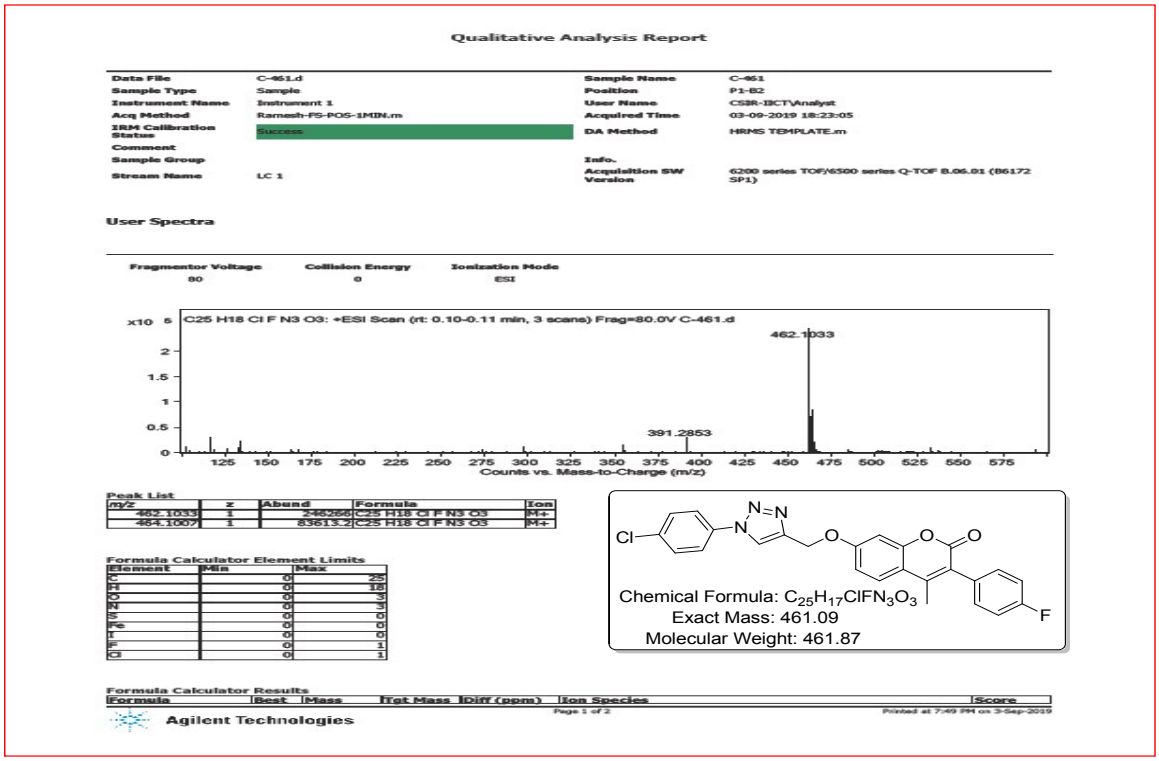
HRMS Spectrum of compound (8b)



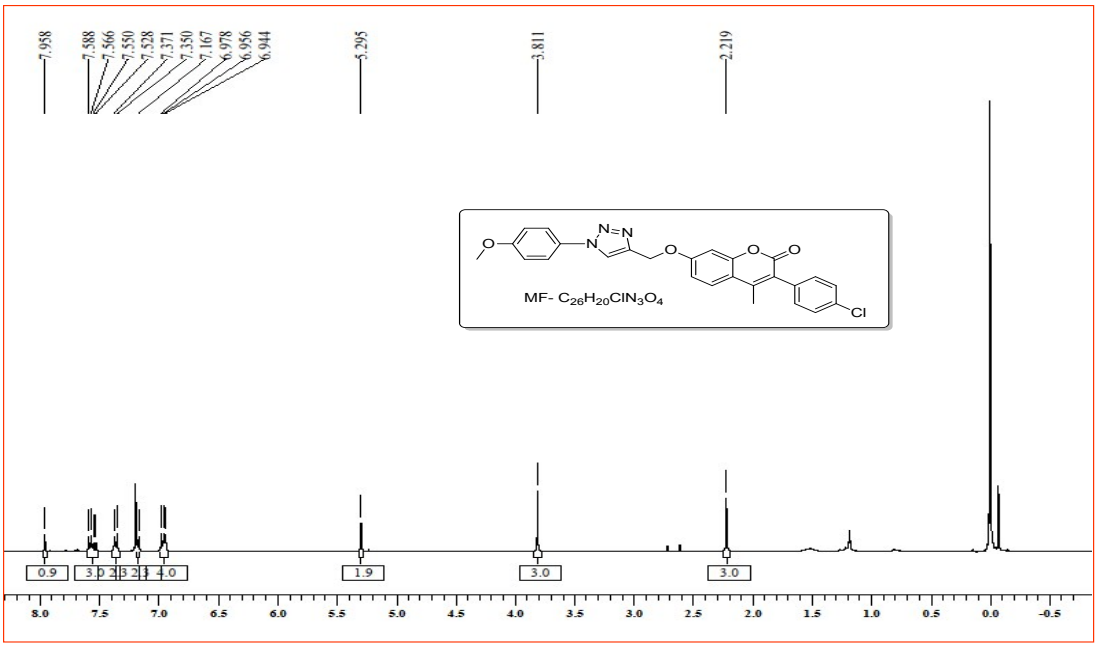
1H NMR (400 MHz, DMSO) Spectrum of compound (8c)



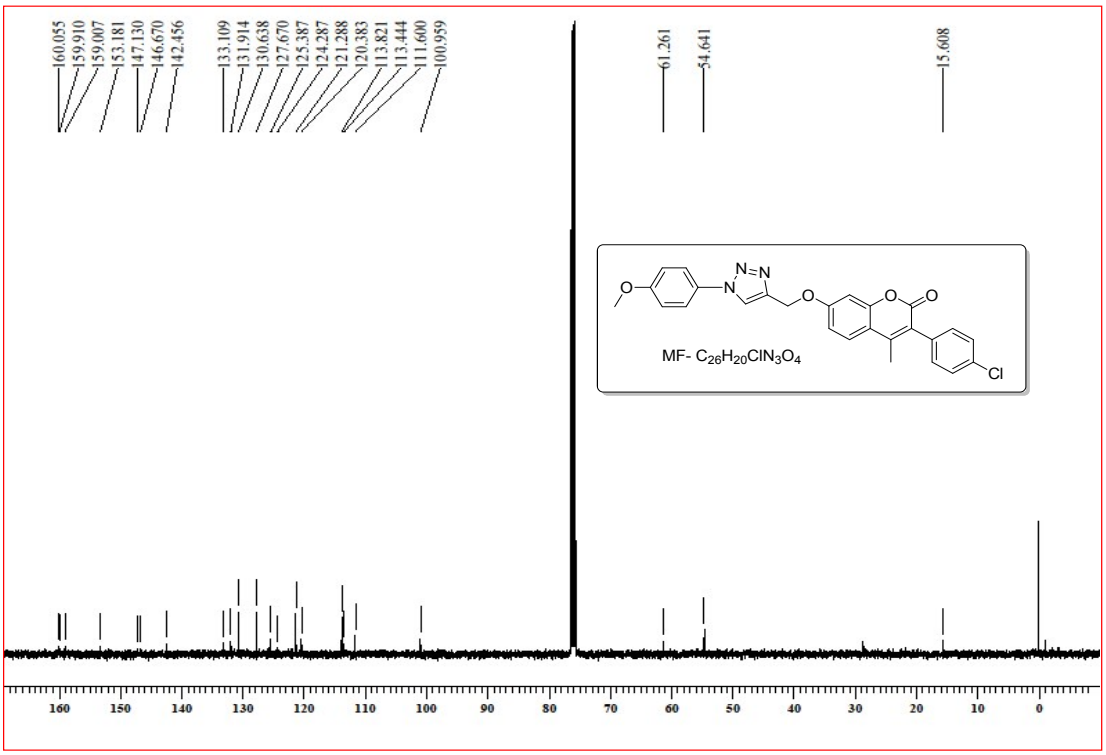
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8c)



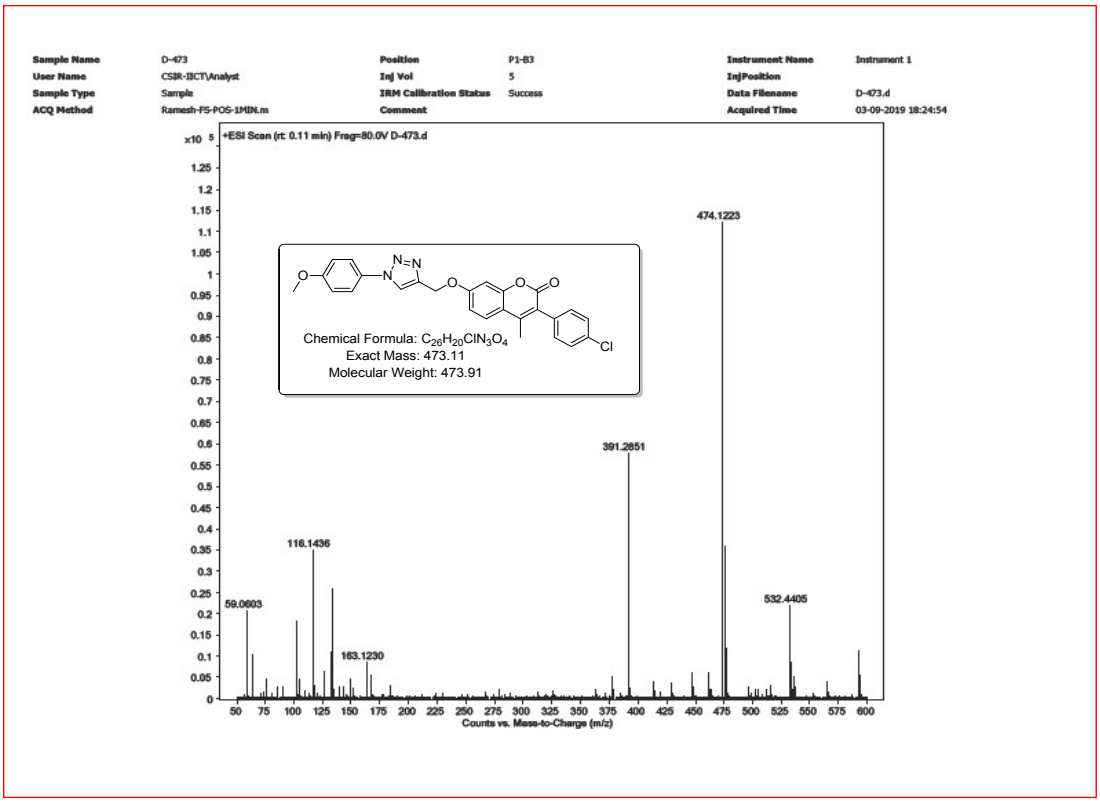
HRMS Spectrum of compound (8c)



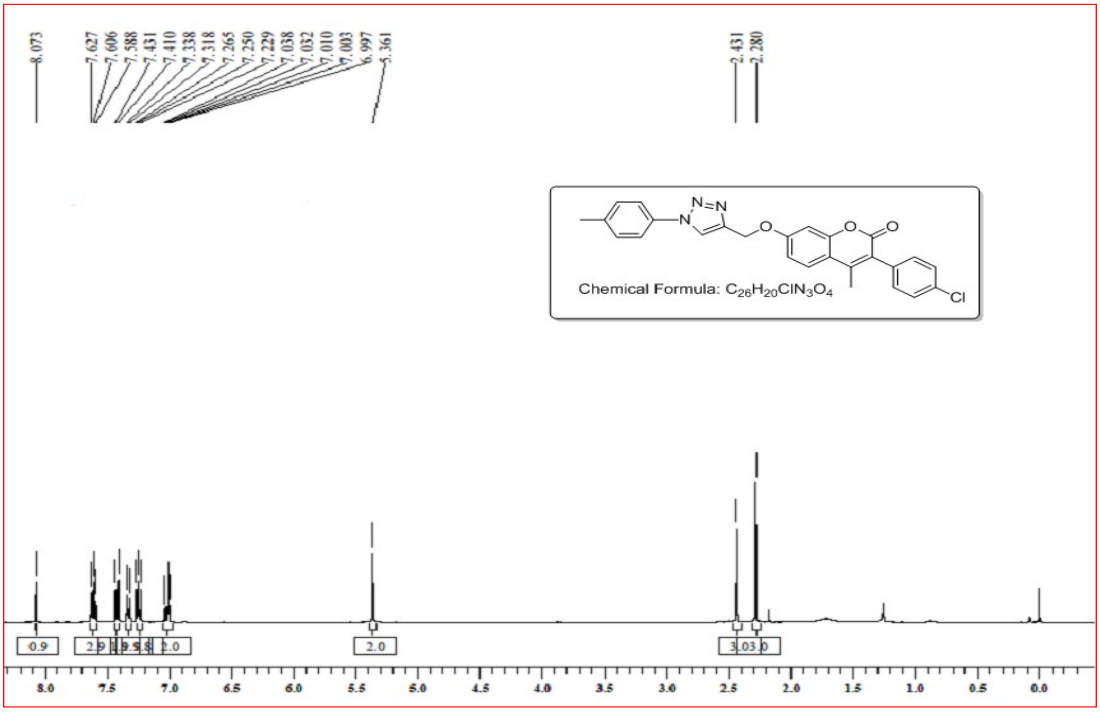
1H NMR (400 MHz, CDCl3) Spectrum of compound (8d)



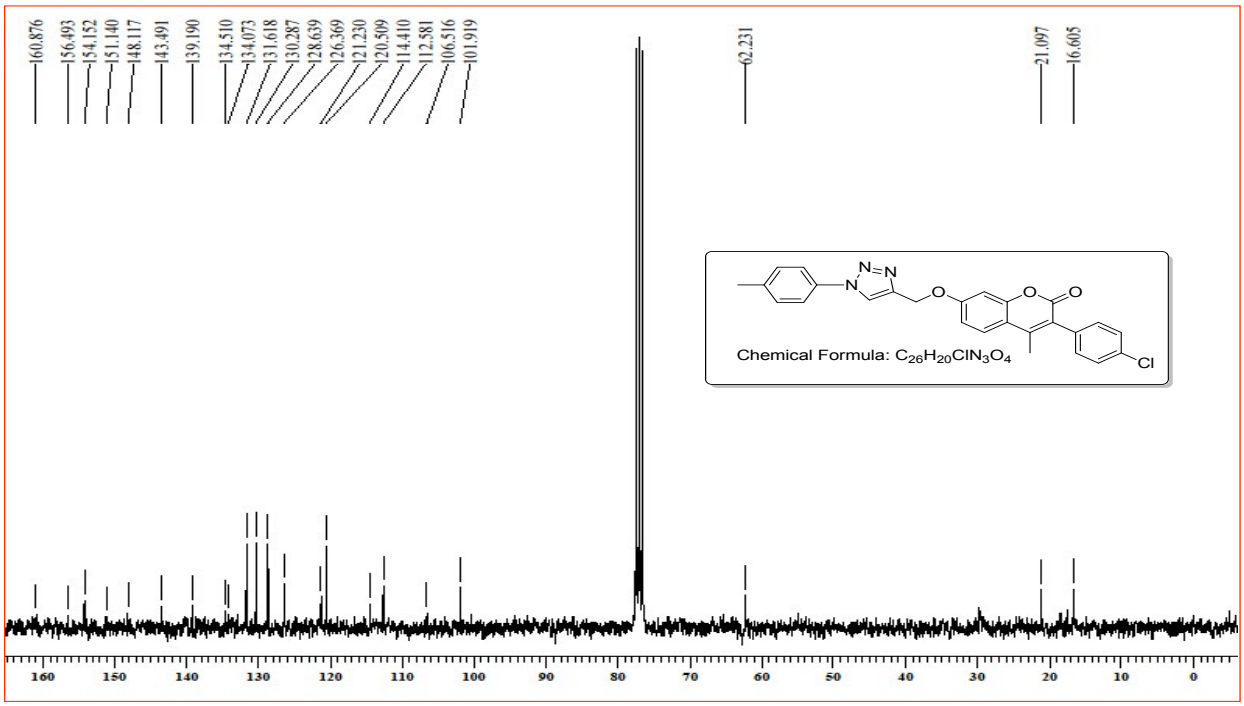
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8d)



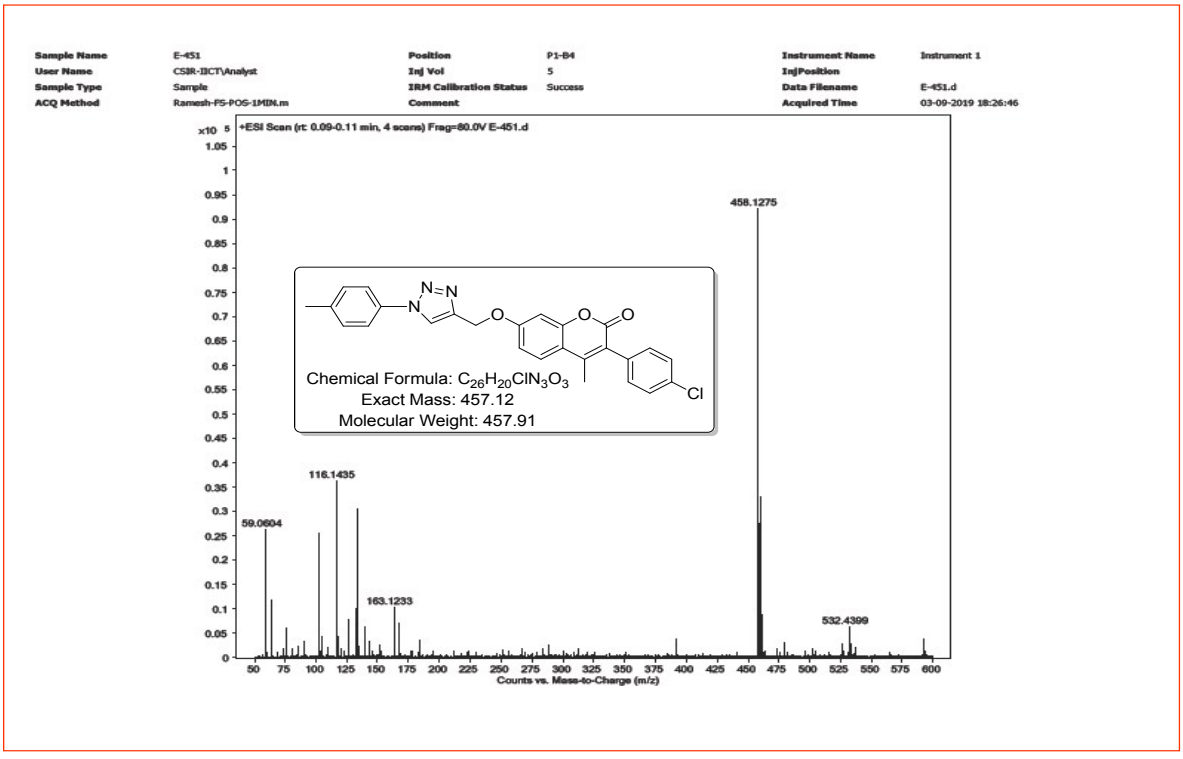
HRMS Spectrum of compound (8d)



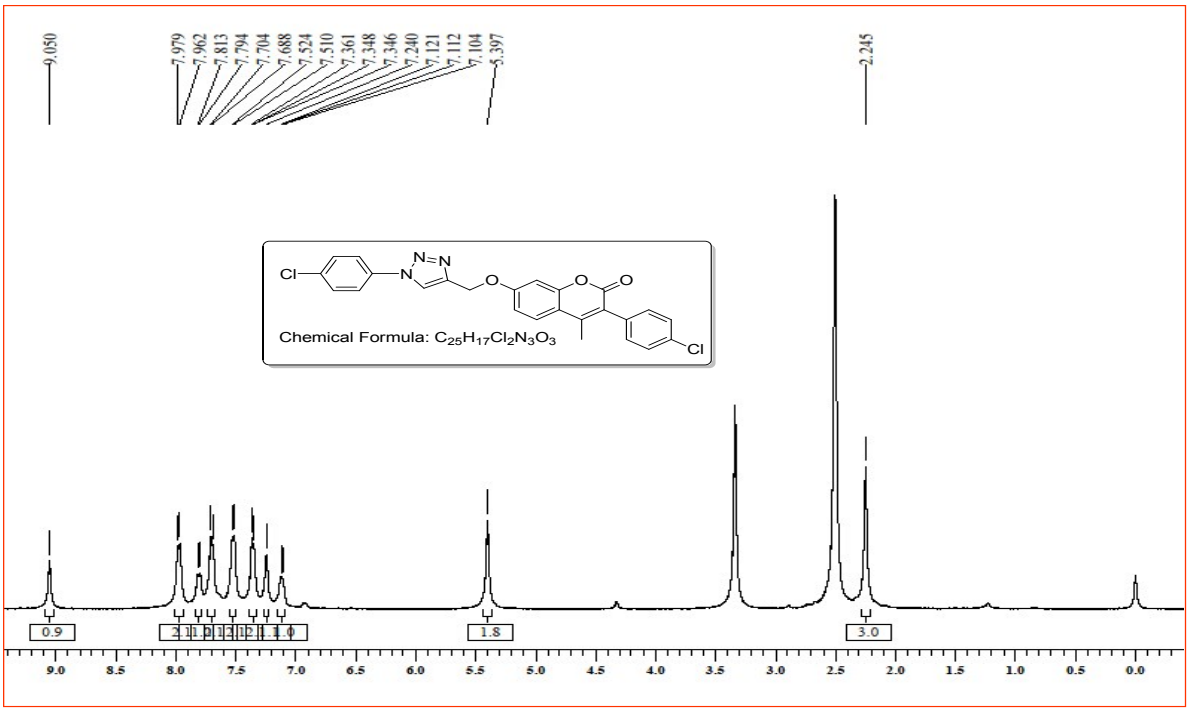
1H NMR (400 MHz, CDCl3) Spectrum of compound (8e)



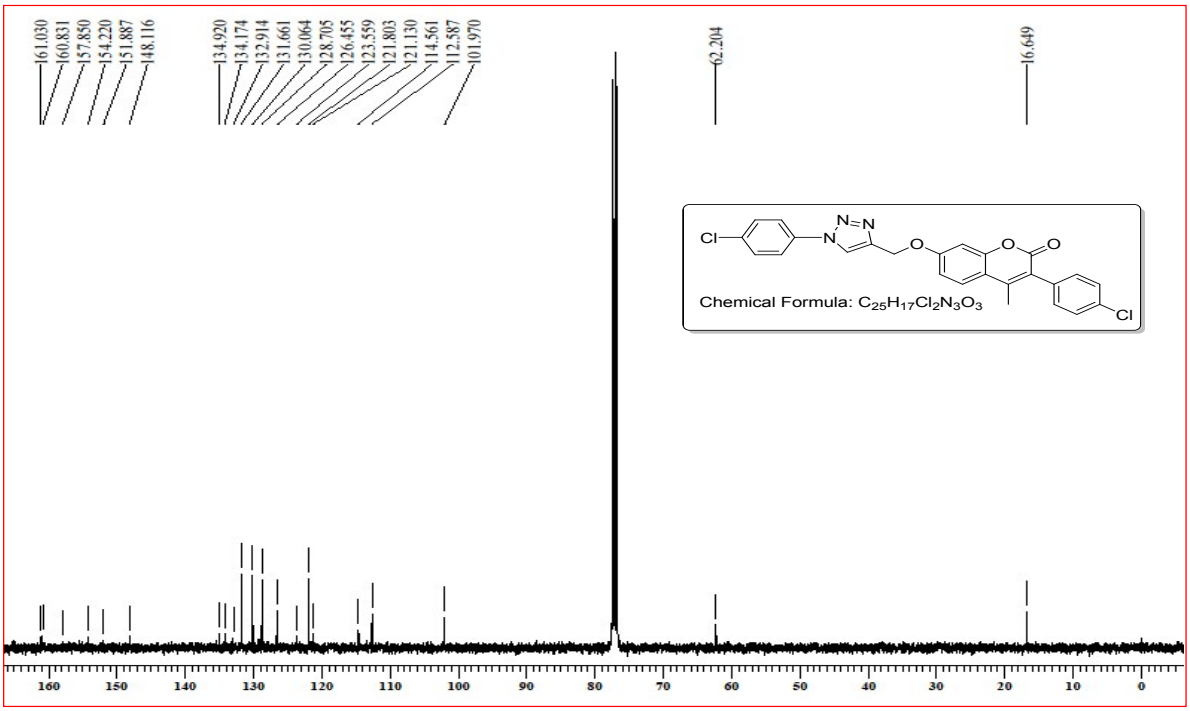
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8e)



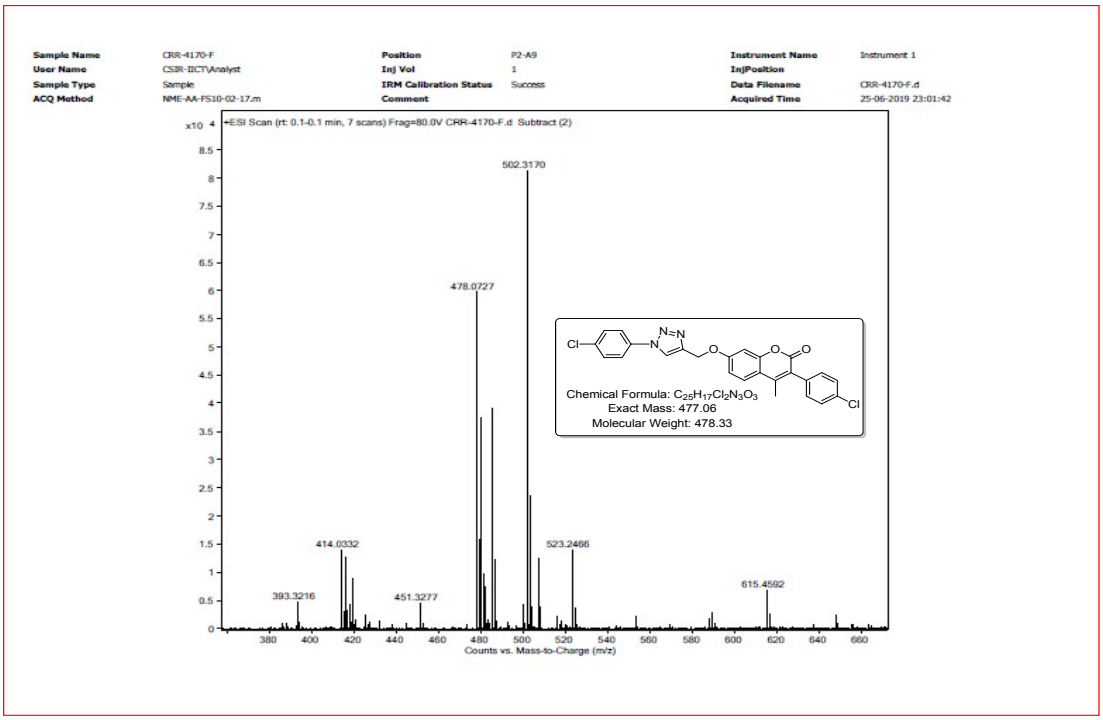
HRMS Spectrum of compound (8e)



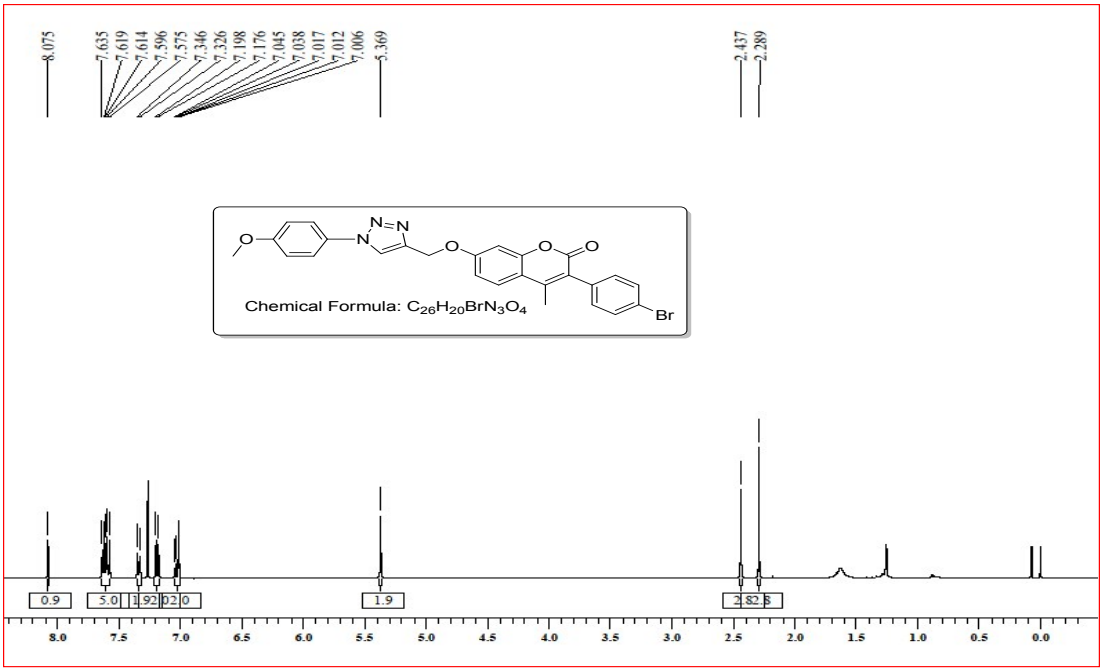
1H NMR (400 MHz, DMSO) Spectrum of compound (8f)



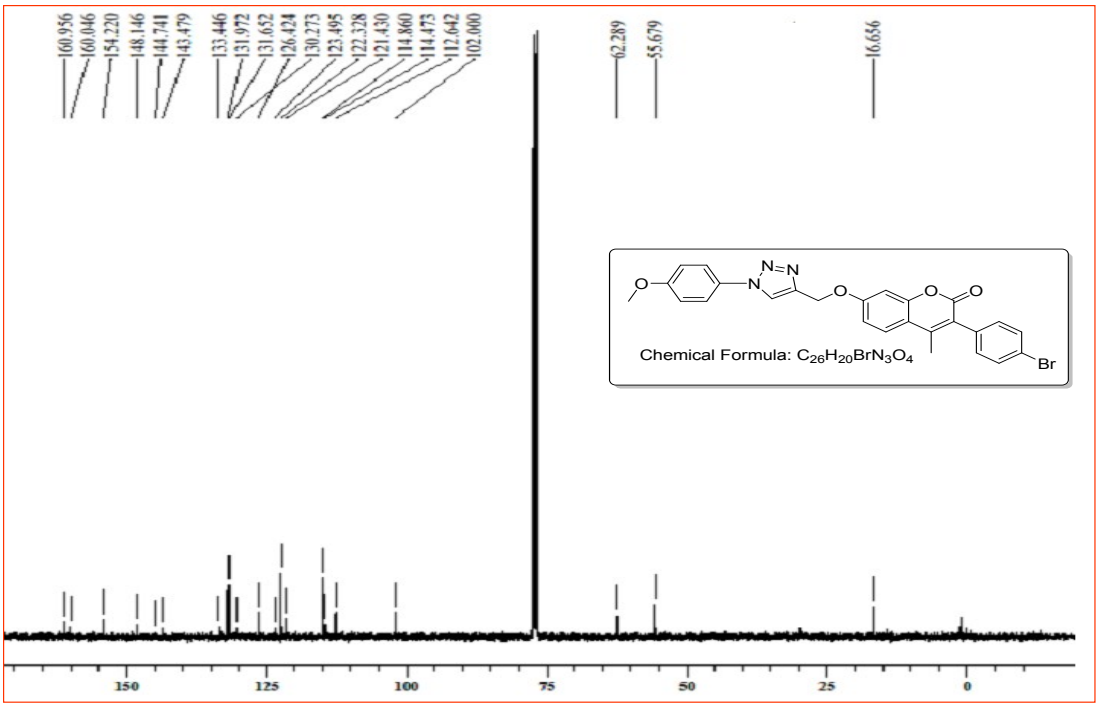
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8f)



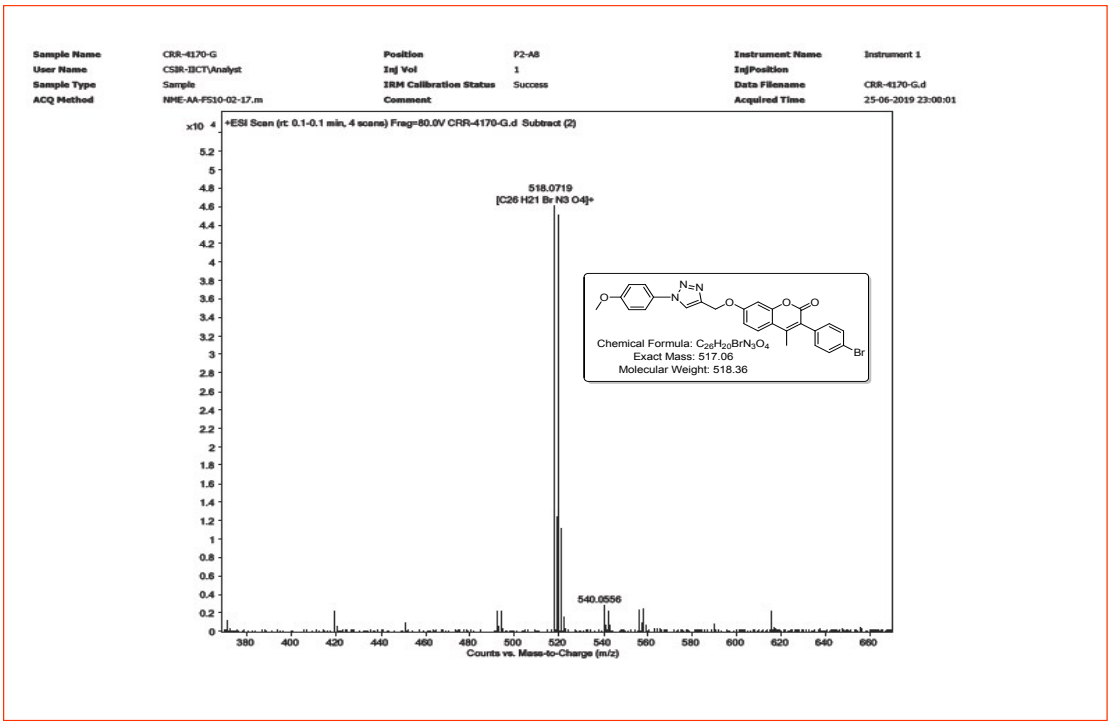
HRMS Spectrum of compound (8f)



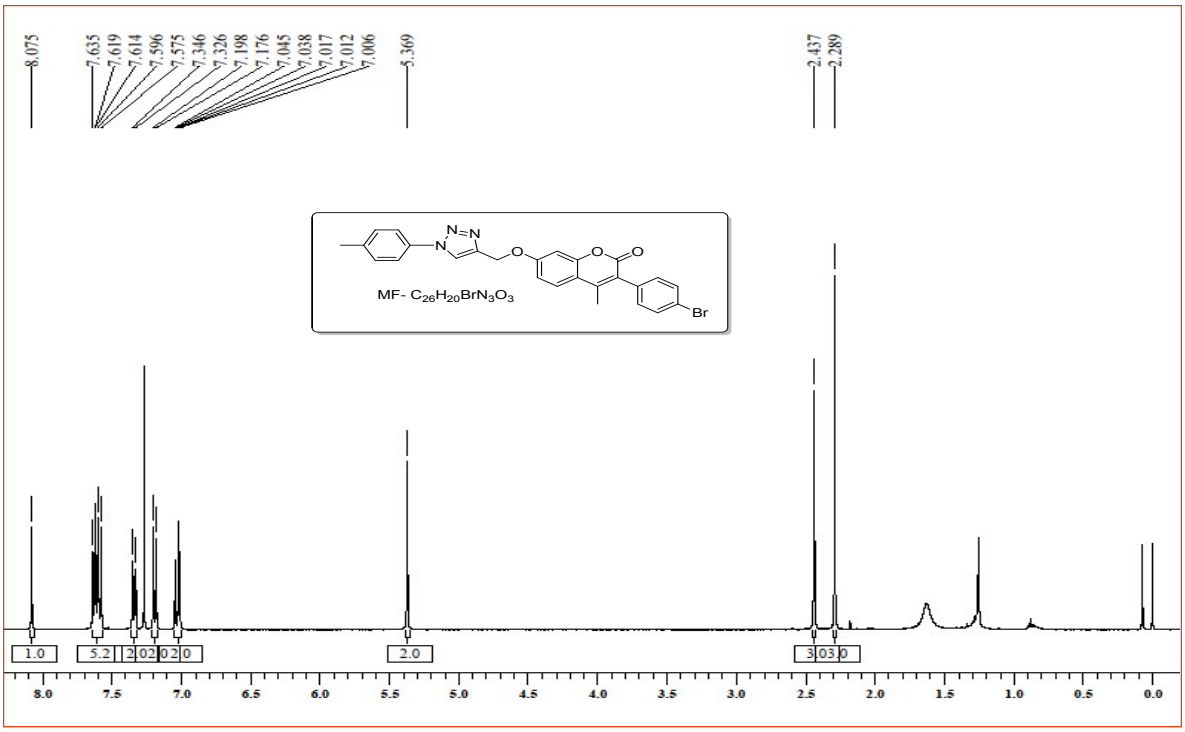
1H NMR (400 MHz, CDCl3) Spectrum of compound (8g)



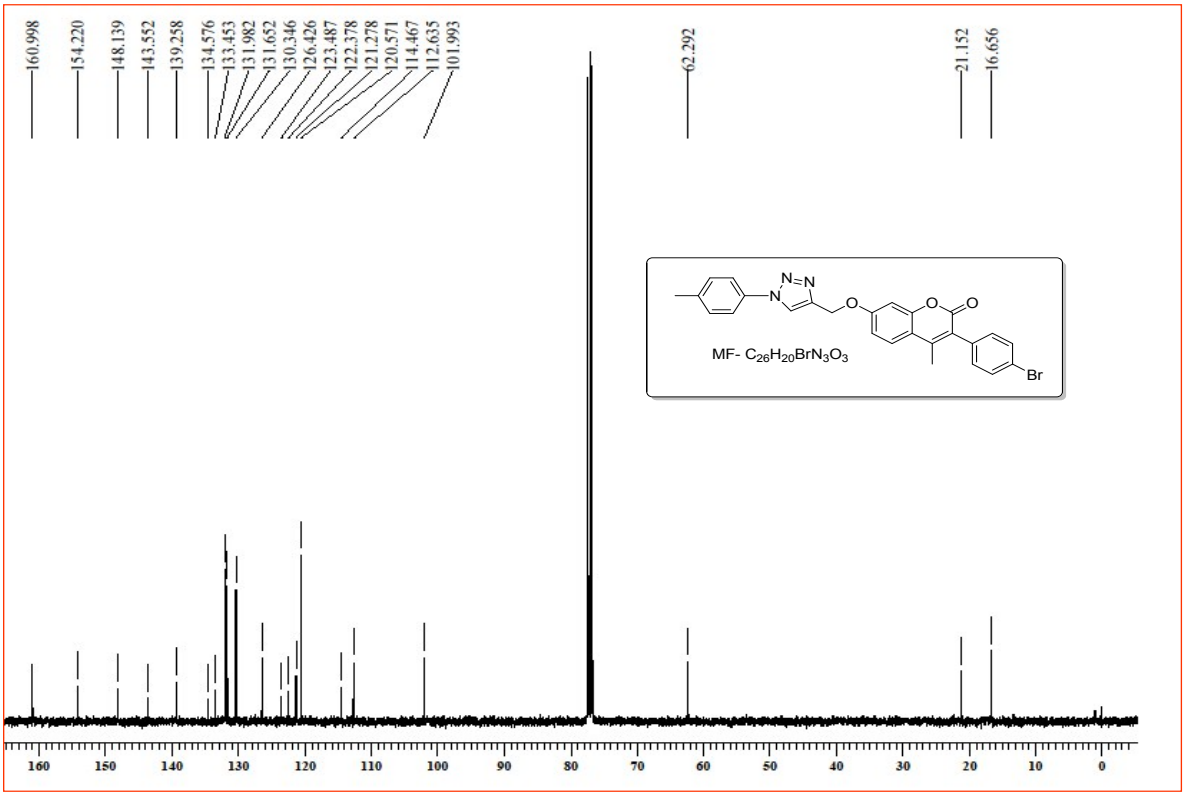
13 C NMR (400 MHz, CDCl3) Spectrum of compound



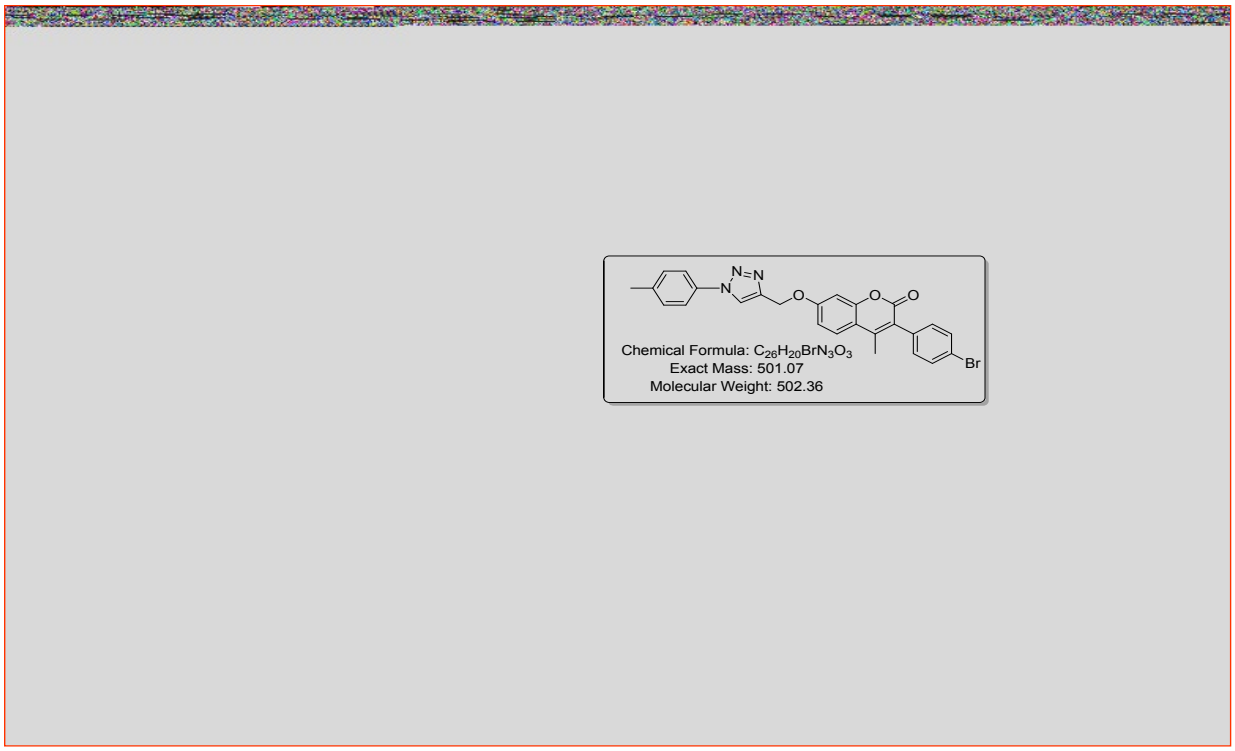
HRMS Spectrum of compound (8g)



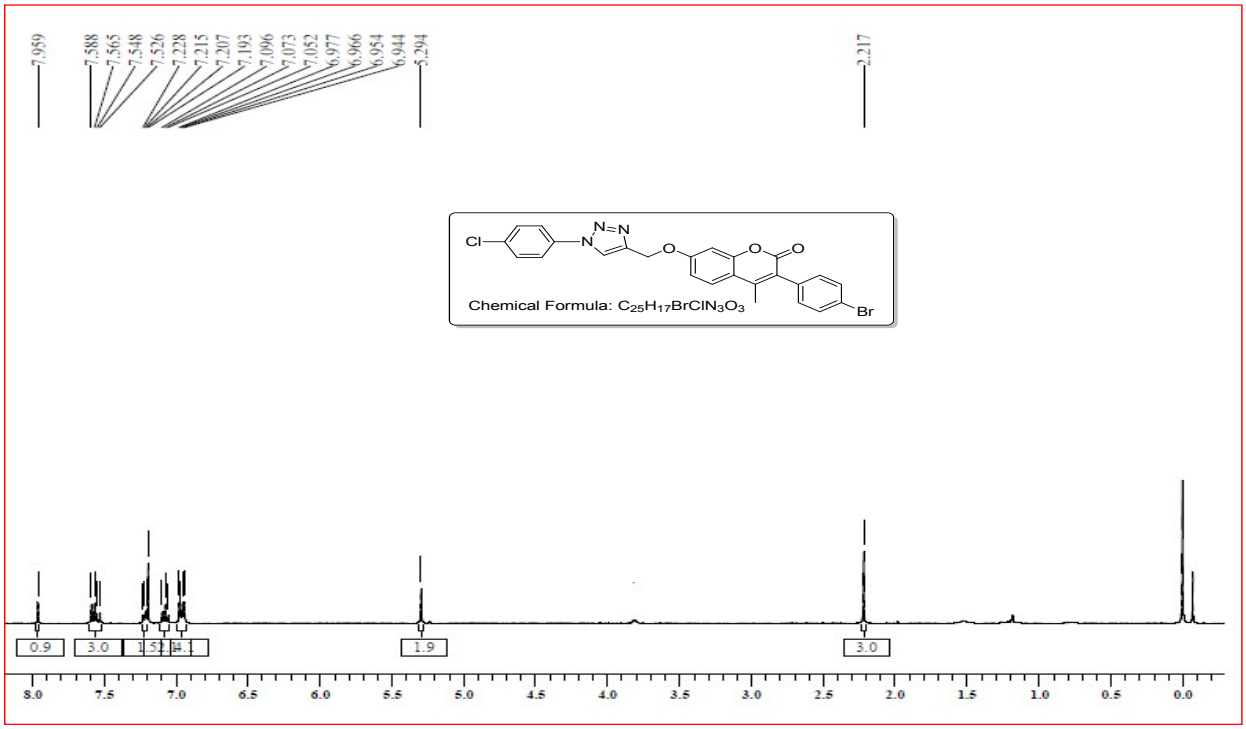
1H NMR (400 MHz, CDCl3) Spectrum of compound (8h)



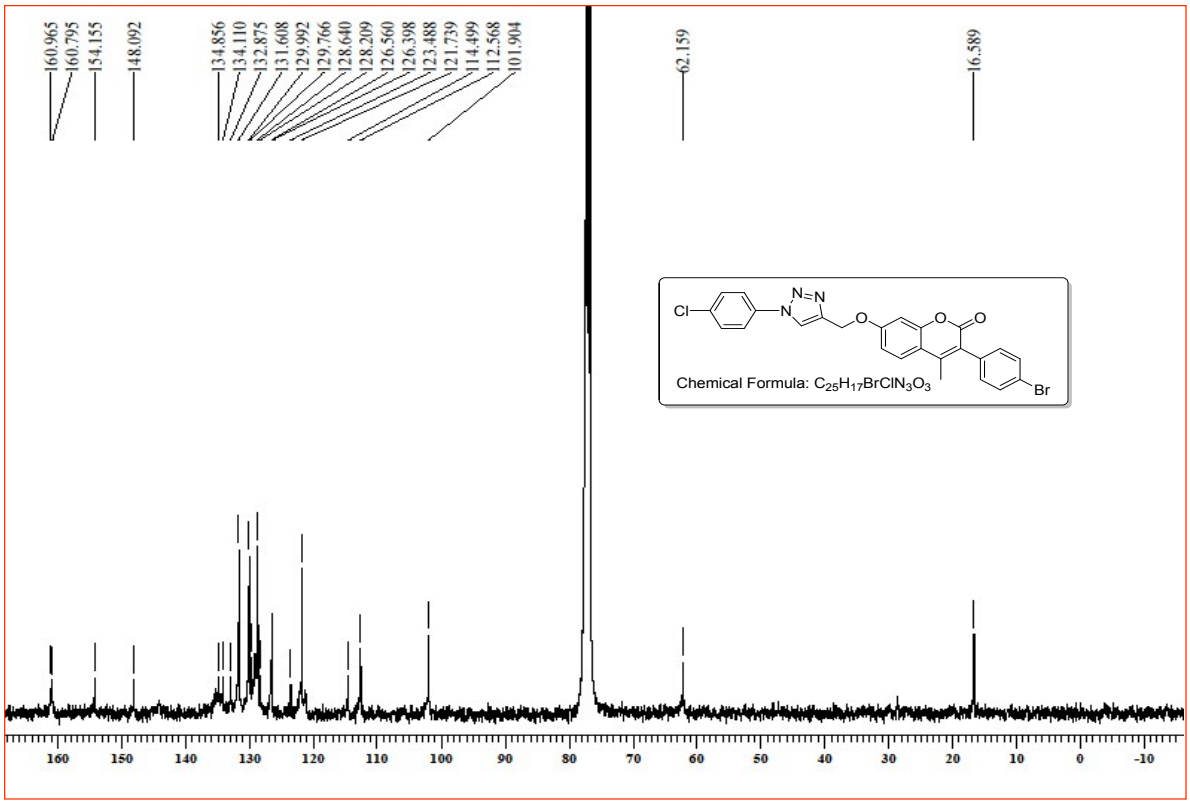
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8h)



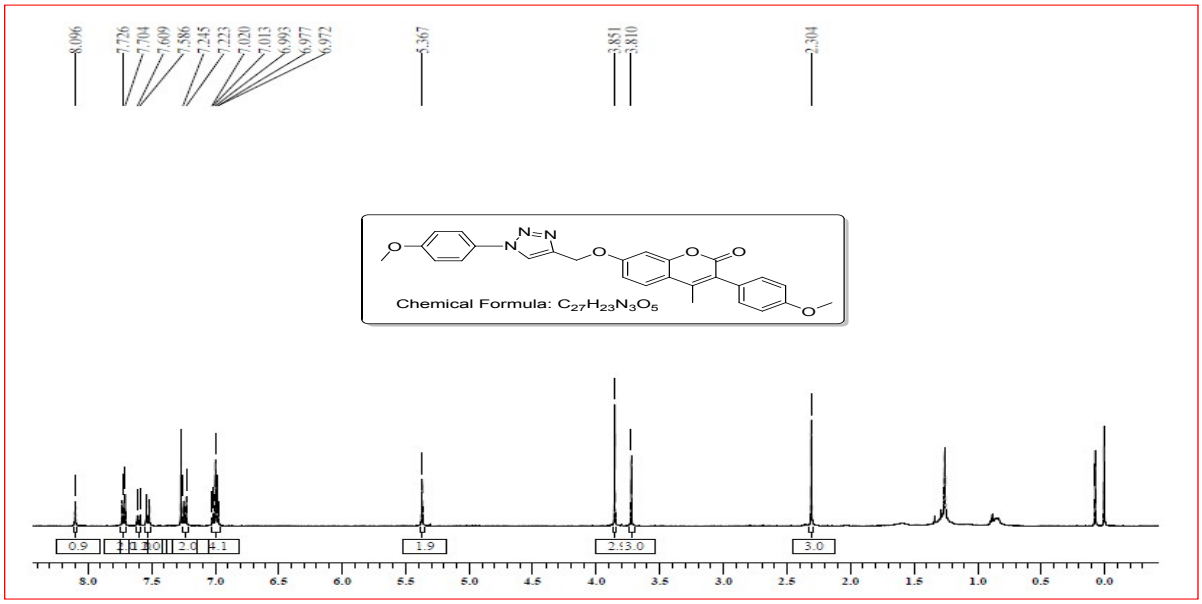
HRMS Spectrum of compound (8h)



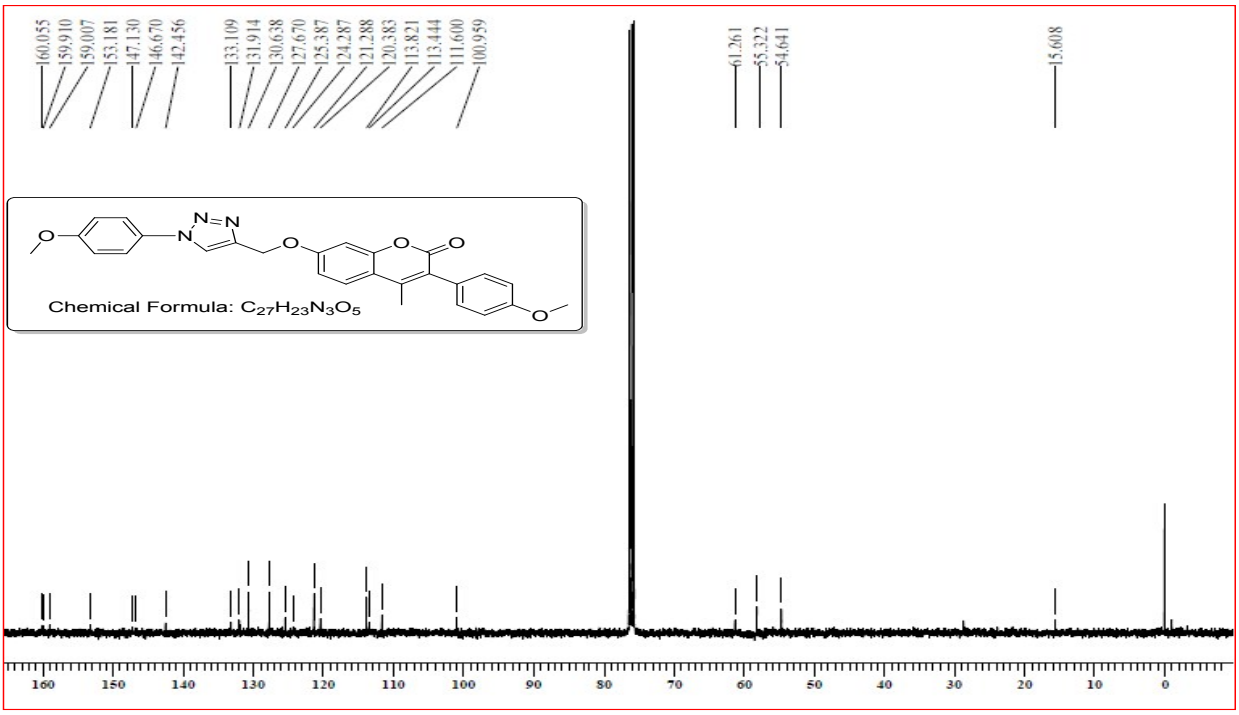
1H NMR (400 MHz, CDCl3) Spectrum of compound (8i)



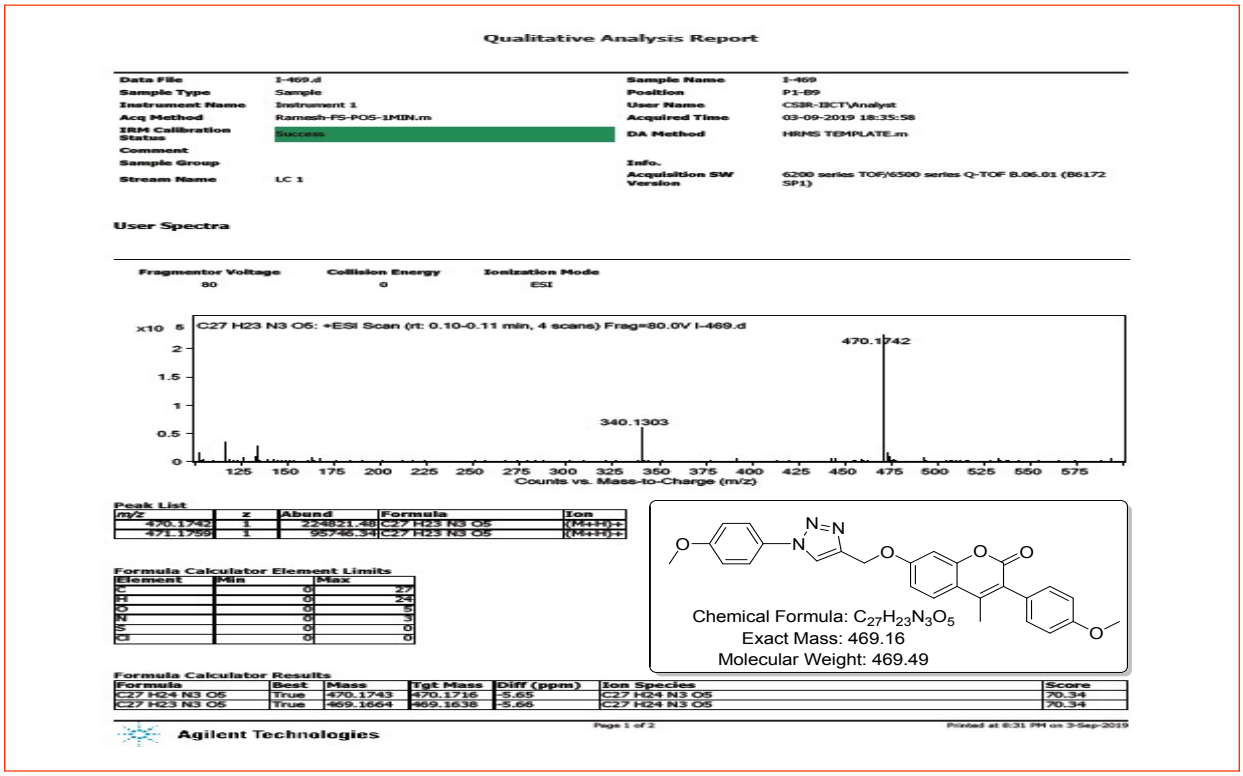
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8i)



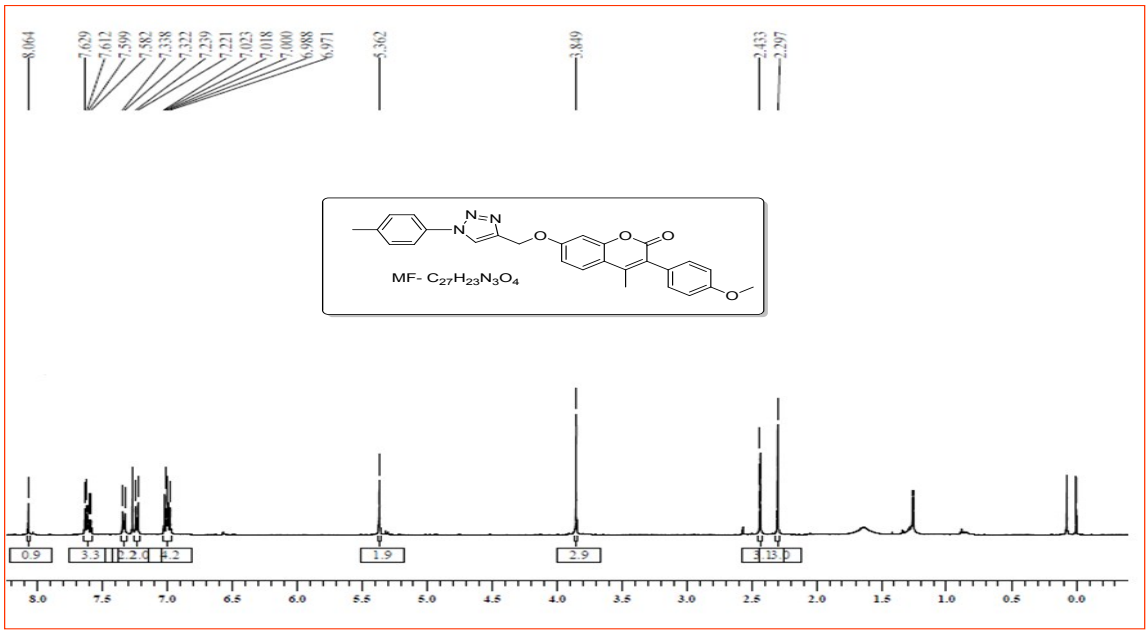
1H NMR (400 MHz, DMSO) Spectrum of compound (8j)



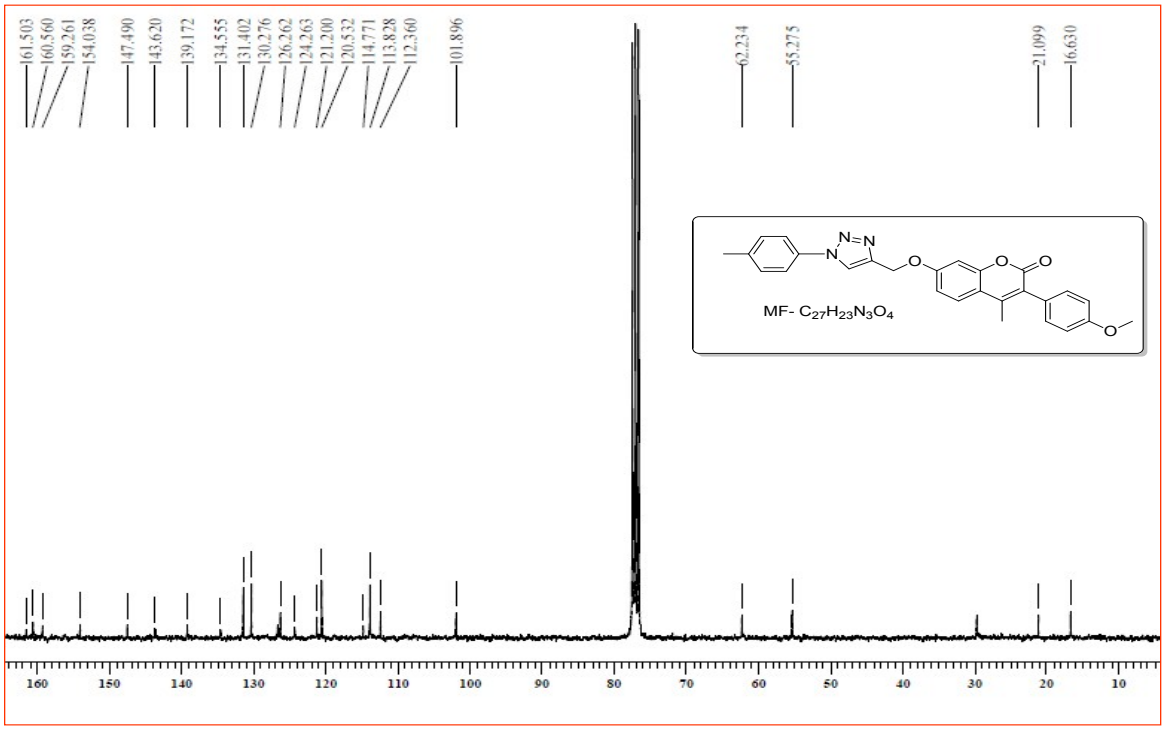
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8j)



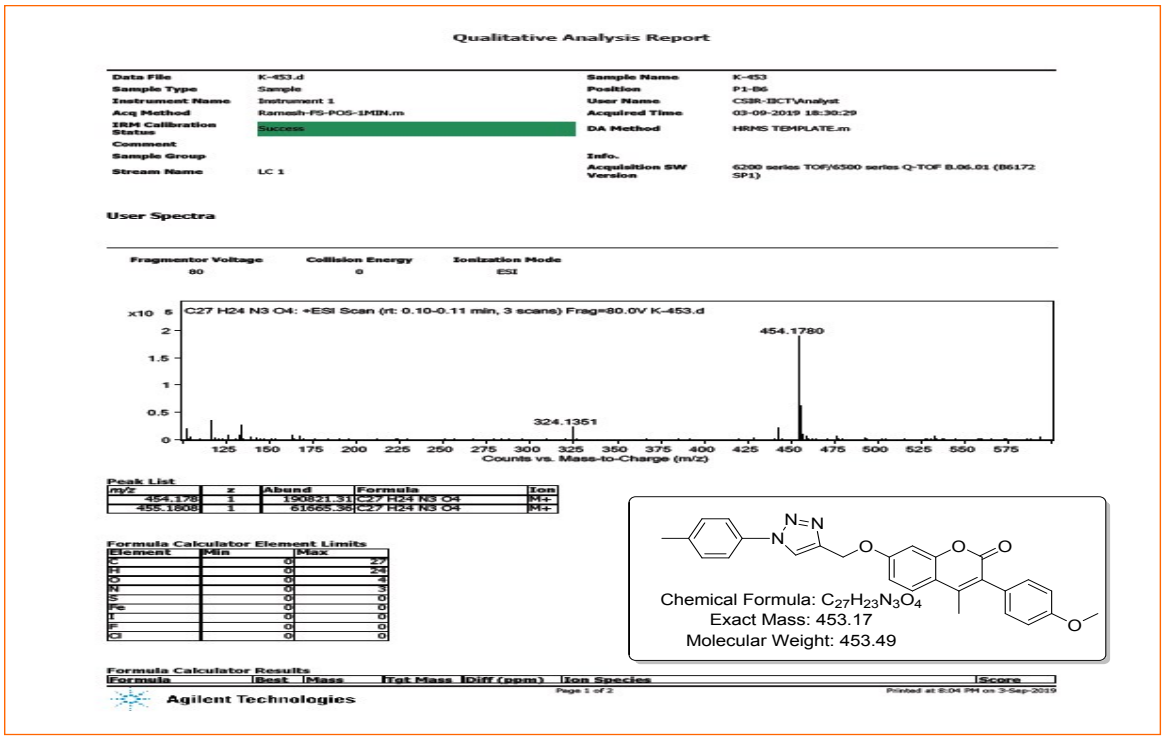
HRMS Spectrum of compound (8j)



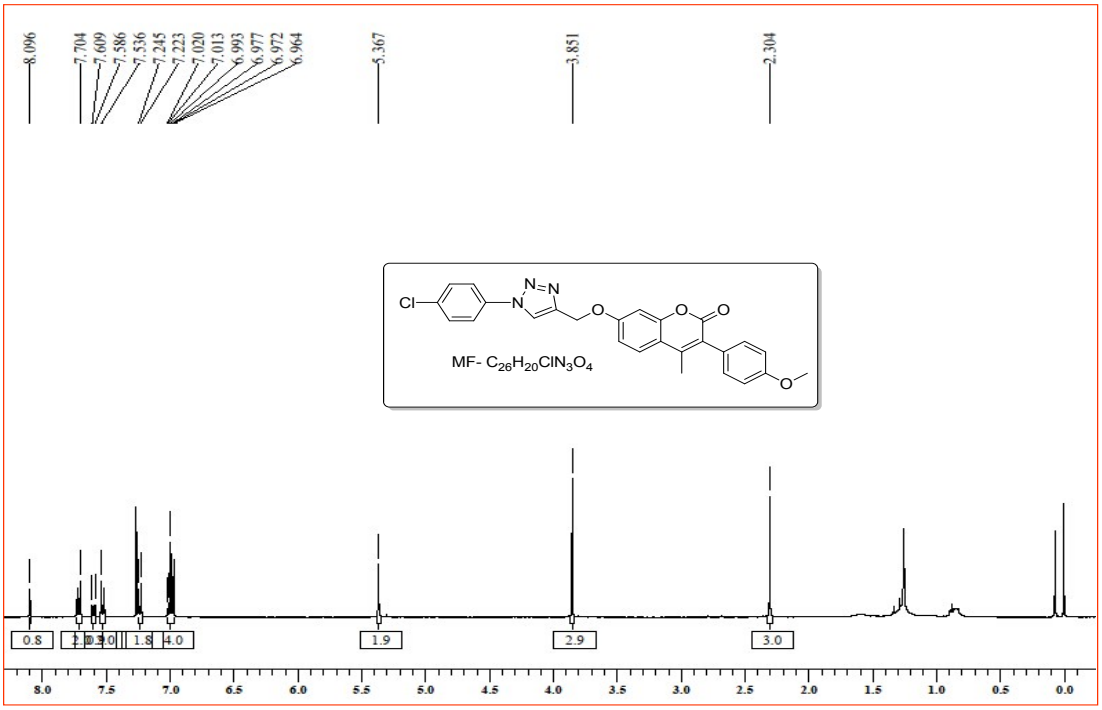
1H NMR (400 MHz, CDCl3) Spectrum of compound (8k)



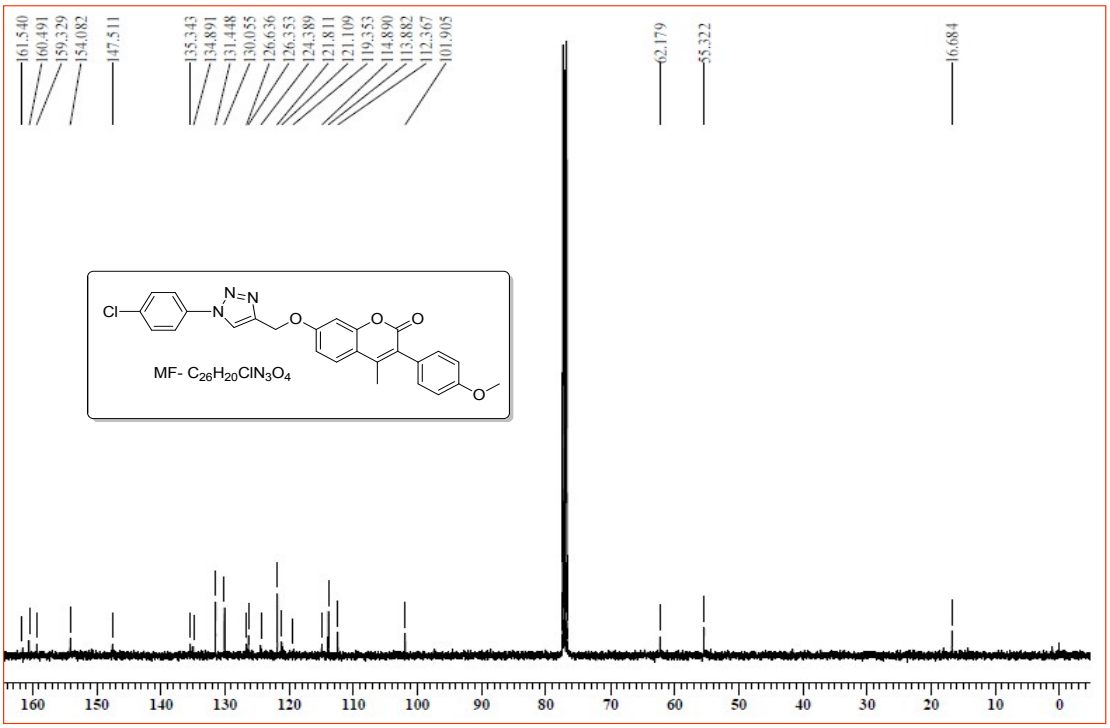
13 C NMR (400 MHz, CDCl3) Spectrum of compound (8k)



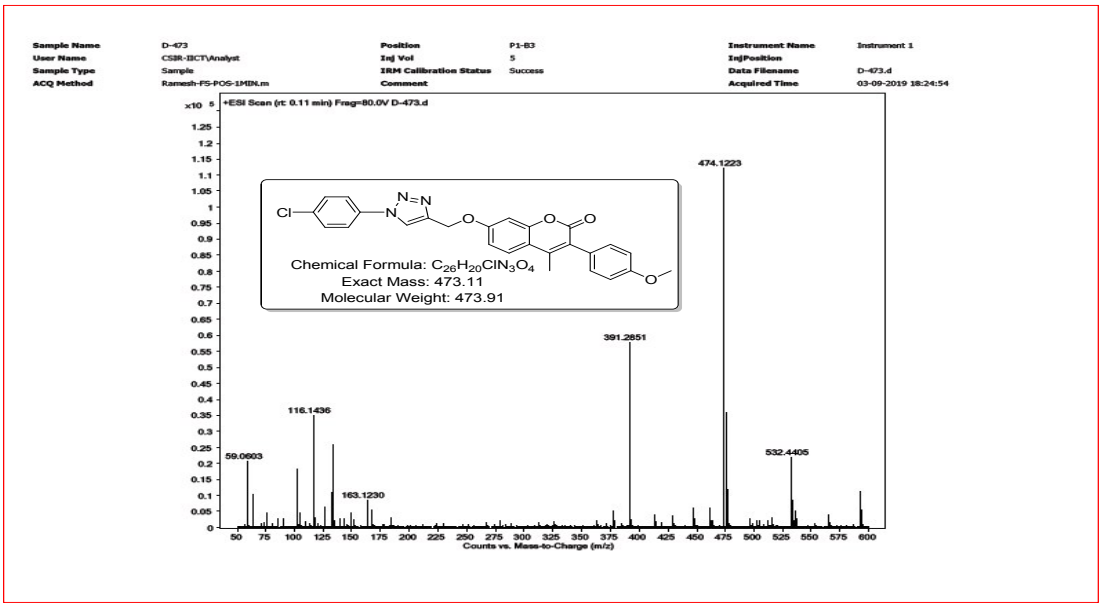
HRMS Spectrum of compound (8k)



1H NMR (400 MHz, CDCl3) Spectrum of compound (8l)



13 C NMR (400 MHz, CDCl3) Spectrum of compound (8l)



HRMS Spectrum of compound (8l)

5. Biological Activities:

i) ***Antioxidant activity:*** The synthesized compounds **6(a-d) & 8(a-l)** were screened for antioxidant activity by

a. DPPH method

b. Hydrogen Peroxide method

a. DPPH Method (2, 2-diphenyl-1-picryl hydrazyl): This is the most widely reported method for screening of antioxidant activity of many plant drugs and synthetic compounds. DPPH is a stable free radical that can accept an electron of hydrogen radical, to become a stable diamagnetic molecule. The scavenging reaction between DPPH and an antioxidant (H-A) can be written as

$$DPPH + H\text{-}A \rightarrow DPPH\text{-}H + A$$

The DPPH shows a strong absorption band at 517 nm antioxidants react with DPPH, which is a stable free radical and reduces DPPH to DPPH-H (2,2-diphenyl-1-picrylhydrazine) and as consequence the absorbance decreases from the DPPH radical (purple) to the DPPH-H (yellow) form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability. The activity is expressed as percentage of inhibition.

Preparation of ascorbic acid stock solution (standard): Ascorbic acid was used as standard. Ascorbic acid stock solution was prepared in methanol in the concentration of 1000µg/ml. From the above stock solution 0.60 ml, 0.12 ml, 0.24 ml, 0.36 ml, 0.48 ml, 0.6 ml was taken and the volume is made to 6 ml using methanol which gives the concentrations of 20, 40, 60, 80, 100 and 120µg/ml respectively and used for antioxidant studies.

Preparation of the test solution: 6 mg test or the standard was weighed and dissolved in 6ml of methanol with aid of sonicator. From the above stock solution 0.60 ml, 0.12 ml, 0.24 ml, 0.36 ml, 0.48 ml, 0.60 ml, was taken and the volume is made to 6 ml using methanol which gives the concentrations of 20, 40, 60, 80, 100 and 120 µg/mL respectively.

Procedure: To 3 ml of various concentrations of test / standard solution, 1 ml solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added. Simultaneously blank samples were prepared for each concentration without addition of 0.1mM of DPPH solution and equal amount of methanol was added to each blank sample. 3 ml of methanol and 1 ml of 0.1 mM DPPH was added and used as control. Ascorbic acid was used as standard for comparison. After incubation

for 20 minutes in dark, absorbance was recorded at 517 nm. % scavenging was calculated using the formula

$$\%\, Scavenging = (A^{\circ} - A)/A^{\circ} \times 100$$

Where A^{O} = Absorbance of the control

A = Absorbance of the test or standard

Graph was plotted by taking concentration (µg/ml) on x-axis and percentage scavenged/ inhibition on y-axis.

Calculation of IC_{50} values: The Half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug is needed to inhibit a given biological process or component of a process by half. The concept of IC_{50} is fundamental to Pharmacology. In case of DPPH method, IC_{50} will corresponds to the concentration of antioxidant that leads to a loss of 50% of DPPH absorbance.

IC_{50} was calculated by using Graphpad prism software. In order to calculate IC_{50} initially XY data table was created. Then the logarithm of the concentration of the inhibitor was entered into X and response was entered into Y. From the data table click Analyze, choose nonlinear regression, then choose the panel of equations "Dose response curves-Inhibition" and then choose the equation "log (Inhibitor) v/s normalized response-variable slope". Then we will get IC_{50} values for the given data.

Statistical analysis: All the data was expressed as mean ± SEM. Statistical significance was tested by using one way ANOVA followed by the Tukey's test using computer based fitting program (Graphpad prism 5)

% of Inhibition of Antioxidant Activity by DPPH Method							
Compound	20	40	60	80	100	120	% of Inhibition
6a	19.21	34.56	56.32	97.45	84.78	93.13	64.24
6b	50.48	60.51	53.96	43.01	55.85	72.30	56.01
6c	34.87	42.32	58.53	44.97	49.87	37.79	44.72
6d	46.91	42.02	47.06	52.40	13.54	22.60	37.42

8a	37.63	56.65	93.84	90.30	95.59	93.56	77.92
8b	10.86	22.09	17.34	64.25	23.71	13.91	25.36
8c	50.89	24.69	68.61	72.02	5.09	92.44	52.29
8d	46.59	57.89	76.45	85.9	90.21	91.65	74.78
8e	38.20	49.81	56.08	44.33	47.83	42.17	46.40
8f	48.30	54.30	39.40	42.80	43.57	39.92	44.71
8g	19.92	47.23	32.67	28.97	27.85	36.60	32.20
8h	19.21	34.56	56.32	97.45	84.78	93.13	64.24
8i	34.54	56.34	99.89	89.19	99.01	95.45	79.07
8j	32.13	71.83	89.99	90.78	99.01	98.78	80.42
8k	37.78	68.89	73.23	56.68	89.87	73.59	66.67
8l	46.32	62.12	75.13	80.54	87.67	99.29	75.17
Ascarbic acid	46.67	56.78	78.12	86.45	89.98	90.78	74.79

Table I. Percentage of Inhibition of antioxidant activity by DPPH method

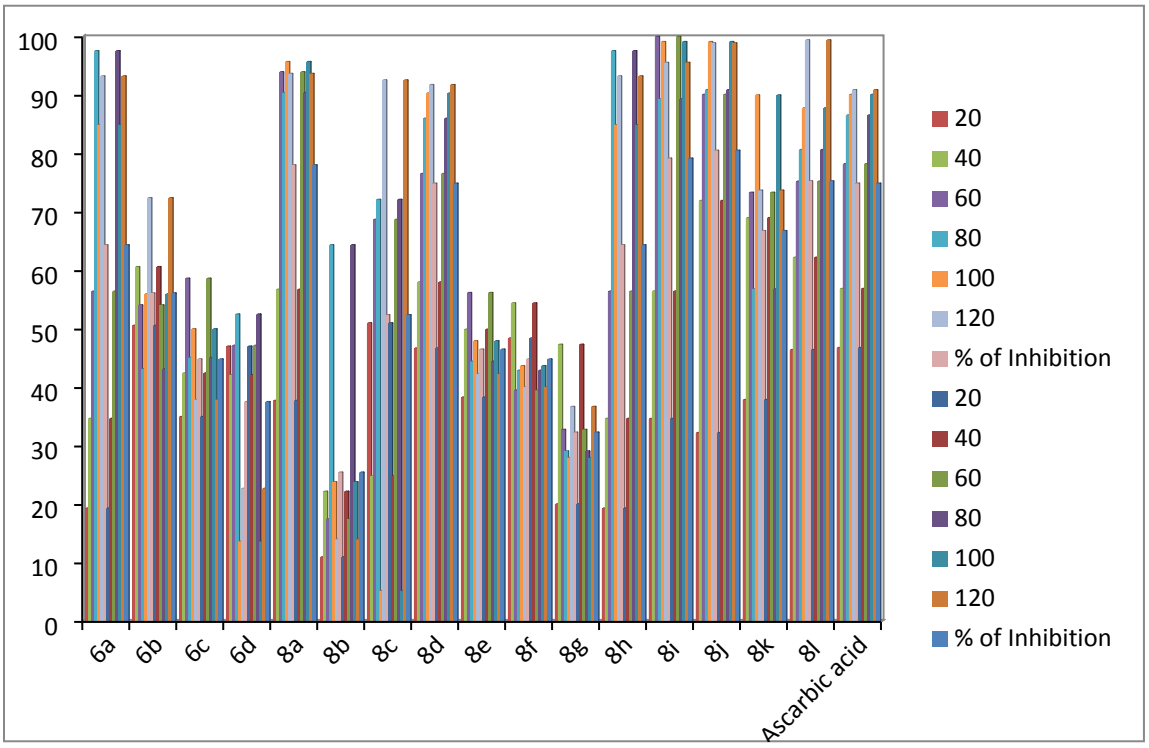


Figure I. Graphical representation of % of Inhibition

b. Hydrogen Peroxide method: The ability of the synthesized compounds to scavenge H_2O_2 was determined by the following procedure. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). The concentration of H_2O_2 was determined by absorption at 230 nm using a UV Visible spectrophotometer. Test solutions were added to a H_2O_2 solution (0.6 ml 40mM). The absorbance of H_2O_2 at 230 nm was determined after 10 minutes against blank solution containing phosphate buffer and test compound without H_2O_2. Control solution was prepared by taking a solution of H_2O_2 in phosphate buffer (pH 7.4) and its absorbance was measured. The percentage of H_2O_2 scavenging by the test and the standard was calculated using the following formula.

$$\% \, Scavenged = [(A - A1)/A]100$$

Where A = absorbance of the control

A1= absorbance of the test /standard

Preparation of 40mM H_2O_2 in pH 7.4 phosphate buffer: 0.45 ml (0.136g) of 30% H_2O_2 w/v is taken and dissolved in 100ml of pH 7.4 buffers to give 40mM H_2O_2 solution.

Preparation of the test solution: 6mg test or the standard was weighed and dissolved in 6ml of methanol with aid of sonicator.

From the above stock solution 0.60ml 0.12 ml, 0.24 ml, 0.36 ml, 0.48 ml, 0.60 ml was taken and the volume is made to 6 ml using methanol which gives the concentrations of 20, 40, 60, 80, 100 and 120 µg/ml respectively.

Procedure: To 1.4 ml of the test or standard solution, 0.6ml of the 40mM H_2O_2 is added and allowed to stand for 10 minutes. The absorbance of the above solution is measured at 230 nm. Ascorbic acid was taken as standard. Graph was plotted by taking concentration (µg/ml) on x-axis and percentage scavenged/ inhibition on y-axis.

Calculation of IC_{50} values: The Half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug is needed to inhibit a given

biological process or component of a process by half. The concept of IC_{50} is fundamental to Pharmacology. In case of DPPH method, IC_{50} will correspond to the concentration of antioxidant that leads to a loss of 50% of DPPH absorbance.

IC_{50} was calculated by using Graphpad prism software. In order to calculate IC_{50} initially XY data table was created. Then the logarithm of the concentration of the inhibitor was entered into X and response was entered into Y. From the data table click Analyze, choose nonlinear regression, then choose the panel of equations "Dose response curves-Inhibition" and then choose the equation "log (Inhibitor) v/s normalized response-variable slope". Then we will get IC_{50} values for the given data.

Statistical analysis: All the data was expressed as mean ± SEM. Statistical significance was tested by using one way ANOVA followed by the Tukey's test using computer based fitting program (Graph pad prism 5)

% of Inhibition of Antioxidant Activity by Hydrogen Peroxide Method							
Compound	20	40	60	80	100	120	% of Inhibition
6a	37.56	56.65	93.84	90.3	95.59	93.56	77.91
6b	45.65	49.87	56.85	59.47	63.78	79.25	59.14
6c	43.89	56.78	69.89	76.89	78.89	84.56	68.48
6d	33.13	71.83	89.99	90.78	99.01	98.78	80.58
8a	45.65	59.56	76.12	86.45	88.98	91.34	74.68
8b	36.58	39.54	38.56	39.45	48.56	46.25	41.49
8c	46.67	56.78	78.12	86.45	89.98	90.78	74.79
8d	36.89	35.68	48.96	46.87	49.78	56.89	45.84
8e	11.89	24.45	17.34	64.25	23.71	13.91	25.92
8f	44.91	59.56	76.12	86.45	88.98	91.34	74.56
8g	19.92	47.23	32.67	28.97	27.85	36.6	34.664
8h	50.89	24.69	68.61	72.02	5.09	92.44	52.29
8i	34.54	56.34	99.89	89.19	99.01	95.45	79.07
8j	79.42	71.83	89.99	90.78	99.01	98.78	88.3
8k	37.78	68.89	73.23	56.68	89.87	73.59	66.67

8l	43.11	58.25	60.25	68.25	69.87	65.12	62.8
Ascarbic acid	50.53	59.45	68.56	83.61	86.59	92.56	73.55

Table II. Percentage of Inhibition of Antioxidant Activity by Hydrogen Peroxide Method

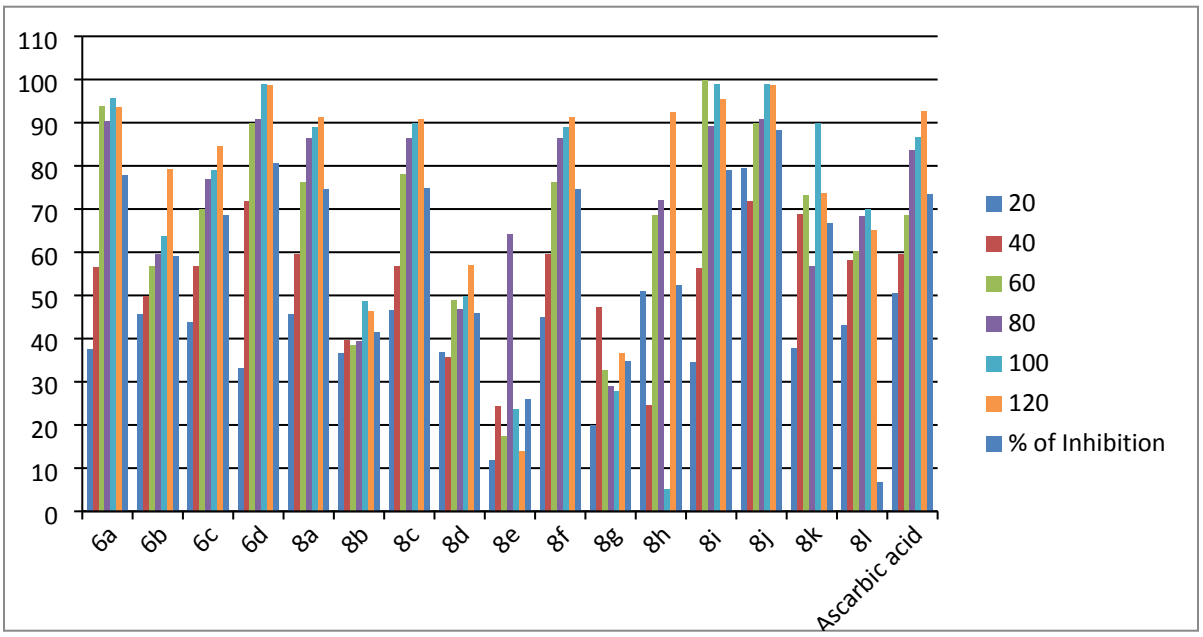


Figure II. Graphical Representation of % of Inhibition

ii) Anti-inflammatory activity: The synthesized compounds **6(a-d) & 8(a-l)** were screened for *in-vitro* anti-inflammatory activity by

a. Inhibition of egg albumin denaturation method.

b. Heat induced hemolytic method.

a. Inhibition of Egg Albumin Denaturation Method[34,35,36]

The *in vitro* anti-inflammatory studies were carried out by the method of inhibition of thermally induced protein denaturation. Inflammation is the reaction of living tissue to injury, infection or irritation. Protein denaturation is a process in which protein lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. So, based on this property *in-vitro* anti-inflammatory activity was performed.

Preparation of Diclofenac sodium stock solution (standard): Diclofenac sodium was used as standard. Diclofenac stock solution was prepared using ethanol in the concentration of 1000µg/ml. From the stock solution various dilutions viz. 20, 40, 60, 80, 100 and 120 µg/ml were prepared using ethanol and used for anti-inflammatory studies.

Preparation of test solution: 6 mg of test or standard was weighed and dissolved in 6ml of ethanol with aid of sonicator. From the above stock solution 0.12 ml, 0.24 ml, 0.36 ml, 0.48ml, 0.6 ml, 0.72ml was taken and the volume is made to 6 ml using ethanol which gives the concentration of 20, 40, 60, 80, 100, 120 µg/ml respectively.

Procedure: To 2ml of various concentrations of test or standard solutions 2.8ml of normal saline (pH=7.4) and 0.2ml of 1% egg albumin solution was added. Simultaneously blank samples were prepared for each concentration without addition of 1% egg albumin solution and equal volume of normal saline (pH7.4) was added to each blank sample. To 4.8ml of normal saline (pH 7.4), 0.2ml of 1% egg albumin solution was added and used as control. The test/standard samples were incubated for 15 min at 70^0C.Then the tubes were cooled under running tap water and then absorbance was recorded at 660nm. % inhibition of denaturation of egg albumin was calculated using the formula

$$\% \, Inhibition = [(A - A1)/A]100$$

Where A = absorbance of the control,

A1= absorbance of the test /standard

Graph was plotted by taking concentrations (µg/ml) on x-axis and % inhibition on y-axis.

Calculation of IC_{50} values: The Half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug is needed to inhibit a given biological process or component of a process by half. The concept of IC_{50} is fundamental to Pharmacology. In case of DPPH method, IC_{50} will correspond to the concentration of antioxidant that leads to a loss of 50% of DPPH absorbance.

IC_{50} was calculated using Graphpad prism software. In order to calculate IC_{50} initially XY data table was created. Then the logarithm of the concentration of the inhibitor was entered into X and response was entered into Y. From the data table click Analyze, choose nonlinear regression, then choose the panel of equations "Dose response curves-Inhibition" and then choose the equation "log (Inhibitor) v/s normalized response-variable slope". Then we will get IC_{50} values for the given data.

Statistical analysis: All the data was expressed as mean ± SEM. Statistical significance was tested by using one way ANOVA followed by the Tukey's test using computer based fitting program (Graph pad prism 5)

Anti-inflammatory Activity by Egg Albumin Method							
Compound	20	40	60	80	100	120	% of Inhibition
6a	79.36	79.3	45.3	24.32	63.07	76.42	61.29
6b	34.64	43.2	72.56	24.07	30.28	20.88	37.06
6c	29.12	53.42	44.04	88.67	40.26	50.67	51.03
6d	73.21	75.76	82	85	90	96	83.66
8a	50.83	51.92	80.53	20.88	92.95	93.45	76.22
8b	31.45	45.8	55.36	59.31	63.33	64.34	53.26
8c	36.91	40.85	44.29	51.29	53.08	63.75	48.36
8d	66.35	10.4	12.75	19.29	19.79	36.07	17.48
8e	31.45	45.8	55.36	59.31	63.33	64.34	53.26
8f	82.71	70.97	66.1	55.7	45.88	38.5	59.97
8g	15.79	22.35	27.09	39.22	42.78	63.75	35.16
8h	21.89	47.56	54.61	71.32	77.18	88.73	60.21
8i	64.51	56.12	21.72	40.26	62.16	86.57	55.22
8j	88.67	86.57	85.48	80.28	79.02	89.26	84.88
8k	38.67	49.58	50.16	78.1	83.89	84.89	64.21
8l	76.09	76.76	55.36	42.36	81.04	77.85	68.24
Diclofenac	75	81	86	94	96	98.5	88.41

Table III. Percentage of Inhibition Anti-inflammatory Activity by Egg Albumin Method

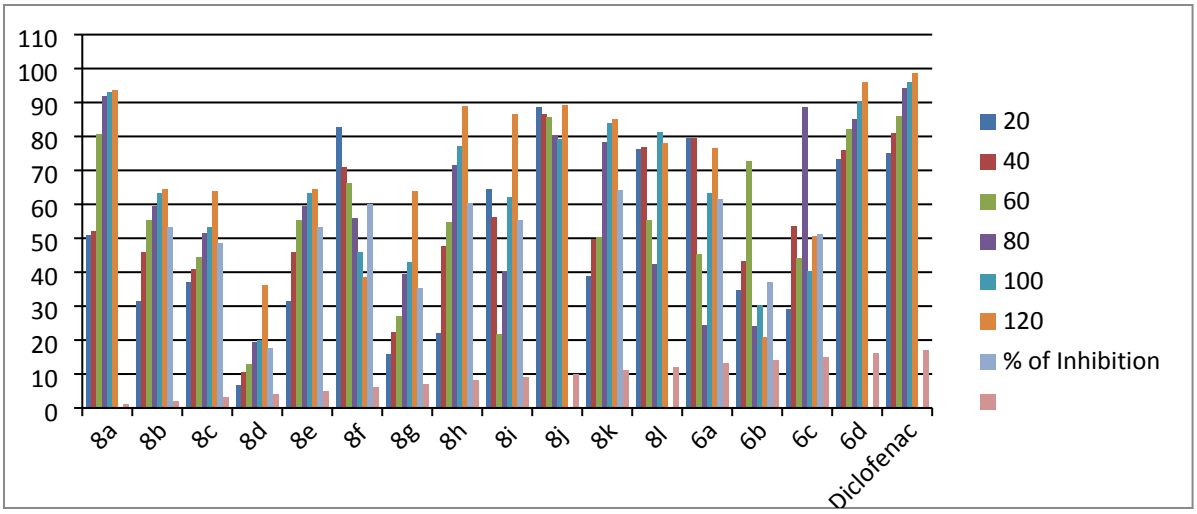


Figure III. Graphical Representation of % of Inhibition

b. Heat induced hemolytic method: The *in vitro* anti-inflammatory studies were carried out by the method of membrane stabilization i.e., heat induced hemolytic method. Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecule and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions such as heart attacks, septic shock and rheumatoid arthritis etc,. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophile such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane. Erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the test compounds may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by heat induced membrane lysis can be taken as an *in vitro* measure of anti-inflammatory activity of test compounds.

Preparation of RBC suspension: Fresh whole human blood (5ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline (pH 7.4). Then 1 ml of blood was measured and reconstituted as 1% v/v suspension with normal saline (pH 7.4).

Preparation of Diclofenac sodium stock solution (standard): Diclofenac sodium was used as standard. Diclofenac stock solution was prepared using ethanol in the concentration of 1000µg/ml. From the stock solution various dilutions viz., 20, 40, 60, 80, 100 and 120 µg/ml were prepared using ethanol and used for anti-inflammatory studies.

Preparation of test solution: 6 mg of test or standard was weighed and dissolved in 6 ml of ethanol with aid of sonicator. From the above stock solution 0.12 ml, 0.24 ml, 0.36 ml, 0.48ml, 0.6 ml, 0.72 ml was taken and the volume is made to 6 ml using ethanol which gives the concentration of 20, 40, 60, 80, 100 and 120 µg/mL respectively.

Procedure: To 1ml of various concentrations of test or standard solutions, 1ml of 1% RBC's suspension was added. Simultaneously blank samples were prepared for each concentration without addition of 1% RBC's solution and equal amount of normal saline was added to each blank sample. Equal amount of 1%RBC's solution and normal saline was added and was used as control.

All these samples were taken into centrifuge tubes and incubated in water bath at 56^0C for 30 min. The tubes were cooled under running tap water and then centrifuged at 2500 rpm for 15 min and absorbance of supernatant was taken at 560 nm.% inhibition was calculated using formula

$$\% \, Inhibition = [(A - A1)/A]100$$

Where A = absorbance of the control,

Al = absorbance of the test / standard

Graph was plotted by taking concentrations (µg/ml) on x-axis and % inhibition on y-axis.

Calculation of IC_{50} values: The Half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug is needed to inhibit a given biological process or component of a process by half. The concept of IC_{50} is fundamental to Pharmacology. In case of DPPH method, IC_{50} will correspond to the concentration of antioxidant that leads to a loss of 50% of DPPH absorbance.

IC_{50} was calculated using Graph pad prism software. In order to calculate IC_{50} initially XY data table was created. Then the logarithm of the concentration of the inhibitor was entered into X and response was entered into Y. From the data table click Analyze, choose nonlinear regression, then choose the panel of equations "Dose response curves-Inhibition" and then choose the equation "log (Inhibitor) v/s normalized response-variable slope". Then we will get IC_{50} values for the given data.

Statistical analysis: All the data was expressed as mean ± SEM. Statistical significance was tested by using one way ANOVA followed by the Tukey's test using computer based fitting program (Graph pad prism 5)

% of Inhibition of Ant-inflammatory Activity by Heat Induced Hemolytic Method							
Compound	20	40	60	80	100	120	% of Inhibition
6a	51.48	58.34	62.58	69.32	72.51	79.25	12.06
6b	24.64	43.20	72.56	24.07	30.28	20.88	22.91
6c	20.56	30.12	56.45	69.79	74.36	85.98	63.72
6d	34.14	50.53	59.83	65.27	73.14	75.14	15.00
8a	21.53	83.78	85.89	89.70	90.78	91.89	17.78
8b	18.59	38.11	58.69	60.45	61.31	63.10	69.89
8c	15.98	36.97	57.78	60.65	62.56	79.12	66.49
8d	5.36	16.35	17.24	19.29	36.07	40.25	38.13
8e	18.59	38.59	58.69	60.45	61.31	62.99	69.80
8f	23.61	83.78	85.89	89.70	90.78	91.89	17.11
8g	52.97	59.24	63.42	69.32	73.82	78.31	13.16
8h	17.37	43.68	85.89	89.70	90.78	91.89	43.77
8i	49.85	58.34	62.58	69.32	72.51	79.25	15.35
8j	49.21	53.14	83.25	86.25	90.54	91.47	15.90
8k	13.24	54.21	75.25	88.12	91.02	81.23	28.90
8l	22.14	83.78	85.89	90.46	90.78	91.89	18.90
Diclofenac	23.34	83.78	85.89	89.70	90.78	91.89	17.52

Table IV. Percentage of Inhibition of Anti-inflammatory Activity by Heat Induced Heamolytic Method

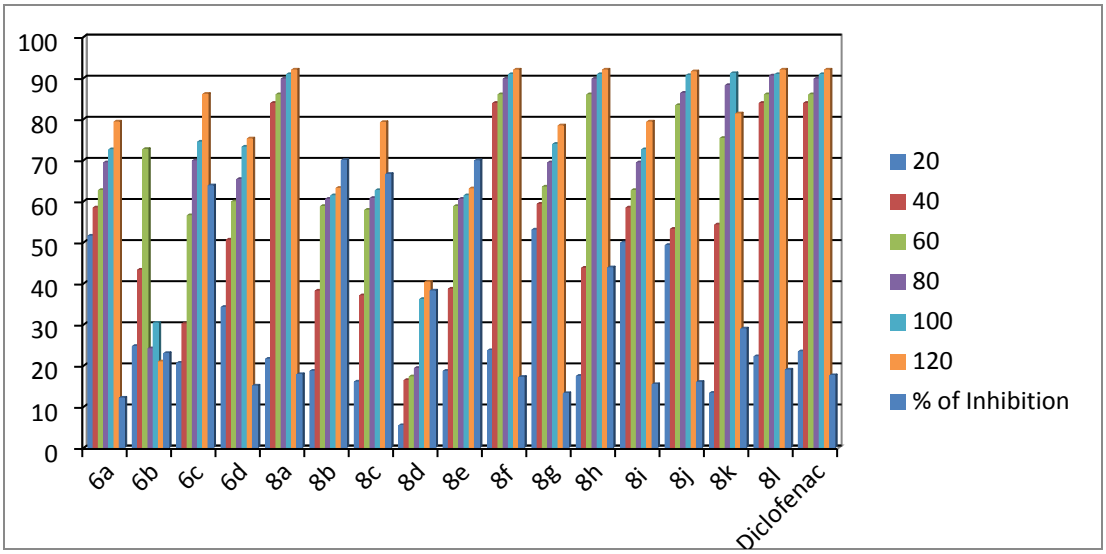


Figure IV. Graphical Representation of % of Inhibition

iii) Antibacterial activity:

The synthesized new compounds **6(a-d) & 8(a-l)** were screened for their antibacterial activity against different types of bacterial strains such as gram-negative bacterial strains of *Klebsiella pneumonia*, *Escherichia coli*, and gram-positive bacterial strains of *Bacillus subtilis, Staphylococcus aeureus* at two different concentrations of 10 and 20 µg/mL. The cultures were diluted with 5 % saline autoclaved, and the final volume was made with concentration approximately 10^5–10^6 CFU/mL. The synthesized compounds were diluted in DMSO for antibacterial biological assays. For agar disc diffusion method, the liquid form of test compound was soaked on to the disc and then allowed to air dry, such that the disc gets completely saturated with test compound. The saturated chemical discs were introduced onto the upper layer of the medium evenly flooded with the bacteria. The discs were dipped in different chemical samples, were placed over the evenly spread bacterial nutrient media, and incubated at 37°C for 24-48 h for better inhibition of bacteria. The zones of inhibition were measured after 24-48 h. All the experiments were carried out in triplicated and the results were expressed as zone of Inhibition in mm. The zones of inhibition of synthesized compounds **6(a-d) & 8(a-l)** were compared to the zone of inhibition of standard antibiotic concentrations of gatifloxacin (10 and 20 µg/mL).

iv) Antifungal activity

The antifungal activity of synthesized compounds **6(a-d) & 8(a-l)** were tested against three pathogenic fungi, namely *Aspergillus niger, Aspergilus flavus* and *Fusariumoxy sporum* by the poison plate technique at a concentration of 50 µg/mL. Three kinds of fungi were incubated in PDA at 25 ± 1 ^{0}C for 5 days to get new mycelium for antifungal assay, then a mycelium as discs of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated in the center of PDA plate. Test compounds were dissolved in DMSO (10 mL) after that added into the Potato Dextrose Agar medium (PDA, 90 mL). The final concentration of compounds into the medium was adjusted to 50µg/mL. The inoculated plates were incubated at 25 ± 1ºC for 5 days. Acetone was diluted with sterilized distilled water and used as control, while clotrimazole (50µg/mL) was used as standard control for each treatment three replicates of experiments were carried out. The radial growth of the fungal colonies was measured on the 5th day.

Molecular Docking studies

Methodology:

Drug activity is an imperative aspect for every synthesized compound. Though the activity exhibited by all synthesized compounds along with the intermediates are commendable, reliability of our compounds have been evaluated through docking studies. To perform this, proteins of desired activity has been retrieved from the protein data bank. Pdb Id: 2VCX, 3VXI, 4GEE and 2XFH. Grid was generated with the aid of crystallized ligand with in the protein and molecular docking has been carried out using Glide mode in Schrodinger. The potential binding mode of all the synthesized compounds along with binding free energies (ΔG) has been quantified. Rationality of compounds has been further evaluated through QikProp for their drug likeness properties.

Results and Discussion:

Molecular docking reveals the ideal binding mode of synthesized compounds with the active site of protein. Comparative docking studies from all the proteins reveal that the compound **8l**

has shown high dock score of **-7.792** and binding energy of -117.95 than the standard Diclofenac in the protein 2VCX, but not with the other proteins. Even the calculated predicted activities for this compound are found to be slightly higher than the standard in all the activities (provided in supplementary table). This clearly enumerates, Compound 8l has shown explicit effect as potential Anti inflammatory, anti microbial and antioxidant. The intermediate compounds predominantly **6b** and **6d** has shown ideal H-Bond interaction with water in all the proteins 2XFH, 4GEE and 3VXI as that of standard Ascorbic acid, Gatifloxacin (as illustrated in **figures 1d, 3d** and **4d**) stating that this compounds can be further exploited to newer chemical agents possessing anti oxidant and antimicrobial activity. (Molecular docking figures and tables abbreviated with **fig.1d – fig 4fd and table 1a – table 6d**)

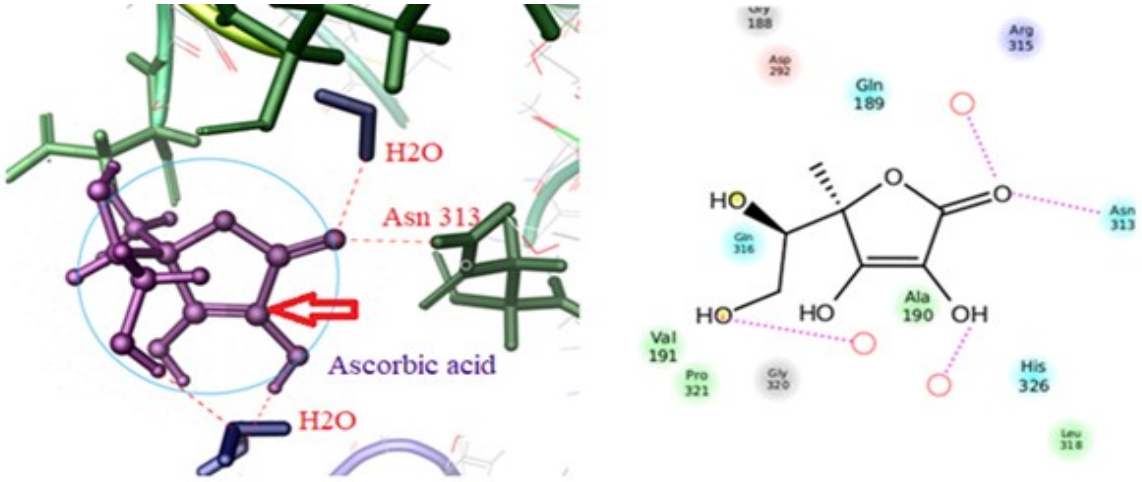


Fig. 1d. Dock pose images of Standard Antioxidant drug Ascorbic acid showing three H-bond interactions with water and one H-Bond interaction with Asn-313.

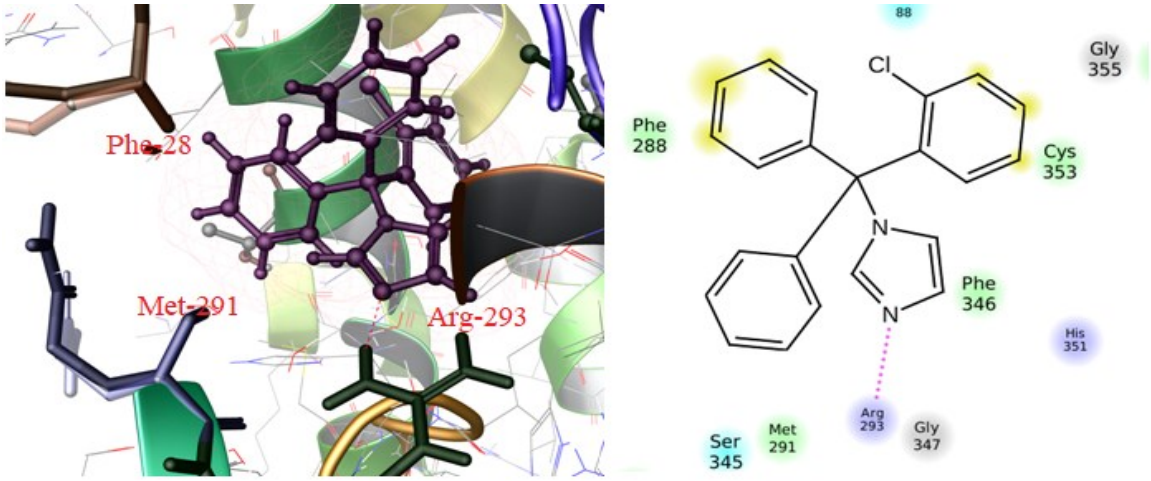


Fig. 2d. Dock pose images of Standard Anti fungal drug Cotrimazole showing H-bond interactions with Arg-293.

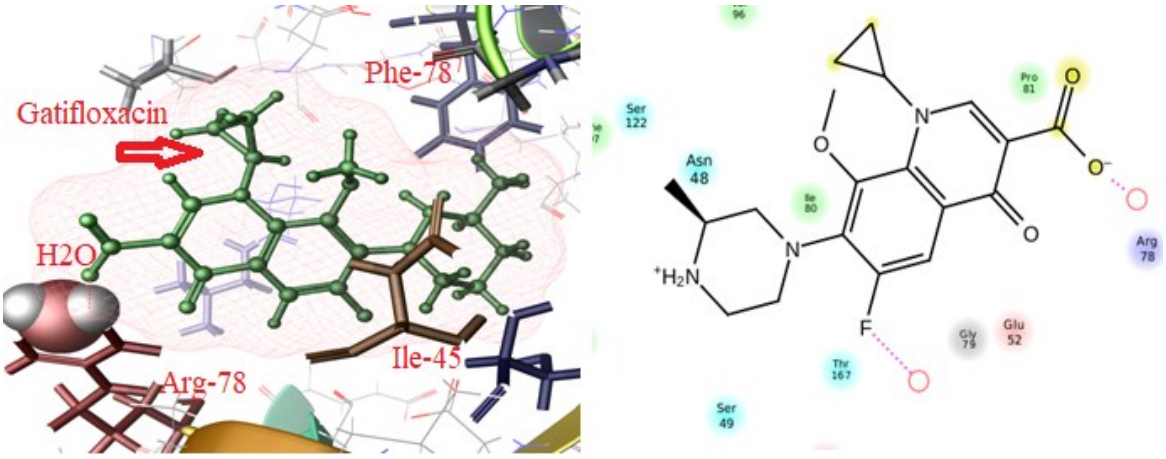


Fig. 3d. Dock pose images of Standard Anti microbial activity showing two H-bond interactions with water.

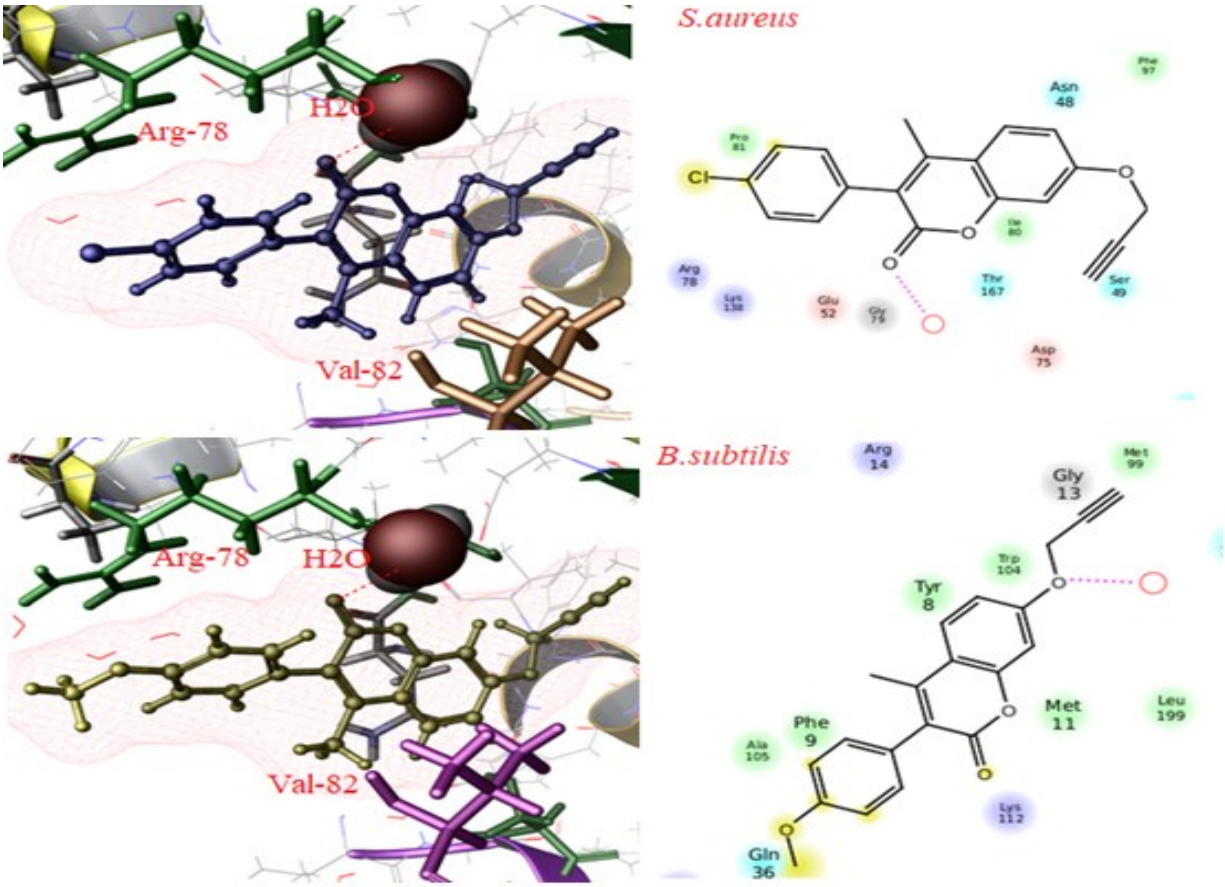


Fig. 4d. Dock pose images of intermediate compounds 6b, 6d showing H-Bond interaction with water in gram positive bacteria.

The standard anti fungal agents Cotrimazole as shown hydrogen bond interaction with Arg-293 depicted in **fig. 2**. None of the molecules succeeded in displaying this interaction, but interacted with other amino acids within the active site such as Val-191. While the final compounds **8d, 8e**, **8h** and **8k** has shown hydrogen bond interaction His-88 **(Fig. 4a)** Val-191 **(4b**) and with water **(4c)** in the proteins 4GEE and 2XFH. This signifies that the synthesized compounds possessing anti bacterial and anti fungal activity in addition to anti oxidant and anti inflammatory activity. Dock scores along with the binding free energies for all the synthesized compounds have been quantified as shown in **table 1d**. The average predicted activities of different chemical methods have been calculated for the synthesized compounds. (**table 3d** to **table 6d).**

A drug is said to be therapeutically active if it possess significant drug likeness as that of standard. Hence pharmacokinetic properties (ADME) have been exemplified using Qik Prop module in Schrodinger. Most of the compounds exhibit excellent 100 % human oral absorption. Solubility (QPlogS), partition coefficient (QPlogPo/w), cell permeability (QPPMDCK) and permeability of blood brain barrier (QPlogBB) is found to be in acceptable range as shown in **table 2d.**

Compounds	Mol MW	QPlogPo/w	QPlogS	QPlogBB	QPPMDCK	% Human Oral Absorption
6a	308.308	4.249	-5.074	-0.091	2930.25	100
6b	324.763	4.489	-5.385	-0.033	4004.915	100
6c	369.214	4.574	-5.521	-0.024	4290.133	100
6d	320.344	4.074	-4.827	-0.272	1610.51	100
8a	457.46	5.321	-6.976	-0.644	1198.461	100
8b	441.461	5.645	-7.428	-0.52	1370.607	100
8c	461.879	5.831	-7.534	-0.306	3591.227	100
8d	473.915	5.659	-7.248	-0.45	2166.729	100
8e	457.915	5.839	-7.845	-0.568	1538.893	100
8f	478.334	6.075	-7.875	-0.244	5005.296	100
8g	518.366	5.46	-7.128	-0.668	1358.178	86.943
8h	502.366	5.915	-7.956	-0.554	1667.039	91.077
8i	522.785	6.18	-7.975	-0.19	5872.723	95.187
8k	453.496	5.252	-7.042	-0.918	470.644	100
8l	473.915	5.489	-7.173	-0.609	1530.718	100

Table 2d. Assessment of drug likeness properties for the synthesized compounds. Where QPlogPo/w- partion coefficient in octane/water system, QPlogS - solubility profile of drug, QPlogBB- blood brain barrier permeability, QPPMDCK – cell permeability.

Compounds	Predicted Anti oxidant activity			Predicted Anti inflammatory activity		
Chemical Method	DPPH Method	H_2O_2 Method	Average predicted anti oxidant activity	Egg Albumin Method	Heat induced Haemolytic method	Average predicted anti inflammatory activity
6a	5.649	5.45	5.549	4.376	4.918	4.647
6b	4.698	4.627	4.662	4.639	4.639	4.639
6c	3.532	5.150	4.341	4.195	4.195	4.195
6d	5.346	5.869	5.607	4.782	4.825	4.802
8a	5.455	5.754	5.604	4.801	4.750	4.77
8b	3.471	5.904	3.687	4.223	4.156	4.189
8c	4.371	5.838	5.104	4.116	4.177	4.146
8d	5.954	4.147	5.050	3.623	3.859	3.741
8e	3.464	3.464	3.464	4.223	4.150	4.189
8f	5.429	5.764	5.596	4.505	4.766	4.635
8g	4.731	4.731	4.731	3.986	4.880	4.433
8h	5.600	4.371	4.985	4.269	4.358	4.313
8i	5.318	5.318	5.318	4.343	4.813	4.578
8j	5.889	5.896	5.937	4.723	4.798	4.760
8k	7.214	7.214	7.214	4.371	4.217	4.294
8l	5.45	5.514	5.482	4.539	4.723	4.631
Ascorbic acid	5.835	5.935	5.885	-	-	-
Diclofenac	-	-	-	4.456	4.756	4.756

Table 3d Calculated average predicted activities for both Anti inflammatory and anti oxidant activities from various chemical methods.

Compound	zone of inhibition (Gram Positive)					
	S. aureus (MTCC 96)			*Bacillus subtilis* (MTCC 121)		
	10µg/mL	20µg/mL	Average	10µg/mL	20µg/mL	Average
6a	4.920	4.744	4.832	4.958	4.677	4.818
6b	5.045	4.721	4.883	4.920	4.721	4.821
6c	5	4.698	4.849	5	4.657	4.828
6d	4.920	4.769	4.845	5.045	4.744	4.895
8a	4.657	4.494	4.576	4.638	4.397	4.518
8b	4.823	4.619	4.721	4.795	4.494	4.645
8c	4.744	4.443	4.594	4.795	4.468	4.632
8d	4.638	4.494	4.566	4.657	4.376	4.517
8e	4.795	4.638	4.717	4.823	4.494	4.659
8f	4.744	4.602	4.673	4.721	4.420	4.570
8g	4.619	4.468	4.544	4.657	4.376	4.517
8h	4.823	4.619	4.721	4.795	4.552	4.674
8i	4.795	4.568	4.682	4.721	4.408	4.565
8j	4.585	4.468	4.526	4.568	4.408	4.488
8k	4.721	4.585	4.653	4.795	4.494	4.645
8l	4.721	4.568	4.644	4.744	4.431	4.588
Gatifloxacin	4.698	4.522	4.610	4.698	4.397	4.548

Table 4d Calculated predicted zone of inhibition for anti microbial activity in Gram positive cell lines like *Stapylococus aeureus, Bacillus subtilis* with the concentrations of 10 and 20µg/mL.

Compound	zone of inhibition (Gram Negative)					
	E.Coli (MTCC 43)			*K.pneumonia* (MTCC 530)		
	10µg/mL	20µg/mL	Average	10µg/mL	20µg/mL	Average
6a	5.096	4.958	5.027	5.221	5	5.110
6b	5	4.920	4.960	5.397	4.958	5.178

6c	4.958	4.823	4.891	5.154	5.045	5.100
6d	5.045	4.920	4.983	4.920	4.853	4.887
8a	4.795	4.677	4.736	4.853	4.698	4.776
8b	4.886	4.795	4.840	5.096	4.853	4.975
8c	4.853	4.744	4.799	5.045	4.795	4.920
8d	4.744	4.638	4.691	4.886	4.698	4.792
8e	4.920	4.795	4.858	5.096	4.886	4.991
8f	4.823	4.721	4.772	4.958	4.769	4.864
8g	4.744	4.602	4.673	4.920	4.657	4.789
8h	4.853	4.769	4.811	5.221	4.886	5.053
8i	4.920	4.698	4.809	5.045	4.769	4.907
8j	4.677	4.537	4.607	4.853	4.657	4.755
8k	4.920	4.853	4.887	5.221	4.920	5.071
8l	4.853	4.602	4.727	5.096	4.823	4.960
Gatifloxacin	4.823	4.698	4.761	5	4.744	4.872s

Table 5d Calculated predicted zone of inhibition for anti microbial activity against Gram Negative cell lines like *Escherichia coli* (MTCC 43), and *Klebsiella pneumonia* (MTCC 530) with the concentrations of 10 and 20µg/mL.

Compound	Zone of Inhibition (mm)		
	Aspergillus niger (50µg/mL)	*Aspergillus flavus* (50µg/mL)	*Fusariumoxy sporum* (50µg/mL)
6a	5.14	5.19	5.14
6b	5.06	5.14	5.18
6c	5.14	5.07	5.04
6d	5.09	5.10	5.19
8a	4.73	4.75	4.73
8b	4.75	4.77	4.75
8c	4.74	4.79	4.72

8d	4.97	4.93	4.94
8e	4.87	4.85	4.83
8f	4.82	4.83	4.79
8g	4.99	4.90	4.90
8h	4.84	4.86	4.84
8i	4.80	4.84	4.80
8j	4.73	4.78	4.72
8k	4.78	4.86	4.79
8l	4.80	4.83	4.84
Clotrimazole	4.77	4.80	4.74

Table 6d Calculated predicted zone of inhibition for anti fungal activity against *Aspergillus niger*, *Aspergillus flavus*, and *Fusariumoxy sporum* at 50 µg/mL concentration.