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Supporting Information

Chemistry, Chemical Biology and Photophysics of Certain New Chromene-Triazole-Coumarin Triads as Fluorescent Inhibitors of CDK2 and CDK4 Induced Cancers

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Figures:

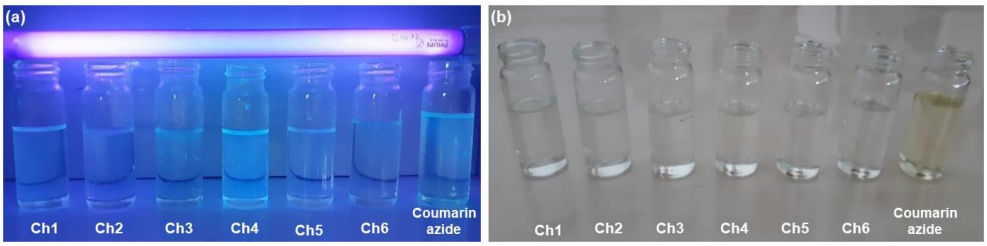


Fig. S1 Starting alkynes Ch1 to Ch6 and Coumarin-azide under UV exposure: (a) samples under UV light (b) samples under Day-light

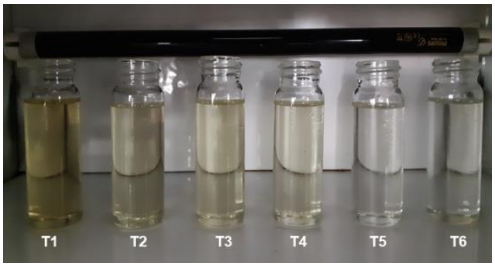
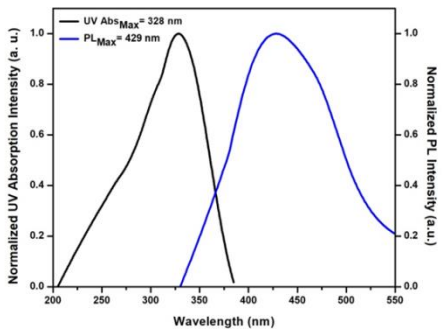


Fig. S2 Compounds **T1** to **T6** (50 ppm solution in DMSO) under Day-light



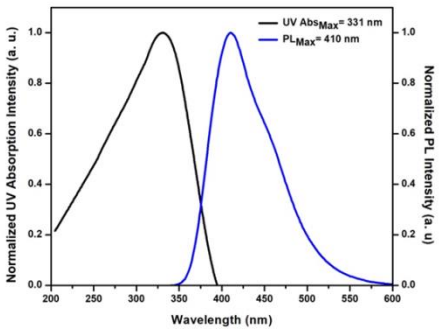


Fig. S3 Absorption and emission spectra of (a) **T4** and (b) **T5**

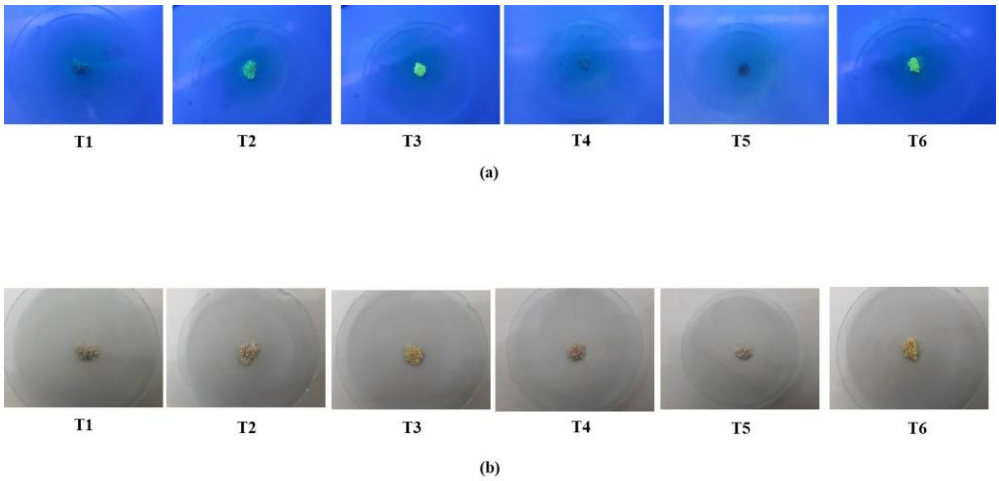


Fig. S4 Triads **T1** to **T6**, solid samples under (a) UV-light (b) Day light

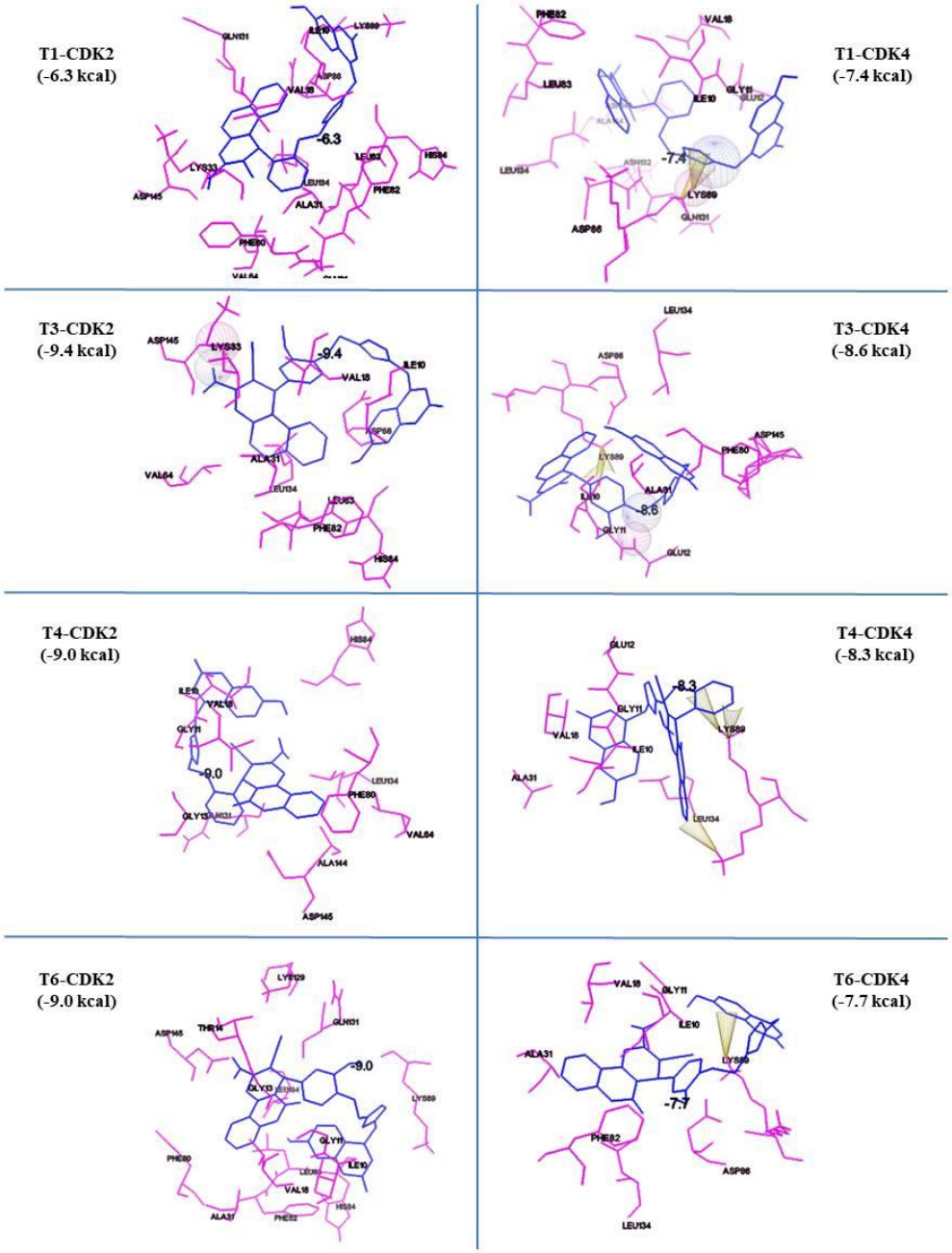


Fig. S5 Docking mode of Chromene-Triazole-Coumarin Triads **T1**, **T3**, **T4** and **T6** in the active site of CDK2 and CDK4 with their corresponding docking score

Experimental Section

Materials and Methods

All materials were obtained from commercial suppliers, and used without further purification. IR spectra were recorded on a JASCO-FT/IR-4100 Fourier- transform infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained by Bruker amx 400/500 MHz spectrometer in DMSO/$CDCl_3$ solutions. The chemical shifts (δ) values are given relative to tetramethylsilane (TMS) and the coupling constants (J) are represented in Hertz (Hz). HRMS was obtained using ESI ionization. Quantum mechanical calculations were done with Gaussian 09 software. UV-Vis absorption spectra were recorded on a Jasco V-550 UV-VIS Spectrophotometer. Fluorescence spectra were measured using Perkin Elmer LS 45 and LS 55 and Horiba Fluoromax-4 Spectrometers.

The human cervical cancer cell line (HeLa) was procured from National Center for Cell Science (NCCS, Pune), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), was purchased from Sigma-Aldrich (Bangalore). Analytical grade reagents were purchased from Sigma-Aldrich (Bangalore). All the samples were prepared in Milli-Q water.

Synthetic Procedures

(1) ***3-amino-1-(2-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[f]chromene-2 carbonitrile (Ch1):*** A mixture of propargylated salicylaldehyde (160 mg, 1mmol), 2-naphthol (144mg, 1 mmol), malononitrile (66mg, 1mmol) and sodium carbonate (0.106 mg, 0.01mmol) were mixed together by using a mortar and pestle. The mixture was heated in an oven at 80°C for 5 minutes and mixed again. The process was repeated thrice. After cooling, the mixture

was washed with hot water and the solid separated was filtered and dried. The product was then recrystallized from hot ethanol to obtain pure **Ch1**: 320mg (Yield 86%) White solid M.P.: 112ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3475, 3317, 3299, 3188, 3066, 2916, 2862, 2172, 2127, 1909, 1646, 1583, 1515, 1490, 1451, 1415, 1376, 1293, 1255, 1232, 1183. ^{1}H-NMR δH (500 MHz, DMSO, δ ppm): 3.319 (1H, s, CH), 3.698 (2H, s, -OCH_2), 5.00 (1H, s, Ar-H), 7.089-7.402 (8H, m, Ar-H), 7.672-8.006 (3H, m, Ar-H), 8.462 (2H, s, Ar-NH_2). **^{13}C NMR** δC(DMSO, 125MHz, δ ppm)**:** 22.30, 56.24, 59.15, 78.17, 79.15, 112.98, 117.05, 117.98, 121.72, 122.72, 126.82, 128.45, 128.48, 129.83, 130.43, 132.66, 133.88, 138.75, 156.42, 171.97, 200.79. EIMS: m/z ($[M+H]^+$) calculated for $C_{23}H_{16}N_2O_2$: 353.12, found: 353.37

(2) ***3-amino-1-(4-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[f] chromene-2-carbonitrile (Ch2):*** A mixture of propargylated 4-hydroxy benzaldehyde (160 mg, 1mmol), 2-naphthol (144mg, 1 mmol), malononitrile (66mg, 1mmol) and sodium carbonate (0.106 mg, 0.01mmol) were mixed together by using a mortar and pestle. The mixture was heated in an oven at 80ºC for 5 minutes and mixed again. The process was repeated thrice. After cooling, the mixture was washed with hot water and the solid separated was filtered and dried. The product was then recrystallized from hot ethanol to obtain pure **Ch2**: 315mg (Yield 85%) White solid M.P.: 113ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3462, 3379, 3272, 3084, 3031, 2930, 2972, 2222, 2129, 1652, 1584, 1554, 1507, 1455, 1429, 1373, 1316, 1257, 1235, 1191. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 3.313 (1H, s, CH), 3.681 (2H, s, Ar-O-CH_2), 4.978 (1H, s, Ar-H), 7.079-7.237 (2H, m, Ar-H), 7.255 (2H, s, Ar-H), 7.373-7.403 (1H, t, Ar-H), 7.671-7.687 (1H, d, 8Hz, Ar-H), 7.748-7.777 (1H, t, Ar-H), 7.990-8.007 (2H, d, J=8Hz, Ar-H), 8.428 (2H, s, Ar-NH_2). ^{13}C NMR δC(DMSO, 100MHz, δ ppm): 22.38, 56.24, 59.15, 78.17, 79.15, 112.98, 117.05, 117.98, 121.72,

122.72, 126.82, 128.45, 128.48, 129.83, 130.43, 132.66, 133.88, 138.75, 156.42, 171.97, 200.79. EIMS: m/z ($[M+H]^+$) calculated for $C_{23}H_{16}N_2O_2$: 353.12, found: 353.35

(3) ***3-amino-1-(3-methoxy-4-(prop-2-yn-1-yloxy) phenyl)-1Hbenzo[f]chromene-2-carbonitrile (Ch3)*****:** A mixture of propargylated 4-hydroxy-3-methoxybenzaldehyde (190 mg, 1mmol), 2-naphthol (144mg, 1 mmol), malononitrile (66mg, 1mmol) and sodium carbonate (0.106 mg, 0.01mmol) were mixed together by using a mortar and pestle. The mixture was heated in an oven at 80°C for 5 minutes and mixed again. The process was repeated thrice. After cooling, the mixture was washed with hot water and the solid separated was filtered and dried. The product was then recrystallized from hot ethanol to obtain pure **Ch3**: 340mg (Yield 85%) as Yellow solid M.P.: 115°C. IR absorptions (KBr, ν_{max}/cm^{-1}): 3502, 3369, 3277, 3068, 3031, 2969, 2928, 2220, 2128, 1673, 1583, 1563, 1510, 1451, 1417, 1377, 1342, 1305, 1288, 1204, 1182, 1143. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 3.339 (1H, s, CH), 3.675 (2H, s, Ar-O-CH_2), 3.827 (3H, s, Ar-O-CH_3), 4.981 (1H, s, Ar-H), 7.089-7.319 (7H, m, Ar-H), 7.372-7.402 (1H, t, Ar-H), 7.672-8.006 (3H, m, Ar-H), 8.466 (2H, s, Ar-NH_2). ^{13}C NMR δC (DMSO, 100MHz, δ ppm): 27.82, 54.24, 56.15, 59.28, 78.17, 79.15, 112.98, 117.05, 117.98, 121.72, 122.72, 128.70, 128.76, 128.93, 129.77, 130.43, 132.66, 134.96, 139.76, 157.30, 172.82. EIMS: m/z ($[M+H]^+$) calculated for $C_{24}H_{18}N_2O_3$: 382.13, found: 382.32

(4) ***2-amino-5-oxo-4-(2-(prop-2-yn-1-yloxy)phenyl)-4,5 dihydropyrano [3,2-c]chromene-3-carbonitrile (Ch4):*** A mixture of propargylated salicylaldehyde (160 mg, 1mmol), 4-hydroxy coumarin (162mg, 1 mmol), malononitrile (66mg, 1mmol) and hexamethylenetetramine (0.140 mg, 0.01mmol) were dissolved in minimum amount of ethanol. The reaction

mixture is refluxed in a round bottom flask for one hour. The reaction mixture was then poured into cooled water and the precipitated solid was washed with water, filtered and dried. The product was recrystallized from hot ethanol to obtain pure **Ch4:** 313 mg (Yield 81%) as White solid, M.P.:122ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3576, 3354, 3278, 3147, 2925, 2892, 2191, 2130, 1675, 1606, 1576, 1489, 1449, 1411, 1377, 1337, 1310, 1272, 1247, 1229, 1212, 1174, 1111, 1064. ^{1}H NMR δH(500 MHz, $CDCl_3$, δ ppm): 3.869 (1H, s, CH), 4.668 (1H, s, Ar-H), 4.779 (2H, s, Ar-O-CH_2), 6.030 (1H, s, Ar-H), 6.911-6.929 (2H, m, Ar-H), 7.076-7.145 (2H, m. Ar-H), 7.257 (2H, s, Ar-H), 7.396-7.412 (1H, d, 8Hz, Ar-H), 7.603-7.638 (1H, m, Ar-H), 7.852 (2H, s, Ar-NH_2). HRMS: m/z ($[M+H]^+$) calculated for $C_{22}H_{14}N_2O_4$: 371.0953, found: 371.1021

(5) ***2-amino-5-oxo-4-(4-(prop-2-yn-1-yloxy)phenyl)-4,5 dihydropyrano [3,2-c]chromene-3-carbonitrile (Ch5):*** A mixture of propargylated 4-hydroxy benzaldehyde (160 mg, 1mmol), 4-hydroxy coumarin (162mg, 1 mmol), malononitrile (66mg, 1mmol) and hexamethylenetetramine (0.140 mg, 0.01mmol) were dissolved in minimum amount of ethanol. The reaction mixture is refluxed in a round bottom flask for one hour. The reaction mixture was then poured into cooled water and the precipitated solid was washed with water, filtered and dried. The product was recrystallized from hot ethanol to obtain pure **Ch5:** 318 mg (Yield 82%) as White solid, M.P.:125ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3388, 3291, 3200, 3073, 2224, 2198, 2122, 1708, 1669, 1602, 1558, 1507, 1454, 1402, 1374, 1352, 1305, 1263, 1232, 1214, 1178, 1092. ^{1}H NMR δH(500 MHz, $CDCl_3$, δ ppm): 3.833 (1H, s, CH), 4.668 (1H, s, Ar-H), 4.779 (2H, s, Ar-O-CH_2), 6.030 (1H, s, Ar-H), 6.911-6.929 (2H, m, Ar-H), 7.076-7.145 (2H, m, Ar-H), 7.257 (2H, s, Ar-H), 7.388-7.412 (1H, d, 12Hz, Ar-H), 7.603-7.663 (2H, m, Ar-H), 7.853 (2H, s, Ar-NH_2). HRMS: m/z (M^+) calculated for $C_{22}H_{14}N_2O_4$: 371.0953, found: 371.0965

(6) ***2-amino-4-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (Ch6):*** A mixture of propargylated 4-hydroxy-3-methoxybenzaldehyde (190 mg, 1mmol), 4-hydroxy coumarin (162mg, 1 mmol), malononitrile (66mg, 1mmol) and hexamethylenetetramine (0.140 mg, 0.01mmol) were dissolved in minimum amount of ethanol. The reaction mixture is refluxed in a round bottom flask for one hour. The reaction mixture was then poured into cooled water and the precipitated solid was washed with water, filtered and dried. The product was recrystallized from hot ethanol to obtain pure **Ch6:** 330 mg (Yield 79%) as Yellow solid, M.P.:123ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3406, 3324, 3278, 3032, 2960, 2931, 2217, 2196, 2118, 1693, 1671, 1601, 1588, 1565, 1509, 1454, 1418, 1380, 1344, 1310, 1266, 1213, 1179, 1141, 1112, 1062, 1011. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 3.449 (2H, s, -O-CH_2), 3.732 (3H, s, Ar-OCH_3), 3.815 (1H, s, CH), 4.424 (1H, s, Ar-H), 6.729-6.974 (3H, m, Ar-H), 7.235-7.256 (1H, d, 10Hz, Ar-H), 7.429-7.495 (1H, m, Ar-H), 7.583-7.933 (1H, m, Ar-H), 8.316 (2H, s, Ar-NH_2). HRMS: m/z ($[M+H]^+$) calculated for $C_{23}H_{16}N_2O_5$: 401.1059, found: 401.1118

(7) ***4-(azidomethyl)-7-hydroxy-2H-chromen-2-one (Coumarin-azide):*** 4-bromomethyl-7-hydroxycoumarin (255 mg, 1mmol) and sodium azide (65 mg, 1mmol) were dissolved in minimum quantity of N, N-Dimethylformamide (DMF) in a round bottomed flask. The reaction mixture was stirred for 18 hrs at 60ºC. The mixture was then poured into excess of ice cold water. The solid product obtained was washed with water, filtered and dried. The product was recrystallized from hot ethanol to obtain pure **coumarin-azide:** 186 mg (Yield 58%) as Brown solid, M.P.:186ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3502, 3068, 2918, 2827, 2744, 2196, 2120, 1682, 1610, 1566, 1515, 1471, 1334, 1362, 1342, 1312, 1253, 1205, 1152,

1098. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 2.142 (2H, s, CH_2), 6.669 (1H, s, Ar-H), 6. 732-6.762 (1H, d, J=12Hz, Ar-H), 6.820-6.841 (1H, d, J=8Hz, Ar-H), 7.361-7.383 (1H, d, J=9Hz, Ar-H), 7.668-7.690 (1H, d, J=9Hz, Ar-H), 8.229 (1H, s, Ar-OH). ^{13}C NMR δC (DMSO, 100MHz, δ ppm): 167.012, 163.011, 156.671, 154.657, 129.314, 127.956, 125.010, 113.657, 112.752, 112.314, 102.132, 56.303. HRMS: m/z (M^+) calculated for $C_{10}H_7\ N_3O_3$: 217.0487, found: 217.0118

(8) ***3-amino-1-(2-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[f]chromene-2-carbonitrile (T1):*** **Ch1** (88 mg, 0.25 mmol) and **coumarin-azide** (54.3 mg, 0.25 mmol) were dissolved in 2 mL of DMSO. To this mixture, 8 mL of *t*-BuOH, 4 mL of water, $CuSO_4\ 5H_2O$ (40 mg), and sodium ascorbate (80 mg) were added and stirred at room temperature for 48 hrs. The reaction mixture was then poured in to cold water. The precipitated solid was collected, washed with water and dried. The dried product was washed with diethyl ether (5 mL) to afford **T1**: 110 mg (Yield 77%) as White solid, M.P.: 192ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3467, 3443, 3359, 3181, 3067, 2921, 2190, 1763, 1727, 1696, 1647, 1598, 1513, 1486, 1455, 1406, 1321, 1292, 1213, 1174, 1136, 1083, 1016. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 5.025 (1H, s, Ar-OH), 5.307 (1H, s, Ar-H), 5.501 (2H, s, ArN-CH_2), 5.586 (2H, s, Ar-O-CH_2), 6.507 (1H, s, Ar-H), 6.879 (2H, s, Ar-H), 7.243 (1H, s, Ar-H), 7.416-7.466 (3H, d, J=20Hz, Ar-H), 7.695-7.982 (4H, m, Ar-H), 8.259 (2H, s, Ar-NH_2), 8.483-8.525 (3H, d, J=16.8Hz, Ar-H). ^{13}C NMR δC(DMSO, 100MHz, δ ppm): 18.954, 55.520, 56.556, 64.033, 78.323, 108.712, 112.096, 114.299, 126.589, 126.888, 127.468, 127.761, 128.892, 128.976, 132.893, 146.034, 152.649, 157.798, 165.299. HRMS (ESI): m/z (M^+) calculated for $C_{33}H_{23}\ N_5O_5$: 569.1699, found: 569.1683

(9) ***3-amino-1-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[f]chromene-2-carbonitrile (T2):*** **Ch2** (88 mg, 0.25 mmol) and **coumarin-azide** (54.3 mg, 0.25 mmol) were dissolved in 2 mL of DMSO. To this mixture, 8 mL of *t*-BuOH, 4 mL of water, $CuSO_4$ $5H_2O$ (40 mg), and sodium ascorbate (80 mg) were added and stirred at room temperature for 48 hrs. The reaction mixture was then poured in to cold water. The precipitated solid was collected, washed with water and dried. The dried product was washed with diethyl ether (5 mL) to afford **T2**: 116 mg (Yield 82%) as White solid, M.P.:196^{o}C. IR absorptions (KBr, ν_{max}/cm^{-1}): 3441, 3405, 3346, 3083, 3030, 2925, 2223, 1729, 1598, 1585, 1509, 1475, 1430, 1375, 1317, 1260, 1236, 1184, 1089, 1048, 1010. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 4.615-4.692(3H, m, Ar-H, ArN-CH_2), 5.151-5.226 (3H, m, Ar-OH, Ar-O-CH_2), 6.935 (1H, s, Ar-H), 7.045 (2H, s, Ar-H), 7.155-7.221 (6H, t, Ar-H), 7.387-7.480 (3H, m, Ar-H), 7.654-7.752 (3H, m, Ar-H), 7.910-7.933 (1H, d, J=9Hz, Ar-H), 8.273 (2H, s, Ar-NH_2). ^{13}C NMR δC(DMSO, 100MHz, δ ppm): 18.982, 53.888, 61.982, 74.020, 108.702, 112.085, 114.315, 127.850, 128.082, 129.354, 132.918, 138.218, 152.692, 156.177, 159.253, 165.292. HRMS (ESI): m/z (M^+) calculated for $C_{33}H_{23}N_5O_5$: 569.1699, found: 569.1685

(10) ***3-amino-1-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1H-benzo[f]chromene-2-carbonitrile (T3):*** **Ch3** (95.6 mg, 0.25 mmol) and **coumarin-azide** (54.3 mg, 0.25 mmol) were dissolved in 2 mL of DMSO. To this mixture, 8 mL of *t*-BuOH, 4 mL of water, $CuSO_4$ $5H_2O$ (40 mg), and sodium ascorbate (80 mg) were added and stirred at room temperature for 48 hrs. The reaction mixture was then poured in to cold water. The precipitated solid was collected, washed with water and dried. The dried product was washed with diethyl ether (5 mL)

to afford **T3**: 112 mg (Yield 75%) as Yellow solid, M.P.:198ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3561, 3472, 3368, 3086, 3028, 2928, 2220, 1729, 1694, 1570, 1509, 1456, 1418, 1376, 1338, 1270, 1201, 1146, 1010. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 3.285 (3H, s, -O-CH3), 4.626-4.690 (3H, m, Ar-H, ArN-CH_2), 5.152-5.229 (3H, m, Ar-OH, Ar-O-CH_2), 6.936 (1H, s, Ar-H), 7.046 (2H, s, Ar-H), 7.157-7.222 (4H, t, Ar-H), 7.388-7.482 (3H, m, Ar-H), 7.652-7.754 (3H,m, Ar-H), 7.914-7.932 (1H, d, J=7Hz, Ar-H), 8.264 (2H, s, Ar-NH_2). ^{13}C NMR δC(DMSO, 100MHz, δ ppm): 21.089, 55.547, 56.209, 64.083, 77.587, 112.461, 113.401, 113.876, 114.645, 124.798, 126.383, 148.959, 151.869, 160.364, 162.216. HRMS (ESI): m/z (M^+) calculated for $C_{34}H_{25}N_5O_6$: 599.1804, found: 599.1839

(11) ***2-amino-4-(2-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (T4):*** **Ch4** (92.6 mg, 0.25 mmol) and **coumarin-azide** (54.3 mg, 0.25 mmol) were dissolved in 2 mL of DMSO. To this mixture, 8 mL of *t*-BuOH, 4 mL of water, $CuSO_4$ $5H_2O$ (40 mg), and sodium ascorbate (80 mg) were added and stirred at room temperature for 48 hrs. The reaction mixture was then poured in to cold water. The precipitated solid was collected, washed with water and dried. The dried product was washed with diethyl ether (5 mL) to afford **T4**: 100 mg (Yield 68%) as White solid, M.P.:198ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3505, 3441, 3386, 2922, 2952, 2611, 2189, 1706, 1674, 1606, 1572, 1491, 1454, 1377, 1329, 1234, 1174. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 4.606 (2H, s, ArN-CH_2), 4.924 (1H, s, Ar-H), 5.152 (1H, s, Ar-OH), 5.369-5.420 (2H, d, J=20.4Hz, Ar-O-CH_2), 6.241 (1H, s, Ar-H), 6.777-7.069 (3H, m, Ar-H), 7.207-7.311 (3H, m, Ar-H), 7.526-7.503 (1H, t, Ar-H), 7.867-7.920 (5H, m, Ar-H), 8.241 (2H, s, Ar-NH_2). ^{13}C NMR δC (DMSO, 100MHz, δ ppm): 33.395, 55.970, 56.593, 67.910, 77.610,

78.715, 103.016, 113.032, 113.156, 116.383, 121.263, 122.536, 124.558, 128.466, 129.785, 130.728, 131.030, 132.692, 152.044, 155.388, 158.522, 159.644. HRMS (ESI): m/z ($[M+H]^+$) calculated for $C_{32}H_{21}N_5O_7$: 588.1441, found: 588.1510

(12) ***2-amino-4-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (T5):*** **Ch5** (92.6 mg, 0.25 mmol) and **coumarin-azide** (54.3 mg, 0.25 mmol) were dissolved in 2 mL of DMSO. To this mixture, 8 mL of *t*-BuOH, 4 mL of water, $CuSO_4$ $5H_2O$ (40 mg), and sodium ascorbate (80 mg) were added and stirred at room temperature for 48 hrs. The reaction mixture was then poured in to cold water. The precipitated solid was collected, washed with water and dried. The dried product was washed with diethyl ether (5 mL) to afford **T5**: 108 mg (Yield 74%) as White solid, M.P.:190ºC. IR absorptions (KBr, ν_{max}/cm^{-1}): 3494, 3477, 3388, 3072, 2916, 2227, 1660, 1603, 1504, 1384, 1311, 1250, 1173, 1116, 1021. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 4.687 (2H, s, ArN-CH_2), 4.949 (1H, s, Ar-H), 5.159 (1H, s, Ar-OH), 5.384-5.409 (2H, d, J=10Hz, Ar-O-CH_2), 6.206 (1H, s, Ar-H), 6.777-7.011 (4H, m, Ar-H), 7.257-7.343 (3H, m, Ar-H), 7.515-7.590 (1H, t, Ar-H), 7.812-7.985 (5H, m, Ar-H), 8.256 (2H, s, Ar-NH_2). ^{13}C NMR δC(DMSO, 100MHz, δ ppm): 30.945, 55.552, 56.208, 73.965, 77.595, 102.152, 112.475, 113.411, 114.645, 115.854, 123.486, 123.622, 124.802, 126.383, 131.453, 135.853, 148.966, 151.869, 152.024, 160.596, 163.416. HRMS (ESI): m/z ($[M+Na]^+$) calculated for $C_{32}H_{21}N_5O_7$: 610.1441, found: 610.1327

(13) ***2-amino-4-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-5-oxo-4,5-***

dihydropyrano[3,2-c]chromene-3-carbonitrile (T6): **Ch6** (100.1 mg, 0.25 mmol) and **coumarin-azide** (54.3 mg, 0.25 mmol) were dissolved in 2 mL of DMSO. To this mixture, 8 mL of *t*-BuOH, 4 mL of water, $CuSO_4$ $5H_2O$ (40 mg), and sodium ascorbate (80 mg) were added and stirred at room temperature for 48 hrs. The reaction mixture was then poured in to cold water. The precipitated solid was collected, washed with water and dried. The dried product was washed with diethyl ether (5 mL) to afford **T6**: 115 mg (Yield 74%) as Yellow solid, M.P.: 202°C. IR absorptions (KBr, ν_{max}/cm^{-1}): 3558, 3440, 3362, 3085, 3028, 2929, 2612, 2220, 1728, 1695, 1563, 1509, 1455, 1420, 1376, 1338, 1269. ^{1}H NMR δH(400 MHz, DMSO, δ ppm): 3.808 (3H, s, -OCH_3), 4.398 (1H, s, Ar-OH), 4.643 (2H, s, ArN-CH_2), 4.953 (2H, s, Ar-O-CH_2), 5.446 (1H, s, Ar-OH), 6.138 (1H, s, Ar-OH), 7.238-7.308 (3H, t, Ar-H), 7.552-7.646 (4H, m, Ar-H), 7.886-7.901 (2H, d, J=6Hz, Ar-H), 8.257 (2H, s, Ar-NH_2), 8.329 (2H, s, Ar-H). ^{13}C NMR δC (DMSO, 100MHz, δ ppm): 35.205, 53.552, 55.202, 57.902, 63.563, 73.566, 77.596, 103.610, 106.724, 114.116, 115.494, 119.463, 123.049, 124.028, 127.564, 131.089, 152.353, 154.694, 164.663, 167.305. HRMS (ESI): m/z (M^+) calculated for $C_{33}H_{23}N_5O_8$: 617.1546, found: 617.1519

Cell culture

Cervical cancer (HeLa) cells and were procured from the National Center for Cell Sciences (NCCS), Pune, India. The cancer cells were maintained in Dulbecco's modified eagles medium (DMEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to

1mL/L. The cells were maintained at 37◦C with 5% CO_2 in a humidified CO_2 incubator.

Evaluation of cytotoxicity [MTT-Assay]

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells and were grown (1×10^4 cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37◦C for 4 h. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 minutes to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multi well plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

$$\%\ \text{of viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

Morphological study

The HeLa cells that were grown on cover slips (1×10^5 cells/cover slip) were incubated with disintegrin at the IC50 concentration, and they were then fixed in an ethanol:acetic acid solution (3:1, v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Three monolayers per experimental group were photo micrographed. The morphological changes of

the HeLa cells were analyzed using Nikon (Japan) bright field inverted light microscopy at 40× magnification.

Cell cycle analysis

HeLa cells ($1x10^5$) were seeded in a 6-well plate. After 24-h incubation at 37˚C (5% CO_2), the medium was changed with fresh, supplemented or not (control) with the compound (1, 2 and 4µM). After 24-h incubation, cells were harvested with trypsin, washed by PBS, fixed in 70% ethanol and stored at -20˚C for 1h. The cellular nuclear DNA was stained by propidium iodide (PI) as described, briefly, followed by removing the ethanol, washed with PBS, the cells were suspended in 0.5 ml PBS containing 50 µg/ml PI and 100 µg/ml RNase and incubated at 37˚C for 30 min. Flow cytometry was performed in duplicate with a BD FACS flow cytometer. From each sample 10,000 events were collected and fluorescent signal intensity was recorded and analyzed by CellQuest and Modifit.

Western Blot Analysis

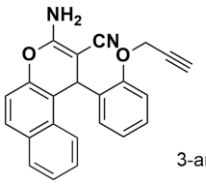
Western blotting was performed to detect the proteins of CDK-2 and CDK4. The HeLa cells (1×10^6) were seeded into 100-mm culture dishes in the presence or absence of compound **T2** and compound **T5** were treated for 48 h. Cells were then washed twice with ice-cold PBS and incubated in lysis buffer. The lysates were centrifuged at 10,000 × g for 5 min at 4 ◦C, and were used as the cell protein extracts. Each of the extracts was applied to 12% SDS polyacrylamide gel electrophoresis after which the proteins were transferred onto a nitrocellulose membrane, and then blocked for 1 h using 10% skim milk in water. After washing in a PBS containing 0.1% Tween 20 for 3 times, the primary antibodies were added at a v/v ratio of 1:1000. After overnight incubation at 4 ◦C, the primary antibodies were washed away and the

secondary antibodies were added for 1 h incubation at room temperature. Finally, the enhanced chemiluminescence detection reagents were used to develop the signal of the membrane

Spectral Details

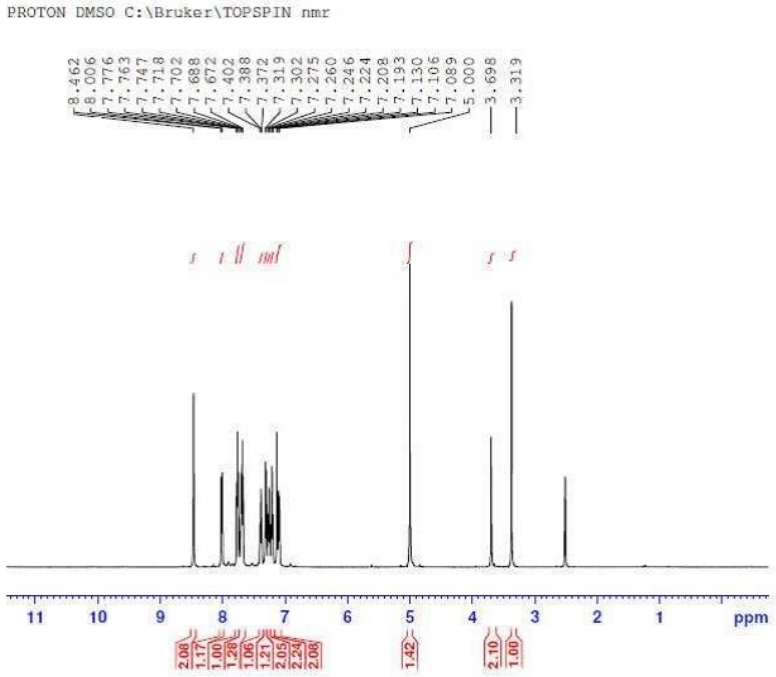
Spectral Details of Chromene-Alkyne 1 (Ch1)

^{1}H-NMR spectra

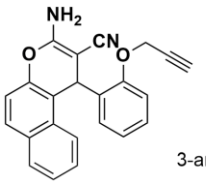


3-amino-1-(2-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{1}H NMR δH(500 MHz, DMSO, δ ppm)**:** 3.319 (1H, s, CH), 3.698 (2H, s, -OCH_2), 5.00 (1H, s, Ar-H), 7.089-7.402 (8H, m, Ar-H), 7.672-8.006 (3H, m, Ar-H), 8.462 (2H, s, Ar-NH_2)

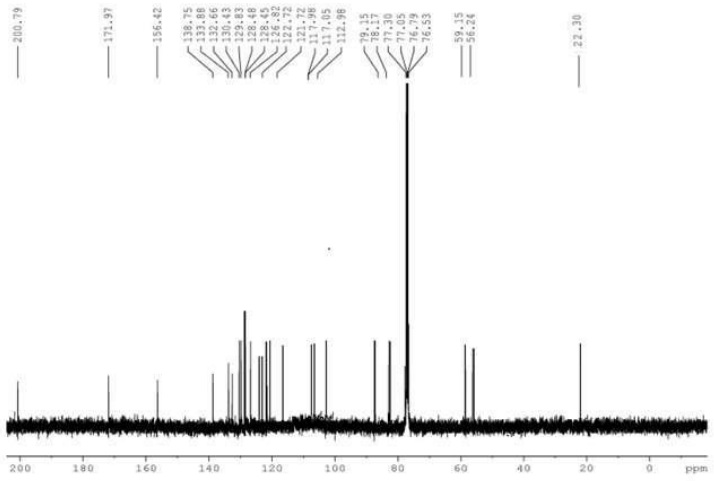


^{13}C-NMR Spectra

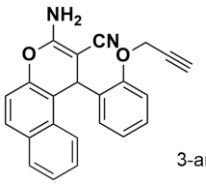


3-amino-1-(2-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{13}C NMR δC(DMSO, 125MHz, δ ppm)**:** 22.30, 56.24, 59.15, 78.17, 79.15, 112.98, 117.05, 117.98, 121.72, 122.72, 126.82, 128.45, 128.48, 129.83, 130.43, 132.66, 133.88, 138.75, 156.42, 171.97, 200.79

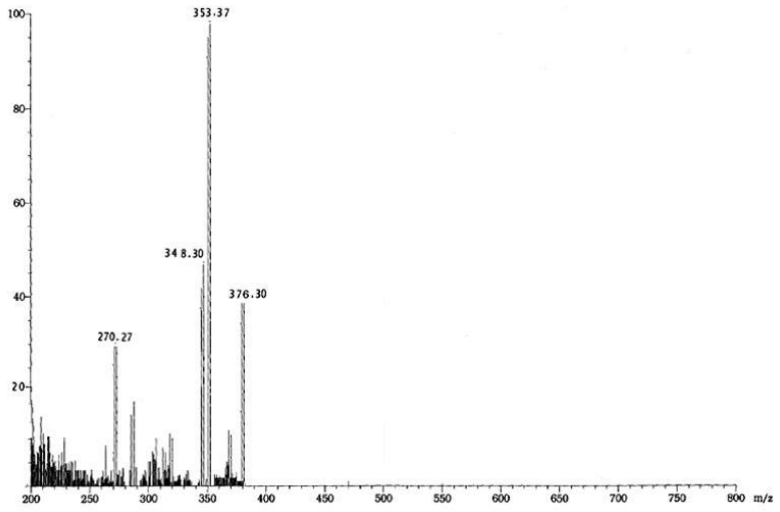


EIMS

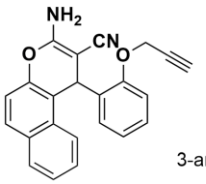


3-amino-1-(2-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

EIMS: m/z ([M+H]$^+$) calculated for $C_{23}H_{16}N_2O_2$: 353.12, found: 353.37

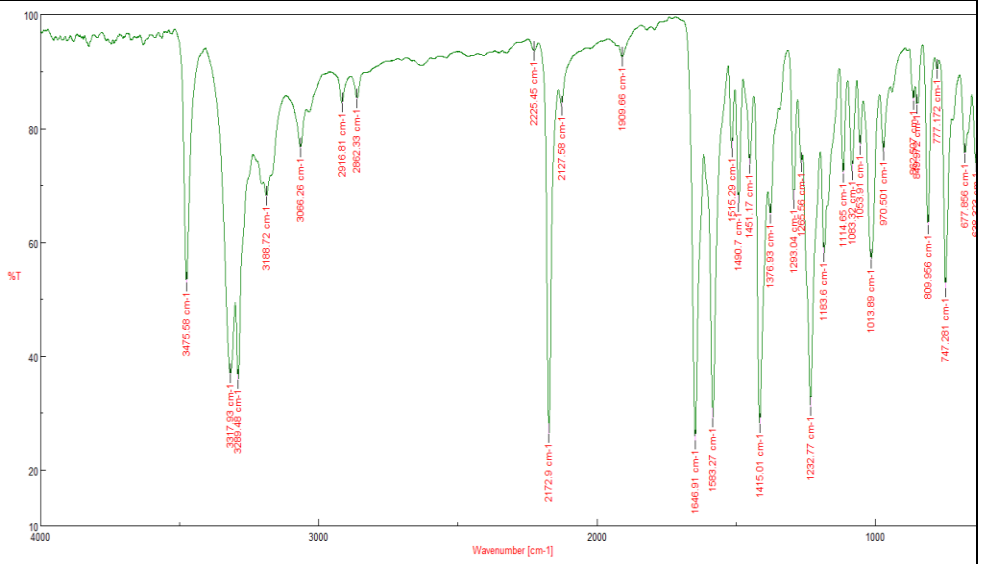


IR Spectra



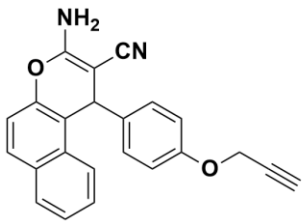
3-amino-1-(2-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3475, 3317, 3299, 3188, 3066, 2916, 2862, 2172, 2127, 1909, 1646, 1583, 1515, 1490, 1451, 1415, 1376, 1293, 1255, 1232, 1183



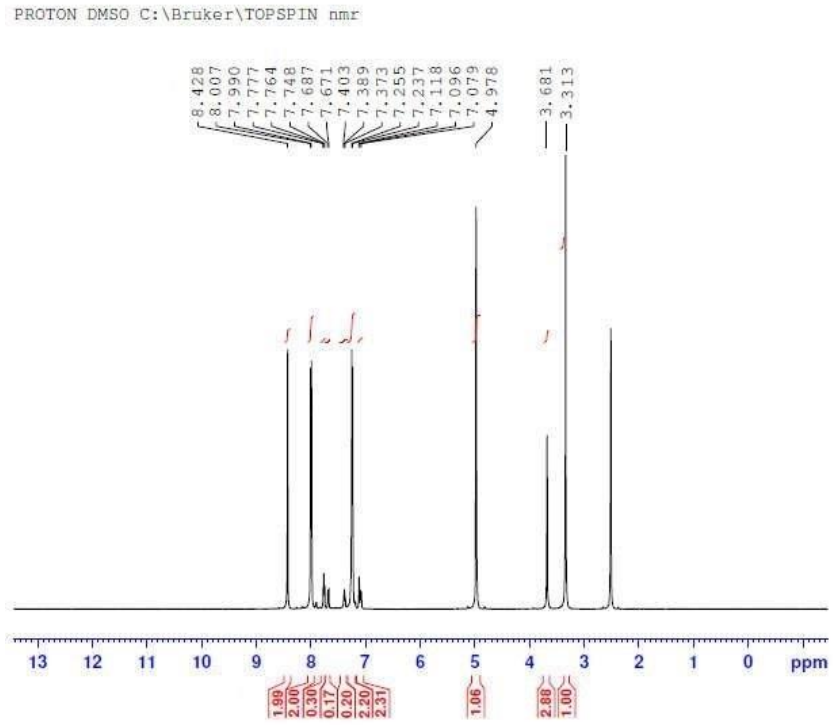
Spectral Details of Chromene-Alkyne 2 (Ch2)

^{1}H-NMR spectra

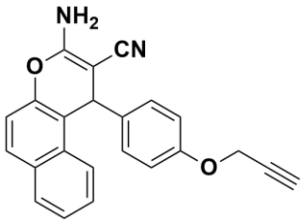


3-amino-1-(4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{1}H NMR δH(400 MHz, DMSO, δ ppm)**:** 3.313 (1H, s, CH), 3.681 (2H, s, Ar-O-CH_2), 4.978 (1H, s, Ar-H), 7.079-7.237 (2H, m, Ar-H), 7.255 (2H, s, Ar-H), 7.373-7.403 (1H, t, Ar-H), 7.671-7.687 (1H, d, 8Hz, Ar-H), 7.748-7.777 (1H, t, Ar-H), 7.990-8.007 (2H, d, J=8Hz, Ar-H), 8.428 (2H, s, Ar-NH_2)

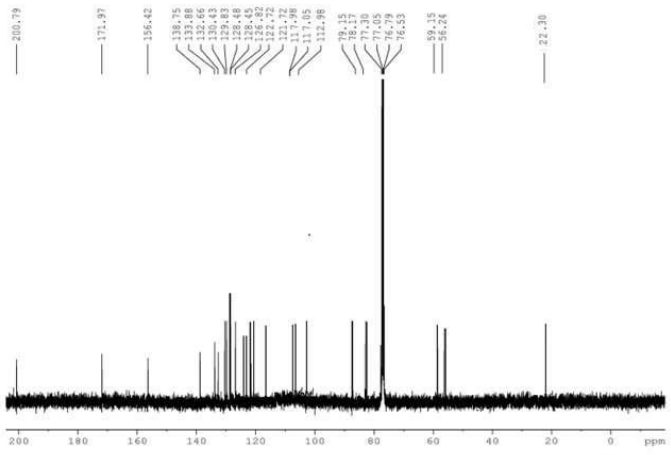


^{13}C-NMR Spectra

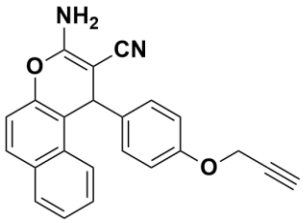


3-amino-1-(4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{13}C NMR δC(DMSO, 100 MHz, δ ppm)**:** 22.38, 56.24, 59.15, 78.17, 79.15, 112.98, 117.05, 117.98, 121.72, 122.72, 126.82, 128.45, 128.48, 129.83, 130.43, 132.66, 133.88, 138.75, 156.42, 171.97, 200.79

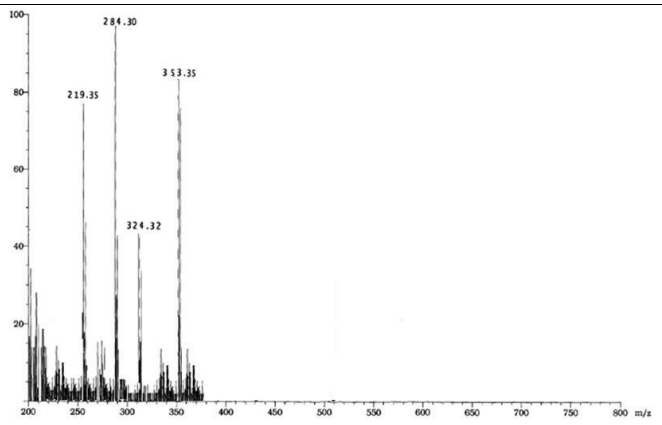


EIMS

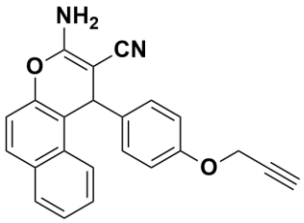


3-amino-1-(4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

EIMS: m/z ([M+H]$^+$) calculated for $C_{23}H_{16}N_2O_2$: 353.12, found: 353.35

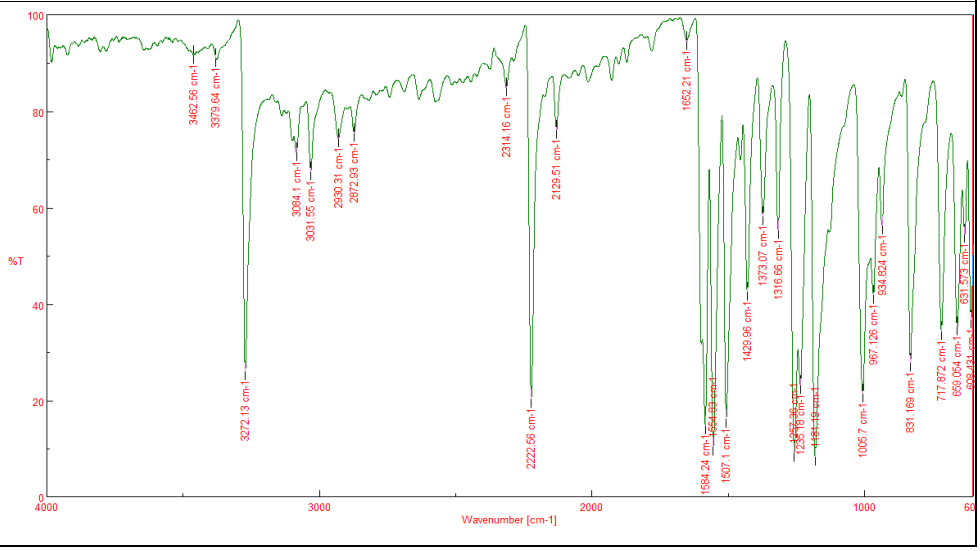


IR Spectra



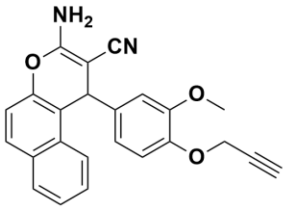
3-amino-1-(4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3462, 3379, 3272, 3084, 3031, 2930, 2972, 2222, 2129, 1652, 1584, 1554, 1507, 1455, 1429, 1373, 1316, 1257, 1235, 1191



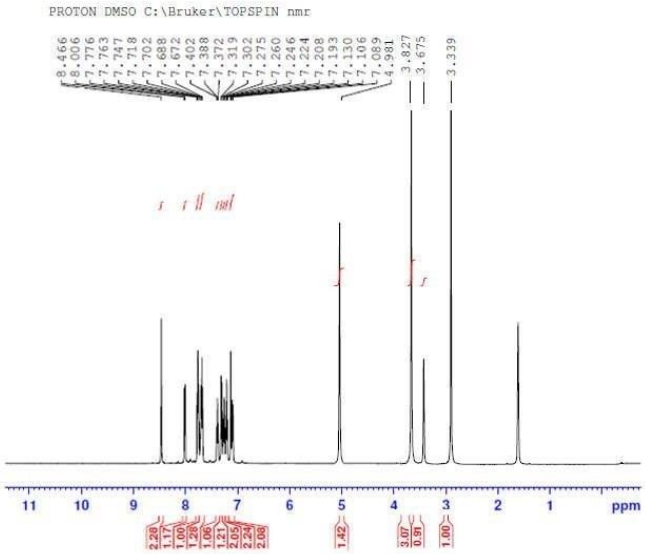
Spectral Details of Chromene-Alkyne 3 (Ch3)

^{1}H-NMR spectra

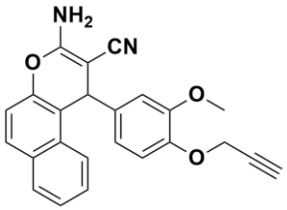


3-amino-1-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{1}H NMR δH(400 MHz, DMSO, δ ppm)**:** 3.339 (1H, s, CH), 3.675 (2H, s, Ar-O-CH_2), 3.827 (3H, s, Ar-O-CH_3), 4.981 (1H, s, Ar-H), 7.089-7.319 (7H, m, Ar-H), 7.372-7.402 (1H, t, Ar-H), 7.672-8.006 (3H, m, Ar-H), 8.466 (2H, s, Ar-NH_2)

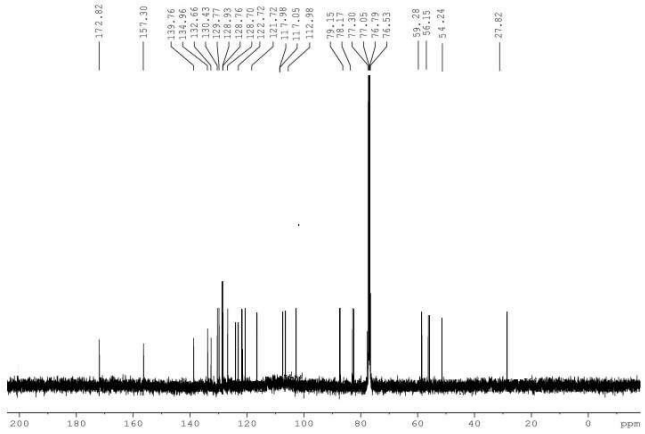


^{13}C-NMR Spectra

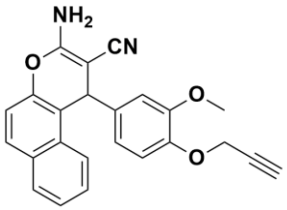


3-amino-1-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 27.82, 54.24, 56.15, 59.28, 78.17, 79.15, 112.98, 117.05, 117.98, 121.72, 122.72, 128.70, 128.76, 128.93, 129.77, 130.43, 132.66, 134.96, 139.76, 157.30, 172.82

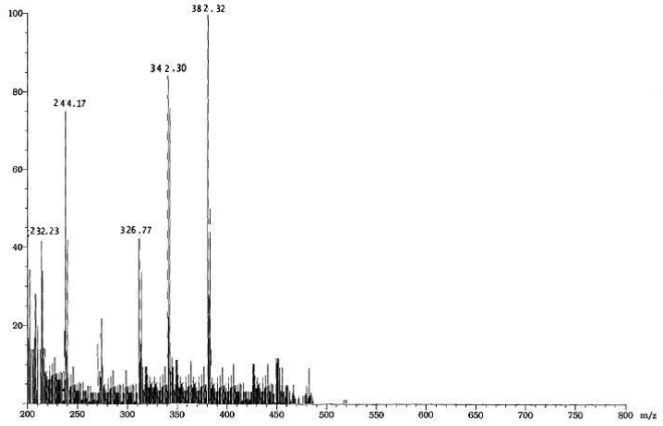


EIMS

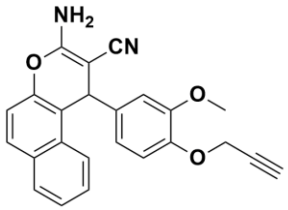


3-amino-1-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-
1*H*-benzo[*f*]chromene-2-carbonitrile

EIMS: m/z ([M+H]$^+$) calculated for $C_{24}H_{18}N_2O_3$: 382.13, found: 382.32

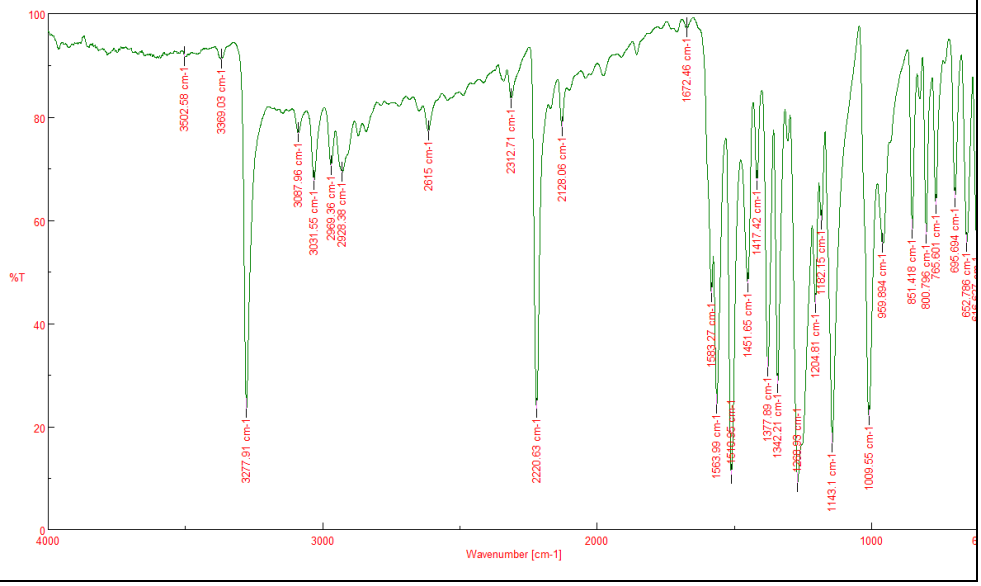


IR Spectra



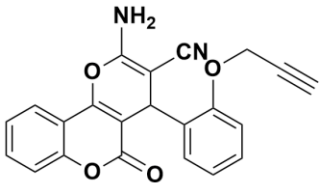
3-amino-1-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3502, 3369, 3277, 3068, 3031, 2969, 2928, 2220, 2128, 1673, 1583, 1563, 1510, 1451, 1417, 1377, 1342, 1305, 1288, 1204, 1182, 1143



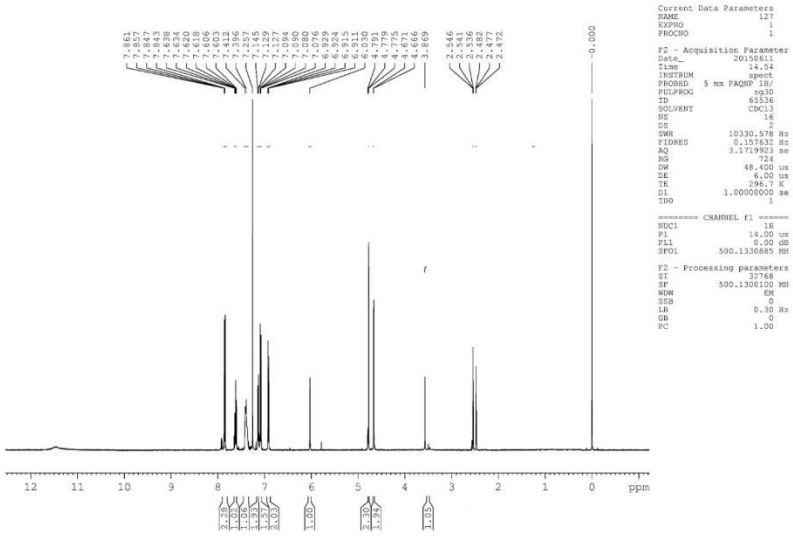
Spectral Details of Chromene-Alkyne 4 (Ch4)

^{1}H-NMR spectra

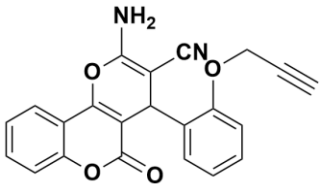


2-amino-5-oxo-4-(2-(prop-2-yn-1-yloxy)phenyl)-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

^{1}H NMR δH(500 MHz, $CDCl_3$, δ ppm)**:** 3.869 (1H, s, CH), 4.668 (1H, s, Ar-H), 4.779 (2H, s, Ar-O-CH_2), 6.030 (1H, s, Ar-H), 6.911-6.929 (2H, m, Ar-H), 7.076-7.145 (2H, m. Ar-H), 7.257 (2H, s, Ar-H), 7.396-7.412 (1H, d, 8Hz, Ar-H), 7.603-7.638 (1H, m, Ar-H), 7.852 (2H, s, Ar-NH_2)

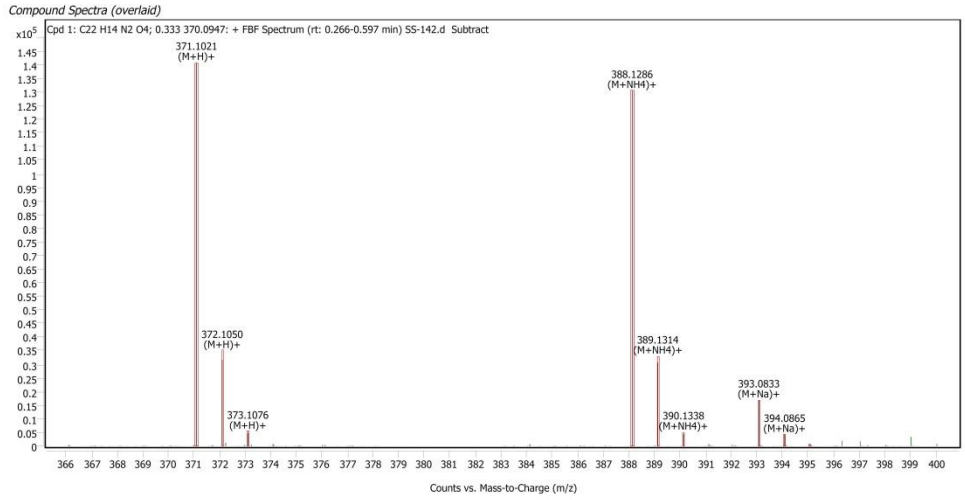


HRMS

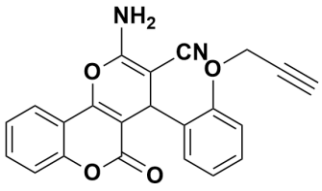


2-amino-5-oxo-4-(2-(prop-2-yn-1-yloxy)phenyl)-
4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

HRMS: m/z ([M+H]$^+$) calculated for $C_{22}H_{14}N_2O_4$: 371.0953, found: 371.1021

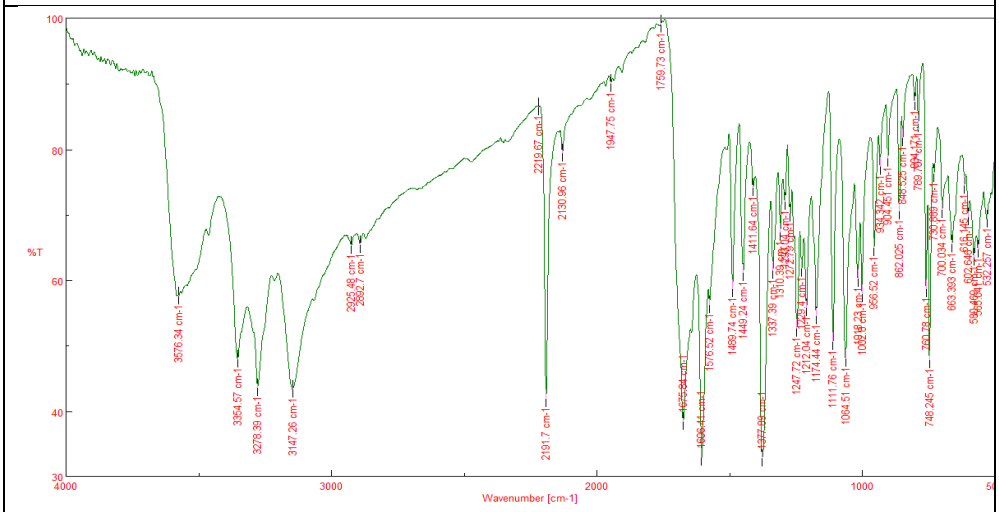


IR Spectra



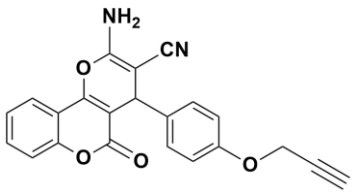
2-amino-5-oxo-4-(2-(prop-2-yn-1-yloxy)phenyl)-
4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3576, 3354, 3278, 3147, 2925, 2892, 2191, 2130, 1675, 1606, 1576, 1489, 1449, 1411, 1377, 1337, 1310, 1272, 1247, 1229, 1212, 1174, 1111, 1064



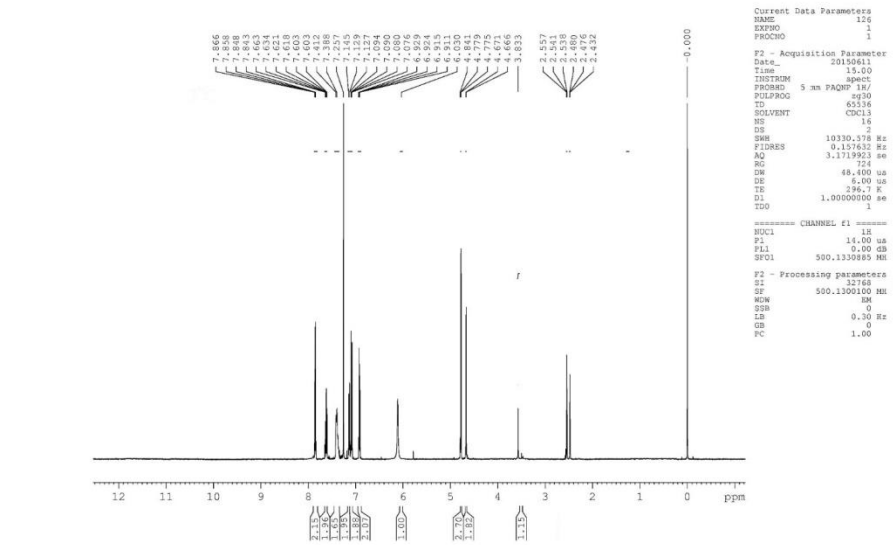
Spectral Details of Chromene-Alkyne 5 (Ch5)

^{1}H-NMR spectra

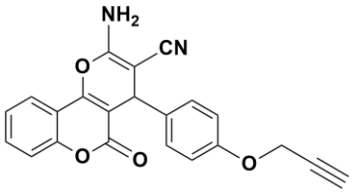


2-amino-5-oxo-4-(4-(prop-2-yn-1-yloxy)phenyl)-
4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

^{1}H NMR δH(500 MHz, $CDCl_3$, δ ppm)**:** 3.833 (1H, s, CH), 4.668 (1H, s, Ar-H), 4.779 (2H, s, Ar-O-CH_2), 6.030 (1H, s, Ar-H), 6.911-6.929 (2H, m, Ar-H), 7.076-7.145 (2H, m, Ar-H), 7.257 (2H, s, Ar-H), 7.388-7.412 (1H, d, 12Hz, Ar-H), 7.603-7.663 (2H, m, Ar-H), 7.853 (2H, s, Ar-NH_2)

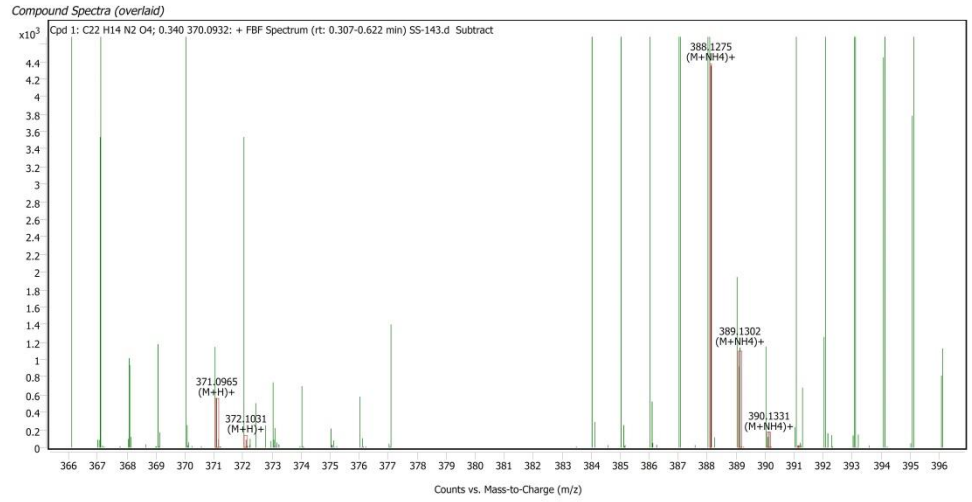


HRMS

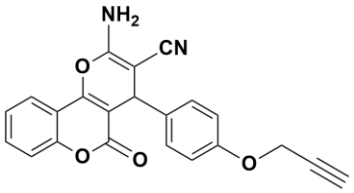


2-amino-5-oxo-4-(4-(prop-2-yn-1-yloxy)phenyl)-
4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

HRMS: m/z ([M+H]$^+$) calculated for $C_{22}H_{14}N_2O_4$: 371.09536, found: 371.0965

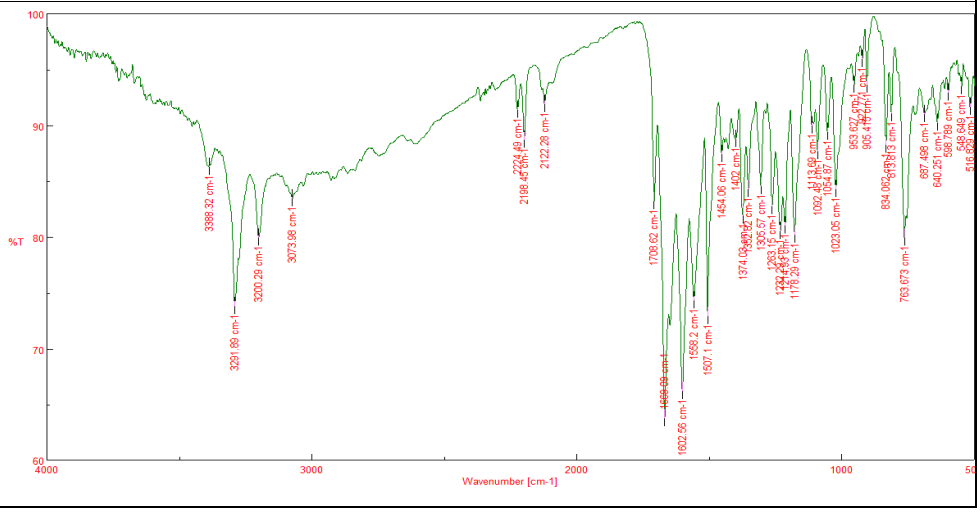


IR Spectra



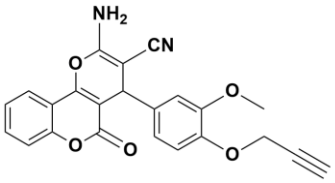
2-amino-5-oxo-4-(4-(prop-2-yn-1-yloxy)phenyl)-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3388, 3291, 3200, 3073, 2224, 2198, 2122, 1708, 1669, 1602, 1558, 1507, 1454, 1402, 1374, 1352, 1305, 1263, 1232, 1214, 1178, 1092



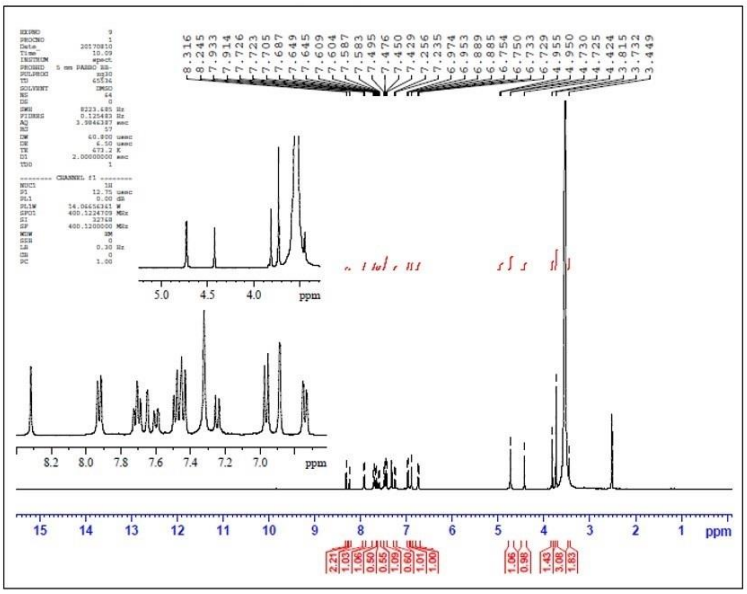
Spectral Details of Chromene-Alkyne 6 (Ch6)

^{1}H-NMR spectra

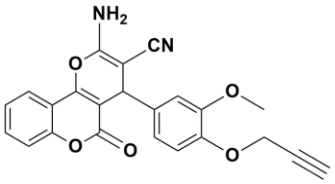


2-amino-4-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-
5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

^{1}H NMR δH(400 MHz, DMSO, δ ppm)**:** 3.449 (2H, s, -O-CH_2), 3.732 (3H, s, Ar-OCH_3), 3.815 (1H, s, CH), 4.424 (1H, s, Ar-H), 6.729-6.974 (3H, m, Ar-H), 7.235-7.256 (1H, d, 10Hz, Ar-H), 7.429-7.495 (1H, m, Ar-H), 7.583-7.933 (1H, m, Ar-H), 8.316 (2H, s, Ar-NH_2)

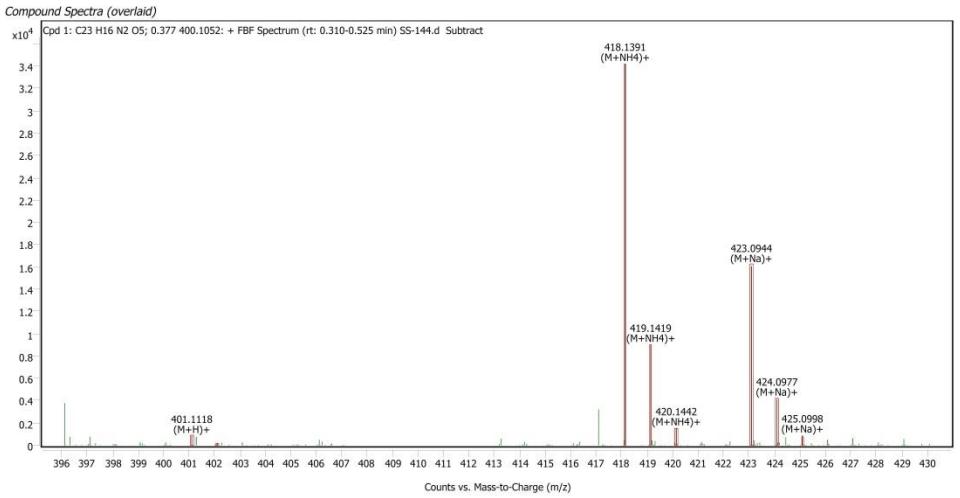


HRMS

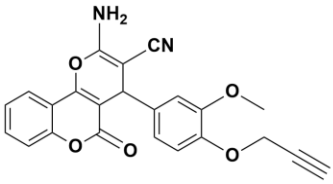


2-amino-4-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-
5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

HRMS: m/z ([M+H]$^+$) calculated for $C_{23}H_{16}N_2O_5$: 401.1059, found: 401.1118

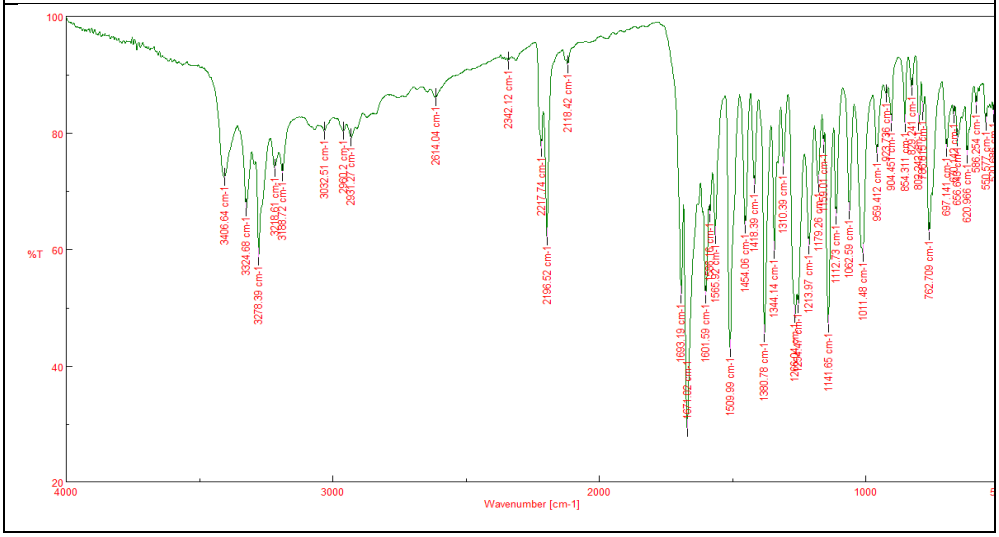


IR Spectra



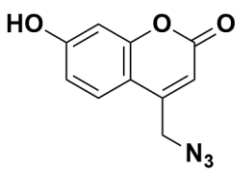
2-amino-4-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-
5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3406, 3324, 3278, 3032, 2960, 2931, 2217, 2196, 2118, 1693, 1671, 1601, 1588, 1565, 1509, 1454, 1418, 1380, 1344, 1310, 1266, 1213, 1179, 1141, 1112, 1062, 1011



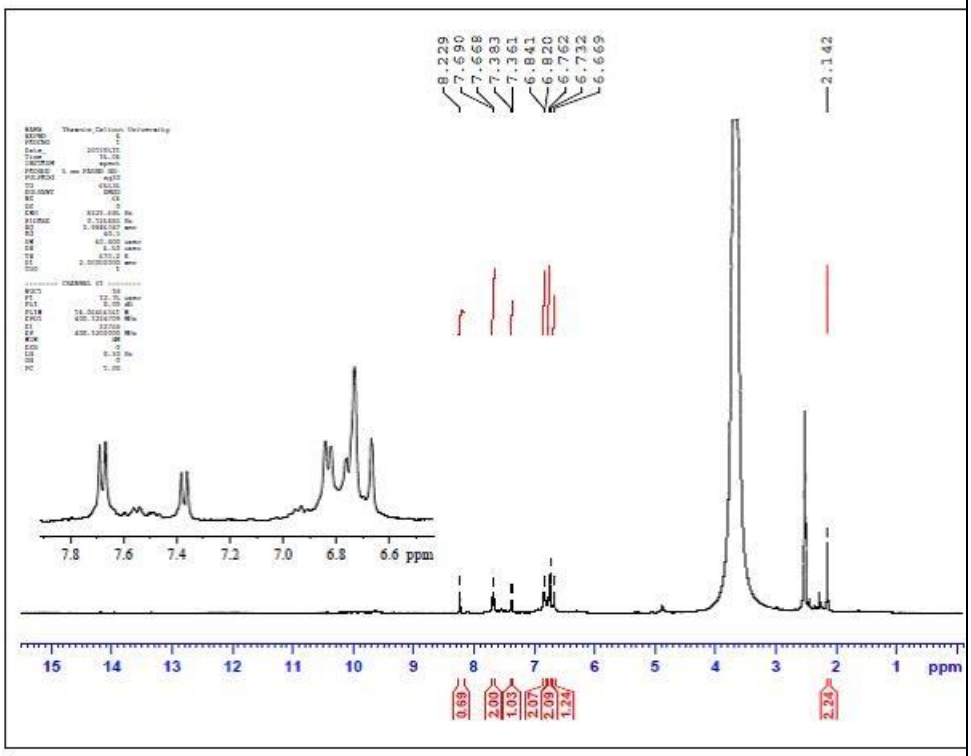
Spectral Details of 4-azidomethylumbelliferone (Coumarin-azide)

^{1}H-NMR spectra

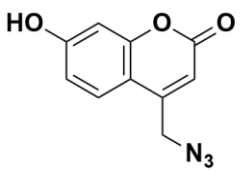


4-(azidomethyl)-7-hydroxy-2*H*-chromen-2-one

^{1}H NMR δH (400 MHz, DMSO, δ ppm)**:** 2.142 (2H, s, CH_2), 6.669 (1H, s, Ar-H), 6. 732-6.762 (1H, d, J=12Hz, Ar-H), 6.820-6.841 (1H, d, J=8Hz, Ar-H), 7.361-7.383 (1H, d, J=9Hz, Ar-H), 7.668-7.690 (1H, d, J=9Hz, Ar-H), 8.229 (1H, s, Ar-OH)

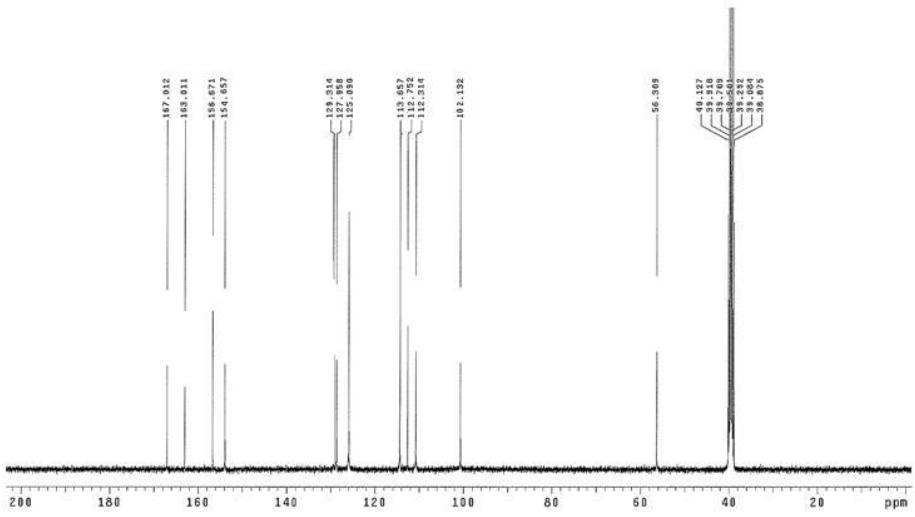


^{13}C-NMR Spectra

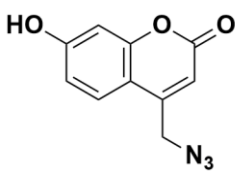


4-(azidomethyl)-7-hydroxy-2*H*-chromen-2-one

^{13}C NMR δC (DMSO, 100MHz, δ ppm)**:** 167.012, 163.011, 156.671, 154.657, 129.314, 127.956, 125.010, 113.657, 112.752, 112.314, 102.132, 56.303

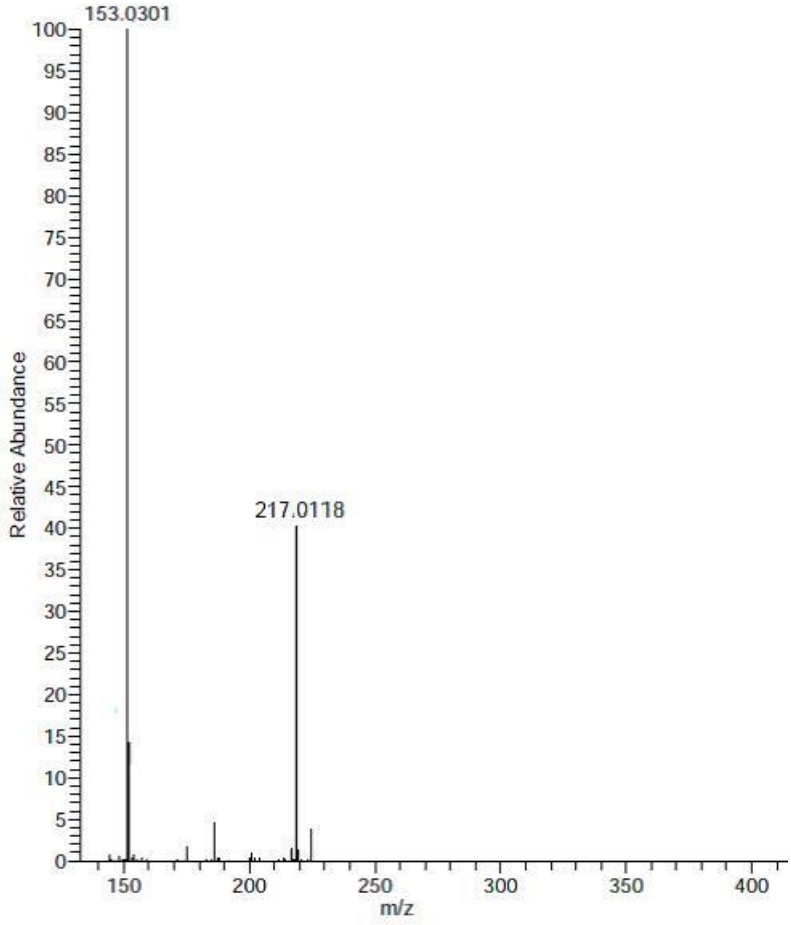


HRMS

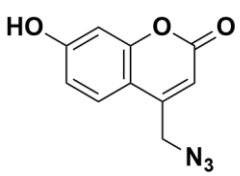


4-(azidomethyl)-7-hydroxy-2*H*-chromen-2-one

HRMS: m/z (M^+) calculated for $C_{10}H_7N_3O_3$: 217.0487, found: 217.0118

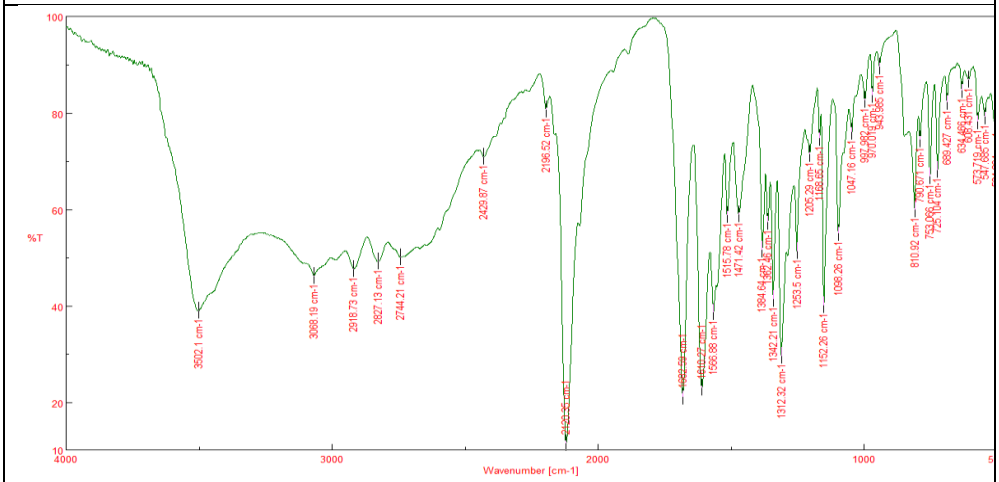


IR Spectra



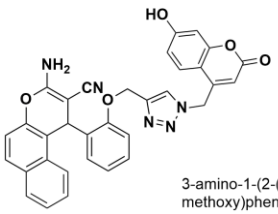
4-(azidomethyl)-7-hydroxy-2*H*-chromen-2-one

IR absorptions (KBr, ν_{max}/cm^{-1}): 3502, 3068, 2918, 2827, 2744, 2196, 2120, 1682, 1610, 1566, 1515, 1471, 1334, 1362, 1342, 1312, 1253, 1205, 1152, 1098



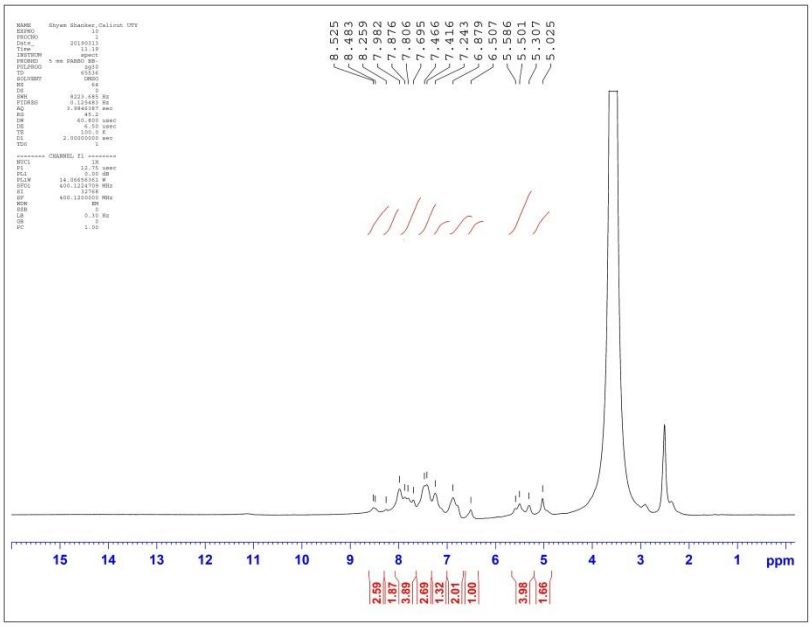
Spectral Details of **T1**:

[1]H-NMR spectra

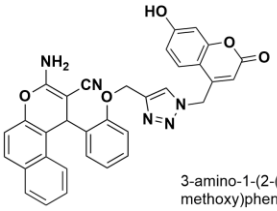


3-amino-1-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

[1]H NMR δH(400 MHz, DMSO, δ ppm)**:** 5.025 (1H, s, Ar-OH), 5.307 (1H, s, Ar-H), 5.501 (2H, s, ArN-CH_2), 5.586 (2H, s, Ar-O-CH_2), 6.507 (1H, s, Ar-H), 6.879 (2H, s, Ar-H), 7.243 (1H, s, Ar-H), 7.416-7.466 (3H, d, J=20Hz, Ar-H), 7.695-7.982 (4H, m, Ar-H), 8.259 (2H, s, Ar-NH_2), 8.483-8.525 (3H, d, J=16.8Hz, Ar-H)

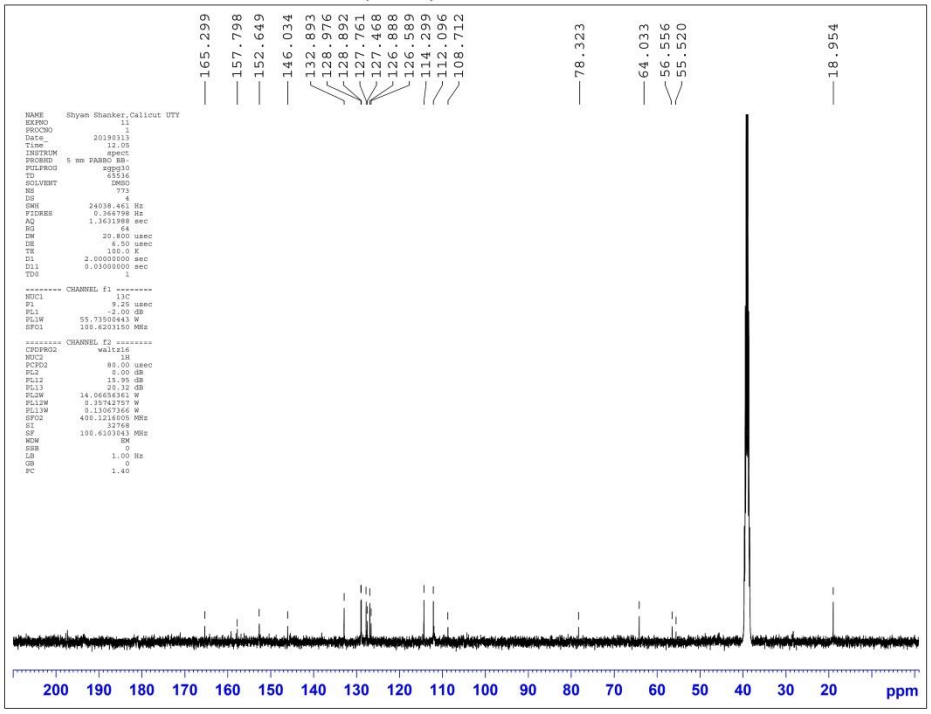


^{13}C-NMR Spectra

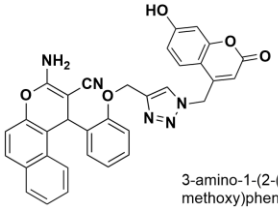


3-amino-1-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 18.954, 55.520, 56.556, 64.033, 78.323, 108.712, 112.096, 114.299, 126.589, 126.888, 127.468, 127.761, 128.892, 128.976, 132.893, 146.034, 152.649, 157.798, 165.299

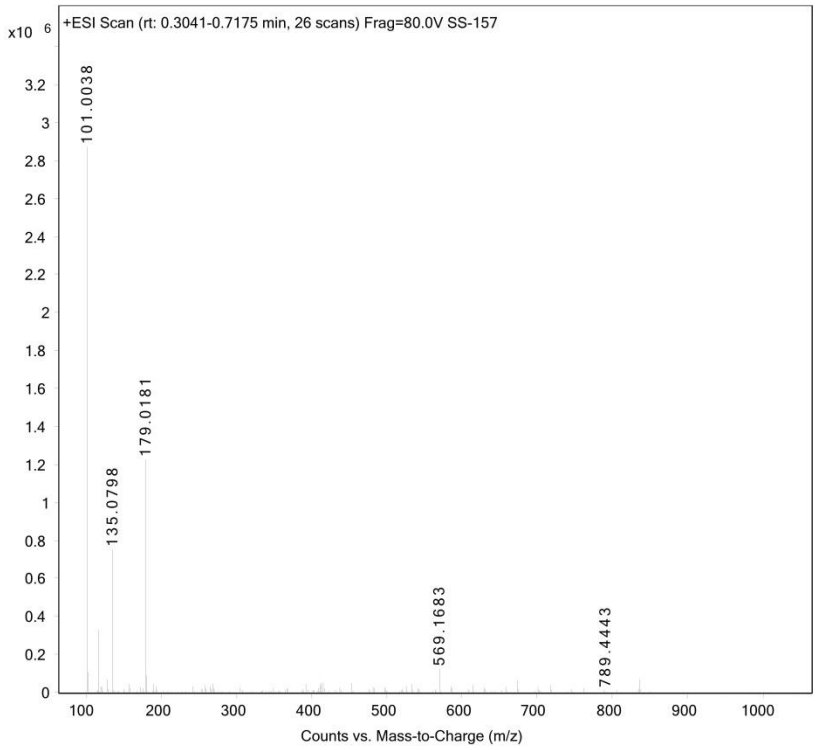


HRMS

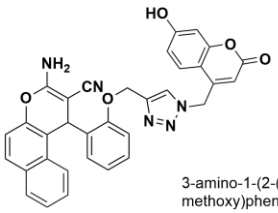


3-amino-1-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

HRMS(ESI): m/z (M^+) calculated for $C_{33}H_{23}N_5O_5$: 569.1699, found: 569.1683

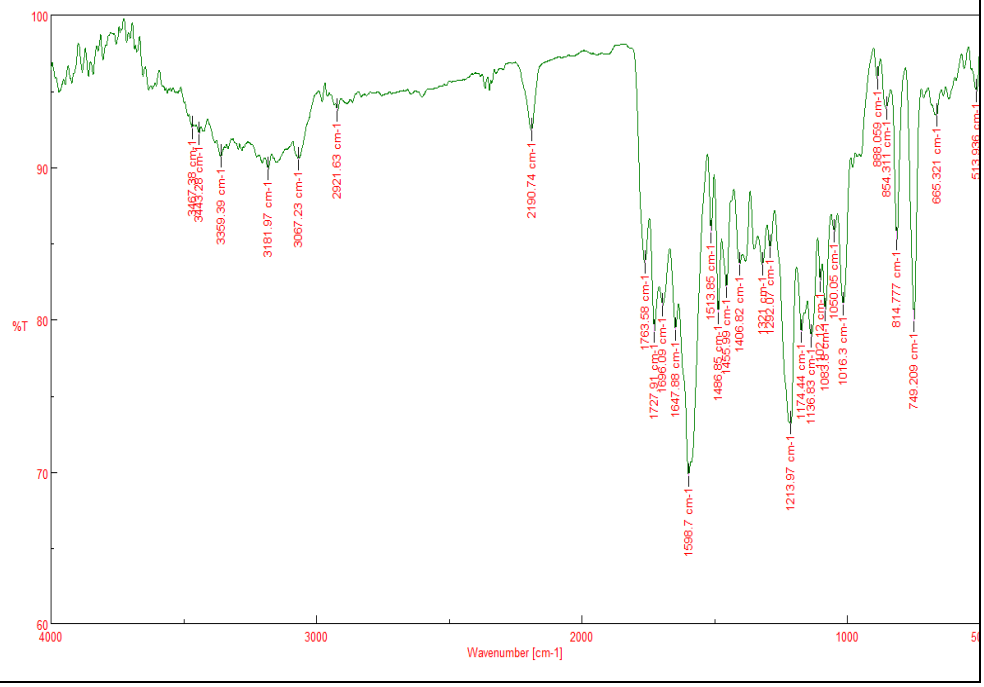


IR Spectra



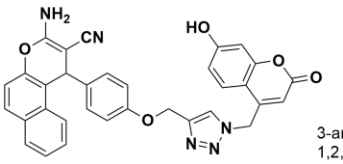
3-amino-1-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3467, 3443, 3359, 3181, 3067, 2921, 2190, 1763, 1727, 1696, 1647, 1598, 1513, 1486, 1455, 1406, 1321, 1292, 1213, 1174, 1136, 1083, 1016



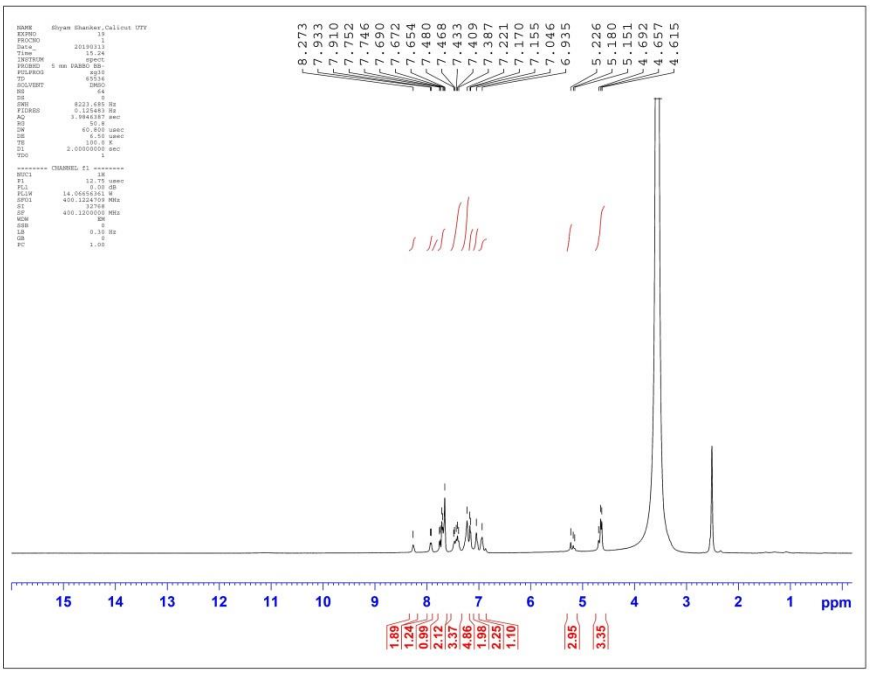
Spectral Details of **T2**:

[1]H-NMR

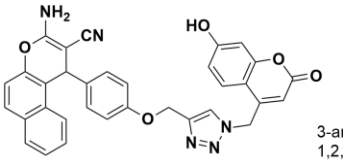


3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

[1]H NMR δH(400 MHz, DMSO, δ ppm)**:** 4.615-4.692(3H, m, Ar-H, ArN-CH_2), 5.151-5.226 (3H, m, Ar-OH, Ar-O-CH_2), 6.935 (1H, s, Ar-H), 7.045 (2H, s, Ar-H), 7.155-7.221 (6H, t, Ar-H), 7.387-7.480 (3H, m, Ar-H), 7.654-7.752 (3H, m, Ar-H), 7.910-7.933 (1H, d, J=9Hz, Ar-H), 8.273 (2H, s, Ar-NH_2)

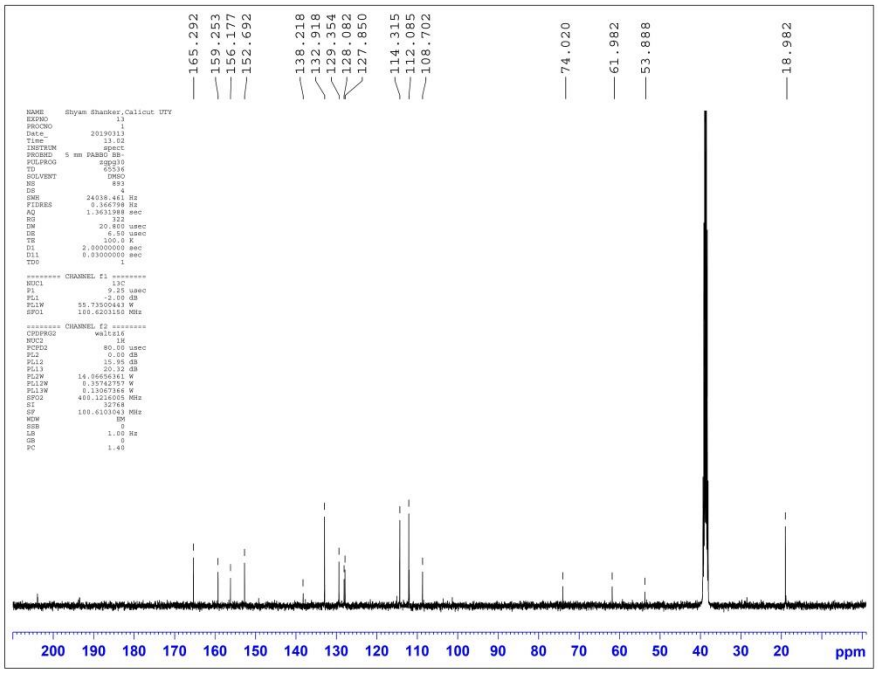


^{13}C-NMAR Spectra

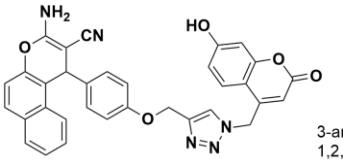


3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 18.982, 53.888, 61.982, 74.020, 108.702, 112.085, 114.315, 127.850, 128.082, 129.354, 132.918, 138.218, 152.692, 156.177, 159.253, 165.292

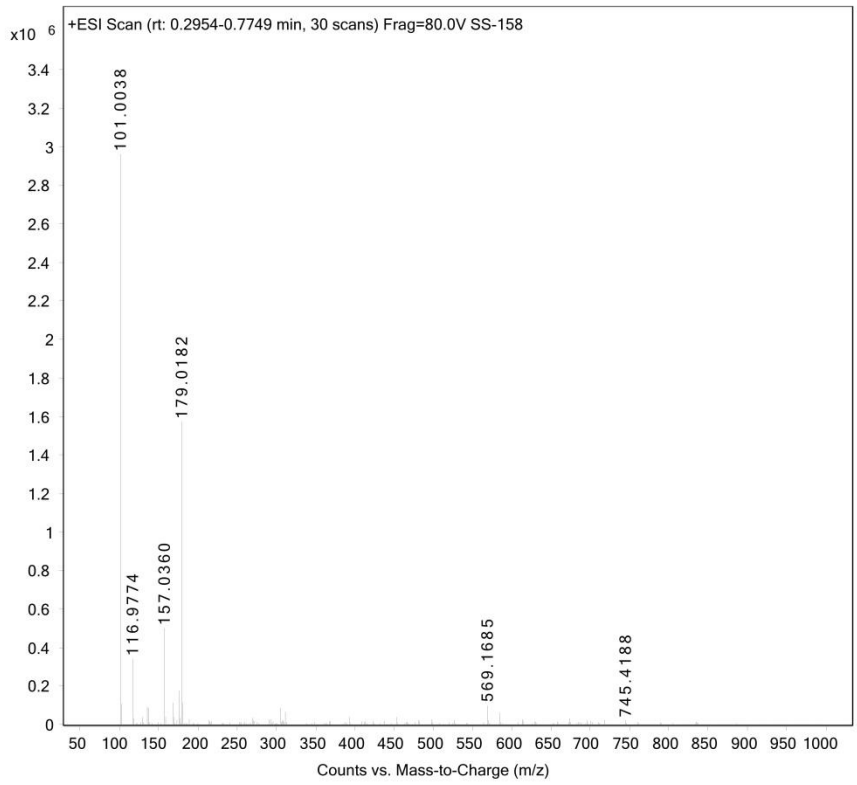


HRMS

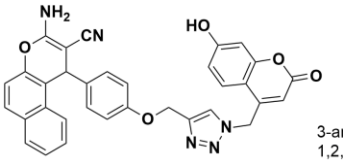


3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

HRMS(ESI): m/z (M^+) calculated for $C_{33}H_{23}N_5O_5$: 569.1699, found: 569.1685

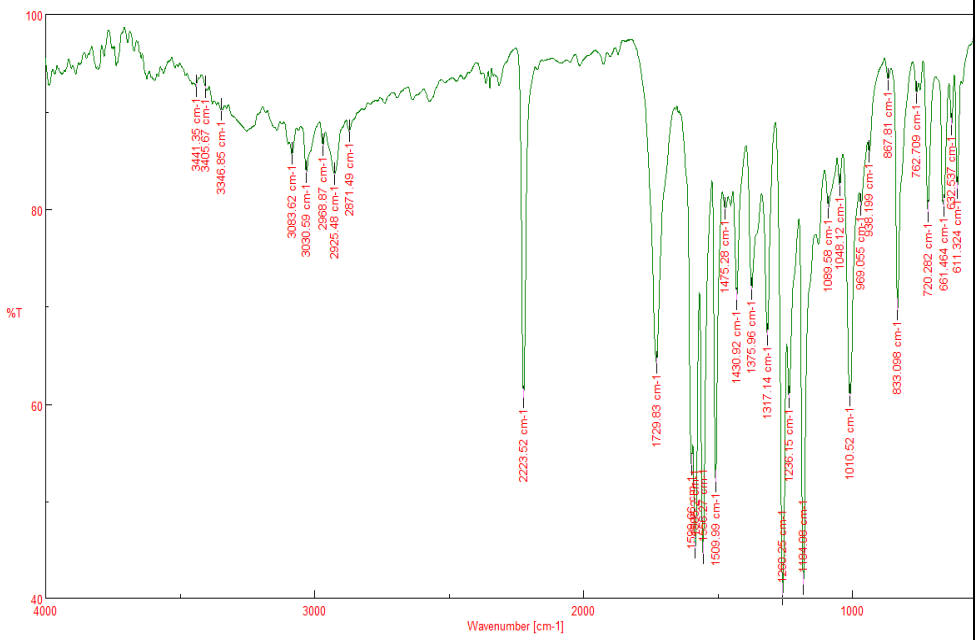


IR Spectra



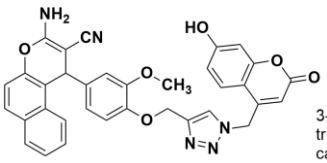
3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3441, 3405, 3346, 3083, 3030, 2925, 2223, 1729, 1598, 1585, 1509, 1475, 1430, 1375, 1317, 1260, 1236, 1184, 1089, 1048, 1010



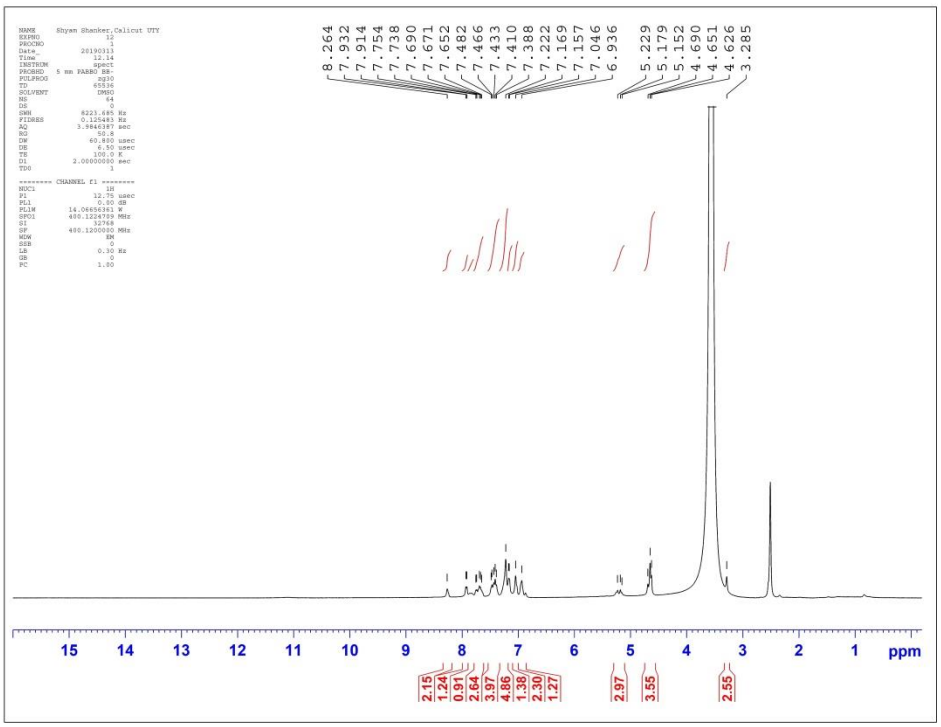
Spectral Details of **T3**:

[1]H-NMR Spectra

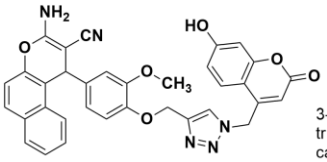


3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

[1]H NMR δH(400 MHz, DMSO, δ ppm)**:** 3.285 (3H, s, -O-CH3), 4.626-4.690 (3H, m, Ar-H, ArN-CH_2), 5.152-5.229 (3H, m, Ar-OH, Ar-O-CH_2), 6.936 (1H, s, Ar-H), 7.046 (2H, s, Ar-H), 7.157-7.222 (4H, t, Ar-H), 7.388-7.482 (3H, m, Ar-H), 7.652-7.754 (3H,m, Ar-H), 7.914-7.932 (1H, d, J=7Hz, Ar-H), 8.264 (2H, s, Ar-NH_2)

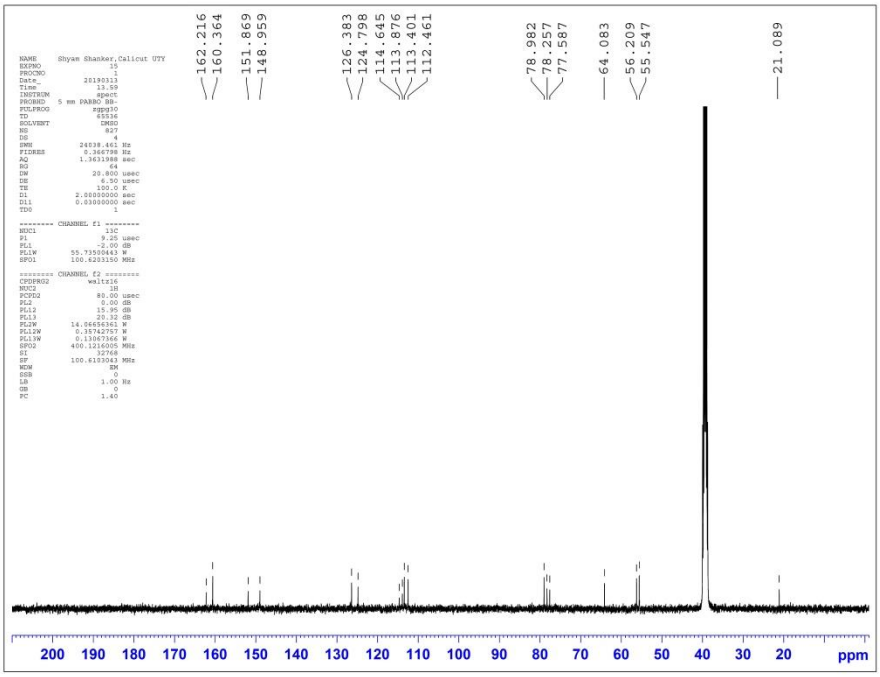


^{13}C-NMR Spectra

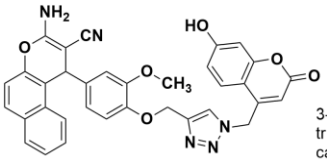


3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 21.089, 55.547, 56.209, 64.083, 77.587, 112.461, 113.401, 113.876, 114.645, 124.798, 126.383, 148.959, 151.869, 160.364, 162.216

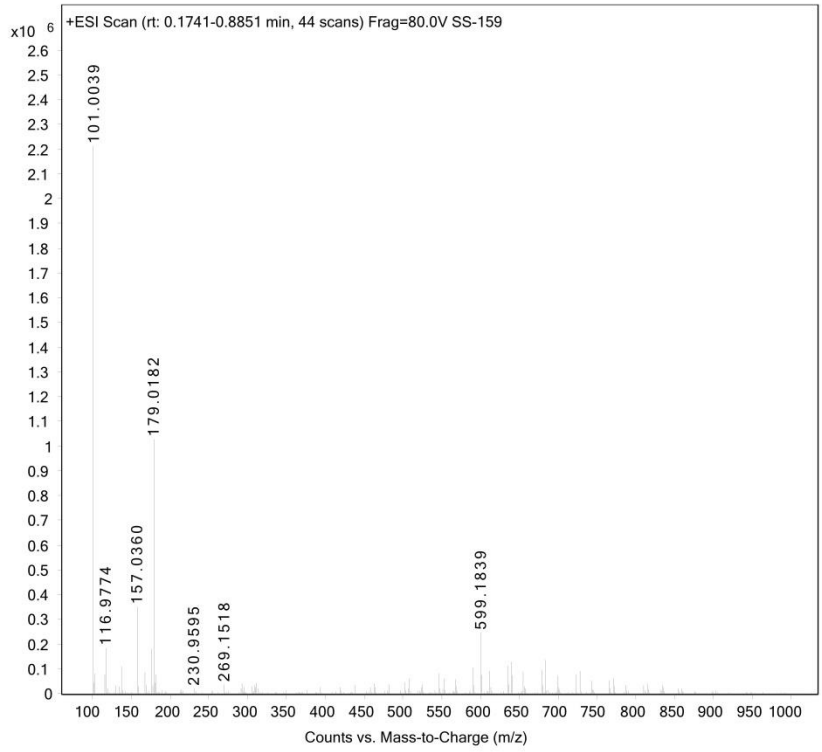


HRMS

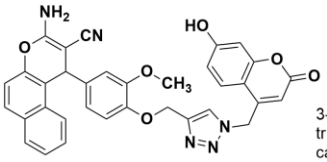


3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

HRMS(ESI): m/z (M^+) calculated for $C_{34}H_{25}N_5O_6$: 599.1804, found: 599.1839

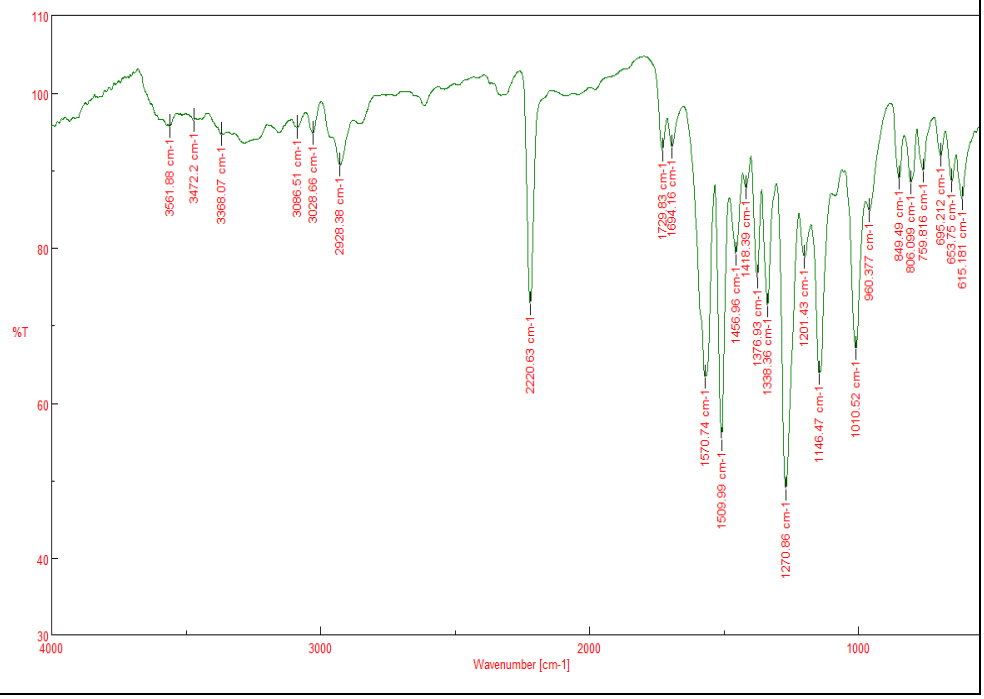


IR Spectra



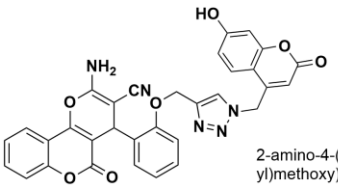
3-amino-1-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3561, 3472, 3368, 3086, 3028, 2928, 2220, 1729, 1694, 1570, 1509, 1456, 1418, 1376, 1338, 1270, 1201, 1146, 1010



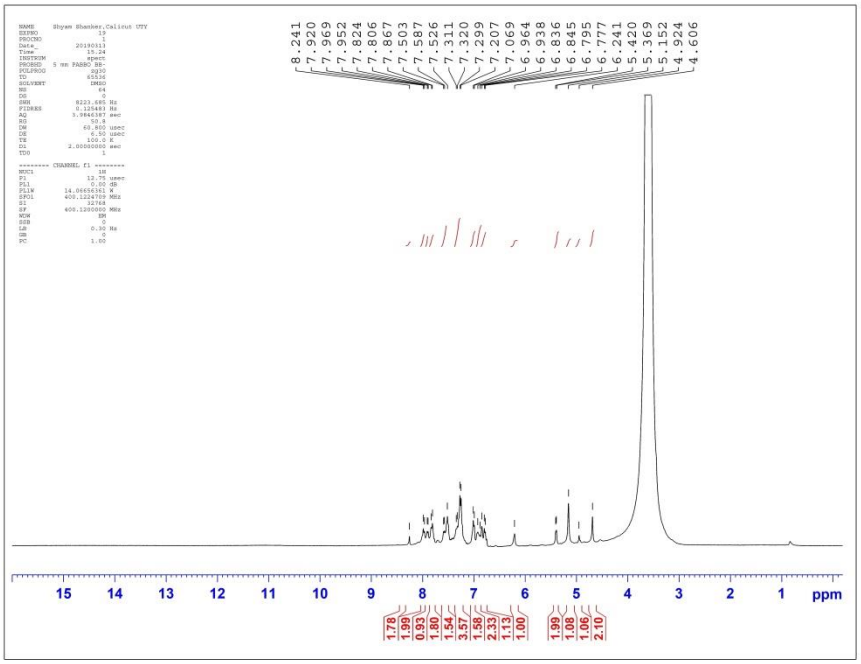
Spectral Details of **T4**:

[1]H-NMR Spectra

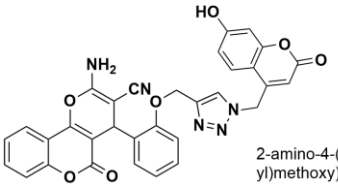


2-amino-4-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

^{1}H NMR δH(400 MHz, DMSO, δ ppm)**:** 4.606 (2H, s, ArN-CH_2), 4.924 (1H, s, Ar-H), 5.152 (1H, s, Ar-OH), 5.369-5.420 (2H, d, J=20.4Hz, Ar-O-CH_2), 6.241 (1H, s, Ar-H), 6.777-7.069 (3H, m, Ar-H), 7.207-7.311 (3H, m, Ar-H), 7.526-7.503 (1H, t, Ar-H), 7.867-7.920 (5H, m, Ar-H), 8.241 (2H, s, Ar-NH_2)

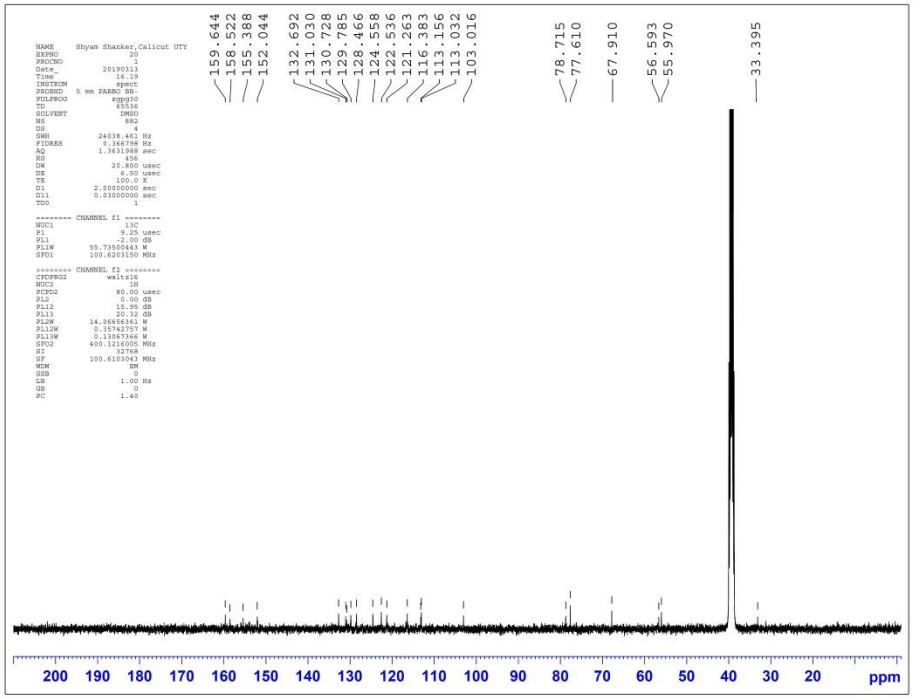


^{13}C-NMR Spectra

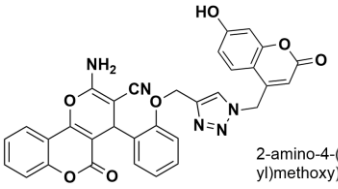


2-amino-4-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 33.395, 55.970, 56.593, 67.910, 77.610, 78.715, 103.016, 113.032, 113.156, 116.383, 121.263, 122.536, 124.558, 128.466, 129.785, 130.728, 131.030, 132.692, 152.044, 155.388, 158.522, 159.644

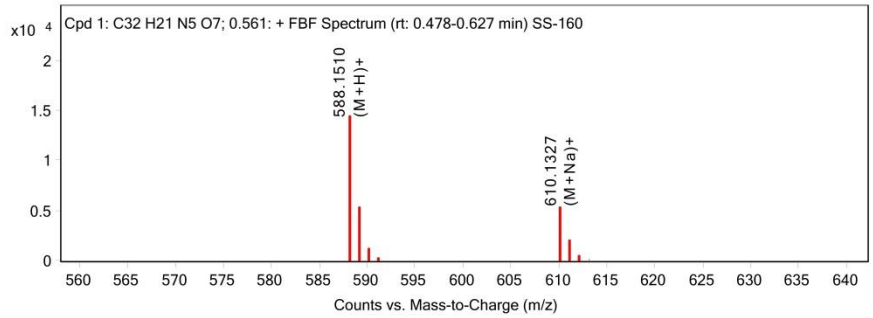


HRMS



2-amino-4-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

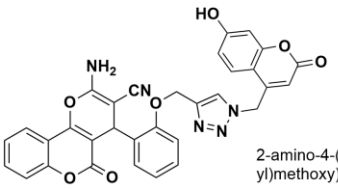
HRMS(ESI): m/z ([M+H]$^+$) calculated for $C_{32}H_{21}N_5O_7$: 588.1441, found: 588.1510



MS Spectrum Peak List

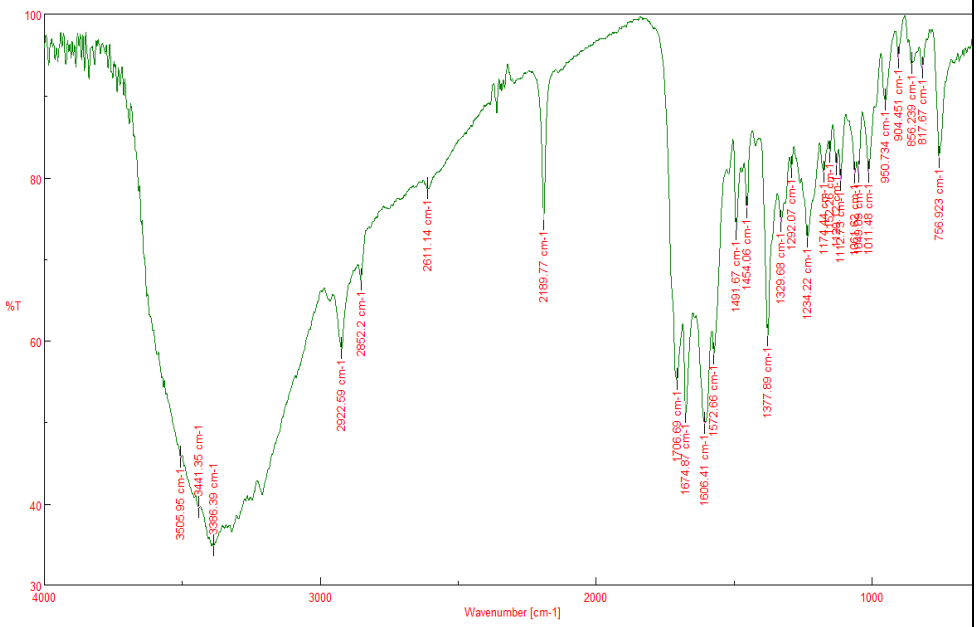
m/z	z	Abund	Ion
588.151	1	14450.09	(M+H)+
589.1536	1	5098.05	(M+H)+
590.1563	1	1088.4	(M+H)+
591.1611	1	148.06	(M+H)+
610.1327	1	5104.92	(M+Na)+
611.136	1	2031.71	(M+Na)+
612.1366	1	536.34	(M+Na)+

IR Spectra



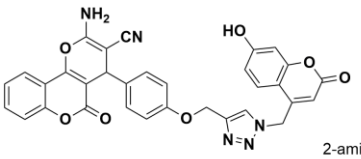
2-amino-4-(2-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3505, 3441, 3386, 2922, 2952, 2611, 2189, 1706, 1674, 1606, 1572, 1491, 1454, 1377, 1329, 1234, 1174



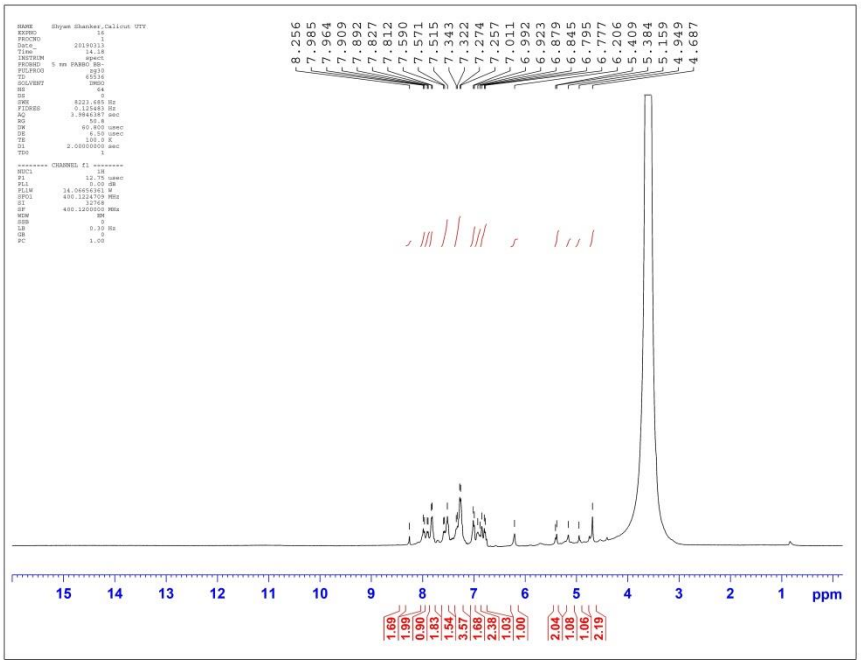
Spectral Details of **T5**:

[1]H-NMR Spectra

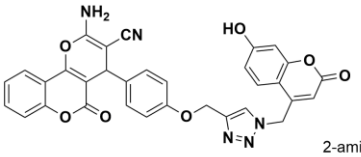


2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

[1]H NMR δH(400 MHz, DMSO, δ ppm)**:** 4.687 (2H, s, ArN-CH_2), 4.949 (1H, s, Ar-H), 5.159 (1H, s, Ar-OH), 5.384-5.409 (2H, d, J=10Hz, Ar-O-CH_2), 6.206 (1H, s, Ar-H), 6.777-7.011 (4H, m, Ar-H), 7.257-7.343 (3H, m, Ar-H), 7.515-7.590 (1H, t, Ar-H), 7.812-7.985 (5H, m, Ar-H), 8.256 (2H, s, Ar-NH_2)

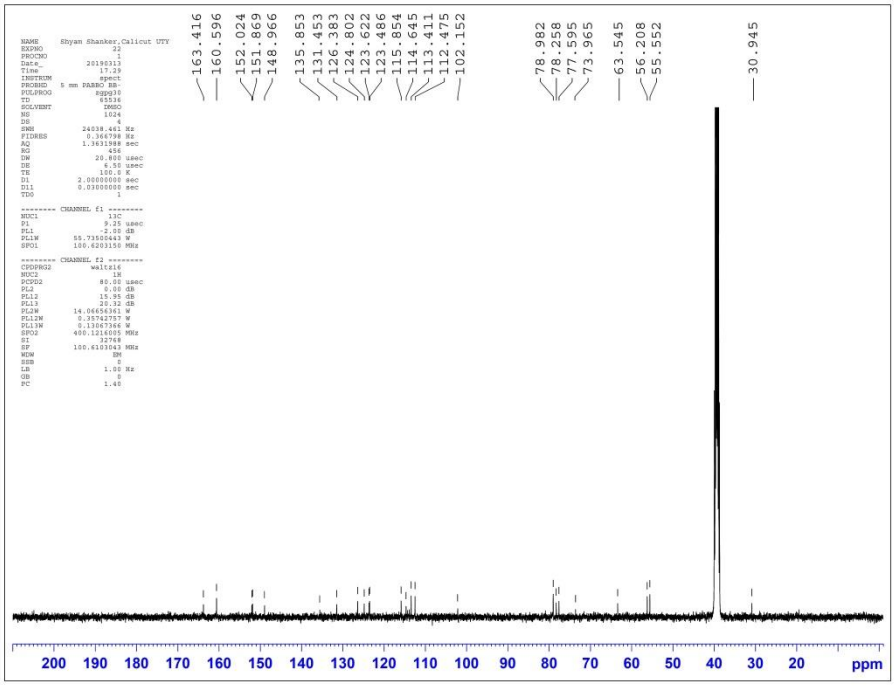


^{13}C-NMR Spectra

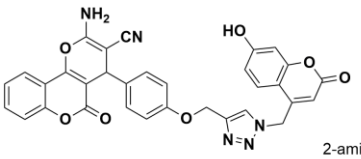


2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 30.945, 55.552, 56.208, 73.965, 77.595, 102.152, 112.475, 113.411, 114.645, 115.854, 123.486, 123.622, 124.802, 126.383, 131.453, 135.853, 148.966, 151.869, 152.024, 160.596, 163.416

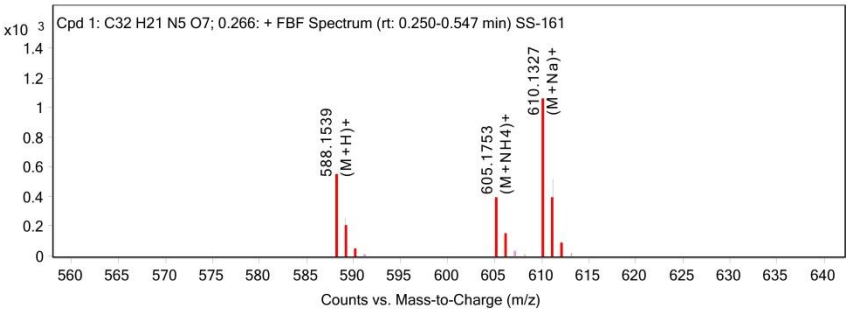


HRMS



2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

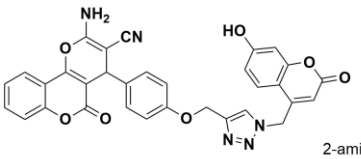
HRMS(ESI): m/z ([M+Na]$^+$) calculated for $C_{32}H_{21}N_5O_7$: 610.1441, found: 610.1327



MS Spectrum Peak List

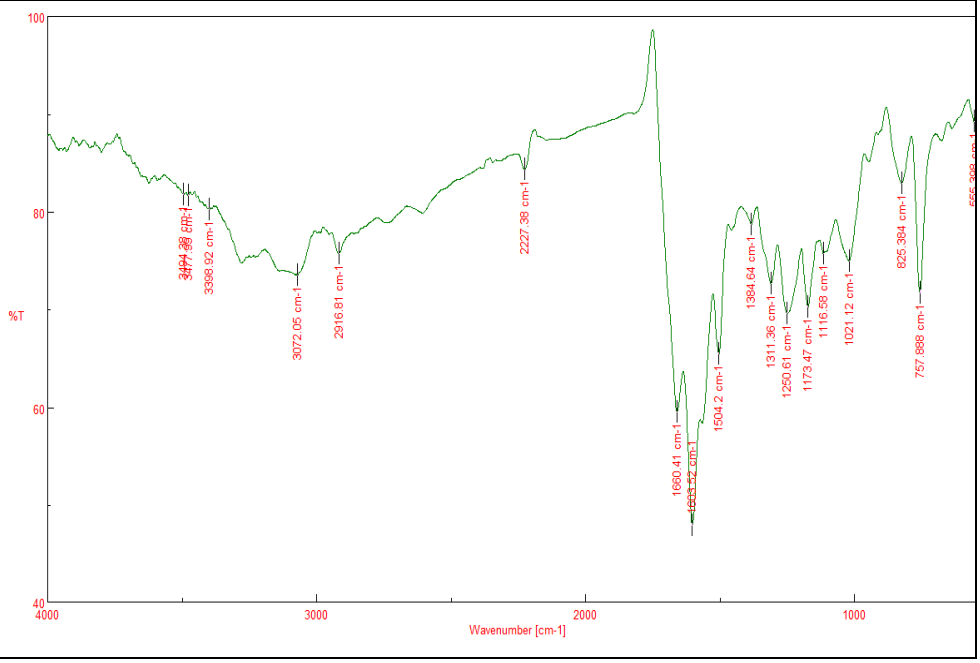
m/z	z	Abund	Ion
588.1539	1	516.73	(M+H)+
589.1548	1	253.31	(M+H)+
590.1597	1	23.36	(M+H)+
605.1753	1	393.75	(M+NH4)+
606.1803	1	114.54	(M+NH4)+
610.1327	1	962.47	(M+Na)+
611.1348	1	521.56	(M+Na)+
612.1346	1	46.68	(M+Na)+

IR Spectra



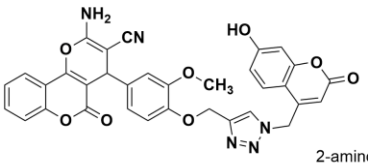
2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3494, 3477, 3388, 3072, 2916, 2227, 1660, 1603, 1504, 1384, 1311, 1250, 1173, 1116, 1021



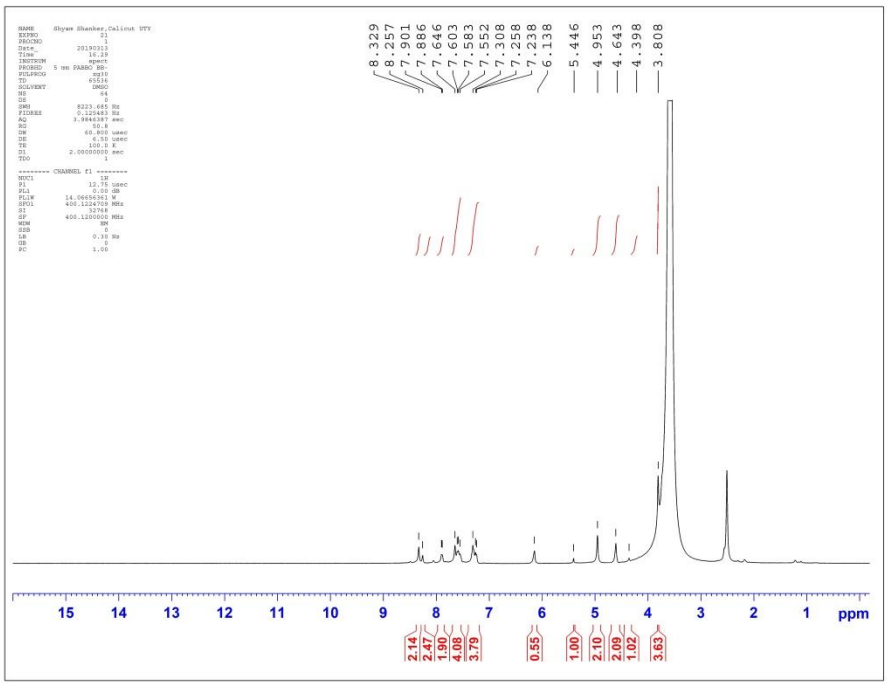
Spectral Details of **T6**:

[1]H-NMR Spectra

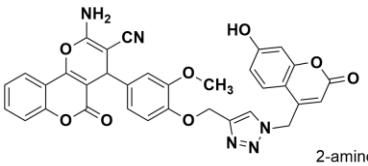


2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*] chromene-3-carbonitrile

[1]H NMR δH(400 MHz, DMSO, δ ppm)**:** 3.808 (3H, s, -OCH_3), 4.398 (1H, s, Ar-OH), 4.643 (2H, s, ArN-CH_2), 4.953 (2H, s, Ar-O-CH_2), 5.446 (1H, s, Ar-OH), 6.138 (1H, s, Ar-OH), 7.238-7.308 (3H, t, Ar-H), 7.552-7.646 (4H, m, Ar-H), 7.886-7.901 (2H, d, J=6Hz, Ar-H), 8.257 (2H, s, Ar-NH_2), 8.329 (2H, s, Ar-H)

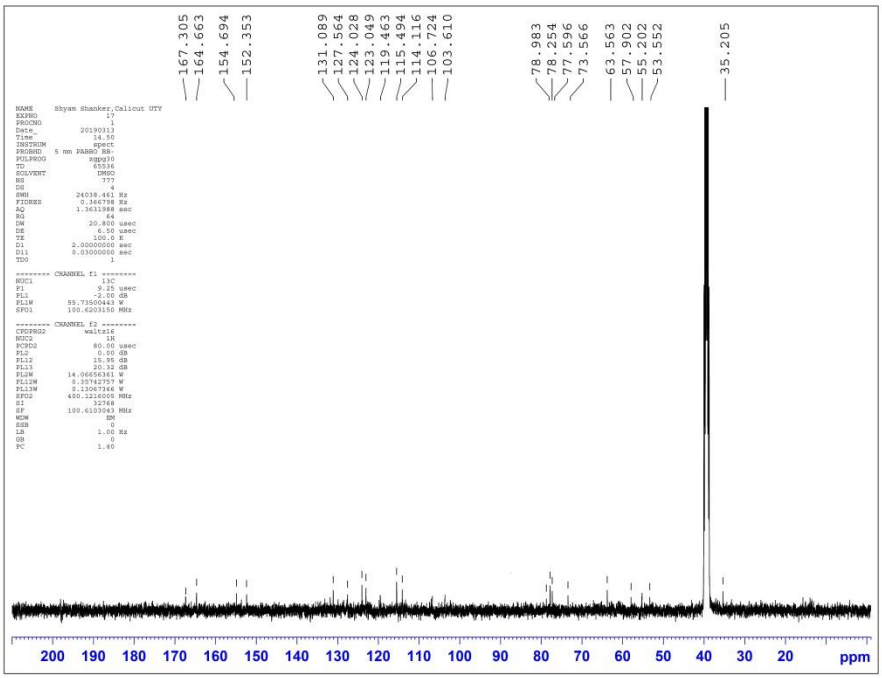


^{13}C-NMR Spectra

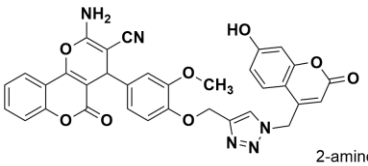


2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*] chromene-3-carbonitrile

^{13}C NMR δC(DMSO, 100MHz, δ ppm)**:** 35.205, 53.552, 55.202, 57.902, 63.563, 73.566, 77.596, 103.610, 106.724, 114.116, 115.494, 119.463, 123.049, 124.028, 127.564, 131.089, 152.353, 154.694, 164.663, 167.305

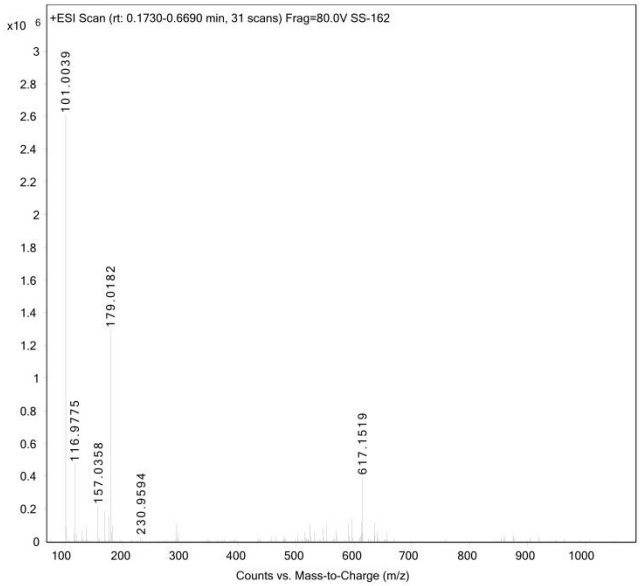


HRMS

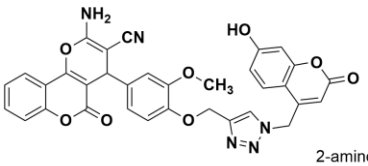


2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile

HRMS(ESI): m/z (M^+) calculated for $C_{33}H_{23}N_5O_8$: 617.1546, found: 617.1519



IR Spectra



2-amino-4-(4-((1-((7-hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-*c*] chromene-3-carbonitrile

IR absorptions (KBr, ν_{max}/cm^{-1}): 3558, 3440, 3362, 3085, 3028, 2929, 2612, 2220, 1728, 1695, 1563, 1509, 1455, 1420, 1376, 1338, 1269