SUPPORTING INFORMATION

Chalcone-Thiazole Hybrids: Rational Design, Synthesis and Lead Identification against 5-Lipoxygenase

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1. **General.** All chemicals, solvents and reagents were procured from commercial sources such as Merck Millipore (Billerica, MA, USA), Sigma-Aldrich Chemie GmbH (Steinheim, Germany), Thermo Fisher Scientific (Waltham, United States), Acros Organics (Geel, Belgium), Sisco Research Laboratories, Spectrochem® and Rankem® and were used without additional purification. Melting points were recorded on GUNA melting point apparatus and are uncorrected. IR spectra were taken in KBr pellets on Perkin Elmer Spectrum1 FT-IR spectrophotometer (resolution of 1.0 cm^{-1} and MIR 450-4000 cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE III 500 MHz (AV-500) NMR Spectrometer. $CDCl_3$ and DMSO-d_6 are used as solvents with tetramethylsilane (TMS) as an internal standard (chemical shift represented in δ ppm and

coupling constants (*J*) in Hz. HRMS were measured by Thermo Scientific Orbitrap Elite Mass spectrometer with high-field orbitrap Mass analyzer. Silica gel 60G F_{254} TLC plates from Merck KGaA (Darmstadt, Germany), Millipore were used to moniter reactions. Silica gel (60−120 mesh) used for coloumn purification. Purities of all final compounds synthesized here were 95 % or higher.

General procedure for chemical synthesis of compounds (4a-w):

The reaction of substituted benzoyl chloride (1.5 mmol) with ammonium thiocyanate (1.9 mmol) in 2 mL acetonitrile in **scheme 1** (in main article) resulted in substituted benzoyl isothiocyanates (**1a-d**). Product in yellow aromatic layer was filtered after stirring 2 h at 65 ^{0}C and treated with ammonium hydroxide (2 equivalents) at 0 ^{o}C and stirred at room temperature to yield various N-carbamothioyl substituted benzamides (**2a-d**). The white solid product obtained was filtered, washed with saturated aqueous sodium bicarbonate solution and dried in vacuo. Equimolar mixture of N-carbamothioyl substituted benzamides and 3-chloropentane-2,4-dione was refluxed in ACN to afford the desired intermediates, N-(5-acetyl-4-methylthiazol-2-yl) substituted benzamides (**3a-d**) via Hantzsch thiazole synthesis. Solvent was removed under reduced pressure. Solid obtained was recrystallized with ethanol and ethyl acetate to get pure compounds. The target compounds, chalcone-thiazole hybrids (**4a-w**) were synthesized by using base catalyzed Claisen–Schmidt condensation reaction. Substituted benzamide thiazoyl ketone, **3a-d** (1 mmol) is reacted with various substituted aromatic/heteroaromatic aldehyde (1 mmol) in ethanol in the presence of 2.5 N NaOH to give the corresponding thiazole-chalcone hybrids (**4a-w**) in good yield. All the synthesized structures were appropriately confirmed by spectroscopic data and analytical methods.

2. **Spectral details (NMR, HRMS) of compounds (4a-w):**

(E)-N-(5-cinnamoyl-4-methylthiazol-2-yl)benzamide (4a). Pale yellow solid, yield 83 %, mp 170-172 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.23 (s, 1H, NH), 7.97 (d, J = 7.4 Hz, 2H, Ar-H), 7.83 (d, J = 15.4 Hz, 1H, alkene-CH), 7.66 (t, J = 6.8 Hz, 3H, Ar-H), 7.54 (t, J = 7.7 Hz, 2H, Ar-H), 7.48 – 7.43 (m, 3H, Ar-H), 7.32 – 7.28 (m, 1H, alkene-CH), 2.50 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 182.55, 165.46, 159.75, 156.40, 144.13, 134.53, 133.51, 131.64, 130.76, 129.18, 129.04, 128.62, 127.80, 125.52, 124.44, 18.15. HRMS (ESI) *m/z* for $C_{20}H_{16}N_2O_2S$ $[M + H]^-$ calcd 349.1005, found 349.1000.

(E)-N-(5-(3-(4-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4b). Light yellow solid, yield 85 %, mp 167-169 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.20 (s, 1H, NH), 7.97 (dd, J = 8.3, 1.2 Hz, 2H, Ar-H), 7.79 (d, J = 15.3 Hz, 1H, alkene-CH), 7.69 – 7.63 (m, 3H, Ar-H), 7.54 (t, J = 7.8 Hz, 2H, Ar-H), 7.21 (d, J = 15.4 Hz, 1H, alkene-CH), 7.14 (t, J = 8.6 Hz, 2H, Ar-H), 2.50 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 182.32, 165.44, 163.20, 159.72, 156.51, 142.78, 133.52, 131.59, 130.81, 130.78, 130.57, 130.50, 129.18, 127.79, 125.35, 124.19, 116.30, 116.13, 18.14. HRMS (ESI) *m/z* for $C_{20}H_{15}FN_2O_2S$ $[M + H]^-$ calcd 367.0911, found 367.0909.

(E)-N-(5-(3-(4-chlorophenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4c). Pale yellow solid, yield 79 %, mp 181-183 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.12 (s, 1H, NH), 8.14 (d, J = 7.5 Hz, 2H, Ar-H), 7.89 (d, J = 8.4 Hz, 2H, Ar-H), 7.72 – 7.64 (m, 2H, Ar-H, alkene-CH), 7.58 (t, J = 7.7 Hz, 2H, Ar-H), 7.53 (d, J = 8.5 Hz, 2H, Ar-H), 7.48 (d, J = 15.5 Hz, 1H, alkene-CH), 2.71 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz,

$CDCl_3$) δ: 182.32, 165.44, 163.20, 159.72, 156.51, 142.78, 133.52, 131.59, 130.81, 130.78, 130.57, 130.50, 129.18, 127.79, 125.35, 124.19, 116.30, 116.13, 18.14. HRMS (ESI) *m/z* for $C_{20}H_{15}ClN_2O_2S$ $[M + H]^+$ calcd 383.0616, found 383.0618.

(E)-N-(5-(3-(4-bromophenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4d). Yellow solid, yield 89 %, mp 201-203 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.16 (d, J = 6.6 Hz, 2H, Ar-H), 7.73 (d, J = 8.5 Hz, 2H, Ar-H), 7.65 (d, J = 8.5 Hz, 2H, Ar-H), 7.54 (d, J = 15.5 Hz, 1H, alkene-CH), 7.45 (tt, J = 8.6, 4.4 Hz, 4H, Ar-H, alkene-CH), 2.63 (s, 3H, thiazole-CH_3).^{13}C NMR (126 MHz, DMSO-d_6) δ: 180.97, 158.53, 139.29, 134.70, 132.42, 131.28, 131.02, 130.66, 128.72, 128.37, 127.64, 123.68, 19.51. HRMS (ESI) *m/z* for $C_{20}H_{15}BrN_2O_2S$ $[M + H]^+$ calcd 427.0110, found 427.0112.

(E)-N-(5-(3-(3-bromophenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4e). Dark yellow solid, yield 66 %, mp 185-186 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.16 (d, J = 6.8 Hz, 2H, Ar-H), 8.01 (s, 1H, Ar-H), 7.79 (d, J = 7.7 Hz, 1H, alkene-CH), 7.62 (d, J = 8.9 Hz, 1H, alkene-CH), 7.56 – 7.40 (m, 6H, Ar-H), 2.64 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 181.13, 170.41, 158.41, 139.17, 137.94, 133.00, 131.51, 131.22, 128.72, 128.45, 128.29, 127.63, 122.85, 19.46. HRMS (ESI) *m/z* for $C_{20}H_{15}BrN_2O_2S$ $[M + H]^+$ calcd 427.0110, found 271.0111.

(E)-N-(5-(3-(4-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)-4-methoxybenzamide (4f). Light yellow solid, yield 88 %, mp 185-186 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.91 (s, 1H, NH), 7.95 (d, J = 8.8 Hz, 2H, Ar-H), 7.78 (d, J = 15.4 Hz, 1H, alkene-CH), 7.64 (dd, J = 8.6, 5.4 Hz, 2H, Ar-H), 7.21 (d, J = 15.3 Hz, 1H, alkene-CH), 7.14 (t, J = 8.6 Hz, 2H, Ar-H), 7.01 (d, J = 8.8 Hz, 2H, Ar-H), 3.90 (s, 3H, OCH_3), 2.59 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 182.35, 166.51, 164.79, 163.14,

162.81, 156.38, 141.38, 131.66, 131.46, 131.40, 130.83, 125.51, 124.77, 116.56, 116.38, 114.29, 56.51, 55.96, 18.92. HRMS (ESI) *m/z* for $C_{21}H_{17}FN_2O_3S$ $[M + H]^-$ calcd 397.1017, found 397.1016.

(E)-N-(5-(3-(4-chlorophenyl)acryloyl)-4-methylthiazol-2-yl)-4-nitrobenzamide (4g). Dark yellow solid, yield 92 %, mp 202-203 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.38 – 8.27 (m, 6H, Ar-H), 8.20 (s, 1H, Ar-H), 7.86 (d, *J* = 8.5 Hz, 1H, alkene-CH), 7.52 (d, *J* = 8.5 Hz, 1H, alkene-CH), 7.49 – 7.37 (m, 1H, Ar-H), 2.69 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 180.97, 158.53, 139.29, 134.70, 132.42, 131.28, 131.02, 130.66, 128.72, 128.37, 127.64, 123.68, 19.51. HRMS (ESI) *m/z* for $C_{20}H_{14}ClN_3O_4S$ $[M + H]^-$ calcd 428.0466, found 428.0464.

(E)-N-(4-methyl-5-(3-(3-nitrophenyl)acryloyl)thiazol-2-yl)benzamide (4h). Pale yellow solid, yield 92 %, mp 214-216 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.13 (s, 1H, NH), 8.67 (s, 1H, Ar-H), 8.32 (d, *J* = 7.8 Hz, 1H, alkene-CH), 8.27 (d, *J* = 8.2 Hz, 1H, alkene-CH), 8.14 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.82 – 7.72 (m, 2H, Ar-H), 7.70 – 7.62 (m, 2H, Ar-H), 7.57 (t, *J* = 7.7 Hz, 2H, Ar-H), 2.72 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 182.59, 148.86, 140.67, 136.76, 135.08, 133.48, 130.90, 129.17, 128.80, 128.10, 125.16, 123.71, 18.83. HRMS (ESI) *m/z* for $C_{20}H_{15}N_3O_4S$ $[M + H]^-$ calcd 394.0856, found 394.0852.

(E)-N-(5-(3-(4-methoxyphenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4i). Pale yellow solid, yield 85 %, mp 191-193 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.27 (s, 1H, NH), 7.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.80 (d, *J* = 15.3 Hz, 1H, alkene-CH), 7.63 (dd, *J* = 14.3, 8.1 Hz, 3H, Ar-CH), 7.52 (s, 2H, Ar-CH), 7.17 (d, *J* = 15.3 Hz, 1H, alkene-CH), 6.97 (d, *J* = 8.7 Hz, 2H, Ar-CH), 3.89 (s, 3H, O-CH_3), 2.49 (s, 3H, thiazole-CH_3). ^{13}C

NMR (126 MHz, $CDCl_3$) δ: 182.61, 165.42, 161.87, 159.52, 155.91, 143.94, 133.43, 131.69, 130.43, 129.15, 127.79, 127.29, 125.68, 122.14, 114.49, 55.47, 18.08. HRMS (ESI) *m/z* for HRMS (ESI) *m/z* for $C_{21}H_{18}N_2O_3S$ $[M + H]^-$ calcd 379.1111, found 379.1108.

(E)-N-(5-(3-(3,4-dimethoxyphenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4j). Pale yellow solid, yield 77 %, mp 159-160 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.79 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.66 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.54 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.24 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.18 – 7.11 (m, 2H, Ar-H), 6.92 (d, *J* = 8.3 Hz, 1H, alkene-CH), 4.01 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 2.50 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 182.33, 165.49, 159.62, 156.42, 153.54, 144.46, 140.62, 133.54, 131.50, 130.00, 129.17, 127.83, 125.11, 123.50, 105.83, 61.06, 56.35, 18.11. HRMS (ESI) *m/z* for $C_{22}H_{20}N_2O_4S$ $[M + H]^-$ calcd 409.1217, found 409.1216.

(E)-N-(4-methyl-5-(3-(3,4,5-trimethoxyphenyl)acryloyl)thiazol-2-yl)benzamide (4k). Light yellow solid, yield 81 %, mp 180-182 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.76 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.66 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.55 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.16 (d, *J* = 15.2 Hz, 1H, alkene-CH), 6.87 (s, 2H, Ar-H), 3.97 (s, 6H, OCH_3), 3.93 (s, 3H, OCH_3), 2.57 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 182.44, 165.51, 159.54, 155.83, 151.67, 149.33, 144.49, 133.53, 131.56, 129.18, 127.83, 127.48, 125.38, 123.73, 122.08, 111.10, 109.94, 56.13, 56.05, 30.51, 18.02. HRMS (ESI) *m/z* for $C_{23}H_{22}N_2O_5S$ $[M + H]^-$ calcd 439.1322, found 439.1318.

(E)-N-(4-methyl-5-(3-p-tolylacryloyl)thiazol-2-yl)benzamide (4l). Light yellow solid, yield 90 %, mp 168-170 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.40 (s, 1H, NH), 7.97 (d, *J*

= 8.4 Hz, 2H, Ar-H), 7.81 (d, *J* = 15.3 Hz, 1H, alkene-CH), 7.65 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.54 (q, *J* = 7.8 Hz, 4H, Ar-H), 7.28 – 7.20 (m, 3H, Ar-H, alkene-CH), 2.47 (s, 3H, thiazole-CH_3), 2.42 (s, 3H, Ar-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) *δ*: 182.64, 165.55, 159.78, 156.14, 144.22, 141.37, 133.46, 131.80, 131.70, 129.77, 129.15, 128.66, 127.84, 125.56, 123.42, 21.60, 18.09. HRMS (ESI) *m/z* for $C_{21}H_{18}N_2O_2S$ $[M + H]^-$ calcd 363.1162, found 363.1156.

(E)-4-fluoro-N-(4-methyl-5-(3-p-tolylacryloyl)thiazol-2-yl)benzamide (4m). Light yellow solid, yield 95 %, mp 183-185 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 13.10 (s, 1H, NH), 8.21 (s, 2H, Ar-H), 7.72 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.66 (d, *J* = 15.4 Hz, 1H, alkene-CH), 7.39 (d, *J* = 15.9 Hz, 3H, Ar-H, alkene-CH), 7.29 (d, *J* = 7.4 Hz, 2H, Ar-H), 2.70 (s, 3H, thiazole-CH_3), 2.36 (s, 3H, Ar-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) *δ*: 182.82, 166.35, 164.36, 143.37, 141.26, 132.11, 131.76, 131.68, 130.13, 129.24, 124.19, 116.32, 116.15, 21.56, 18.66.

HRMS (ESI) *m/z* for $C_{21}H_{17}FN_2O_2S$ $[M + H]^-$ calcd 381.1068, found 381.1062.

(E)-4-methoxy-N-(4-methyl-5-(3-p-tolylacryloyl)thiazol-2-yl)benzamide (4n). Light yellow solid, yield 92 %, mp 207-209 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 12.88 (s, 1H, NH), 8.14 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.65 (d, *J* = 15.4 Hz, 1H, alkene-CH), 7.37 (d, *J* = 15.4 Hz, 1H, alkene-CH), 7.27 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.09 (d, *J* = 9.0 Hz, 2H, Ar-H), 3.86 (s, 3H, OCH_3), 2.69 (s, 3H, thiazole-CH_3), 2.35 (s, 3H, Ar-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) *δ*: 182.76, 163.49, 143.22, 141.21, 132.11, 130.91, 130.11, 129.20, 124.21, 114.47, 56.04, 21.56, 18.75. HRMS (ESI) *m/z* for $C_{22}H_{20}N_2O_3S$ $[M + H]^-$ calcd 393.1267, found 393.1265.

(E)-N-(4-methyl-5-(3-p-tolylacryloyl)thiazol-2-yl)-4-nitrobenzamide (4o). Yellow solid, yield 87 %, mp 208-210 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.28 (d, J = 9.0 Hz, 3H, Ar-H), 8.19 (s, 2H, Ar-H), 7.66 (d, J = 7.7 Hz, 2H, Ar-H), 7.58 (d, J = 16.1 Hz, 1H, alkene-CH), 7.36 (d, J = 15.5 Hz, 1H, alkene-CH), 7.28 (d, J = 7.9 Hz, 2H, Ar-H), 2.65 (s, 3H, thiazole-CH_3), 2.35 (s, 3H, Ar-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 184.47, 130.15, 130.02, 128.98, 124.51, 123.82, 21.52, 19.04. HRMS (ESI) *m/z* for $C_{21}H_{17}N_3O_4S$ $[M + H]^+$ calcd 408.1013, found 408.1013.

(E)-N-(5-(3-(4-(dimethylamino)phenyl)acryloyl)-4-methylthiazol-2-yl)benzamide (4p). Fluorescent yellow solid, yield 82 %, mp 174-176 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.41 (s, 1H, NH), 7.98 – 7.93 (m, 3H, Ar-H), 7.80 (d, J = 15.1 Hz, 1H, alkene-CH), 7.64 (t, J = 7.4 Hz, 1H, Ar-H), 7.56 – 7.50 (m, 4H, Ar-H), 7.09 (d, J = 15.1 Hz, 1H, alkene-CH), 6.71 (d, J = 8.8 Hz, 1H, Ar-H), 3.08 (s, 3H, thiazole-CH_3), 2.55 (s, 3H, N-CH_3), 2.37 (s, 3H, N-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 190.71, 182.70, 165.54, 159.99, 159.41, 155.17, 155.00, 152.21, 145.11, 133.43, 133.33, 131.89, 131.68, 130.65, 129.11, 127.82, 126.07, 125.39, 122.27, 119.10, 111.83, 40.15, 30.51, 17.82. HRMS (ESI) *m/z* for $C_{22}H_{21}N_3O_2S$ $[M + H]^-$ calcd 392.1427, found 392.1425.

(E)-N-(5-(3-(4-(dimethylamino)phenyl)acryloyl)-4-methylthiazol-2-yl)-4-fluorobenzamide (4q). Dark yellow solid, yield 86 %, mp 212-213 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.02 (s, 1H, NH), 8.28 – 8.12 (m, 4H, Ar-H), 7.63 (d, J = 9.2 Hz, 1H, alkene-CH), 7.46 – 7.31 (m, 4H, Ar-H), 6.74 (d, J = 8.8 Hz, 1H, alkene-CH), 3.35 (s, 6H, N-CH_3), 3.01 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 191.23, 182.46, 166.34, 164.34, 152.50, 144.54, 131.67, 131.10, 122.01, 119.11, 116.30, 116.13,

112.27, 111.53, 30.58, 18.40. HRMS (ESI) *m/z* for $C_{22}H_{20}FN_3O_2S$ $[M + H]^-$ calcd 410.1333, found 410.1328.

(E)-N-(5-(3-(furan-2-yl)acryloyl)-4-methylthiazol-2-yl)benzamide (4r). Pale yellow solid, yield 81 %, mp 185-186 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.11 (s, 1H, NH), 8.13 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.92 (d, *J* = 1.4 Hz, 1H, furan-H), 7.67 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.57 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.53 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.13 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.10 (d, *J* = 3.4 Hz, 1H, furan-H), 6.70 (dd, *J* = 3.4, 1.8 Hz, 1H, furan-H), 2.69 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 181.94, 151.25, 146.81, 133.52, 129.79, 129.18, 128.80, 121.73, 117.85, 113.65, 18.72. HRMS (ESI) *m/z* for $C_{18}H_{14}N_2O_3S$ $[M + H]^-$ calcd 339.0796, found 339.0794.

(E)-4-fluoro-N-(5-(3-(furan-2-yl)acryloyl)-4-methylthiazol-2-yl)benzamide (4s). Yellow solid, yield 97 %, mp 126-128 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.11 (s, 1H, NH), 8.21 (dd, *J* = 8.9, 5.4 Hz, 2H, Ar-H), 7.92 (d, *J* = 1.4 Hz, 1H, furan-H), 7.52 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.41 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.12 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.09 (d, *J* = 3.4 Hz, 1H, furan-H), 6.70 (dd, *J* = 3.4, 1.8 Hz, 1H, furan-H), 2.69 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 181.92, 166.37, 164.37, 151.25, 146.79, 131.77, 131.70, 129.79, 121.71, 117.82, 116.34, 116.16, 113.63, 18.68. HRMS (ESI) *m/z* for $C_{18}H_{13}FN_2O_3S$ $[M + H]^-$ calcd 357.0704, found 357.0701.

(E)-N-(4-methyl-5-(3-(thiophen-2-yl)acryloyl)thiazol-2-yl)benzamide (4t). Light yellow solid, yield 77 %, mp 184-186 ^{0}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.40 (s, 1H, NH), 7.98 – 7.93 (m, 3H, Ar-H, alkene-CH), 7.65 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.53 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.47 (d, *J* = 5.0 Hz, 1H, thiofene-H), 7.38 (d, *J* = 3.5 Hz, 1H, thiofene-H), 7.12 (dd, *J* = 5.0, 3.7 Hz, 1H, thiofene-H), 7.07 (d, *J* = 15.0 Hz, 1H, alkene-

CH), 2.46 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 182.10, 165.57, 159.85, 156.15, 140.00, 136.55, 133.49, 132.36, 131.66, 129.27, 129.16, 128.41, 127.84, 125.47, 123.13, 18.08. HRMS (ESI) *m/z* for $C_{18}H_{14}N_2O_2S_2$ $[M + H]^-$ calcd 355.0569, found 355.0566.

(E)-4-fluoro-N-(4-methyl-5-(3-(thiophen-2-yl)acryloyl)thiazol-2-yl)benzamide (4u). Light yellow solid, yield 86 %, mp 211-213 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.11 (s, 1H, NH), 8.21 (dd, *J* = 8.9, 5.4 Hz, 2H, Ar-H), 7.86 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.79 (d, *J* = 5.0 Hz, 1H, thiofene-H), 7.66 (d, *J* = 3.4 Hz, 1H, thiofene-H), 7.40 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.20 (dd, *J* = 5.0, 3.7 Hz, 1H, thiofene-H), 7.09 (d, *J* = 15.1 Hz, 1H, alkene-CH), 2.68 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 182.01, 166.37, 164.36, 139.85, 136.07, 133.54, 131.76, 131.69, 130.81, 129.32, 123.40, 116.33, 116.15, 18.62. HRMS (ESI) *m/z* for $C_{18}H_{13}FN_2O_2S_2$ $[M + H]^+$ calcd 373.0475, found 373.0475.

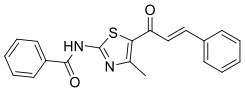
(E)-N-(5-(3-(6-bromobenzo[d][1,3]dioxol-5-yl)acryloyl)-4-methylthiazol-2-yl)benzamide (4v). Yellow solid, yield 95 %, mp 203-205 ^{0}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.14 (d, *J* = 7.2 Hz, 2H, methylenedioxy Ar-H), 7.84 (d, *J* = 15.2 Hz, 1H, alkene-CH), 7.72 (s, 1H, alkene-CH), 7.59 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.52 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.38 (d, *J* = 13.8 Hz, 2H, Ar-H), 6.18 (s, 2H, dioxy-CH_2), 2.67 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 150.64, 148.41, 139.71, 128.81, 128.75, 127.80, 126.94, 118.30, 113.30, 107.54, 103.12, 19.15. HRMS (ESI) *m/z* for $C_{21}H_{15}BrN_2O_4S$ $[M + H]^-$ calcd 471.0009, found 471.0003.

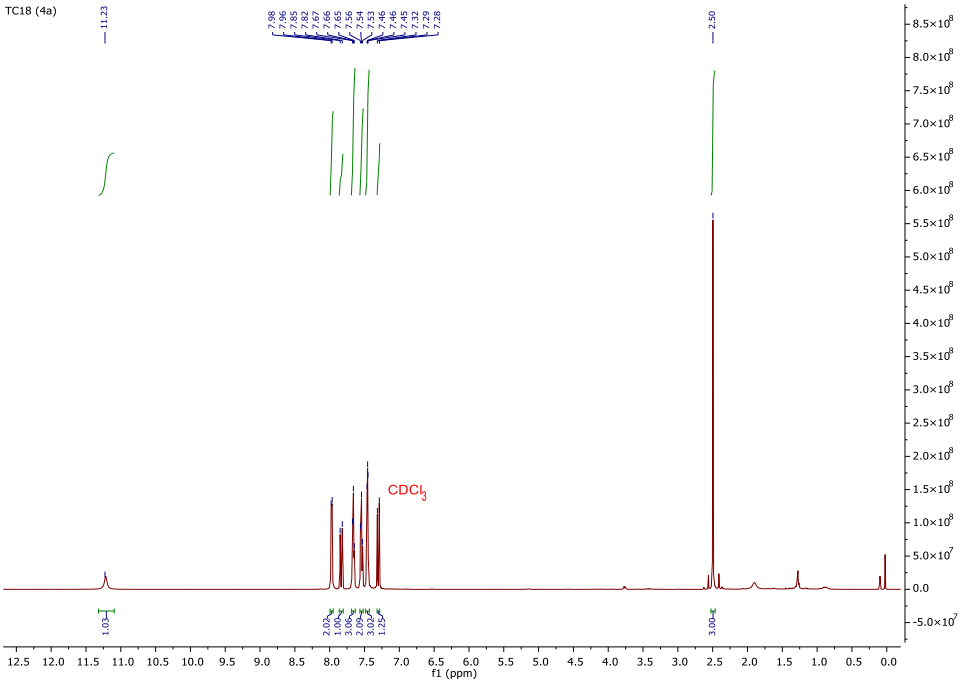
(E)-N-(5-(3-(6-bromobenzo[d][1,3]dioxol-5-yl)acryloyl)-4-methylthiazol-2-yl)-4-fluorobenzamide (4w). Dark yellow solid, yield 94 %, mp 217-219 ^{0}C. ^{1}H NMR (500

MHz, DMSO-d_6) δ: 8.21 (dd, J = 8.7, 5.6 Hz, 2H, methylenedioxy Ar-H), 7.85 (d, J = 15.2 Hz, 1H, alkene-CH), 7.72 (s, 1H, alkene-CH), 7.36 (d, J = 3.6 Hz, 4H, Ar-H), 6.17 (s, 2H, dioxy-CH_2), 2.68 (s, 3H, thiazole-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 181.89, 150.66, 148.51, 139.82, 131.52, 131.44, 127.75, 126.79, 118.35, 115.89, 115.72, 113.28, 107.53, 103.13, 19.08. HRMS (ESI) *m/z* for $C_{21}H_{14}BrFN_2O_4S$ $[M + H]^+$ calcd 488.9914, found 488.9914.

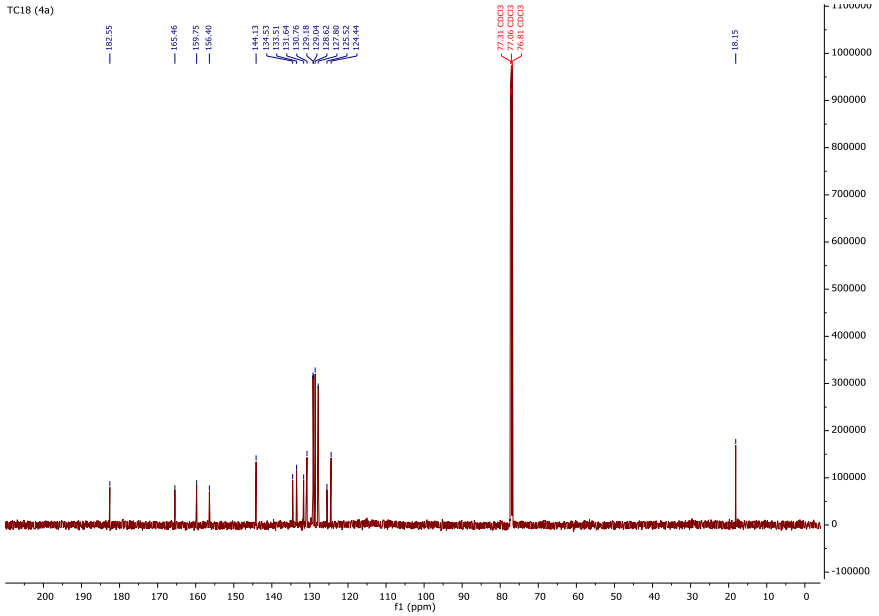
3. ^{1}H and ^{13}C NMR spectra for the target compounds (4a-w):

4a



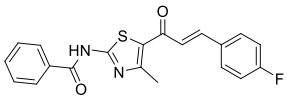


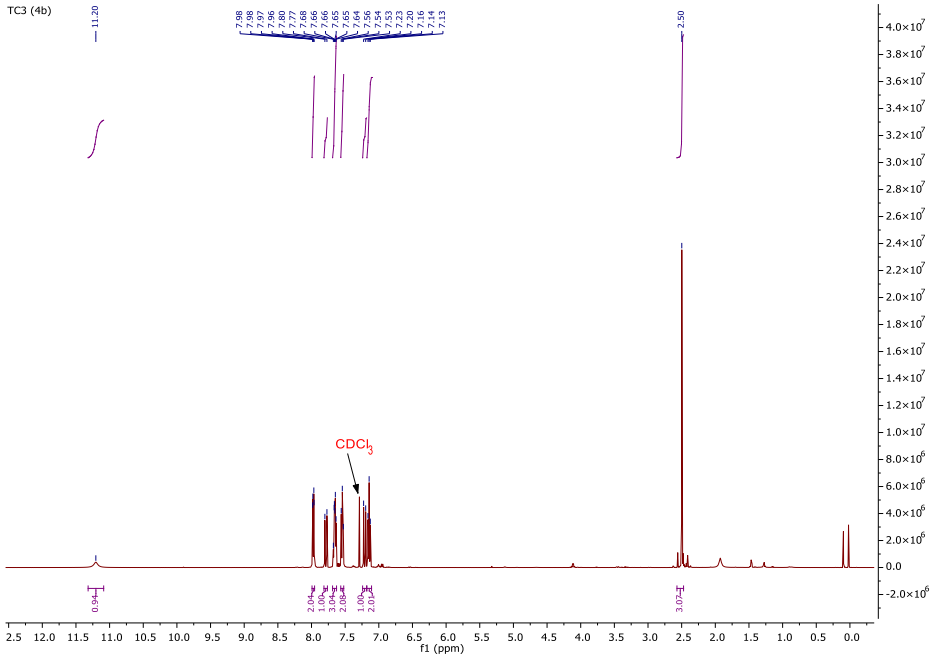
^{1}H NMR spectrum of **4a** (500 MHz, $CDCl_3$)



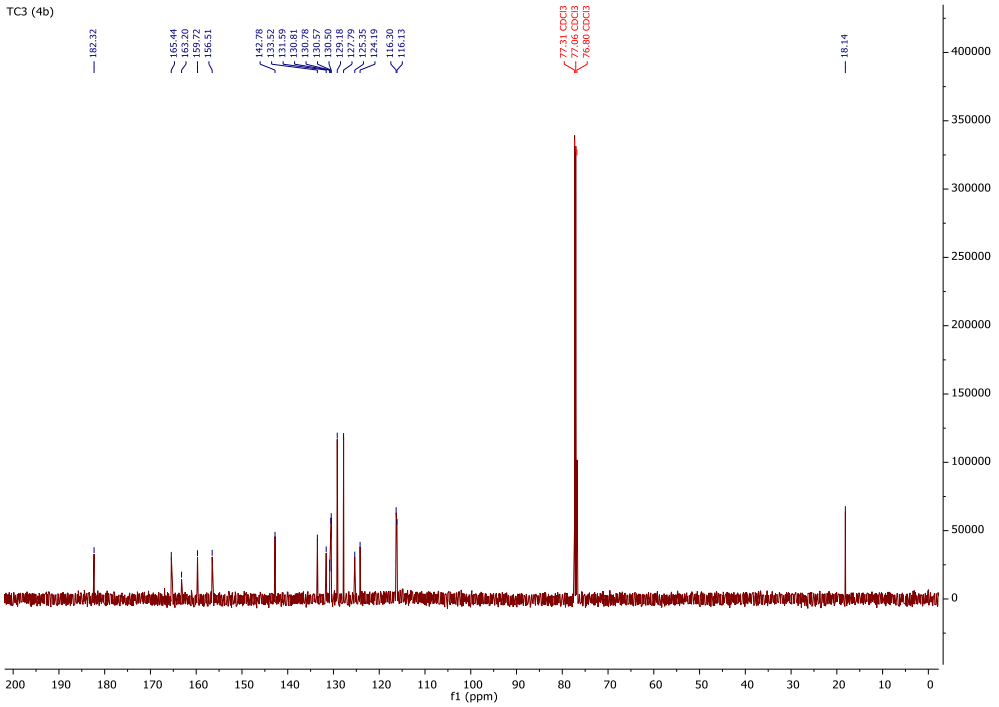
^{13}C NMR spectrum of **4a** (126 MHz, $CDCl_3$)

4b



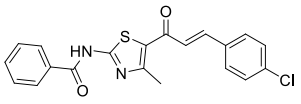


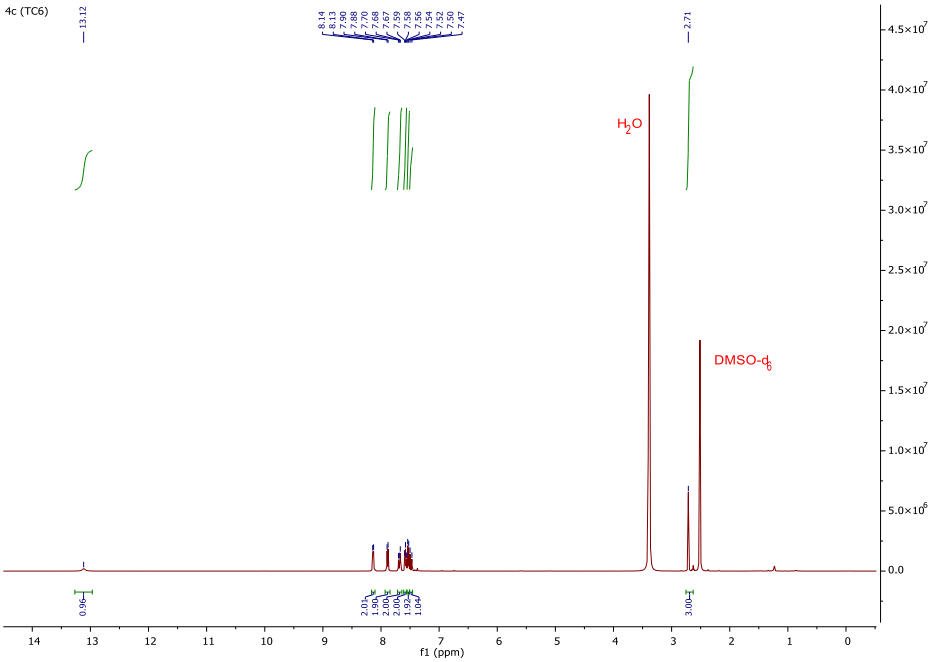
^{1}H NMR spectrum of **4b** (500 MHz, $CDCl_3$)



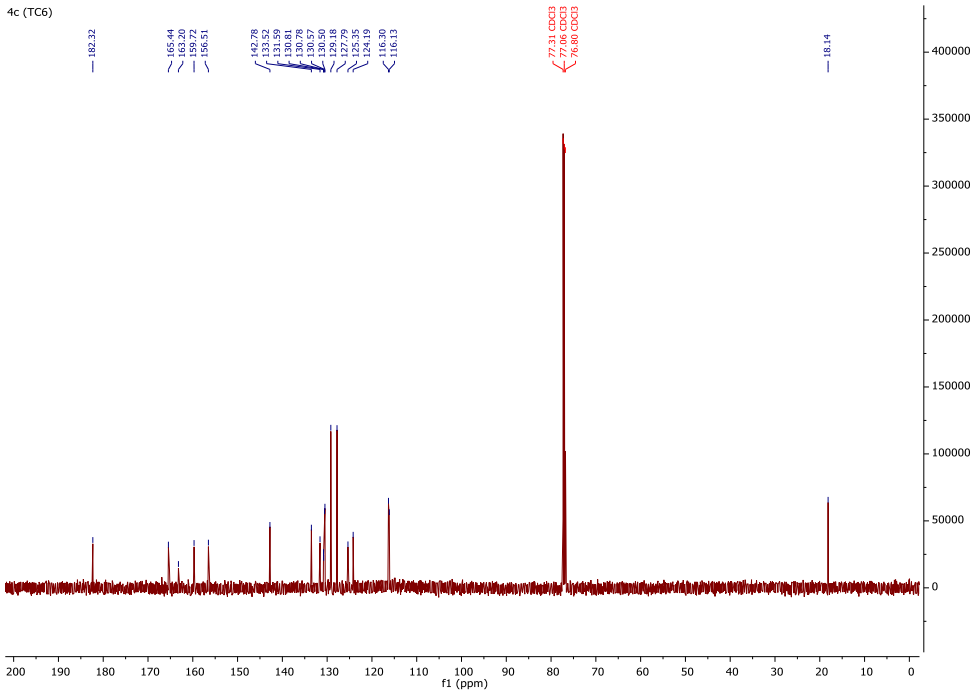
^{13}C NMR spectrum of **4b** (126 MHz, $CDCl_3$)

4c



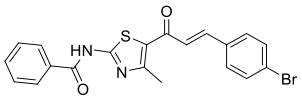


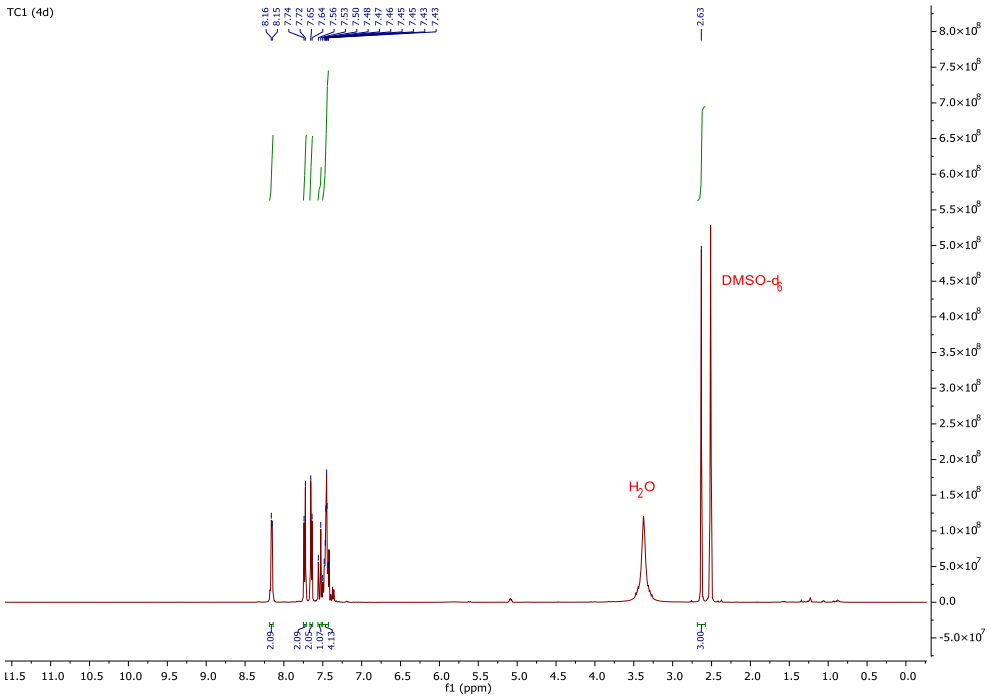
^{1}H NMR spectrum of **4c** (500 MHz, DMSO-d_6)



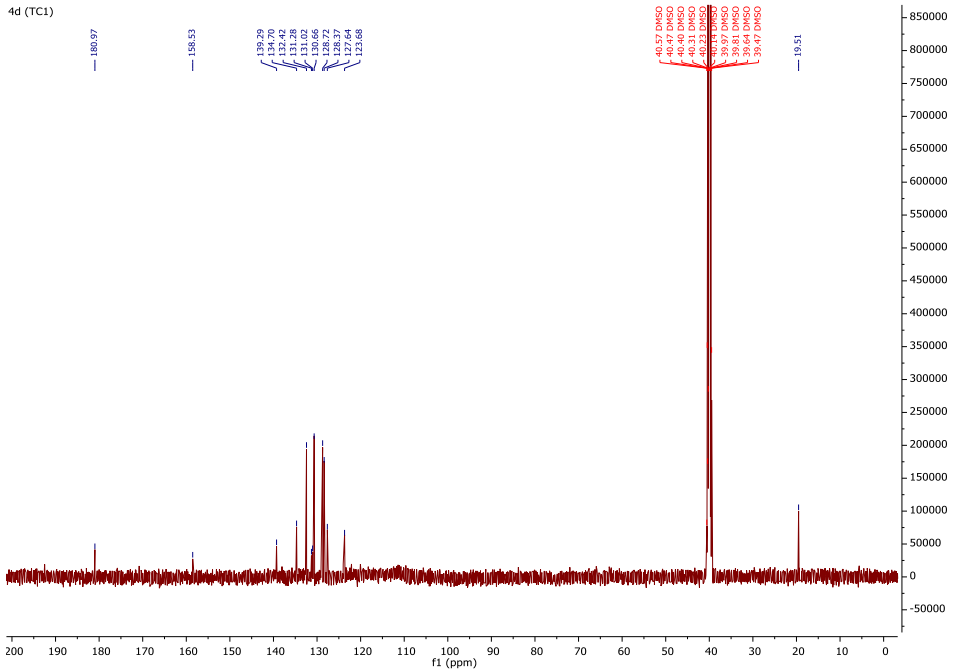
^{13}C NMR spectrum of **4c** (126 MHz, $CDCl_3$)

4d



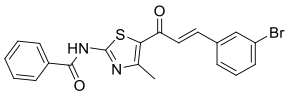


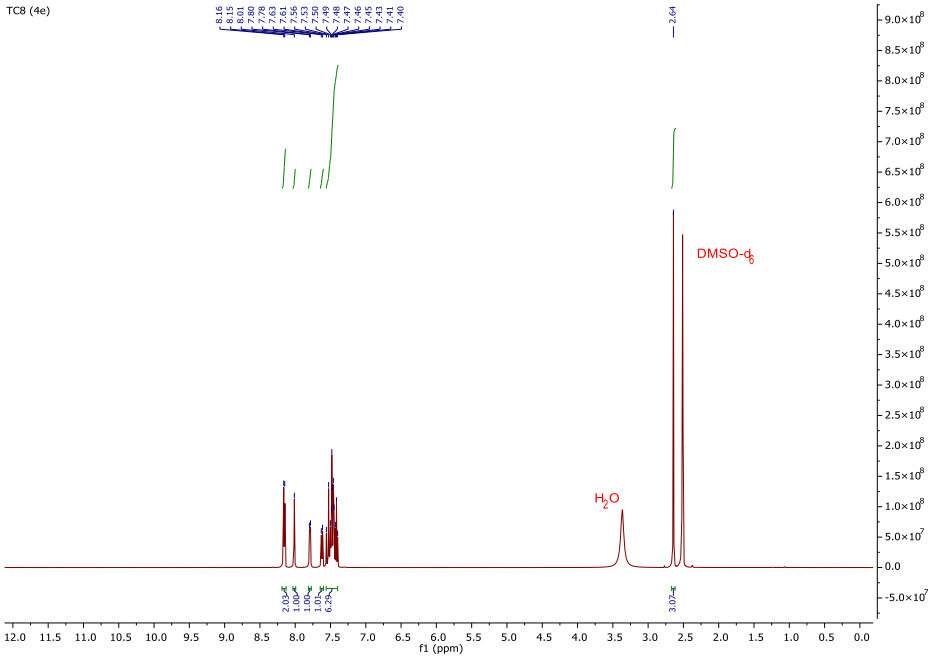
^{1}H NMR spectrum of **4d** (500 MHz, DMSO-d_6)



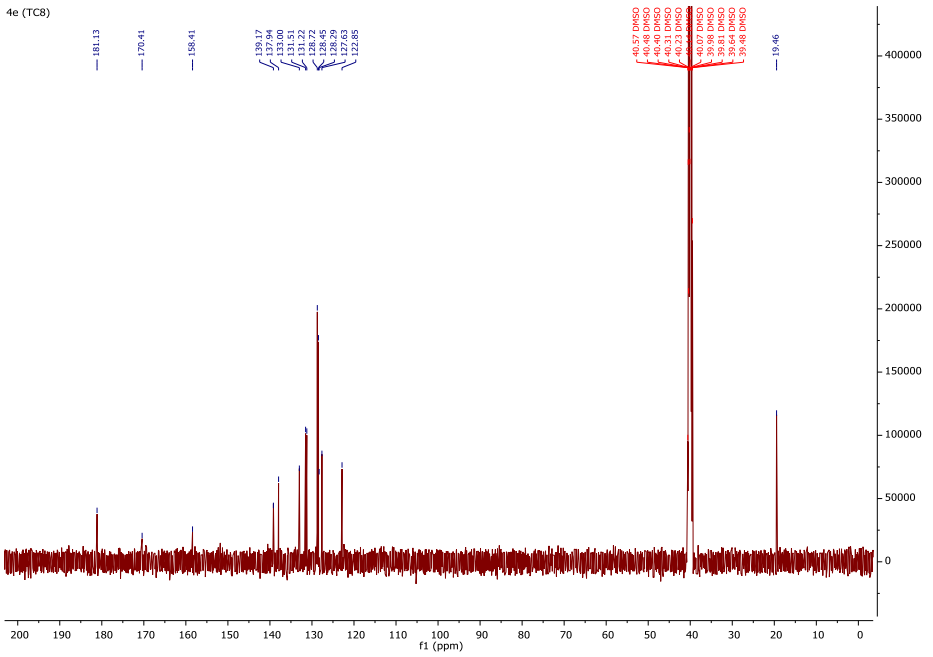
^{13}C NMR spectrum of **4d (**126 MHz, DMSO-d_6)

4e



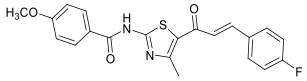


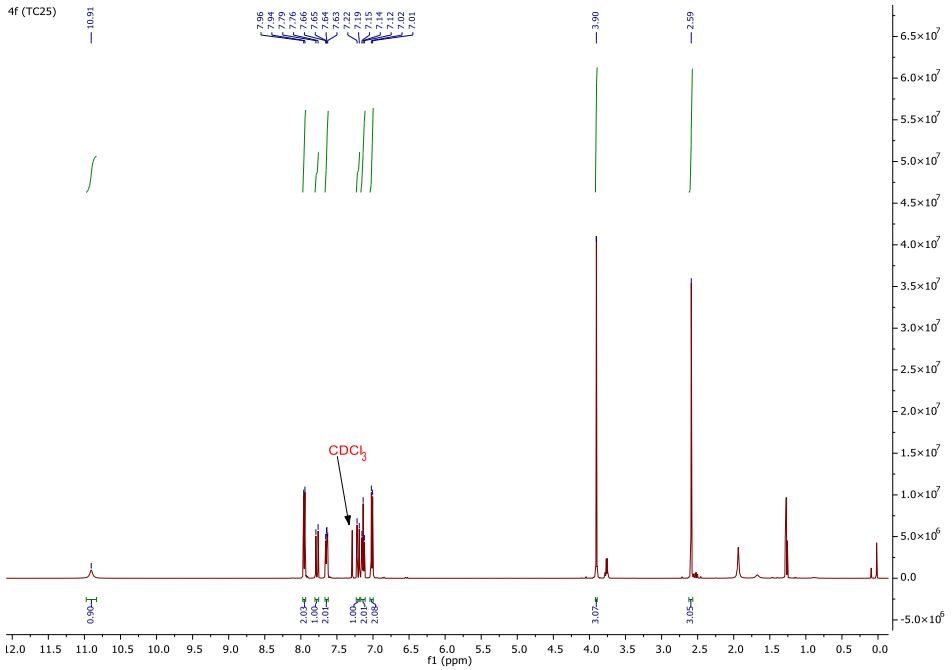
^{1}H NMR spectrum of **4e** (500 MHz, DMSO-d_6)



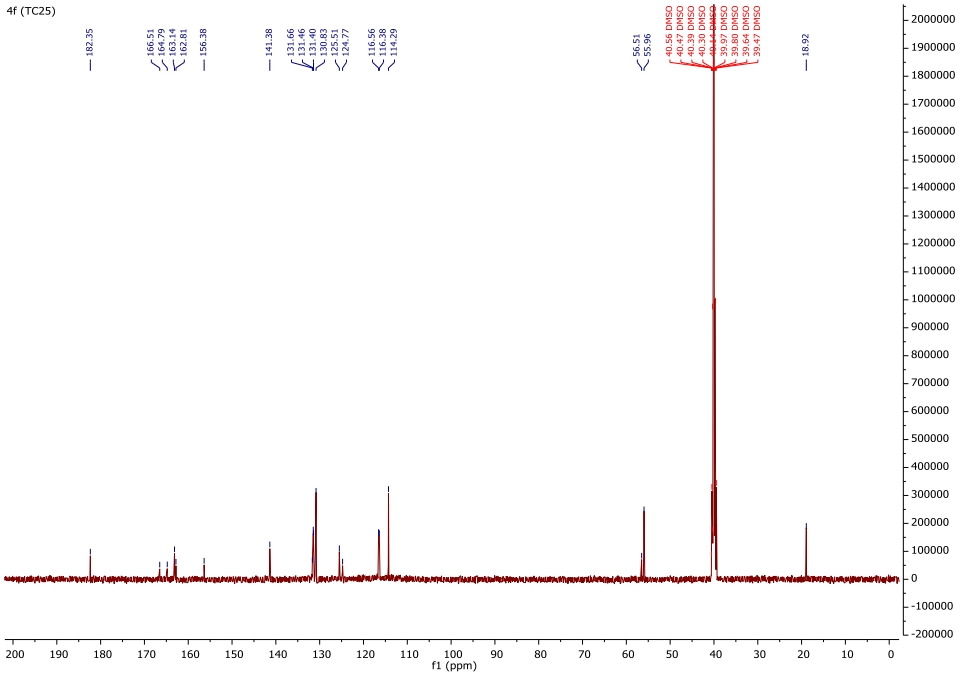
^{13}C NMR spectrum of **4e** (126 MHz, DMSO-d_6)

4f



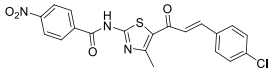


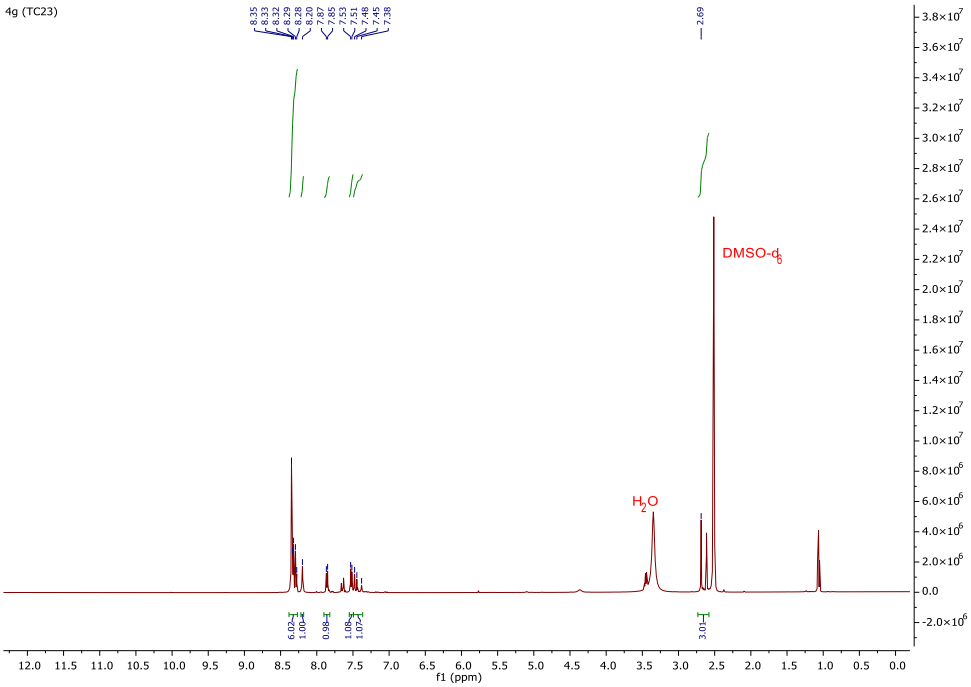
^{1}H NMR spectrum of **4f** (500 MHz, $CDCl_3$)



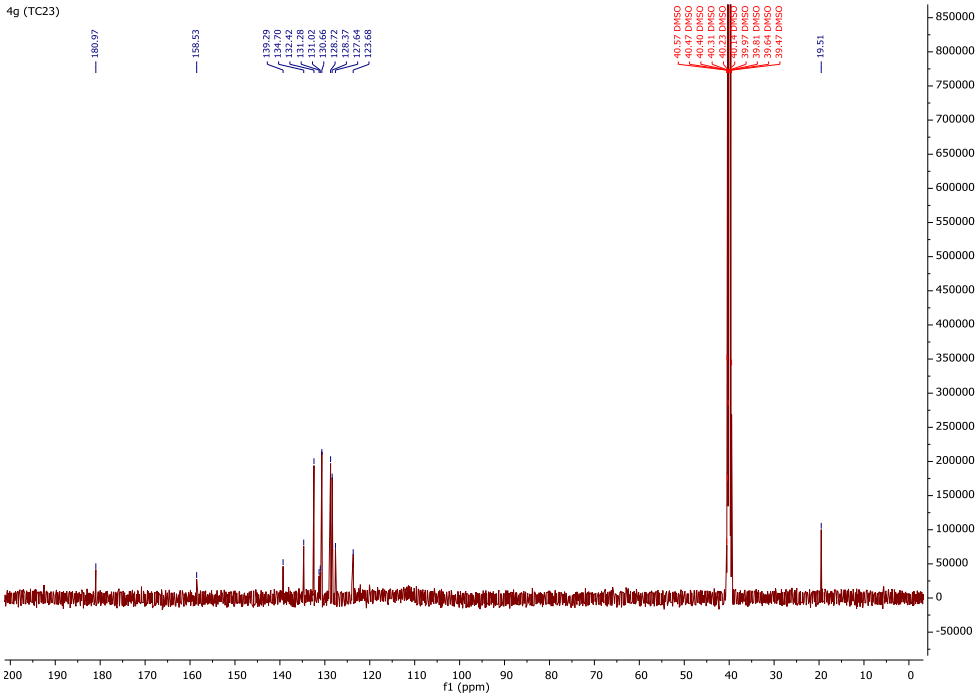
^{13}C NMR spectrum of **4f** (126 MHz, DMSO-d_6)

4g



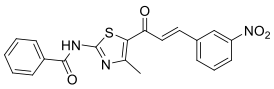


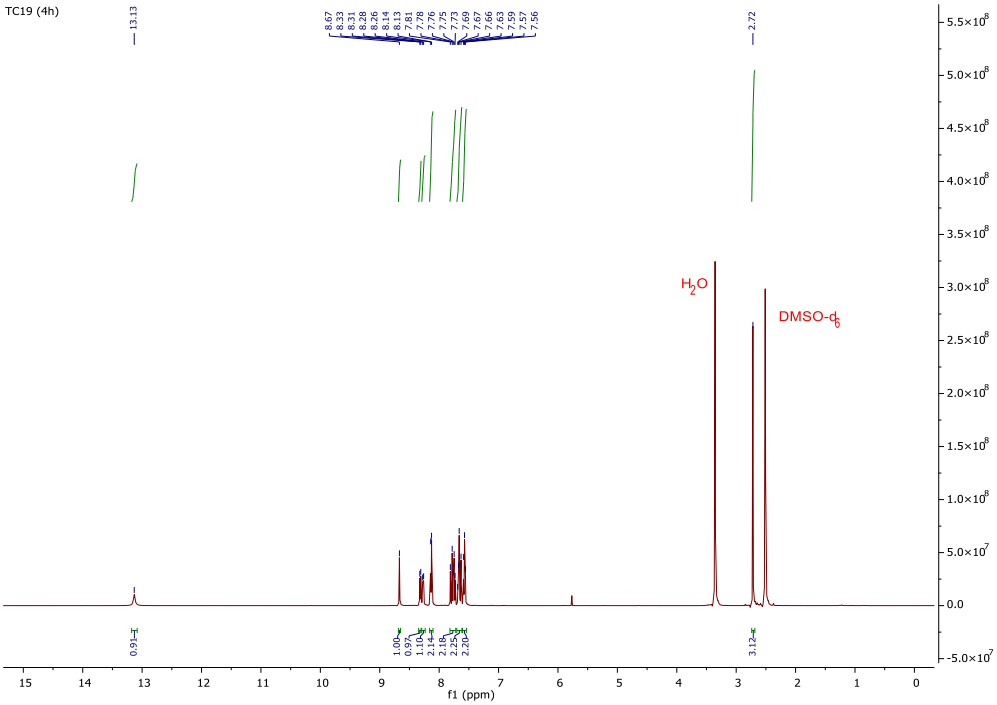
^{1}H NMR spectrum of **4g** (500 MHz, DMSO-d_6)



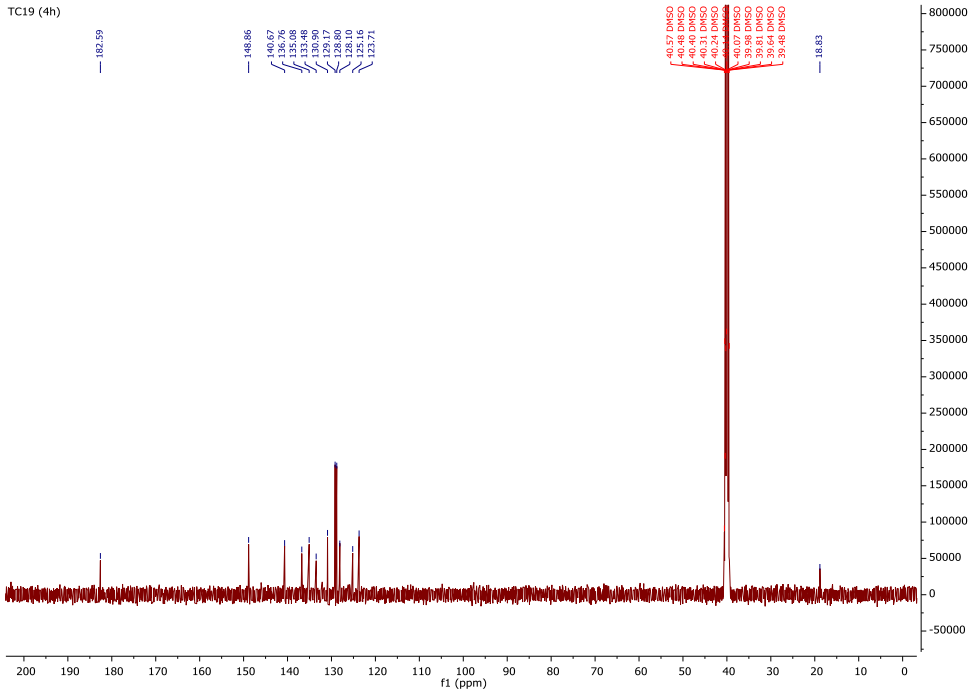
^{13}C NMR spectrum of **4g** (126 MHz, DMSO-d_6)

4h



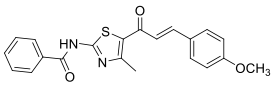


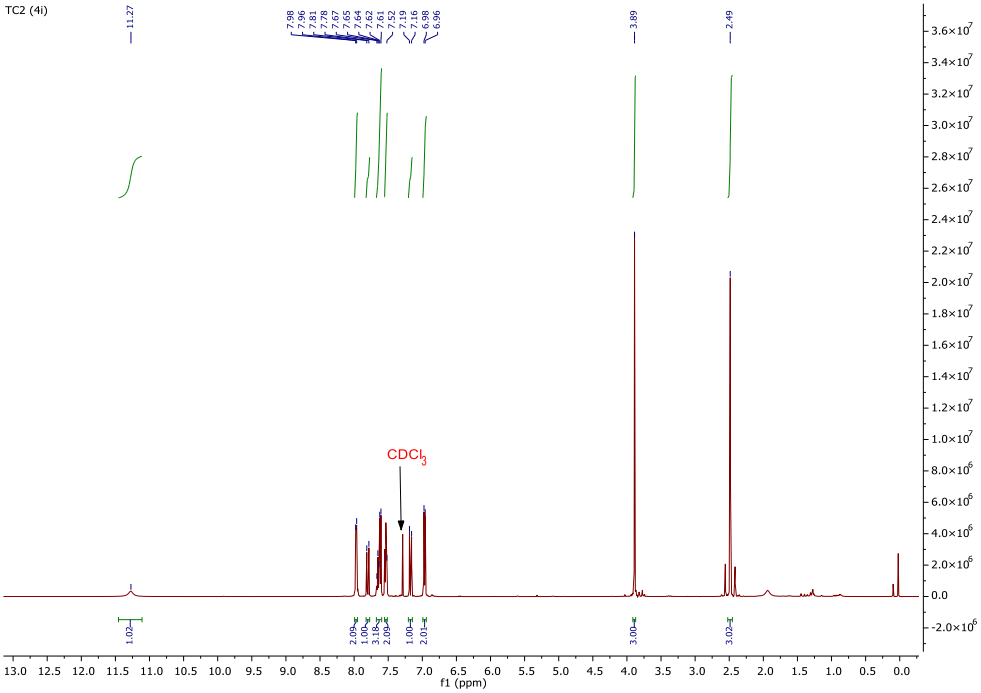
^{1}H NMR spectrum of **4h** (500 MHz, DMSO-d_6)



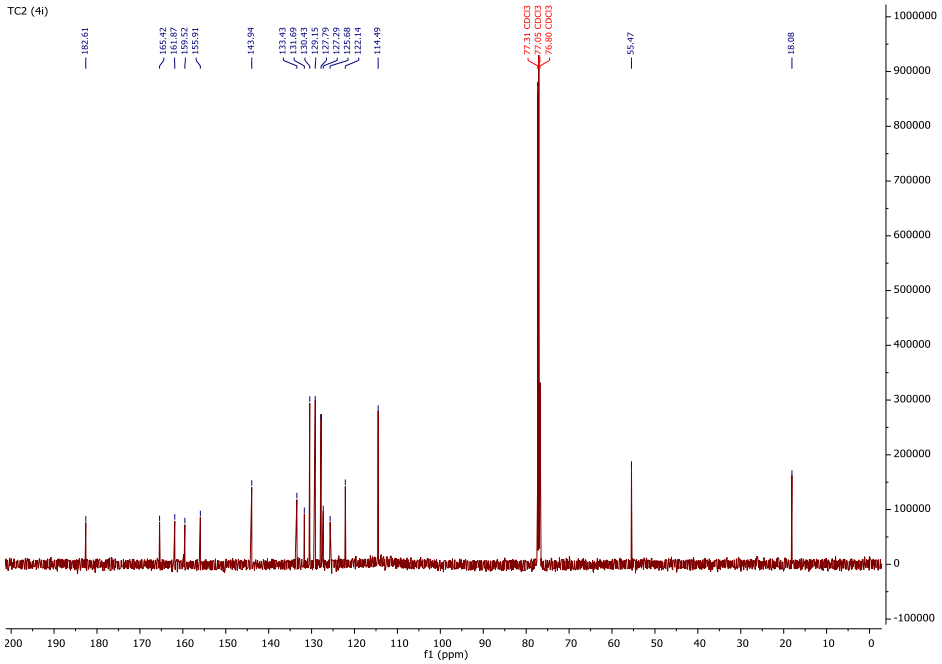
^{13}C NMR spectrum of **4h** (126 MHz, DMSO-d_6)

4i



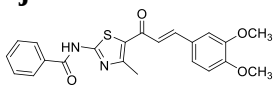


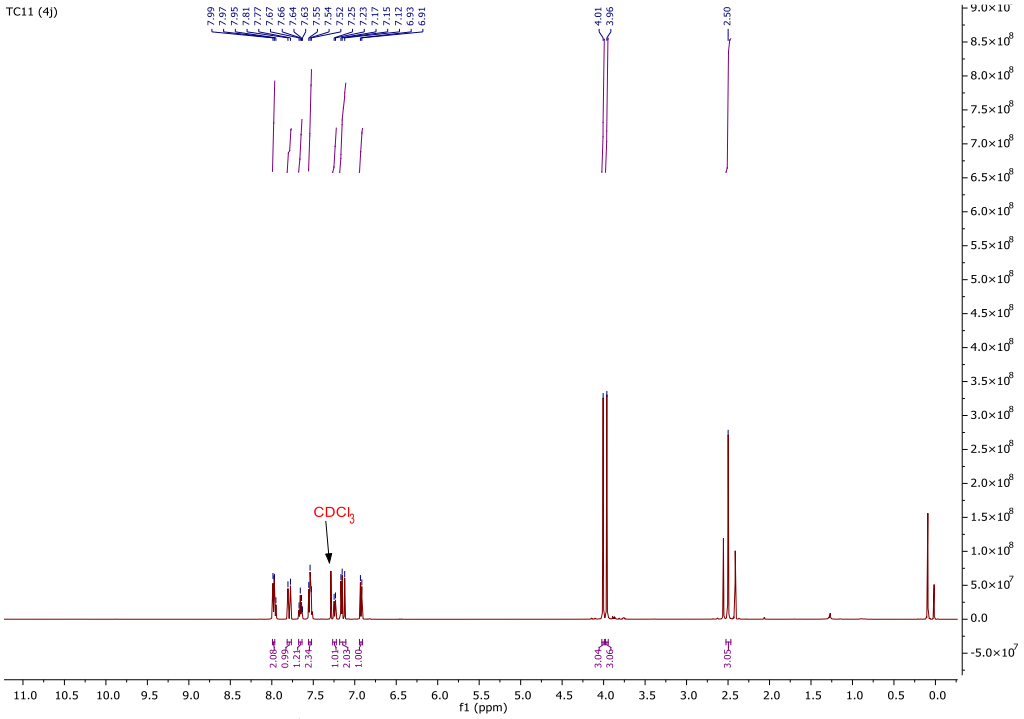
^{1}H NMR spectrum of **4i** (500 MHz, $CDCl_3$)



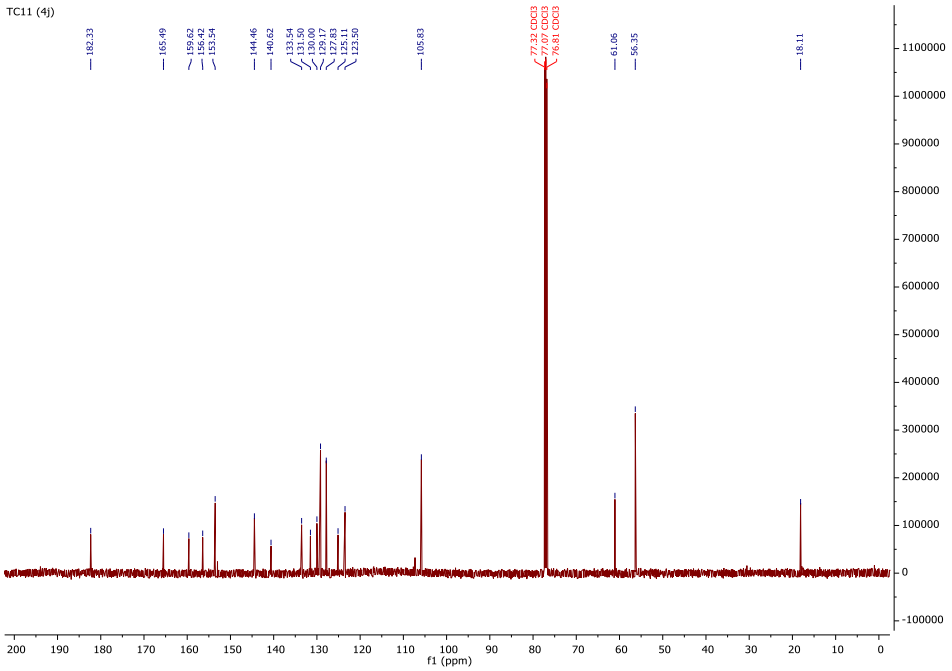
^{13}C NMR spectrum of **4i** (126 MHz, $CDCl_3$)

4j



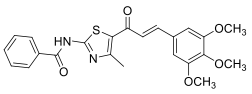


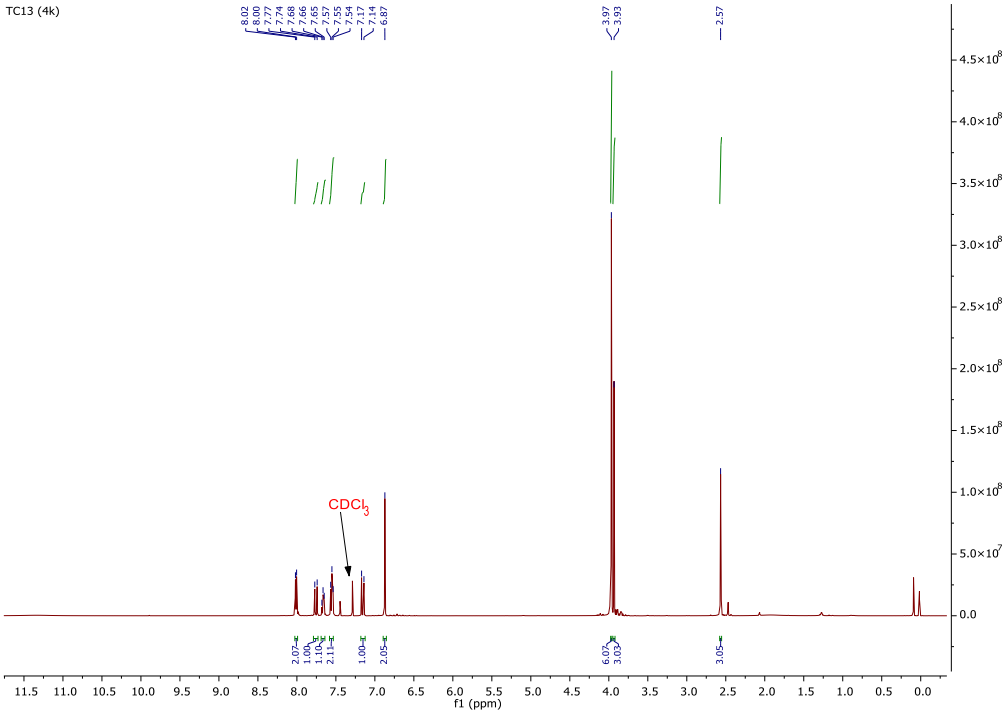
^{1}H NMR spectrum of **4j** (500 MHz, $CDCl_3$)



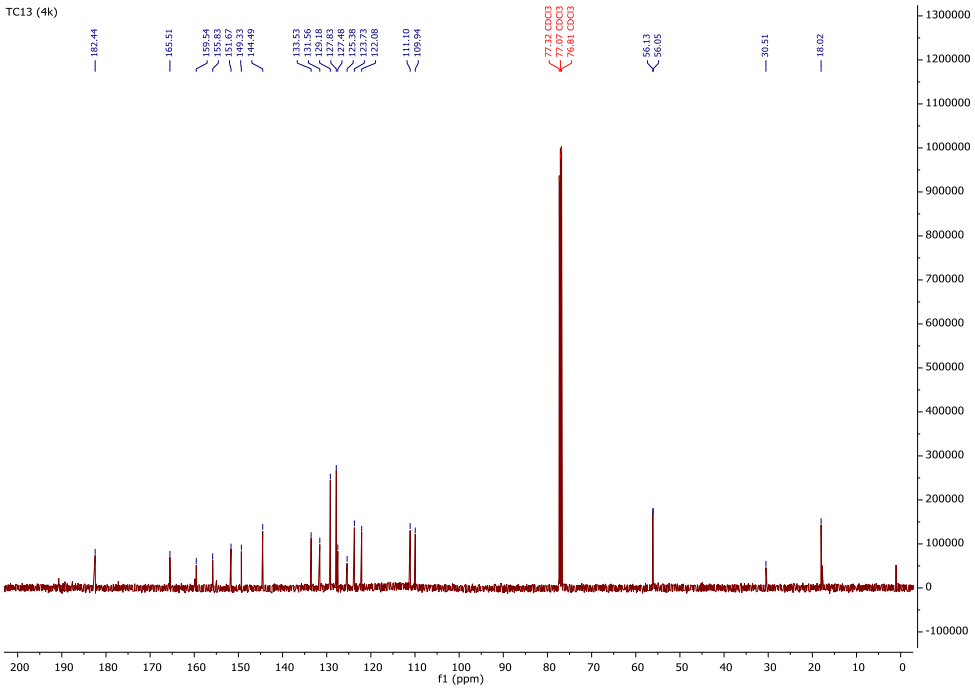
^{13}C NMR spectrum of **4j** (126 MHz, $CDCl_3$)

4k



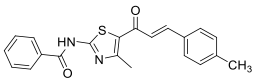


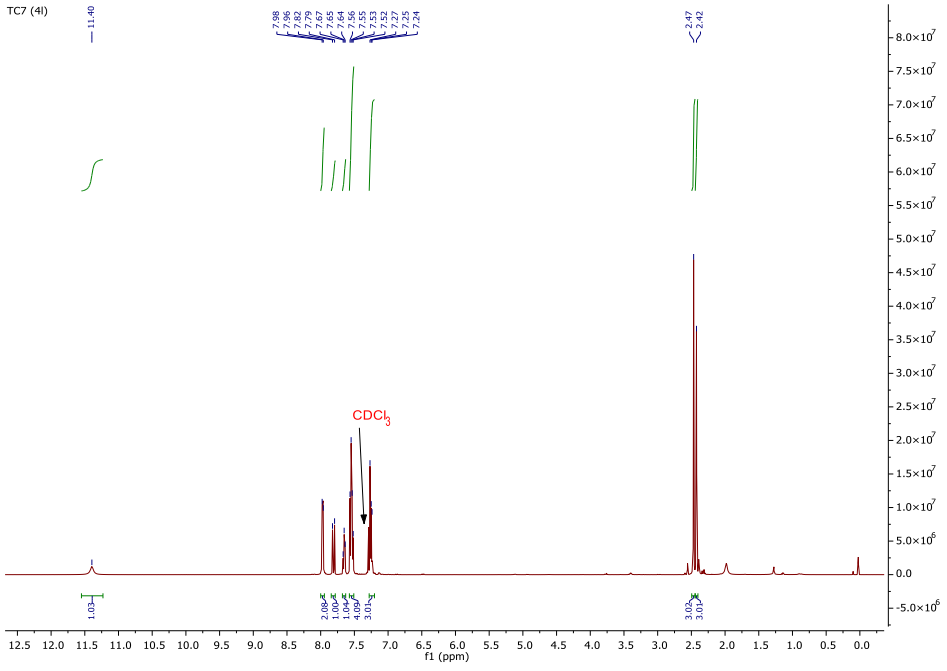
^{1}H NMR spectrum of **4k** (500 MHz, $CDCl_3$)



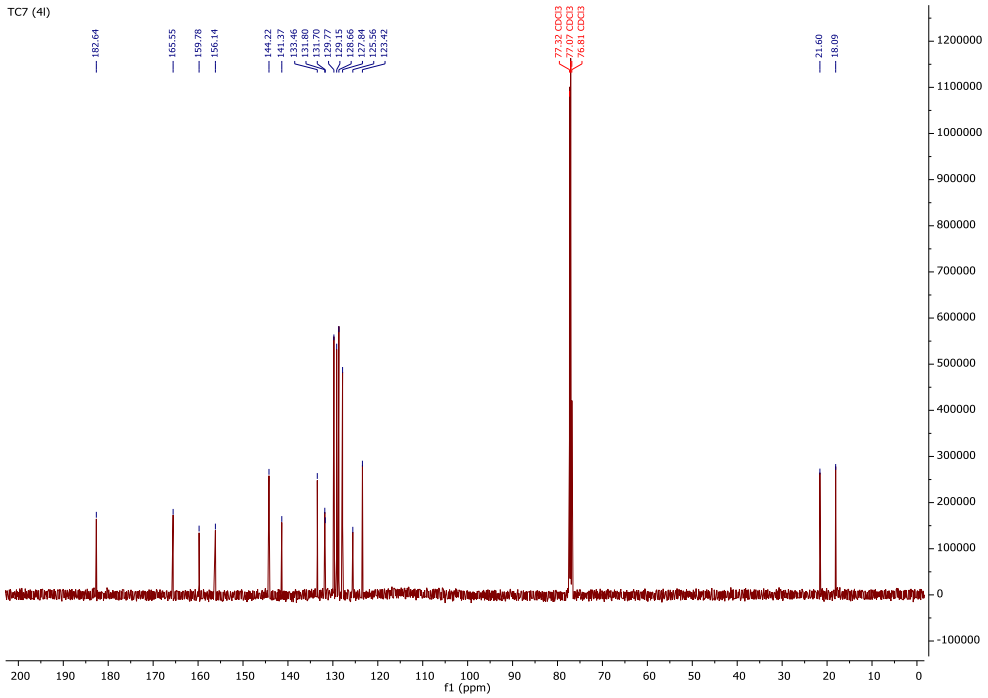
^{13}C NMR spectrum of **4k** (126 MHz, $CDCl_3$)

4l



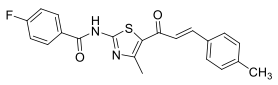


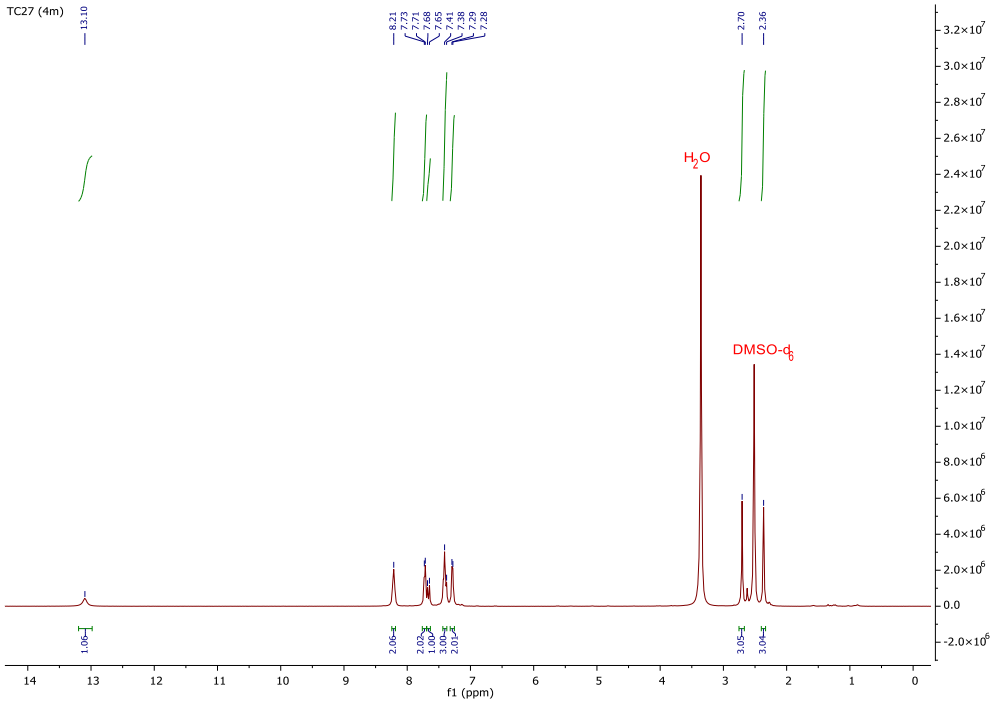
^{1}H NMR spectrum of **4l** (500 MHz, $CDCl_3$)



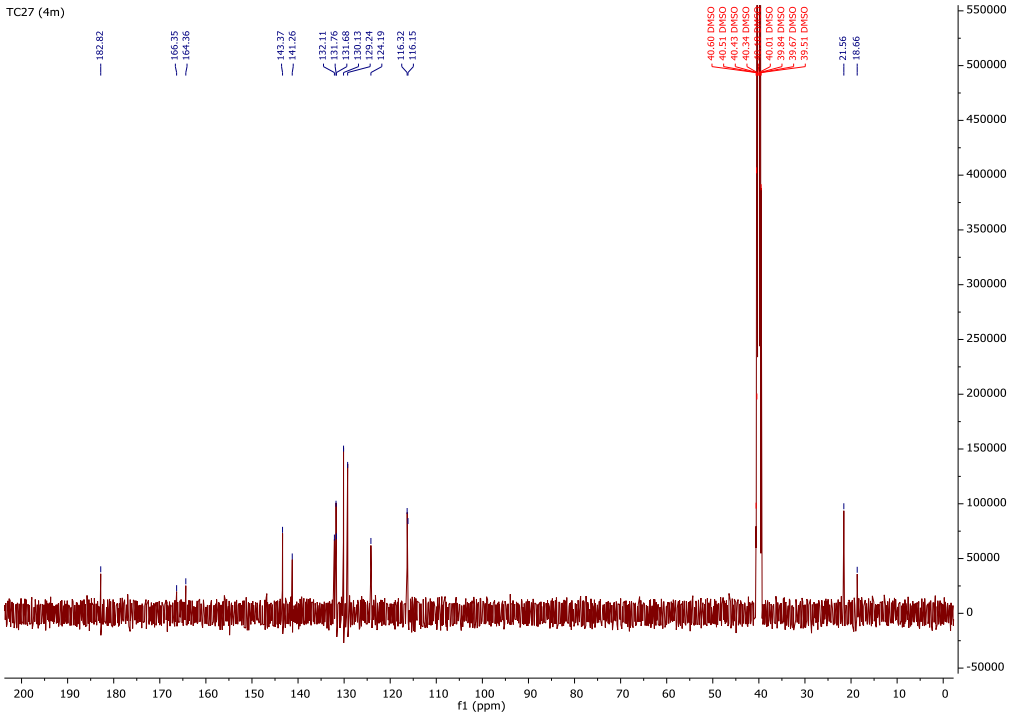
^{13}C NMR spectrum of **4l** (126 MHz, $CDCl_3$)

4m



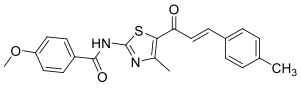


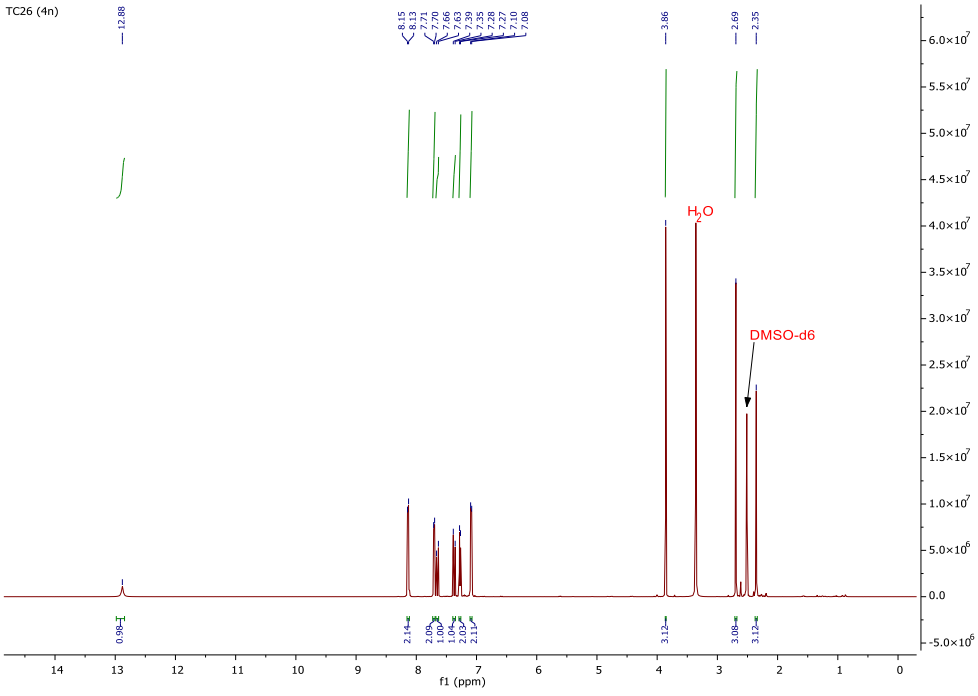
^{1}H NMR spectrum of **4m** (500 MHz, DMSO-d_6)



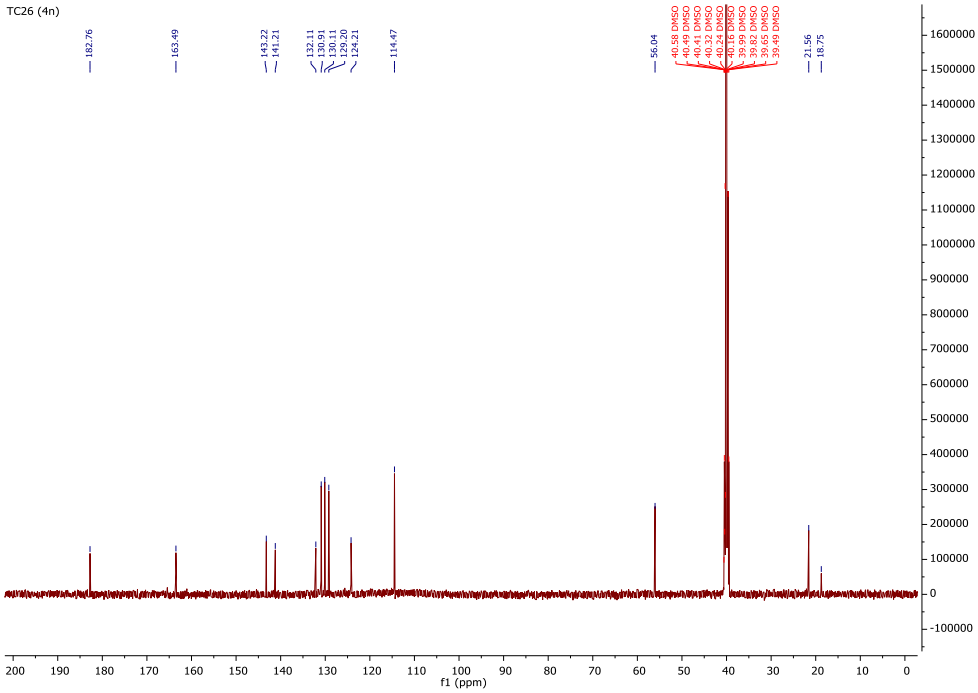
^{13}C NMR spectrum of **4m** (126 MHz, DMSO-d_6)

4n



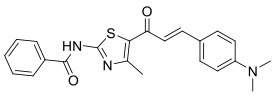


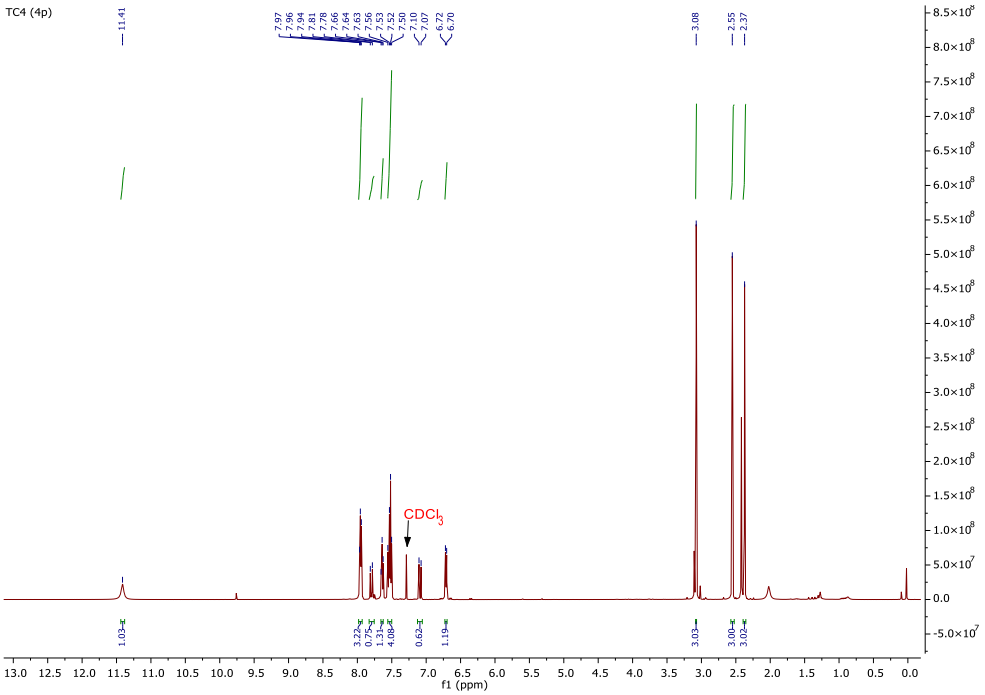
^{1}H NMR spectrum of **4n** (500 MHz, DMSO-d_6)



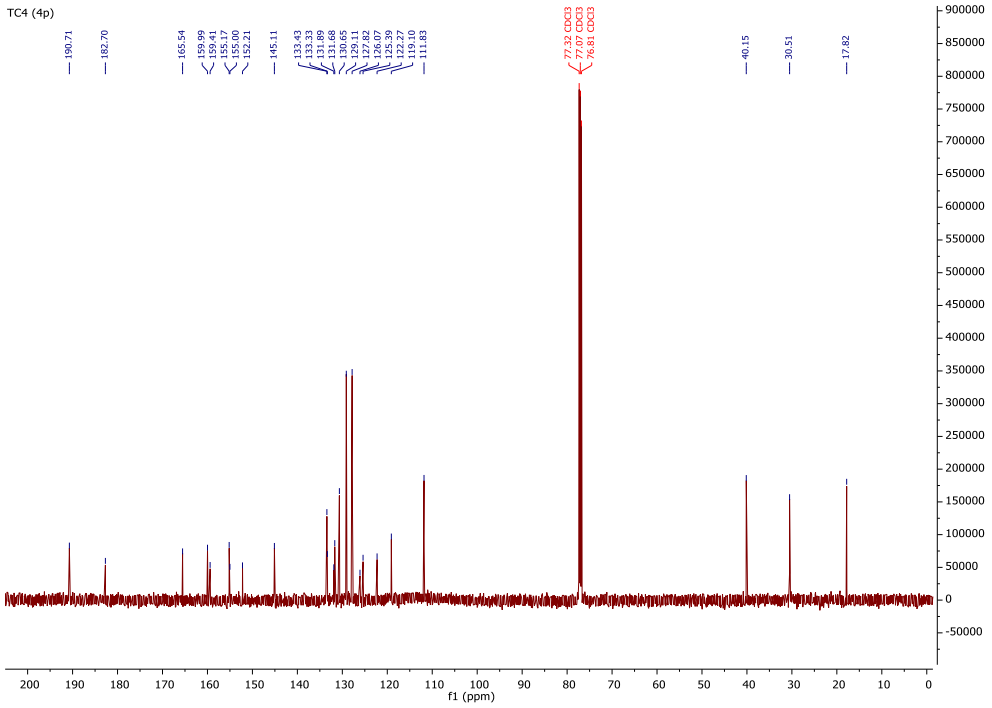
^{13}C NMR spectrum of **4n** (126 MHz, DMSO-d_6)

4p



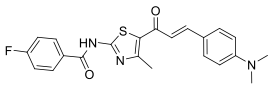


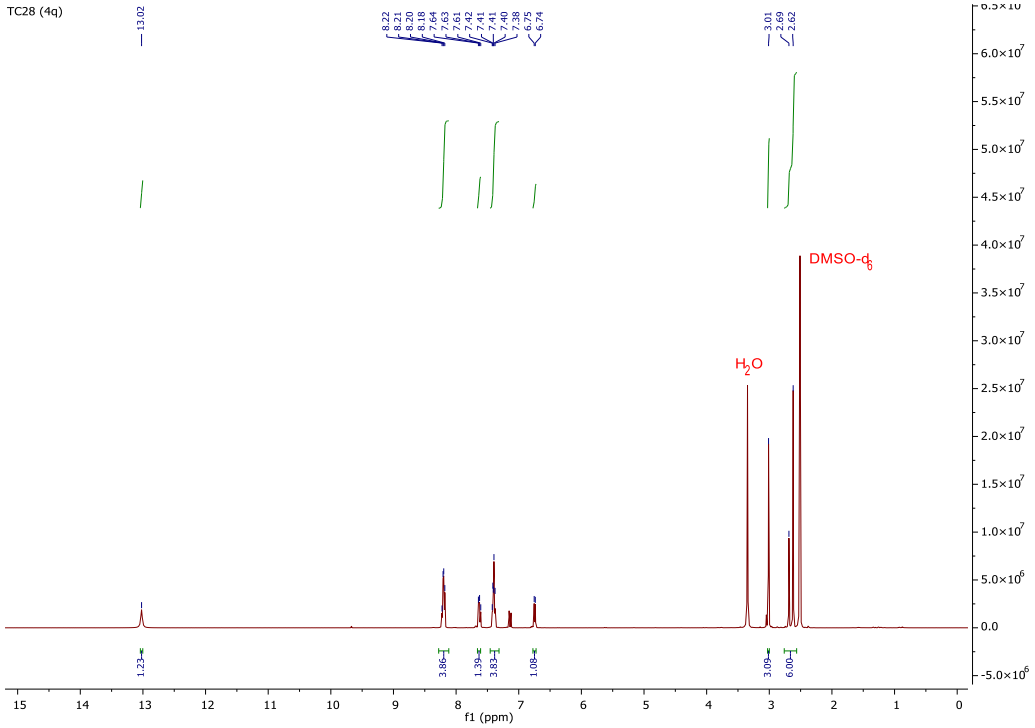
^{1}H NMR spectrum of **4p** (500 MHz, $CDCl_3$)



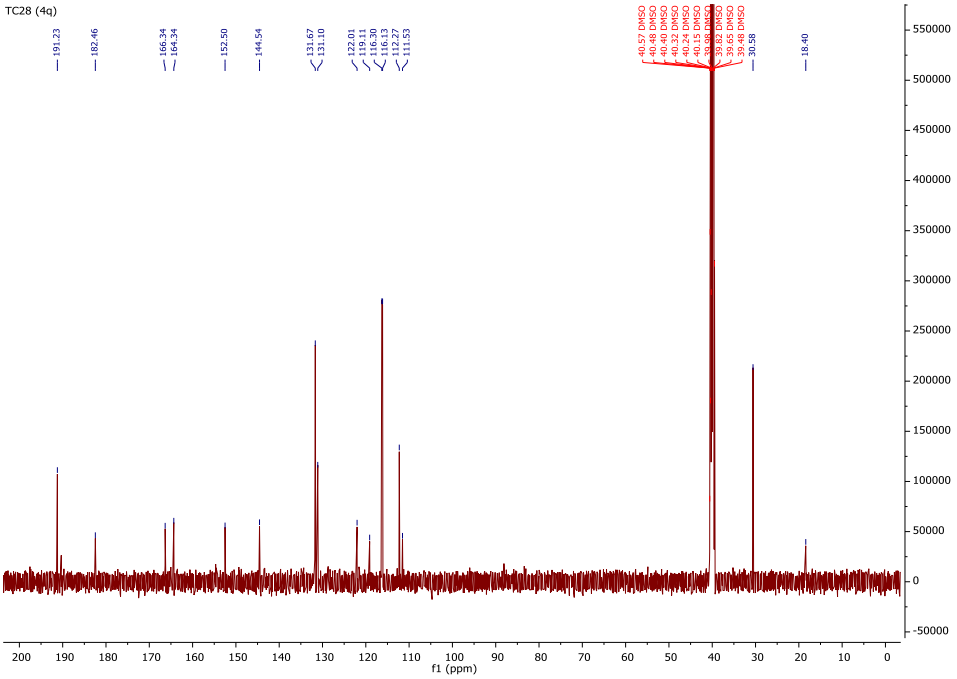
^{13}C NMR spectrum of **4p** (126 MHz, $CDCl_3$)

4q



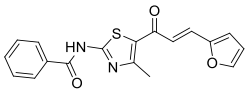


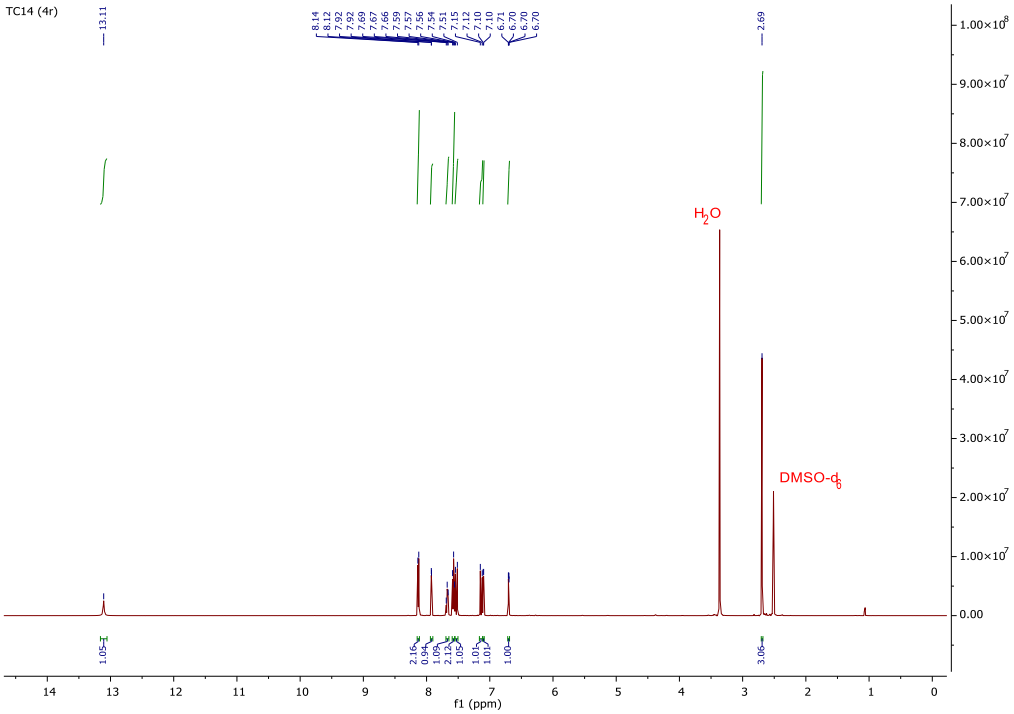
^{1}H NMR spectrum of **4q** (500 MHz, DMSO-d_6)



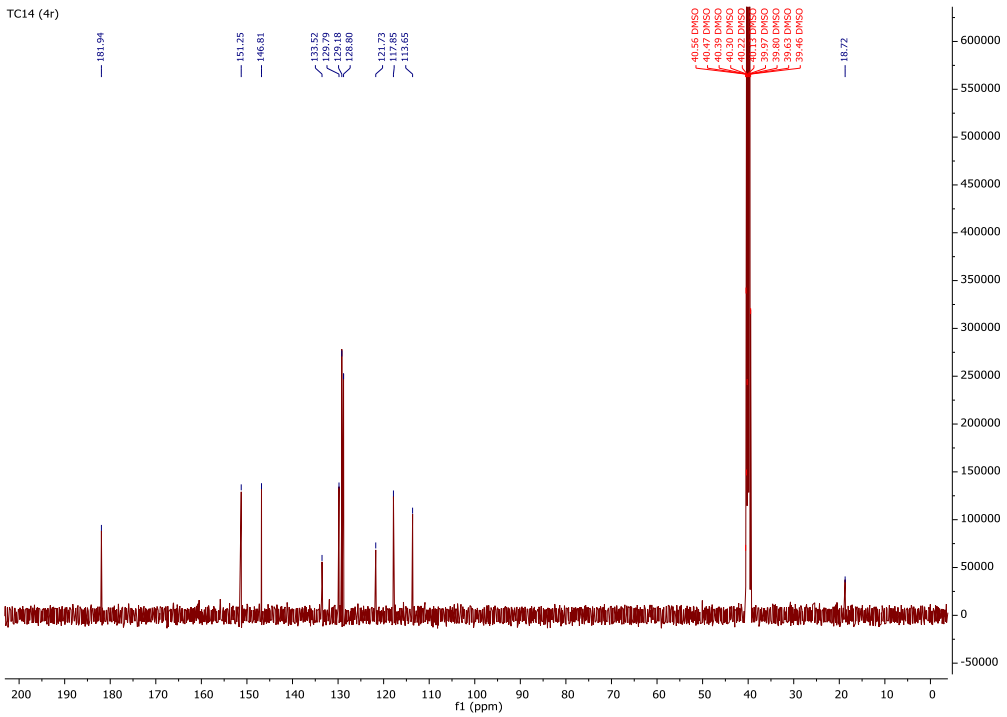
^{13}C NMR spectrum of **4q** (126 MHz, DMSO-d_6)

4r



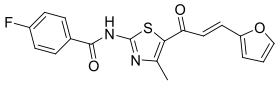


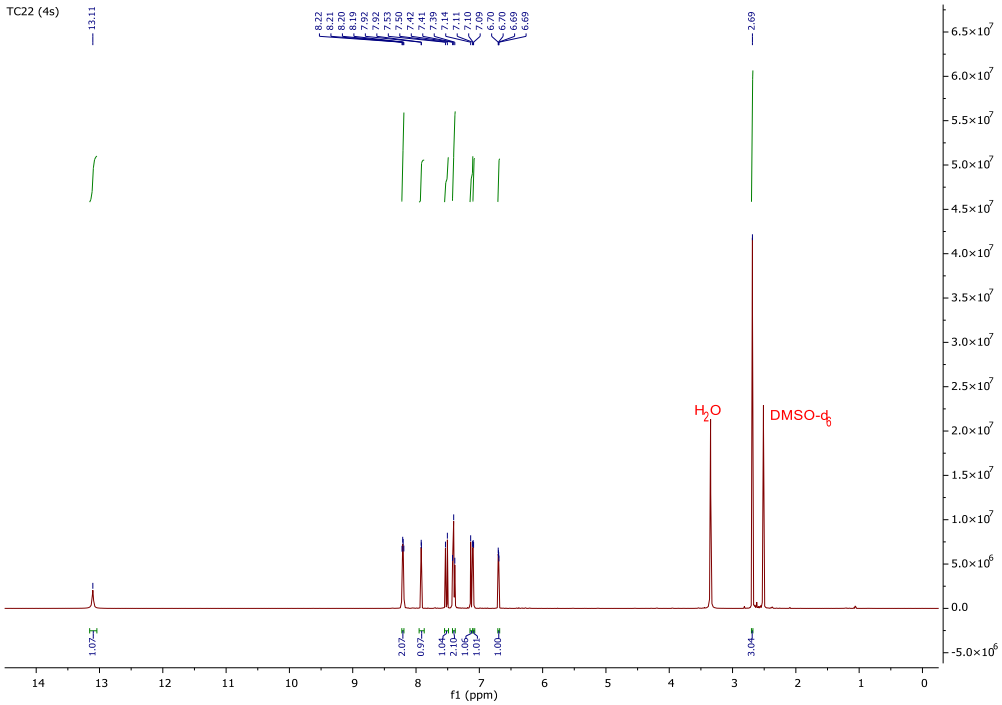
^{1}H NMR spectrum of **4r** (500 MHz, DMSO-d_6)



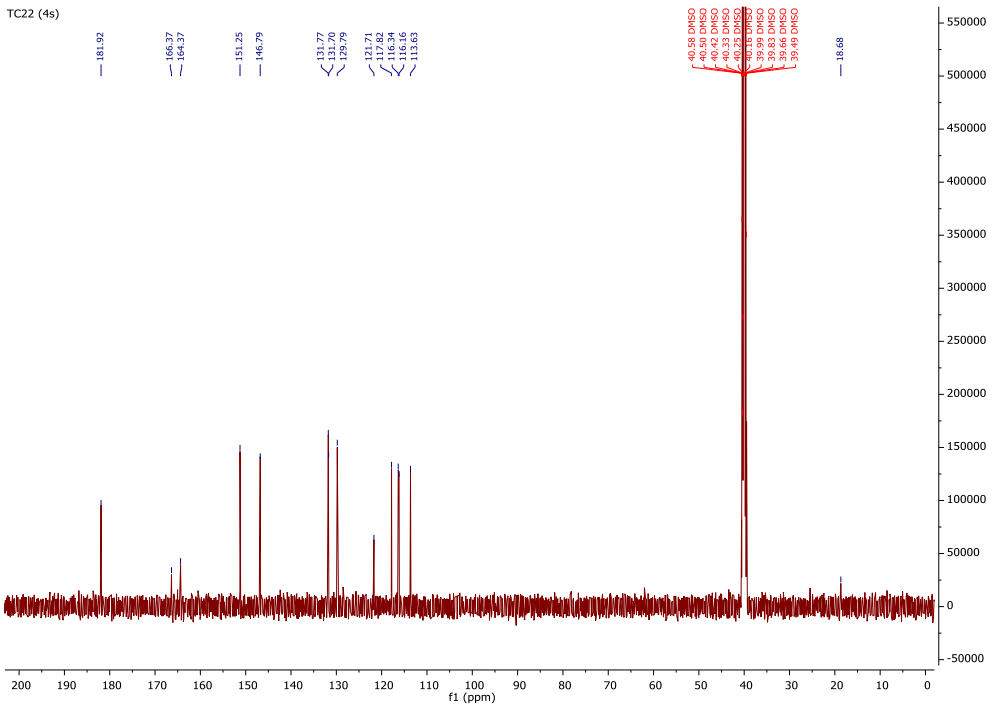
^{13}C NMR spectrum of **4r** (126 MHz, DMSO-d_6)

4s



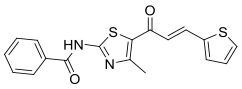


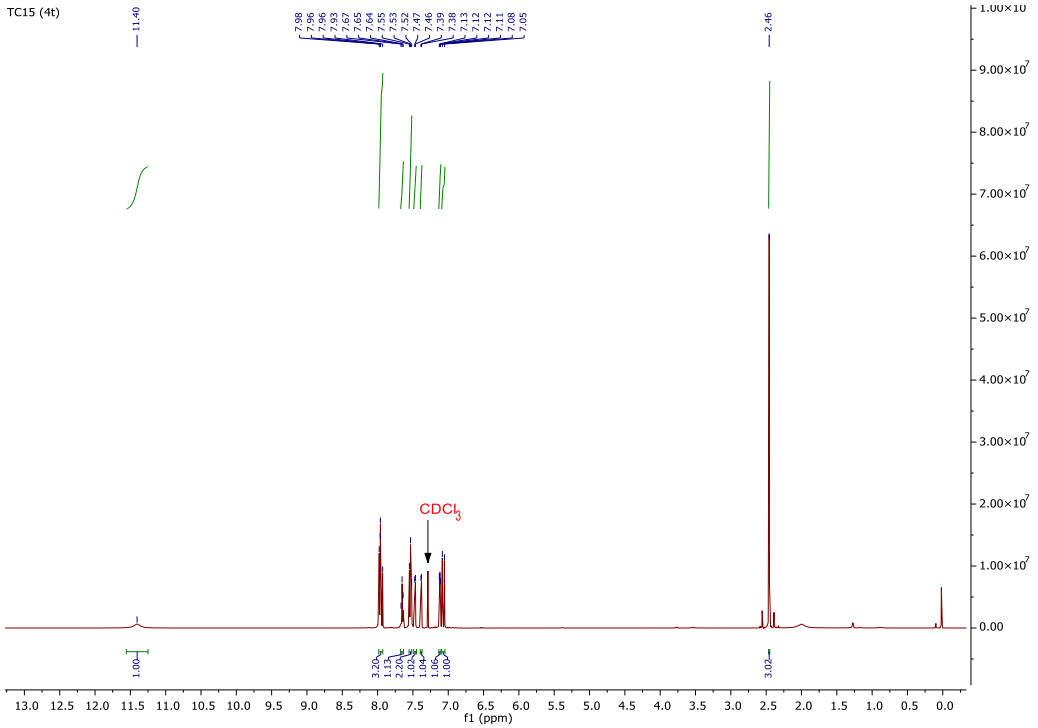
^{1}H NMR spectrum of **4s** (500 MHz, DMSO-d_6)



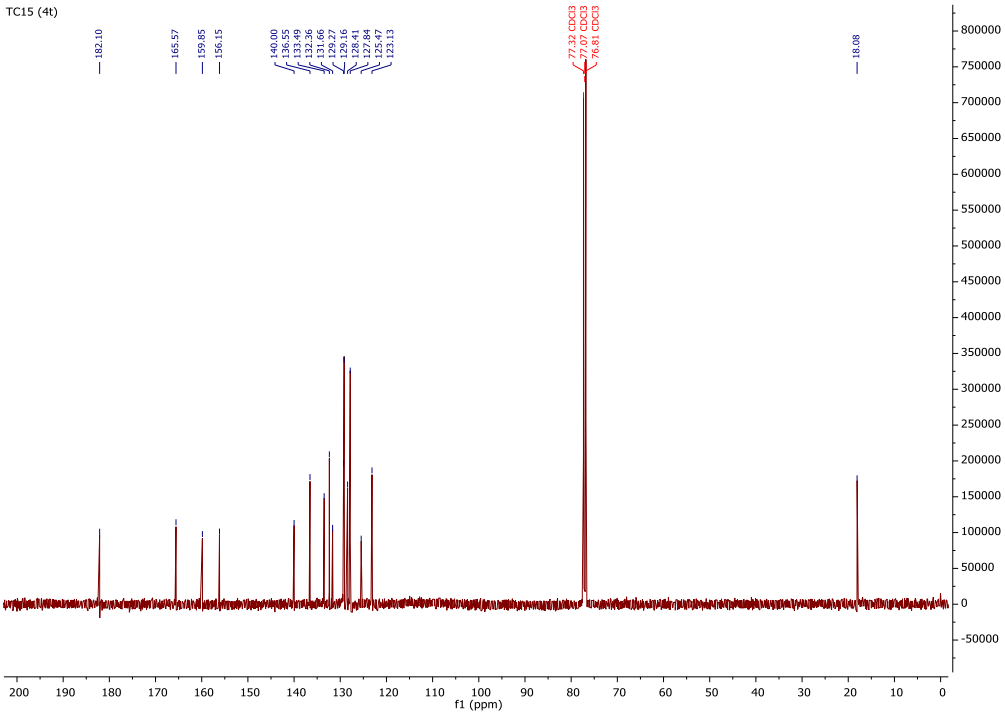
^{13}C NMR spectrum of **4s** (126 MHz, DMSO-d_6)

4t



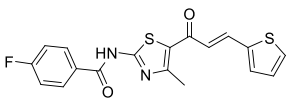


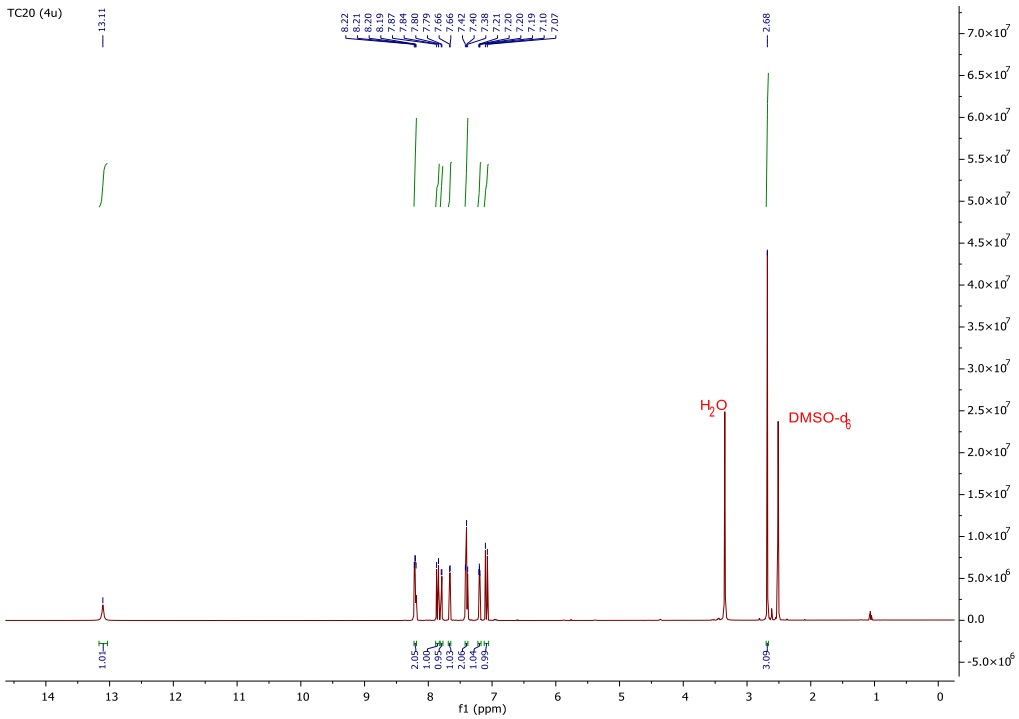
^{1}H NMR spectrum of **4t** (500 MHz, $CDCl_3$)



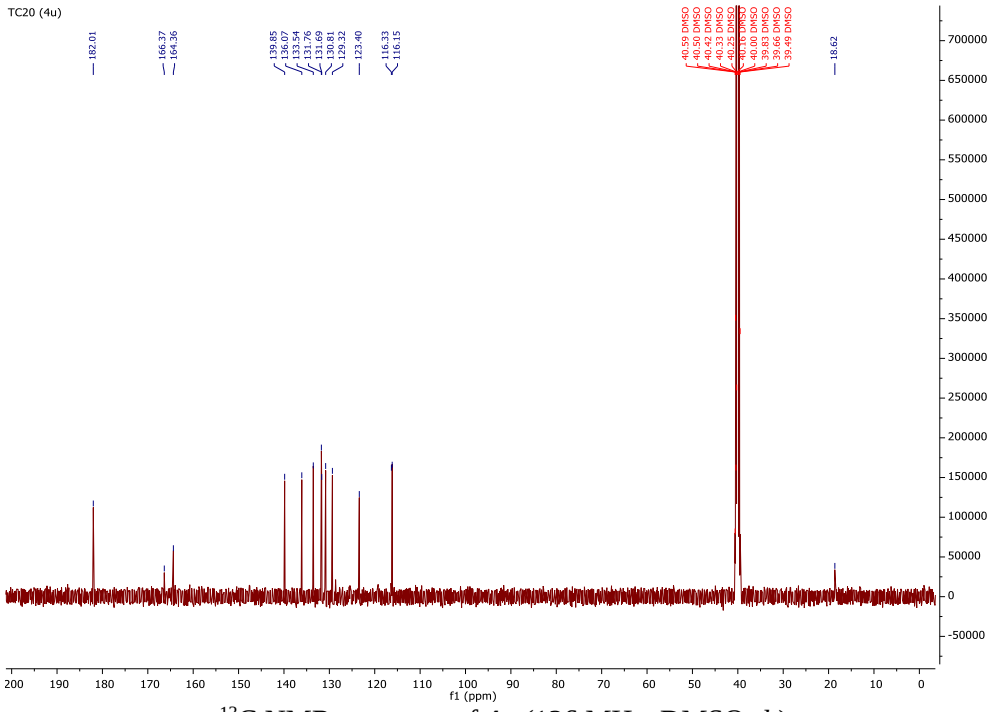
^{13}C NMR spectrum of **4t** (126 MHz, $CDCl_3$)

4u



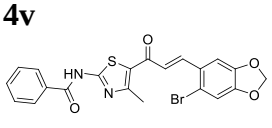


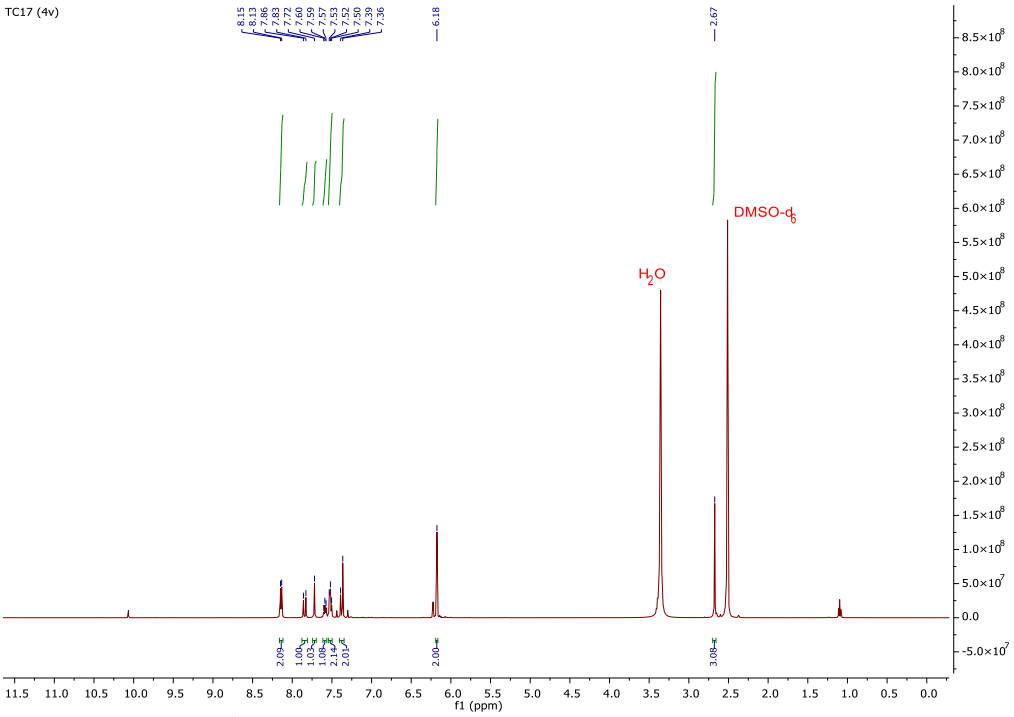
^{1}H NMR spectrum of **4u** (500 MHz, DMSO-d_6)



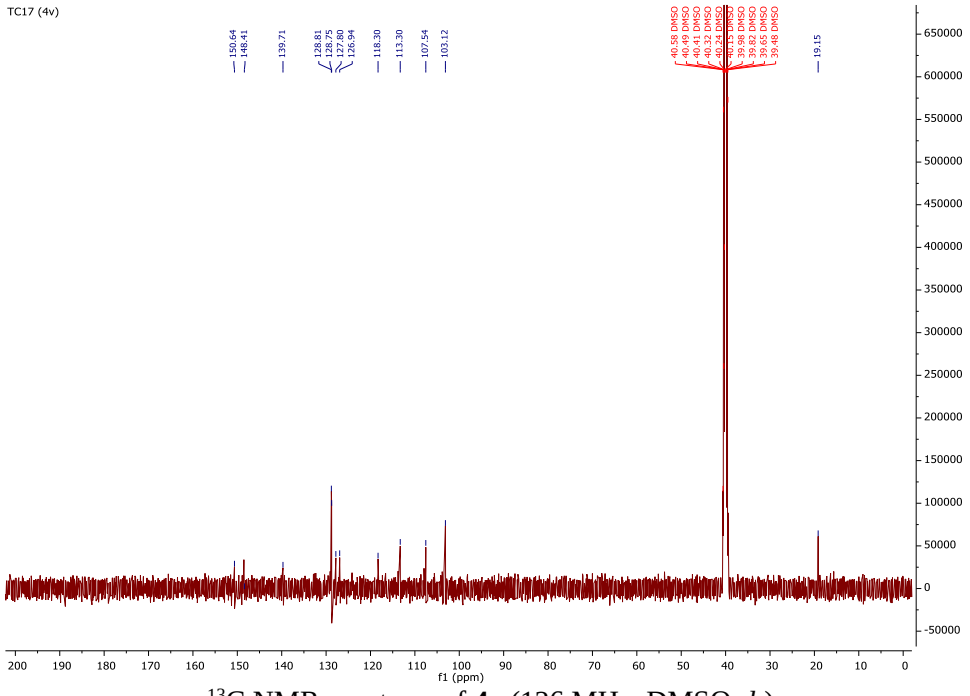
^{13}C NMR spectrum of **4u** (126 MHz, DMSO-d_6)

4v



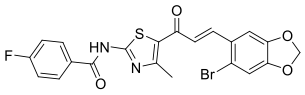


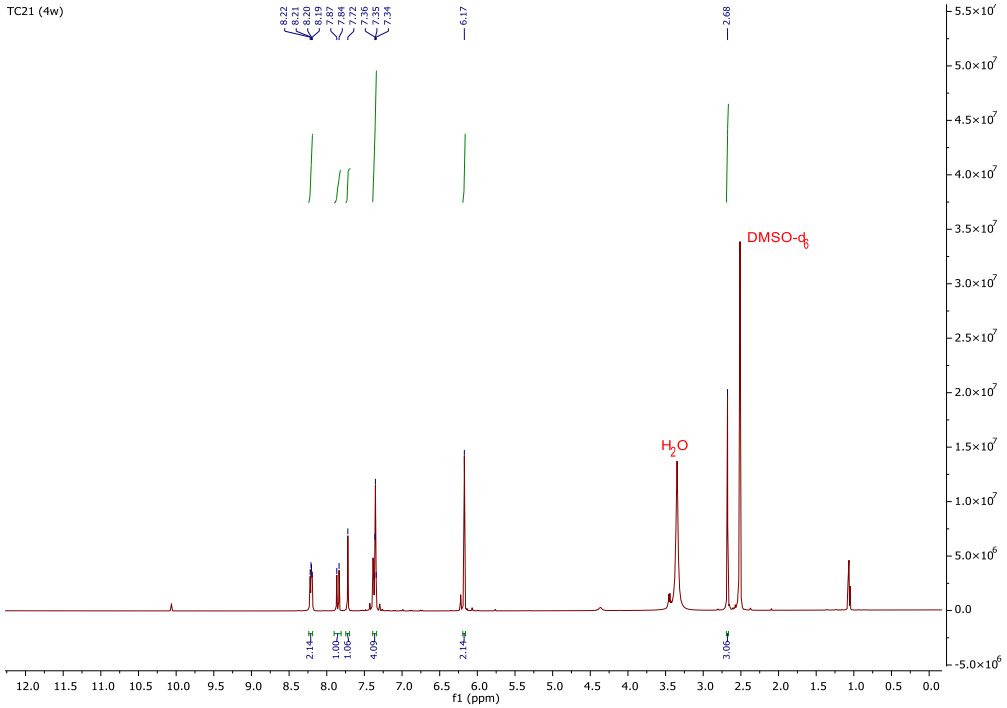
^{1}H NMR spectrum of **4v** (500 MHz, DMSO-d_6)



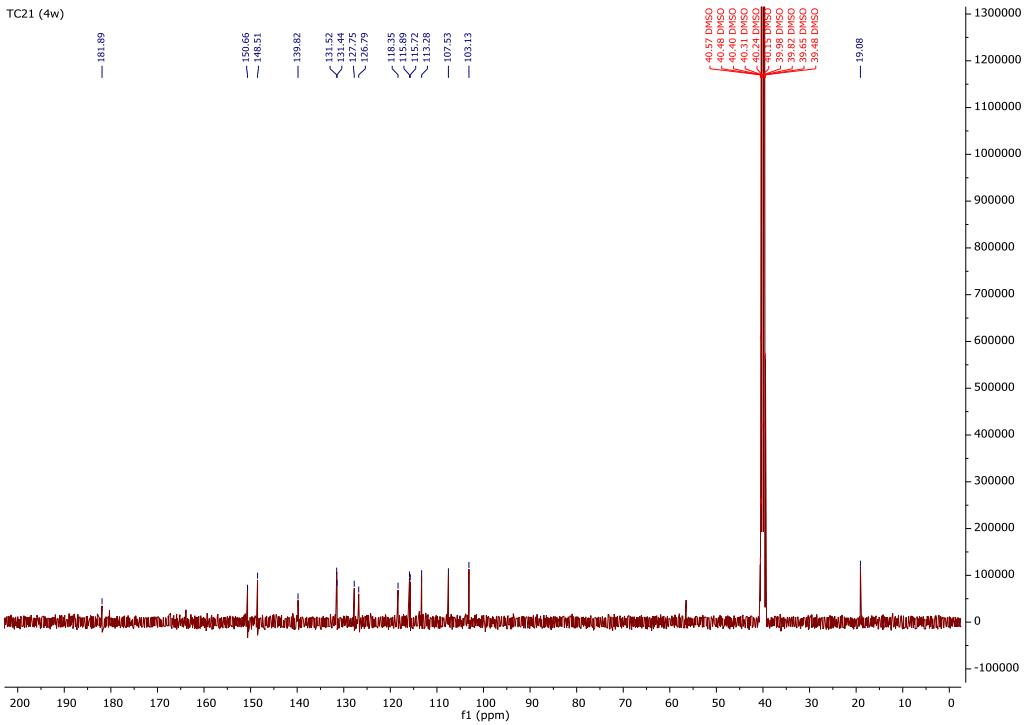
^{13}C NMR spectrum of **4v** (126 MHz, DMSO-d_6)

4w





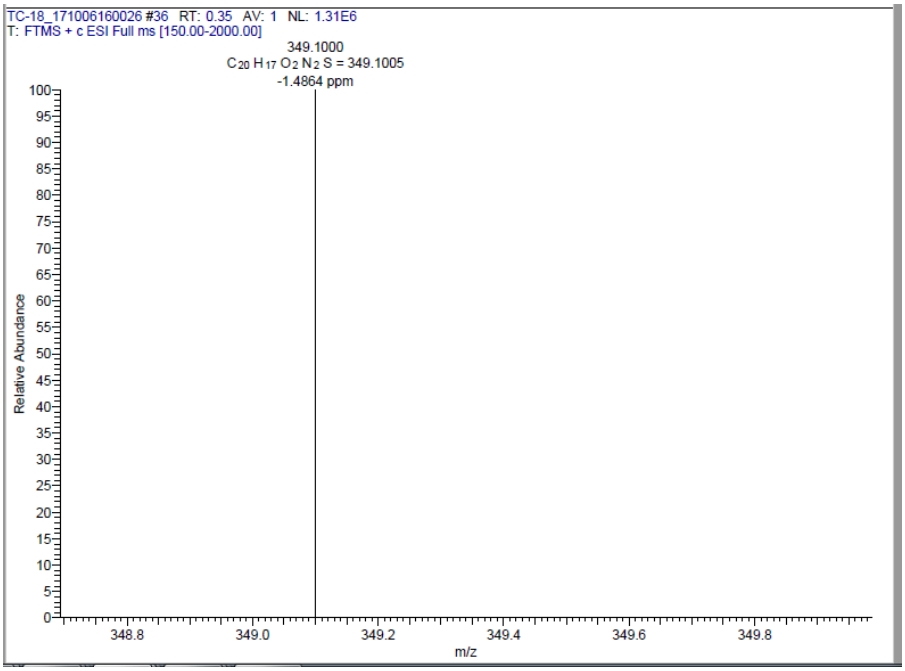
^{1}H NMR spectrum of **4w** (500 MHz, DMSO-d_6)



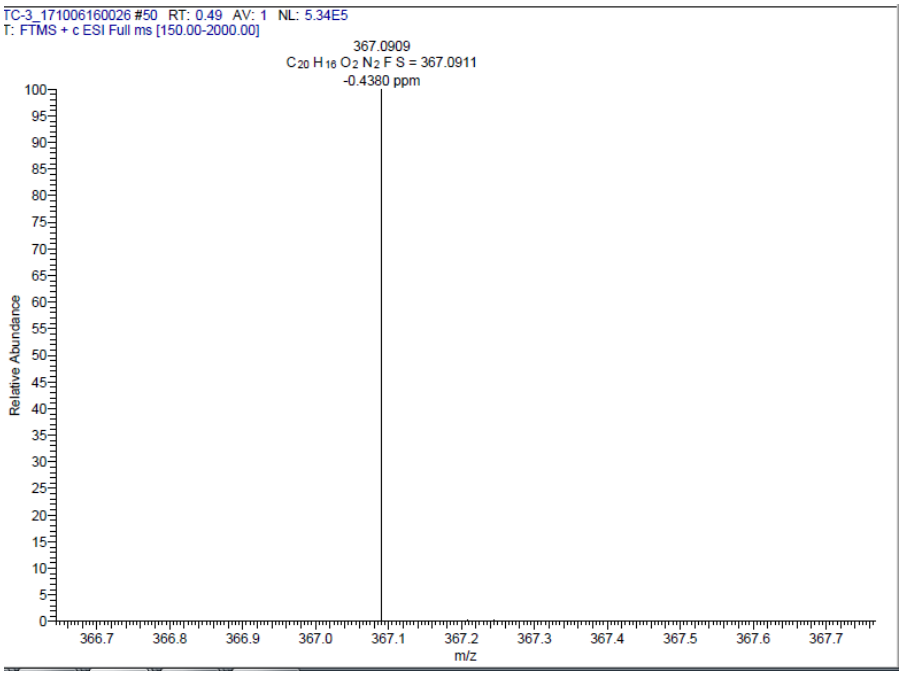
^{13}C NMR spectrum of **4w** (126 MHz, DMSO-d_6)

4. HRMS spectra for the target compounds (4a-w):

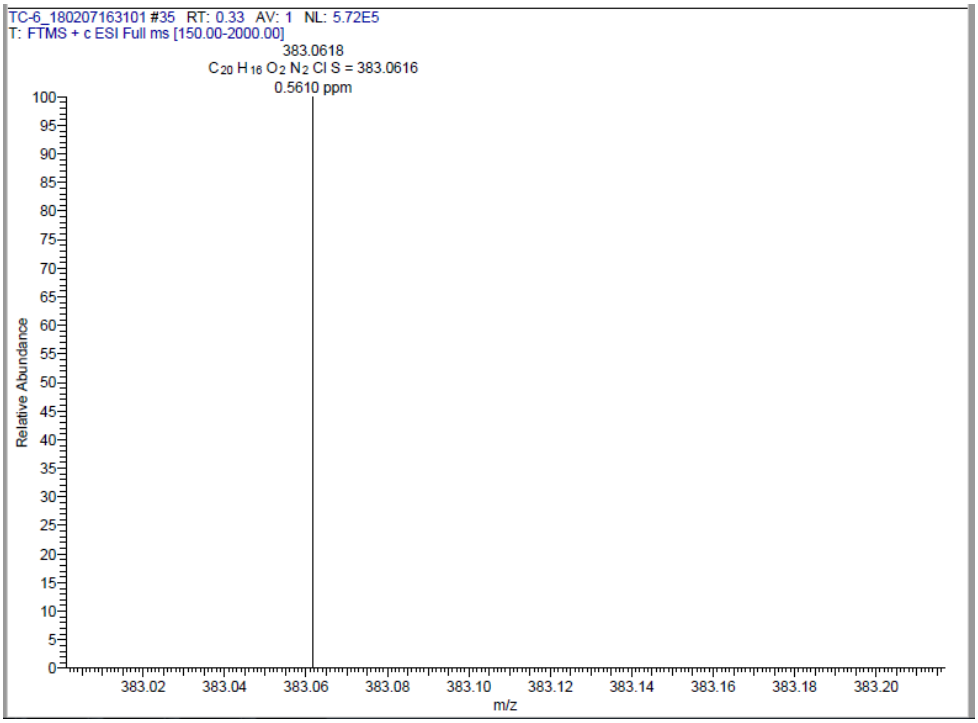
4a : $C_{20}H_{16}N_2O_2S$



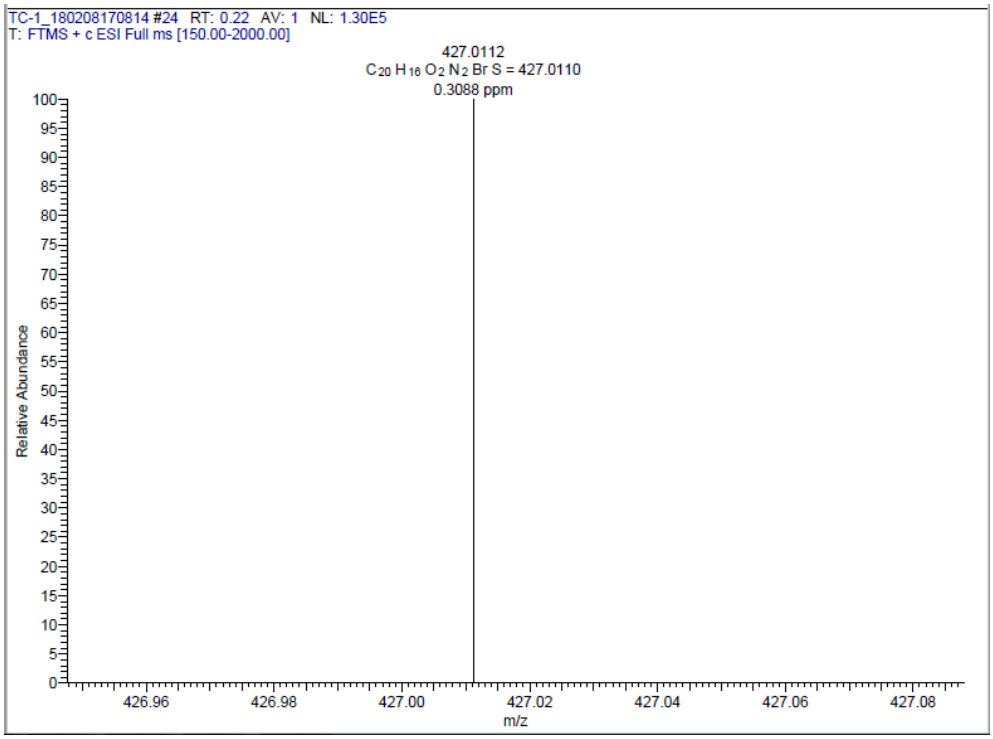
4b : $C_{20}H_{15}FN_2O_2S$



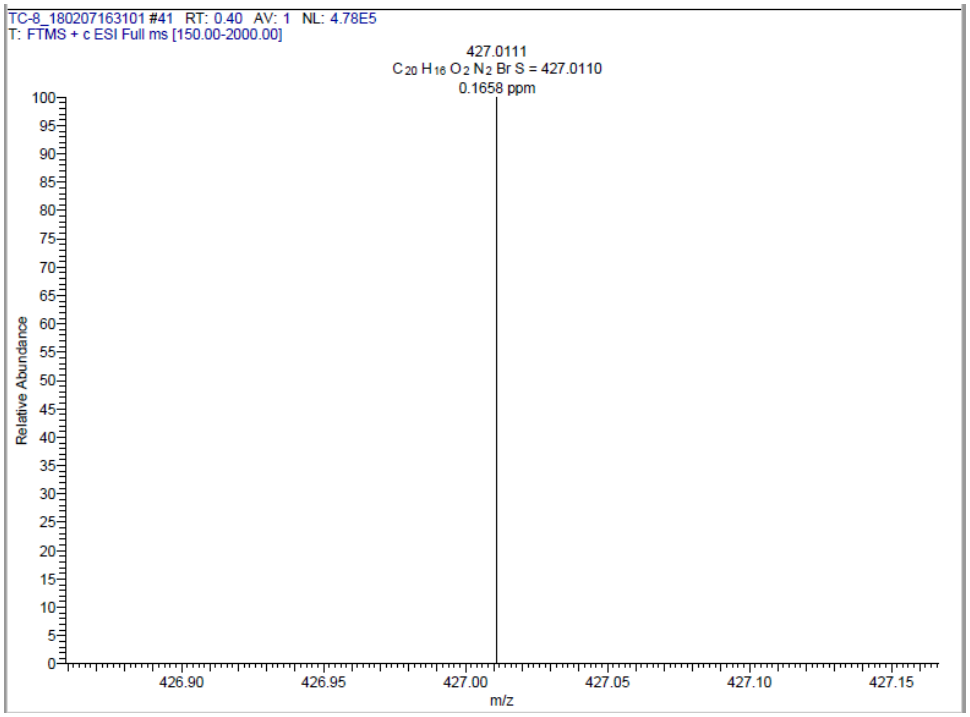
4c : $C_{20}H_{15}ClN_2O_2S$



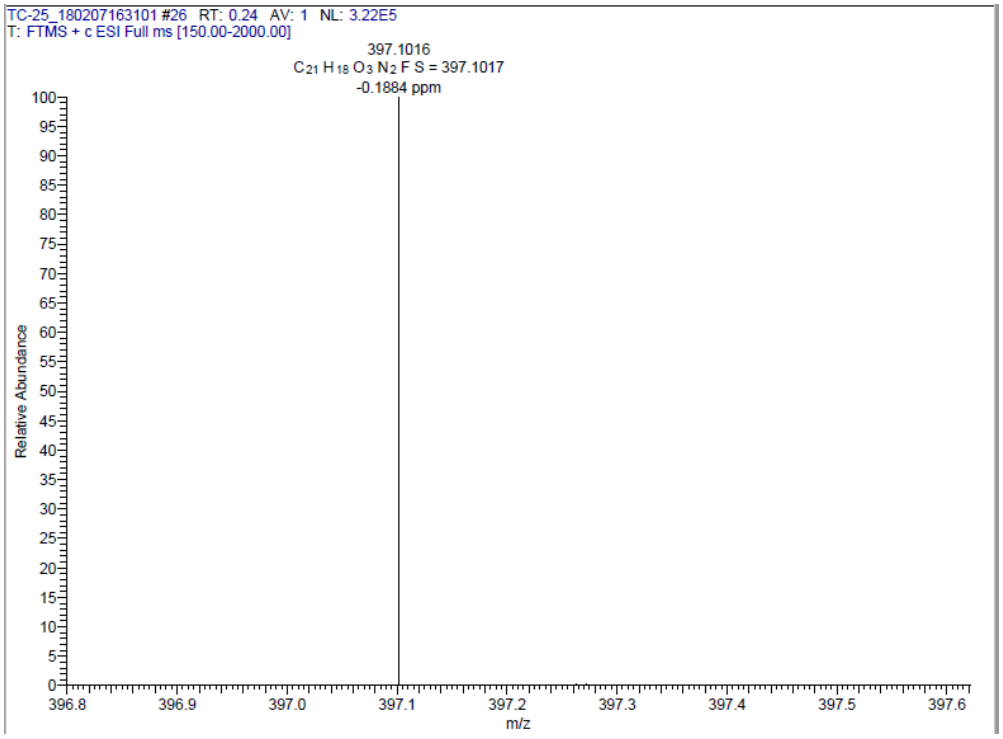
4d : $C_{20}H_{15}BrN_2O_2S$



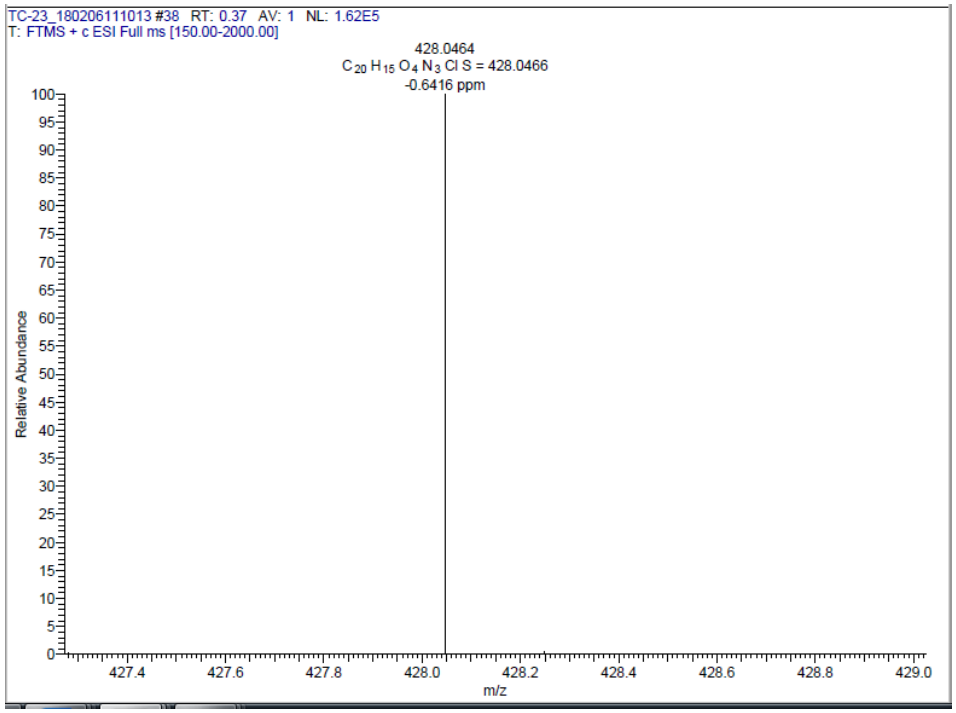
4e : $C_{20}H_{15}BrN_2O_2S$



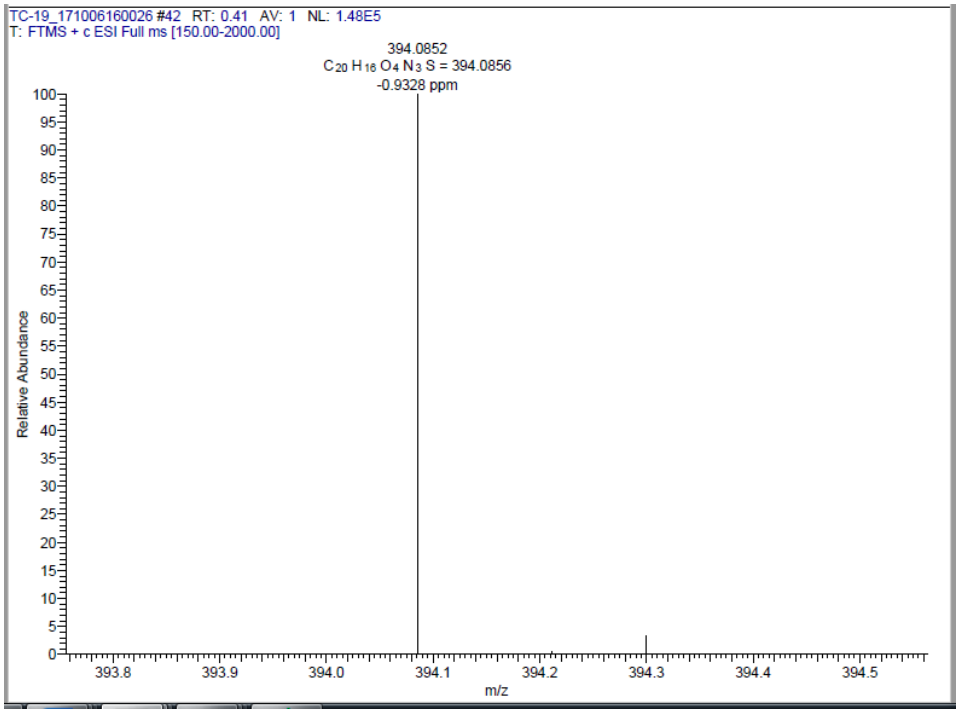
4f : $C_{21}H_{17}FN_2O_3S$



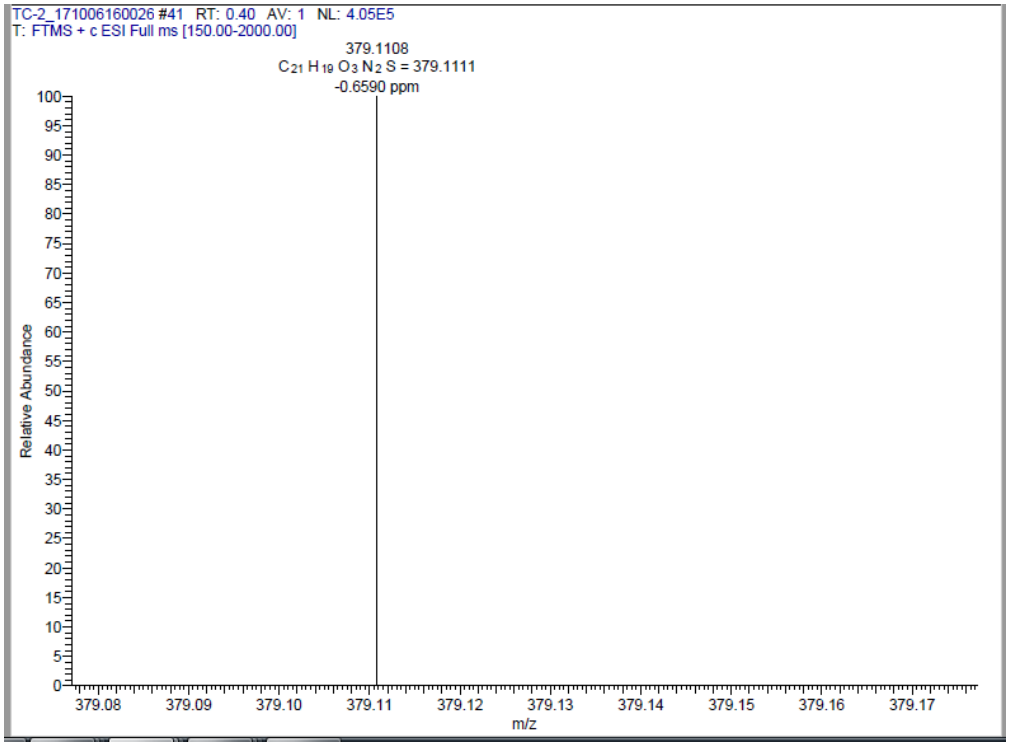
4g : $C_{20}H_{14}ClN_3O_4S$



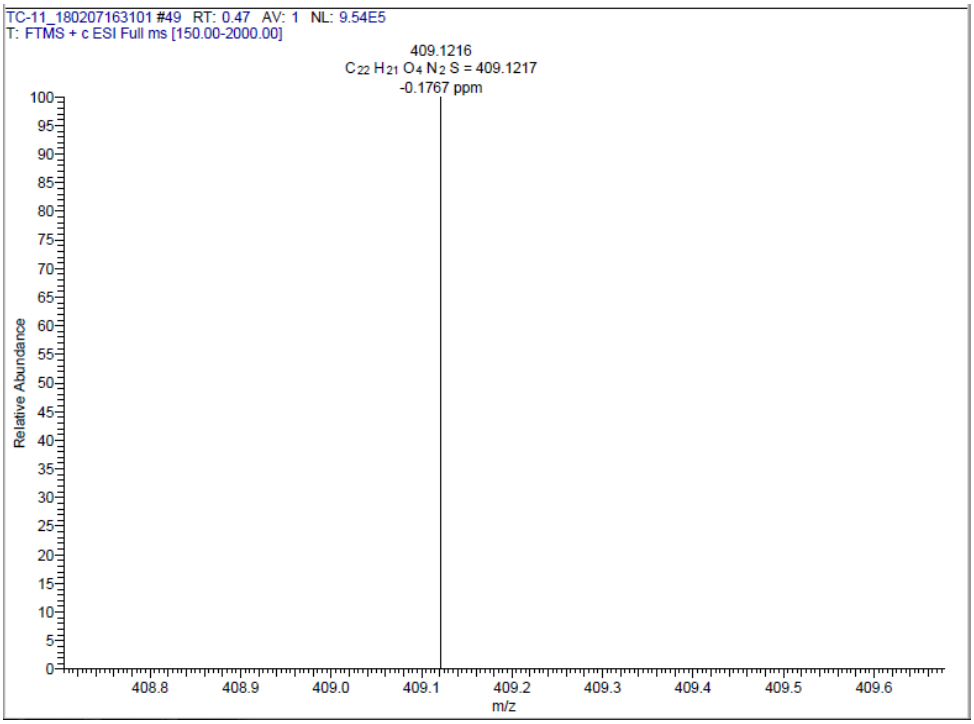
4h : $C_{20}H_{15}N_3O_4S$



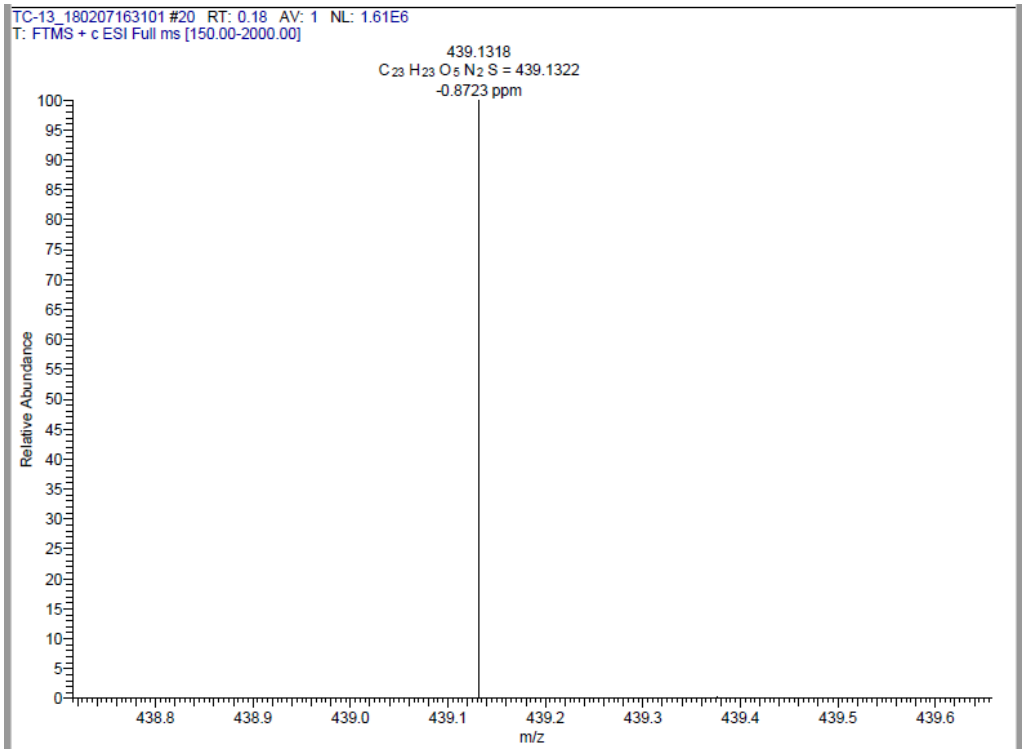
4i : $C_{21}H_{18}N_2O_3S$



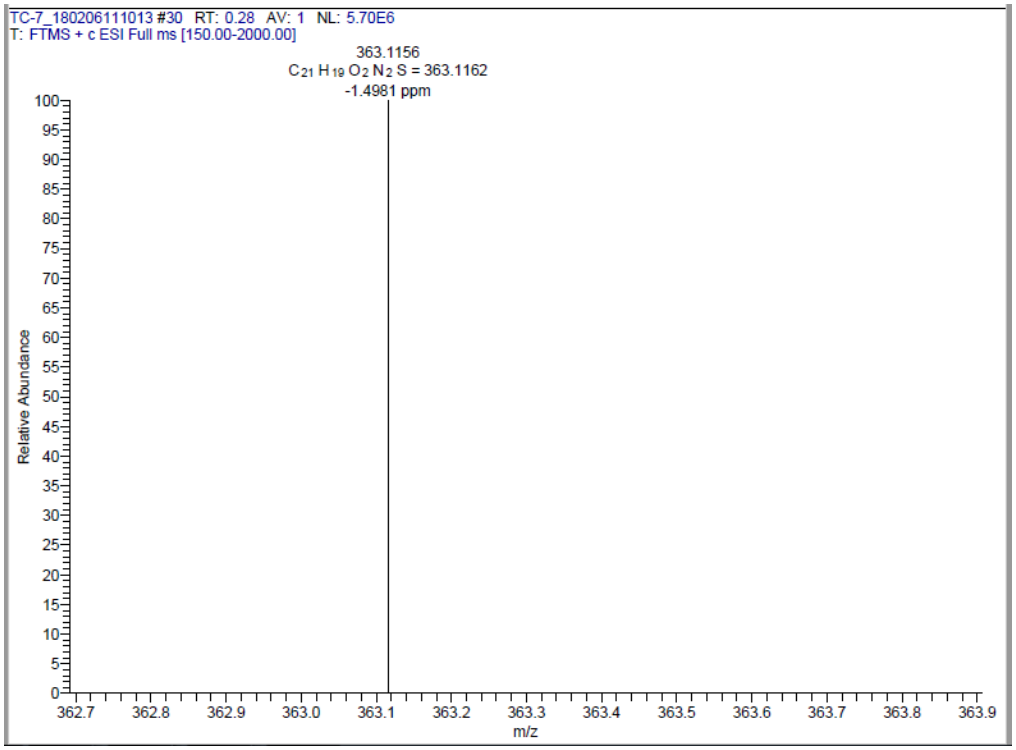
4j : $C_{22}H_{20}N_2O_4S$



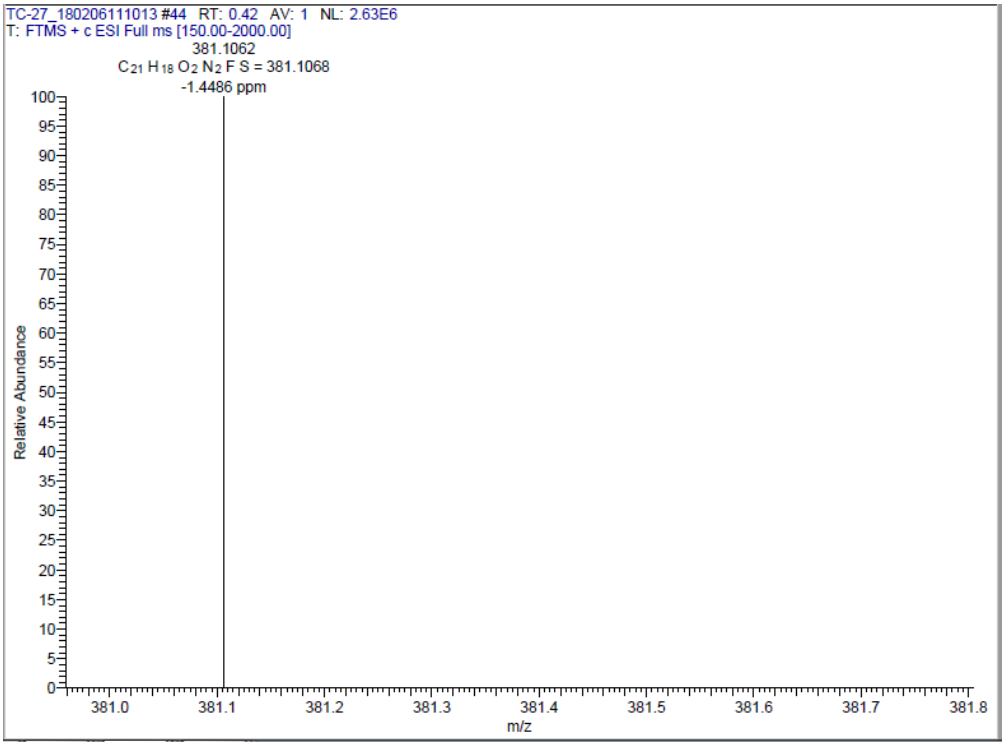
4k : $C_{23}H_{22}N_2O_5S$



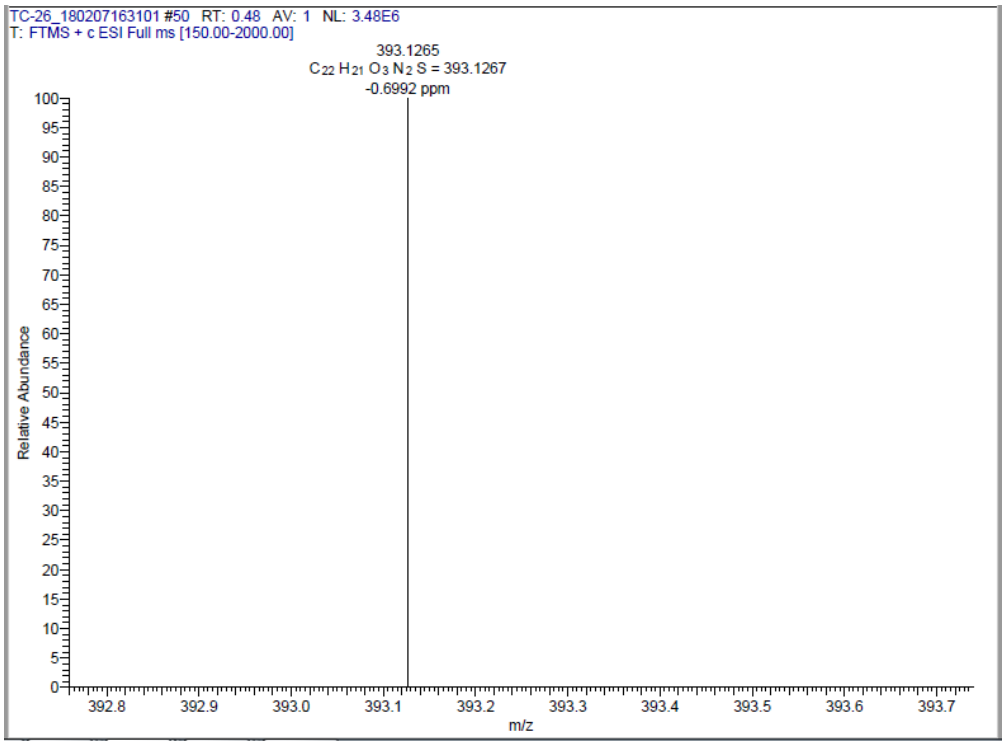
4l : $C_{21}H_{18}N_2O_2S$



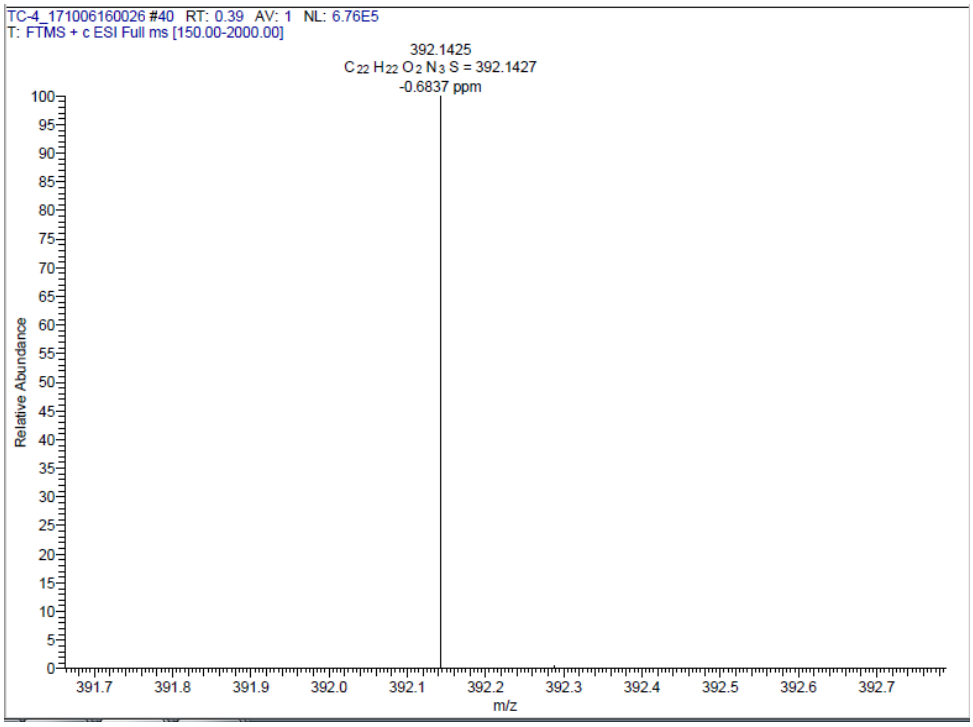
4m : $C_{21}H_{17}FN_2O_2S$



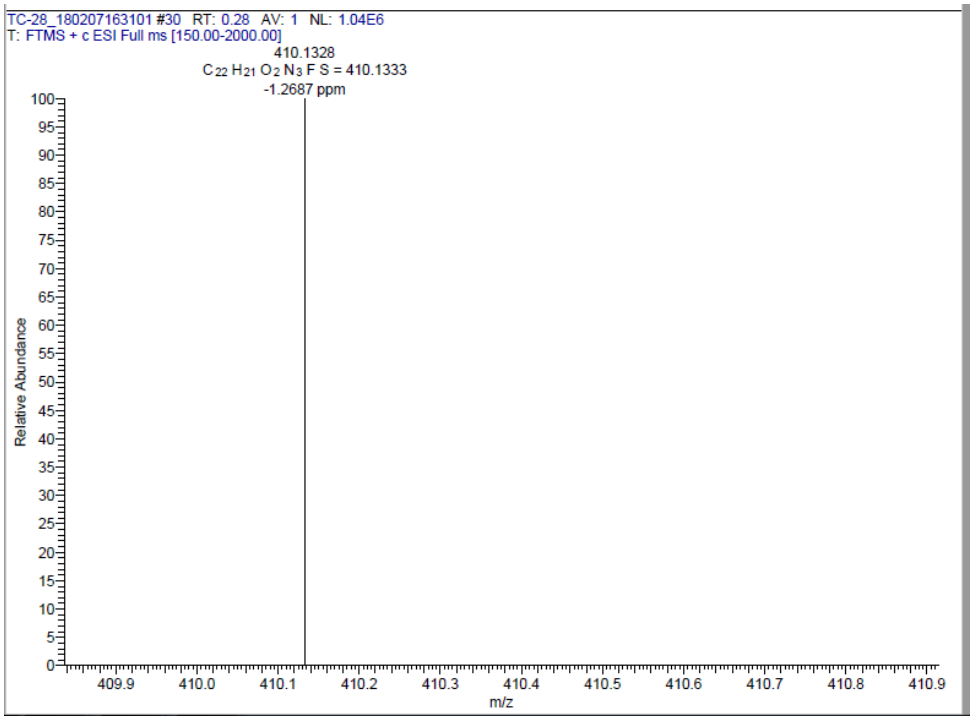
4n : $C_{22}H_{20}N_2O_3S$



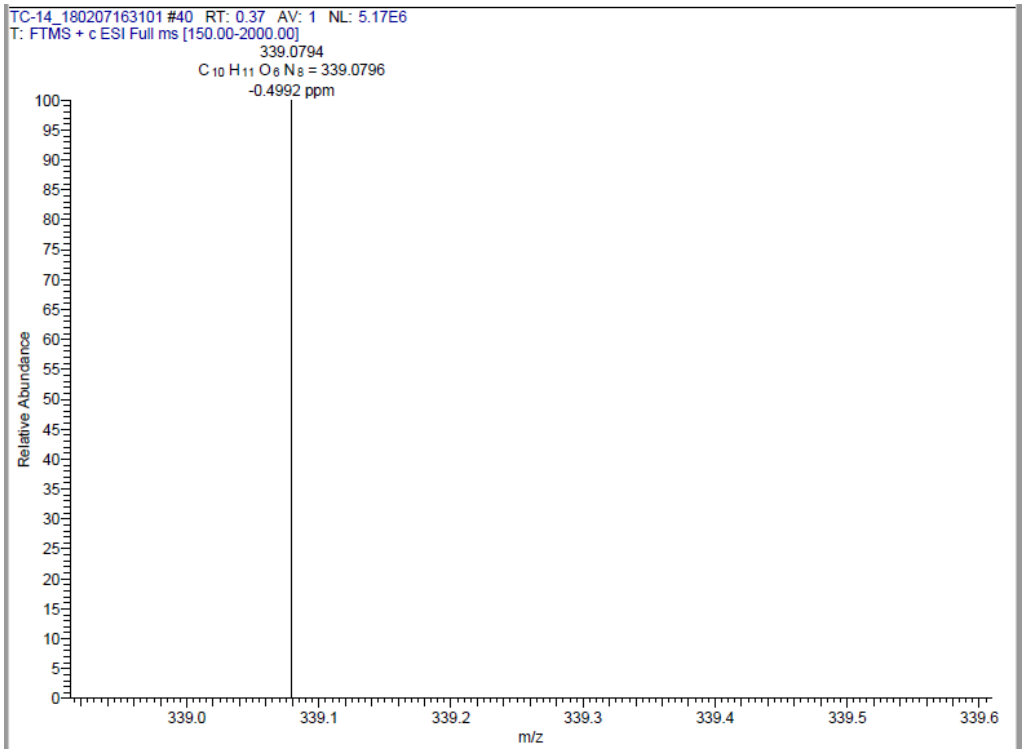
4p : $C_{22}H_{21}N_3O_2S$



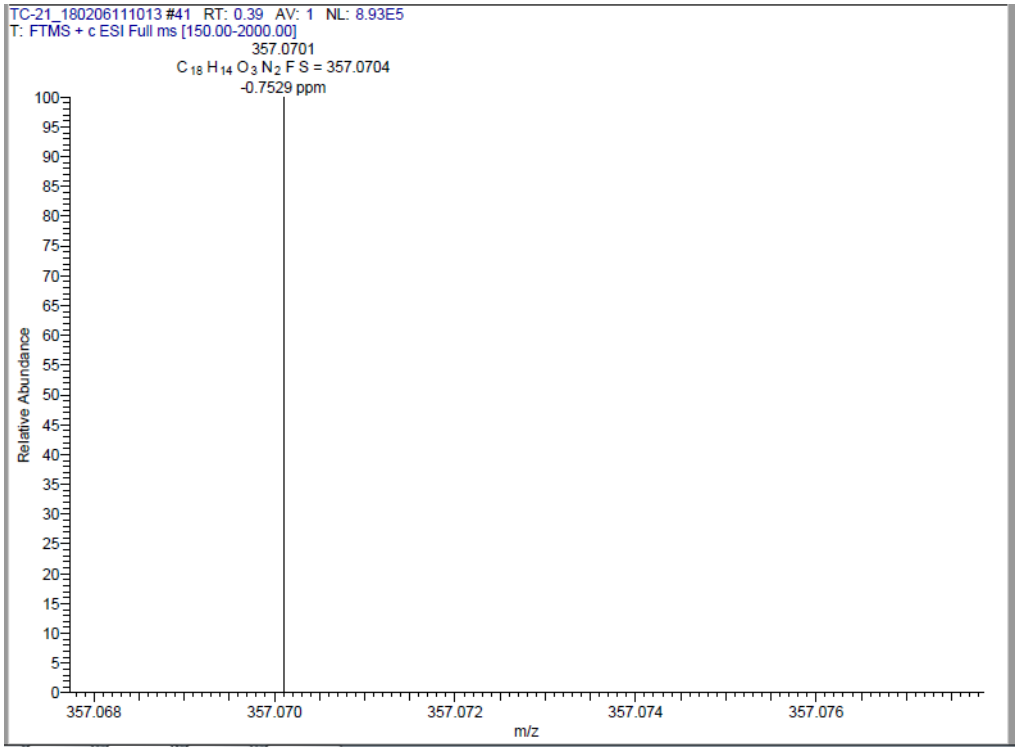
4q : $C_{22}H_{20}FN_3O_2S$



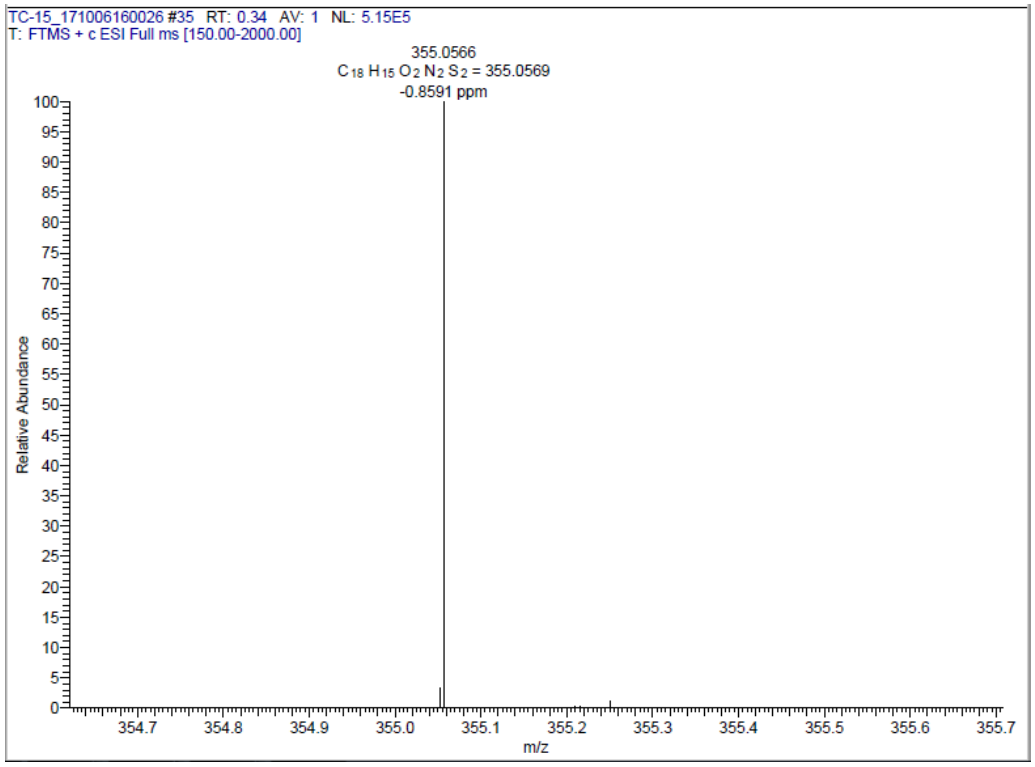
4r : $C_{18}H_{14}N_2O_3S$



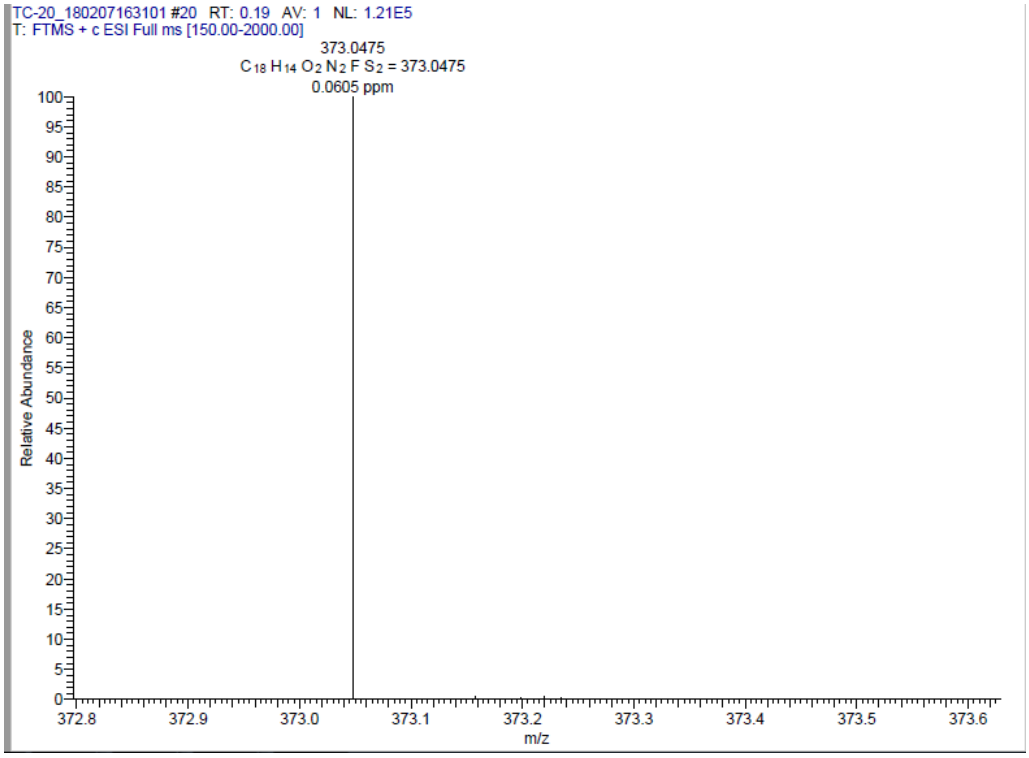
4s : $C_{18}H_{13}FN_2O_3S$



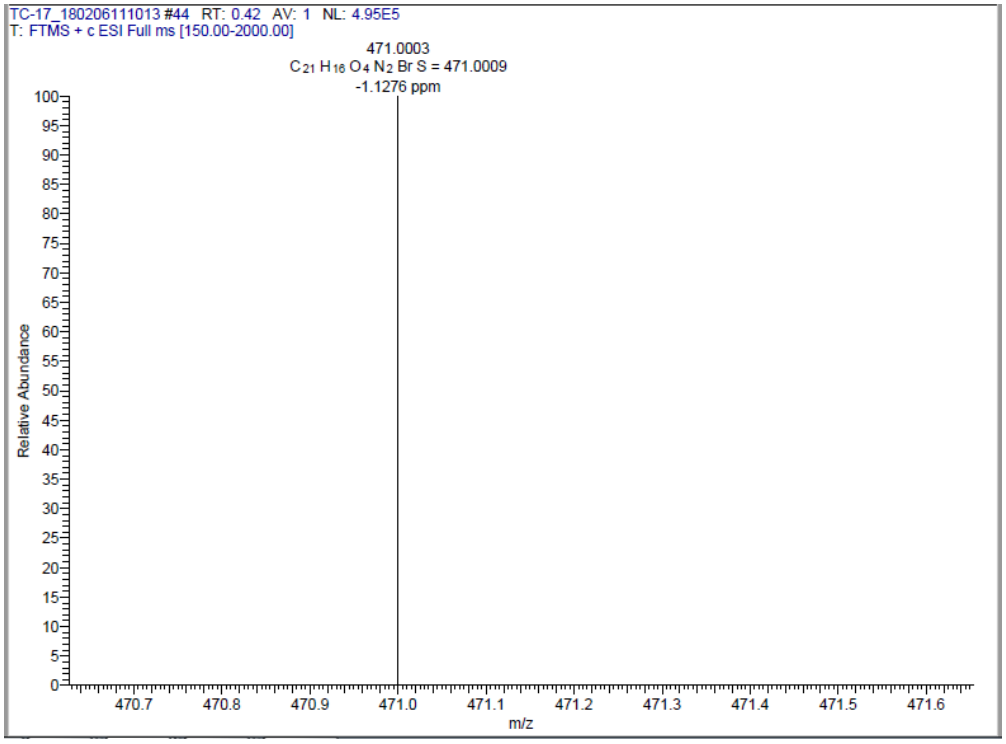
4t : $C_{18}H_{14}N_2O_2S_2$



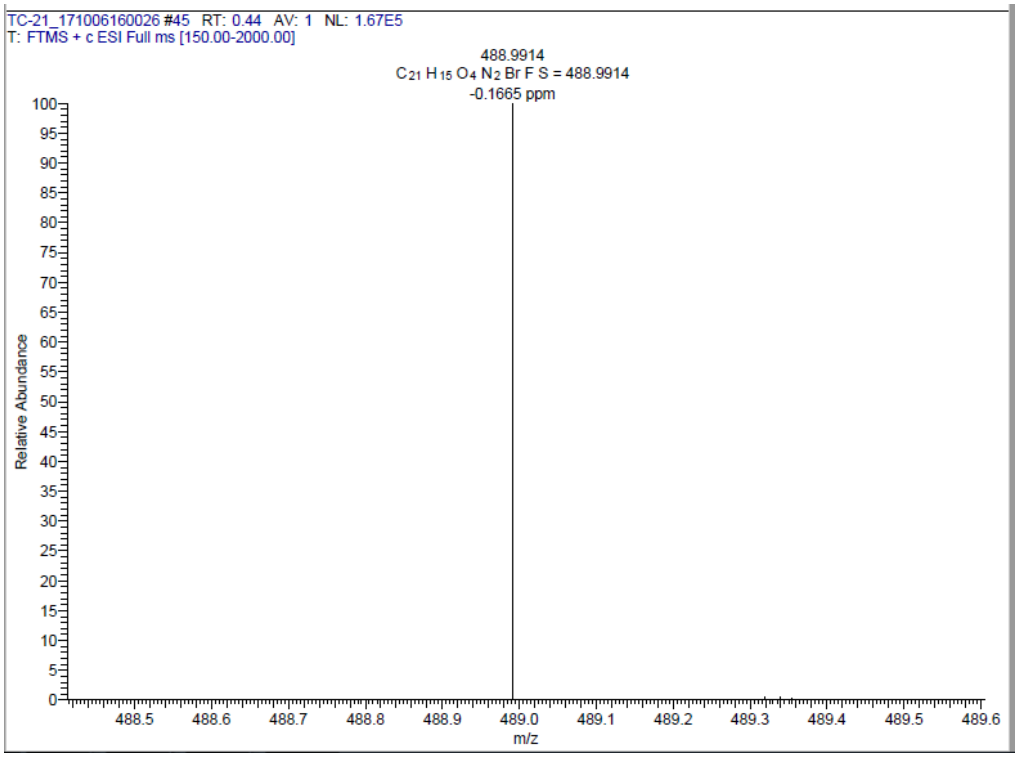
4u : $C_{18}H_{13}FN_2O_2S_2$



4v : $C_{21}H_{15}BrN_2O_4S$



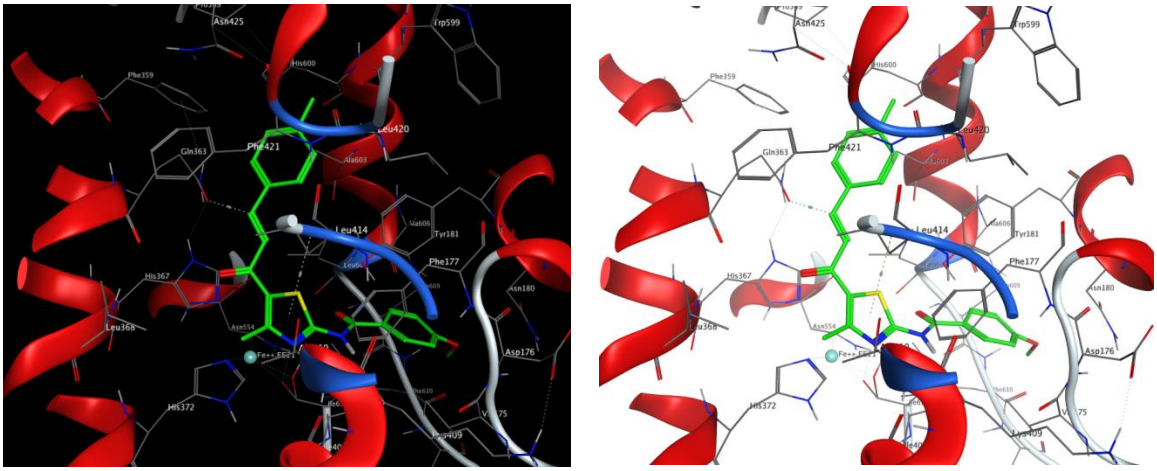
4w : $C_{21}H_{14}BrFN_2O_4S$



5. Docking protocol, images and PDB ID number

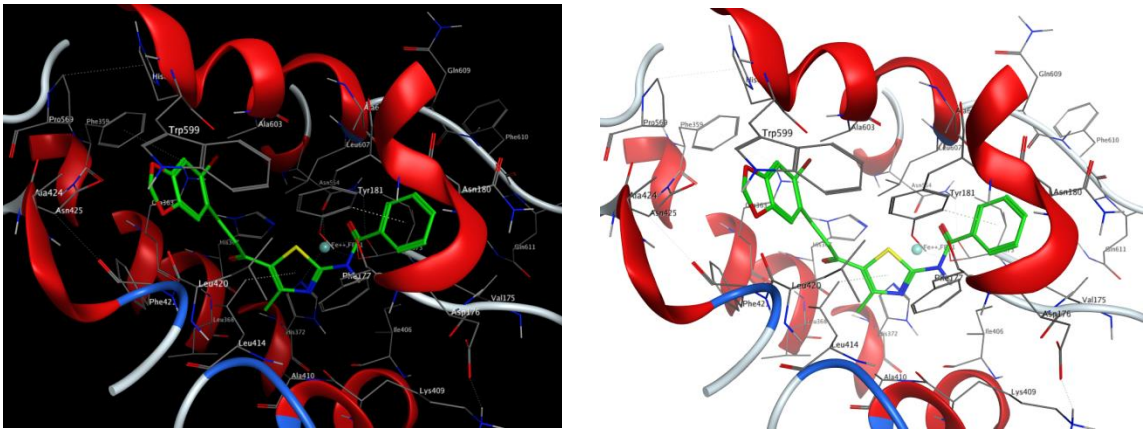
The software used for docking was Molecular operating environment (MOE; version 2016.0801, Chemical Computing Group, Suite 910, Canada). The X-ray crystal structure of protein 5-LOX was obtained from Protein Data Bank (PDB Code: 3O8Y). The protein structure was then processed for protein preparation by removing duplicate chain B, excess water molecules and non-receptor ions. In the next step, kollmann charges were assigned and polar hydrogens were added. Then, energy of the protein was minimized to a least possible energy state using AMBER99 force field. Ligand preparation was done by drawing their structures in MOE and minimizing energies to the lowest energy state using MMFF94x force field. The active site of the enzyme 5-LOX was selected as per previous literature reports comprising of amino acids: Trp147, Phe177, Tyr181, Thr364, His367, Leu373, Ile406, Asn407, Leu414, Leu420, Phe421, His432, His550, Trp599, His600, Ala606, Ile673.[1] In the selected active site, the ligands **4n** and **4v** were docked and the binding energy was calculated using LondondG and GBVI/WSAdG rescoring methodologies. The ligand-receptor interaction was analysed for the best fit pose and the lowest binding energy with H-bonds.

Ref. 1. Gilbert, N. C.; Bartlett, S. G.; Waight, M. T.; Neau, D. B.; Boeglin, W. E.; Brash, A. R.; Newcomer, M. E. The structure of human 5-lipoxygenase. *Science* **2011,** 331, 217-219.



(a) (b)

Figure1. Docked pose of compound **4n** in stick model with enzyme 5-LOX (PDB Code: 3O8Y) (a) black (b) white background with ionic interactions.



(a) (b)

Figure2. Docked pose of compound **4v** in stick model with enzyme 5-LOX (PDB Code: 3O8Y) (a) black (b) white background with ionic interactions.

6. Experimental section of biological activity

Arachidonic acid, Zileuton and 13(S) HpODE) and were obtained from Cayman Chemicals (Inalco, Milan, Italy). 5-LOX-pT3 plasmid was received as a generous gift from Prof. Olof Rådmark, Karolinska Institute, Stockholm, Sweden.

Expression of Recombinant 5-LOX Enzyme. The expression and purification of human recombinant 5-LOX were performed as per previously reported methodology.[1,2] 5-LOX in a pT3 plasmid was transformed into *E. coli* BL21 bacteria and grown overnight with 150 µg/mL ampicillin in LB medium at 37 °C. The culture was induced with 0.5 mM of isopropyl-D-thiogalactopyranoside (IPTG) when OD_{600} attained between 0.5-1. The medium was shaked overnight at 18 ºC and cells were pelleted out at 5000 rpm and 4 °C by centrifugation (Centrifuge 5804 R, Eppendorf AG). Cell pellets were incubated for lysis in buffer solution of 50 mM of triethanolamine/HCl at a pH 8.0 containing 5 mM of ethylenediaminetetraacetic acid (EDTA), 1 mM of phenylmethylsulphonylfluoride (PMSF), 60 µg/mL of trypsin inhibitor and 500 µg/mL of lysozyme. Further, sonication for 42 s and centrifugation (Centrifuge 5418 R, Eppendorf AG) at 19000 × g and 4 °C for 15 min of cell lysate were performed. The supernatant collected was precipitated with 50 % w/v ammonium sulphate solution, centrifuged at 16000 × g and 4 °C for 30 min and pellet was resuspended in Phosphate buffered saline (PBS) containing 1 mM EDTA and 1 mM PMSF. Further supernatant collected from centrifugation at 100000 x g and 4 °C for 70 min were immediately used for 5-LOX enzyme activity assays.

***In vitro* 5-LOX Inhibition Assay.** The activity of enzyme was measured in cell free system by determining the product, 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE) converted from AA in presence of 5-LOX at λ_{236}. An assay reaction mixture used for 5-LOX inhibition studies was 25 mM of HEPES buffer, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (pH 7.3) containing 10 mM of $CaCl_2$, 0.4 M of ethylenediaminetetraacetic acid (EDTA), 4 mM of adenosine 5'-triphosphate (ATP). Test compounds were dissolved in DMSO (2 % v/v) for the assay. For 5-LOX enzyme activity assay, test compound (10 μM) was mixed in the assay buffer and reaction was started by the addition of the substrate AA (30 μM). The UV absorbance was taken at λ_{max}, 236 nm (Jasco V-550 UV–vis spectrophotometer) for the activity which was measured in terms of the product, 5-HPETE formed from AA. The positive control used was Zileuton. IC_{50} of the active compounds were determined using GraphPad Prism version 5.01. Each assay was repeated three times and means ± SEM were determined.

Kinetics of 5-LOX Enzyme. The kinetics of enzyme inhibition was determined to elucidate the mode of action of inhibitors. The methodology followed was same as 5-LOX activity assay with varying substrate (AA) concentration between 1–30 μM and three different constant concentrations (0, 2 and 5 μM) of inhibitors, **4k, 4n** and **4v.**[3] Lineweaver–Burk plot was plotted against rate of reaction and substrate concentrations. K_m and V_{max} values were determined from the non-linear curve fitting graphs. Calculations were done in GraphPad Prism 5.01. All assays were performed thrice.

Pseudoperoxidase Activity Assay. The redox behaviour of inhibitors is determined by pseudoperoxidase activity assay in presence of enzyme 5-LOX and substrate 13-HPODE.

The activity is measured at 234 nm (Jasco V-550 UV-Vis spectrophotometer) in terms of UV absorbance. The decrease in the amount of the substrate 13-HPODE with respect to time is the measure of redox nature of inhibitors. The assay buffer used is 50 mM potassium phosphate (pH 7.4) containing 0.1 mM EDTA, 0.3 mM $CaCl_2$, 200 μM ATP. The reaction was started by the addition of same concentration, 10 μM of inhibitor and substrate (1:1 ratio to 13-HPODE).[4,5] Standard used for this assay is one of the known redox inhibitors, Zileuton.

Radical Scavenging Assay. DPPH radical scavenging property of inhibitors was evaluated using 10 μM of test compounds, and 0.1 mM of methanolic solution of DPPH.[6] The mixture was incubated for 30 min in dark at room temperature and absorbance taken at 517 nm in Multimode Plate reader (Enspire Perkin Elmer, version 4.10.3005.1440). Ascorbic acid was used as positive control. Each assay was done in triplicates and data are expressed as mean ± SEM. The antioxidant behaviour was calculated in terms of percentage using formula:

$$\% \text{ Inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

where, A_0 = absorbance of blank and A_t = absorbance of test compound .

Pharmacophore Model Elucidation. The essential features in the active molecules required for biological activity was evaluated through pharmacophore modeling and elucidation studies. The software used was MOE; version 2016.0801, Chemical Computing Group, Quebec, Canada. All the chemical structures were drawn and their energies minimized in MOE. A conformational library of all the structures has been generated. A pharmacophore model was developed from the known inhibitors for the common essential features. Then a pharmacophore search against Ph model was

performed on pharmacophore editor in MOE for pharmacophore mapping of the hit molecules.[7] The results obtained were saved in .ph4 format and viewed using database viewer.

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7. *In silico* pharmacokinetic parameters from Swiss ADME software

In silico study including pharmacokinetic parameters has been done using Swiss ADME (http://www.swissadme.ch) software. The active inhibitors are having more total polar

surface area (TPSA) than Zileuton (Table 3 in manuscript), indicating chances of less adverse effects in compounds (http://www.asteris-app.com/technical-info/core-properties/logp.htm). The inhibitors also followed Lipinski rule of five as well as Ghose, Veber, Egan, Muegge rules signifying that they have good druglikeness properties along with *C log P* value more than Zileuton indicating their higher lipophilic nature.

The preliminary experimental study for **4k, 4n** and **4v** in normal L929 (mouse fibroblast) cells showed no cytotoxicity below 1 µM inhibitor concentration. Hence, these molecules could be explored further for in vivo related studies in future as potential anti-inflammatory agents targeting 5-LOX.

In silico pharmacokinetic parameters of **4k** in pictorial form as below as obtained from Swiss ADME software:

4k