

Supporting Information for
Inhibitors of the Diadenosine Tetraphosphate Phosphorylase
Rv2613c of *Mycobacterium tuberculosis*

Kathrin H. Götz[‡], Stephan M. Hacker[‡], Daniel Mayer[†], Jan-Niklas Dürig[‡], Steffen Stenger[†], Andreas Marx^{*‡}

[‡] Department of Chemistry, Konstanz Research School Chemical Biology, Universität Konstanz, Universitätsstrasse 10, D-78464 Konstanz, Germany

[†] Institute for Medical Microbiology and Hygiene, University Hospital of Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany

* Correspondence: andreas.marx@uni-konstanz.de

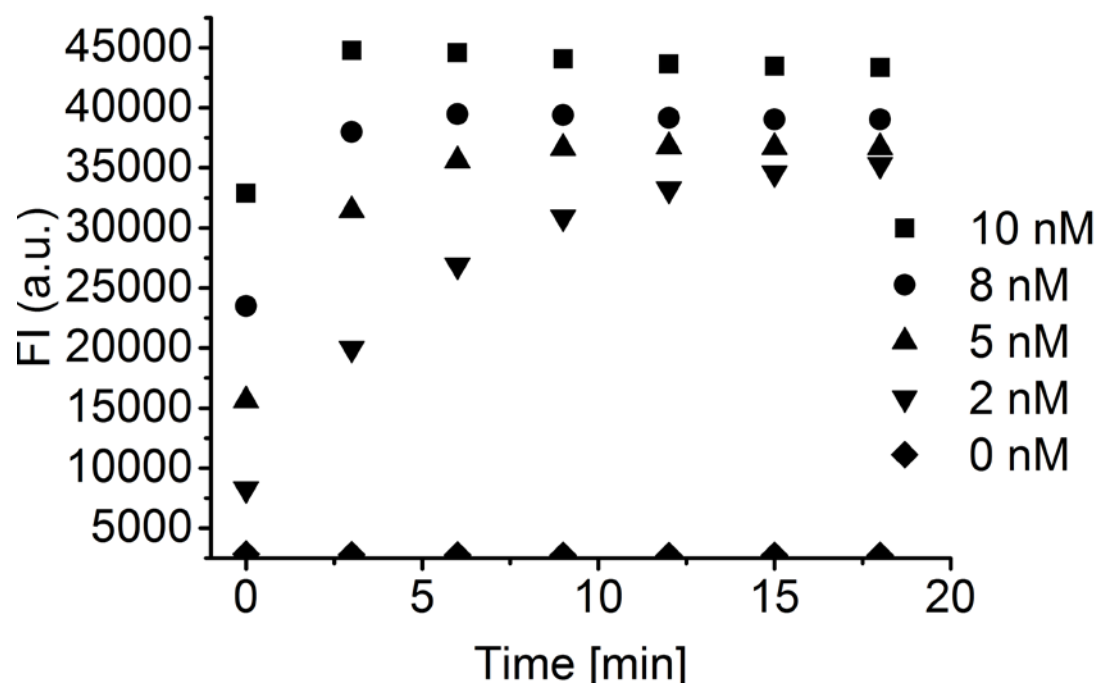


Figure S1 10 μ M of probe **1** were incubated with Rv2613c (concentrations are indicated) in buffer containing 0.2 mg/mL BSA, 2 mM DTT, 5 mM NaH_2PO_4 , 5 mM MgCl_2 and 20 mM Tris-HCl (pH 8.0) in a total volume of 30 μ L at 25°C. Fluorescence measurements were continuously performed over 20 min using an infinite f500 plate reader (TECAN) with excitation at 535 nm and emission at 595 nm. a.u.= arbitrary units

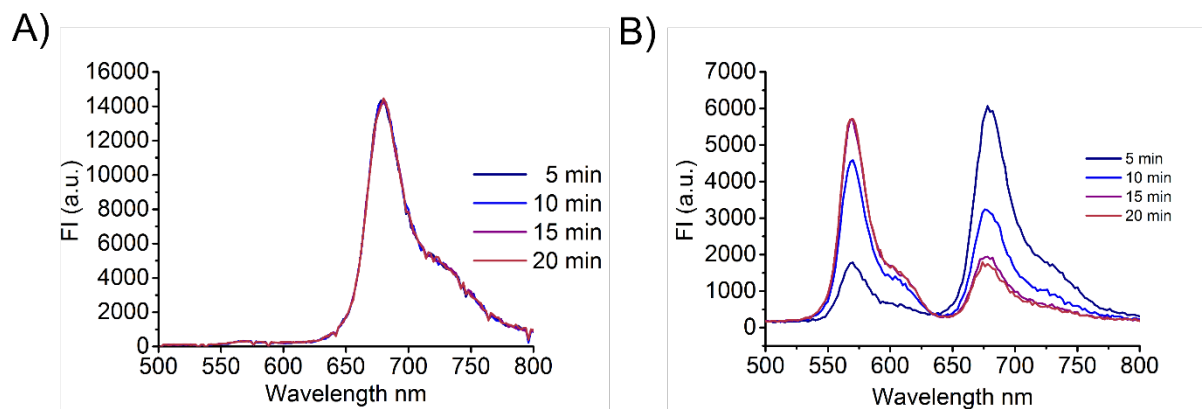


Figure S2 Change of the fluorescence spectra upon incubation of probe **1** in the absence (A) or presence (B) of Rv2613c. 10 μ M probe **1** were incubated in the presence or absence of 5 nM recombinant Rv2613c in buffer containing 20 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM DTT, 0.2 mg/mL BSA, 5 mM NaH_2PO_4 , 5 mM MgCl_2 in a total volume of 2 mL at 25°C. Fluorescence measurements were performed with excitation at 490 nm over 20 min.

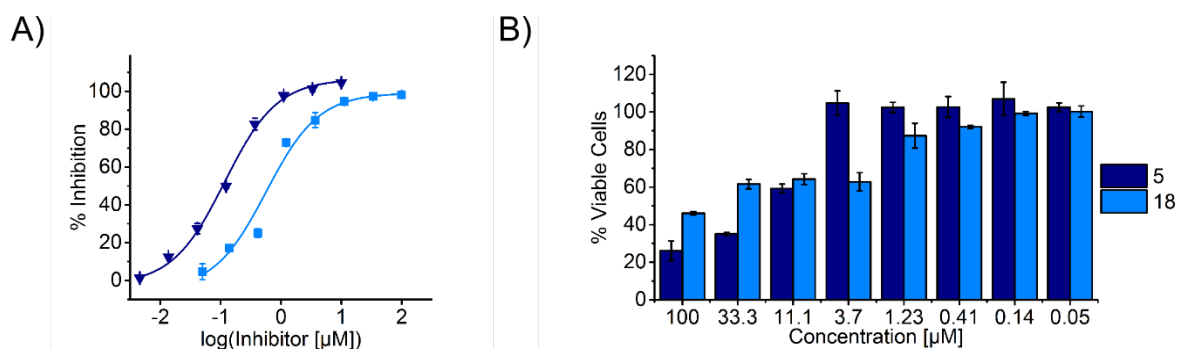


Figure S3 A) Dose response curves against Rv2613c with **1** as the substrate for derivatives **5** and **18**. 8 nM of Rv2613c were preincubated in buffer (20 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM DTT, 0.2 mg/mL BSA, 5 mM NaH₂PO₄, 5 mM MgCl₂) with indicated amounts of compound solved in DMSO at 25°C for 30 min. Substrate **1** (2 μM in Tris-HCl buffer pH 8.0) was added. Measurement of the fluorescence at 570 nm was conducted every 3 min over 1 h. Turnover of probe **1** was calculated by normalization to the intensity of controls. The rate of probe **1** turnover was obtained by linear fitting of probe **1** turnover over time. % Inhibition was calculated relative to the controls. The IC₅₀ was calculated by a non-linear curve fit/dose response curve in Origin^{®1}. All data represent mean ± standard error of triplicates. The IC₅₀ values were determined as 0.12 ± 0.02 μM for compound **5** and as 0.57 ± 0.17 μM for compound **18**. B) Column diagram of the MTT assay assessing the cytotoxicity (EC₅₀) against HeLa cells for derivatives **5** and **18**. 2 μL of each inhibitor solved in DMSO at the indicated concentrations were added to HeLa cells which were seeded into a 96-well plate one day before treatment. The cells were incubated 24 h at 37°C and 5% CO₂. Cells treated with 1% DMSO instead of an inhibitor and medium without cells and 1% DMSO served as controls. MTT (5 mg/mL in water) was diluted with DMEM in a 1:10 ratio and 100 μL were added into each well. After 1 h incubation at room temperature, the medium was removed and 100 μL of DMSO were added. The plate was incubated at room temperature on a shaker until the crystals were fully dissolved. Absorbance was measured at 570 nm. The cell viability was calculated by normalization to the intensity of the controls. The EC₅₀ was calculated by a non-linear curve fit/dose response curve in Origin^{®1}. All data represent mean ± standard error of triplicates. The EC₅₀ values were determined as 13.3 ± 4.49 μM for compound **5** and as >30 μM for compound **18**.

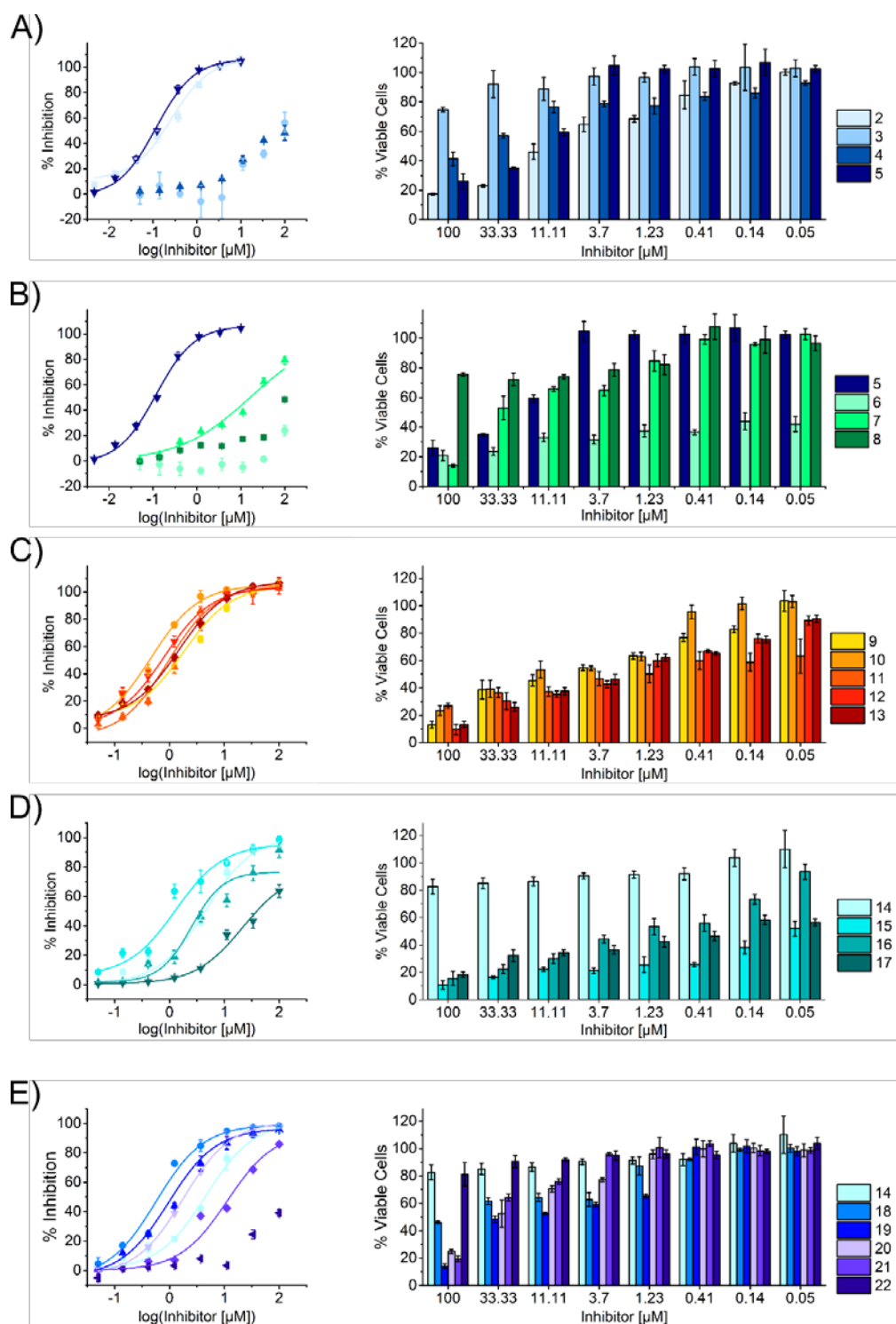


Figure S4 Dose response curves of FRET assays to determine the IC_{50} values against Rv2613c in vitro (left panel) and column diagrams of the MTT assay assessing the cytotoxicity (EC_{50}) against HeLa cells (right panel). The compounds were grouped based on the structural characteristics, which were the basis for the investigation of the structure-activity relationship, as described in the main text. The experiments were performed as for Figure S3.

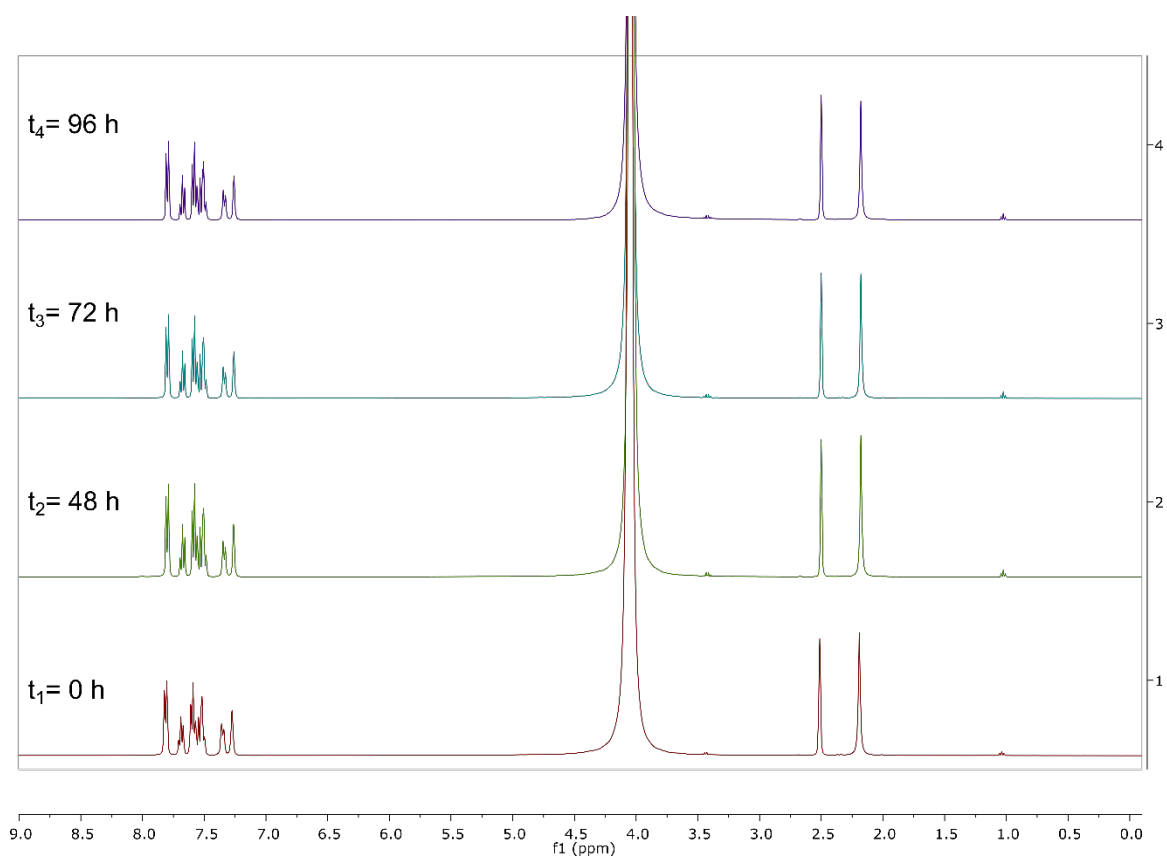


Figure S5 NMR stability study. **5** was dissolved in a mixture of d_6 -DMSO and 30% 1x PBS. ^1H spectra were recorded for 8 days every 48 h. The NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer.

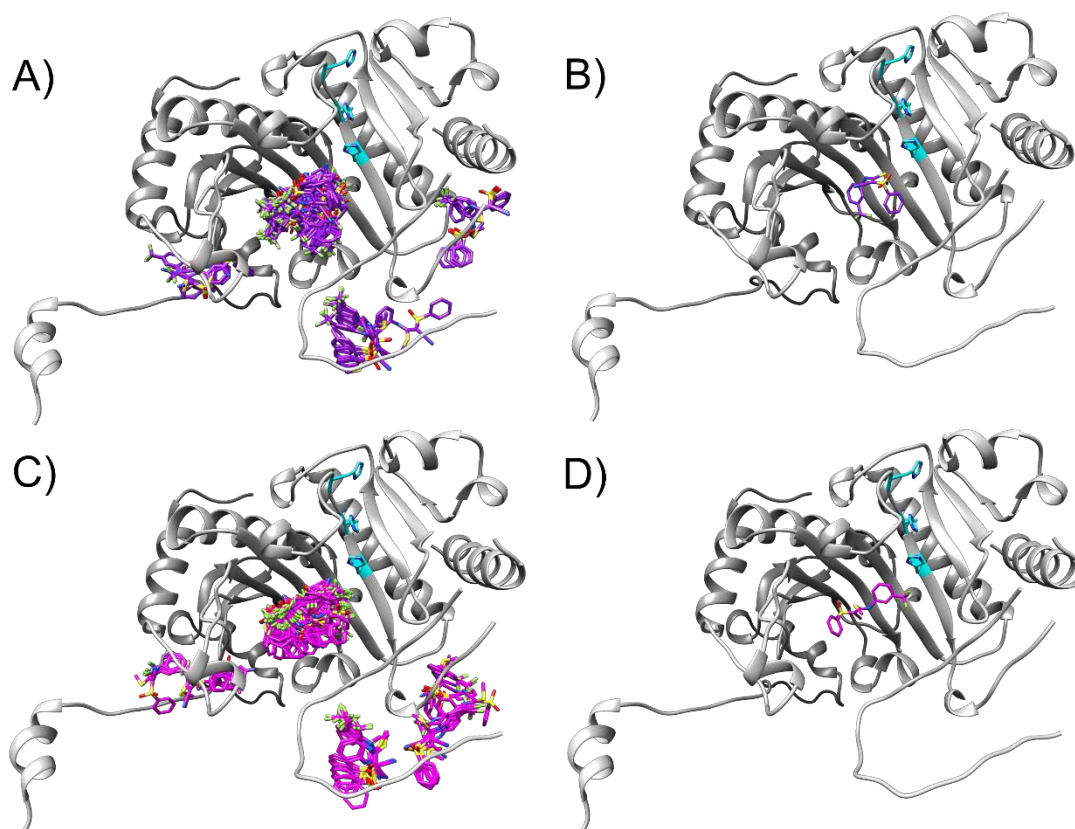
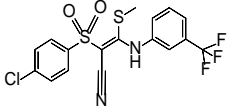
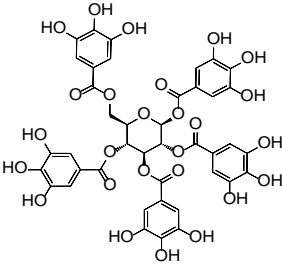
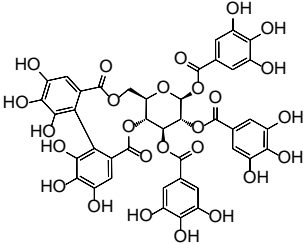
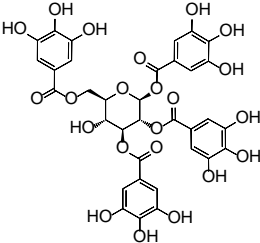
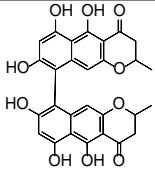
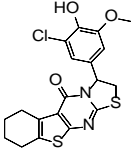
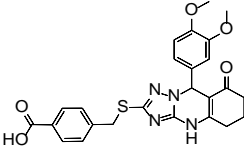
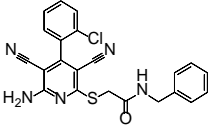
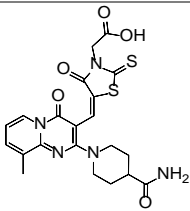
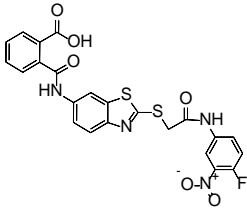
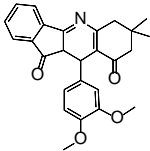
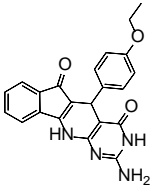
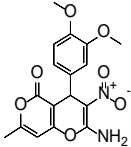
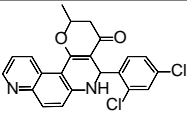
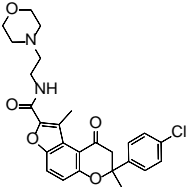
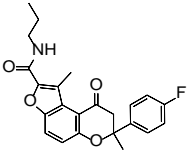
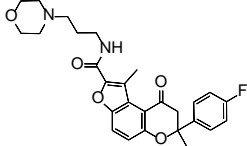
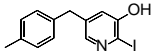
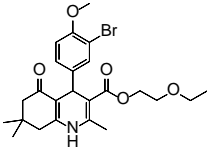
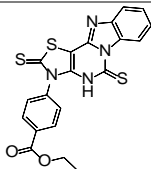
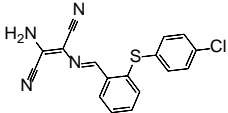


Figure S6 Docking models for inhibitor **5** in Rv2613c. The modeling was performed with the SwissDock web service^{2, 3}. Rv2613c is shown in grey and active site Histidine residues are shown in cyan. A) All possible results docking the E- isomer of **5** (purple) into Rv2613c. B) Best docking result of the E-isomer of **5** into Rv2613c. C) All possible results docking the Z- isomer of **5** (magenta) into Rv2613c. D) Best docking result of the Z-isomer of **5** into Rv2613c.

Table 1S Structure and residual activity in percent of all small molecules, which were identified as hits during the screening.

NR	Structure	Residual activity [%]
2		1.59
31		35.81
32		29.96
33		47.50
34*		-0.38
35		43.92
36		33.53

37		42.76
38		28.09
39		43.56
40		24.76
41		48.87
42		48.50
43		42.23
44		32.08
45		48.72

46		47.73
47		29.63
48		31.80
49		42.80
50		46.29

*: This compound was poorly soluble in aqueous solutions and was therefore not followed further.

MATERIALS AND METHODS

General Experimental Procedures:

All reagents are commercially available and were used without further purification. Solvents were dried over molecular sieves as needed and used directly without further purification. Reactions were conducted under exclusion of air and moisture as needed. Purification of nucleotides by anion-exchange chromatography was performed on an Äkta purifier (GE Healthcare) using a DEAE Sephadex™ A-25 (GEHealthcare Bio-SciencesAB) column and a linear gradient of 0.1 M to 1.0 M triethylammonium hydrogencarbonate buffer (TEAB, pH 7.5). 1.0 M TEAB buffer was made by passing carbon dioxide through a suspension of 5 mol triethylamine in 5 L water until the pH was 7.5. The buffer was diluted to 0.1 M with water as needed. Reversed phase high pressure liquid chromatography (RP-HPLC) was performed using a Shimadzu Prominence system having preparative LC-20AP pumps. For the purification of nucleotides, a VP 250/21 NUCLEODUR C18 HTec, 5 µm (Macherey-Nagel) column and a linear gradient of 5% to 100% acetonitrile in 50 mM triethylammonium acetate buffer (TEAA, pH 7.0) were used. 1.0 M TEAA was made by adding 1 mol triethylamine to a solution of 1 mol acetic acid in 1 L water and adjusting the pH to 7.0. The buffer was diluted to 50 mM as needed. NMR spectra were recorded on a Bruker Avance III 400 MHz or 600 MHz spectrometer. ¹H and ¹³C chemical shifts are reported relative to the residual solvent peak and are given in ppm (δ). s: singlet, d: duplet, t: triplet, q: quartet, bs: broad signal, m: multiplet. HR-ESI-MS was measured on a Bruker Daltonics micrOTOF-Q II ESI-Q-TOF. The reported yield refers to the analytically pure substance and is not optimized. All temperatures quoted are uncorrected. Buffers were filtered through a 0.45 µm Whatman™ RC55 Membrane filter (GE Healthcare Life Sciences) under sterile conditions. If not in use they were stored at 4°C

Expression and Purification of the Rv2613 Protein

Cloning and expression of Rv2613c has been described before⁴. We started from the gene for the full length Rv2316c of *M. tuberculosis* that was synthesized (IDT) with the following sequence.

```
TTAGCCATATGAGCGATGAAGACCGCACGGATCGCGCAACCGAAGATCATACGA
TTTTCGACCGTGGAGTTGGTCAACGTGATCAATTACAGCGTCTTTGGACGCCGT
ATCGTATGAATTACCTTGCCGAAGCCCCCGTGAAACGTGATCCGAACAGTTCGG
CGAGCCCTGCGCAACCGTTCACGGAAATTCCGCAGCTGAGCGATGAGGAAGGT
CTCGTTGTGGCTCGTGGCAAACCTGGTATACGCAGTGCTGAACCTGTACCCGTAT
AATCCTGGTCACTTAATGGTTCGTGCCTTATCGCCGCGTGTCCGAACTGGAAGAC
CTGACCGATCTGGAATCGGCTGAACTGATGGCCTTTACGCAGAAAGCTATCCGT
GTTATTAAAAATGTCAGTCGCCCCGCACGGTTTCAATGTCGGTTTGAACCTGGGAA
CCAGCGCGGGCGGGTCGTTGGCGGAACATCTGCACGTTACGTGGTGCCACG
CTGGGGTGGCGACGCCAATTTTATCACTATTATTGGCGGTAGTAAAGTGATTCCC
CAATTACTGCGCGATACTCGCCGCCTGTTAGCTACTGAGTGGGCACGTCAACCG
TAA GGATCCTAAGCT
```

It was cloned into pET15b using the NdeI and BamHI restriction sites and expressed as a His-tagged protein in BL21(D3) *E. coli* cells. The cells were grown overnight in LB (lysogeny broth) medium containing carbenicillin (0.1 mg/mL) at 37°C, diluted by a factor of 100 and grown until the OD₆₀₀ = 0.6 was reached. The cells were transferred to 25°C and the expression was induced by addition of 1 mM IPTG. After 12 h at 25°C the cells were collected by centrifugation (4,400 g, 25 min., 4°C). The pellet was stored at -20°C over night. The pellet was resuspended in 40 mL lysis buffer (20 mM NaH₂PO₄, 500 mM NaCl and imidazole (5 mM) at pH 7.4 containing 1:1000 Pefabloc®

SC and Aprotinin/Leupeptin). The cells were lysed by sonification and the solution was cleared by centrifugation (14,000 rpm, 45 min, 4°C). The supernatant was loaded onto a His-Trap-FF (5 mL column by GE Healthcare) that was pre-equilibrated with buffer containing 20 mM NaH₂PO₄, 0.5 M NaCl and 5 mM imidazole. The pH of this buffer was adjusted with HCl to 7.4. The protein was eluted with a linear gradient with a buffer containing 20 mM NaH₂PO₄, 0.5 M NaCl and 0.5 M imidazole at pH 7.4. 2.5 mL of the fractions containing protein were loaded onto PD-10 Desalting Columns (GE Healthcare Life Sciences), which were preequilibrated with 20 mM HEPES buffer containing 0.5 mM DTT and 10% glycerol for buffer exchange and eluted with 3.5 mL of the same buffer. Fractions containing Rv2613c were identified by 15% SDS-PAGE, pooled and concentrated using a Merck Millipore Amicon Ultra-15, 10 kDa. The protein concentration was determined by NanoDrop™ 1000 (peqlab Biotechnologie GmbH).

Growth of *M. tuberculosis* (H37Rv)

To prepare LL37 media, 21.8 mg NaHCO₃ were dissolved in 200 µL of a solution of L-glutamine in PBS (200 mM). This mixture was then filled up to 20 mL with RPMI Media 1640 from Gibco (31870-25 from Thermo Fisher). For further usage 12.5 mL of this media mixture were further diluted with 37.5 mL MQ water. The cells were inoculated directly from a cryo-stock of *M. tuberculosis* 20% glycerol in 7H9-media.

Measurement of the Fluorescence Spectra over Time

10 µM probe **1** were incubated in the presence or absence of 5 nM Rv613c in buffer containing 0.2 mg/mL BSA, 2 mM DTT, 5 mM NaH₂PO₄, 5 mM MgCl₂ and 20 mM Tris-HCl (pH 8.0) in a total volume of 2 mL at 25°C. Fluorescence measurements were performed with excitation at 490 nm in a FluoTime 300 (Pico Quant). The emission was recorded from 500-800 nm every 5 min over 20min.

NMR stability study over time

10 mg of **5** were dissolved in a mixture of d_6 -DMSO and 30%1x PBS(pH 7.4). ^1H and spectra were recorded for 8 days each 48 h on a Bruker Avance III 400 MHz spectrometer. In the meantime the sample was stored at room temperature

Docking studies

The crystal structure of Rv2613c was downloaded from RCSB Protein Data Bank (PDB ID: 3ANO). The PDB file of Ap₄A was downloaded and converted to a mol2 file in Chimera⁵. Both isomers of **5** were drawn in Chemdraw to obtain a SMILES string and saved as a mol2 file in Avogadro⁶. We then used the SwissDock web service^{2, 3} which is based on EADock DSS to run docking calculations using the default settings. Therefore the PDB file (3ANO) of Rv2613c was uploaded as target and one of the respective mol2 files was uploaded as the ligand. The generated csv file was downloaded and analyzed in Chimera. The different binding clusters were visualized and the percentage of Ap₄A or inhibitor binding in the active site was obtained by counting how many of the clusters show binding within or outside the pocket near the active site. All docking studies were performed twice and the mean of both was used. 75% of Ap₄A is binding near the active site; 65% of the Z-Isomer of **5** is binding near the active site and 73% of the E-Isomer of **5** is binding near the active site. Figures showing either the best docking solution (Element 0 of cluster 0) or all docking solutions (Figures S3 and S7) were created in Chimera.

Chemical synthesis

Synthesis of the Alkylsulfanylacrylonitriles

General procedure a: Synthesis of Sulfonylacetonitriles

The substituted aryl sulfonyl acetonitril was synthesized over two steps starting from the substituted aryl thiol. Bromoacetonitrile (1.1 eq) was dissolved in DMF (0.4 M), cooled to 0°C treated with substituted benzenethiole (1.0 eq) and K₂CO₃ (2.0 eq) and allowed to stir for 2 to 3 h at 0°C. The reaction mixture was taken up in excess of water and extracted with diethyl ether (3 x 50 mL). The combined organic layers were washed twice with water (2x 50 mL) and twice with brine (2 x 50 mL) before they were dried over MgSO₄ and concentrated under reduced pressure to give the intermediate aryl sulfide acetonitrile as a crude product in approximately 90% yield. The sulfide (1.0 eq) was dried overnight under reduced pressure, dissolved in dry DCM (~50 mL for 2 g crude product) and treated with mCPBA (2.0 eq) at 0°C. The reaction was stirred for 2 h at 0°C and afterwards at r.t. until TLC (1:4 EtOAc:Hexane) showed completion of the reaction. The reaction mixture was quenched with excess of saturated Na₂SO₃ solution and extracted three times with DCM (3 x 40 mL). The combined organic phases were washed with brine (1 x 40 mL), dried over Na₂SO₄ and concentrated in vacuo. The residue was recrystallized from EtOH to yield the respective sulfonyl acetonitrile as colorless crystals in moderate to high yield⁷. In case of the unsubstituted aryl sulfonylacetonitrile the commercial available compound (Sigma- Aldrich) was used.

General procedure b: Synthesis of Isothiocyanates

The respective modified aniline (2.0 g, 1.0 eq) was dissolved in dry THF (50 mL). 2.4 eq NEt₃ were added under inert atmosphere. At 0°C, 1.2 eq thiophosgene were added dropwise over 15 min. The ice bath was removed and the reaction mixture was stirred for ~2 h until TLC monitoring showed completion of reaction. The solvent was evaporated under reduced pressure, the residue was dissolved in ethyl acetate and washed with saturated NaHCO₃ (1 x 40 mL) solution and brine (1 x 40 mL). The organic layer was dried over MgSO₄ and concentrated in vacuum. The desired isothiocyanate was purified by flash column chromatography (silica gel, hexane, if not stated differently)⁸.

General procedure c: Synthesis of Alkylsulfanylacrylonitrils

For the coupling, 2.0 eq of potassium carbonate were added under inert atmosphere to a solution of respective sulfonylacetonitril (1.0 eq) in dry acetone (20 mL), followed by 1.1 eq of isothiocyanate. The resulting mixture was stirred for 2 h at r.t., potassium carbonate was removed by filtration and 3.0 eq alkyl halogenide were added. The reaction solution was stirred for ~2 h until TLC showed completion of the reaction. After evaporation of the solvent under reduced pressure the residue was dissolved in ethyl acetate and washed with water (2 x 40 mL), 0.1 M HCl (1 x 40 mL) and 1 M NaHCO₃ (1 x 40 mL). The organic layer was dried over MgSO₄ and concentrated in vacuum. After recrystallization from EtOH the desired alkylsulfanylacrylonitrils were obtained in moderate to high yields^{9, 10}.

3-(Trifluoromethyl)phenyl isothiocyanate(2a)⁸

Following the general reaction procedure b, 3-(trifluoromethyl)phenyl isothiocyanate was synthesized and obtained in 89% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ= 7.85 (s, 1H, H-2), 8.06-8.03 (H-2), 7.76-7.66 (m, 3H, H-4, H-5, H-6) ppm.

¹⁹F-NMR (CDCl₃, 376 MHz): δ -61.45 ppm.

(4-Chlorobenzenesulphonyl)-acetonitrile (2b)⁷

Following the general reaction procedure a (4-chloro-benzenesulphonyl)-acetonitrile was obtained in 57% yield.

¹H-NMR (CDCl₃, 400 MHz) δ= 7.89 (d, J=8.49 Hz, 2H, H -2, H-6), 7.54 (d, J=8.49 Hz, 2H, H-3, H-5), 4.21 (s, 2H, H-7) ppm.

¹³C-NMR (CDCl₃, 151 MHz) δ= 142.20 (C-4), 135.15(C-1), 133.28 (C-2, C-6), 130.10 (C-3, C-5), 110.53 (C≡N), 45.65 (CH₂) ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(4-chlorophenyl-sulfonyl)-3-methylsulfanylacrylonitrile (2)^{10, 11}

3-[3-Trifluoromethyl-phenylamino]-2-(4-chlorophenyl-sulfonyl)-3-methylsulfanylacrylonitrile **2** was synthesized according to the general reaction procedure c using 3.0 eq methyl iodide as alkylation reagent. After recrystallization, **2** was obtained as pale yellow crystals in 49% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ= 10.39 (bs, 1H, N-H), 7.88-7.84 (m, 2H, H-3, H-5), 7.72-7.68 (m, 2H, H-2, H-6), 7.62-7.54 (m, 2H, H-4', H-5'), 7.46-7.40 (m, 2H, H-2', H-6'), 2.26 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz) δ= 168.92 (CNH), 140.6 (C-1'), 140.08 (C-1), 138.80 (C-4), 130.66 (C-5'), 130.29 (q, J²=31.8 Hz, C-3'), 130.08 (q, J²=31.8 Hz, C-3'), 129.87 (q, J²=31.8 Hz, C-3'), 129.81 (C-3, C-5), 129.65 (q, J²=31.8 Hz, C-3'), 128.77 (C-2, C-

6), 127.26 (C-2'), 126.53 (q, $J^1=271.7$ Hz, -CF₃), 124.72 (q, $J^1=271.7$ Hz, -CF₃), 122.92 (C-6'), 121.12 (q, $J^1=271.7$ Hz, -CF₃), 119.88 (C-4'), 115.11 (C≡N), 88.25 (C=C), 16.35 (S-CH₃) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ -61.41 ppm.

HR-ESI-MS: found: 433.0007; calculated: 433.0059 (M+H⁺, C₁₇H₁₂ClF₃N₂O₂S₂⁺); deviation: 5.2 ppm.

Phenyl isothiocyanate (3a)⁸

Phenyl isothiocyanate was synthesized starting from aniline following the general reaction procedure b in 60% yield.

¹H-NMR (CDCl₃, 400 MHz) δ= 7.37-7.33 (m, 2H, H-3, H-5), 7.30-7.26 (m, 1H, H-4), 7.23-7.20 (m, 2H, H-2, H-6) ppm.

¹³C-NMR (CDCl₃, 100 MHz) δ= 135.48 (NCS), 131.34 (C-1), 129.62 (C-3, C-5), 127.37 (C-4), 125.80 (C-2, C-6) ppm.

3-Phenylamino-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (3)⁹

Following the general reaction procedure c **3** could be obtained as pale yellow crystals in 70% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ=10.24 (s(b), 1H, N-H), 7.90-7.87 (m, 2H, H-2, H-6), 7.76-7.71 (m, 1H, H-4), 7.67-7.62 (m, 2H, H-3, H-5), 7.39-7.35 (m, 2H, H-3', H-5'), 7.24 (t, $J=7.47$, 1H, H-4'), 7.17 (d, $J=7.74$, 2H, H-2', H-6'), 2.12 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz) δ= 167.67 (CNH), 141.86 (C-1'), 138.83 (C-1), 133.61 (C-4), 129.56 (C-3,C-5), 129.32 (C-3',C-5'), 126.66 (C-2,C-6), 126.31(C-2', C-6'), 123.57 (C-4'), 115.56 (C≡N), 87.17 (C=C), 16.12 (S-CH₃) ppm.

HR-ESI-MS: found: 331.0542; calculated: 331.0575 (M+H⁺, C₁₆H₁₄N₂O₂S₂⁺); deviation: 3.3 ppm.

3-Phenylamino-2-(4-chlorophenylsulfonyl)-3-methylsulfanylacrylonitrile (4)⁹

Following the general reaction procedure c **4** could be obtained as pale yellow crystals in 31% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ=10.31 (s(b), 1H, N-H), 7.90-7.86 (m, 2H, H-3, H-5), 7.74-7.70 (m, 2H, H-2, H-6), 7.40-7.35 (m, 2H, H-3', H-5'), 7.27-7.23 (m, 1H, H-4'), 7.20-7.18 (m, 2H, H-2', H-6'), 2.15 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz) δ= 168.09 (CNH), 140.72 (C-1), 138.79 (C-4), 138.44 (C-1'), 129.62 (C-3,C-5), 129.25 (C-3',C-5'), 128.52 (C-2,C-6), 126.32 (C-2',C-6'), 123.57 (C-4'), 115.33 (C≡N), 86.47 (C=C), 16.22 (S-CH₃) ppm.

HR-ESI-MS: found: 365.0165; calculated: 365.0180 (M+H⁺, C₁₆H₁₄ClN₂O₂S₂⁺); deviation: 4.1 ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (5)⁹

Following the general reaction procedure c **5** could be obtained as pale yellow crystals in 60% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ = 10.36 (s(b), 1H, N-H), 7.86 (d, 2H, H-2, H-6), 7.73-7.70 (m, 1H, H-4), 7.64-7.53 (m, 4H, H-3, H-5, H-4', H-5'), 7.42-7.38 (m, 2H, H-2', H-6'), 2.21 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ= 167.87 (CNH), 141.52 (C-1'), 139.85 (C-1), 133.66 (C-4), 130.45 (C-5'), 130.15 (q, J²=32.03 Hz, C-3'), 129.94 (q, J²=32.03 Hz, C-3'), 129.73 (q, J²=32.03 Hz, C-3'), 129.63 (q, J²=32.03 Hz, C-3'), 129.46 (C-3, C-5), 126.66 (C-2, C-6), 126.36 (q, J¹=272.8 Hz, -CF₃), 124.56 (q, J¹=272.8 Hz, -CF₃), 122.75 (q, J¹=272.8 Hz, -CF₃), 122.30 (C-2', C-6'), 120.95 (q, J¹=272.8 Hz, -CF₃), 119.77 (C-4'), 115.15 (C≡N), 89.04 (C=C), 16.12 (S-CH₃) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ= -61.40 ppm.

HR-ESI-MS: found: 399.0404; calculated: 399.0449 (M+H⁺, C₁₇H₁₃F₃N₂O₂S₂⁺); deviation: 4.5 ppm.

2-(Trifluoromethyl)phenyl isothiocyanate (6a)¹²

2-(Trifluoromethyl)phenyl isothiocyanate was synthesized starting from 2-trifluoromethyl aniline following the general reaction procedure b in 34% yield.

¹H-NMR (CDCl₃, 400 MHz) δ= 7.66 (d, J=7.89 Hz, 1H, H-3), 7.57 (t, J=7.65 Hz, 1H, H-6), 7.41-7.35 (m, 2H, H-4, H-5) ppm.

3-[2-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (6)⁹

Following the general reaction procedure c **6** could be obtained as colourless crystals in 57% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ= 10.09 (s(b), 1H, N-H), 7.91 (d, J=7.17 Hz, 2H, H-2, H-6), 7.82 (d, J=7.95 Hz, 1H, H-3'), 7.78-7.66 (m, 4H, H-3, H-4, H-5, H-4'), 7.60 (t, J=7.69 Hz, 1H, H-5'), 7.48 (d, J=7.95 Hz, 1H, H-6'), 2.29 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz) δ= 168.49 (CNH), 141.44 (C-1'), 135.58 (C-1), 133.82 (C-4), 133.59 (C-5'), 129.55 (C-3, C-5, C-3'), 128.82 (C-4'), 126.92 (q, C-2'), 126.89 (q, C-2'), 126.50 (C-2, C-6), 125.97 (q, J¹=271.7 Hz, -CF₃), 124.99 (C-6'), 124.16 (q, J¹=271.7 Hz, -CF₃), 122.34 (q, J¹=271.7 Hz, -CF₃), 120.53 (q, J¹=271.7 Hz, -CF₃), 115.09 (C≡N), 86.81 (C=C), 16.87 (S-CH₃) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ= -59.50 ppm.

HR-ESI-MS: found: 399.0410; calculated: 399.0449 (M+H⁺, C₁₇H₁₃F₃N₂O₂S₂⁺); deviation: 3.9 ppm.

4-(Trifluoromethyl)phenyl isothiocyanate (7a)⁸

4-(Trifluoromethyl)phenyl isothiocyanate was synthesized starting from 4-trifluoromethyl aniline following the general reaction procedure b in 67% yield.

¹H-NMR (CDCl₃, 400 MHz): δ= 7.65 (d, J=8.38, 2H), 7.35 (d, J=8.38, 2H) ppm.

3-[2-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (7)⁹

Following the general reaction procedure c **7** could be obtained as colourless crystals in 50% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ = 10.42 (s(b), 1H, N-H), 7.87-7.85 (m, 2H, H-2, H-6), 7.76-7.69 (m, 3H, H-3, H-4, H-5), 7.66-7.62 (m, 2H, H-3', H-5'), 7.33 (s, 2H, H-2', H-6'), 2.21 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ = 167.68 (CNH), 142.85 (C-1), 141.43 (C-1'), 133.68 (C-4), 129.49 (C-3,C-5), 126.67 (C-2,C-6), 126.41 (C-3', C-5'), 125.62 (q, J²=35.0 Hz, C-4), 125.39 (q, J²=35.0 Hz, C-4), 124.94 (q, J¹=270.0 Hz, -CF₃), 123.14 (q, J¹=270.0 Hz, -CF₃), 122.69 (C-2', C-6'), 121.34 (q, J¹=270.0 Hz, -CF₃), 115.26 (C≡N), 89.94 (C=C), 16.17 (S-CH₃) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ = -60.67 ppm.

HR-ESI-MS: found: 399.0402; calculated: 399.0449 (M+H⁺, C₁₇H₁₃F₃N₂O₂S₂⁺); deviation: 4.7 ppm.

3,5-Bis(trifluoromethyl)phenyl isothiocyanate (8a)⁸

3,5-Bis(trifluoromethyl)phenyl isothiocyanate was synthesized starting from 3,5-bis(trifluoromethyl)aniline following the general reaction procedure b in 46% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 7.76 (s, 1H, H-4), 7.64 (s, 2H, H-2, H-6) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ= -63.49 ppm.

3-[3,5-Bis(trifluoromethyl)-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (8)⁹

Following the general reaction procedure c **8** could be obtained as colourless crystals in 36% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.43 (s(b), 1H, N-H), 7.88-7.85 (m, 3H, H-2, H-6, H-4'), 7.75-7.71 (m, 3H, H-3, H-4, H-5) 7.65-7.61(m, 2H, H-2', H-6'), 2.32 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ= 168.18 (CNH), 141.48 (C-1), 141.20 (C-1'), 133.92 (C-4), 131.42 (q, J²=33.08 Hz, C-3'), 131.20 (q, J²=33.08 Hz, C-3'), 130.98 (q, J²=33.08 Hz, C-3'), 130.76 (q, J²=33.08 Hz, C-3'), 129.58 (C-3, C-5), 126.81. (C-2, C-6), 125.68 (q, J¹=271.68 Hz, -CF₃), 123.87 (q, J¹=271.68 Hz, -CF₃), 123.08 (C-2', C-6'), 122.06 (q, J¹=271.68 Hz, -CF₃), 120.25 (q, J¹=271.68 Hz, -CF₃), 118.58 (C-4'), 115.05 (C≡N), 90.44 (C=C), 16.15 (S-CH₃) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ= -61.61 ppm.

HR-ESI-MS: found:467.0253; calculated: 467.0322 (M+H⁺, C₁₈H₁₂F₆N₂O₂S₂⁺); deviation: 6.9 ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-ethylsulfanylacrylonitrile (9)⁹

Following the general reaction procedure c using 3.0 eq ethyl iodide as alkylating agent **9** could be obtained as colourless crystals in 72% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.42 (s(b), 1H, N-H), 7.92 (d, 2H, H-2, H-6), 7.77-7.72 (m, 1H, H-4), 7.67-7.55 (m, 4H, H-3, H-5, H-4', H-5'), 7.49-7.45 (m, 2H, H-2', H-6'), 2.68 (m, 2H, S-CH₂), 0.91 (m, 3H, -CH₃) ppm.

¹³C-NMR (DMSO-d₆, 100 MHz): δ= 164.98 (CNH), 141.77 (C-1'), 139.82 (C-1), 133.81 (C-4), 130.59 (C-5'), 130.21 (q, J²= 33.47 Hz, C-3'), 1300.00 (q, J²= 33.47 Hz, C-3'), 19.78 (q, J²= 33.47 Hz, C-3'), 129.56 (q, J²= 33.47 Hz, C-3'), 129.46 (C-3, C-5), 127.00 (C-2, C-6) 126.40 (q, J¹= 272.1 Hz, -CF₃), 124.60 (q, J¹= 272.1 Hz, -CF₃), 122.79 (q,

$J^1 = 272.1$ Hz, $-\text{CF}_3$), 122.45 (C-2'), 120.99 (q, $J^1 = 272.1$ Hz, $-\text{CF}_3$), 120.46 (C-6'), 118.04 (C-4'), 115.52 (C \equiv N), 90.44 (C=C), 27.70 (S-CH₂), 14.03 ($-\text{CH}_3$) ppm.

^{19}F -NMR (DMSO- d_6 , 376 MHz): $\delta = -61.42$ ppm.

HR-ESI-MS: found: 413.0602; calculated: 413.0600 ($\text{M}+\text{H}^+$, $\text{C}_{18}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_2\text{S}_2^+$); deviation: 0.5 ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-propylsulfanylacrylonitrile (10**)⁹**

Following the general reaction procedure c using 3.0 eq 1-iodopropane as alkylating agent **10** could be obtained as colourless crystals in 33% yield.

^1H -NMR (DMSO- d_6 , 400 MHz): $\delta = 10.40$ (s(b), 1H, N-H), 7.91-7.89 (m, 2H, H-2, H-6), 7.77-7.72 (m, 1H, H-4), 7.67-7.58 (m, 4H, H-3, H-5, H-4', H-5'), 7.42-7.38 (m, 2H, H-2', H-6'), 2.68-2.61 (m, 2H, S-CH₂), 1.30 (m, 2H, CH₂), 0.70 (m, 3H, $-\text{CH}_3$) ppm.

^{13}C -NMR (DMSO- d_6 , 100 MHz): $\delta = 169.19$ (CNH), 141.20 (C-1'), 139.80 (C-1), 133.81 (C-4), 130.56 (C-5'), 130.17 (q, $J^2 = 31.52$ Hz, C-3'), 129.96 (q, $J^2 = 31.52$ Hz, C-3'), 129.75 (q, $J^2 = 31.52$ Hz, C-3'), 129.51 (C-3, C-5), 127.86 (C-2'), 126.90 (C-2, C-6), 126.40 (q, $J^1 = 270.68$ Hz, $-\text{CF}_3$), 125.75 (C-6'), 124.60 (q, $J^1 = 270.68$ Hz, $-\text{CF}_3$), 122.79 (q, $J^1 = 270.68$ Hz, $-\text{CF}_3$), 120.99 (q, $J^1 = 270.68$ Hz, $-\text{CF}_3$), 118.14 (C-4'), 115.53 (C \equiv N), 90.34 (C=C), 35.02 (S-CH₂), 22.11 ($-\text{CH}_2$), 12.61 ($-\text{CH}_3$) ppm.

^{19}F -NMR (DMSO- d_6 , 376 MHz): $\delta = -61.41$ ppm.

HR-ESI-MS: found: 427.0735; calculated: 427.0756 ($\text{M}+\text{H}^+$, $\text{C}_{19}\text{H}_{17}\text{F}_3\text{N}_2\text{O}_2\text{S}_2^+$); deviation: 4.9 ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-isopropylsulfanylacrylonitrile (11)⁹

Following the general reaction procedure c using 6.0 eq 2-iodopropane as alkylating agent **11** could be obtained as colourless crystals in 38% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ = 10.50 (s(b), 1H, N-H), 7.94-7.92 (m, 2H, H-3, H-5), 7.77-7.72 (m, 1H, H-4), 7.68-7.55 (m, 5H, H-2, H-6, H-4', H-5', H-6'), 7.45 (m, 1H, H-2'), 3.09 (m, 1H, S-CH), 0.97 (m, 6H, 2x-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ = 166.71 (CNH), 142.05 (C-1'), 140.04 (C-1), 133.81 (C-4), 130.58 (C-5'), 130.13 (q, J^2 =31.7 Hz, C-3'), 129.92 (q, J^2 =31.7 Hz, C-3'), 129.71 (q, J^2 =31.7 Hz, C-3'), 129.41 (C-3, C-5), 127.28 (C-2, C-6), 126.39 (q, J^1 =273.0 Hz, -CF₃), 124.58 (q, J^1 =273.0 Hz, -CF₃), 122.77 (q, J^1 =273.0 Hz, -CF₃), 122.17 (C-2', C-6'), 120.97 (q, J^1 =273.0 Hz, -CF₃), 118.30 (C-4') 115.43 (C \equiv N), 92.39 (C=C), 54.87 (S-CH), 21.88 (-CH₃) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ = -61.49 ppm.

HR-ESI-MS: found: 427.0770; calculated: 427.0756 (M+H⁺, C₁₉H₁₇F₃N₂O₂S₂⁺); deviation: 3.3 ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-benzylsulfanylacrylonitrile (12)⁹

Following the general reaction procedure c using 3.0 eq benzyl bromide as alkylating agent **12** could be obtained as colourless crystals in 44% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.38 (s(b), 1H, N-H), 7.82-7.75 (m, 3H, H-2, H-6, H-4), 7.66-7.63 (m, 2H, H-3, H-5), 7.53-7.49 (m, 2H, H-4', H-5'), 7.32-6.56 (m, 7H, H-2', H-6', 5x-BzH), 4.24-3.97 (m, 2H, S-CH₂) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ= 169.07 (CNH), 142.30 (C-1'), 140.34 (C-1), 140.22 (C-1''), 136.10 (C-4), 134.20 (C-5'), 130.73 (C-3, C-5), 130.40 (q, J²=32.4 Hz, C-3'), 130.19 (q, J²=32.4 Hz, C-3'), 129.97 (q, J²=32.4 Hz, C-3'), 129.33 (C-3'', C-5''), 129.76 (q, J²=32.4 Hz, C-3'), 129.00 (C-2, C-6), 127.76 (C-4''), 127.26 (C-2'', C-6''), 126.79 (q, J¹=273.1 Hz, -CF₃), 124.98 (q, J¹=273.1 Hz, -CF₃), 123.18 (q, J¹=273.1 Hz, -CF₃), 122.23 (C-2'), 121.37 (q, J¹=273.1 Hz, -CF₃), 118.58 (C-6'), 116.24 (C-4'), 114.26 (C≡N), 91.22 (C=C), 37.89 (S-CH₂) ppm.

¹⁹F-NMR (DMSO-d₆, 376 MHz): δ= -61.56 ppm.

HR-ESI-MS: found: 475.0743; calculated: 475.0756 (M+H⁺, C₂₃H₁₈F₃N₂O₂S₂⁺); deviation: 2.7 ppm.

3-[3-Trifluoromethyl-phenylamino]-2-(phenylsulfonyl)-3-allylsulfanylacrylonitrile (13**)⁹**

Following the general reaction procedure c using 3.0 eq allyl bromide as alkylating agent **13** could be obtained as colourless crystals in 46% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.45 (s(b), 1H, N-H), 7.91-7.89 (m, 2H, H-2, H-6), 7.78-7.73 (m, 1H, H-4), 7.68-7.52 (m, 4H, H-3, H-5, H-4', H-5'), 7.52-7.20 (m, 2H, H-2', H-6'), 5.54-5.22 (m, 1H, CH), 5.02-4.77 (m, 2H, -S-CH₂), 3.48 (m, 2H, -CH₂) ppm.

¹³C-NMR (DMSO-d₆, 100 MHz): δ= 165.08 (CNH), 141.54 (C-1'), 139.68 (C-1), 133.80 (C-4), 131.98 (C-5'), 130.42 (-CH), 130.19 (q, J²=32.0 Hz, C-3'), 129.87 (q, J²=32.0 Hz, C-3'), 129.55 (q, J²=32.0 Hz, C-3'), 129.42 (C-3, C-5), 129.23 (q, J²=32.0 Hz, C-

3'), 127.78 (q, $J^1=273.0$ Hz, $-\text{CF}_3$), 127.00 (C-2, C-6), 126.26 (C-2', C-6'), 125.03 (q, $J^1=273.0$ Hz, $-\text{CF}_3$), 122.32 (q, $J^1=273.0$ Hz, $-\text{CF}_3$), 122.22 ($-\text{CH}_2$), 119.61 (q, $J^1=273.0$ Hz, $-\text{CF}_3$), 119.03 (C-4'), 115.57 ($\text{C}\equiv\text{N}$), 90.86 ($\text{C}=\text{C}$), 35.89 (S- CH_2) ppm.

^{19}F -NMR (DMSO- d_6 , 376 MHz): $\delta = -61.44$ ppm.

HR-ESI-MS: found: 425.0617; calculated: 425.0600 ($\text{M}+\text{H}^+$, $\text{C}_{19}\text{H}_{17}\text{F}_3\text{N}_2\text{O}_2\text{S}_2^+$); deviation: 4.0 ppm.

3-(Methyl)phenyl isothiocyanate(14a)⁸

3-(Methyl)phenyl isothiocyanate was synthesized starting from 3-methyl aniline following the general reaction procedure b in 85% yield.

^1H -NMR (DMSO- d_6 , 400 MHz) $\delta = 7.25$ (t, $J=7.75$ Hz, 1H, H-2), 7.12-7.04 (m, 3H, H-4, H-5, H-6), 2.37 (s, 3H, CH_3) ppm.

3-[3-methyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (14)⁹

Following the general reaction procedure c **14** could be obtained as colourless crystals in 20% yield.

^1H -NMR (DMSO- d_6 , 400 MHz) $\delta = 10.17$ (s(b), 1H, N-H), 7.88-7.84 (m, 2H, H-2, H-6), 7.75-7.71 (m, 1H, H-4), 7.67-7.62 (m, 2H, H-3, H-5), 7.25 (t, 1H, $J=7.76$ Hz, H-5'), 7.05 (d, 1H, $J=7.54$ Hz, H-4'), 6.97-6.92 (m, 2H, H-2', H-6'), 2.25 (s, 3H, $-\text{CH}_3$), 2.14 (s, 3H, S- CH_3) ppm.

^{13}C -NMR (DMSO- d_6 , 100 MHz) $\delta = 167.83$ (CNH), 141.93 (C-1'), 138.86 (C-3'), 138.83 (C-1), 133.63 (C-4), 129.59 (C-3, C-5), 129.18 (C-5'), 127.02 (C-2'), 126.57 (C-2, C-6), 123.84 (C-4'), 120.66 (C-6'), 115.58 ($\text{C}\equiv\text{N}$), 87.40 ($\text{C}=\text{C}$), 20.92 ($-\text{CH}_3$), 16.26 (S- CH_3) ppm.

HR-ESI-MS: found: 345.0690; calculated: 345.0731 ($M+H^+$, $C_{17}H_{16}N_2O_2S_2^+$);
deviation: 4.1 ppm.

3-(Nitro)phenyl isothiocyanate (15a)⁸

3-(Nitro)phenyl isothiocyanate was synthesized starting from 3-nitro aniline following the general reaction procedure b in 81% yield.

¹H-NMR (DMSO- d_6 , 400 MHz): δ = 8.14-8.11 (m, 1H), 8.06-8.05 (m, 1H), 7.58-7.52 (m, 2H) ppm.

3-[3-Nitro-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (15)⁹

Following the general reaction procedure c **15** could be obtained as yellow crystals in 57% yield.

¹H-NMR (DMSO- d_6 , 400 MHz): δ = 10.44 (s(b), 1H, N-H), 8.04-8.02 (m, 1H, H-2'), 7.94-7.92 (m, 1H, H-4'), 7.86-7.84 (m, 2H, H-2, H-6), 7.74-7.70 (m, 1H, H-5'), 7.66-7.56 (m, 4H, H-3, H-5, H-4', H-6'), 2.26 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO- d_6 , 151 MHz): δ = 168.02 (CNH), 148.06 (C-3'), 141.41 (C-1'), 140.48 (C-1), 133.81 (C-4), 130.72 (C-5'), 129.60 (C-3, C-5), 129.14 (C-6'), 126.70 (C-2, C-6) 120.28 (C-4'), 117.46 (C-2'), 115.39 (C \equiv N), 89.43 (C=C), 16.24 (S-CH₃) ppm.

HR-ESI-MS: found: 376.0392; calculated: 376.0425 ($M+H^+$, $C_{16}H_{13}N_3O_4S_2^+$);
deviation: 3.3 ppm.

3-(Cyano)phenyl isothiocyanate (16a)⁸

3-(Cyano)phenyl isothiocyanate was synthesized starting from 3-aminobenzonitrile following the general reaction procedure b in 70% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 7.56-7.54 (m, 1H, H-2), 7.50-7.43 (m, 3H, H-3, H-4, H-5) ppm.

3-[3-Cyano-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (16**)¹²**

Following the general reaction procedure c **16** could be obtained as fine yellow crystals in 27% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.10 (s(b), 1H, N-H), 7.84 (d, J=7.57, 2H, H-2, H-6), 7.69-7.64 (m, 1H, H-4), 7.58-7.55 (m, 3H, H-3, H-5, H-4'), 7.50-7.46 (m, 2H, H-5', H-6'), 7.42-7.40 (m, 1H, H-2'), 2.13 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ= 167.86 (CNH), 141.47 (C-1'), 140.11 (C-1), 133.81 (C-4), 130.66 (C-5'), 129.62 (C-3, C-5), 129.52 (C-4'), 128.17 (C-2'), 126.70 (C-2, C-6), 126.49 (C-6'), 117.12 (C≡N²), 115.51 (C≡N), 112.10 (C-3'), 88.95 (C=C), 16.34 (S-CH₃) ppm.

HR-ESI-MS: found: 356.0492; calculated: 356.0527 (M+H⁺, C₁₇H₁₃N₃O₂S₂⁺); deviation: 3.5 ppm.

3-(Amino)methylbenzoate (17a**)**

3-amino benzoic acid (1.0 eq) was dissolved in dry MeOH (10.0 mL per 1.0 g) and cooled to 0°C. Thionyl chloride (2.5 eq) was added dropwise. After stirring for 2 h at 0°C the reaction mixture was refluxed for two days until TLC showed completion of the reaction. The reaction mixture was concentrated in vacuum. The residue was neutralized with NaHCO₃, washed with ethyl acetate, and the organic layer was dried over MgSO₄, concentrated and purified by flash chromatography (1:5 hexane: ethyl acetate) to yield 86% of the benzoate.

3-Carbomethoxy isothiocanate (17b)⁸

17b was synthesized starting from 3-(amino)methylbenzoate (**17a**) following the general reaction procedure b. Instead of THF ethyl acetate was used as solvent. Instead of washing the residue during work up with sodium hydrogen carbonate solution the residue was washed with water (2 x 50mL) and brine (2 x 50mL) yielding 94% of the pure isothiocyanate.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 7.94-7.92 (m, 1H), 7.88-7.87 (m, 1H), 7.45-7.37 (m, 2H), 3.93 (s, 3H) ppm.

3-[3-carbomethoxy-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (17)⁹

Following the general reaction procedure c **17** could be obtained as yellow crystals in 56% yield.

¹H-NMR (DMSO-d₆, 400 MHz): δ= 10.31 (s(b), 1H, N-H), 7.87-7.85 (m, 2H, H-2, H-6), 7.79-7.77 (m, 1H, H-2'), 7.75-7.71 (m, 1H, H-4), 7.67-7.61 (m, 3H, H-3, H-5, H-5'), 7.51 (t, J=7.9, 1H, H-4'), 7.43-7.41 (m, 1H, H-6'), 7.36 (s, 3H, Ph-COOMe), 2.20 (s, 3H, S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz): δ 167.82 (CNH), 165.45 (-CO), 141.61 (C-1'), 139.51 (C-1), 133.59 (C-4), 130.54 (C-3'), 129.81 (C-5'), 129.50 (C-3, C-5), 127.82 (C-6'), 126.62 (C-2, C-6), 126.49 (C-4'), 123.67 (C-2'), 115.44 (C≡N), 88.26 (C=C), 52.40 (COOCH₃), 16.14 (S-CH₃) ppm.

HR-ESI-MS: found: 389.0593; calculated: 389.0629 (M+H⁺, C₁₈H₁₆N₂O₄S₂⁺); deviation: 3.6 ppm.

3-(Ethyl)phenyl isothiocyanate (18a)⁸

3-(Ethyl)phenyl isothiocyanate was synthesized starting from 3-ethyl aniline following the general reaction procedure b in 65% yield.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.25 (t, J=7.77 Hz, 1H, H-5), 7.12-7.10(m,1H, H-4), 7.09-7.06 (m, 1H, H-3), 7.05-7.02 (m, 1H, H-6), 2.64 (q, J=7.62 Hz, 2H,-CH₂), 1.23 (t, J=7.77 Hz, 3H, -CH₃) ppm.

¹³C-NMR (CDCl₃, 151 MHz) δ = 146.03 (C-3), 134.70 (NCS), 131.04 (C-1), 129.38 (C-5), 127.08 (C-4), 125.20 (C-2), 122.94 (C-1), 28.54 (CH₂), 15.28 (CH₃) ppm

HR-ESI-MS: found: 345.0690; calculated: 345.0731 (M+H⁺, C₁₇H₁₆N₂O₂S₂⁺); deviation: 4.1 ppm.

3-[3-Ethyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (18)⁹

Following the general reaction procedure c **18** could be obtained as colourless crystals in 70% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ = 10.18 (s(b), 1H, N-H), 7.75-7.71 (m, 2H, H-2, H-6), 7.75-7.71 (m, 1H, H-4), 7.66-7.62 (m, 2H, H-3, H-5), 7.27 (t, 1H, J= 7.45 Hz, H-5'), 7.08 (d, 1H, J= 7.45 Hz, H-4'), 6.99-6.95 (m, 2H, H-2', H-6'), 2.55 (q, 2H, J= 7.71 Hz, -CH₂), 2.14 (s, 3H, S-CH₃), 1.13 (t, 3H, J= 7.71 Hz, -CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz) δ = 167.65 (CNH), 145.01 (C-3'), 141.88 (C-1'), 138.74 (C-1), 133.50 (C-4), 129.47 (C-3, C-5), 129.13 (C-5'), 126.50 (C-2, C-6), 125.74 (C-4'), 122.68 (C-2'), 120.81 (C-6'), 115.42 (C \equiv N), 87.03 (C=C), 27.88 (-CH₂), 16.15 (S-CH₃), 15.29 (-CH₃) ppm.

HR-ESI-MS: found: 359.0876; calculated: 359.0882 ($M+H^+$, $C_{18}H_{19}N_2O_2S_2^+$); deviation: 1.6 ppm.

3-Allylnitrobenzene (19a)¹³

3-Nitroaniline (1.0 g, 1.0 eq) was added portionwise over 20 min to a solution of *tert*-butylnitrile (2.0 eq) and allyl bromide (15.0 eq) in acetonitrile (60.0 mL), during that time the temperature was kept between 34-36°C. The reaction mixture was stirred for 1-2 h at 35°C. The reaction was concentrated under reduced pressure. **19a** was purified by flash chromatography (hexane to hexane with 6% ethyl acetate) in 37% yield.

¹H-NMR ($CDCl_3$, 400 MHz) δ = 8.05-8.02 (m, 2H, H-2, H-6), 7.53-7.50 (m, 1H, H-4), 7.46-7.41 (m, 1H, H-5), 5.99-5.69 (m, 1H, H-8), 5.16-5.09 (m, 2H, H-9), 3.49-3.46 (d, 2H, J = 6.57 Hz, H-7) ppm.

HR-ESI-MS: found: 164.0712; calculated: 164.0706 ($M+H^+$, $C_9H_{10}NO_2^+$); deviation: 3.6 ppm.

3-*n*-Propylaniline (19b)¹⁴

To a solution of **19a** (0.5 g) in ethyl acetate (20.0 mL) 10% Pd/C were added. The reaction mixture was shaken under H_2 -pressure (50 psi). After 5 h TLC monitoring showed the completion of reaction. The Pd/C was filtered off over cellite. The cellite was washed with ethyl acetate until the flow through was colorless. The combined organic layers were concentrated and 3-*n*-proplaniline was purified by flash column chromatography (hexane: ethyl acetate 10:1) in 20% yield.

¹H-NMR (CDCl₃, 400 MHz) δ= 7.13-7.09 (m, 1H, H-5), 6.65 (d, 1H, J= 7.50 Hz, H-2), 6.57-6.54 (m, 2H, H-4, H-6), 3.68 (s(b), 2H,NH), 2.54 (t, 2H, J= 7.50 Hz, CH₂), 1.64 (sx, 2H, J= 7.50 Hz, CH₂-CH₂), 0.99 (t, 3H, J= 7.3 Hz, CH₃) ppm.

¹³C-NMR (CDCl₃, 100 MHz) δ= 146.15 (C-1), 144.08 (C-3), 129.19 (C-5), 119.17 (C-4), 115.57 (C-2), 112.78 (C-6), 38.18 (-CH₂), 24.51 (CH₂-CH₂), 14.00 (CH₃) ppm.

HR-ESI-MS: found: 136.1119; calculated: 136.1121 (M+H⁺, C₉H₁₄N⁺); deviation: 1.5 ppm.

3-*n*-Propyl isothiocanate (19c)⁸

3-*n*-Propyl isothiocanate was synthesized starting from 3-*n*-propylaniline (**19b**) following the general reaction procedure b in 57% yield.

¹H-NMR (CDCl₃, 400 MHz): δ= 7.26-7.22 (m, 1H, H-5), 7.10-7.08 (m, 1H, H-4), 7.05-7.02 (m, 2H, H-2, H-6), 2.57 (t, J=7.20 Hz, 2H, -CH₂), 1.63 (sx, J=7.46 Hz, 2H, CH₂-CH₂), 0.94 (t, J=7.40 Hz, 3H, -CH₃) ppm.

¹³C-NMR (CDCl₃, 100 MHz) δ= 144.66 (C-3), 135.04 (NCS), 131.16 (C-1), 129.40 (C-5), 127.76 (C-4), 125.86 (C-2), 123.07 (C-1), 37.72 (CH₂), 24.36 (CH₂-CH₂), 13.80 (-CH₃) ppm

HR-ESI-MS: found: 178.0665; calculated: 178.0685 (M+H⁺, C₁₀H₁₆NS⁺); deviation: 1.3 ppm.

3-[3-*n*-Propyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (19)⁹

Following the general reaction procedure c **19** could be obtained. For purification the crude material was purified by flash column chromatography (Hexane: ethyl acetate 3:1) to give **19** as light yellow oil in 72% yield.

¹H-NMR (CDCl₃, 400 MHz) δ = 9.87 (s(b), 1H, NH), 7.94-7.91 (m, 2H, H-2, H-6), 7.66-7.62 (m, 1H, H-4), 7.55-7.52 (m, 2H, H-3, H-5), 7.29 (t, 1H, J= 7.70 Hz, H-5'), 7.11 (d, 1H, J= 7.92 Hz, H-4'), 7.04-7.00 (m, 2H, H-2', H-6'), 2.58 (t, J= 7.58 Hz, 2H, -CH₂), 2.08 (s, 3H, S-CH₃), 1.63 (dt, J= 14.94 Hz, J= 7.43 Hz, 2H, -CH₂-CH₂) 0.92 (t, J= 7.31 Hz, 3H, -CH₃)ppm.

¹³C-NMR (CDCl₃, 151 MHz) δ = 167.68 (CNH), 143.39 (C-3'), 141.98 (C-1'), 138.86 (C-1), 133.55 (C-4), 129.52 (C-3, C-5), 129.12 (C-5'), 126.55 (C-2, C-6), 126.34 (C-4'), 123.24 (C-2'), 120.90 (C-6'), 115.55 (C \equiv N), 86.89 (C=C), 36.87 (-CH₂), 23.87 (-CH₂-CH₂), 16.12 (-S-CH₃), 13.47 (-CH₃) ppm.

HR-ESI-MS: found: 373.1036; calculated: 373.1039 (M+H⁺, C₁₉H₂₁N₂O₂S₂⁺); deviation: 0.8 ppm.

3-(*iso*-Propyl)phenyl isothiocyanate (20a**)⁸**

3-(*iso*-Propyl)phenyl isothiocyanate was synthesized starting from 3-isopropyl aniline following the general reaction procedure b in 72% yield.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.31-7.28 (m, 1H, H-5), 7.19-7.17 (m, 1H, H-4), 7.13-7.11 (m, 1H, H-2), 7.09-7.07(m, 1H, H-6), 2.92 (sept, J=7.00 Hz, 1H,-CH), 1.28 (d, J=7.00 Hz, 6H, -CH₃) ppm.

¹³C-NMR (CDCl₃, 100 MHz) δ = 150.88 (C-3), 134.66 (NCS), 131.15 (C-1), 129.56 (C-5), 125.86 (C-4), 123.97 (C-2), 123.18 (C-6), 34.05 (-CH), 23.88 (-CH₃) ppm

HR-ESI-MS: found: 178.0681; calculated: 178.0685 ($M+H^+$, $C_{10}H_{16}NS^+$); deviation: 2.3 ppm.

3-[3-*iso*-Propyl-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (20)⁹

Following the general reaction procedure c **20** could be obtained as colourless crystals in 50% yield.

¹H-NMR (DMSO- d_6 , 400 MHz) δ = 10.18 (s(b), 1H, NH), 7.90-7.87 (m, 2H, H-2, H-6), 7.76-7.71 (m, 1H, H-4), 7.66-7.63 (m, 2H, H-3, H-5), 7.28 (t, 1H, J = 7.95 Hz, H-5'), 7.11 (d, 1H, J = 7.95 Hz, H-4'), 7.00-6.97 (m, 2H, H-2', H-6'), 2.84 (sept, , J = 7.00 Hz, 2H, -CH), 2.13 (s, 3H, S-CH₃), 1.15 (d, J = 7.00 Hz, 6H, -CH₃) ppm.

¹³C-NMR (DMSO- d_6 , 151 MHz) δ = 167.65 (CNH), 149.64 (C-3'), 141.92 (C-1'), 138.73 (C-1), 133.57 (C-4), 129.52 (C-3, C-5), 129.18 (C-5'), 126.56 (C-2, C-6), 124.37 (C-4'), 121.19 (C-2', C-6'), 155.44 (C \equiv N), 86.98 (C=C), 33.19 (-CH), 23.63 (-CH₃), 16.12 (S-CH₃) ppm.

HR-ESI-MS: found: 373.1031; calculated: 373.1039 ($M+H^+$, $C_{19}H_{21}N_2O_2S_2^+$); deviation: 2.1 ppm.

3-(Methoxy)phenyl isothiocyanate (21a)⁸

3-(Methoxy)phenyl isothiocyanate was synthesized starting from 3-methoxy aniline following the general reaction procedure as described before in 73% yield.

¹H-NMR (CDCl₃, 400 MHz): δ = 7.22 (t, J = 8.00 Hz, 1H, H-5), 6.83-6.79 (m, 2H, H-2, H-4), 6.71 (t, J = 2.20 Hz, 1H, H-6), 3.78 (s, 3H, -CH₃) ppm.

¹³C-NMR (CDCl₃, 100 MHz) δ = 160.27 (C-3), 135.41 (NCS), 132.06 (C-1), 130.20 (C-5), 118.11 (C-4), 113.60 (C-2), 111.09 (C-6), 55.44 (-CH₃) ppm

HR-ESI-MS: found: 166.0319; calculated: 166.0321 (M+H⁺, C₈H₈NOS⁺); deviation: 1.2 ppm.

3-[3-Methoxy-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (21)⁹

Following the general reaction procedure c **21** could be obtained as colourless crystals in 19% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ = 10.19 (s(b), 1H, NH), 7.89-7.85 (m, 2H, H-2, H-6), 7.75-7.71 (m, 1H, H-4), 7.67-7.62 (m, 2H, H-3, H-5), 7.27 (t, 1H, J= 8.00 Hz, H-5'), 6.81 (dd, 1H, J= 8.00 Hz, J= 1.90 Hz, H-4'), 6.75-6.672 (m, 2H, H-2', H-6'), 3.70 (s, 3H, -O-CH₃), 2.16 (s, 3H, -S-CH₃) ppm.

¹³C-NMR (DMSO-d₆, 151 MHz) δ = 161.57 (CNH), 159.76 (C-3'), 141.80 (C-1'), 139.96 (C-1), 133.61 (C-4), 130.13 (C-5'), 129.54 (C-3, C-5), 126.60 (C-2, C-6), 115.41 (C \equiv N), 111.80 (C-4', C-6'), 108.93 (C-2'), 87.46 (C=C), 55.28 (-O-CH₃), 16.18 (-S-CH₃) ppm.

HR-ESI-MS: found: 373.1033; calculated: 373.1039 (M+H⁺, C₁₉H₂₁N₂O₂S₂⁺); deviation: 1.6 ppm.

3,5-Bis(methyl)phenyl isothiocyanate (22a)⁸

3,5-Bis(methyl)phenyl isothiocyanate was synthesized starting from 3,5-dimethyl aniline following the general reaction procedure b in 94% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ = 6.93 (s, 1H, H-4), 6.88(s, 2H, H-2, H6), 2.32 (s, 6H, -CH₃) ppm.

3-[3,5-Bis(methyl)-phenylamino]-2-(phenylsulfonyl)-3-methylsulfanylacrylonitrile (22)⁹

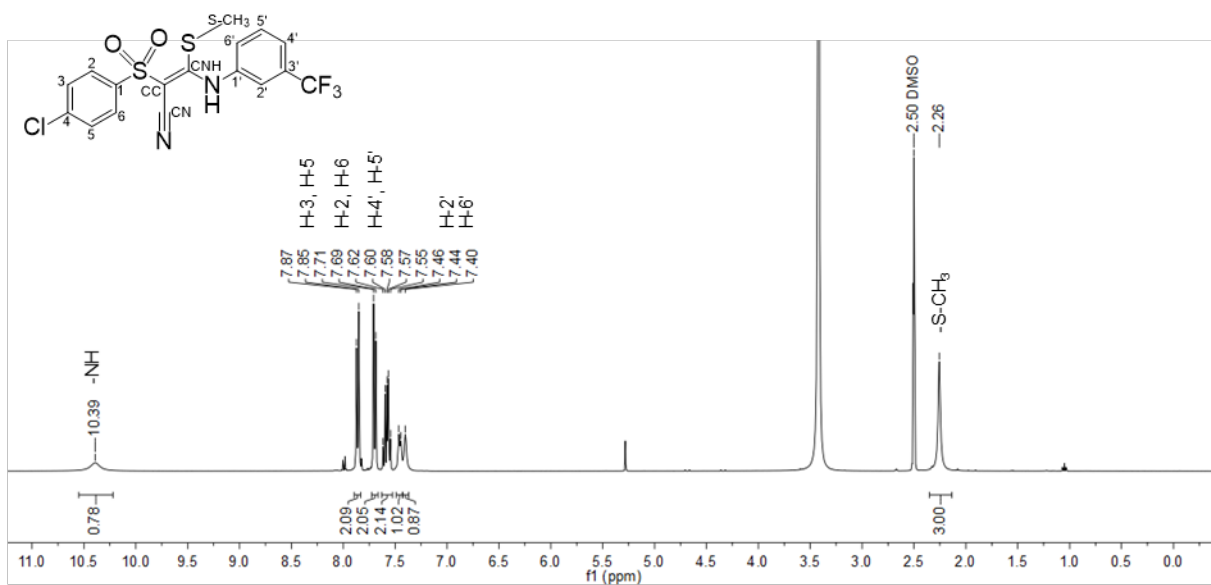
Following the general reaction procedure c **22** could be obtained as colourless crystals in 20% yield.

¹H-NMR (DMSO-d₆, 400 MHz) δ 10.09 (s(b), 1H, N-H), 7.86-7.84 (m, 2H, H-2, H-6), 7.76-7.71 (m, 1H, H-4), 7.67-7.62 (m, 2H, H-3, H-5), 6.86 (s, 1H, H-4'), 6.72 (s, 2H, H-2', H-6'), 2.21 (s, 6H, -CH₃), 2.17 (s, 3H, S-CH₃) ppm.

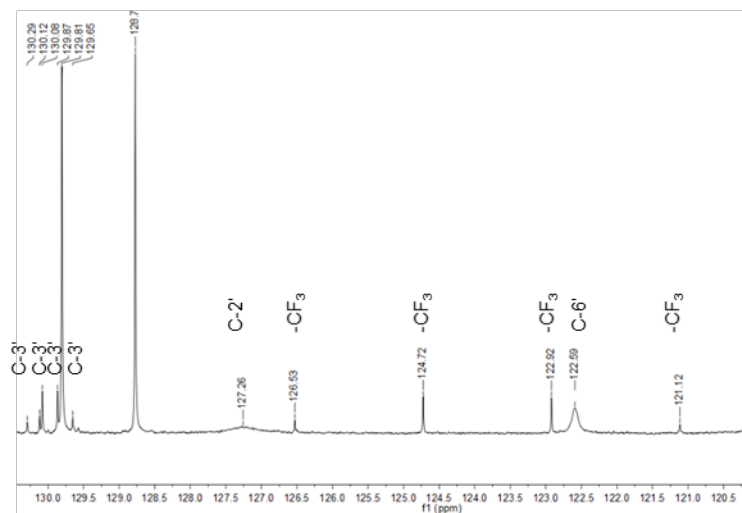
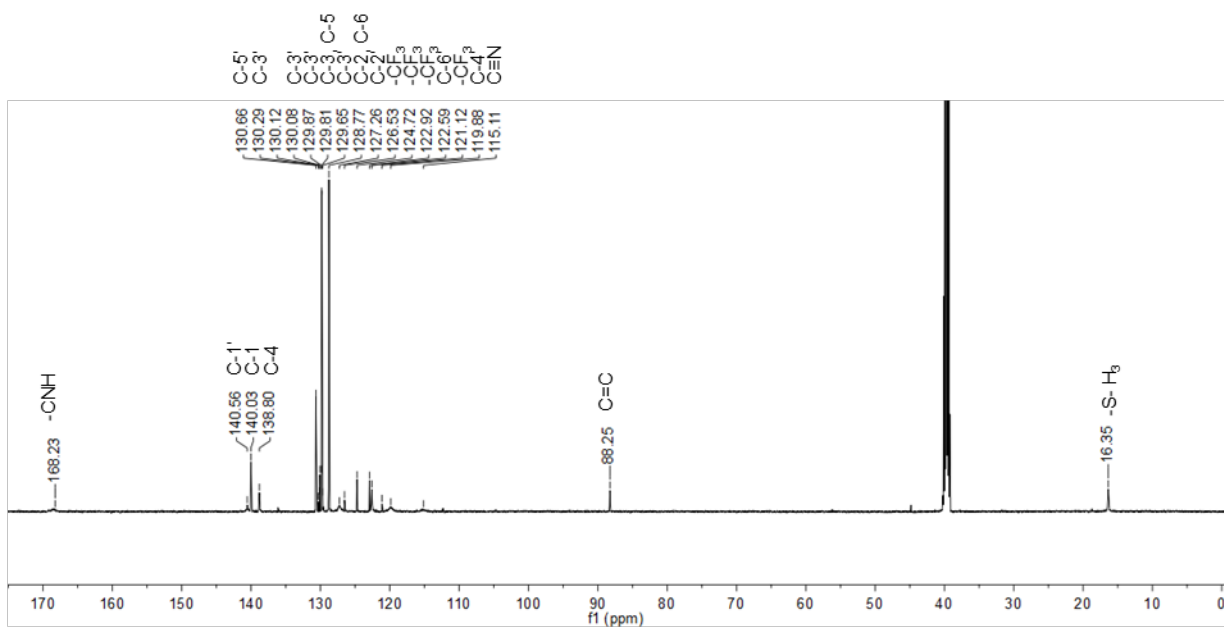
¹³C-NMR (DMSO-d₆, 151 MHz) δ = 168.20 (CNH), 142.34 (C-1'), 139.20 (C-1), 138.96 (C-3', C-5'), 133.98 (C-4), 129.97 (C-3, C-5), 128.14 (C-4'), 126.94 (C-2, C-6), 121.32 (C-2', C-6'), 116.62 (C \equiv N), 87.21 (C=C), 21.26 (2x-CH₃), 16.67 (S-CH₃) ppm.

HR-ESI-MS: found: 359.0852; calculated: 359.0888 (M+H⁺, C₁₈H₁₈N₂O₂S₂⁺); deviation: 3.6 ppm.

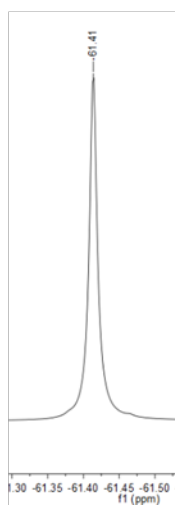
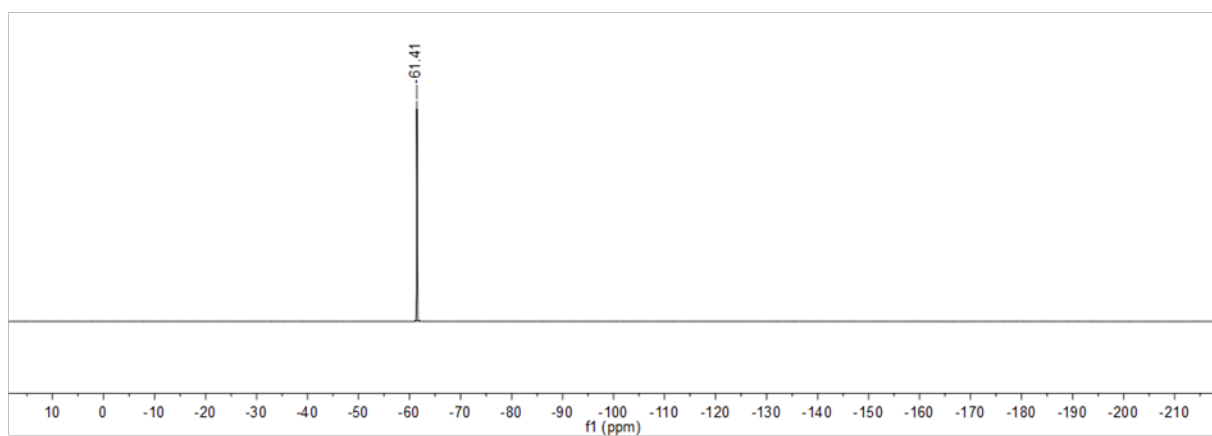
^1H NMR (DMSO- d_6 , 400 MHz)



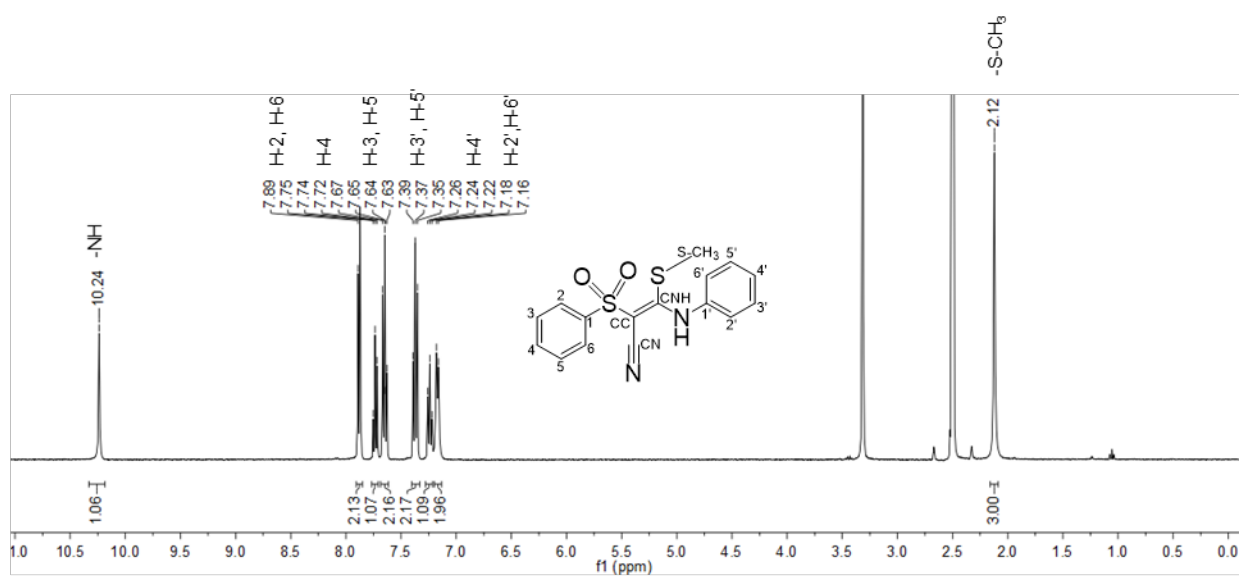
^{13}C NMR (DMSO- d_6 , 115 MHz)



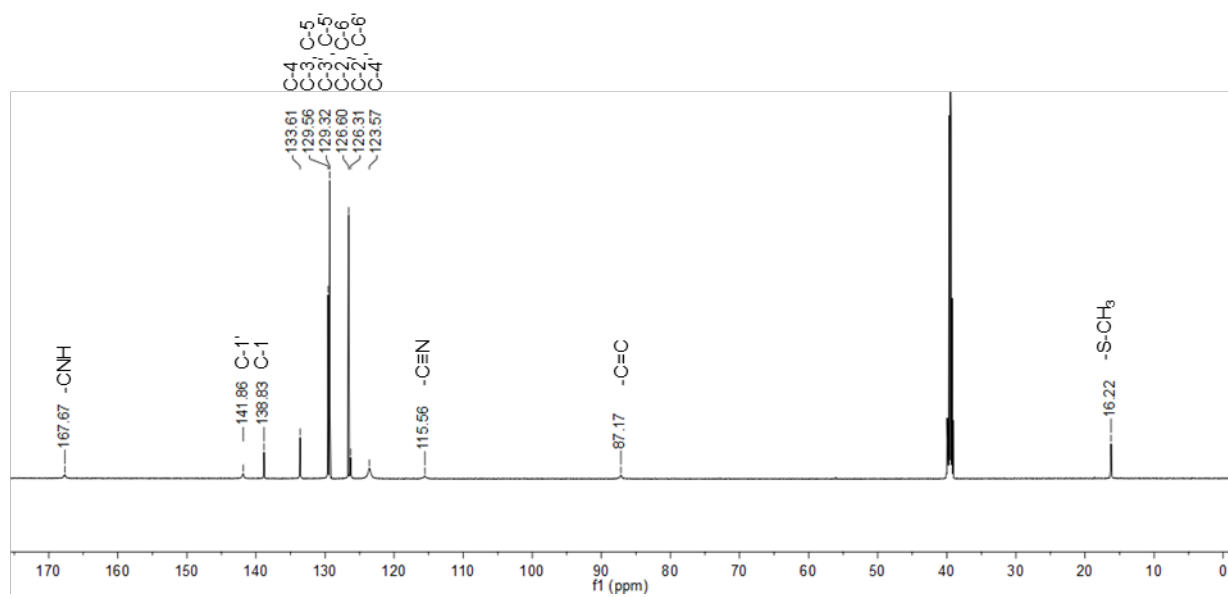
^{19}F NMR (DMSO- d_6 , 376 MHz)



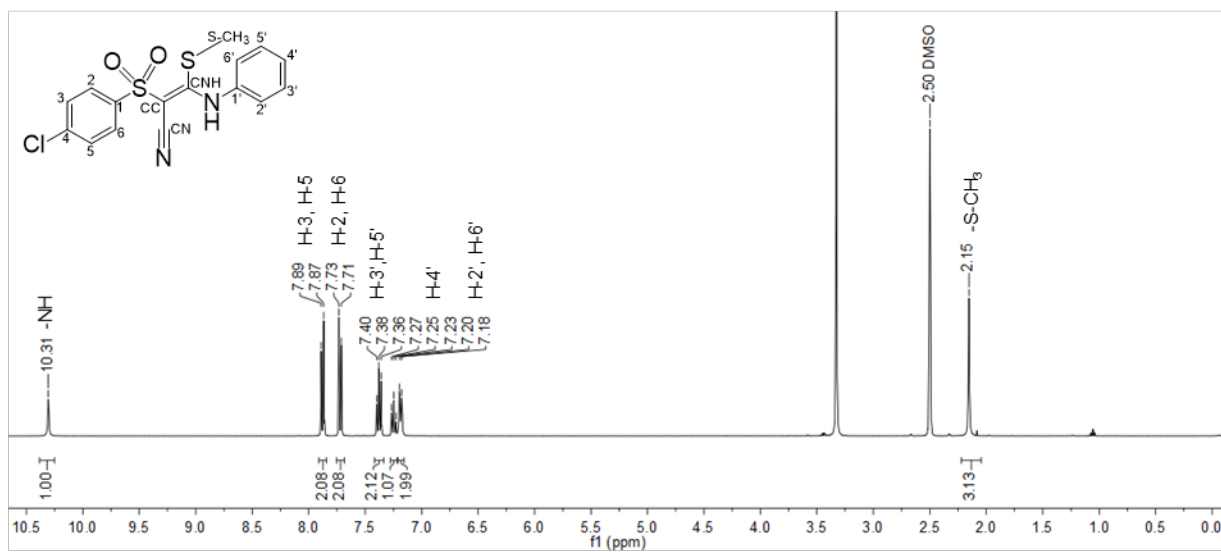
^1H NMR (DMSO- d_6 , 400 MHz)



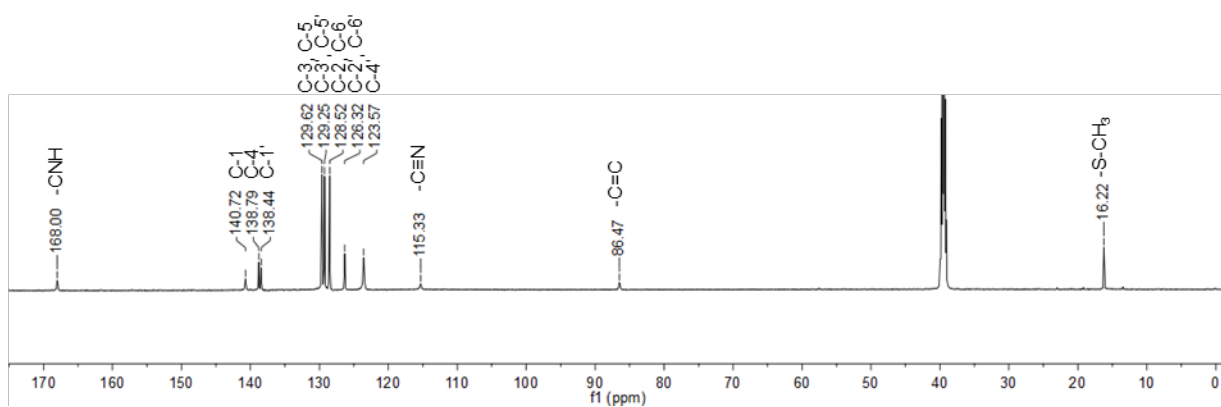
^{13}C NMR (DMSO- d_6 , 151 MHz)



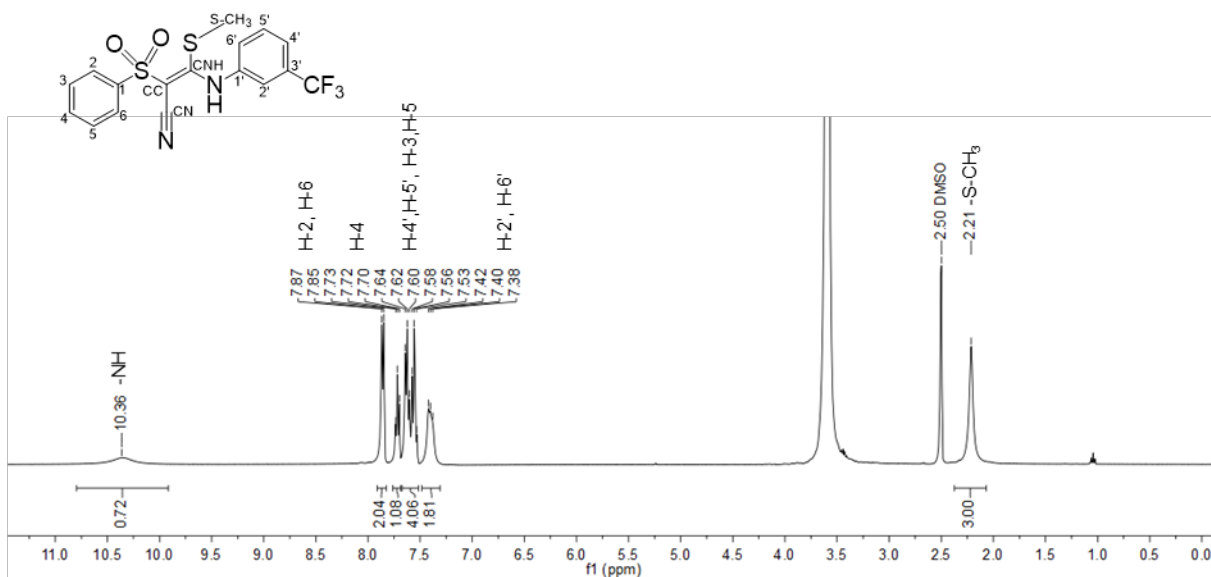
^1H NMR (DMSO- d_6 , 400 MHz)



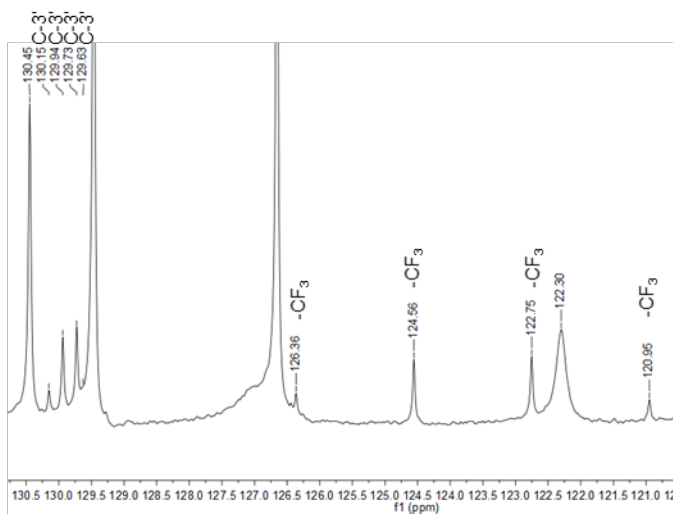
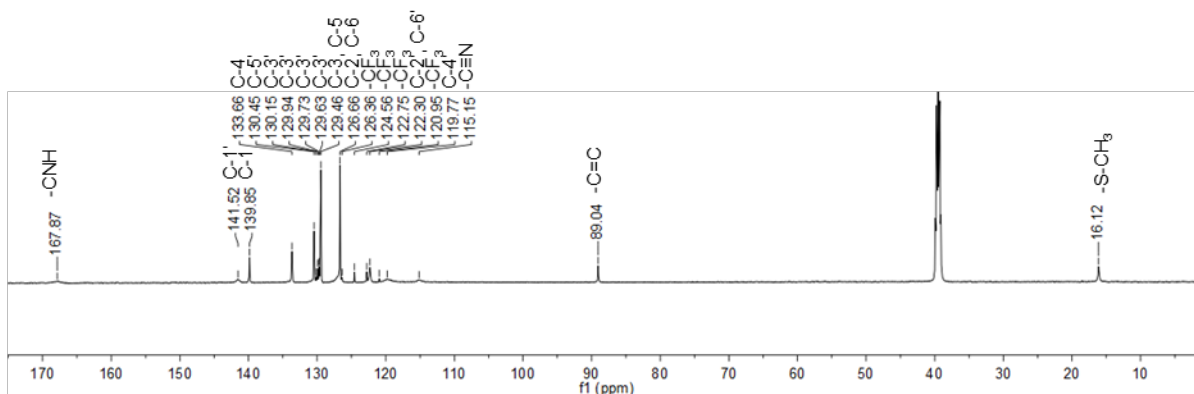
^{13}C NMR (DMSO- d_6 , 151 MHz)



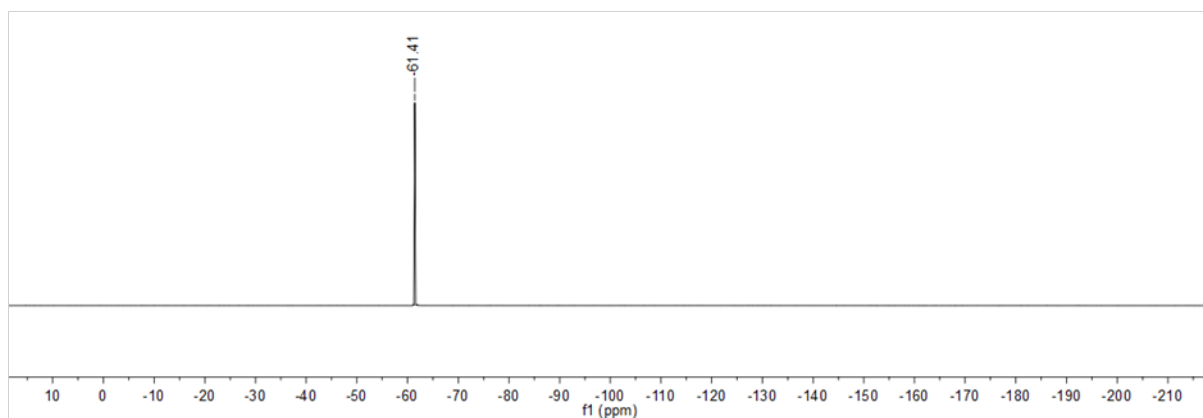
^1H NMR (DMSO- d_6 , 400 MHz)



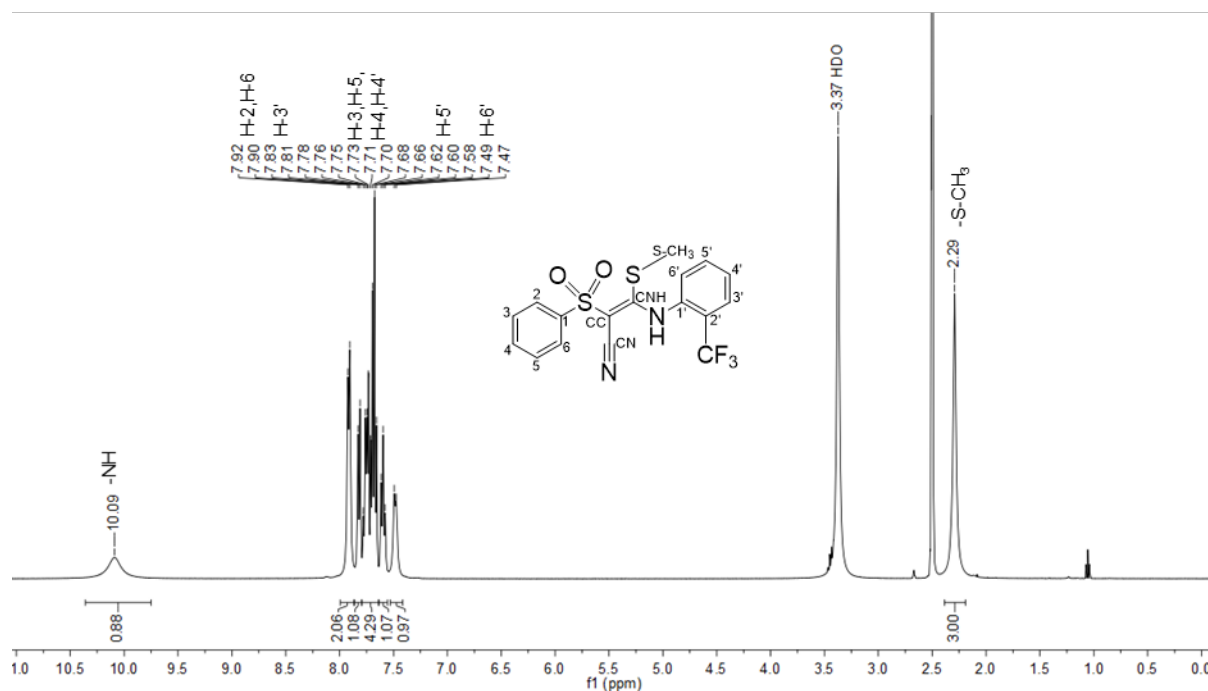
^{13}C NMR (DMSO- d_6 , 100 MHz)



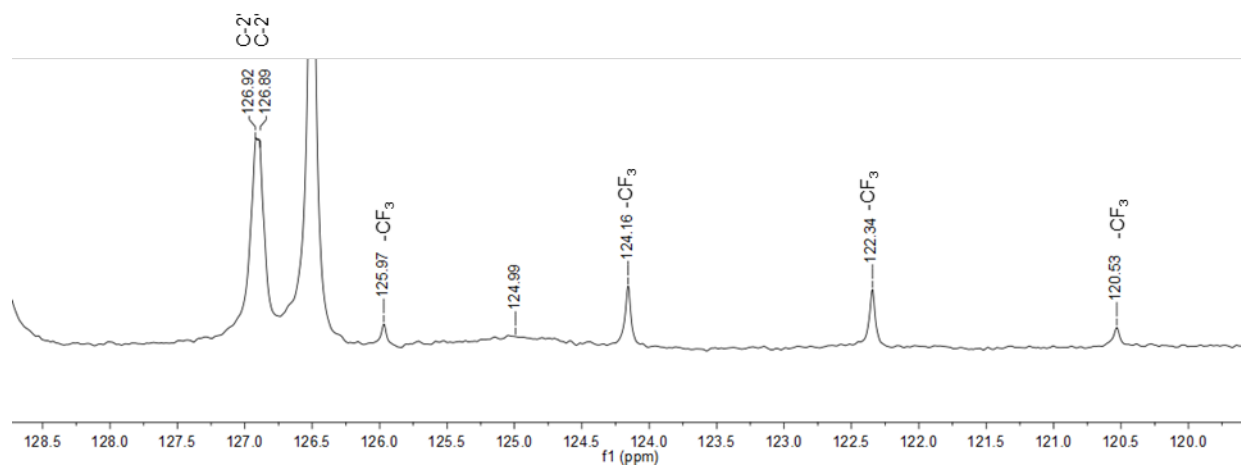
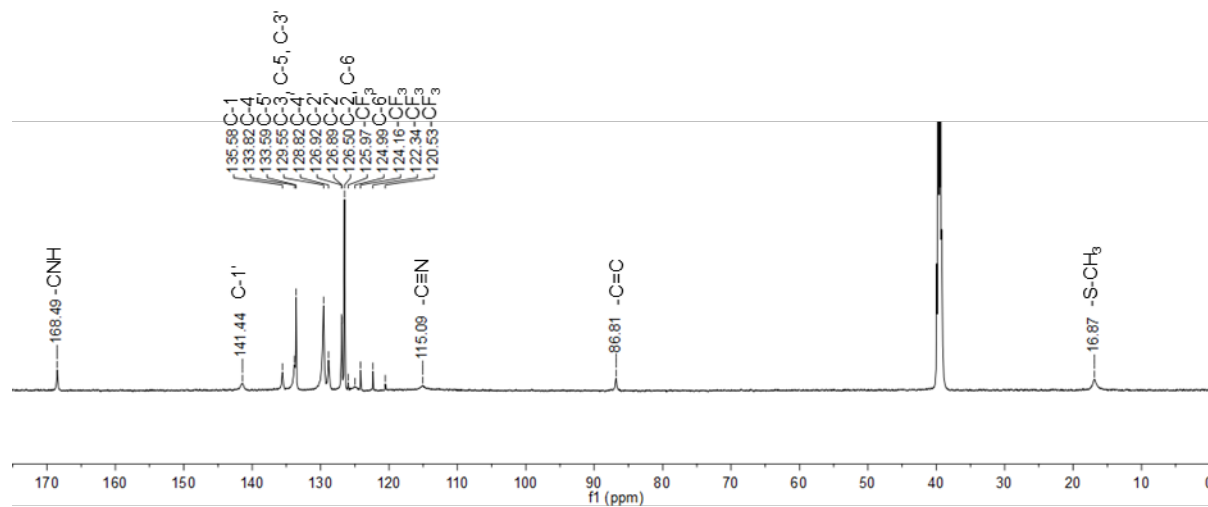
^{19}F NMR (DMSO- d_6 , 376MHz)



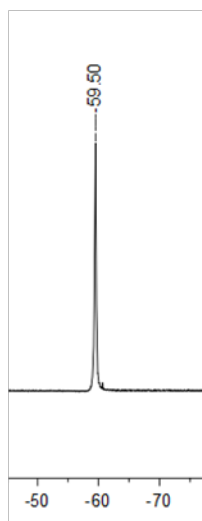
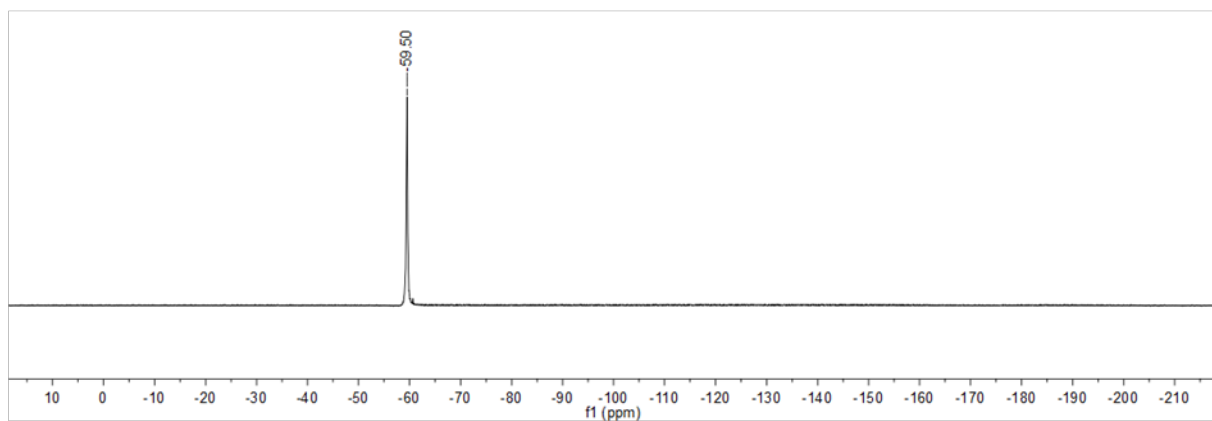
^1H NMR (DMSO- d_6 , 400 MHz)



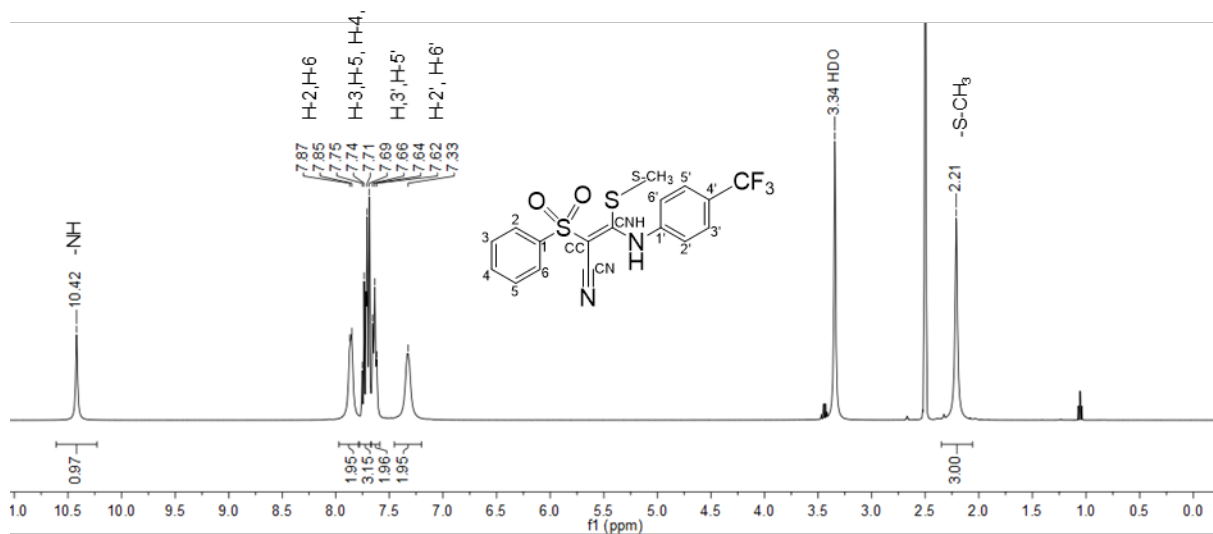
^{13}C NMR (DMSO- d_6 , 151 MHz)



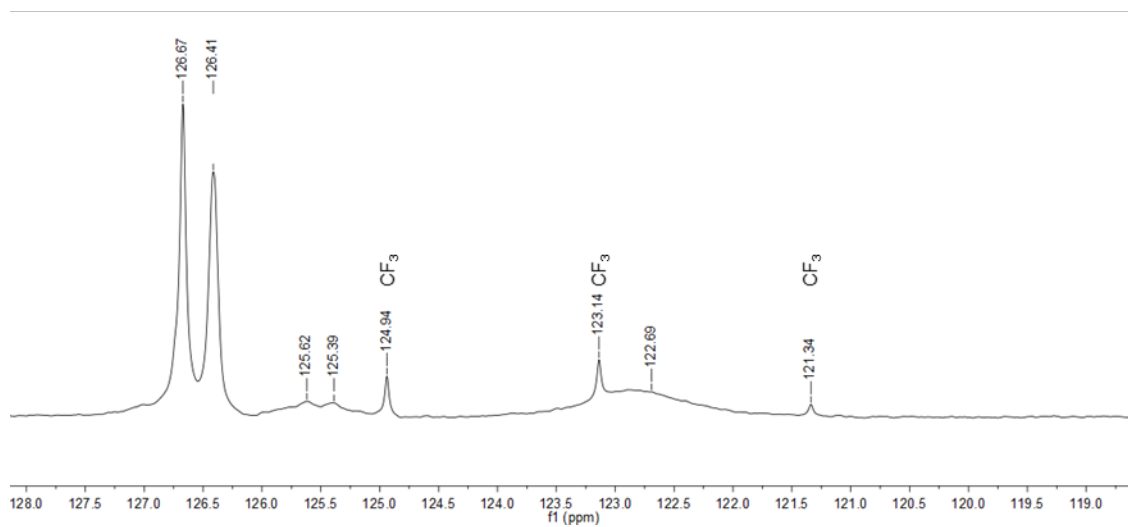
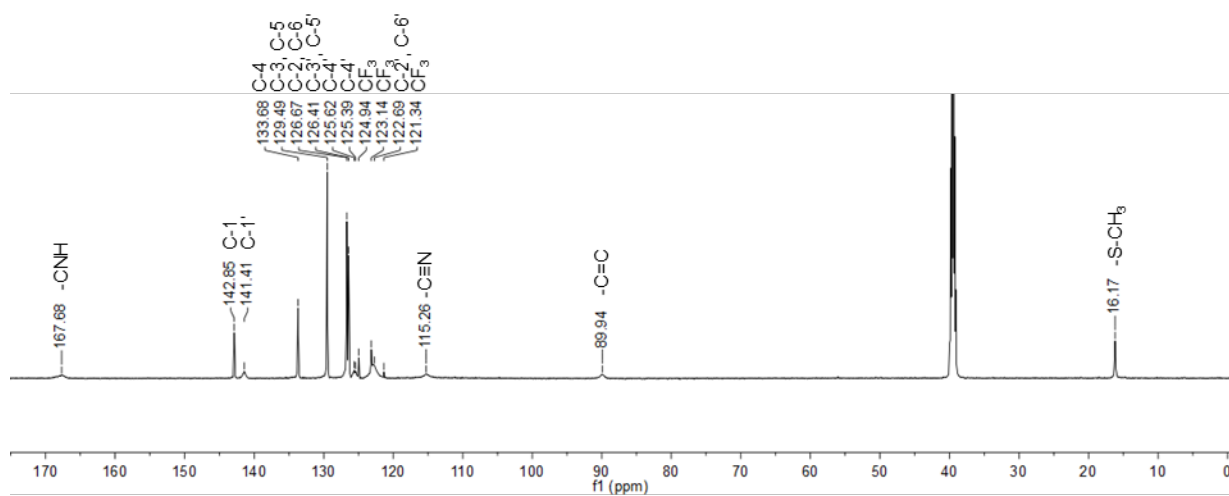
^{19}F NMR (DMSO- d_6 , 376 MHz)



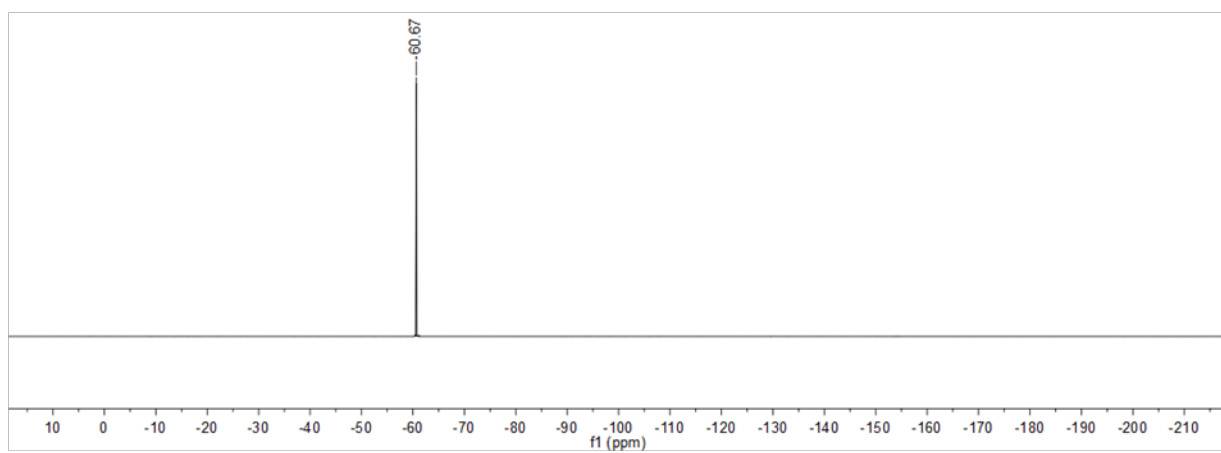
¹H NMR (DMSO-d₆, 400 MHz)



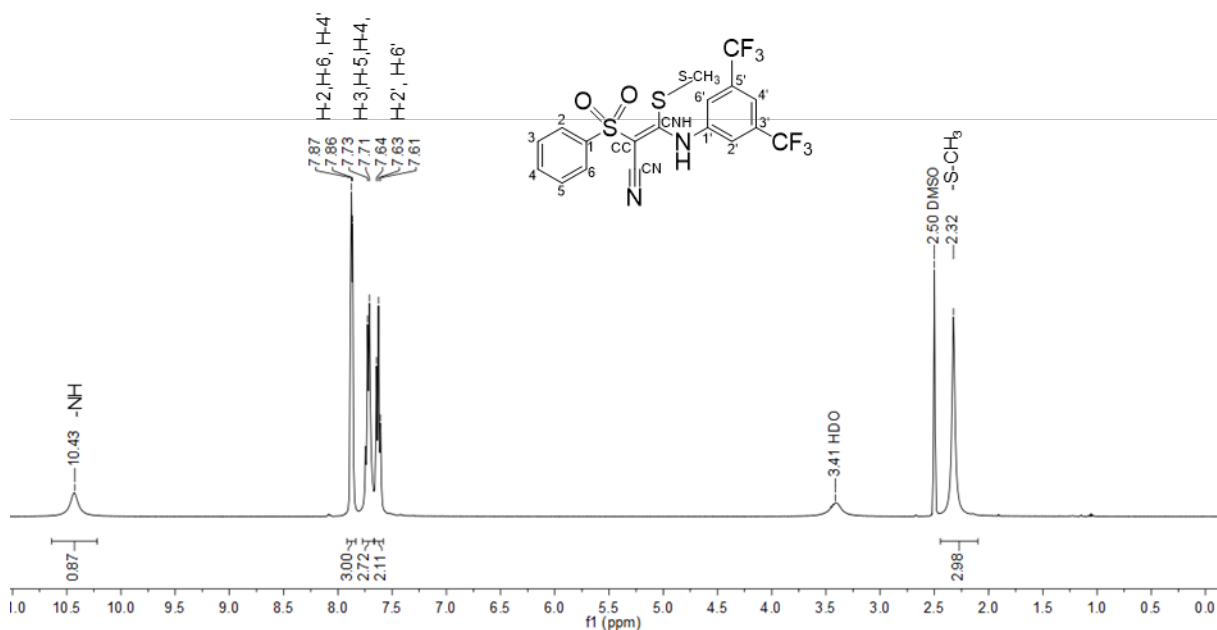
¹³C NMR (DMSO-d₆, 151 MHz)



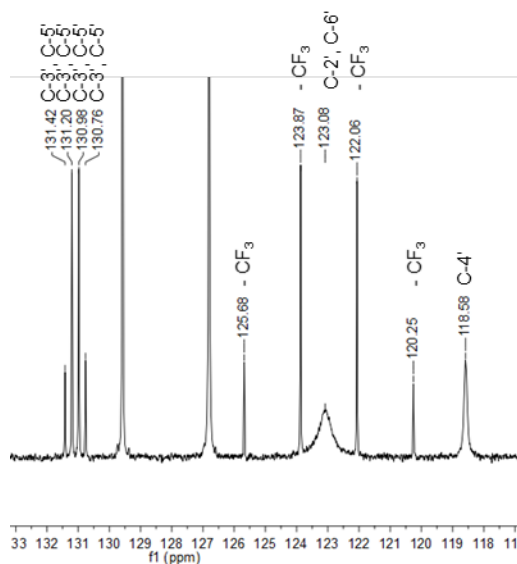
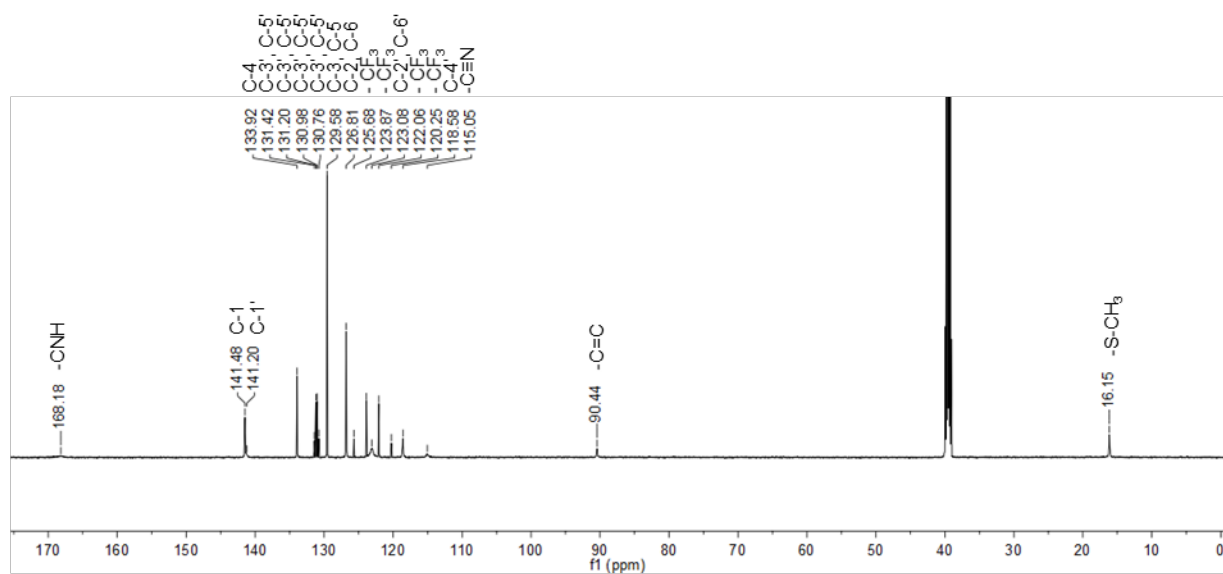
^{19}F NMR (DMSO- d_6 , 376 MHz)



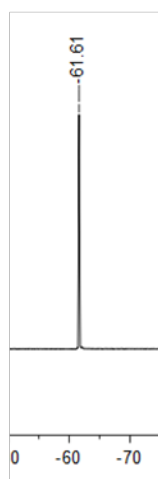
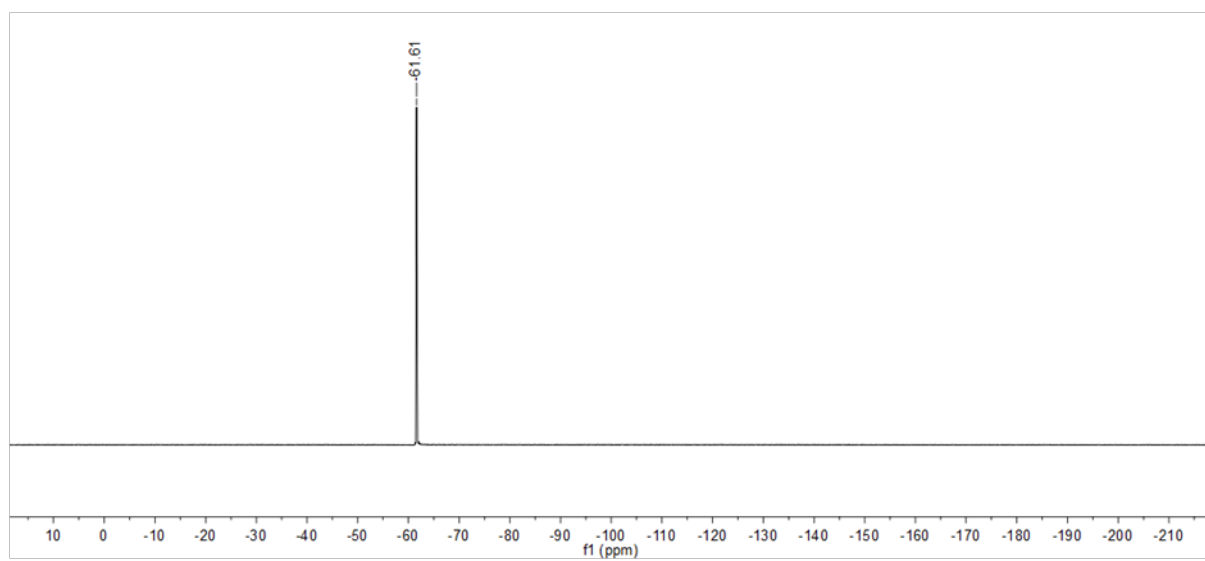
¹H NMR (DMSO-d₆, 400 MHz)



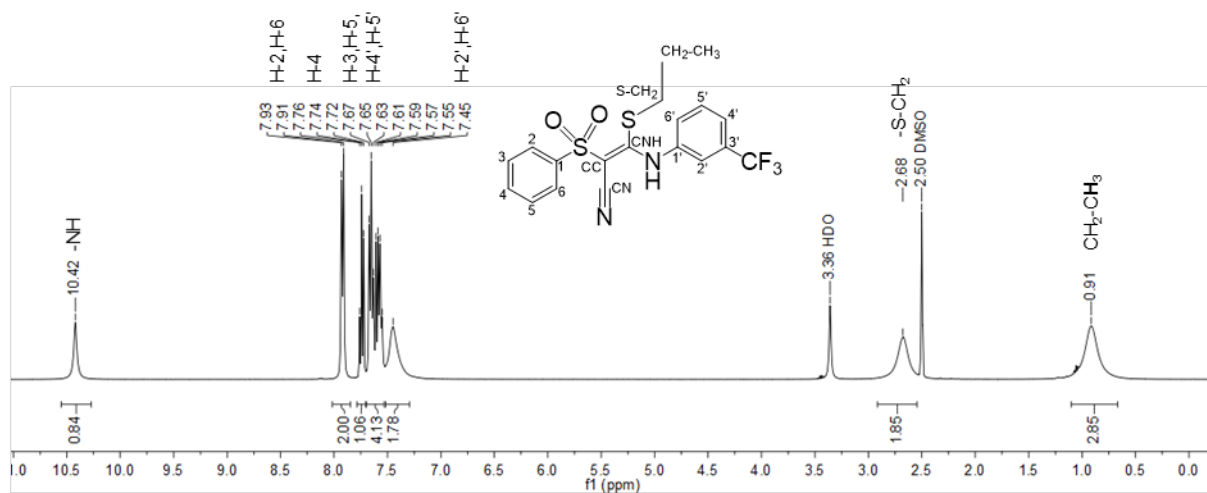
¹³C NMR (DMSO-d₆, 151 MHz)



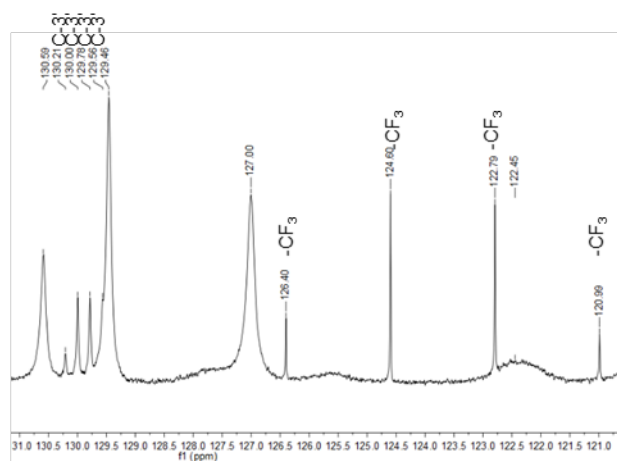
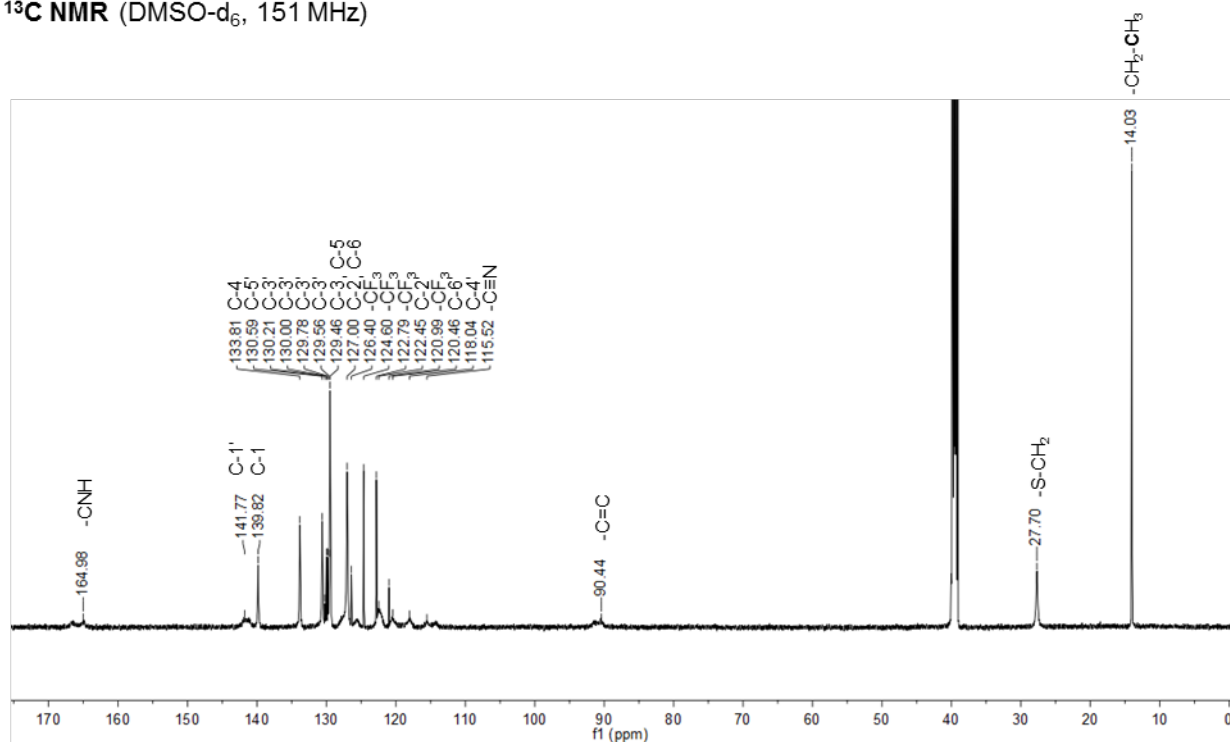
^{19}F NMR (DMSO- d_6 , 376 MHz)



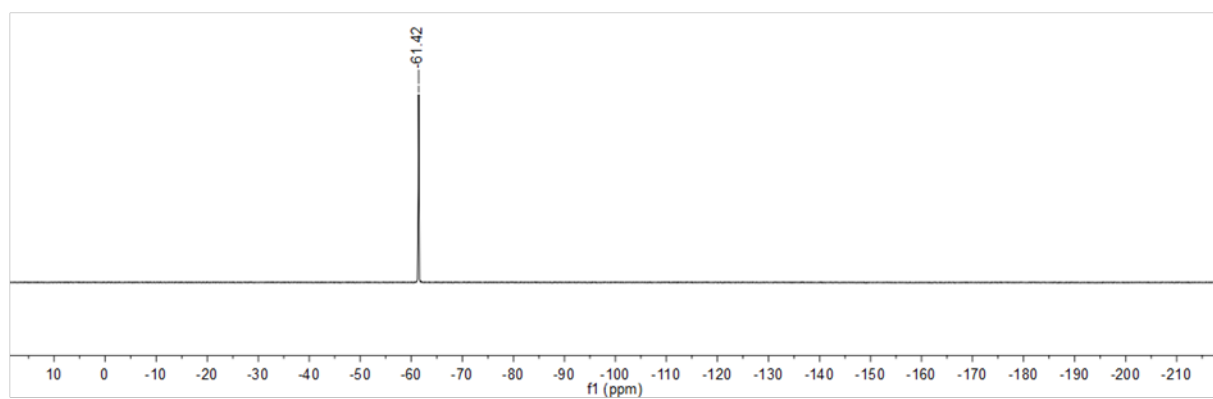
^1H NMR (DMSO- d_6 , 400 MHz)



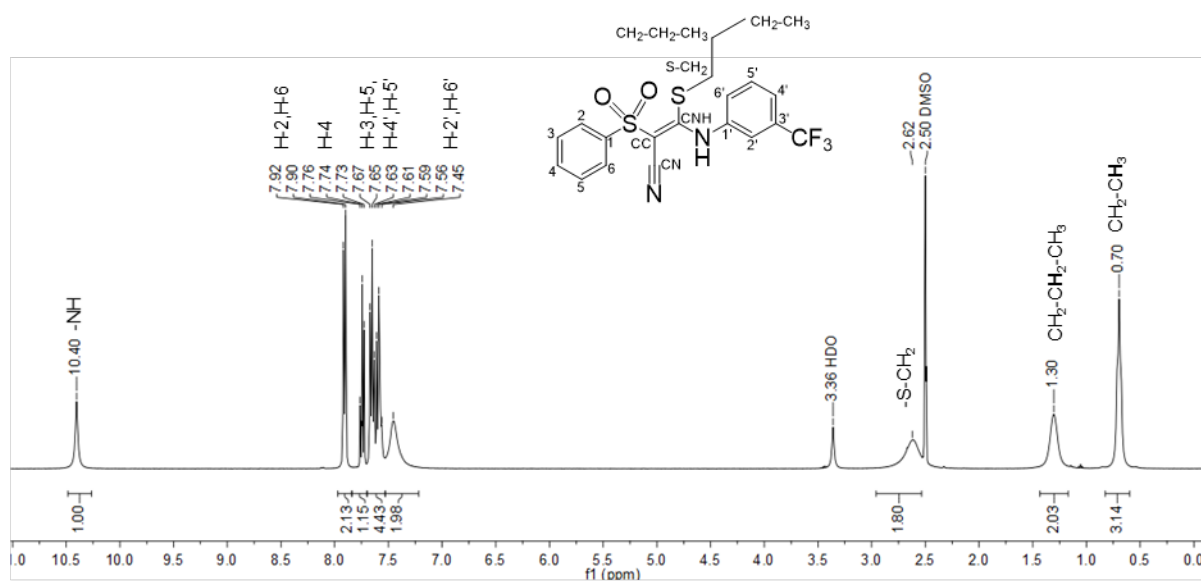
^{13}C NMR (DMSO- d_6 , 151 MHz)



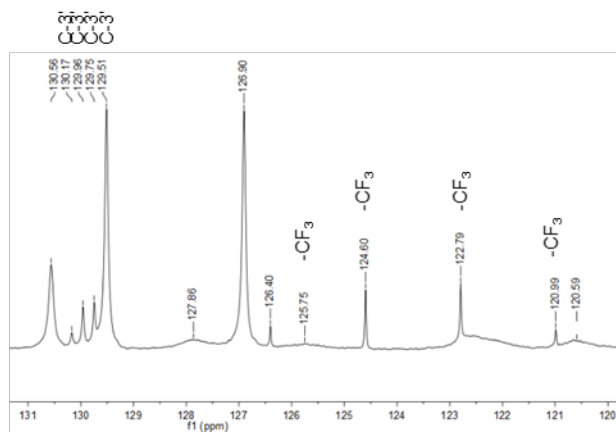
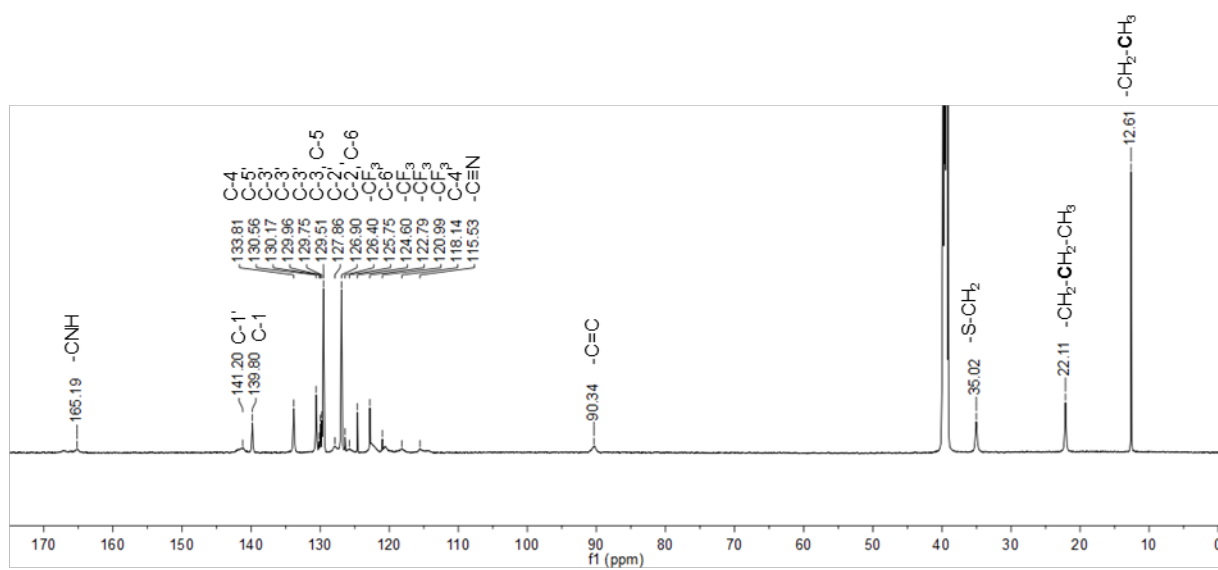
^{18}F NMR (DMSO- d_6 , 376 MHz)



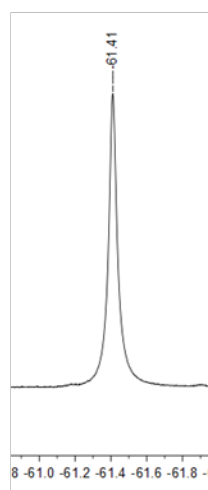
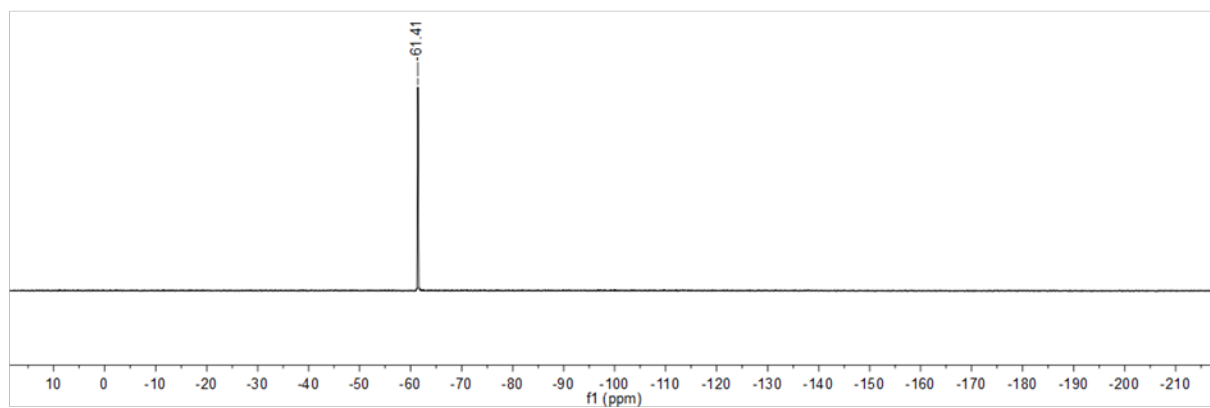
¹H NMR (DMSO-d₆, 400 MHz)



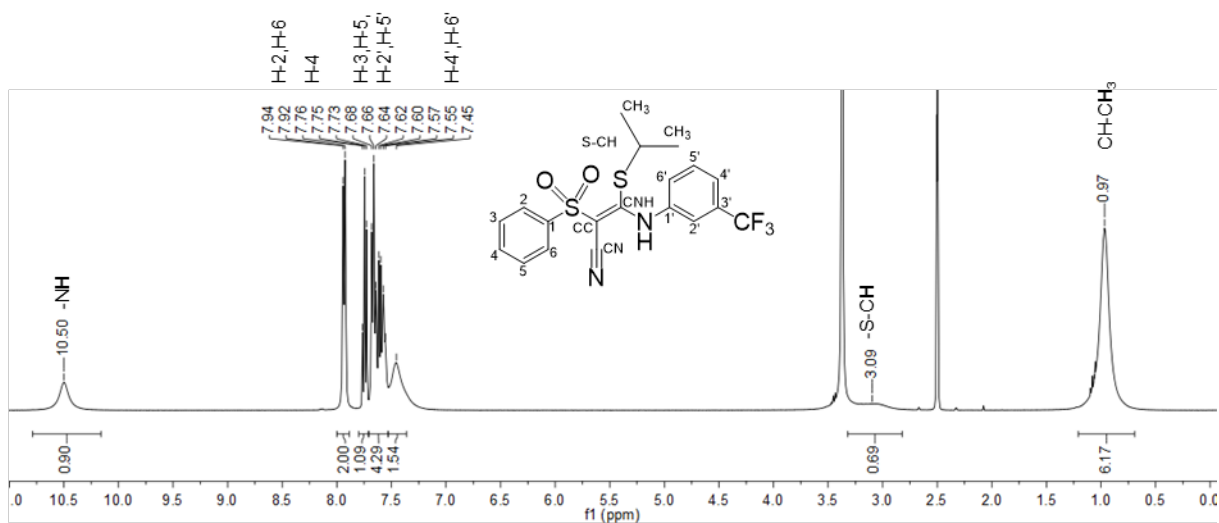
¹³C NMR (DMSO-d₆, 151 MHz)



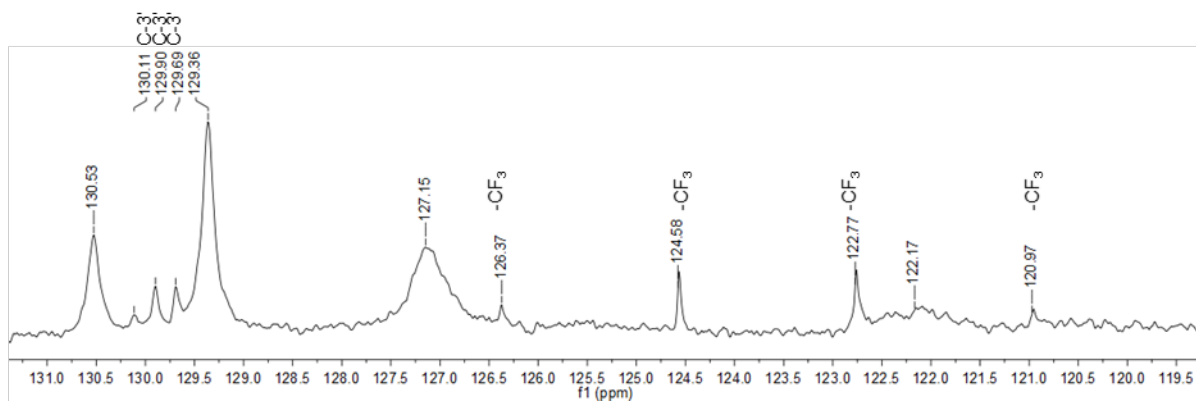
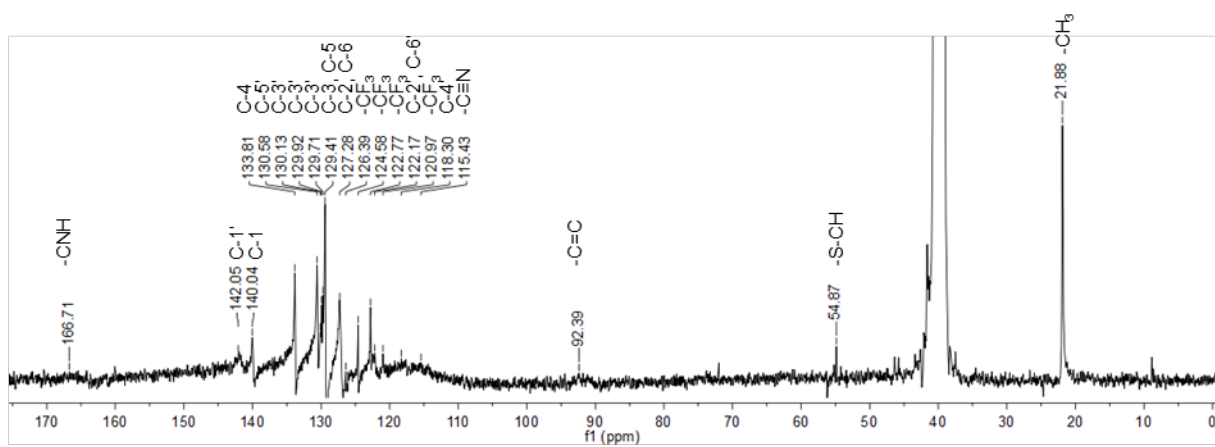
^{18}F NMR (DMSO- d_6 , 376 MHz)



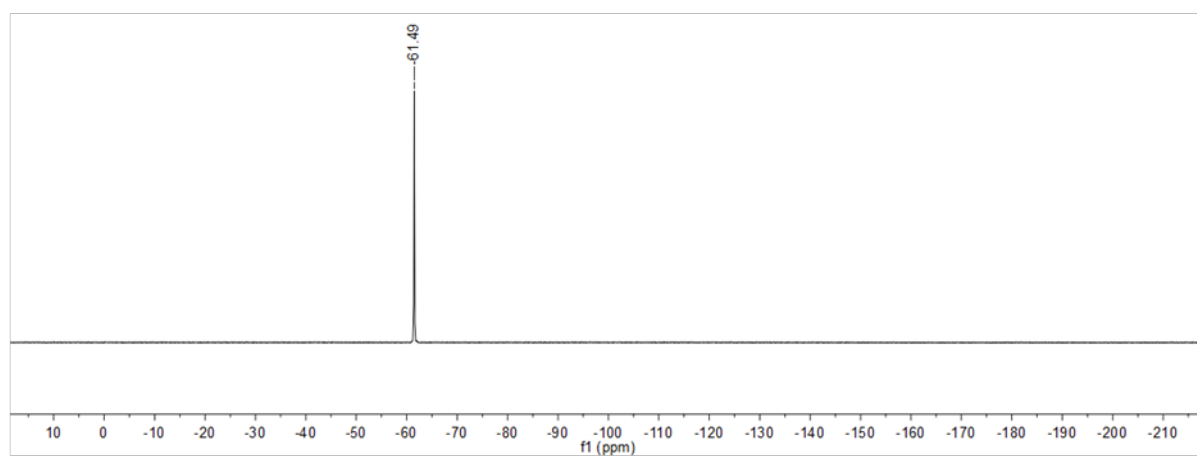
^1H NMR (DMSO- d_6 , 400 MHz)



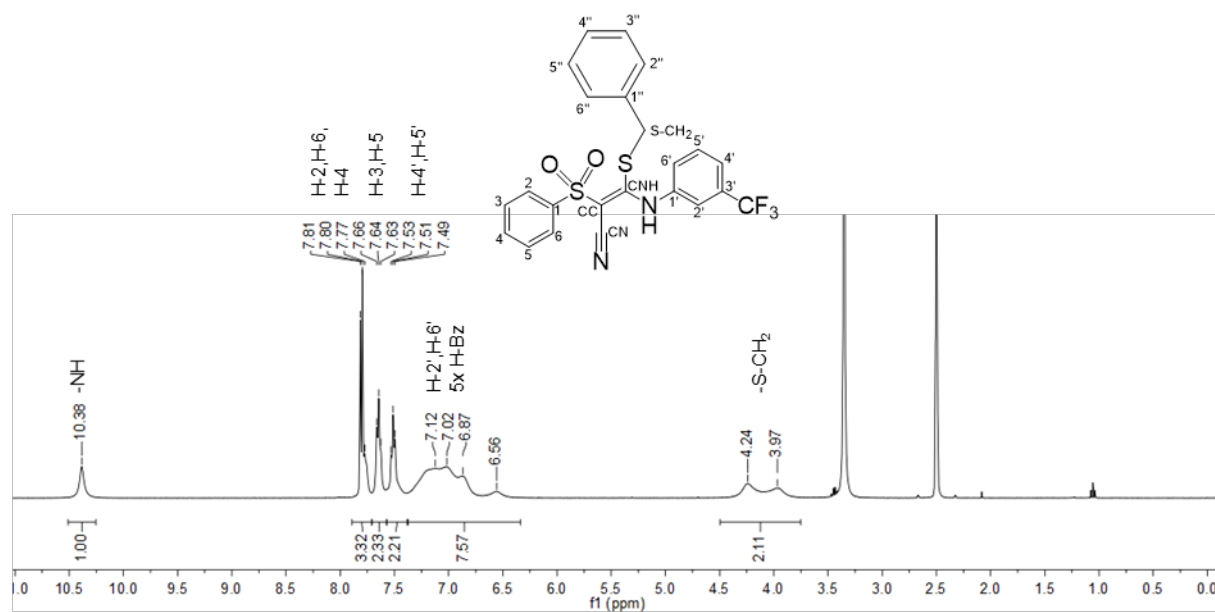
^{13}C NMR (DMSO- d_6 , 151 MHz)



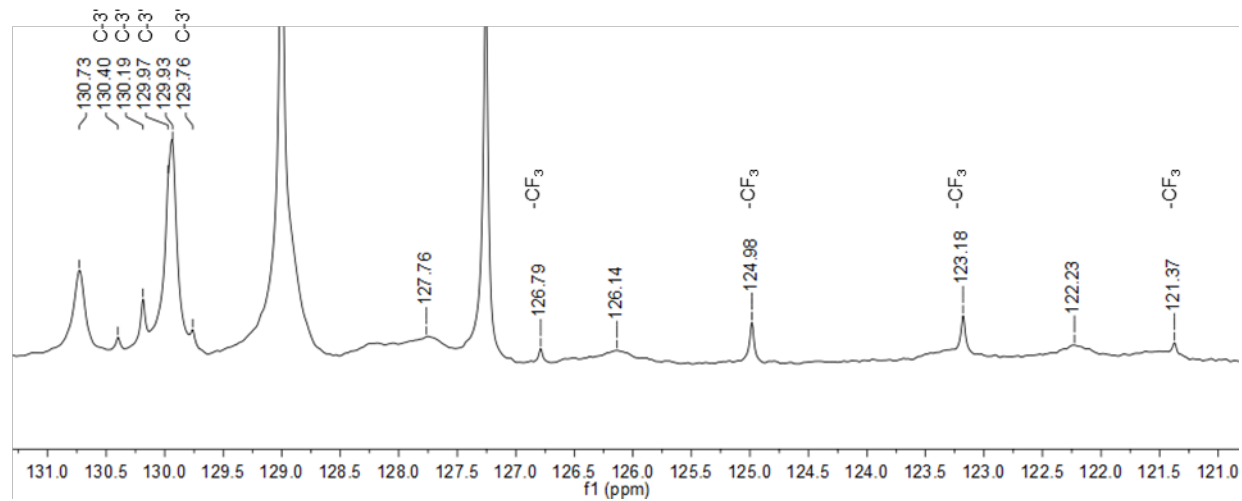
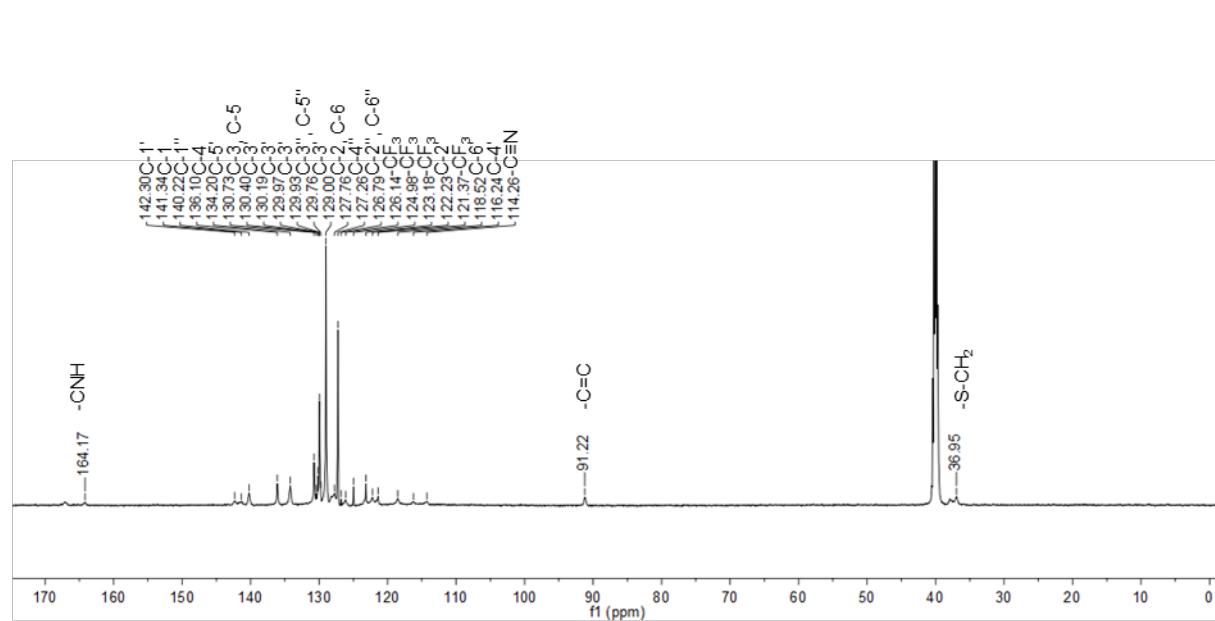
^{18}F NMR (DMSO- d_6 , 376 MHz)



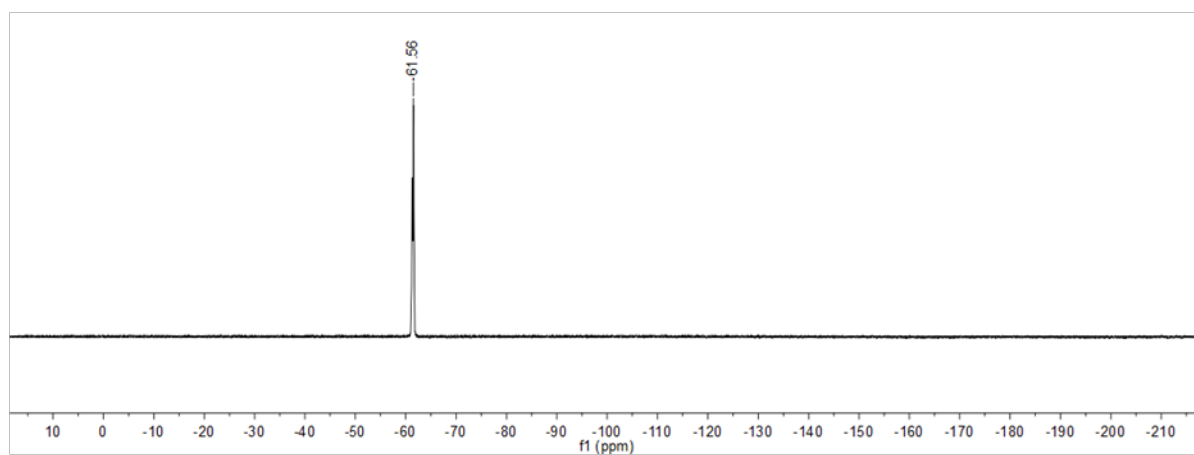
^1H NMR (DMSO- d_6 , 400 MHz)



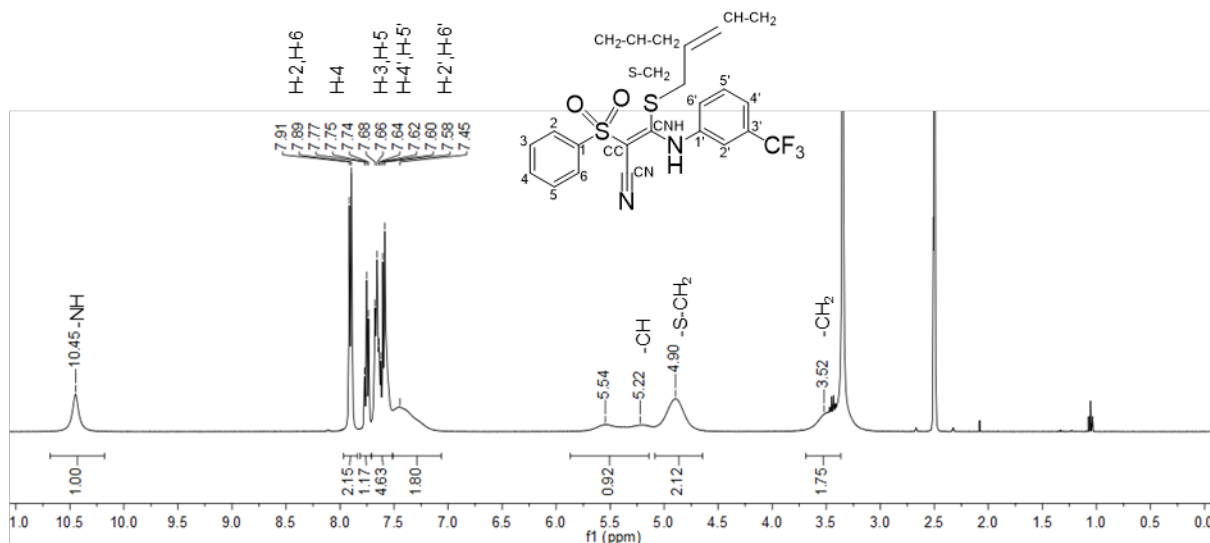
^{13}C NMR (DMSO- d_6 , 151 MHz)



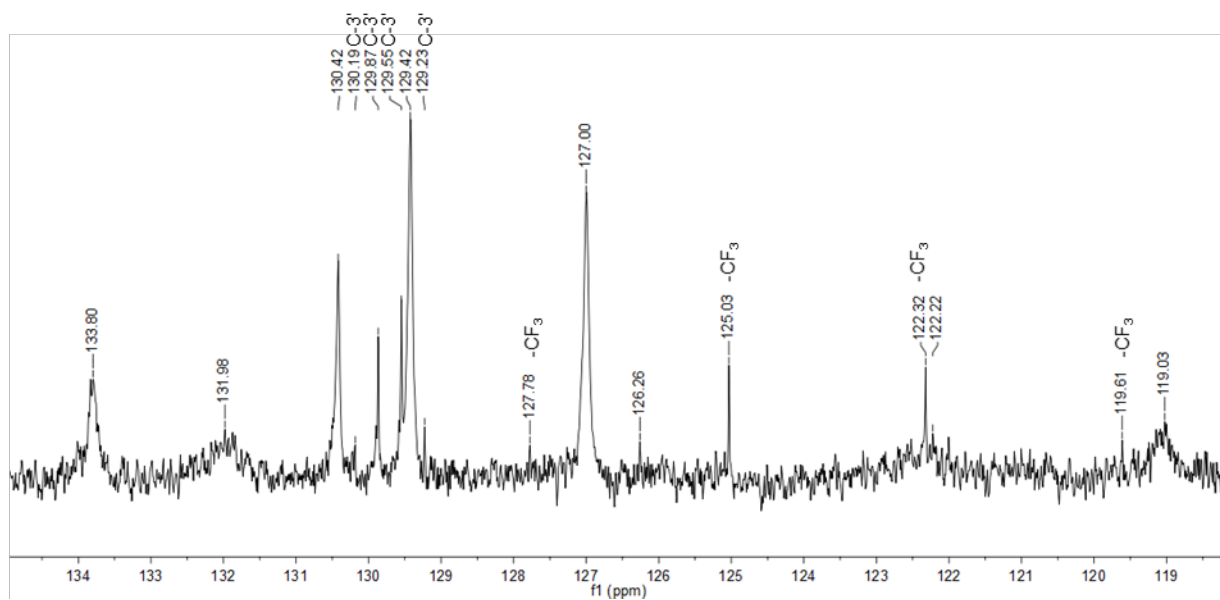
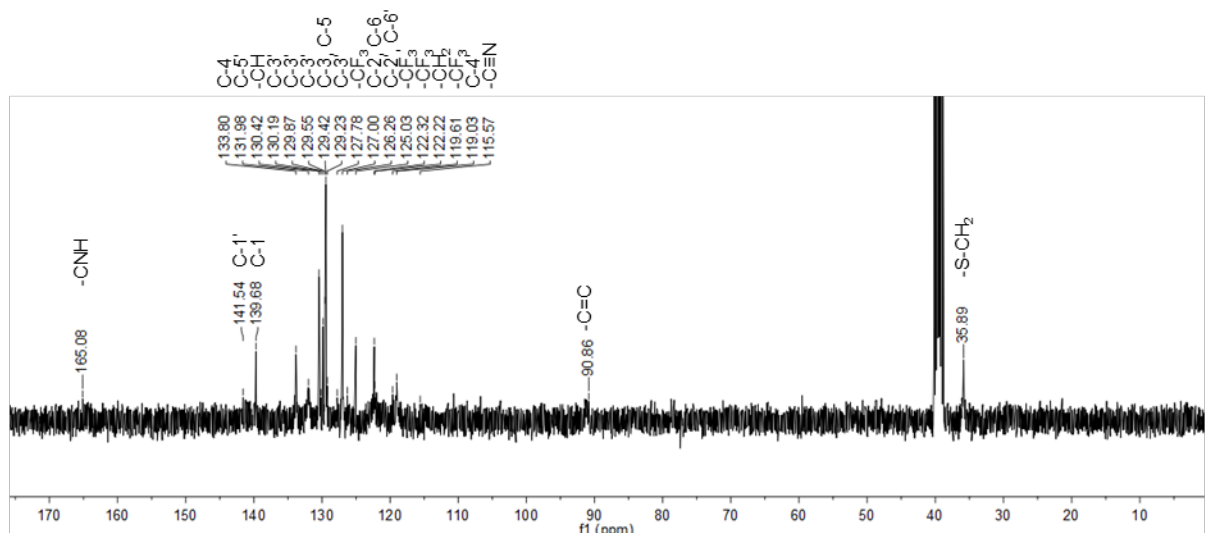
^{18}F NMR (DMSO- d_6 , 376 MHz)



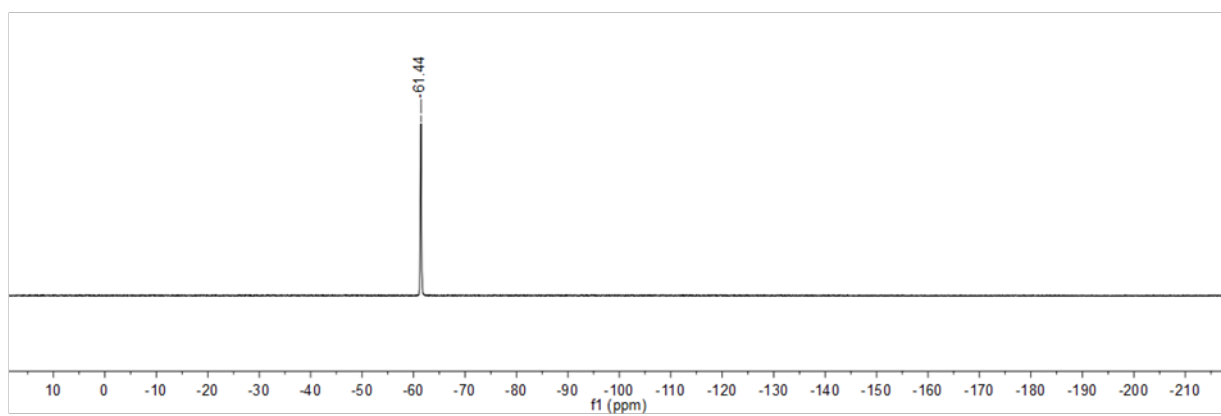
¹H NMR (DMSO-d₆, 400 MHz)



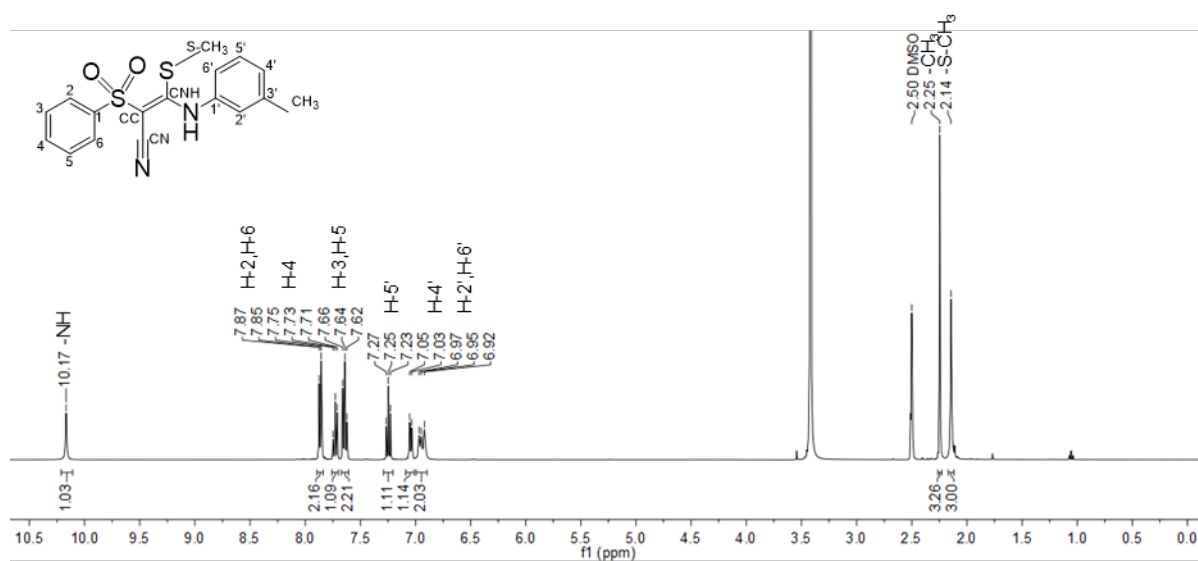
¹³C NMR (DMSO-d₆, 100 MHz)



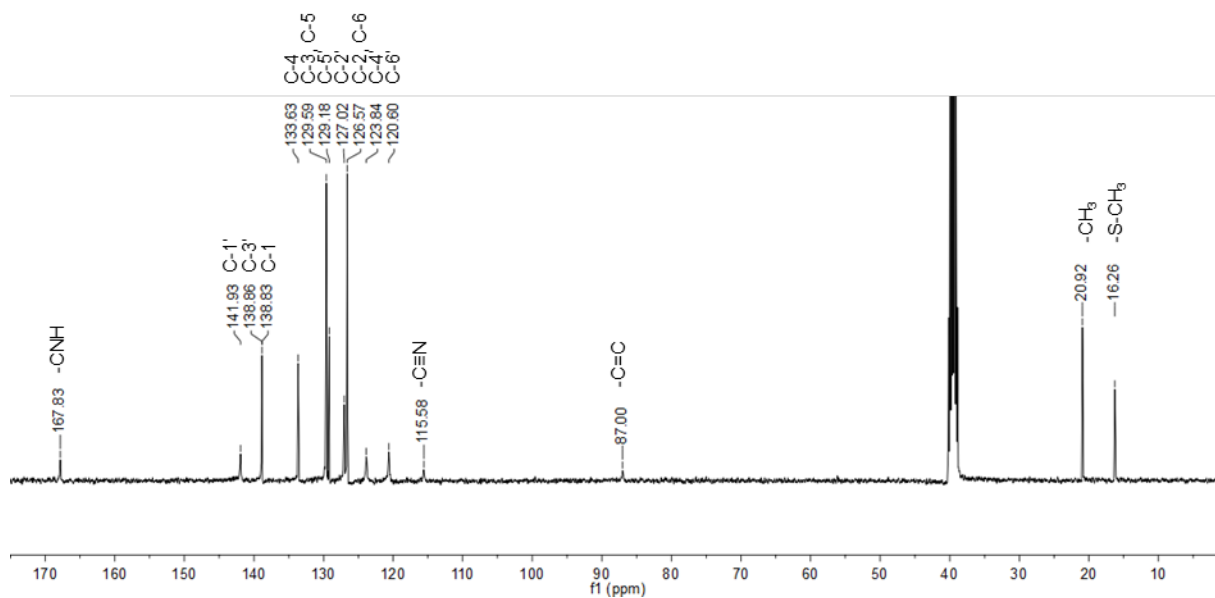
^{18}F NMR (DMSO- d_6 , 376 MHz)



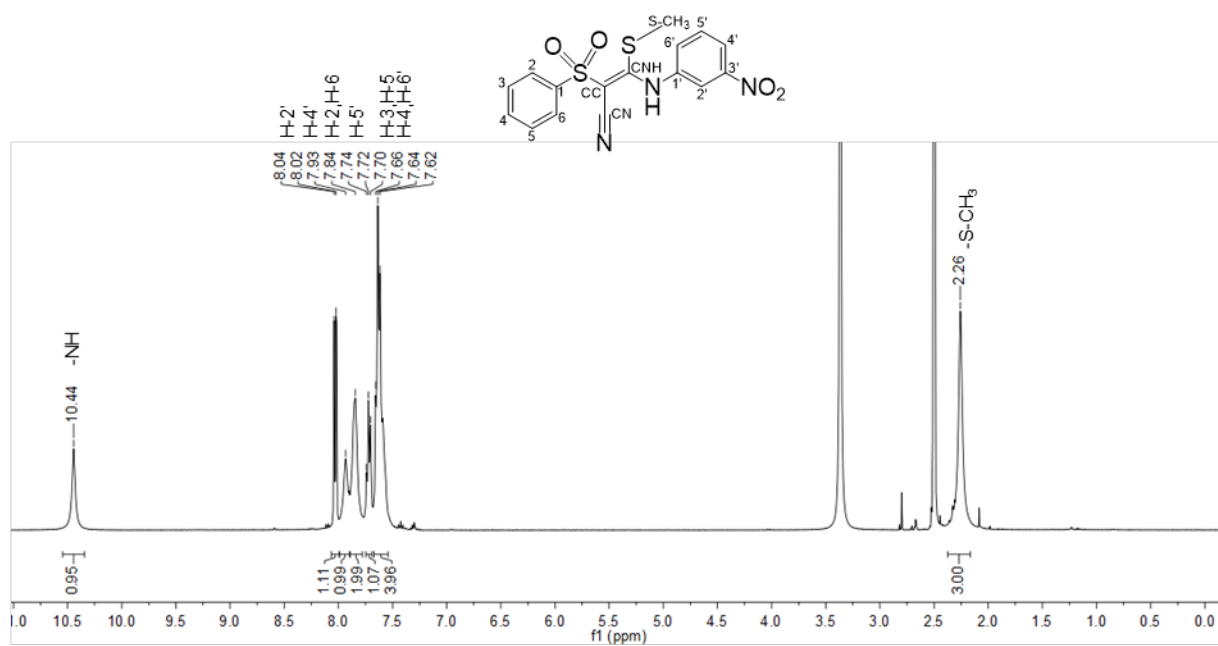
¹H NMR (DMSO-d₆, 400 MHz)



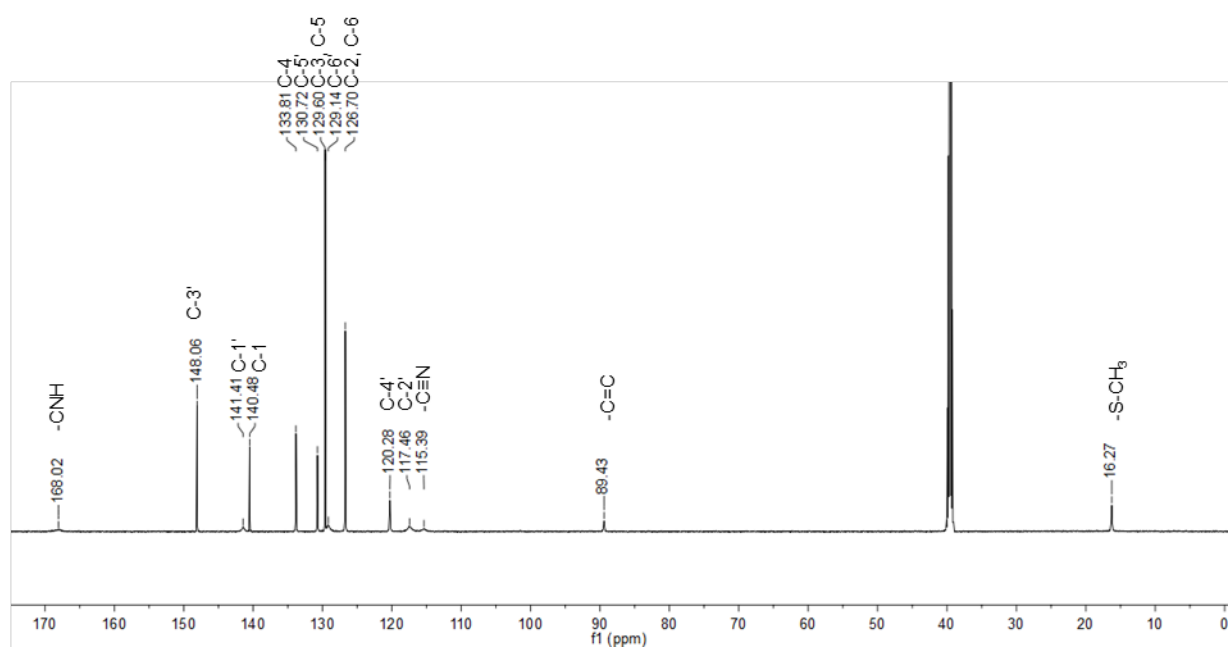
¹³C NMR (DMSO-d₆, 100 MHz)



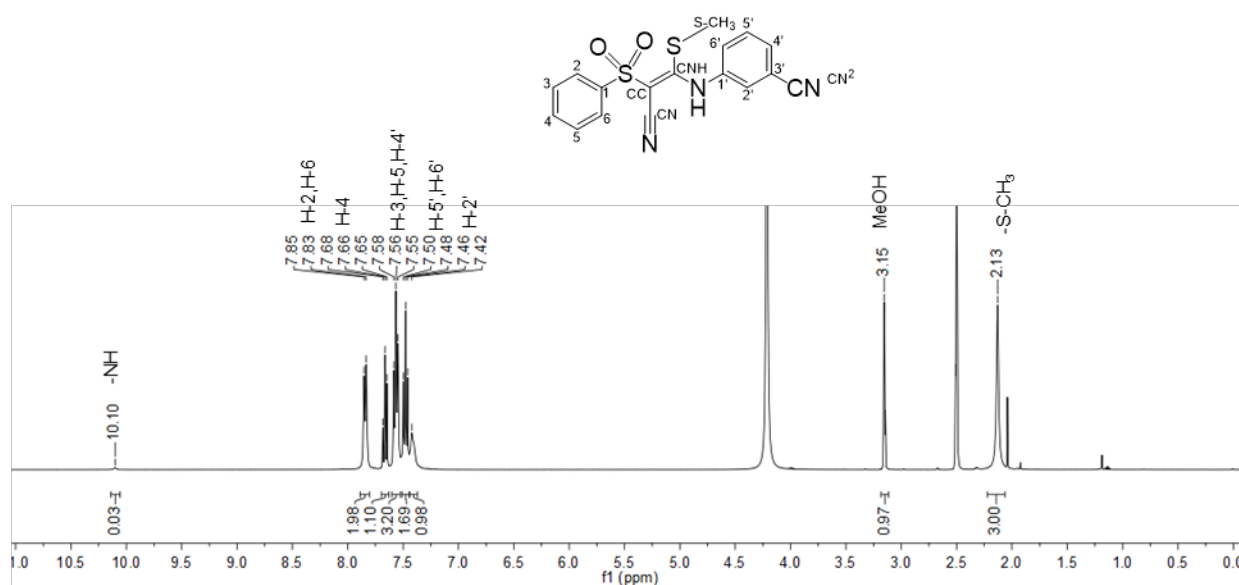
¹H NMR (DMSO-d₆, 400 MHz)



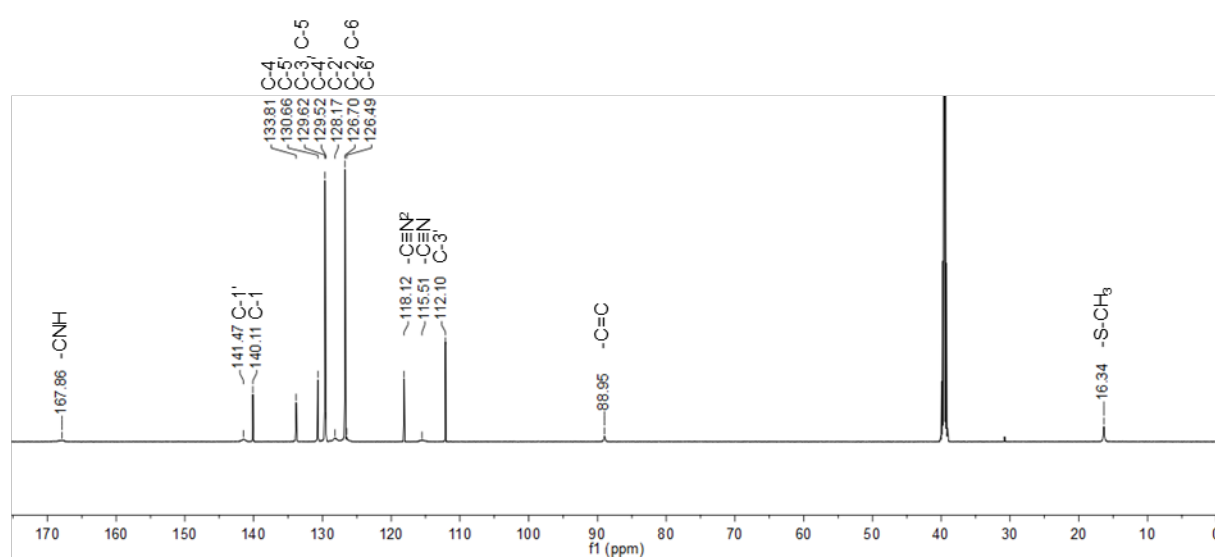
¹³C NMR (DMSO-d₆, 151 MHz)



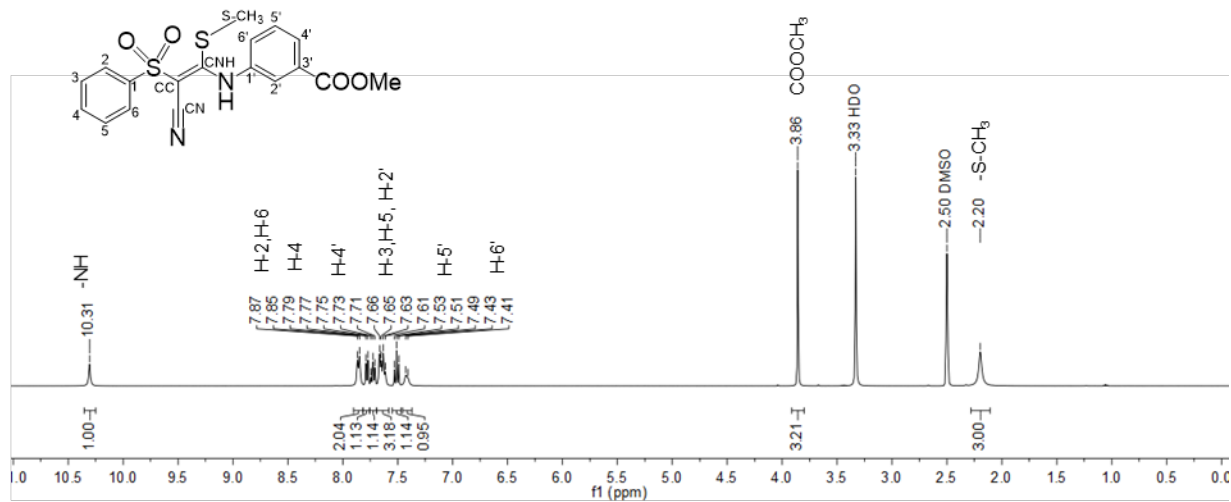
¹H NMR (DMSO-d₆, 400 MHz)



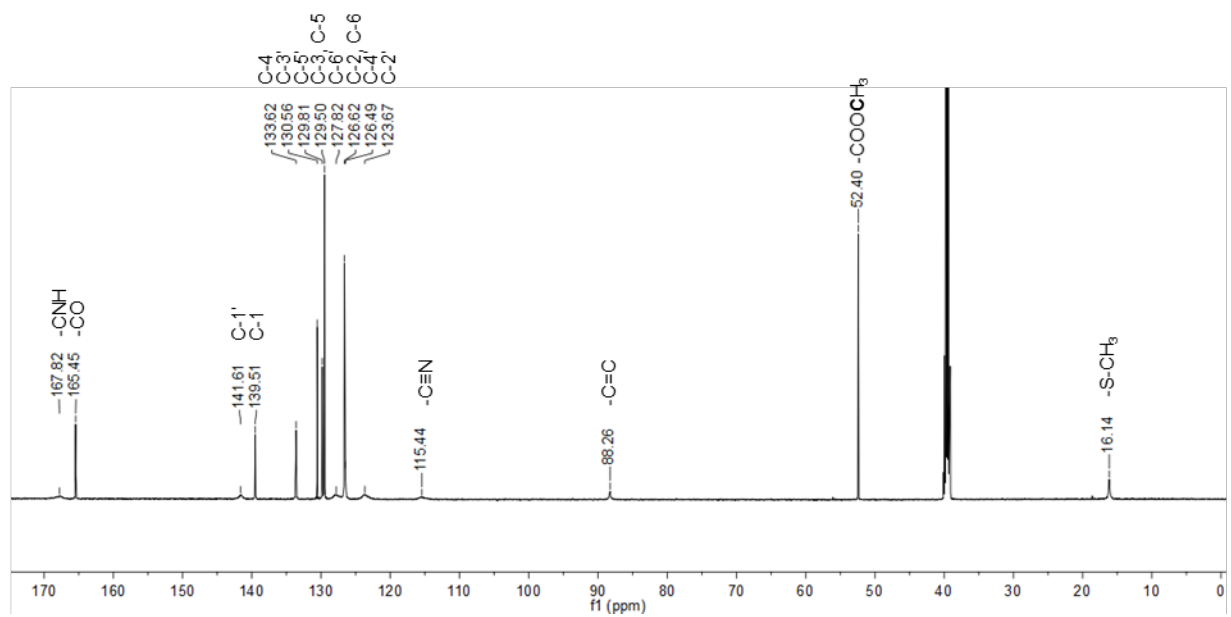
¹³C NMR (DMSO-d₆, 151 MHz)



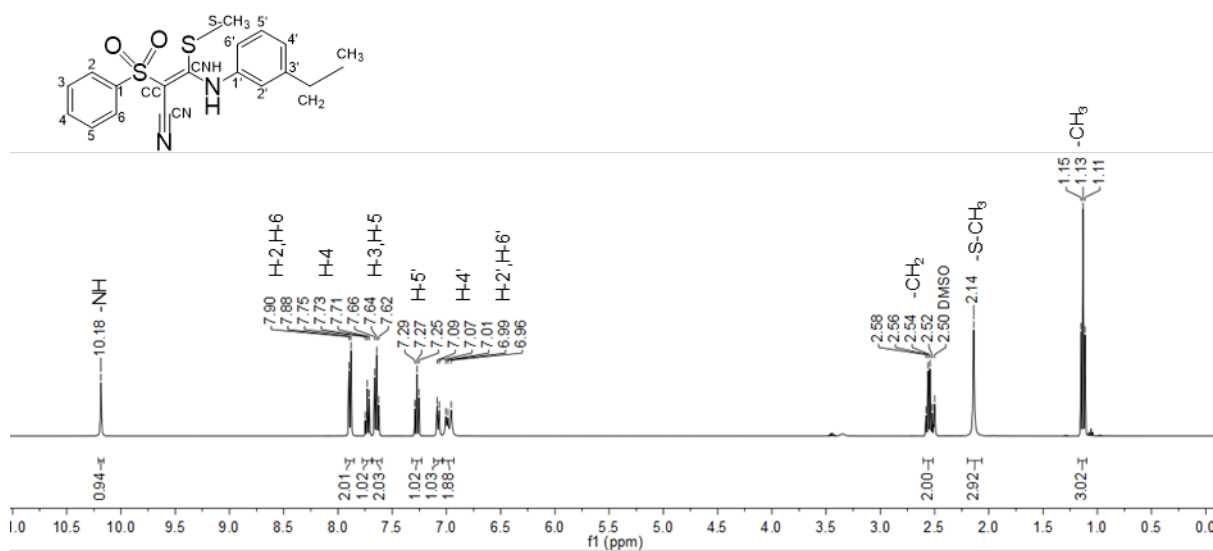
^1H NMR (DMSO- d_6 , 400 MHz)



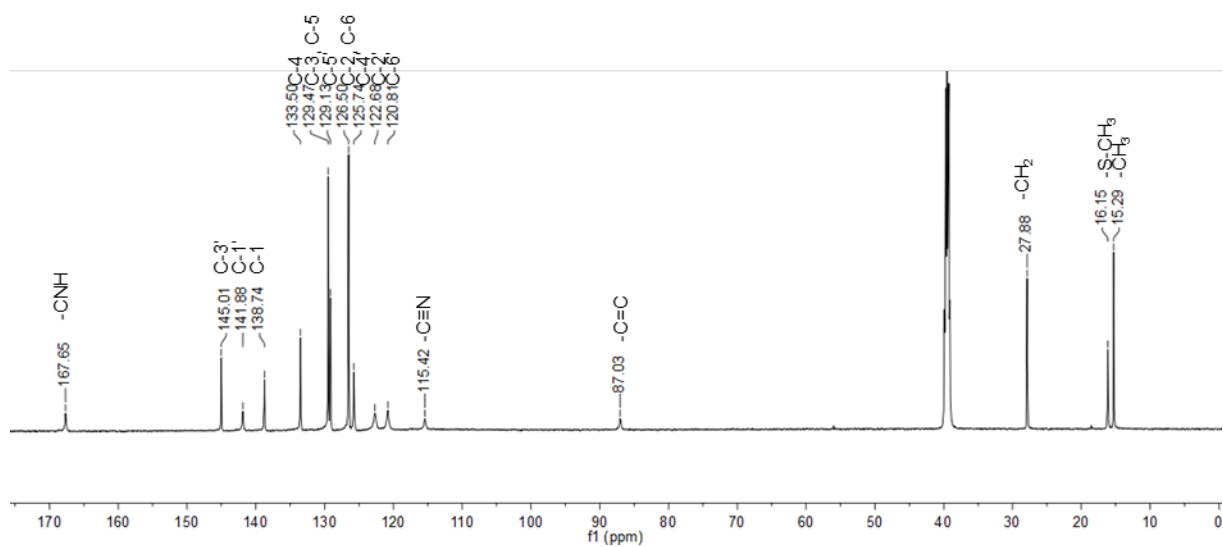
^{13}C NMR (DMSO- d_6 , 151 MHz)



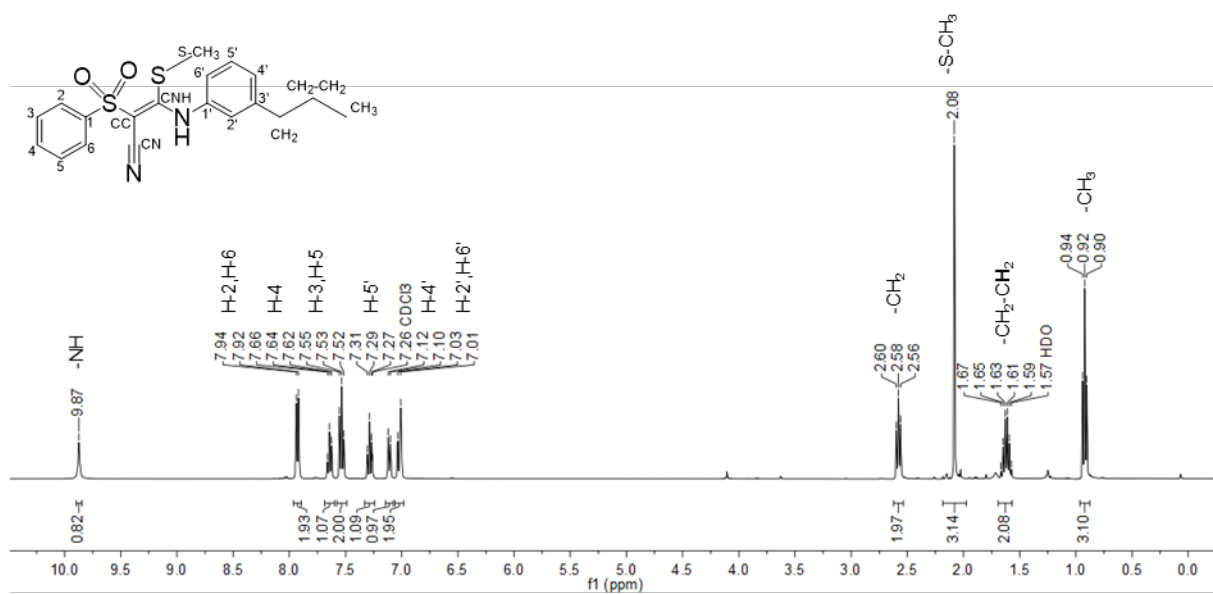
^1H NMR (DMSO- d_6 , 400 MHz)



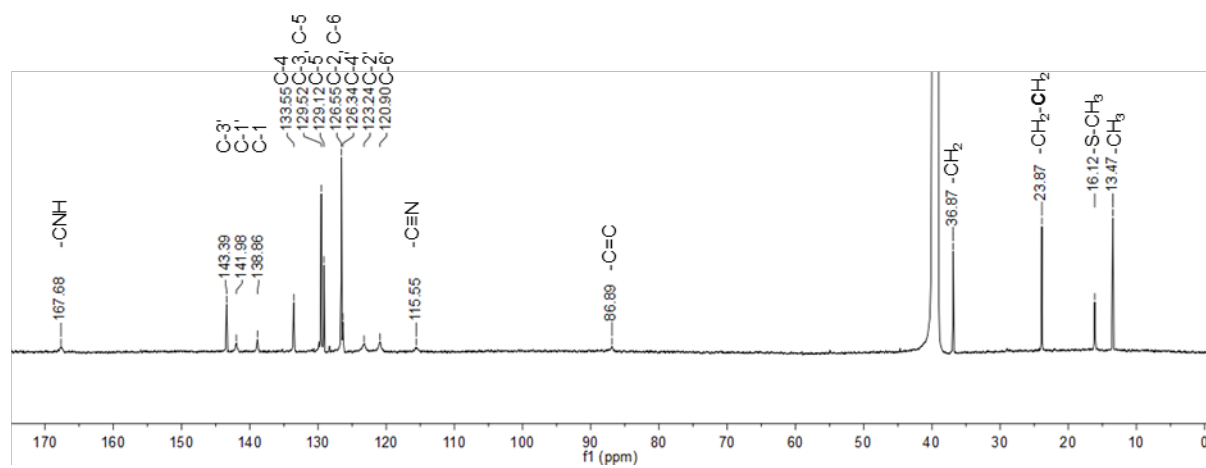
^{13}C NMR (DMSO- d_6 , 151 MHz)



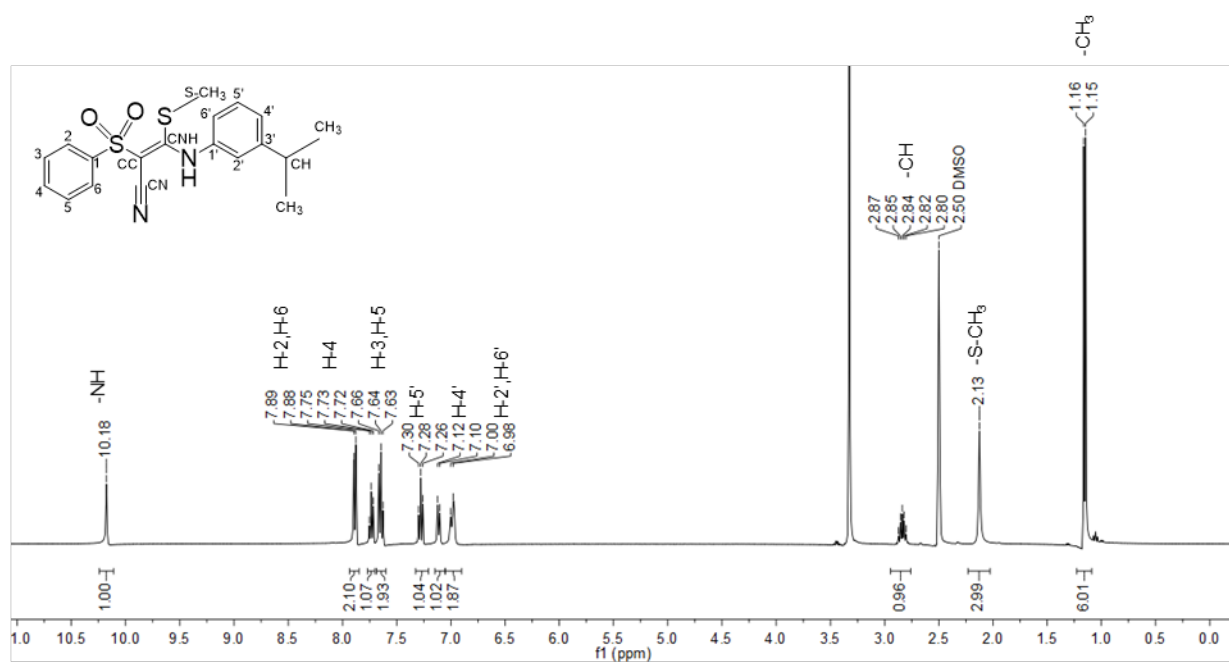
^1H NMR (CDCl_3 , 400 MHz)



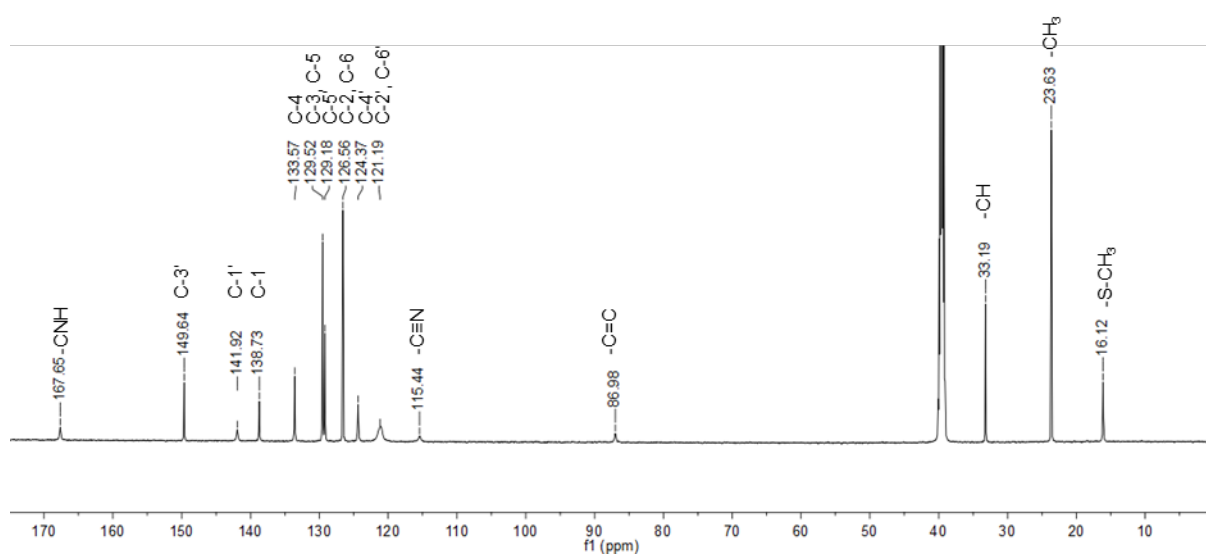
^{13}C NMR (CDCl_3 , 151 MHz)



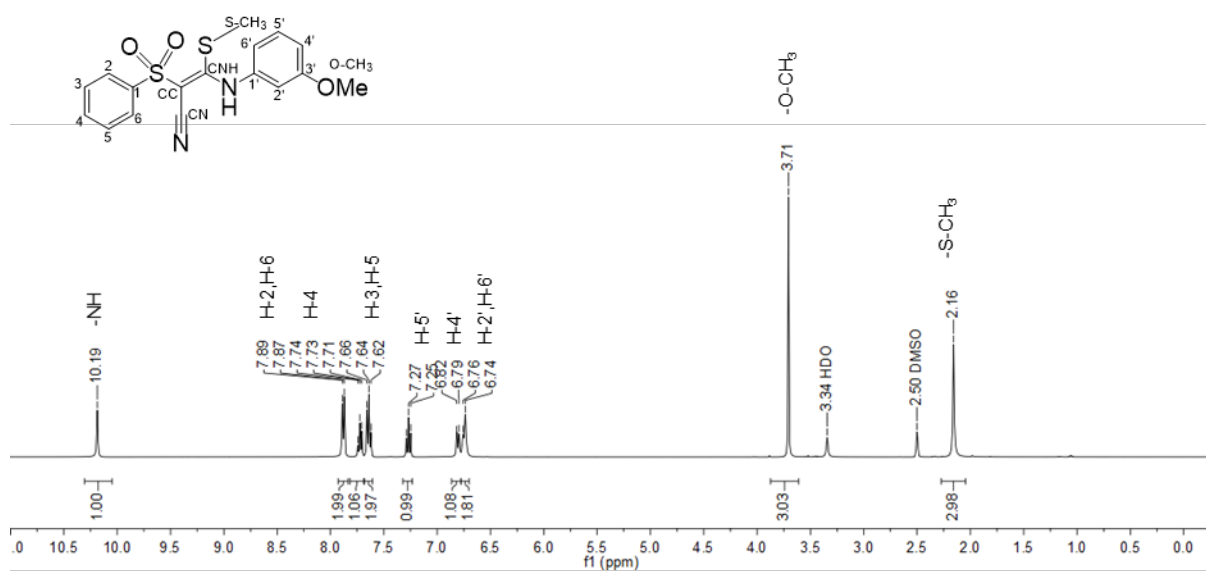
^1H NMR (DMSO- d_6 , 400 MHz)



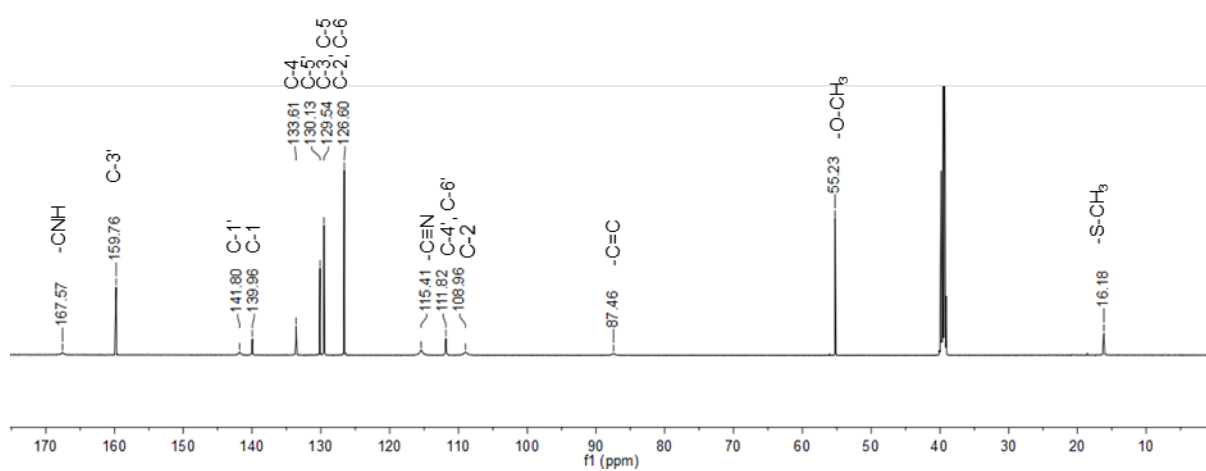
^{13}C NMR (DMSO- d_6 , 151 MHz)



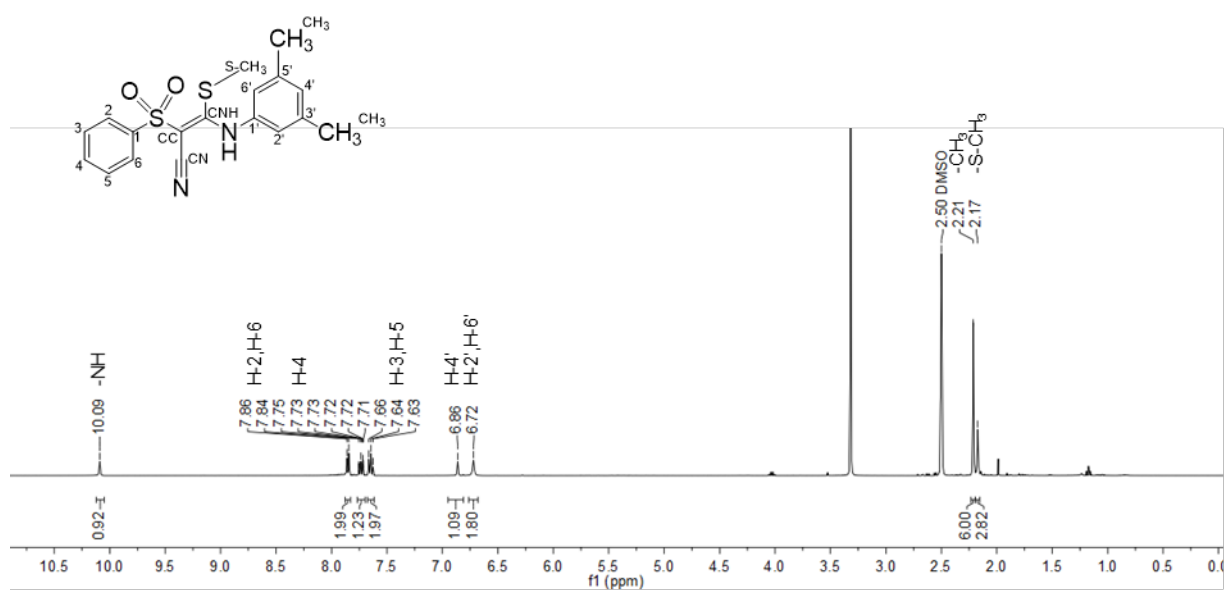
^1H NMR (DMSO- d_6 , 400 MHz)



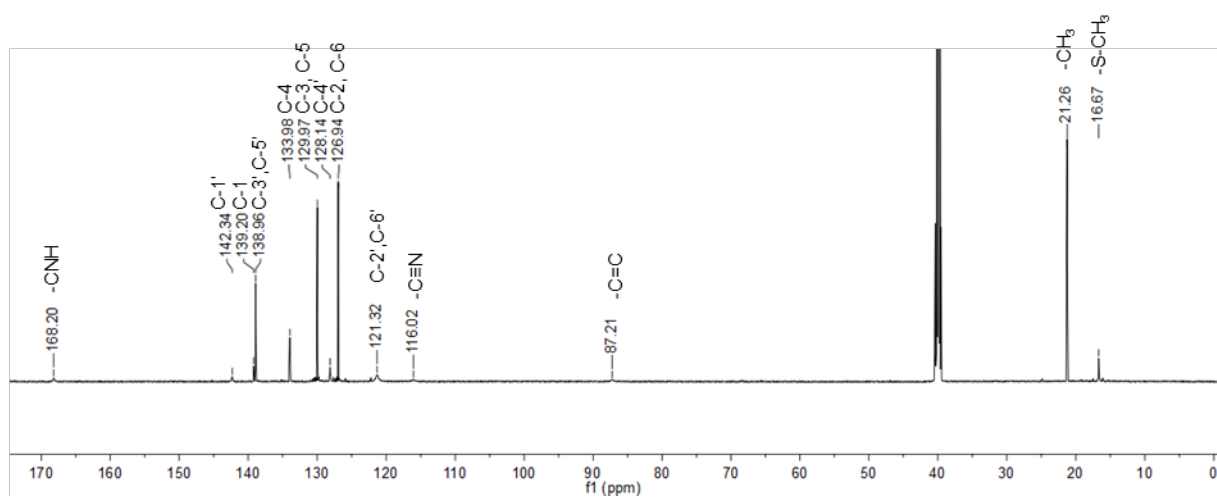
^{13}C NMR (DMSO- d_6 , 151 MHz)



^1H NMR (DMSO- d_6 , 400 MHz)



^{13}C NMR (DMSO- d_6 , 151 MHz)



References

1. Corporation, O. (1991-2015) Origin, Origin 2015 (64bit) Sr2 ed., OriginLab Corporation, One Roundhouse Plaza PriginLab Cooperation, Northampton MA 01060 USA.
2. Grosdidier, A., Zoete, V., and Michielin, O. (2011) SwissDock, a protein-small molecule docking web service based on EADock DSS, *Nucleic Acids Res.* 39, W270-277.
3. Grosdidier, A., Zoete, V., and Michielin, O. (2011) Fast docking using the CHARMM force field with EADock DSS, *J.Comput.Chem.* 32, 2149-2159.
4. Mori, S., Shibayama, K., Wachino, J.-i., and Arakawa, Y. (2010) Purification and molecular characterization of a novel diadenosine 5',5'''-P1,P4-tetraphosphate phosphorylase from Mycobacterium tuberculosis H37Rv, *PREP* 69, 99-105.
5. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera--a visualization system for exploratory research and analysis, *J. Comput. Chem.* 25, 1605-1612.
6. Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., and Hutchison, G. R. (2012) Avogadro: an advanced semantic chemical editor, visualization, and analysis platform, *J. Cheminform.* 4, 17.
7. Anderson, M., and Verkman, A. S. (2013) Triazolothienopyrimidine compound inhibitors of urea transporters and methods of using inhibitors, Anderson, M. The Regents of the University of California.
8. Munch, H., Hansen, J. S., Pittelkow, M., Christensen, J. B., and Boas, U. (2008) A new efficient synthesis of isothiocyanates from amines using di-tert-butyl dicarbonate, *Tetrahedron Lett.* 49, 3117-3119.
9. Tagmose, T. M., Zaragoza, F., Boonen, H. C. M., Worsaae, A., Mogensen, J. P., Nielsen, F. E., Jensen, A. F., and Hansen, J. B. (2003) Synthesis and biological activity of 3,3-Diamino-sulfonylacrylonitriles as novel inhibitors of glucose induced insulin secretion from beta cells, *Bioorg. Med. Chem.* 11, 931-940.
10. Hansen, J. B., Tagmose, T. M., Mogensen, J. P., Dörwald, F. Z., and Jørgensen, A. S. (2000) Substituted 3,3-diamino-2-propenenitriles, their preparation and use, (AS, N. N., Ed.), Espacenet.
11. Gaymes, T. J., Shall, S., MacPherson, L. J., Twine, N. A., Lea, N. C., Farzaneh, F., and Mufti, G. J. (2009) Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes, *Haematologica* 94, 638-646.

12. Frieden, M., Aviñó, A., and Eritja, R. (2003) Convenient Synthesis of 8-Amino-2'-deoxyadenosine, *Nucleosides, Nucleotides and Nucleic Acids* 22, 193-202.
13. Ek, F., and Wistrand, L. G. (2001) Preparation of allylic aromatic compounds, (As, N. I., Ed.), Escapenet, United Staates of America.
14. Dohme, M. S. a. (1993) (Dohme, M. S. a., Ed.), Escapenet, US.