Supporting Information

Design, Syntheses and Anti-TB activity of 1,3-Benzothiazinone (BTZ) Azide and Click Chemistry Products Inspired by BTZ043

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General Experimental Section

All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased from either Aldrich Chemical Co. (Milwaulkee, WI) or Fisher Scientific (Suwanee, GA). All reactions were conducted under argon unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. All compounds are >98% pure by HPLC analysis and MIC values reported are the average of three individual measurements. Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). General methods of purification of compounds involved the use column chromatography with silica gel (230-400 mesh) purchased from Silicycle, Quebec city, Canada. Trimethylsilyl azide was purchased from Alfa Aesar and *tert*-butyl nitrite was purchased from Acros Organics. All other reagents were purchased either from Sigma Aldrich Limited or VWR. The reactions were monitored by TLC on precoated Merck 60 F_{254} silica gel plates and visualized using UV light (254 nm). All compounds were analyzed for purity by HPLC and characterized by ^{1}H and ^{13}C NMR using Varian 300MHz, 500 MHz NMR and/or Bruker 400 MHz NMR spectrometers. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform δ 7.26 and δ 77.23, methanol δ 3.31 and δ 49.00 and coupling constants (*J*) are reported in hertz (Hz) (where, s = singlet, bs = broad singlet, d = doublet, dd = double doublet, bd = broad doublet, ddd = double doublet of dublet, t = triplet, tt – triple triplet, q = quartet, m = multiplet) and analyzed using 1D NMR processor (ACD/SpecManager) purchased from ACD labs (Product version 11.03). The Mass spectra values are reported as *m/z* and HRMS analyses were carried out with a Bruker MicroOTOF-Q II, electrospray ionization time-of-flight mass spectrometer. The liquid chromatography mass spectrum ("LC/MS") analyses were carried out on Waters ZQ instrument consisting of chromatography module Alliance HT, photodiode array detector 2996, and mass spectrometer Micromass ZQ, using a 3 x 50 mm Pro C18 YMC reverse phase column. Mobile phases: 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 minutes at 0.7 mL/min. The MS electrospray source operated at capillary voltage 3.5 kV and a desolvation temperature 300^{o}C.

(*S*)-8-Amino-2-(2-Methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (6)[1]

Compound **6** was synthesized in 87 % yield from compound **1** (10 g, 23.2 mmol) by treatment with Fe (3.88 g) in acetic acid (100 mL) at 120°C for 2 h. The reaction material was then cooled to rt and excess acetic acid was removed by vacuum distillation. The reaction mixture was then carefully neutralized by adding a cold saturated solution of $NaHCO_3$. The reaction mixture (thick slurry) was then partitioned between dichloromethane and water. The organic layer was separated and the aq layer was additionally washed with dichloromethane (2 x 200 mL). The combined organic layers was dried ($MgSO_4$), filtered and evaporated *in vacuo*. The crude residue was purified by column chromatography (hexanes: EtOAC, 4:6) to obtain **6** (8.1 g, 20.19 mmol, 87 %). ^{1}H NMR ($CDCl_3$) δ 8.19 (s, 1 H), 7.09 (d, *J* = 1.5 Hz, 1 H), 4.34 - 4.20 (m, 1 H), 4.08 (dd, *J* = 5.8, 8.1 Hz, 2 H), 3.98 (br. s., 3 H), 3.47 (t, *J* = 7.9 Hz, 1 H), 1.89 - 1.69 (m, 4 H), 1.28 (d, *J* = 6.0 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 169.1, 159.9, 142.2, 124.0, 118.1, 114.4, 106.9, 72.7, 71.1, 44.4, 36.6, 35.3, 18.6. MS (HR-ESI) $C_{17}H_{18}F_3N_3O_3S$ $(M + H)^+$ calcd 402.1194, found 402.1122.

(*S*)-8-Azido-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3)

In a 100 mL round bottom flask, (*S*)-8-amino-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4 g, 10.0 mmol) was dissolved in acetonitrile (50 mL) and cooled to 0°C using an ice bath. To this stirred solution was added *t*-BuONO (1.8 mL, 15.0 mmol) followed by $TMSN_3$ (1.6 mL, 12.0 mmol) dropwise. The reaction mixture was stirred at 0°C for an additional 30 min before warming to the room temperature and it was then stirred at rt for another 30 min. The reaction mixture was then evaporated *in vacuo* and the crude product was purified by silica gel chromatography (hexanes:ethyl acetate, 4:6) to give the product, **3** (2.64 g, 6.2 mmol, 62%). ^{1}H NMR ($CDCl_3$) δ 8.53 (s, 1 H), 7.51 (d, *J* = 1.2 Hz, 1 H), 4.29 (dt, *J* = 5.9, 7.8 Hz, 1 H), 4.11 (dd, *J* = 5.6, 8.0 Hz, 1 H), 4.0 – 3.7 (br. s, 4H), 3.50 (t, *J* = 7.9 Hz, 1 H),

1.92 - 1.69 (m, 4 H), 1.31 (d, *J* = 6.1 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 167.7, 160.7, 137.0, 130.5, 130.1, 123.6 (q), 121.7, 116.7 (q), 106.6, 72.5, 70.9, 44.3, 36.3, 35.1, 18.3. MS (HR-ESI) $C_{15}H_{17}F_3N_2O_6S$ (M + Na) $^+$ $C_{17}H_{16}F_3N_5O_3S$ (M+H), calcd. 428.0999, found. 428.1084. HPLC t_R = 8.40 min

(S)-2-(2-Methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one4-imino-9-((S)-2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-5-(trifluoromethyl)-4H-[1,3]thiazino[5',6':5,6]benzo[1,2-d][1,2,3]triazin-7(4aH)-one (7)

To a solution of **3** (0.100 g, 0.23 mmol) in 4 mL of acetonitrile and 1 mL of water was added KCN (31 mg, 0.47 mmol) in a 20 mL scintillation vial. The reaction mixture was then stirred at room temperature for 3-4 h and monitored by TLC and LC/MS. The reaction mixture was then filtered through a cotton plug. The filtrate was concentrated to obtain the pure product, **7** (0.100 g, 0.22 mmol) 94% yield. ^{1}H NMR (CD_3OD) δ 8.26 (s, 1 H), 7.81 (br. s., 1 H), 4.31 - 4.25 (m, 1 H), 4.15 – 3.86 (m, 5 H), 3.49 (t, *J* = 7.8 Hz, 1 H), 2.15 (br. s., 1 H), 1.82 (br. s., 4 H), 1.26 (d, *J* = 6.1 Hz, 3 H). ^{13}C NMR (CD_3OD) δ 171.4, 165.8, 149.1, 133.9, 130.8, 130.4, 126.8, 124.6, 124.1, 123.0 (q), 115.7 (q), 108.0, 73.9, 72.0, 45.6, 37.4, 36.1, 18.9. MS (HR-ESI) $C_{18}H_{17}F_3N_6O_3S$ (M+H), calcd. 455.1108, found. 455.1105. HPLC t_R = 6.03 min

(*S*)-2-(2-Methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one

(**8**)

In a 100 mL round bottom flask, (*S*)-8-amino-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3.65 g, 9.1 mmol) was dissolved in THF (30 mL) and cooled to 0°C using an ice bath. To this stirred solution was added *t*-BuONO (4.3 mL, 15.0 mmol) dropwise. The reaction mixture was stirred at 0°C for additional 30 min before warming to rt and it was then stirred at rt for another 30 min. The reaction mixture was then evaporated *in vacuo* and the crude product was purified by silica gel chromatography (hexanes: EtOAc, 5:5) to afford **8** (1.1 g, 2.85 mmol, 32 %). ^{1}H NMR ($CDCl_3$) δ 8.69 (s, 1 H), 7.69 (dd, *J* = 1.5, 8.3 Hz, 1 H), 7.43 (d, *J* = 8.3 Hz, 1 H), 4.25 (dt, *J* = 5.9, 7.8 Hz, 1 H), 4.08 (dd, *J* = 5.6, 8.1 Hz, 1 H), 3.9 – 3.6 (br.s, 4H), 3.46 (t, *J* = 7.9 Hz, 1 H), 1.89 - 1.74 (m, 4 H), 1.27 (d, *J* = 6.1 Hz, 3 H). ^{13}C

NMR ($CDCl_3$) δ 167.9, 161.0, 136.4, 130.5, 130.1, 128.0 (q), 127.5 (q), 126.2, 124.7, 123.1, 122.0, 106.5, 72.4, 70.7, 44.2, 36.2, 35.0, 18.2. MS (HR-ESI) $C_{17}H_{17}F_3N_2O_3S$ (M+H), calcd. 387.1028, found. 387.0985. HPLC t_R = 7.55 min

(*S*)-8-(4-((Dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (9)

To a solution of **3** (0.100 g, 0.23 mmol), in acetonitrile (4 mL) and water (1mL) was added *N*,*N*-dimethyl propargylamine (0.027 mL, 0.026 mmol), a 2 mL aq solution of $CuSO_4.5H_2O$ (0.03 g, 0.12 mmol) and 2 mL of an aq solution of sodium ascorbate (0.093 g, 0.47 mmol). The reaction was stirred overnight at room temperature. The reaction mixture was filtered and the filtrate was concentrated. The crude residue was purified by silica gel chromatography (DCM: MeOH, 10:1) to obtain **9** (0.12 g, 0.24 mmol, 99%). ^{1}H NMR (CD_3OD) δ 8.76 (s, 1 H), 8.47 - 8.42 (m, 1 H), 8.23 (s, 1 H), 4.32 - 4.23 (m, 1 H), 4.11 (dd, *J* = 5.7, 8.1 Hz, 1 H), 3.90 – 3.65 (m, 6 H), 3.49 (s, 1 H), 2.49 - 2.26 (s, 6 H), 1.85 - 1.76 (m, 4 H), 1.27 (d, *J* = 6.2 Hz, 3 H). ^{13}C NMR (CD_3OD) δ 169.7, 162.9, 136.0, 131.3, 131.1, 128.9 (q), 127.6 (q), 126.4, 125.5, 123.7, 107.8, 73.9, 72.0, 37.2, 36.0, 18.8. MS (HR-ESI) $C_{22}H_{25}F_3N_6O_3S$ (M+H), calcd. 511.1734, found. 511.1762. HPLC t_R = 5.80 min

(*S*)-8-(4-Benzyl-1H-1,2,3-triazol-1-yl)-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**10**)

Compound **10** was synthesized from **3** (0.100g, 0.23 mmol) by following the same procedure as described for the synthesis of **9**. The crude **10** was purified by column chromatography (hexanes: EtOAc, 4:6) to obtain **10** (0.096 g, 0.18 mmol, 76 %) ^{1}H NMR ($CDCl_3$) δ 8.88 (s, 1 H), 7.76 (s, 1 H), 7.54 (s, 1 H), 7.39 - 7.33 (m, 3 H), 7.31 - 7.28 (m, 2 H), 4.28 (d, *J* = 6.5 Hz, 1 H), 4.25 – 4.15 (m, 4H), 4.11 (dd, *J* = 5.9, 7.9 Hz, 1 H), 3.8 – 3.6 (br.s, 2H), 3.49 (t, *J* = 8.1 Hz, 1 H), 1.86 - 1.73 (m, 4 H), 1.31 (d, *J* = 5.9 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 167.3, 160.6, 149.1, 138.1, 133.6, 133.5, 130.1, 129.9, 128.8, 128.7, 128.4 (q), 126.9, 125.6, 124.9 (q), 123.6, 122.8,

121.8, 106.4, 72.5, 70.8, 32.2, 30.9, 18.3. MS (HR-ESI) $C_{26}H_{24}F_3N_5O_3S$ (M+H), calcd. 544.1625, found. 544.1577. HPLC t_R = 8.73 min

Ethyl (*S*)-1-(2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-8-yl)-1H-1,2,3-triazole-4-carboxylate (**11**)

Compound **11** was synthesized from **3** (0.100g, 0.23 mmol) by following the same procedure as described for the synthesis of **9**. Crude **11** was purified by column chromatography (hexanes: EtOAc, 4:6) to obtain **11** (0.084 g, 0.16 mmol, 68 %) ^{1}H NMR ($CDCl_3$) δ 8.96 (s, 1 H), 8.45 (s, 1 H), 7.86 (s, 1 H), 4.52 (q, *J* = 7.1 Hz, 2 H), 4.32 – 4.15 (m, 2 H), 4.11 (m, 2 H), 3.80 – 3.60 (br.s, 2H), 3.49 (t, *J* = 7.9 Hz, 1 H), 1.86 - 1.70 (m, 4 H), 1.48 (t, *J* = 7.2 Hz, 3 H), 1.31 (d, *J* = 6.2 Hz, 3 H). ^{13}C NMR ($CDCl_3$) δ 167.0, 160.0, 159.7, 141.3, 134.0, 132.7, 130.5, 129.4 (q), 128.9, 125.7, 125.6 (q), 106.3, 72.5, 70.9, 62.0, 18.3, 14.3. MS (HR-ESI) $C_{22}H_{22}F_3N_5O_3S$ (M+H), calcd. 526.1367, found. 526.1327. HPLC t_R = 7.73 min

Docking details with Autodock Vina

DprE1 from *M.tb* (PDB # 4NCR) and ligand Protein Data Bank (PDB) files were prepared for docking using MGLTools 1.5.6. The PDB structure of DprE1 is reported as dimer so one of the monomer (B) was deleted and to the remaining monomer, hydrogen were added, the autodock 4 atom types were assigned and Kollmann charges were added. The protein was saved as a "pdbqt" file. The hydroxylamine form of pBTZ169 was downloaded from the PDB website, visualized using ChemBio3D Ultra and saved as a mol2 file. The file was opened using MGLTOOls 1.5.6, Kollmann charges were added and processed using "Ligand" module and saved as a pdbqt file. The structures of BTZ-N3 (**3**) and compound **9** were drawn using ChemBioDraw Ultra 12.0, minimized using AM1 method and saved as a mol2 files. Both **3** and **9** were then processed the same way using the "Ligand" module of MGLTools and saved as pdbqt files prior to docking. Docking was conducted with a grid spacing of 0.375 and xyz of 40 × 40 × 40 using a rigid receptor generating 9 docked poses.

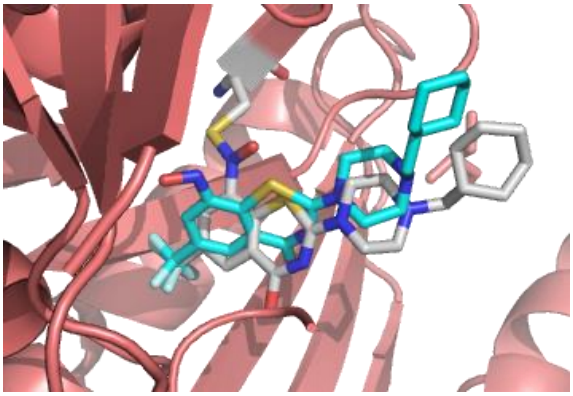


Figure S1: Overlay of the docked pose of hydroxylamine form of **2** on the semimercaptal adduct of **2** with DprE1**.** The carbons of **2** are colored in white whereas carbons of its hydroxylamine form are in cyan.

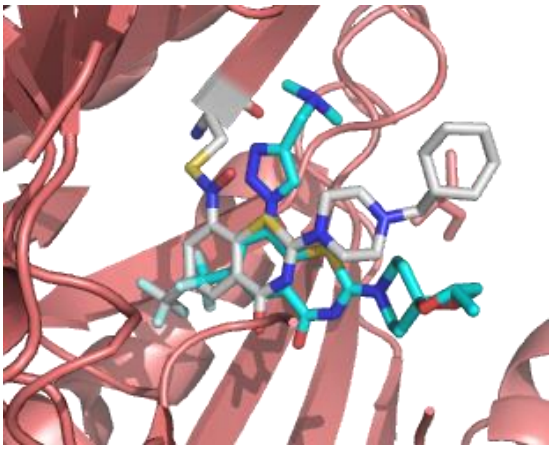


Figure S2: Overlay of the docked pose of **9** on the semimercaptal adduct of **2** with DprE1**.** The carbons of **2** are colored in white whereas carbons of **9** are in cyan.

Enzyme reactions with P-^{14}C-RPP

Effects of the compounds **3** and **8** on P-^{14}C-RPP incorporation into ^{14}C-DPA and its precursors were tested with the mixture of membrane and cell envelope enzyme fractions prepared from *M. smegmatis* mc^{2}155 as described.[2] Membranes (300 μg of protein) together with cell envelope (500 μg of protein) were incubated in 50 mM MOPS, pH 7.9, 10 mM $MgCl_2$ and 5 mM β–mercaptoethanol with 15,000 dpm of P-^{14}C-RPP [synthetised and purified according to Scherman et al., 1996[3] from ^{14}C-glucose (ARC, specific activity 300 mCi/mmol)] for 1 hr at 37°C in the final volume of reaction mixture 80 μl. Radiolabeled lipid products were extracted with $CHCl_3$/CH_3OH (2:1), analysed by TLC and autoradiography using Biomax MR-1 film (Kodak) by published procedures.[2] All compounds [**3, 8** and BTZ-043 (gift from V. Makarov, A. N. Bakh Institute of Biochemistry, Russian Academy of Science, Moscow, Russia], were used in the concentrations: 2.5; 12.5; 25; 50 and 100 μg/ml in the final reaction mixture. These were added as DMSO stock solutions; final concentration of DMSO in the reaction mixtures was 6.25% in all tubes, including the controls.

References:

1. Tiwari, R.; et al. Thiolates chemically induce redox activation of BTZ043 and related potent nitroaromatic anti-tuberculosis agents. *J Am Chem Soc* **2013,** 135, 3539-3549.

2. Mikusova K, Huang H, Yagi T, Holsters M, Vereecke D, D'Haeze W, Scherman MS, Brennan PJ, McNeil MR, Crick DC. Decaprenylphosphoryl arabinofuranose, the donor of the D-arabinofuranosyl residues of mycobacterial arabinan, is formed via a two-step epimerization of decaprenylphosphoryl ribose. J Bacteriol, 2005; 187, 8020-8025.

3. Scherman MS, Kalbe-Bournonville L, Bush D, Xin Y, Deng L, McNeil M. Polyprenylphosphate-pentoses in mycobacteria are syn-thesized from 5-phosphoribose pyrophosphate. J Biol Chem, 1996; 271, 29652-29658.

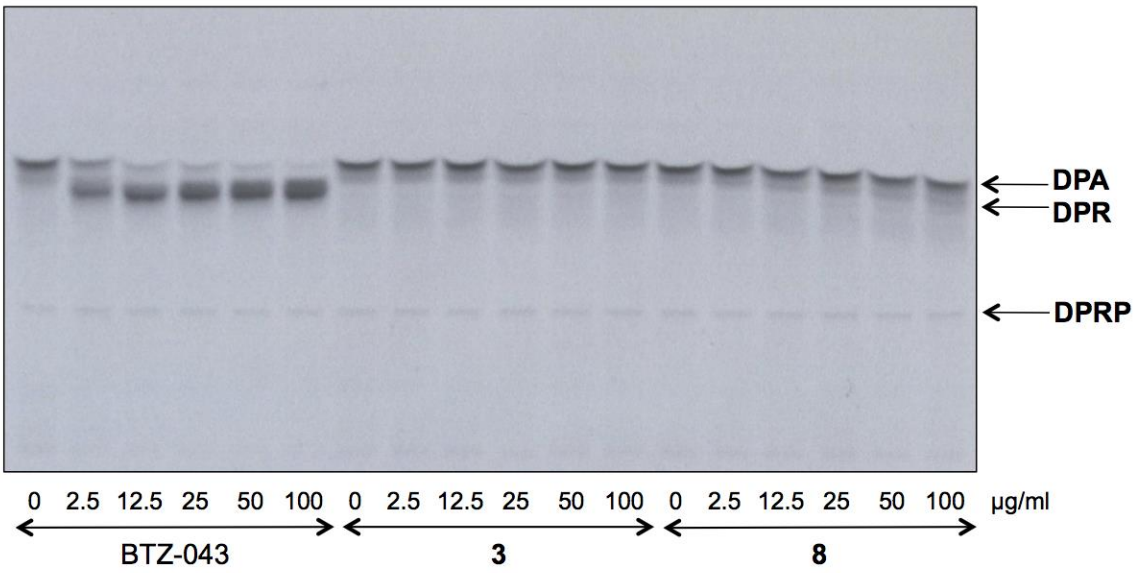
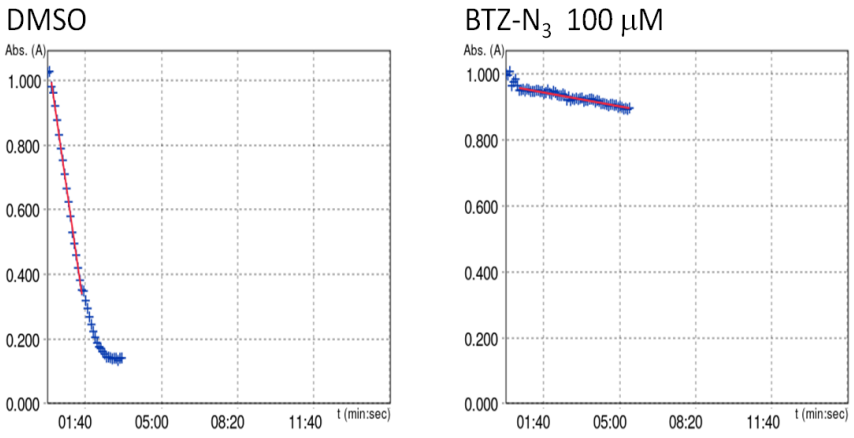


Figure S3. Evaluation of the effects of **3** and **8** on DprE1 activity in the assay with mycobacterial enzyme fractions. The reactions with P-^{14}C-RPP and membrane and cell envelope fractions prepared from *M. smegmatis* mc^{2}155 were performed as described above. Extracted lipids were separated on Silica gel 60 TLC plate in $CHCl_3$/CH_3OH/concentrated NH_4OH/1 M ammonium acetate/H_2O (180:140:9:9:23,v/v). The plate was exposed to autoradiography film at -80°C for 3 days. DPRP-decaprenylphosphoryl ribose-5 phosphate, DPR-decaprenylphosphoryl ribose, DPA-decaprenylphosphoryl arabinose

Detailed enzymatic study of BTZ-N_3 with recombinant DprE1

DprE1 recombinant enzyme was prepared as previously[1].The enzyme activity of the recombinant DprE1 was spectrophotometrically determined at 30°C, using farnesylphosphoryl-β-D-ribofuranose (FPR) as substrate and 2,6-dichlorophenolindophenol (DCPIP) as electron acceptor, measuring the decrease in absorbance at 600 nm of DCPIP (ε= 19100 M^{-1} cm^{-1})[2]. The standard reaction mixture contained 20 mM glycylglycine buffer pH 8.5, 500 mM FPR, 50 mM DCPIP, and the reaction was started by adding the enzyme solution. For blank control DMSO (in which the compound was dissolved) was used.

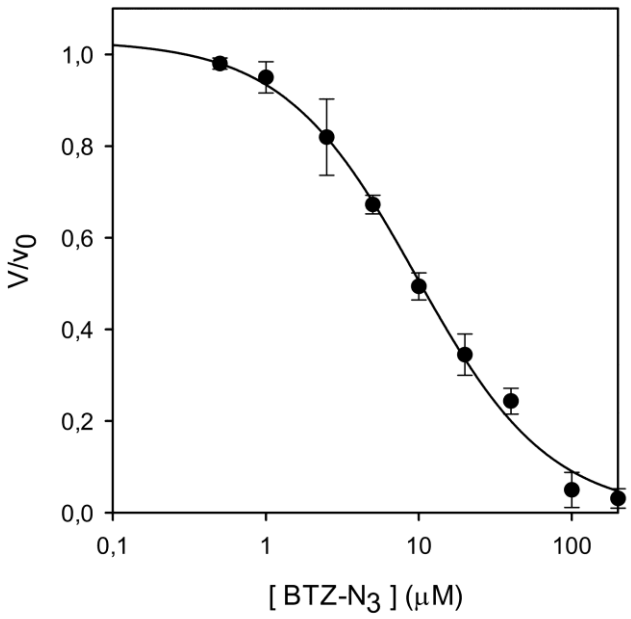
BTZ-N3, firstly tested at a concentration of 100 µM, inhibits almost 90% of the enzyme activity.



The IC_{50} was then determined. The enzyme activities were measured in presence of different concentration of the compound **3**, ranging from 0.1 to 200 µM performed in triplicate, and value was obtained by plotting the initial velocities with the following equation using Origin 8 software:

$$A_{[I]} = A_{[0]}\left(1 - \frac{[I]}{[I] + IC_{50}}\right)$$

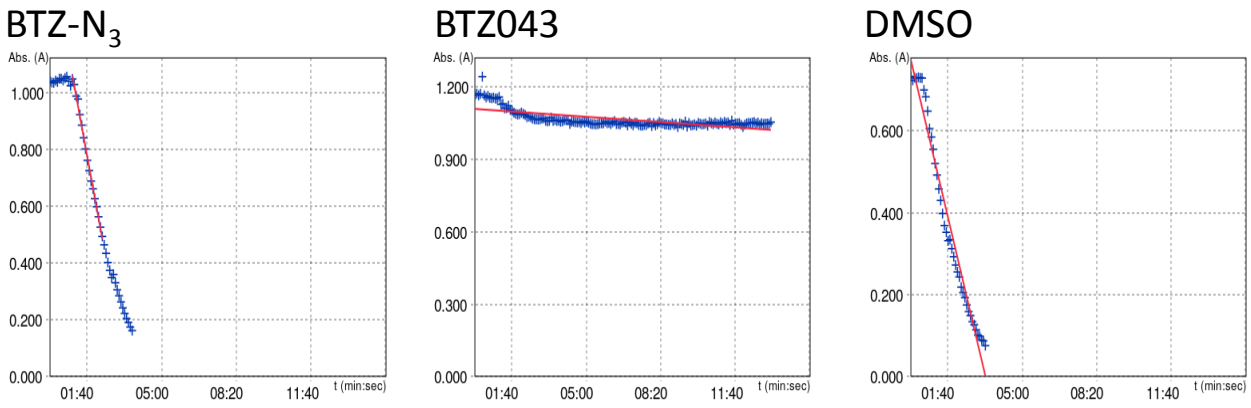
The compound showed an IC_{50} value of 9.6±0.5 µM, which is similar to that of the others benzothiazinones[2].



In the in vitro reaction BTZ-N_3 does not act as a covalent inhibitor

To determine if BTZ-N3 shows a irreversible mode of action, DprE1(20 mg/ml) was incubated for 30 minutes with 100 mM FPR and 50 μM of BTZ-N3 or 50 μM of BTZ043; after the incubation the enzyme was diluted 100 fold in the reaction mixture and its residual enzymatic activity was determined.

Whereas the incubation with BTZ043 leads to an irreversible inhibition of DprE1, the enzyme incubated with BTZ-N_3 once diluted is still active, indicating that this compound does not form covalent adduct in the in vitro conditions. The following graphs are representative of three independent experiments.



The *M. tuberculosis* NTB1 strain resistant to BTZ043, is not resistant to BTZ-N3

The MIC value of the *M. tuberculosis* H37Rv or NTB1 (harboring Cys387Ser mutation in DprE1) strains to BTZ-N3 compound was determined in solid media. To this end, a single colony of was inoculated in complete Middlebrook 7H9 medium. The cell cultures were grown at 37°C until exponential growth phase was reached (~10^8 CFU/ml). After dilution to a final concentration of ~10^7 CFU/ml, 1 μl of the mycobacterial cell cultures was streaked onto complete Middlebrok 7H11 agar plates containing two-fold serial dilutions of the compound. The MIC value was defined as the lowest concentration of compound that prevented colony formation on solid medium.

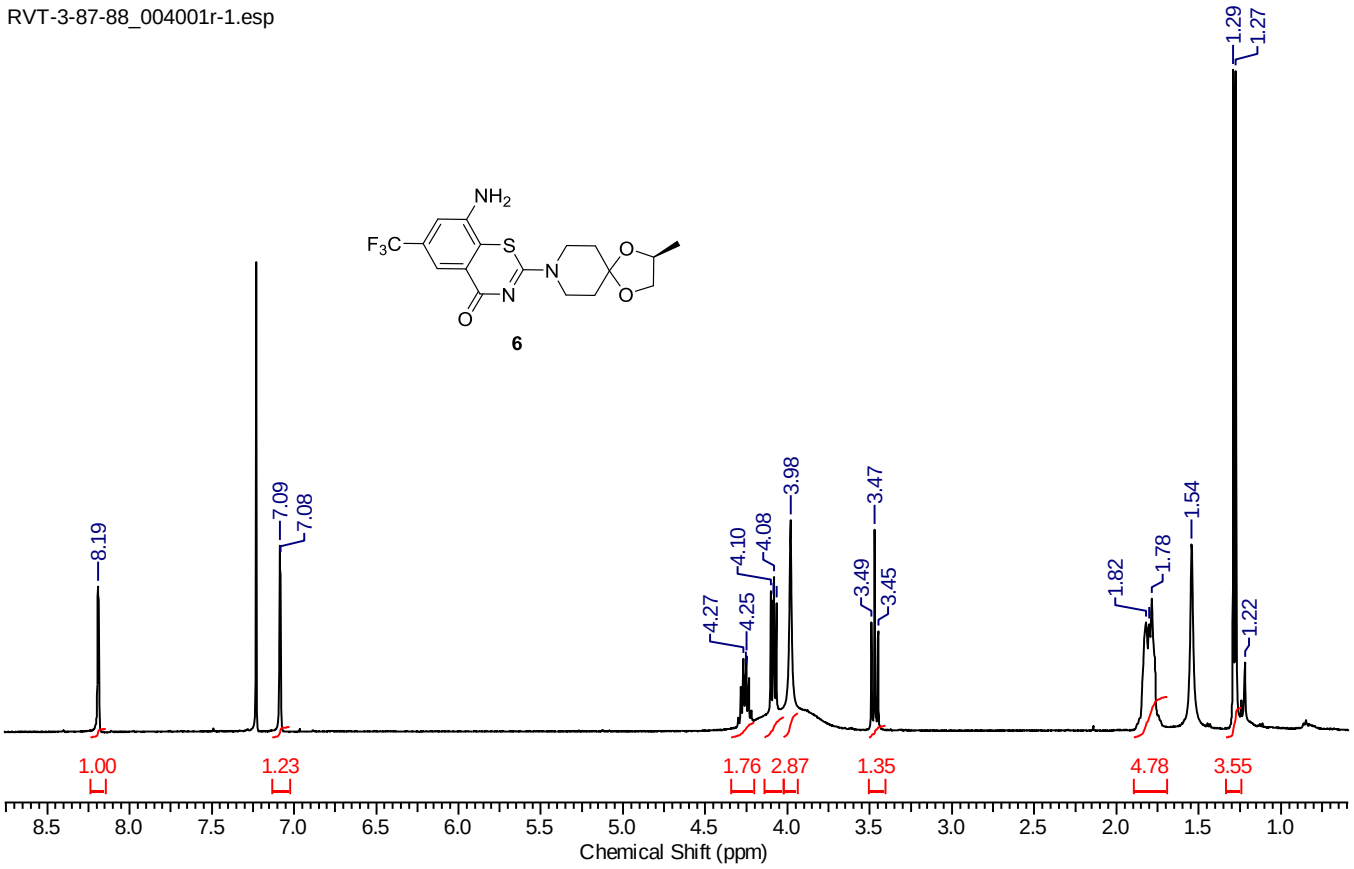
The C387S mutation in DprE1 does not confer resistance to the compound, similarly to others non covalent DprE1 inhibitors[3,4]. Moreover this results is in agreement with that obtained by the in vitro DprE1 inhibition experiments, which showed that the compound is an effective DprE1 inhibitor (IC50 9 μM), but differently from the BTZ043, in such condition, it is reversible and non covalent.

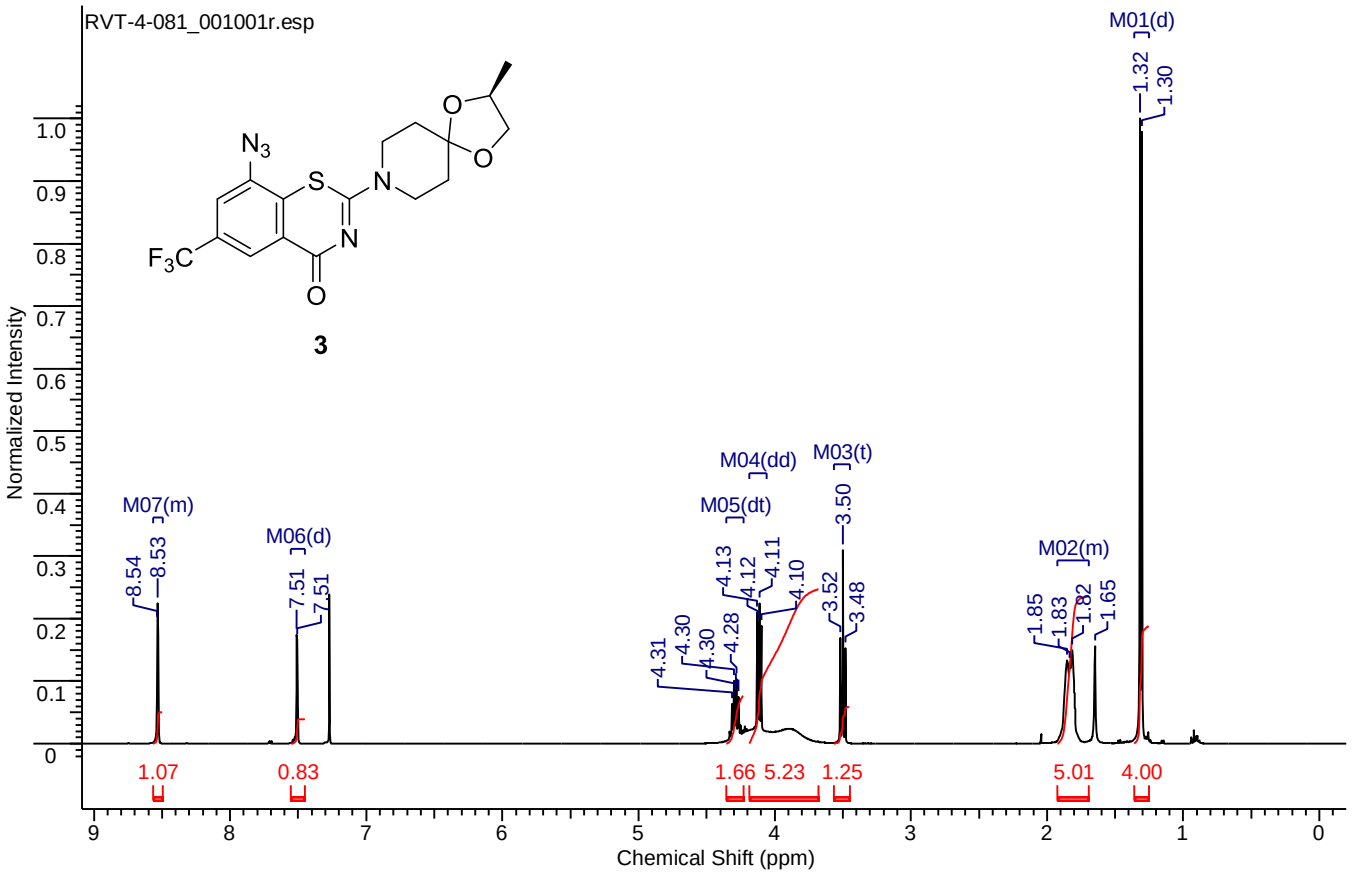
***M . tuberculosis* strain**	**BTZ-N3 MIC (μg/ml)**
H37Rv	0.5
NTB1	0.5

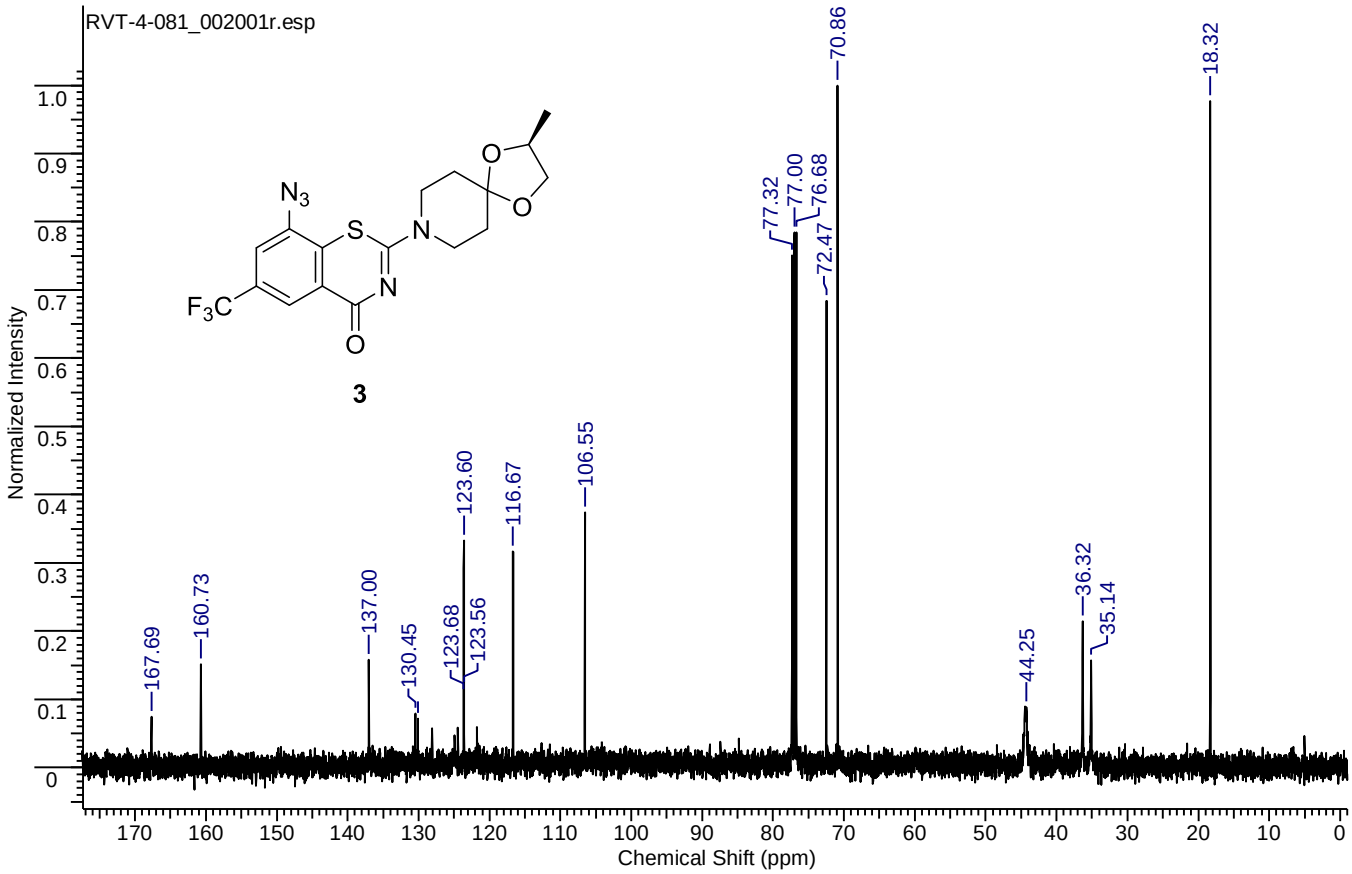
1-Neres J et al. ACS Chem Biol. 2015 10:705-14.
2-Neres J et al. Sci Transl Med. 2012 4:150ra121.
3-Shirude PS et al. J Med Chem. 2014, 57:5728-37.

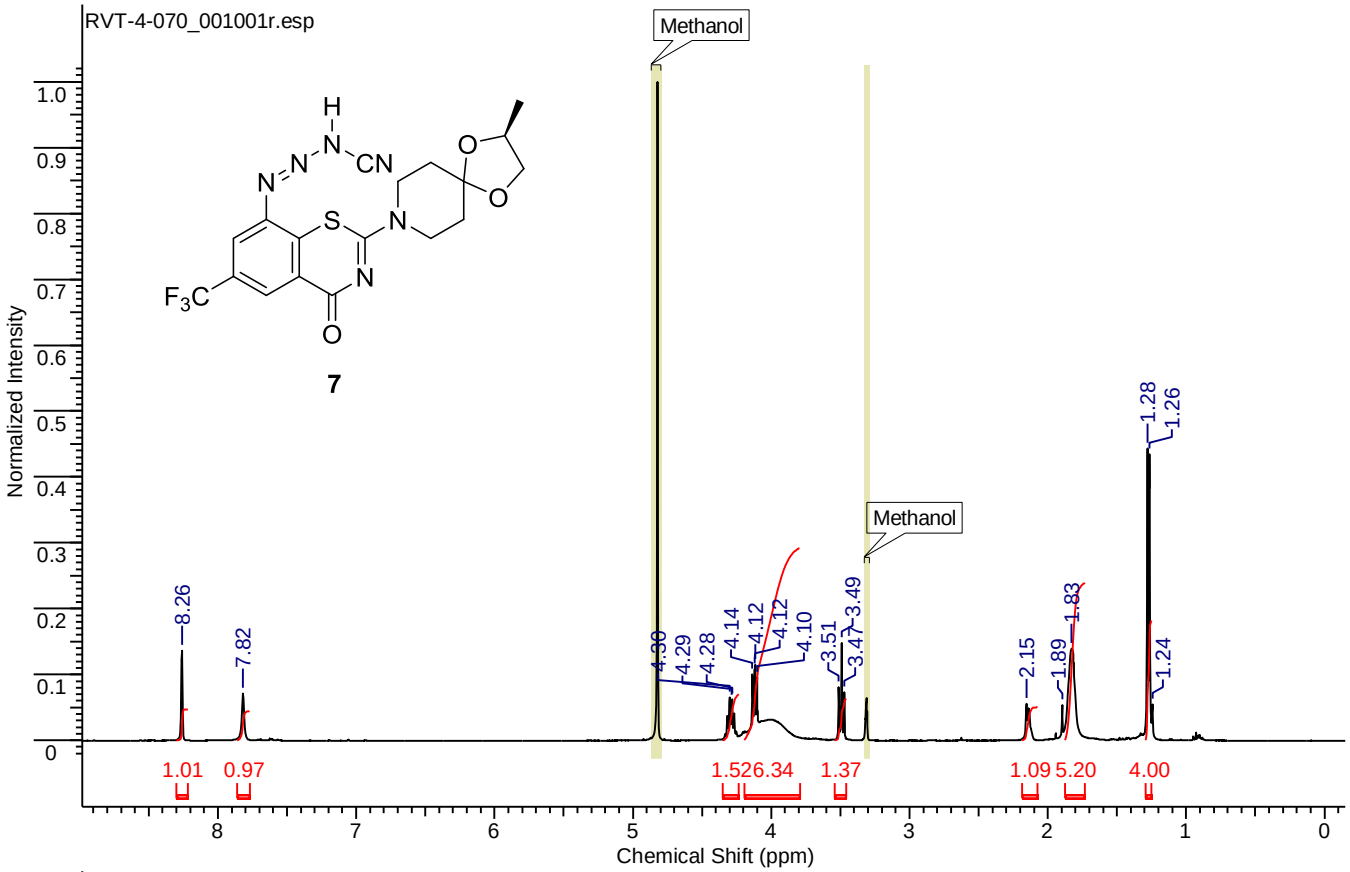
4-Panda M et al. J Med Chem. 2014, 57:4761-71.

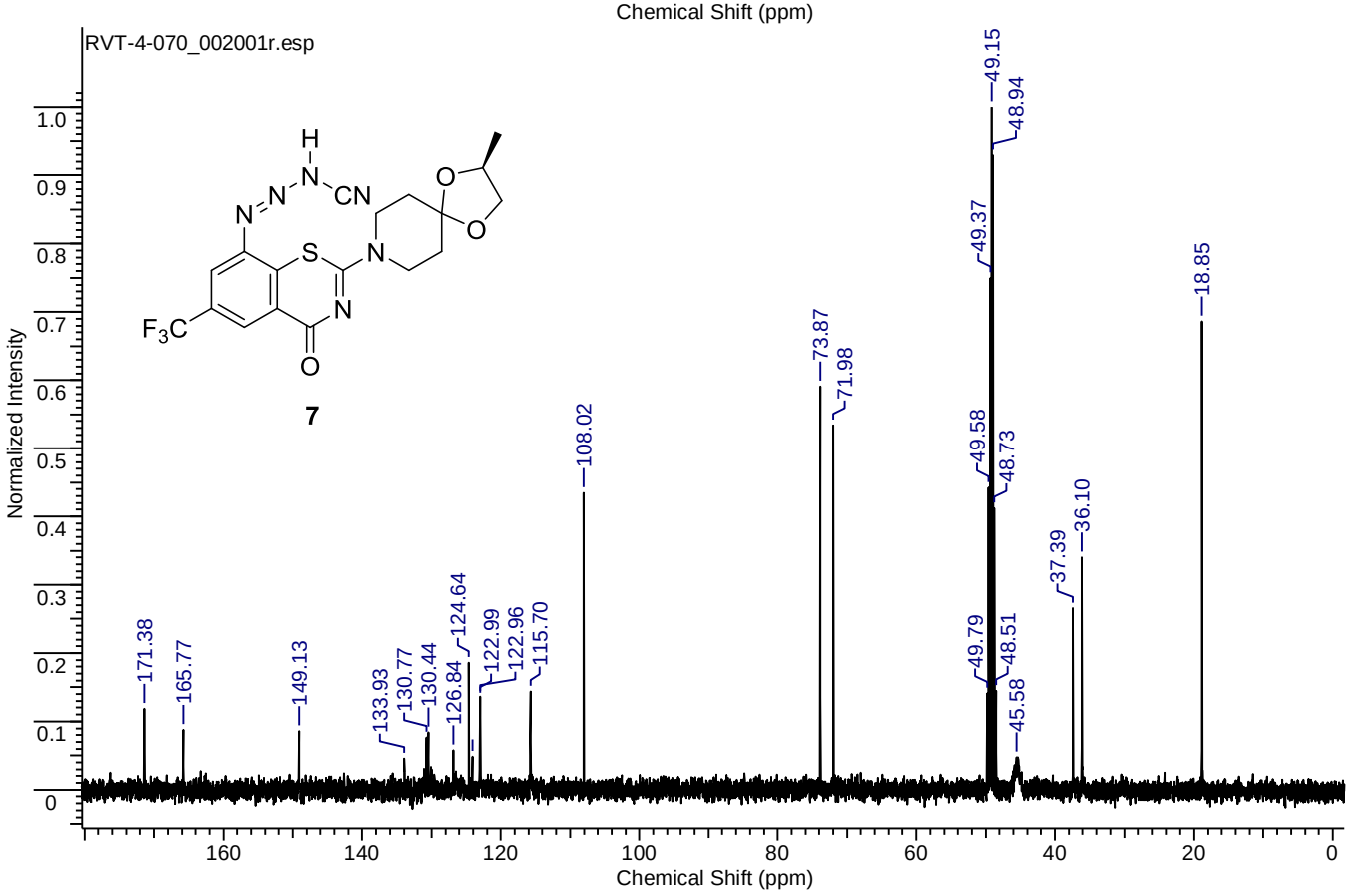
RVT-3-87-88_004001r-1.esp



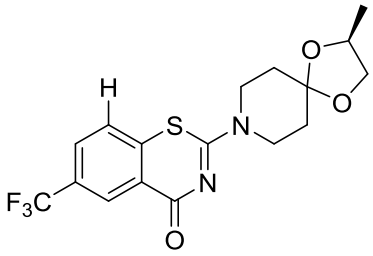

RVT-4-081_001001r.esp
M01(d)
1.32
1.30
N3
S
N
O
O
N
F3C
N
O
3
M07(m)
8.54
8.53
M06(d)
7.51
7.51
M04(dd)
M03(t)
M05(dt)
3.50
4.13
4.12
4.11
4.10
3.52
3.48
4.31
4.30
4.30
4.28
M02(m)
1.85
1.83
1.82
1.65
1.07
0.83
1.66
5.23
1.25
5.01
4.00
Normalized Intensity
Chemical Shift (ppm)


RVT-4-081_002001r.esp
N3
S
N
O
O
N
F3C
N
O
3
70.86
18.32
77.32
77.00
76.68
72.47
106.55
123.60
116.67
167.69
160.73
137.00
130.45
123.68
123.56
44.25
36.32
35.14
Normalized Intensity
Chemical Shift (ppm)


RVT-4-070_001001r.esp
Methanol
Methanol
7
Normalized Intensity
Chemical Shift (ppm)
8.26
7.82
4.30
4.29
4.28
4.14
4.12
4.12
4.10
3.51
3.47
3.49
2.15
1.89
1.83
1.28
1.26
1.24
1.01
0.97
1.52
6.34
1.37
1.09
5.20
4.00

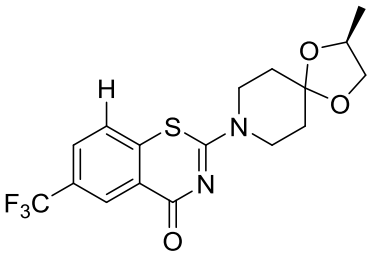

RVT-4-070_002001r.esp
7
Normalized Intensity
Chemical Shift (ppm)
171.38
165.77
149.13
133.93
130.77
130.44
126.84
124.64
122.99
122.96
115.70
108.02
73.87
71.98
49.15
48.94
49.37
49.58
48.73
49.79
48.51
45.58
37.39
36.10
18.85

RVT-3-245-3_001000fid.esp

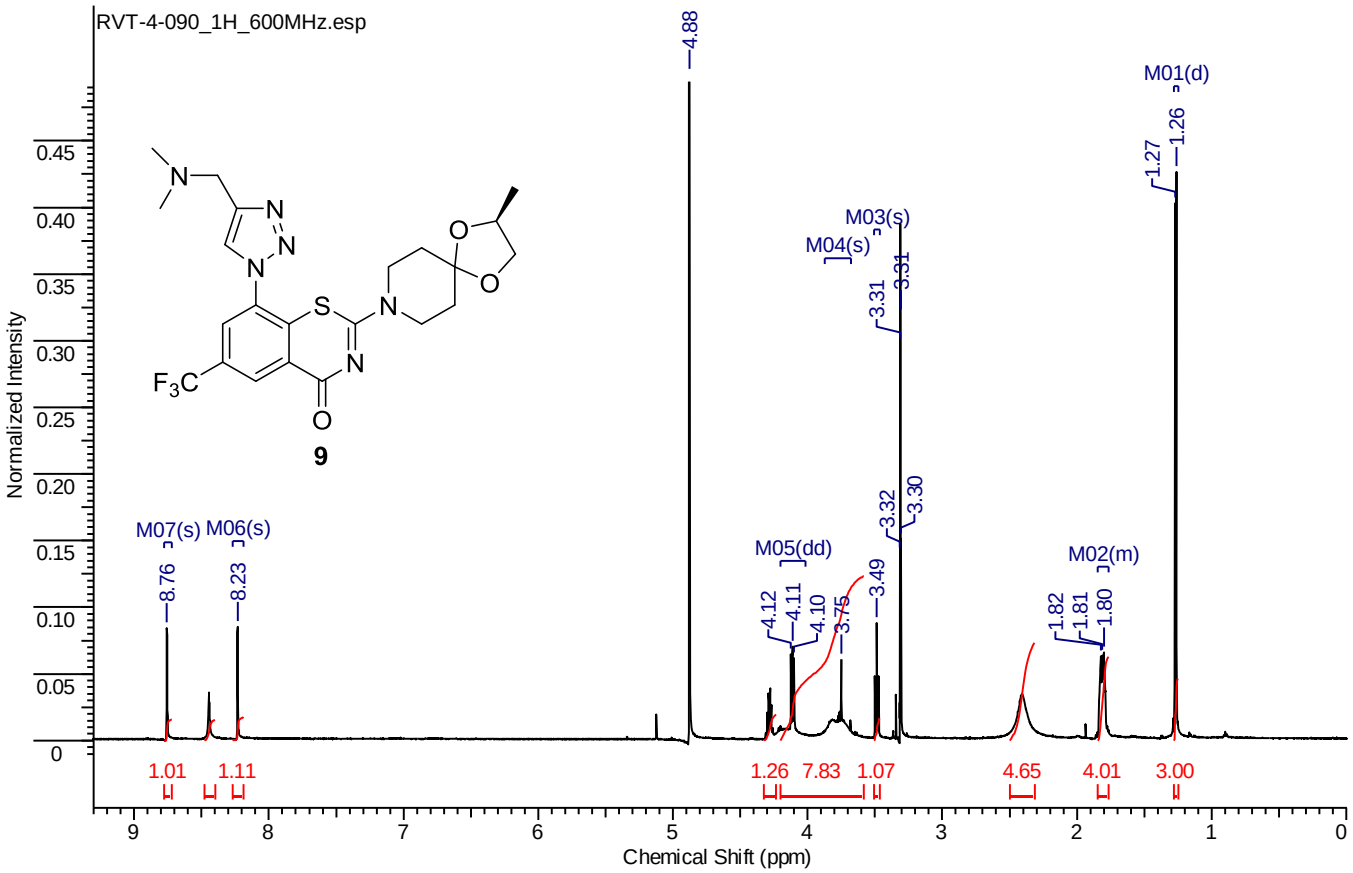


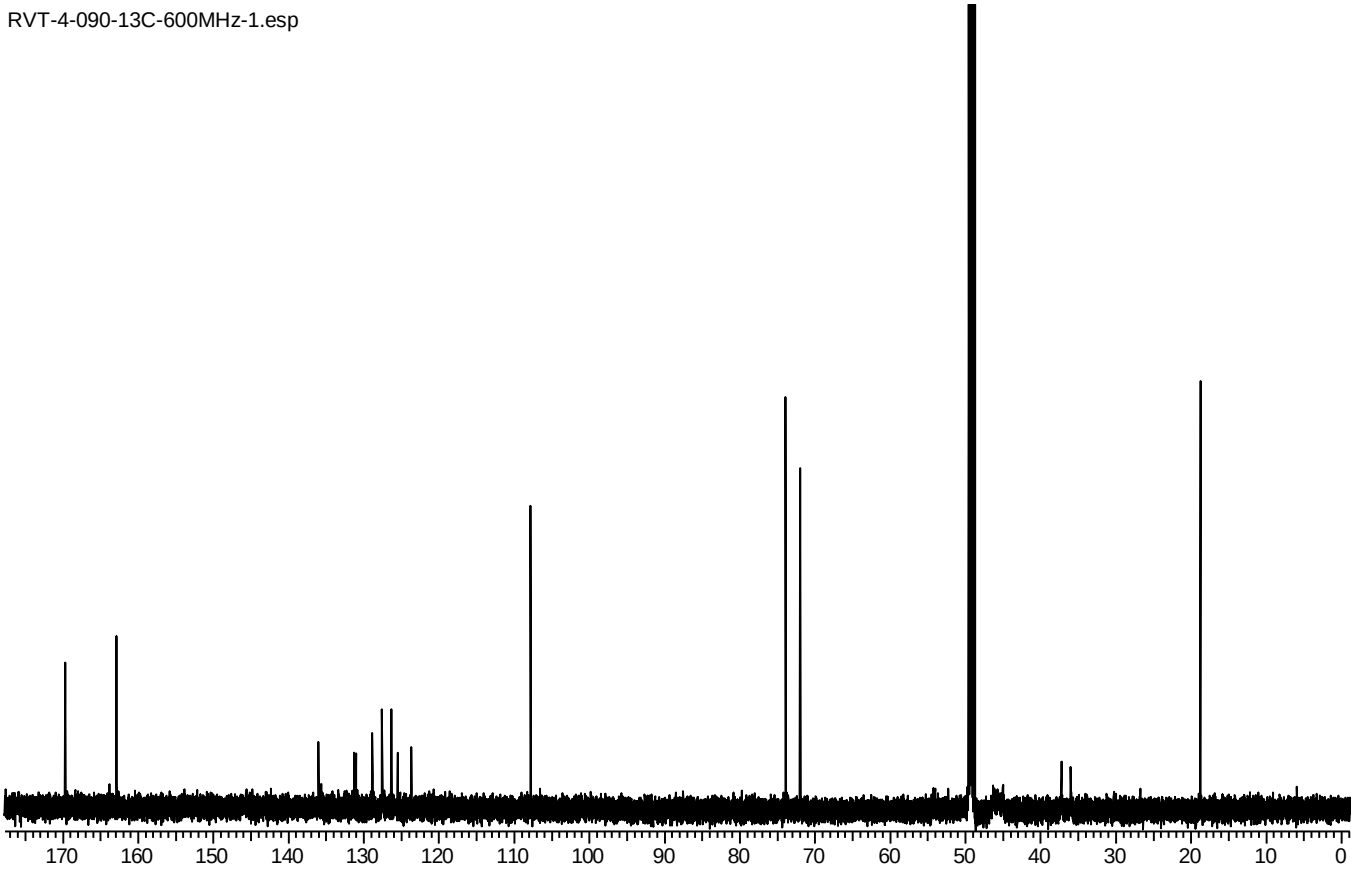
8

RVT-3-245-3_002000fid.esp

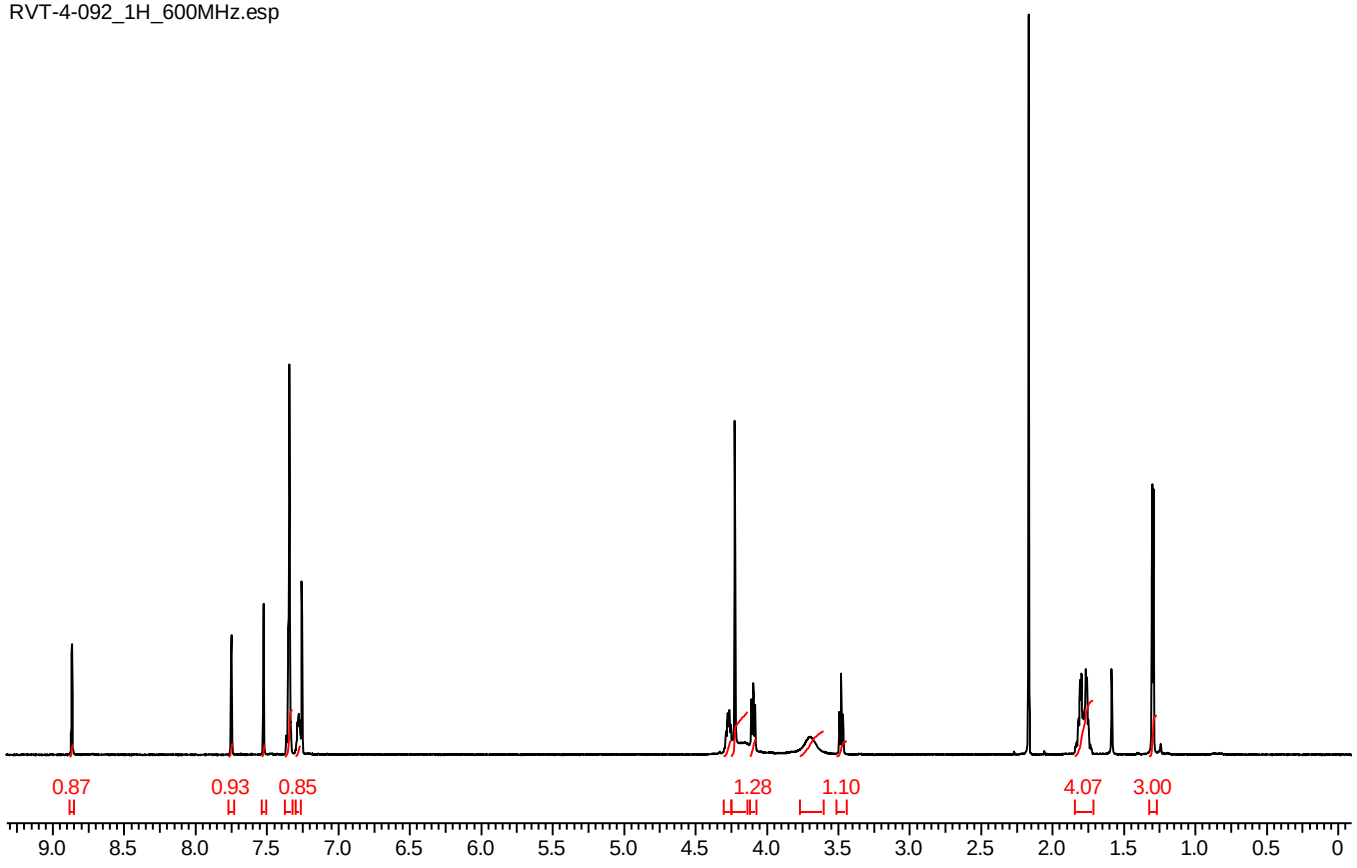


8

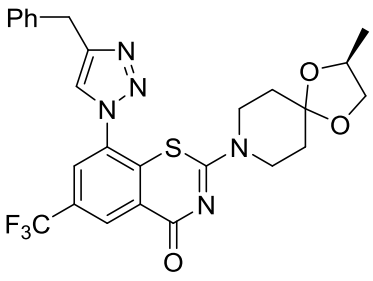

RVT-4-090_1H_600MHz.esp
9
M07(s)
M06(s)
M05(dd)
M04(s)
M03(s)
M02(m)
M01(d)
8.76
8.23
4.88
4.12
4.11
4.10
3.75
3.49
3.32
3.31
3.31
3.30
1.82
1.81
1.80
1.27
1.26
1.01
1.11
1.26
7.83
1.07
4.65
4.01
3.00
Normalized Intensity
Chemical Shift (ppm)


RVT-4-090-13C-600MHz-1.esp

RVT-4-092_1H_600MHz.esp

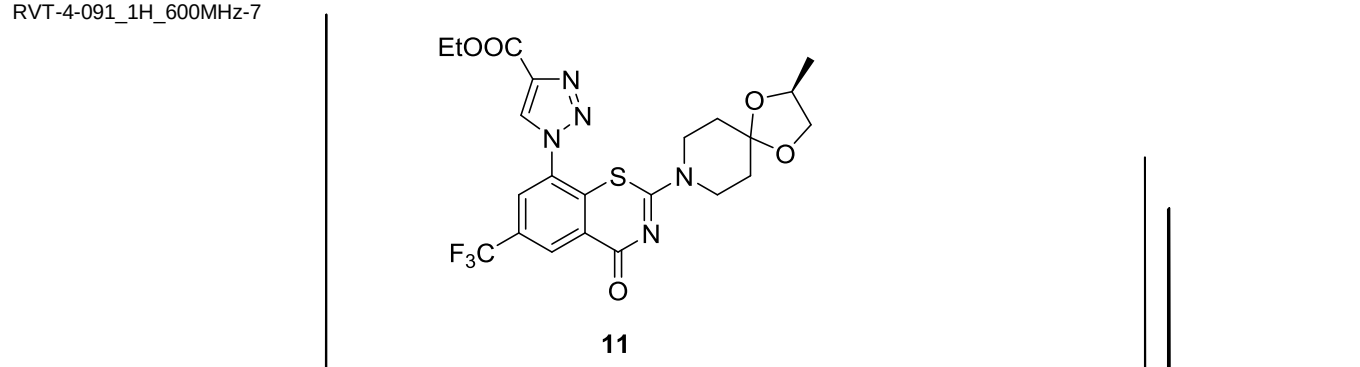


RVT-4-092-2_13C_600MHz

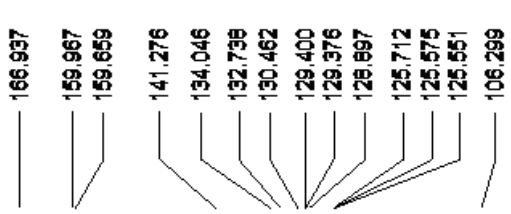


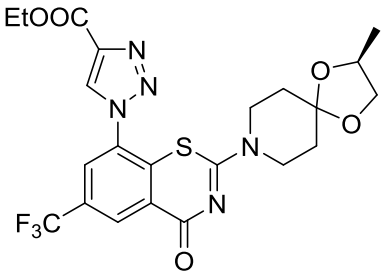
10

RVT-4-091_1H_600MHz-7

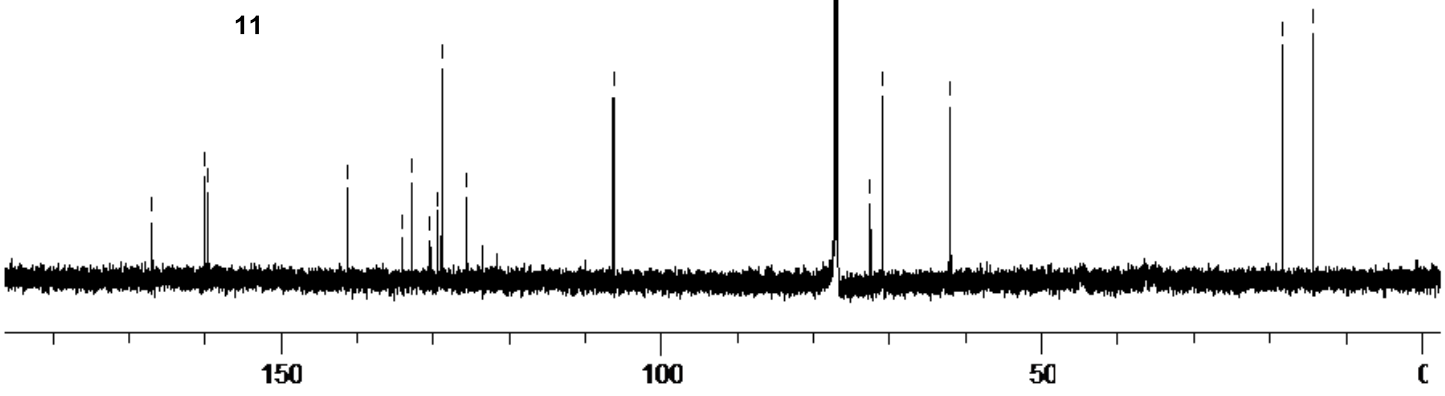


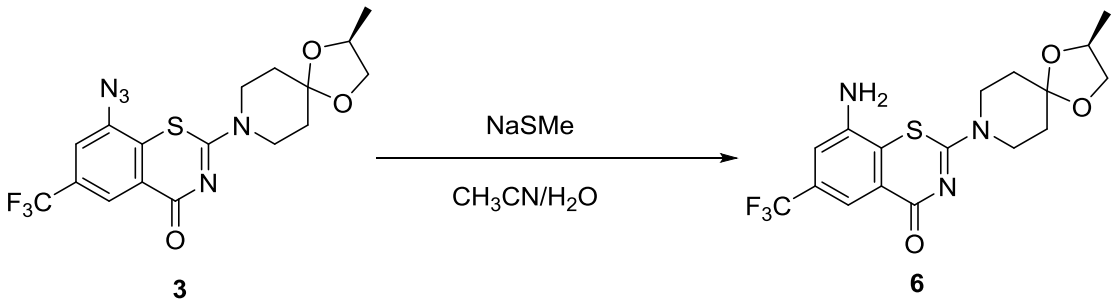
11





11

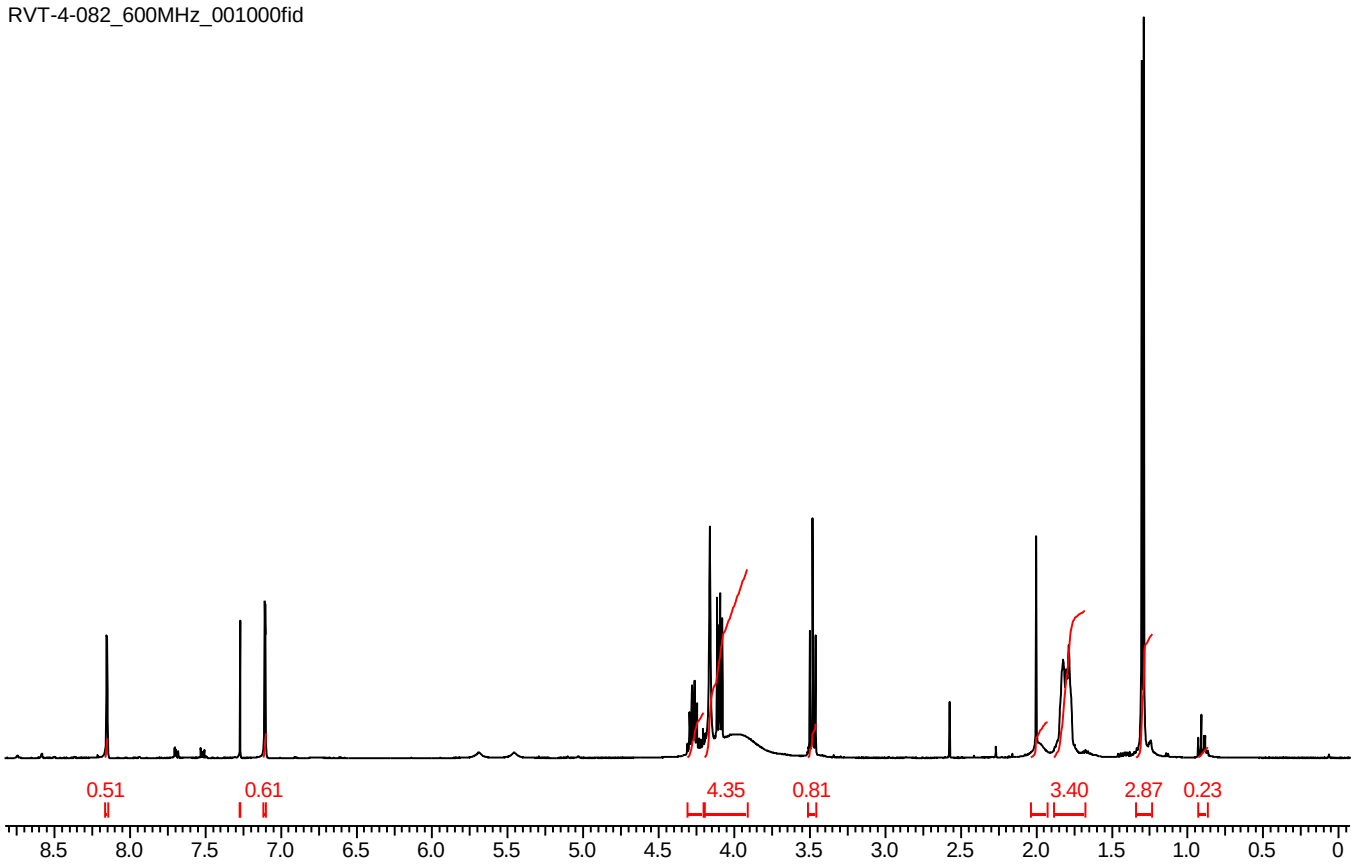


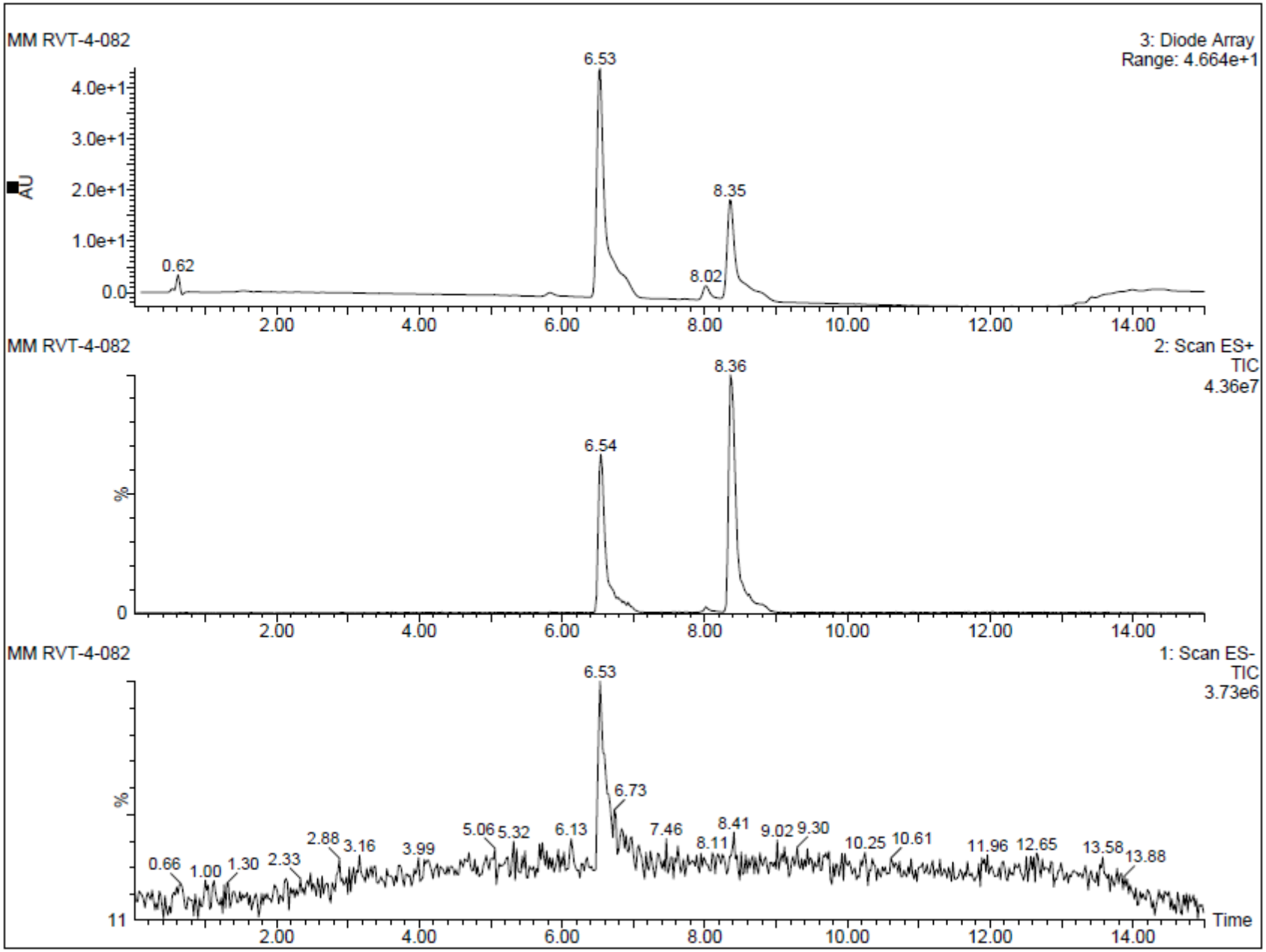


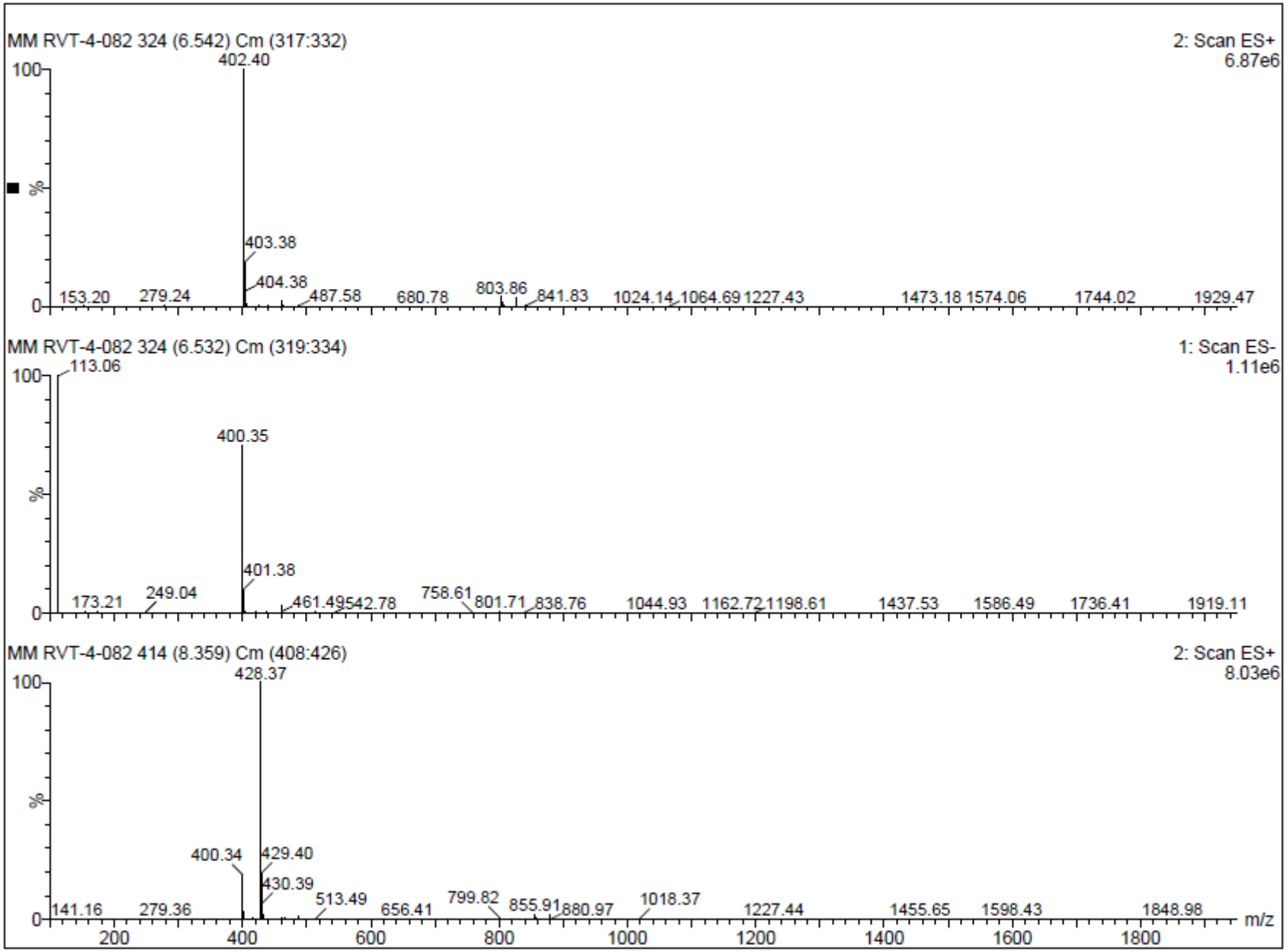
Chemical Formula: $C_{17}H_{16}F_3N_5O_3S$
Molecular Weight: 427.4022

Chemical Formula: $C_{17}H_{18}F_3N_3O_3S$
Molecular Weight: 401.4042

The crude NMR of the reaction mixture after work up confirmed that the product obtained in the above reaction was compound **6**. Please refer to the 1H NMR of authentic **6** above for comparison purposes. Additionally LC/MS spectra of the reaction mixture while in progress also confirmed the formation of **6** from **3**. See LC/MS on the next pages.




MM RVT-4-082
3: Diode Array
Range: 4.664e+1
AU
4.0e+1
3.0e+1
2.0e+1
1.0e+1
0.0
6.53
8.35
0.62
8.02
2.00
4.00
6.00
8.00
10.00
12.00
14.00
MM RVT-4-082
2: Scan ES+
TIC
4.36e7
%
0
8.36
6.54
MM RVT-4-082
1: Scan ES-
TIC
3.73e6
%
11
6.53
6.73
0.66
1.00
1.30
2.33
2.88
3.16
3.99
5.06
5.32
6.13
7.46
8.11
8.41
9.02
9.30
10.25
10.61
11.96
12.65
13.58
13.88
Time


MM RVT-4-082 324 (6.542) Cm (317:332)
2: Scan ES+
6.87e6
402.40
403.38
404.38
153.20
279.24
487.58
680.78
803.86
841.83
1024.14
1064.69
1227.43
1473.18
1574.06
1744.02
1929.47
MM RVT-4-082 324 (6.532) Cm (319:334)
1: Scan ES-
1.11e6
113.06
400.35
401.38
173.21
249.04
461.49
542.78
758.61
801.71
838.76
1044.93
1162.72
1198.61
1437.53
1586.49
1736.41
1919.11
MM RVT-4-082 414 (8.359) Cm (408:426)
2: Scan ES+
8.03e6
428.37
400.34
429.40
430.39
141.16
279.36
513.49
656.41
799.82
855.91
880.97
1018.37
1227.44
1455.65
1598.43
1848.98
%
m/z
100
0
200
400
600
800
1000
1200
1400
1600
1800