Supporting information

Hitting a moving target: simulation and crystallography study of ATAD2 bromodomain blockers

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1. Methods

Protein expression and purification

The plasmid expressing the 6-His-tagged bromodomain of ATAD2 was a gift from the laboratory of Nicola Burgess-Brown (obtained through Addgene - #38916). It was used to express and purify the protein for crystallization in two chromatographic steps, as described before. The GST-tagged protein for HTRF experiments was obtained from the Reaction Biology Corporation (RD-11-236) and used as received.

Crystallization and structure determination of ligand-protein complexes

The crystals of the unliganded form of the protein were obtained in by mixing the protein solution (concentrated do 5 mg/mL) with the mother liquor solution containing 2.0 M ammonium sulfate and 0.1 M Bis-Tris at pH = 5.5 in the 1:1 ration and incubating at 4 °C. Hexagonal crystals typically formed within 24-48 hours. Co-crystals of the ATAD2 bromodomain complexed with ligands were obtained by soaking. To this end, protein crystals were transferred to drops containing 30 % PEG 3350 and 0.1 M Tris at pH 8.0 supplemented with ligand at the concentration of 100 mM (or saturating concentration, if lower) and soaked for 16-24 hours. Crystals were then harvested and flash-frozen in liquid nitrogen. Data collection was performed at the beamlines PX or PXIII of the Swiss Light Source (Viligen, Switzerland). Details of data collection and refinement are summarized in Supporting Table 1.

HTRF assay for ATAD2 bromodomain

HTRF measurements (time-resolved FRET) were conducted following the guidelines for ATAD2 HTRF kit from Cisbio (62BDCPEG) with small modifications. All reagents were dissolved in the assay buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.5 mM TCEP, 0.1 % BSA) to the final concentrations described in the kit manual. Compounds were serially diluted in a 1:3 dilution series and were added to the GST-tagged ATAD2 bromodomain (RD-11-236, Reaction Biology Corp.). The biotinylated peptide (SGRGK(Ac)GGKGLGKGGAKRHRKVLRDNGSGS-[biotin] – REF 10.1042/BJ20140933) was used as the binding partner. After 3 h incubation at room temperature, signal was detected using Tecan Infinite M1000 plate reader. The HTRF ratio was calculated, normalized and analyzed using nonlinear curve fit, supplied with the Graph Prism Software to obtain the IC_{50} value. The dose-response curves are shown in supporting Figure 3.

ITC assay for ATAD2 bromodomain

Isothermal Titration Calorimetry experiment was performed using a VP-ITC instrument (GE Healthcare, formerly MicroCal, Inc.). Protein sample was purified in the same batch of buffer in order to minimize artifacts due to minor differences in buffer composition. Compound (500 µM) dissolved in ITC buffer (50 mM HEPES pH 7.5, 150 mM NaCl,0.5 mM TCEP, 1% DMSO) was injected into the 1.4 mL sample cell containing the bromodomain of ATAD2 (50 µM). The titration experiments were carried out at 10°C while stirring at 300 rpm: after a control injection of 2 µL, 24 12-µL injections (12 s duration each, with a 4 min interval between) were

performed. The raw data were integrated, normalized for concentration, and analyzed using a single binding site model, supplied with the MicroCal Origin software package to obtain the K_D value. The dose-response curve for compound **17** is shown in supporting Figure 5.

MD simulations with compound 8

Multiple molecular dynamics (MD) runs with explicit solvent were carried out to further validate the binding mode suggested by the docking protocol for compound **8**. In absence of exploitable crystallographic data concerning the 3-aminopyridino orientation of compound **8** within the ATAD2 binding site, three initial docked poses of compound **8** bound to the ATAD2 bromodomain were generated by tethered docking (Figure S4) and used as starting conformations for MD: a first pose (green) from which four trajectories with different velocities were generated; a second pose (cyan) with three trajectories, a third pose (magenta) with three trajectories. All production simulations were carried out using GROMACS versions 5.1.4 and 2018.3 with the CHARMM36/CGenFF force field. Each system was solvated with TIP3P water molecules in a cubic box with boundaries at 1.0 nm distance from the solute. Na^+ and Cl^- ions concentration was set at 0.15 M including excess Na^+ counterions for neutralization. A 10-Å cutoff was used for van der Waals interactions and the particle Ewald method (PME) was used to treat long-range electrostatics. Equilibration steps in the NVT and then NPT ensembles were run for 10 ns and 100 ps, respectively. The temperature was maintained constant by modified Berendsen thermostat (0.1 ps coupling) at 310K while keeping the pressure constant by Parrinello-Rahman barostat at 1 bar (2 ps coupling). The time step was 2 fs and snapshots were saved for analysis every 200 ps for an overall run of 200 ns (1000).

The results for trajectories **A** (Supporting Figures 5A to 8A, 200 ns, unbinding observed after ~140 ns, initial pose in magenta in Figure S4), **B** (Supporting Figures 5B to 8B, 200 ns, no unbinding observed, initial pose in green in Figure S4) and **C** (Supporting Figures 5C to 8C, 200 ns, unbinding observed after ~100 ns, initial pose in green in Supporting Figure 4) are presented herein. Most trajectories led to a rapid unbinding of the acetylthiazole ring from the binding pocket (Supporting Figure 5C). For most trajectories however the salt bridge between the ammonium of compound **8** and ATAD2's Asp1071 was stable for longer simulation time (Supporting Figure 6).

Considering the dihedral angle N1-C2-C3-O4 (Supporting Figure 9A; rotation of the acetyl group relatively to the thiazole ring), the values near 0° correspond to the *cis*-conformation. On the one hand, such conformer involves the intramolecular repulsion of the lone-pairs of the oxygen O4 and the nitrogen N1 but, on the other hand, it enables favorable intermolecular interactions with the conserved Asn1164 and Tyr1121 of ATAD2 through a direct and water-bridge H-bond respectively. In contrast, the *trans*-conformation (θ = 180°) allows the oxygen O4 and the nitrogen N1 to be antiperiplanar to one another but prevents H-bonding of the C=O to the structural water bridged to the conserved Tyr1021. Instead the polar oxygen points toward both the C=O of Val1008 and the lipophilic sidechain of Val1013 leading to respectively repulsive or unfavorable interactions. Analysis of the absolute value of this dihedral angle N1-C2-C3-O4 throughout the trajectory **A** reveled a preference for values tending toward 180° (Supporting Figure 9B). MD simulations of the free compound **8** in a water box (Cubix box, TIP3P-water model, with boundaries at 1.5 nm distance from the

solute at 0.15 M ions concentration, 100 ns, 500 frames, other parameters as described above) provided similar results (Supporting Figure 9C).

To compare the distribution observed in the simulations with experimental data, a substructure search was performed in the Cambridge Structural Database (CSD). A total of 427 structures were retrieved from the CSD starting from the SMARTS substructures in Figure S5D. For only 75 structures, the carbon (7) was not in a ring. Most structures displayed an O-C-C-N dihedral angle above 160° (Figure S5D), which is consistent with the MD results.

MD simulations with compound 5

In order to maintain the *cis*-orientation (namely O of acetyl moiety and N of thiazole ring both pointing toward the Asn1064 and Tyr1021 and hence building direct and water-bridged H-bonds with those residues, respectively) the bicyclic compound **5** was synthesized. The ligand was docked in three different ATAD2 structures and the one with the most favorable binding energy was then used for the MD simulations. Four trajectories (118 ns, 76 ns, 76 ns, 76 ns, figure S6D) each with different initial velocities were started from this pose. The methylene moieties of the 6-membered ring occupies the lipophilic pocket formed by Val1008 and Val1013 (Supporting Figure 10, A to C). While the MD simulations for compound **8** are characterized by the instability of the ZA loop (Figure S6,A to C), the lipophilic interactions in the ATAD2-compound **5** complex stabilize the ZA loop, hereby preventing its unfolding (RMSD of selected Cα atoms in the ZA loop, Supporting Figures 10D and 11).

Orientation of gatekeeper Ile1074 and Val1008

Analysis of the Val1008 and Ile1074 sidechain dihedrals via multivariate kernel density estimation (Supporting Figure 12) reveals that the ligand **5** promotes the conformation with the biggest opening of the binding pocket.

Characterization of compounds

NMR spectra were recorded on AV 300, AV2 400 or AV2 500 MHz Bruker spectrometers. Chemical shifts are given in ppm. The spectra are calibrated to the residual 1H and 13C signals of the solvents. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), doublet-doublet (dd), quintet (quint), multiplet (m), and broad (br). Melting points were determined on a Mettler Toledo MP70 melting point instrument. Infrared spectra were recorded on a JASCO FT/IR-4100 spectrometer. High-resolution electrospray ionization mass spectrometry was performed on a Finnigan MAT 900 (Thermo Finnigan, San Jose, CA, USA) double-focusing magnetic sector mass spectrometer. Ten spectra were acquired. A mass accuracy ≤2 ppm was obtained in the peak matching acquisition mode by using a solution containing 2 µL PEG200, 2 µL PPG450, and 1.5 mg NaOAc (all obtained from Sigma-Aldrich, Buchs, Switzerland) dissolved in 100 mL MeOH (HPLC Supra grade, Scharlau, E-Barcelona) as internal standard. The purity of all tested compounds was determined by one out of the 4 methods. Method 1: HPLC on a Waters Acquity UPLC (Waters, Milford, MA) Top spectrometer using an Acquity BEH C18 HPLC column (1.7 µm, 1 × 50 mm, Waters) with a mixture of H_2O + 0.1% HCOOH (A) and CH_3CN + 0.1% HCOOH (B) solvent (0.1 mL flow rate, linear gradient from 5% to 98% B within 4 min, followed by flushing with 98% B for 1 min). Method 2 – 4: HPLC

using an Agilent 1200 Series HPLC (Santa Clara, CA, USA) and a Gemini C18 column (5 µm, 150 × 4.6 mm, *Phenomenex, Germany*). The temperature was kept constant at 50 °C. The eluent at flow rate of 1.5 mL/min was consisting of H_2O + 0.01% TFA (A) and MeOH + 0.01% TFA (B) (Method 2, 3) or CH_3CN + 0.01% TFA (B) (Method 4). The gradient elution was starting at 0% of B for 3 min and increased to 90% within 6 min, followed by flushing with 90% B for 3 minutes. An Evaporative Light Scattering Detector (ELSD) (Method 2, 4) or UV-Vis Detector (Method 3) was used for detection. The injected sample amount was always 2 µL at 1 mg/mL concentration. All compounds showed ≥ 95 % purity. Marvin was used for drawing, displaying, and characterizing chemical structures and protonation states as well as for generating conformers, Marvin version 16.2.15.0, 2016, ChemAxon (http://www.chemaxon.com)

Synthetic methods

Unless otherwise stated, reactions were carried out under a nitrogen atmosphere using standard Schlenk-techniques. All reagents were used as received unless otherwise noted. Solvents were purchased in the best quality available, degassed by purging thoroughly with nitrogen and dried over activated molecular sieves of appropriate size. Alternatively, they were purged with argon and passed through alumina columns in a solvent purification system (Innovative Technology). Reactions were monitored by thin layer chromatography (TLC) using Merck TLC silica gel 60 F_{254}. Flash column chromatography was performed over silica gel (230-400 mesh). For schemes detailing the numbering of the different reaction intermediates, see SI.

General procedure for amide formation

To a stirred solution of the corresponding carboxylic acid (1.0 equiv) in dimethylformamide (0.3 M), EDC·HCl (2 equiv), HOBt (1 equiv) and the corresponding amine (1 equiv) were added. The reaction mixture was stirred at 25 °C for 18-22 h and it was subsequently diluted with ethyl acetate, washed two times with $NaHCO_3$ saturated aqueous solution, two times with HCl (1 M) and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography affording the desired amide in pure form. For amide **38**, the reaction mixture was stirred at 25 °C for 15 h, then heated to 70 °C for an additional 15 h.

General procedure for bromination

To a stirred solution of the corresponding thiazole in acetic acid (1 M), *N*-bromosuccinimide (1.1 equiv) was added. The reaction mixture was stirred at 70 °C for 1-3 h and it was subsequently diluted with ethyl acetate, washed three times with $NaHCO_3$ saturated aqueous solution and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography affording the desired product in pure form.

General procedure for Suzuki cross-coupling reactions

To a solution of the corresponding halide (1 equiv) in dioxane (0.25 M) and water (5 equiv) under a nitrogen atmosphere, the corresponding pinacol boronic ester or boronic acid (1-1.2

equiv), K_2CO_3 (3.0 equiv) and $Pd(dppf)Cl_2$ (10 mol %) were added. Nitrogen gas was bubbled through the reaction for two minutes and the reaction mixture was stirred at 90 °C for 15-22 h and diluted with ethyl acetate. It was then concentrated under reduced pressure and purified by flash column chromatography.

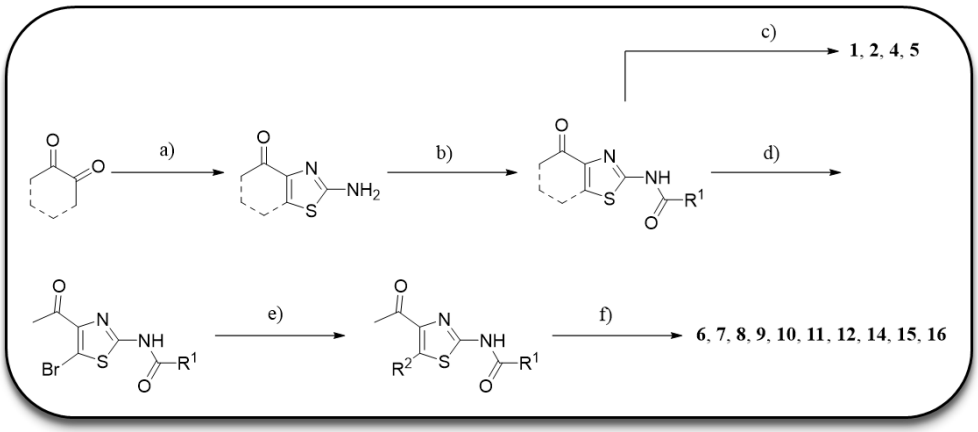
General procedure A for Boc deprotection

To a stirred solution of the corresponding Boc protected amine in methanol (0.2 M), HCl (3 equiv, 4 M in dioxane) was added. The reaction mixture was stirred at 25 °C for 4 h and if not completed, additional HCl was added until completion. The reaction mixture was concentrated under reduced pressure and the obtained residue was triturated, filtered and dried to afford the desired amine in pure form.

General procedure B for Boc deprotection

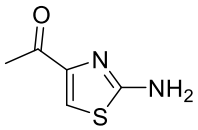
To a stirred solution of the corresponding Boc protected amine in dichloromethane (0.3 M), TFA (3 equiv) was added. The reaction mixture was stirred at 25 °C for 4 h and if not completed, additional TFA was added until completion. The reaction mixture was concentrated under reduced pressure and the obtained residue was dissolved in methanol, HCl (32 %, 4 equiv) was added and it was concentrated under reduced pressure. These steps were repeated twice and the crude residue was triturated, filtered and dried to afford the desired amine in pure form.

A. Synthesis of compounds 1, 2, 4-12, 14-16:



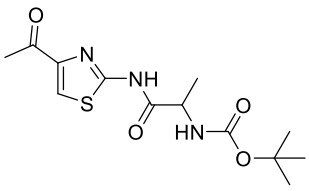
Scheme 1: General synthetic route for the compounds **1**, **2**, **4**-**12** and **14**-**16**. Reagents and conditions: (a) (i) Br_2, DCM, 0-25 °C, 2 h; (ii) thiourea, ethanol, 25 °C, 4 h, 57 % over two steps; (b) R^1-COOH, EDC.HCl, HOBt, DMF, 25-70 °C, 18-30 h, 46-82 % or HN(Boc)-$C(Me)_2$-COOH, HATU, DIPEA, DMF, 25-70 °C, 24 h, 34 %; (c) HCl, methanol, 25 °C, 20 h - 3 d, 70-84 % or TFA, DCM, 25 °C, 4 h, 70 %; (d) NBS, acetic acid, 70 °C, 1-3 h, 46-96 %; (e) R^2-Bpin/$B(OH)_2$, K_2CO_3, $Pd(dppf)Cl_2$, dioxane/H_2O, 90 °C, 15-18 h; (f) HCl, MeOH, 25 °C, 8 h – 8 d, 11-47 % or TFA, DCM, 25 °C, 3-16 h, 3-54 %.

1-(2-Aminothiazol-4-yl)ethan-1-one (19)



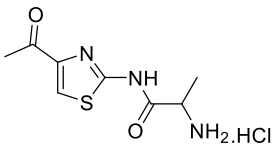
To a stirred solution of butane-2,3-dione (15 mL, 171 mmol) in dry dichloromethane (30 mL) at 0 °C under a nitrogen atmosphere, a solution of bromine (8.8 mL, 171 mmol) in dry dichloromethane (9 mL) was added dropwise over twenty minutes. At the end of the addition, the ice bath was removed and the dark red solution was stirred at 25 °C for 1.5 h. The reaction mixture was washed three times with brine, dried over $MgSO_4$, concentrated under reduced pressure and the obtained residue was engaged in the next step without further purification.
The crude mixture was added dropwise to a stirred solution of thiourea (13.5 g, 171 mmol) in ethanol (198 mL) and stirred at 25 °C for 4 h. The resulting slurry was slowly added to NaOH (4 M, 90 mL) at 0 °C, stirred for 0.5 h, filtered and washed with water until the washings were colorless. The obtained precipitate was dried under vacuum to afford the desired product as a yellow solid (13.8 g, 57 % yield over two steps). Mp: 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.48 (s, 1 H), 7.19 (s, 2 H), 2.39 (s, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.7, 168.1, 150.7, 115.6, 27.5; IR (neat): ũ = 3329, 3088, 1653, 1629, 1549, 1484, 1341, 1306, 1203, 1070, 977, 720, 608, 567 cm^{-1}; HRMS (ESI): m/z: calcd for $C_5H_7N_2OS^+$: 143.0279, found: 143.0273.

***tert*-Butyl (1-((4-acetylthiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (20)**



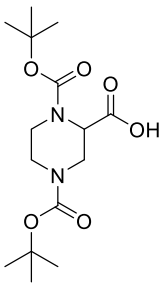
Amide **20** was obtained following the general procedure for amide formation (chromatography: Hexane/EtOAc = 1:1). White solid; Yield: 82 %; Mp: 84-86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.03 (br. s., 1 H), 7.80 (s, 1 H), 4.93 (br. s, 1 H), 4.47 (br. s, 1 H), 2.58 (s, 3 H), 1.53 - 1.45 (12 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.2, 171.3, 157.2, 155.8, 149.5, 121.2, 81.3, 50.3, 28.3 (3 C), 27.3, 17.4; IR (neat): ũ = 1684, 1671, 1557, 1541, 1522, 1507, 1457, 1363, 1159 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{13}H_{20}N_3O_4S^+$: 314.1175 found: 314.1170.

***N*-(4-Acetylthiazol-2-yl)-2-aminopropanamide hydrochloride (1)**



Amine **1** was obtained following the general procedure A for Boc deprotection (trituration: Acetonitrile). White solid; Yield: 84 %; Mp: 268-271 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.99 (br. s., 1 H), 8.56 (br. s., 3 H), 8.16 (s, 1 H), 4.19 - 4.08 (m, 1 H), 2.53 (s, 3 H), 1.49 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.7, 169.2, 157.1, 149.1, 121.9, 48.5, 27.5, 16.7; IR (neat): ũ = 3396, 3115, 3078, 2880, 1703, 1655, 1557, 1489, 1191 cm^{-1}; HRMS (ESI): m/z: calcd for $C_8H_{12}N_3O_2S^+$: 214.0650 found: 214.0647.

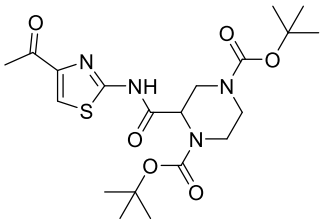
1,4-Bis(*tert*-butoxycarbonyl)piperazine-2-carboxylic acid (21)



To a stirred solution of piperazine-2-carboxylic acid (2 g, 15 mmol) in a mixture of tetrahydrofuran (50 mL) and water (100 mL), Na_2CO_3 (6.4 g, 60 mmol) and Boc_2O (12 g, 57 mmol) were added. The reaction mixture was stirred at 25 °C for 16 h and concentrated under reduced pressure. The obtained residue was extracted with diethyl ether and the aqueous layer was acidified with HCl (5 M) until pH = 1. The aqueous layer was extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and concentrated under reduce pressure to afford the desired product as a white solid (5.18 g, quantitative yield). Mp: 147-150 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.80 - 4.51 (m, 2 H), 4.01 (br. s., 1 H), 3.93 - 3.76 (m, 1 H), 3.23 (br. s., 1 H), 3.12 (dd, J = 13.7, 4.3 Hz, 1 H), 2.87 (br. s., 1 H), 1.55 - 1.40 (m, 18 H); ^{13}C

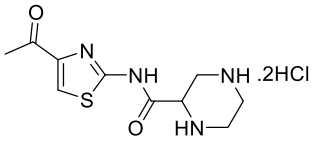
NMR (101 MHz, $CDCl_3$): δ = 174.5, 155.8, 155.0 (rot., 0.5 C), 154.5 (rot., 0.5 C), 81.0, 80.6, 54.6 (rot., 0.5 C), 53.4 (rot., 0.5 C), 43.4, 41.4, 40.1, 28.2 (6 C); IR (neat): ũ = 1692, 1425, 1390, 1365, 1223, 1166, 1108 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{26}N_2NaO_6^+$: 353.1689 found: 353.1683.

Di-*tert*-butyl 2-((4-acetylthiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (22)



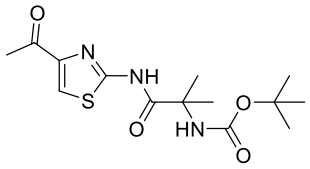
Amide **22** was obtained following the general procedure for amide formation. After work-up, the obtained residue was triturated in EtOAC/Hexane = 4:6, filtered and dried to afford the desired product as a white solid. Yield: 73 %; Mp: 103-105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.79 (s, 1 H), 4.90 (br. s, 1 H), 4.59 (d, *J* = 13.3 Hz, 1 H), 3.95 (br. s., 2 H), 3.30 - 3.15 (m, 2 H), 3.02 (br. s., 1 H), 2.58 (s, 3 H), 1.52 (s, 9 H), 1.46 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 191.9, 168.3, 157.1, 155.7, 154.5, 149.1, 121.1, 82.2, 80.8, 54.6, 43.1, 42.2, 28.3 (6 C), 27.3. One carbon is missing due to overlapping or broadening; IR (neat): ũ = 3206, 1684, 1554, 1365, 1252, 1160, 1108, 752 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{20}H_{31}N_4O_6S^+$: 455.1964 found: 455.1959.

***N*-(4-acetylthiazol-2-yl)piperazine-2-carboxamide hydrochloride (2)**



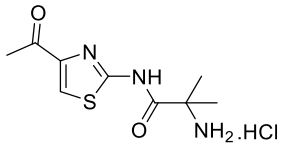
Piperazine **2** was obtained following the general procedure A for Boc deprotection (trituration: MeOH). White solid; Yield: 70 %; Mp: decomposition; ^{1}H NMR (400 MHz, D_2O): δ = 8.23 (s, 1 H), 4.50 (dd, *J* = 10.4, 3.6 Hz, 1 H), 3.91 (dd, *J* = 13.6, 3.8 Hz, 1 H), 3.70 - 3.57 (m, 2 H), 3.51 (dd, *J* = 13.7, 10.4 Hz, 1 H), 3.46 - 3.31 (m, 2 H), 2.58 (s, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.7, 164.3, 157.0, 148.9, 122.2, 53.0, 41.5, 27.5. Two carbons are missing due to overlapping; IR (neat): ũ = 3382, 2969, 1685, 1561, 1371, 1294, 1202 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{10}H_{15}N_4O_2S^+$: 255.0910 found: 255.0910.

***tert*-Butyl (1-((4-acetylthiazol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (23)**



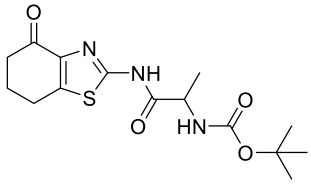
To a stirred solution of amine **19** (2.2 g, 15.7 mmol) and 2-((*tert*-butoxycarbonyl)amino)-2-methylpropanoic acid (3.2 g, 15.7 mmol) in dimethylformamide (31 mL), HATU (6 g, 15.7 mmol) and DIPEA (8.2 mL, 47.1 mmol) were added. The reaction mixture was stirred at 25 °C for 16 h, heated at 70 °C for an additional 8 h and cooled down to 25 °C. The mixture was diluted with ethyl acetate, washed two times with $NaHCO_3$ saturated aqueous solution, two times with HCl (1 M) and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc/Hexane = 2:8 to 6:4) to afford the desired product as a white solid (1.74 g, 34 %). Mp: 150-151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.22 (br. s., 1 H), 7.79 (s, 1 H), 4.95 (s, 1 H), 2.58 (s, 3 H), 1.58 (s, 6 H), 1.42 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.0, 173.3, 158.0, 154.7, 149.4, 121.4, 81.4, 57.0, 28.2 (3 C), 27.1, 25.3 (2 C); IR (neat): ũ = 1722, 1673, 1532, 1488, 1250, 1230, 1154, 1078 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{21}N_3O_4NaS^+$: 350.1151 found 350.1144.

***N*-(4-Acetylthiazol-2-yl)-2-amino-2-methylpropanamide hydrochloride (4)**



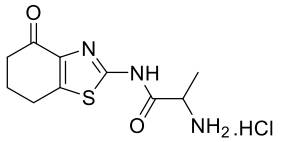
Amine **4** was obtained following the general procedure B for Boc deprotection (trituration: Acetone). White solid; Yield: 70 %; Mp: 192-195 °C; ^{1}H NMR (400 MHz, D_2O): δ = 8.21 (s, 1 H), 2.58 (s, 3 H), 1.68 (s, 6 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 191.6, 171.3, 157.6, 149.1, 122.4, 56.9, 27.5, 22.8 (2 C); IR (neat): ũ = 1684, 1557, 1490, 1252, 1217, 1169, 654 cm^{-1}; HRMS (ESI): m/z: calcd for $C_9H_{14}N_3O_2S^+$: 228.0807 found: 228.0803.

***tert*-Butyl (1-oxo-1-((4-oxo-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)propan-2-yl)carbamate (24):**



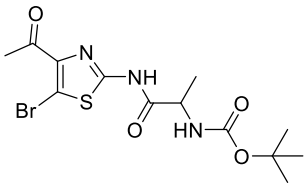
Amide **24** was obtained following the general procedure for amide formation (chromatography: Hexane/EtOAc = 1:1 to 1:3). White foam; Yield: 55 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.08 (s, 1H), 4.95 (s, 1H), 4.44 (br. s., 1H), 3.04 (t, *J* = 6.1 Hz, 2H), 2.69 - 2.60 (t, *J* = 6.53 Hz, 2H), 2.32 - 2.19 (m, 2H), 1.49 (d, *J* = 7.2 Hz, 3H), 1.44 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.5, 171.8, 155.9, 155.5, 147.1, 144.1, 81.1, 50.6, 38.3, 28.4 (3 C), 24.3, 23.9, 18.0; IR (neat): ũ = 2974, 2927, 1670, 1555, 1366, 1269, 1247, 1161, 1124, 1019 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{22}N_3O_4S^+$: 340.1326 found: 340.1325.

2-Amino-*N*-(4-oxo-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)propanamide hydrochloride (5):



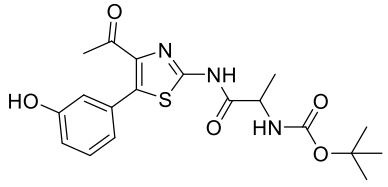
Amine **5** was obtained following the general procedure A for Boc deprotection (trituration: Acetone). White solid; Yield: 80 %; Mp: decomposition; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.92 (br. s., 1 H), 8.53 (br. s., 3 H), 4.11 (br. s., 1 H), 3.04 (t, *J* = 5.8 Hz, 2 H), 2.53 (t, *J* = 6.3 Hz, 2 H), 2.12 (quin, *J* = 6.2 Hz, 2 H), 1.48 (d, *J* = 7.3 Hz, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 190.2, 169.1, 154.6, 146.9, 143.7, 48.5, 38.0, 23.8, 23.1, 16.6; IR (neat): ũ = 2879, 1658, 1557, 1492, 1379, 1276, 1201, 1172, 1126, 1109, 962 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{10}H_{14}N_3O_2S^+$: 240.0801 found: 240.0802.

***tert*-Butyl (1-((4-acetyl-5-bromothiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (25)**



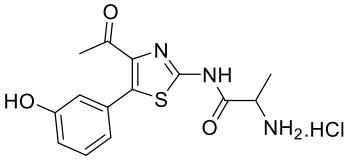
Bromide **25** was obtained following the general procedure for bromination (chromatography: Hexane/EtOAc = 1:1). White solid; Yield: 96 %; Mp: 166-168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.97 (br. s, 1 H), 4.88 (d, *J* = 6.4 Hz, 1 H), 4.42 (br. s., 1 H), 2.61 (s, 3 H), 1.53 - 1.46 (12 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.9, 171.6, 156.2, 155.5, 143.8, 110.1, 81.5, 50.0, 28.9, 28.3 (3 C), 17.4; IR (neat): ũ = 1683, 1671, 1557, 1541, 1522, 1507, 1473, 1364, 1251, 1156 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{13}H_{19}BrN_3O_4S^+$: 392.0280 found: 392.0267.

***tert*-Butyl (1-((4-acetyl-5-(3-hydroxyphenyl)thiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (26)**



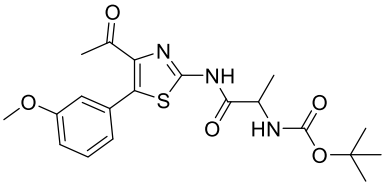
Phenol **26** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH = 5:5:3% to 5:5:10%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(3-hydroxyphenyl)thiazol-2-yl)-2-aminopropanamide hydrochloride (6)**



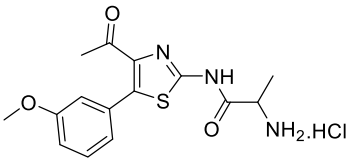
Amine **6** was obtained following the general procedure A for Boc deprotection (trituration: Et_2O). Brown solid; Yield: 11 % over two steps; Mp: 206-207 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.95 (br. s., 1 H), 9.64 (s, 1 H), 8.34 (br. s., 2 H), 7.22 (t, J = 7.9 Hz, 1 H), 6.93 - 6.89 (m, 1 H), 6.86 - 6.79 (m, 1 H), 4.14 (br. s., 1 H), 2.52 (s, 3 H), 1.49 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 193.3, 169.2, 157.1, 153.9, 141.7, 137.6, 131.1, 129.4, 120.4, 116.6, 116.0, 48.4, 29.4, 16.7; IR (neat): ũ = 3349 2929, 1670, 1557, 1277, 1197, 1150, 1094 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{16}N_3O_3S^+$: 306.0912 found: 306.0907.

***tert*-Butyl (1-((4-acetyl-5-(3-methoxyphenyl)thiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (27)**



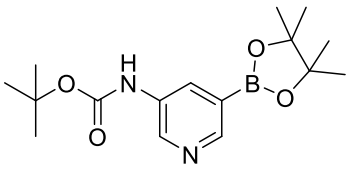
Methoxybenzene **27** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH = 8:2:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(3-hydroxyphenyl)thiazol-2-yl)-2-aminopropanamide hydrochloride (7)**



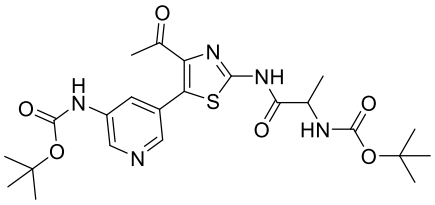
Amine **7** was obtained following the general procedure A for Boc deprotection (trituration: Et_2O). Yellow solid; Yield: 47 % over two steps; Mp: 210-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.01 (s, 1 H), 8.50 (br. s., 3 H), 7.35 (t, J = 7.9 Hz, 1 H), 7.10 - 7.04 (m, 2 H), 7.01 (dd, J = 8.3, 2.3 Hz, 1 H), 4.16 (br. s., 1 H), 3.78 (s, 3 H), 1.49 (d, J = 7.2 Hz, 3 H). Three protons are missing due to overlapping with the DMSO peak; ^{13}C NMR (101 MHz, DMSO-d_6): δ = 193.3, 169.3, 158.9, 154.1, 141.9, 137.3, 131.3, 129.5, 122.1, 115.5, 114.4, 55.2, 48.4, 29.4, 16.7; IR (neat): ũ = 3141, 1653, 1559, 1486, 1283, 1154, 1041 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{18}N_3O_3S^+$: 320.1069 found: 320.1064.

***tert*-Butyl (5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)carbamate (28)**



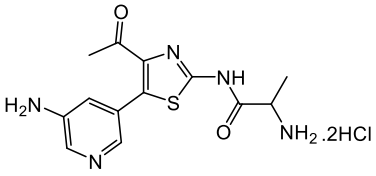
To a solution of *tert*-butyl (5-bromopyridin-3-yl)carbamate (2.0 g, 7.3 mmol) in dry dioxane (37 mL) under a nitrogen atmosphere, bis(pinacolato)diboron (2.2 g, 8.76 mmol), KOAc (2.87 g, 29 mmol) and Pd(dppf)Cl_2 (534 mg, 0.73 mmol) were added. Nitrogen gas was bubbled through the reaction for ten minutes and the reaction mixture was stirred at 90 °C for 16 h. The resulting mixture was diluted with dichloromethane, filtered over a pad of celite and concentrated under reduced pressure. The obtained residue was dissolved in ethyl acetate, filtered over a pad of silica and concentrated under reduced pressure. The crude residue was triturated (Hexane/Et_2O = 9:1), filtered and dried to afford the desired product as an off-white solid (1.61 g, 69 % yield). Mp: 170-172 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.63 (d, J = 1.4 Hz, 1 H), 8.59 (d, J = 2.7 Hz, 1 H), 8.21 (br. s., 1 H), 6.48 (br. s., 1 H), 1.54 (s, 9 H), 1.35 (s, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 152.8, 148.0, 142.4, 135.9, 129.9, 84.0 (2 C), 79.7, 28.0 (3 C), 24.6 (4 C). One carbon is missing due to overlapping; IR (neat): ũ = 1720, 1584, 1558, 1447, 1363, 1244, 1156, 1142 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{16}H_{26}BN_2O_4^+$: 321.1986 found: 321.1977.

***tert*-Butyl (1-((4-acetyl-5-(5-((*tert*-butoxycarbonyl)amino)pyridin-3-yl)thiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (29)**



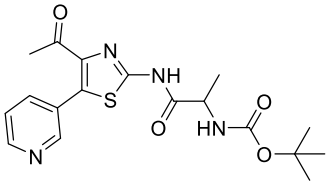
Boc amino pyridine **29** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH/Et_3N = 7:3:1%:1% to 7:3:5%:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)-2-aminopropanamide hydrochloride (8)**



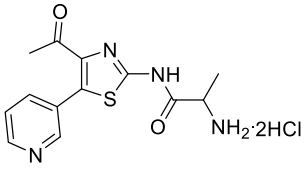
Amine **8** was obtained following the general procedure A for Boc deprotection (trituration: Acetone). Brown solid; Yield: 39 % over two steps; Mp: 233-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.25 (br. s., 1 H), 8.64 (br. s., 3 H), 8.17 (d, J = 1.0 Hz, 1 H), 8.04 (d, J = 2.0 Hz, 1 H), 7.67 (t, J = 2.0 Hz, 1 H), 4.19 (br. s., 1 H), 2.56 (s, 3 H), 1.51 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 193.6, 169.6, 155.6, 147.1, 143.4, 130.4, 130.0, 129.0, 127.6, 125.6, 48.4, 29.1, 16.7; IR (neat): ũ = 3367, 1684, 1570, 1544, 1507, 1362, 1269, 1169 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{13}H_{16}N_5O_2S^+$: 306.1025 found: 306.1012.

***tert*-Butyl (1-((4-acetyl-5-(pyridin-3-yl)thiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (30)**



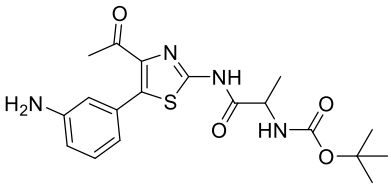
Pyridine **30** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH = 7:3:1% to 4:4:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(pyridin-3-yl)thiazol-2-yl)-2-aminopropanamide hydrochloride (9)**



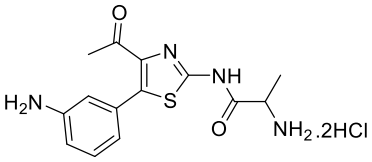
Amine **9** was obtained following the general procedure A for Boc deprotection (trituration: DCM). Brown solid; Yield: 22 % over two steps; Mp: 200-202 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.20 (s, 1 H), 8.90 (d, J = 2.2 Hz, 1 H), 8.75 (dd, J = 5.2, 1.4 Hz, 1 H), 8.66 - 8.55 (m, 3 H), 8.28 (d, J = 7.9 Hz, 1 H), 7.76 (dd, J = 8.0, 5.2 Hz, 1 H), 4.21 - 4.18 (m, 1 H), 2.56 (s, 3 H), 1.51 (d, J = 7.3 Hz, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.6, 169.6, 155.4, 146.6, 145.9, 143.0, 141.4, 131.8, 128.3, 124.7, 48.5, 29.1, 16.7; IR (neat): ũ = 3406, 1684, 1544, 1489, 1272, 1171 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{13}H_{15}N_4O_2S^+$: 291.0916 found: 291.0909.

***tert*-Butyl (1-((4-acetyl-5-(3-aminophenyl)thiazol-2-yl)amino)-1-oxopropan-2-yl)carbamate (31)**



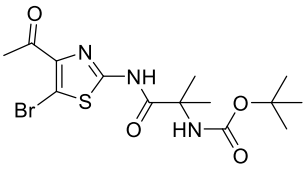
Aniline **31** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH = 3:7:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(3-aminophenyl)thiazol-2-yl)-2-aminopropanamide hydrochloride (10)**



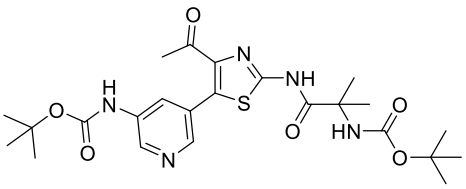
Amine **10** was obtained following the general procedure A for Boc deprotection (trituration: DCM). Brown solid; Yield: 25 % over two steps; Mp: 181-184 °C; ^{1}H NMR (400 MHz, D_2O): δ = 7.46 (t, *J* = 7.5 Hz, 1 H), 7.29 - 7.26 (m, 1 H), 7.24 (t, *J* = 1.9 Hz, 1 H), 7.23 - 7.19 (m, 1 H), 4.39 (q, *J* = 7.2 Hz, 1 H), 2.44 (s, 3 H), 1.67 (d, *J* = 7.2 Hz, 3 H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.4, 169.4, 154.4, 142.0, 136.3, 135.4, 131.4, 129.6, 127.1, 122.5, 121.9, 48.4, 29.4, 16.7; IR (neat): ũ = 3374, 2891, 1686, 1557, 1487, 1277, 1155 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{17}N_4O_2S^+$: 305.1072 found: 305.1069.

***tert*-Butyl (1-((4-acetyl-5-bromothiazol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (32)**



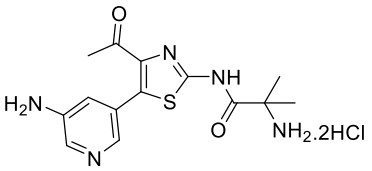
Bromide **32** was obtained following the general procedure for bromination (chromatography: Hexane/EtOAc = 8:2 to 3:7). Pale yellow solid; Yield: 46 %; Mp: 153-154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.84 (s, 1 H), 2.61 (s, 3 H), 1.58 (s, 6 H), 1.45 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.8, 173.3, 156.2, 155.1, 143.9, 110.0, 81.7, 57.0, 29.0, 28.2 (3 C), 25.3 (2 C); IR (neat): ũ = 1684, 1537, 1365, 1281, 1252, 1158, 1074 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{20}BrN_3O_4S^+$: 406.0436 found 406.0430.

***tert*-Butyl (5-(4-acetyl-2-(2-((*tert*-butoxycarbonyl)amino)-2-methylpropanamido)thiazol-5-yl)pyridin-3-yl)carbamate (33)**



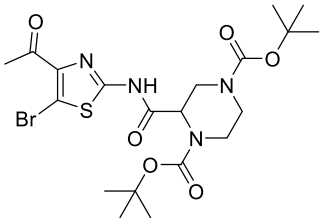
Boc amino pyridine **33** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH/ = 8:2:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)-2-amino-2-methylpropanamide hydrochloride (11)**



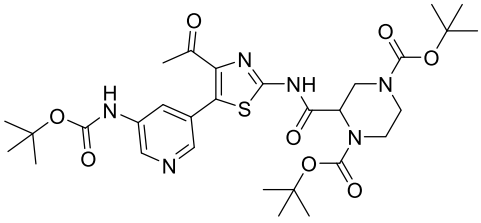
Amine **11** was obtained following the general procedure B for Boc deprotection (trituration: Acetone). Brown solid; Yield: 54 % over two steps; Mp: 239-240 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.12 (br. s., 1 H), 8.65 (br. s., 2 H), 8.14 (s, 1 H), 8.02 (d, *J* = 2.3 Hz, 1 H), 7.58 (s, 1 H), 2.57 (s, 3 H), 1.66 (s, 6 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.7, 171.6, 156.1, 147.1, 143.5, 130.6, 129.9, 129.1, 127.4, 125.9, 57.0, 29.2, 22.8 (2 C); IR (neat): ũ = 1684, 1635, 1557, 1542, 1508, 1362, 1263, 1166 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{17}N_5O_2S^+$: 320.1181, found 320.1175.

Di-*tert*-butyl 2-((4-acetyl-5-bromothiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (34)



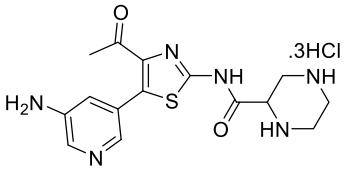
Bromide **34** was obtained following the general procedure for bromination (chromatography: Hexane/EtOAc = 1:1). White solid; Yield: 82 %; Mp: 97-100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.87 (br. s., 1 H), 4.57 (d, *J* = 13.6 Hz, 1 H), 3.94 (br. s., 2 H), 3.27 (dd, *J* = 13.9, 4.5 Hz, 1 H), 3.20 (br. s., 1 H), 3.04 (br. s., 1 H), 2.59 (s, 3 H), 1.52 (s, 9 H), 1.47 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.8 , 168.7 , 155.5 , 143.7 , 110.1 , 82.1 (br. s.), 81.0 (br. s.), 54.1 (br. s.), 43.4 (br. s.), 42.2 (br. s.), 41.6 (br. s.), 28.8 , 28.3 (6 C) Two carbons are missing due to broadening; IR (neat): ũ = 2918, 2848, 1485, 1240, 819, 743, 729, 667, 595, 519 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{20}H_{29}BrN_4NaO_6S^+$: 555.0889 found: 555.0889.

Di-*tert*-butyl 2-((4-acetyl-5-(5-((*tert*-butoxycarbonyl)amino)pyridin-3-yl)thiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (35)



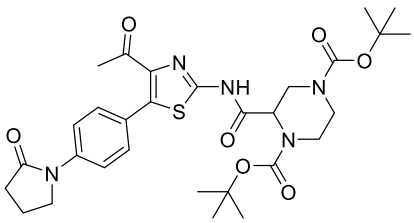
Boc amino pyridine **35** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH/Et_3N = 5:5:1%:1% to 6:4:1%:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-Acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)piperazine-2-carboxamide hydrochloride (12)**



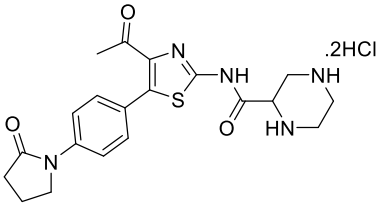
Piperazine **12** was obtained following the general procedure B for Boc deprotection (trituration: Acetone). The crude product was further purified by dissolving it in a MeOH/DCM = 1:1 mixture containing 0.025M HCl. The product was precipitated by the addition of Et_2O and the mother liquor was pipetted off three times to furnish an off-white solid; Yield: 3 % over two steps; Mp: 300 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.15 (s, 1 H), 8.03 (d, *J* = 2.2 Hz, 1 H), 7.59 (s, 1 H), 4.62 (dd, *J* = 11.0, 3.2 Hz, 1 H), 3.89 (dd, *J* = 12.3, 1.9 Hz, 1 H), 3.56 - 3.49 (m, 1 H), 3.47 - 3.34 (m, 2 H), 3.34 - 3.20 (m, 2 H), 2.56 (s, 3 H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 193.6, 164.7, 155.3, 146.9, 143.3, 136.5, 131.1, 129.6, 127.0, 53.1, 41.5, 29.1. Three carbons are missing due to overlapping; IR (neat): ũ = 3323, 1685, 1558, 1367, 1298, 1168, 983 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{19}N_6O_2S^+$: 347.1290 found: 347.1285.

Di-*tert*-butyl 2-((4-acetyl-5-(4-(2-oxopyrrolidin-1-yl)phenyl)thiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (36):



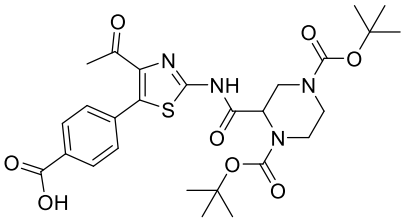
Pyrrolidine **36** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH = 7:3:1%). The impure desired product was engaged in the next step without further purification.

N-(4-Acetyl-5-(4-(2-oxopyrrolidin-1-yl)phenyl)thiazol-2-yl)piperazine-2-carboxamide hydrochloride (15):



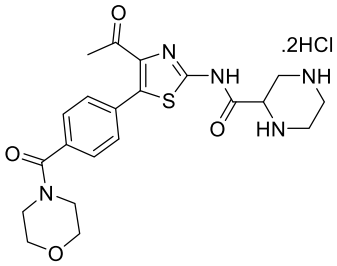
Piperazine **15** was obtained following the general procedure B for Boc deprotection (trituration: Acetone). Brown solid; Yield: 43 % over two steps; Mp: 220-221 °C; ^{1}H NMR (400 MHz, D_2O): δ = 7.56 (s, 4 H), 4.11 (dd, *J* = 7.2, 3.4 Hz, 1 H), 3.97 (t, *J* = 7.2 Hz, 2 H), 3.56 (dd, *J* = 12.4, 3.4 Hz, 1 H), 3.40 - 3.23 (m, 3 H), 3.19 - 3.08 (m, 2 H), 2.65 (t, *J* = 7.9 Hz, 2 H), 2.42 (s, 3 H), 2.24 - 2.14 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.3, 174.2, 164.4, 153.6, 141.4, 140.1, 137.8, 130.2 (2 C), 125.0, 118.7 (2 C), 53.1, 48.0, 41.6, 32.4, 29.4, 17.4. Two carbons are missing due to overlapping; IR (neat): ũ = 1684, 1558, 1524, 1419, 1386, 1217 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{20}H_{24}N_5O_3S^+$: 414.1600 found: 414.1601.

4-(4-Acetyl-2-(1,4-bis(tert-butoxycarbonyl)piperazine-2-carboxamido)thiazol-5-yl)benzoic acid (37):



Acid **37** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH = 1:1:1%). The impure desired product was engaged in the next step without further purification.

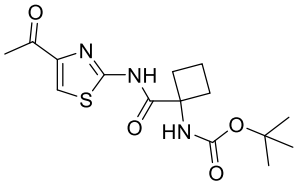
***N*-(4-Acetyl-5-(4-(morpholine-4-carbonyl)phenyl)thiazol-2-yl)piperazine-2-carboxamide hydrochloride (16):**



To a stirred solution of acid **37** (90 mg, 0.16 mmol) in dimethylformamide (0.8 mL), EDC.HCl (46 mg, 0.24 mmol), HOBt (22 mg, 0.16 mmol) and morpholine (14 µL, 0.16 mmol) were added. The reaction mixture was stirred at 25 °C for 17 h and it was subsequently diluted with ethyl acetate, washed two times with $NaHCO_3$ saturated aqueous solution, two times with HCl (1 M) and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was directly engaged in the next step without further purification.

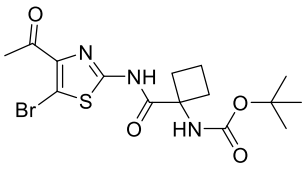
Piperazine **16** was obtained following the general procedure B for Boc deprotection (trituration: Acetone). Beige solid; Yield: 11 % over two steps; Mp: 229-232 °C; ^{1}H NMR (400 MHz, D_2O): δ = 7.66 - 7.59 (m, 2 H), 7.55 - 7.49 (m, 2 H), 4.23 (dd, *J* = 8.5, 3.6 Hz, 1 H), 3.86 - 3.75 (m, 4 H), 3.73 - 3.63 (m, 3 H), 3.56 - 3.50 (m, 2 H), 3.45 - 3.33 (m, 3 H), 3.25 - 3.15 (m, 2 H), 2.43 (s, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.4, 168.5, 164.6, 154.2, 142.0, 136.8, 135.9, 131.3, 129.9 (2 C), 127.0 (2 C), 66.1 (2 C), 53.1, 47.7, 42.0, 41.6, 29.3. Two carbons are missing due to overlapping; IR (neat): ũ = 1684, 1558, 1436, 1355, 1275 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{21}H_{26}N_5O_4S^+$: 444.1706 found: 444.1703.

***tert*-Butyl (1-((4-acetylthiazol-2-yl)carbamoyl)cyclobutyl)carbamate (38)**



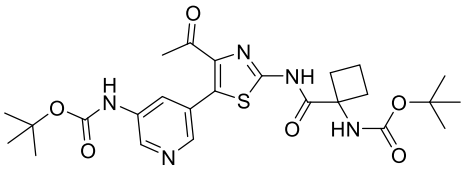
Amide **38** was obtained following the general procedure for amide formation (chromatography: Hexane/EtOAc = 6:4). White solid; Yield: 46 %; Mp: 187-188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.30 (br. s., 1 H), 7.79 (s, 1 H), 5.20 (br. s., 1 H), 2.88 - 2.75 (m, 2 H), 2.58 (s, 3 H), 2.25 - 2.13 (m, 2 H), 2.11 - 1.98 (m, 2 H), 1.45 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.2, 172.3, 158.0, 155.1, 149.4, 121.3, 81.5, 59.0, 31.7, 31.1, 28.2 (3 C), 27.2, 14.8; IR (neat): ũ = 3296, 1674, 1548, 1509, 1369, 1291, 1275, 1257, 1202, 1187, 1166, 1095, 1050 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{21}N_3O_4NaS^+$: 362.1150, found 362.1147.

***tert*-butyl (1-((4-acetyl-5-bromothiazol-2-yl)carbamoyl)cyclobutyl)carbamate (39)**



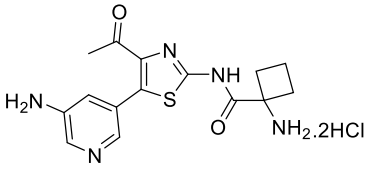
Bromide **39** was obtained following the general procedure for bromination (chromatography: Hexane/EtOAc = 1:1). White solid; Yield: 89 %; Mp: 157-158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.49 (br. s., 1 H), 5.29 (br. s., 1 H), 2.82 - 2.76 (m, 2 H), 2.61 (s, 3 H), 2.24 - 2.14 (m, 2 H), 2.09 - 1.99 (m, 2 H), 1.44 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.9, 172.2, 156.4, 155.5, 144.0, 110.1, 81.8, 58.9, 31.0 (2 C), 29.0, 28.2 (3 C), 15.0; IR (neat): ũ = 1713, 1702, 1685, 1541, 15123, 1508, 1474, 1365, 1311, 1278, 1262, 1178, 1169, 1160, 1010 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{20}BrN_3O_4S^+$: 418.0436, found 418.0433.

***tert*-butyl (1-((4-acetyl-5-(5-((*tert*-butoxycarbonyl)amino)pyridin-3-yl)thiazol-2-yl)carbamoyl)cyclobutyl)carbamate (40)**



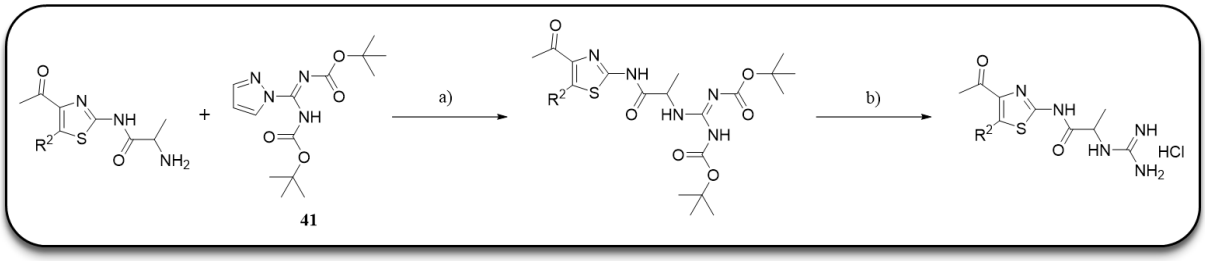
Boc protected amine **40** was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH/ = 7:2:1%). The impure desired product was engaged in the next step without further purification.

***N*-(4-acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)-1-aminocyclobutane-1-carboxamide hydrochloride (14)**



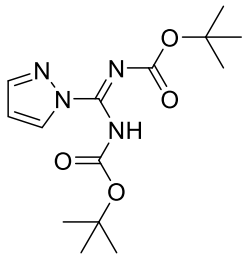
Amine **14** was obtained following the general procedure A for Boc deprotection (trituration: Et_2O). Brown solid; Yield: 22 % over two steps; Mp: 238-239 °C; ^{1}H NMR (400 MHz, MeOD-d_4): δ = 8.19 (s, 1 H), 8.01 (d, J = 2.3 Hz, 1 H), 7.79 (s, 1 H), 3.03 - 2.99 (m, 2 H), 2.67 (s, 3 H), 2.51 - 2.41 (m, 4 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.8, 169.9, 156.3, 147.0, 143.5, 130.7, 129.9, 129.5, 127.3, 126.3, 58.4, 29.4 (2 C), 29.1, 14.2; IR (neat): ũ = 1739, 1684, 1679, 1672, 1658, 1653, 1650, 1646, 1643, 1635, 1612, 1607, 1557, 1553, 1542, 1527, 1507, 1359, 1344, 1338, 1277, 1233, 1202, 1144 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{15}H_{18}N_5O_2S^+$: 332.1181, found 332.1180.

B. Synthesis of compounds 3 and 13:



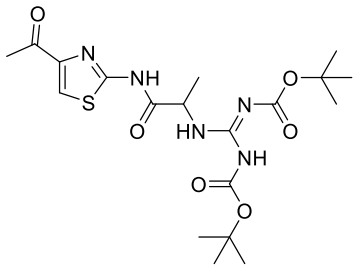
Scheme 2: Synthetic route for compounds **3** and **13**. (a) DIPEA, acetonitrile, 25 °C, 24 h, 64-71 %; (b) TFA, DCM, 25°C, 4 h - 3 d, 71 %.

tert-Butyl (((*tert*-butoxycarbonyl)imino)(1*H*-pyrazol-1-yl)methyl)carbamate (41)



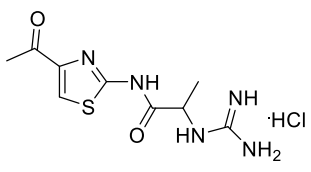
To a stirred solution of *N*-Boc-1*H*-pyrazole-1-carboxamidine (3 g, 20 mmol) in tetrahydrofuran at 0 °C, under a nitrogen atmosphere, NaH (2 g, 51 mmol, 60 % in mineral oil) was added portionwise and the slurry was stirred for 30 min. Boc_2O (10.9 mL, 47.6 mmol) was added, the ice bath was removed and the reaction was stirred for 2 h at 25 °C. The reaction mixture was concentrated under reduced pressure and the obtained residue was purified by flash column chromatography (EtOAc/Hexane = 0:10 to 5:5) to afford the desired product as a white solid (5.29 g, 98 %). Mp: 76-80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.94 (br. s., 1 H), 8.34 (dd, *J* = 2.6, 0.8 Hz, 1 H), 7.65 (dd, *J* = 1.5, 0.8 Hz, 1 H), 6.44 (dd, *J* = 2.8, 1.5 Hz, 1 H), 1.55 (br. s., 18 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 156.9, 149.1, 142.4, 138.8, 128.5, 109.5, 82.8, 80.8, 27.8 (3 C), 27.6 (3 C); IR (neat): ũ = 3250, 2982, 1753, 1706, 1666, 1495, 1300, 1132, 906 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{23}N_4O_4^+$: 311.1719 found: 311.1712.

tert-Butyl *N*-[{[1-[(4-acetyl-1,3-thiazol-2-yl)carbamoyl]ethyl]amino}({[(tert-butoxy)carbonyl]imino})methyl]carbamate (42)



To a stirred slurry of amine **1** (120 mg, 0.48 mmol) in acetonitrile (1.6 mL), DIPEA (84 µL, 0.48 mmol) and compound **41** (149 mg, 0.48 mmol) were added. The reaction mixture was stirred at 25 °C for 24 h and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc/Hexane = 0:10 to 2:8) to afford the desired product as a white solid (155 mg, 71 %). Mp: 100-101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.41 (s, 1 H), 8.83 (br. s., 1 H), 7.80 (s, 1 H), 5.09 (br. s., 1 H), 2.60 (s, 3 H), 1.58 - 1.55 (m, 12 H), 1.52 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 193.1, 169.5, 161.8, 157.0, 156.1, 152.8, 149.8, 120.3, 84.5, 80.7, 49.7, 28.2 (3 C), 28.0 (3 C), 27.6, 15.7; IR (neat): ũ = 3281, 1725, 1608, 1542, 1366, 1308, 1217, 1148, 1123, 1053, 753 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{30}N_5O_6S^+$: 456.1917 found: 456.1913.

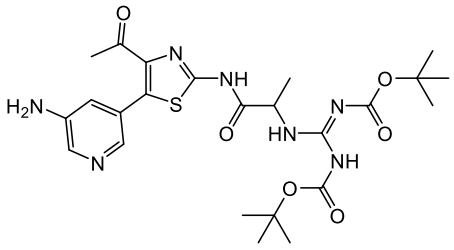
N-(4-Acetylthiazol-2-yl)-2-guanidinopropanamide hydrochloride (3)



Guanidine **3** was obtained following the general procedure B for Boc deprotection (trituration: MeCN/*i*PrOH = 99:1). Yellow solid; Yield: 71 %; Mp: 98-102 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.70 (s, 1 H), 8.14 (s, 1 H), 7.89 (d, *J* = 8.7 Hz, 1 H), 7.22 (br. s., 3 H), 4.45 (quin, *J* = 7.5 Hz, 1 H), 2.52 (s, 3 H), 1.46 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR

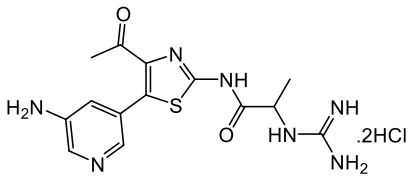
(126 MHz, DMSO-d_6): δ = 191.7, 170.3, 157.6, 156.8, 149.0, 121.7, 50.0, 27.4, 18.1; IR (neat): ũ = 3147, 1663, 1543, 1262, 1174 cm^{-1}; HRMS (ESI): m/z: calcd for $C_9H_{14}N_5O_2S^+$: 256.0868 found: 256.0862.

***tert*-Butyl *N*-[(1Z)-{[1-[(4-acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)-carbamoyl]ethyl]amino}({[(tert-butoxy)carbonyl]imino})methyl]carbamate (43):**



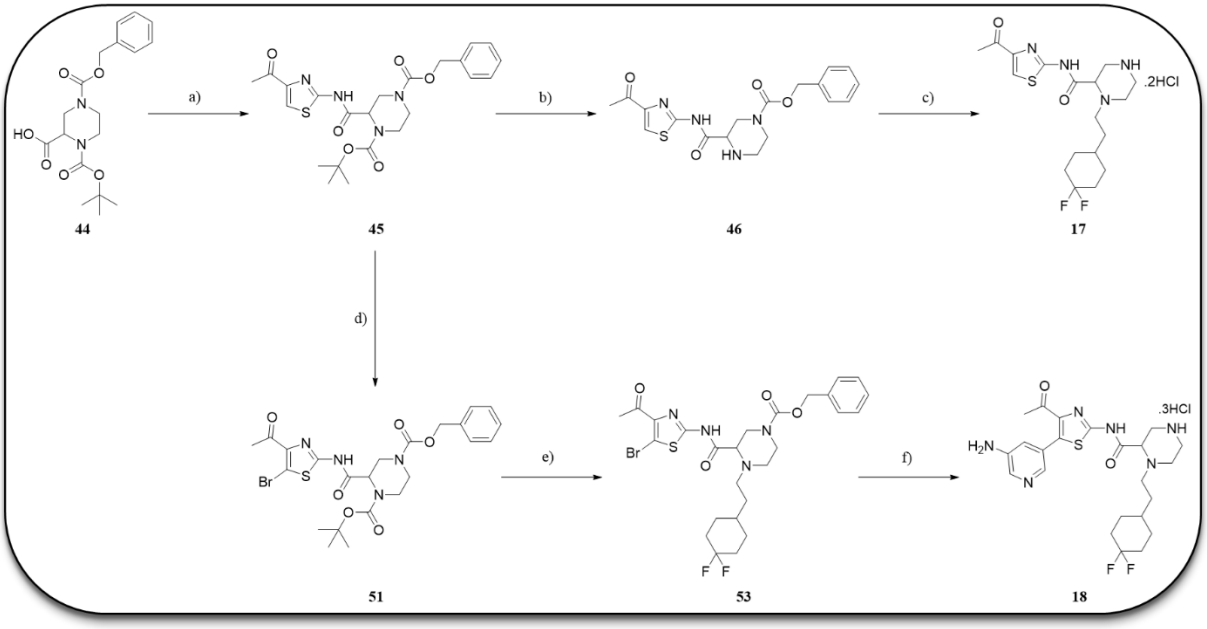
To a stirred slurry of amine **8** (228 mg, 0.55 mmol) in acetonitrile (2 mL), DIPEA (0.29 µL, 1.65 mmol) and compound **41** (171 mg, 0.55 mmol) were added. The reaction mixture was stirred at 25 °C for 24 h and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc/Hexane/MeOH = 3:7:1% to 10:0:5%) to afford the desired product as a white solid (194 mg, 64 %). Mp: decomposition; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.80 (br. s., 1 H), 11.42 (s, 1 H), 8.66 (d, *J* = 6.8 Hz, 1 H), 8.12 (dd, *J* = 8.5, 2.1 Hz, 2 H), 7.24 (t, *J* = 2.1 Hz, 1 H), 4.91 (quin, *J* = 7.0 Hz, 1 H), 3.79 (br. s., 2 H), 2.60 (s, 3 H), 1.58 - 1.56 (m, 12 H), 1.53 (s, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 194.3, 169.5, 162.3, 156.5, 154.6, 152.8, 142.9, 141.8, 140.3, 137.4, 135.2, 127.2, 122.9, 84.3, 80.2, 49.5, 29.3, 28.2 (3 C), 28.0 (3 C), 15.2; IR (neat): ũ = 1644, 1609, 1556, 1412, 1298, 1149, 1121, 752 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{24}H_{34}N_7O_6S^+$: 548.2291 found: 548.2288.

***N*-(4-Acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)-2-guanidinopropanamide hydrochloride (13):**



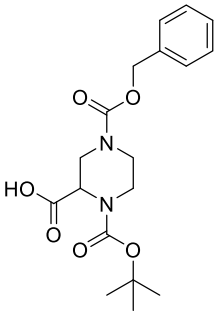
Guanidine **13** was obtained following the general procedure B for Boc deprotection (trituration: Acetone). Yellow solid; Yield: 71 %; Mp: 199-200 °C; ^{1}H NMR (400 MHz, D_2O): δ = 8.14 (s, 1 H), 8.09 (d, *J* = 2.6 Hz, 1 H), 7.76 (s, 1 H), 4.58 (q, *J* = 6.9 Hz, 1 H), 2.58 (s, 3 H), 1.61 (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 193.8, 170.9, 156.8, 156.1, 147.0, 143.3, 130.3, 129.9, 129.5, 127.1, 126.3, 50.1, 29.1, 18.2; IR (neat): ũ = 3310, 3141, 1667, 1633, 1543, 1456, 1357, 1268, 1167 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{18}N_7O_2S^+$: 348.1243 found: 348.1236.

C. Synthesis of compounds 17 and 18:



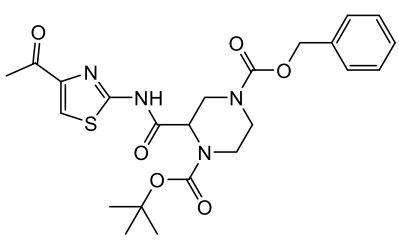
Scheme 3: Synthetic route for compounds **17** and **18**. Reagents and conditions: (a) **44**, HATU, DIPEA, DMF, 25 °C, 16 h, 66 %; (b) TFA, DCM, 25 °C, 2.5 d, 74 %; (c) (i) 2-(4,4-difluorocyclohexyl)ethyl trifluoromethanesulfonate, DIPEA, DCM, 25 °C, 17 h; (ii) HBr, acetic acid, 25 °C, 15 min, 0.3 %. (d) NBS, AcOH, 70 °C, 2 h, 33 %; (e) (i) TFA, DCM, 25 °C, 26 h, 97 %; (ii) 2-(4,4-difluorocyclohexyl)ethyl trifluoromethanesulfonate, DIPEA, DCM, 25 °C, 17 h, 35 %; (f) (i) **28**, K_2CO_3, Pd(dppf)Cl_2, dioxane/H_2O, 90 °C, 15-18 h; (ii) HBr, AcOH, 25 °C, 15 min, 4 % over two steps.

4-((Benzyloxy)carbonyl)-1-(*tert*-butoxycarbonyl)piperazine-2-carboxylic acid (44):



Acid **44** was prepared according to the previously reported procedure.[i] Yellow viscous oil; Yield: 86 % over three steps; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39 - 7.28 (m, 5 H), 5.23 - 5.05 (m, 2 H), 4.88 - 4.59 (m, 2 H), 4.07 (br. s., 1 H), 3.97 - 3.77 (m, 1 H), 3.32 - 3.11 (m, 2 H), 2.94 (br. s, 1 H), 1.58 - 1.32 (m, 9 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.3, 155.8, 155.3, 155.0, 136.2, 128.5 (2 C), 128.1 (2 C), 127.9, 81.3 (rot.), 81.2 (rot.), 67.6, 54.5 (rot.), 53.3 (rot.), 44.4 (rot.), 44.1 (rot.), 43.0, 41.3 (rot.) , 40.1 (rot.), 28.2 (3 C). Two peaks out of three at 155 ppm are rotamers and belong to the same carbon; IR (neat): ũ = 2979, 1697, 1365, 1224, 1163, 1109, 750 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{23}N_2O_6^-$: 363.1556 found: 363.1564.

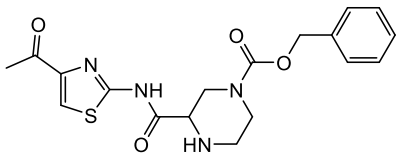
4-Benzyl 1-(*tert*-butyl) 2-((4-acetylthiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (45):



To a stirred solution of amine **19** (2 g, 14 mmol) and acid **44** (5.1 g, 14 mmol) in dimethylformamide (31 mL), HATU (5.7 g, 15.4 mmol) and DIPEA (14.7 mL, 84.14 mmol) were added. The reaction mixture was stirred at 25 °C for 16 h and it was diluted with ethyl acetate, washed two times with $NaHCO_3$ saturated aqueous solution, two times with HCl (1 M) and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc/Hexane = 4:6 to 1:1) to afford the desired product as a white solid (4.56 g, 66 %). Mp: 111-115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.97 (br. s., 1 H), 7.81 (s, 1 H),

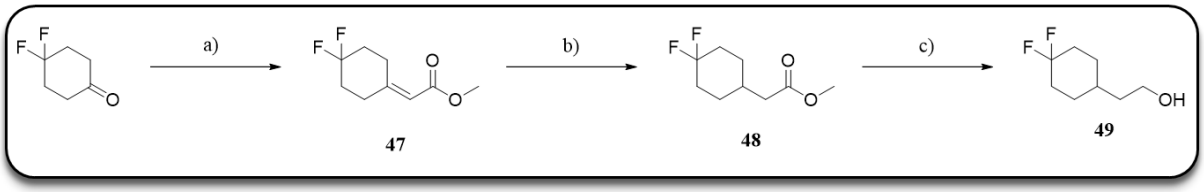
7.44 - 7.29 (m, 5 H), 5.22 - 5.11 (m, 2 H), 4.96 (br. s., 1 H), 4.73 (d, *J* = 13.6 Hz, 1 H), 4.01 (br. s., 2 H), 3.38 - 3.21 (m, 2 H), 3.10 (br. s., 1 H), 2.60 (s, 3 H), 1.53 (s, 9 H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 192.3, 168.4, 157.2, 155.5, 149.3, 136.0, 128.4 (2 C), 128.0, 127.7 (2 C), 121.0, 81.9, 67.7, 54.2, 43.3, 41.4, 28.2 (3C), 27.2. Two carbons are missing due to overlapping; IR (neat): ũ = 3167, 3083, 2984, 2364, 1685, 1656, 1558, 1251, 1221 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{23}H_{29}N_4O_6S^+$: 489.1808 found: 489.1806.

Benzyl 3-((4-acetylthiazol-2-yl)carbamoyl)piperazine-1-carboxylate (46):



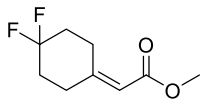
To as stirred solution of Boc piperazine **45** (4.9 g, 10 mmol) in dichloromethane (33 mL), TFA (4.63 mL, 60 mmol) was added. The reaction mixture was stirred at 25 °C for 2.5 days and concentrated under reduced pressure. The obtained residue was dissolved in ethyl acetate, washed two times with $NaHCO_3$ saturated aqueous solution and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc/Hexane/MeOH/Et_3N = 2:8:1%:1% to 10:0:3%:1%) to afford the desired product as a colorless viscous oil (2.89 g, 74 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 7.81 (s, 1 H), 7.40 - 7.31 (m, 5 H), 5.21 - 5.12 (m, 2 H), 4.07 - 3.91 (m, 1 H), 3.80 - 3.51 (m, 3 H), 3.37 - 3.29 (m, 1 H), 3.05 - 2.89 (m, 2 H), 2.59 (s, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 191.9, 169.2, 155.0, 149.1, 136.3, 128.5 (2 C), 128.1, 128.0 (2 C), 121.6, 111.9, 67.6, 56.9, 44.9, 43.2, 29.7, 27.1; IR (neat): ũ = 3260, 3203, 2922, 2359, 1681, 1537, 1426, 1243, 750 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{21}N_4O_4S^+$: 389.1284 found: 389.1277.

D. Synthesis of alcohol 49:



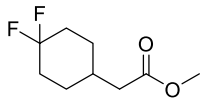
Scheme 4: Synthetic route for alcohol **49**. Reagents and conditions: (a) methyl (triphenylphosphoranylidene)acetate, THF, 65 °, 20 h, quantitative; (b) H2, Pd/C, methanol, 25 °C, 3 h, quantitative; (c) $LiBH_4$, THF, 0-80 °C, 3 h, 77 %.

Methyl 2-(4,4-difluorocyclohexylidene)acetate (47):



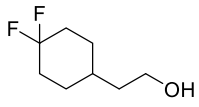
To as stirred solution of 4,4-difluorocyclohexan-1-one (2 g, 14.9 mmol) in dry tetrahydrofuran (40 mL) under a nitrogen atmosphere, methyl (triphenylphosphoranylidene)acetate (7.5 g, 22.4 mmol) was added. The reaction mixture was stirred at 65 °C for 20 h and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc/Hexane = 1:10) to afford the desired product as a colorless liquid (2.92 g, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* = 5.74 (s, 1 H), 3.71 (s, 3 H), 3.04 (t, *J* = 6.8 Hz, 2 H), 2.41 (t, *J* = 6.6 Hz, 2 H), 2.11 - 1.96 (m, 4 H); ^{13}C NMR (101 MHz, $CDCl_3$): *δ* = 166.5, 156.9, 122.4 (t, *J* = 242.0 Hz), 115.5, 51.1, 34.5 (t, *J* = 23.8 Hz), 33.8 (t, *J* = 23.8 Hz), 33.1 (t, *J* = 5.4 Hz), 24.6 (t, *J* = 6.0 Hz); IR (neat): ũ = 1716, 1656, 1435, 1357, 1208, 1169, 1097, 939, 867, 692 cm^{-1}; HRMS (EI): m/z: calcd for $C_9H_{12}F_2O_2^+$: 190.0805 found: 190.0801.

Methyl 2-(4,4-difluorocyclohexyl)acetate (48):



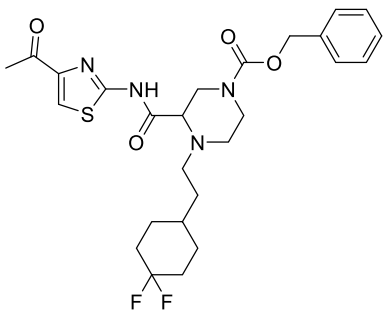
To as stirred solution of alkene **47** (3.6 g, 19 mmol) in methanol (38 mL), Pd/C (202 mg, 0.19 mmol, 10 % wt) was added. The reaction mixture was stirred at 25 °C under a hydrogen balloon for 3 h. It was filtered through a pad of celite, washed with methanol and concentrated under reduced pressure to afford the desired product as a colorless liquid (3.65 g, quantitative yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.69 (s, 3 H), 2.27 (d, *J* = 6.8 Hz, 2 H), 2.14 - 2.02 (m, 2 H), 1.94 - 1.86 (m, 1 H), 1.86 - 1.78 (m, 3 H), 1.78 - 1.66 (m, 1 H), 1.41 - 1.28 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.9, 123.2 (t, *J* = 239.6 Hz), 51.5, 40.1 (d, *J* = 2.4 Hz), 33.5 - 33.0 (m, 2 C), 32.7, 28.7 (d, *J* = 9.5 Hz, 2 C); IR (neat): ũ = 1734, 1684, 1436, 1358, 1242, 1171, 1113, 966, 753 cm^{-1}; HRMS (EI): m/z: calcd for $C_9H_{14}F_2NaO_2^+$: 215.0860 found: 215.0856.

2-(4,4-difluorocyclohexyl)ethan-1-ol (49):



To as stirred solution of ester **48** (3.3 g, 17.1 mmol) in dry tetrahydrofuran (64 mL) at 0 °C, under a nitrogen atmosphere, $LiBH_4$ (16.1 mL, 32.2 mmol, 2 M in THF) was added. The reaction mixture was stirred at 80 °C for 3 h, cooled down to 25 °C and quenched by the slow addition of water. The aqueous layer was extracted three times with ethyl acetate, washed once with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/Hexane = 2:8) to afford the desired product as a colorless liquid (2.38 g, 77 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.72 (t, *J* = 6.4 Hz, 2 H), 2.16 - 2.02 (m, 2 H), 1.85 - 1.62 (m, 4 H), 1.57 - 1.53 (m, 2 H), 1.38 - 1.25 (m, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 123.6 (t, *J* = 242.0 Hz), 60.6, 38.5 (d, *J* = 2.4 Hz), 33.7 - 33.2 (m, 2 C), 32.3, 28.9 (d, *J* = 9.5 Hz, 2 C); IR (neat): ũ = 3356, 3336, 1448, 1379, 1359, 1115, 1052, 1013, 956, 755 cm^{-1}; HRMS (EI): m/z: calcd for (M-H_2O) $C_8H_{12}F_2^-$: 146.0907 found: 146.0901.

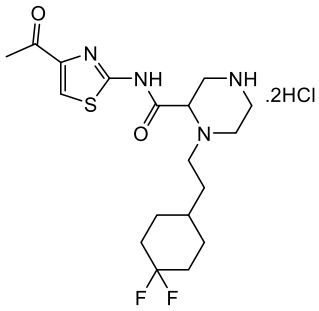
Benzyl 3-((4-acetylthiazol-2-yl)carbamoyl)-4-(2-(4,4-difluorocyclohexyl)ethyl)piperazine-1-carboxylate (50):



To a stirred solution of alcohol **49** (500 mg, 3.05 mmol) in dry dichloromethane (10 mL), under a nitrogen atmosphere, dry Et_3N (0.56 mL, 4 mmol) and Tf_2O (0.67 mL, 4 mmol) were added. The reaction mixture was stirred at 25 °C for 1 h and quenched by the slow addition of water. The two phases were separated and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford the corresponding triflate derivative which was engaged in the next step without further purification.

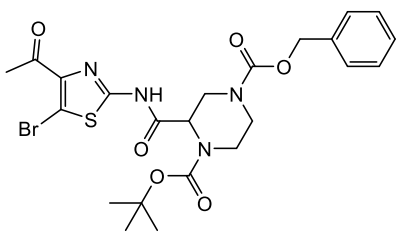
To a stirred solution of piperazine **46** (200 mg, 0.51 mmol) in dry dichloromethane (1.2 mL), under a nitrogen atmosphere, dry DIPEA (0.11 mL, 0.61 mmol) was added, followed by a solution of the triflate (231.3 mg, 0.61 mmol) in dry dichloromethane (0.5 mL). The reaction mixture was stirred at 25 °C for 17 h and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/Hexane = 3:7) to afford the impure desired product which was engaged in the next step without further purification.

_N_-(4-Acetylthiazol-2-yl)-1-(2-(4,4-difluorocyclohexyl)ethyl)piperazine-2-carboxamide hydrochloride (17):



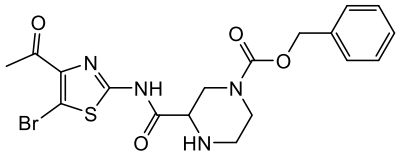
Benzyl piperazine **50** (79 mg, 0.15 mmol) was dissolved in a dry solution of HBr in acetic acid (33 %, 0.75 mL). The reaction mixture was stirred at 25 °C for 15 min and quenched by the addition of diethyl ether. The resulting precipitate was filtered, washed with diethyl ether, poured into K_2CO_3 saturated aqueous solution and extracted three times with chloroform. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was dissolved in methanol, HCl (32 %) was added and it was concentrated under reduced pressure. The obtained residue was triturated with acetonitrile, filtered and dried under vacuum to afford 28 mg of impure product. 1 mg of the latter was further purified by reverse phase preparative HPLC using an *AEKTA Purifier* system (C_{18} *Agilent* Zorbax Exlipse XDB, 250 x 21.2 mm, pore diameter 80 Å, particle size 7 µm) using a gradient of 15-90 % MeCN (+0.1% TFA) in H_2O (+0.1 % TFA) over 5 column volumes at a flow rate of 10 mL/min. The fractions were lyophilized and then dissolved three times in MeOH/DCM = 1:1 containing 0.025 M HCl and evaporated to obtain the desired product as a white solid (0.2 mg, 0.3 % yield). Mp: 198-200 °C; ^{1}H NMR (400 MHz, D_2O): δ = 8.28 (s, 1 H), 4.09 (dd, *J* = 9.4, 3.4 Hz, 1 H), 3.76 (dd, *J* = 13.7, 3.2 Hz, 1 H), 3.66 - 3.55 (m, 2 H), 3.50 (dd, *J* = 13.6, 9.4 Hz, 1 H), 3.44 - 3.35 (m, 1 H), 3.11 - 2.94 (m, 2 H), 2.90 - 2.81 (m, 1 H), 2.62 (s, 3 H), 2.00 (br. s., 2 H), 1.84 - 1.67 (m, 4 H), 1.66 - 1.56 (m, 2 H), 1.51 - 1.41 (m, 1 H), 1.30 - 1.13 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 191.7, 157.0, 148.9, 124.2 (t, *J* = 239.9 Hz), 122.3, 59.9, 52.2, 45.6, 42.7, 41.1, 32.9 - 32.3 (m, 2 C), 32.3, 28.6 (d, *J* = 9.0 Hz), 28.0 (d, *J* = 10.0 Hz), 27.4. Two carbons are missing due to overlapping.; IR (neat): ũ = 1684, 1558, 1362, 1202, 1113, 939 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{27}F_2N_4O_2S^+$: 401.1823 found: 401.1814.

4-Benzyl 1-(*tert*-butyl) 2-((4-acetylthiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (51):



Bromide **51** was obtained following the general procedure for bromination (chromatography: Hexane/EtOAc = 1:1 to 4:6). Brown solid; Yield: 33 %; Mp: 87-89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41 - 7.28 (m, 5 H), 5.20 - 5.07 (m, 2 H), 5.06 - 4.55 (m, 2 H), 4.10 - 3.85 (m, 2 H), 3.48 - 3.25 (m, 2 H), 3.11 (br. s., 1 H), 2.63 - 2.45 (m, 3 H), 1.50 (br. s., 9 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.5, 168.5, 155.7, 154.9, 143.7, 135.9, 128.5 (2 C), 128.3, 127.9 (2 C), 110.2, 82.2, 67.8, 55.9, 54.0, 43.3, 41.5, 28.8, 28.3 (3 C). One carbon is missing due to overlapping; IR (neat): ũ = 1687, 1551, 1365, 1225, 1162, 1107, 970, 696 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{23}H_{27}BrN_4NaO_6S^+$: 589.0732 found: 589.0726.

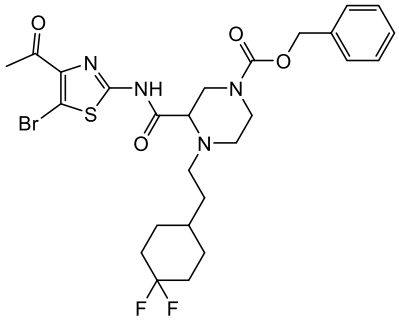
Benzyl 3-((4-acetyl-5-bromothiazol-2-yl)carbamoyl)piperazine-1-carboxylate (52):



To as stirred solution of 4-benzyl 1-(*tert*-butyl) 2-((4-acetylthiazol-2-yl)carbamoyl)piperazine-1,4-dicarboxylate (**51**, 1.5 g, 2.6 mmol) in dichloromethane (8.7 mL), TFA (0.61 mL, 7.9 mmol) was added. The reaction mixture was stirred at 25 °C for 26 h and concentrated under reduced pressure. The obtained residue was dissolved in ethyl acetate, washed two times with $NaHCO_3$ saturated aqueous solution, once

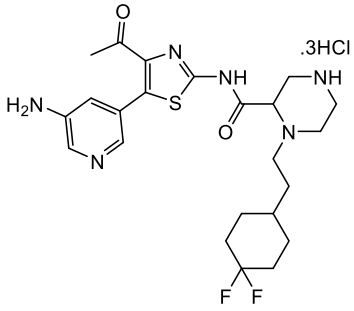
with water and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford the desired product as a yellow solid (1.2 g, 97 % yield). Mp: 75-78 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41 - 7.30 (m, 5 H), 5.23 - 5.13 (m, 2 H), 4.19 (br. s., 1 H), 3.91 (br. s., 2 H), 3.66 (br. s., 1 H), 3.47 (br. s., 1 H), 3.15 (br. s., 2 H), 2.63 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.3, 169.3, 155.0, 144.0, 136.2, 128.6, 128.2, 128.1, 67.7, 56.9, 44.8, 43.2, 29.2. Three carbons are missing due to overlapping; IR (neat): ũ = 3260, 1684, 1532, 1426, 1241, 1174, 1121, 1011, 749, 696 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{20}BrN_4O_4S^+$: 467.0389 found: 467.0376.

Benzyl 3-((4-acetyl-5-bromothiazol-2-yl)carbamoyl)-4-(2-(4,4-difluorocyclohexyl)ethyl)piperazine-1-carboxylate (53):



To a stirred solution of Benzyl 3-((4-acetyl-5-bromothiazol-2-yl)carbamoyl)piperazine-1-carboxylate (**52**, 350 mg, 0.75 mmol) in dry dichloromethane (2 mL), under a nitrogen atmosphere, dry DIPEA (0.2 mL, 1.12 mmol) was added, followed by a solution of 2-(4,4-difluorocyclohexyl)ethyl trifluoromethanesulfonate (332 mg, 1.12 mmol) in dry dichloromethane (0.5 mL). The reaction mixture was stirred at 25 °C for 17 h and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/Hexane = 1:1) to afford the desired product as a white solid (160 mg, 35 % yield). Mp: 71-73 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.02 (br. s., 1 H), 7.36 (s, 5 H), 5.19 - 5.10 (m, 2 H), 4.09 - 3.98 (m, 1 H), 3.87 (d, J = 12.4 Hz, 1 H), 3.57 - 3.44 (m, 1 H), 3.32 - 3.19 (m, 2 H), 3.14 - 3.02 (m, 1 H), 2.62 (s, 3 H), 2.55 - 2.44 (m, 2 H), 2.15 - 1.98 (m, 2 H), 1.81 - 1.61 (m, 4 H), 1.60 - 1.50 (m, 3 H), 1.46 - 1.36 (m, 1 H), 1.31 - 1.23 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.4, 170.1, 155.1, 154.9, 144.0, 136.2, 128.5 (2 C), 128.2, 128.0 (2 C), 123.2 (t, J = 238.4 Hz), 110.4, 67.6, 64.4, 54.1, 48.5, 43.6, 42.2, 33.8, 33.7 - 32.7 (m, 2 C), 29.3 (d, J = 9.5 Hz), 28.9, 28.7 (d, J = 9.5 Hz). One carbon is missing due to overlapping; IR (neat): ũ = 1685, 1531, 1431, 1356, 1230, 1113, 1010, 959, 754, 696 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{26}H_{31}BrF_2N_4O_4S^+$: 613.1296 found: 613.1282.

***N*-(4-Acetyl-5-(5-aminopyridin-3-yl)thiazol-2-yl)-1-(2-(4,4-difluorocyclohexyl)ethyl)piperazine-2-carboxamide hydrochloride (18):**



Boc amino pyridine was obtained following the general procedure for Suzuki cross-coupling reactions (chromatography: alumina neutral, Hexane/EtOAc/MeOH/Et_3N = 7:3:1%:1%). The impure desired product was engaged in the next step without further purification.

The crude mixture was dissolved in a dry solution of HBr in acetic acid (33 %, 0.2 M). The reaction mixture was stirred at 25 °C for 15 min and quenched by the addition of diethyl ether. The resulting precipitate was filtered, washed with diethyl ether, poured into K_2CO_3 saturated aqueous solution and extracted three times with chloroform. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was dissolved in methanol, HCl (32 %) was added and it was concentrated under reduced pressure. The obtained residue was triturated with acetone, filtered and dried under vacuum to afford 58 mg of impure product. 10 mg of the latter were further purified by

dissolving it in a MeOH/DCM = 1:1 mixture containing 0.025M HCl. The product was precipitated by the addition of Et_2O and the mother liquor was pipetted off three times to obtain a light-brown solid (4 mg, 4 % yield over two steps). Mp: 228-229 °C; ^{1}H NMR (400 MHz, D_2O): δ = 8.19 (s, 1 H), 8.12 (d, *J* = 2.3 Hz, 1 H), 7.86 (s, 1 H), 4.10 (dd, *J* = 9.2, 3.6 Hz, 1 H), 3.76 (dd, *J* = 13.7, 3.2 Hz, 1 H), 3.61 - 3.46 (m, 3 H), 3.41 - 3.33 (m, 1 H), 3.09 - 2.91 (m, 2 H), 2.88 - 2.78 (m, 1 H), 2.61 (s, 3 H), 1.97 (br. s., 2 H), 1.80 - 1.69 (m, 4 H), 1.64 - 1.56 (m, 2 H), 1.52 - 1.42 (m, 1 H), 1.29 - 1.20 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 194.2, 156.0, 147.8, 143.9, 130.7, 130.6, 129.0, 128.4, 125.4, 124.8 (t, *J* = 239.9 Hz), 60.8, 52.5, 46.0, 43.4, 42.0, 33.5 - 32.9 (m, 2 C), 32.7, 31.3, 29.5, 29.2 (d, *J* = 10.0 Hz), 28.5 (d, *J* = 9.0 Hz). One carbon is missing due to overlapping; IR (neat): ũ = 1735, 1684, 1558, 1363, 1229, 1217, 959 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{23}H_{31}F_2N_6O_2S^+$: 493.2197 found: 493.2193.

2. Crystallographic Data

Table S1. Summary of crystallographic data collection, model building and refinement.

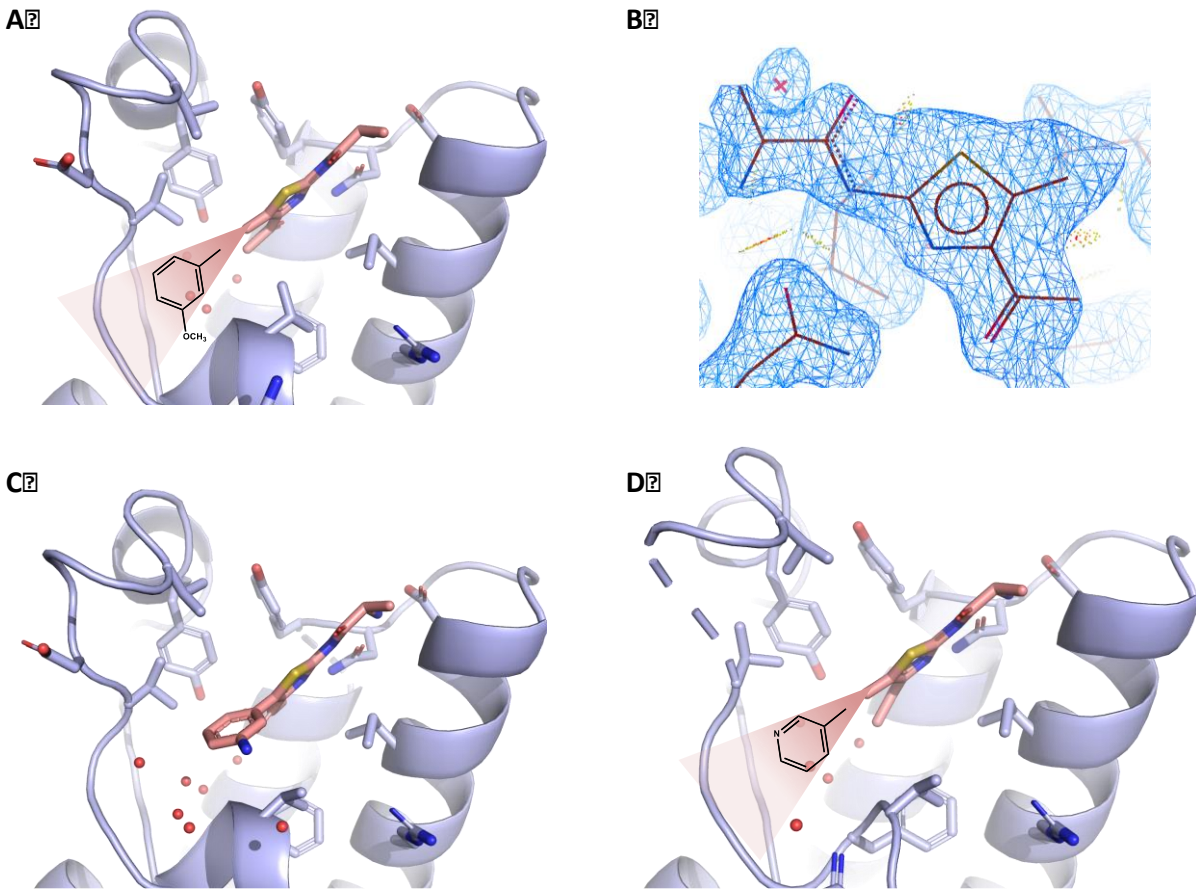
	1	**2**	**3**	**4**	**5**
PDB code	5F36	6EPU	6EPX	6HI3	6EPV
Data Collection					
SLS Beamline	PX	PX	PX	PX	PX
Space group	P6522	P6522	P6522	P6522	P6522
Cell dimensions a, b, c (Å)	79.80 79.80 137.200	79.765 79.765 136.533	79.575 79.575 138.187	78.839 78.839 134.831	79.482 79.482 138.006
Cell dimensions alpha beta gamma	90.0 90.0 120.0	90.0 90.0 120.0	90.0 90.0 120.0	90.0 90.0 120.0	90.0 90.0 120.0
Resolution (Å)	69.11 - 1.50 (1.58 - 1.50)	48.56 - 1.80 (1.91 - 1.80)	48.79 - 1.84 (1.95 - 1.84)	47.97 - 2.40 (2.54 - 2.40)	48.73 - 1.79 (1.90 - 1.79)
Unique observations	42035 (5906)	44912 (7110)	42520 (6773)	18376 (2956)	45731 (7320)
Completeness	99.7 (98.2)	99.6 (97.9)	99.7 (98.3)	99.9 (99.9)	99.8 (99.1)
Redundancy	18.9 (17.7)	5.17 (4.84)	4.33 (2.64)	6.64 (7.05)	5.85 (5.71)
Rmerge	0.043 (0.818)	0.073 (0.925)	0.035 (0.651)	0.187 (1.024)	0.049 (0.961)
CC (1/2)		0.998 (0.687)	1.00 (0.697)	0.993 (0.839)	0.999 (0.961)
I/sigmaI		12.83 (1.79)	23.05 (1.64)	12.43 (1.66)	19.4 (1.76)
Refinement					
Rwork/Rfree	0.168 / 0.190	0.192 / 0.218	0.205 / 0.219	0.292 / 0.337	0.210 / 0.217
RMS deviations bonds (Å)	0.009	0.006	0.008	0.009	0.007
RMS deviations angles	1.11	0.87	1.59	1.17	0.927
Ramachandran Favored	100	100	98.44	96.85	100
Ramachandran Disallowed	0	0	0	0	0

Compound	**6**	**7**	**8**	**9**
PDB code	6EPJ	6HI4	6HI5	6HI6
Data Collection				
SLS Beamline	PX	PX	PX	PX
Space group	P6522	P6522	P6522	P6522
Cell dimensions a, b, c (Å)	80.191 80.191 137.444	79.669 79.669 137.884	79.322 79.322 136.484	79.34 79.34 136.212
Cell dimensions alpha beta gamma	90.0 90.0 120.0	90.0 90.0 120.0	90.0 90.0 120.0	90.0 90.0 120.0
Resolution (Å)	40.10 - 1.65 (1.75 - 1.65)	48.77 - 1.69 (1.79 - 1.69)	39.66 - 1.59 (1.69 - 1.59)	48.37 - 1.64 (1.74 - 1.64)
Unique observations	59272 (9476)	54799 (8790)	64390 (10212)	58570 (9239)
Completeness	99.7 (98.6)	99.8 (99.2)	99.6 (97.5)	99.5 (97.2)
Redundancy	10.4 (9.54)	18.27 (1.39)	17.06 (9.30)	10.34 (9.45)
Rmerge	0.056 (0.828)	0.058 (1.177)	0.081 (1.735)	0.071 (2.025)
CC (1/2)	1.00 (0.828)	0.999 (0.595)	0.999 (0.565)	1.000 (0.471)
I/sigmaI	24.89 (2.88)	18.27 (1.39)	17.06 (1.27)	19.33 (1.24)
Refinement				
Rwork/Rfree	0.177 / 0.212	0.202 / 0.221	0.201 / 0.218	0.211 /0.235
RMS deviations bonds (Å)	0.007	0.008	0.009	0.008
RMS deviations angles	1.17	1.03	1.37	1.11
Ramachandran Favored	100	98.44	100	100
Ramachandran Disallowed	0	0	0	0

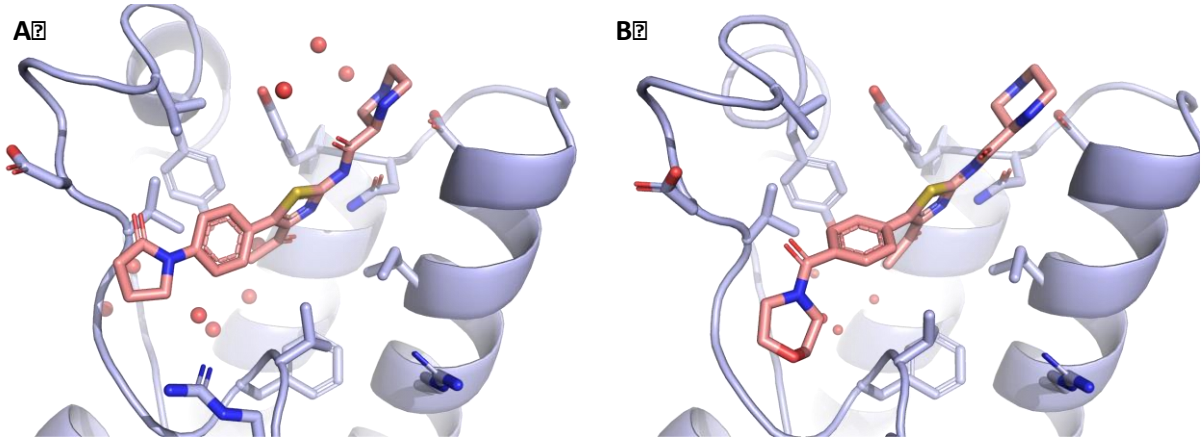
Compound	**10**	**11**	**12**	**13**
PDB code	6HI7	6HI8	6EPT	6HIA
Data Collection				
SLS Beamline	PX	PXIII	PX	PXIII
Space group	P6522	P6522	P6522	P6522
Cell dimensions a, b, c (Å)	80.516 80.516 137.85	79.404 79.404 137.325	79.74 79.74 139.211	79.139 79.139 138.031
Cell dimensions alpha beta gamma	90.0 90.0 120.0	90.0 90.0 120.0	90 90 120	90 90 120
Resolution (Å)	49.02 - 1.74 (1.85 - 1.74)	39.71 - 1.90 (2.01 - 1.90)	49.02-1.65 (1.75-1.65)	39.57 - 1.90 (2.01 - 1.90)
Unique observations	50938 (8125)	38218 (6119)	59426 (9409)	38116 (6072)
Completeness	99.7 (98.5)	99.8 (99.1)	99.6 (97.7)	99.4 (98.1)
Redundancy	10.41 (10.31)	5.75 (5.29)	6.07 (3.22)	5.75 (5.59)
Rmerge	0.059 (1.78)	0.069 (1.065)	0.044 (0.964)	0.060 (0.857)
CC (1/2)	1.000 (0.606)	0.999 (0.603)	1.00 (0.645)	1.000 (0.773)
I/sigmaI	21.66 (1.31)	19.33 (1.63)	21.33 (1.17)	21.15 (1.95)
Refinement				
Rwork/Rfree	0.194 / 0.215	0.188 / 0.218	0.202 / 0.226	0.205 / 0.232
RMS deviations bonds (Å)	0.007	0.007	0.007	0.007
RMS deviations angles	1.02	0.96	1.75	1.07
Ramachandran Favored	100	98.44	99.22	98.44
Ramachandran Disallowed	0	0	0	0

Compound	**14**	**15**	**16**	**17**
PDB code	6HIB	6HIC	6HID	6HIE
Data Collection				
SLS Beamline	PXIII	PX	PX	PXIII
Space group	P6522	P6522	P6522	P6522
Cell dimensions a, b, c (Å)	80.02 80.02 138.337	79.852 79.852 138.123	79.831 79.831 138.001	80.23 80.23 137.472
Cell dimensions alpha beta gamma	90 90 120	90 90 120	90 90 120	90 90 120
Resolution (Å)	48.96 - 2.03 (2.15 - 2.03)	39.93 - 1.77 (1.87 - 1.77)	39.92 - 1.78 (1.88 - 1.77)	48.87 - 2.05 (2.17 - 2.05)
Unique observations	32104 (5177)	48152 (7608)	47738 (7333)	31256 (5041)
Completeness	99.8 (99.2)	99.6 (97.6)	99.0 (94.1)	99.9 (99.4)
Redundancy	5.67 (5.04)	6.03 (2.84)	6.09 (2.99)	6.93 (7.05)
Rmerge	0.107 (1.36)	0.071 (1.39)	0.046 (1.923)	0.061 (0.846)
CC (1/2)	0.999 (0.523)	0.999 (0.533)	1.000 (0.221)	0.999 (0.827)
I/sigmaI	13.42 (1.21)	16.02 (0.72)	18.51 (0.61)	18.24 (2.29)
Refinement				
Rwork/Rfree	0.208 / 0.231	0.202 / 0.225	0.207 / 0.243	0.202 / 0.239
RMS deviations bonds (Å)	0.008	0.008	0.008	0.007
RMS deviations angles	1.14	1.3	1.05	0.99
Ramachandran Favored	100	100	100	99.22
Ramachandran Disallowed	0	0	0	0

3. Supporting Figures, table and MD simulation movie



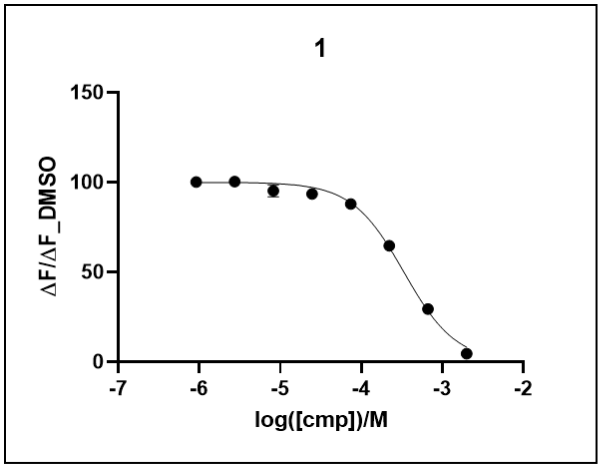
Supporting Figure 1. A. Crystal structure of compound **7** bound to ATAD2 bromodomain, disordered fuzzy binding mode of the aromatic ring is highlighted. B. Electron density of compound **8** bound to ATAD2 bromodomain – the aminopyridine ring is not visible in the density. C. Crystal structure of compound **10** bound to ATAD2 bromodomain. D. Crystal structure of compound **9** bound to ATAD2 bromodomain, disordered fuzzy binding mode of the aromatic ring is highlighted.



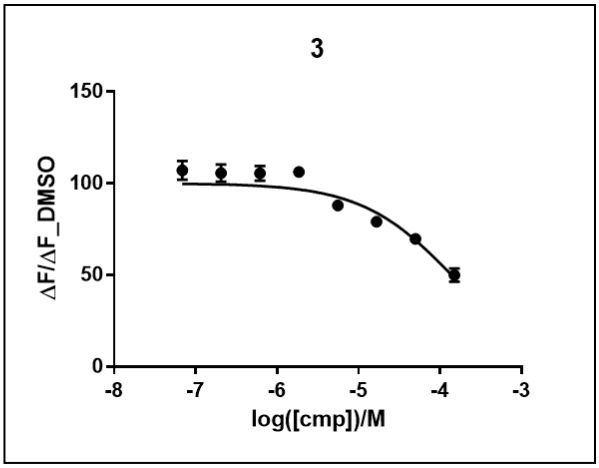
Supporting Figure 2. Crystal structures of compounds A. **15** and B. **16** bound to ATAD2 bromodomain.

	dF/dF_DMSO (1st measurement)	dF/dF_DMSO (2nd measurement)
1	65	68
2	59.4	66.2
3	52.5	47.4
4	72.2	85.9
5	95.5	88.6
6	62.5	65.6
7	108.2	92.3
8	74.4	64.0
9	42.2	37.5
10	60.9	62.4
11	100.4	80.7
12	109.5	116.5
13	86.8	74.1
14	45.1	44.1
15	78.7	78.3
16	97.9	82.8
17	14.2	13.9
18	70.7	69.9

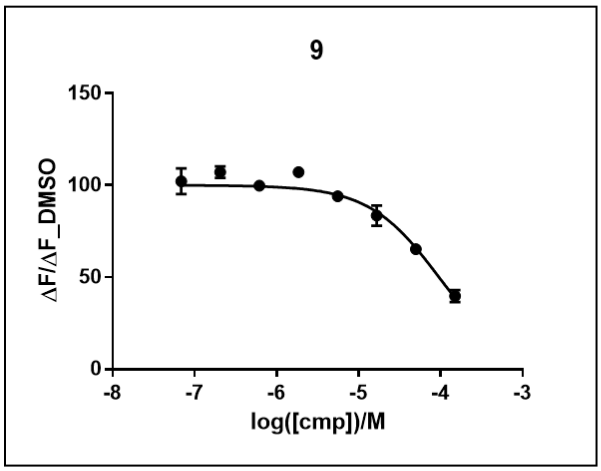
Table S2. HTRF single dose measurements at 150 µM for all ATAD2 ligands.



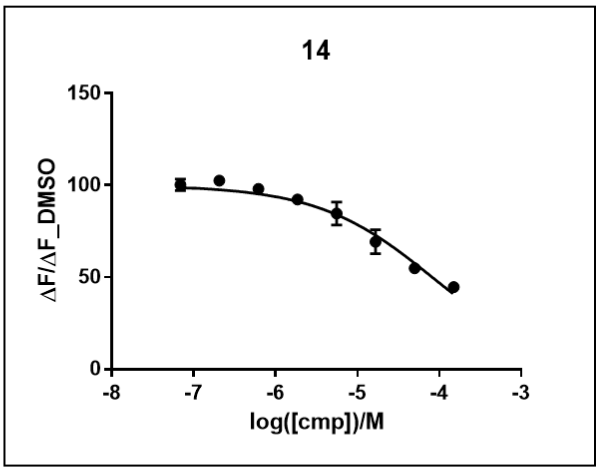
IC_{50} = 336 µM
Hill coeff. = - 1.35



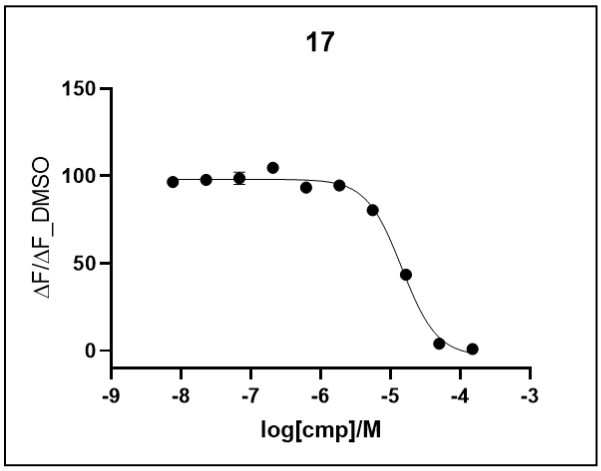
IC_{50} = 140 µM
Hill coeff. = - 0.78



IC_{50} = 95 µM
Hill coeff. = - 1.02

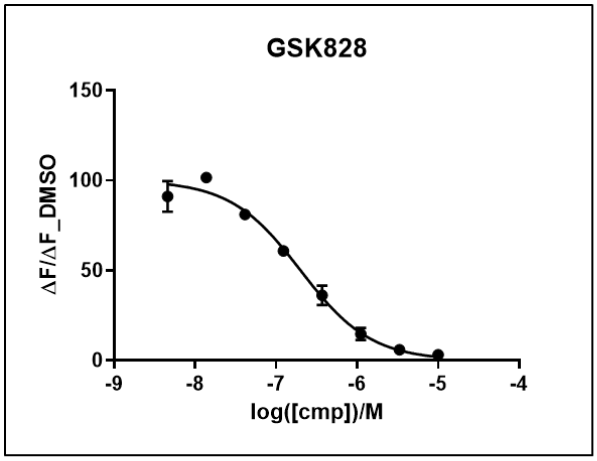


IC_{50} = 80 µM
Hill coeff. = - 0.62

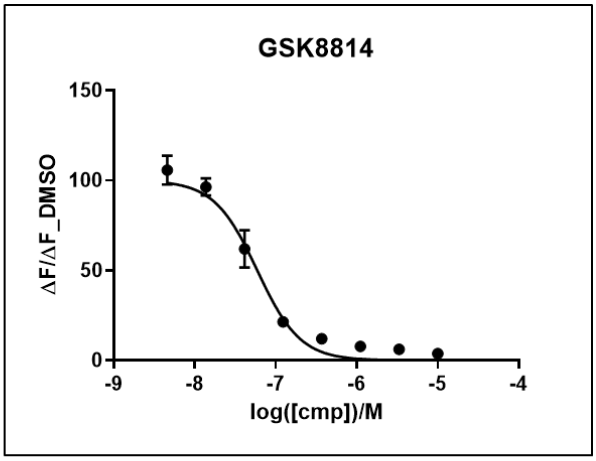


IC_{50} = 15 µM
Hill coeff. = - 1.72

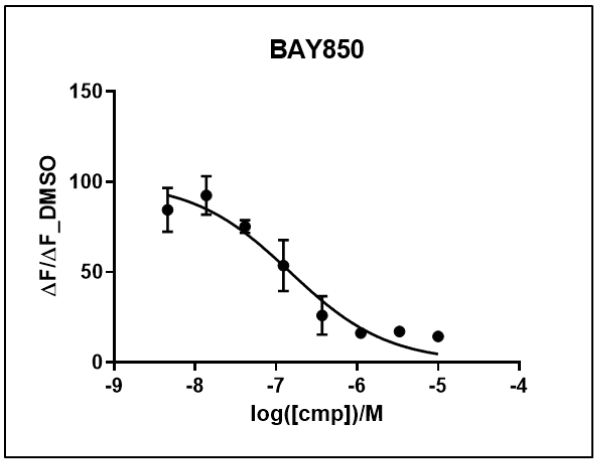
Supporting Figure 3. HTRF measurements for ATAD2 ligands **1**, **3**, **9**, **14** and **17** (IC_{50} values and Hill coefficients are given).



IC_{50} = 0.2 µM
Hill coeff. = - 1.0

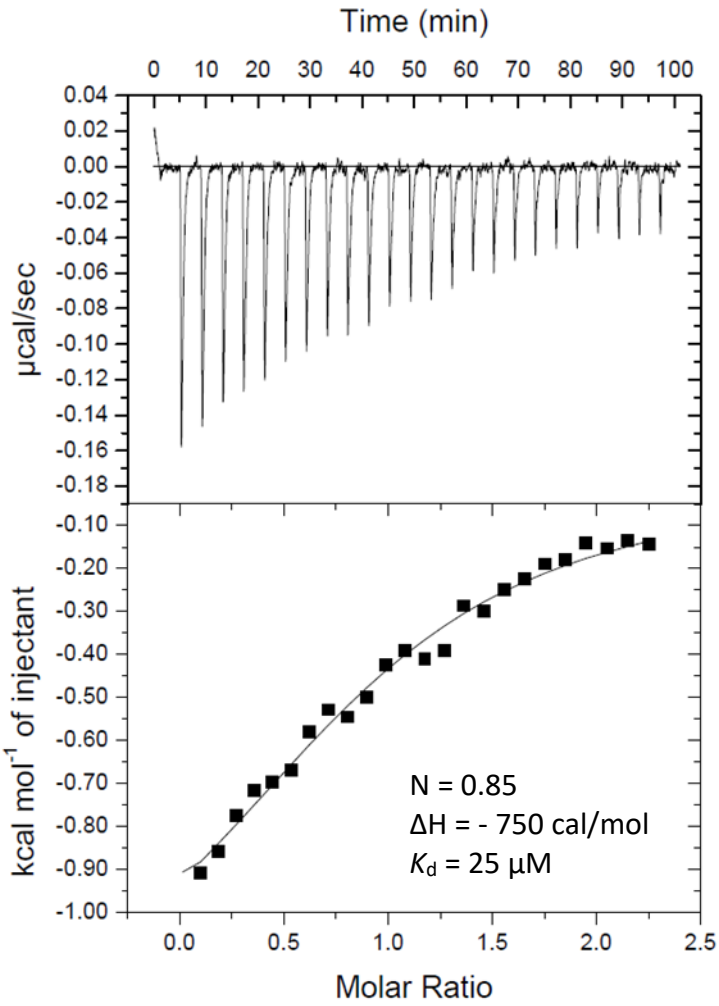


IC_{50} = 0.059 µM
Hill coeff. = - 1.6

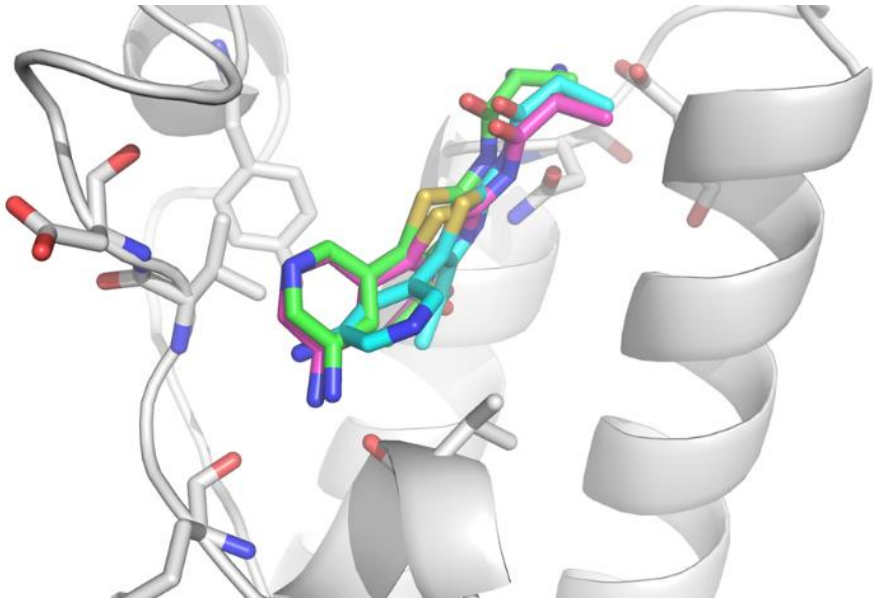


IC_{50} = 0.15 µM
Hill coeff. = - 0.7

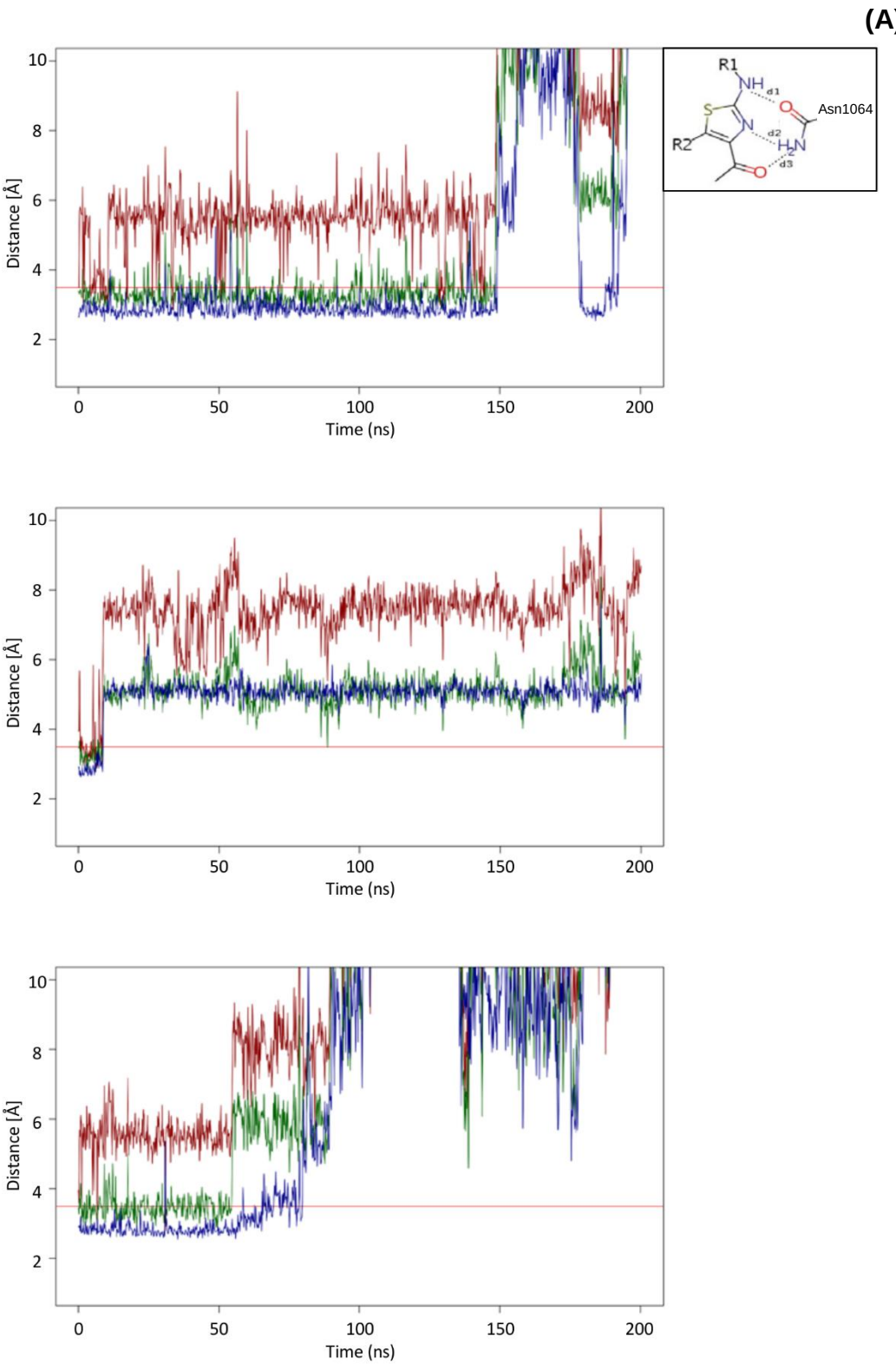
Supporting Figure 4. HTRF measurements for ATAD2 reported ligands **GSK828**, **GSK8814** and **BAY850** (IC_{50} values and Hill coefficients are given).



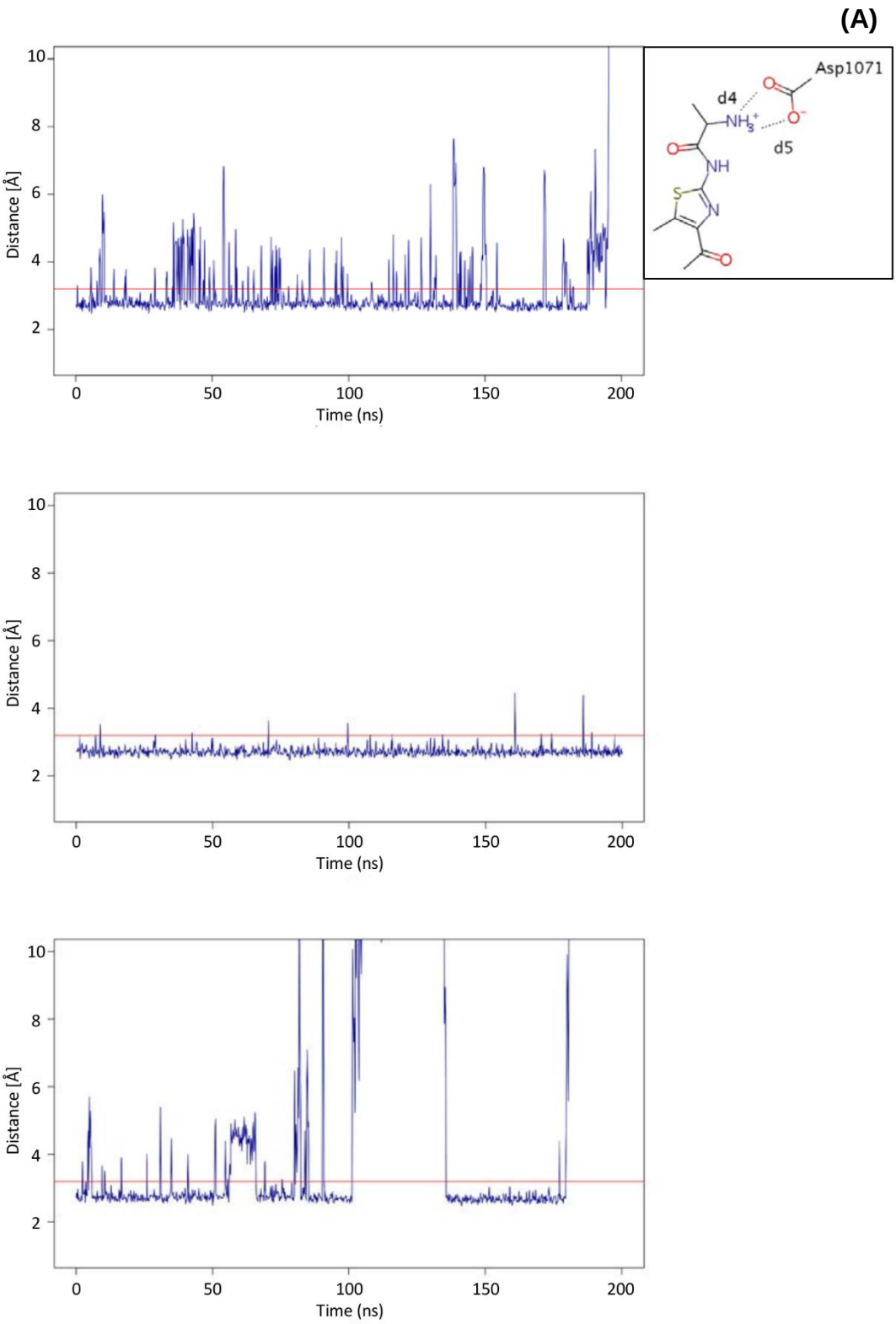
Supporting Figure 5. ITC measurement for ATAD2 ligand **17**.



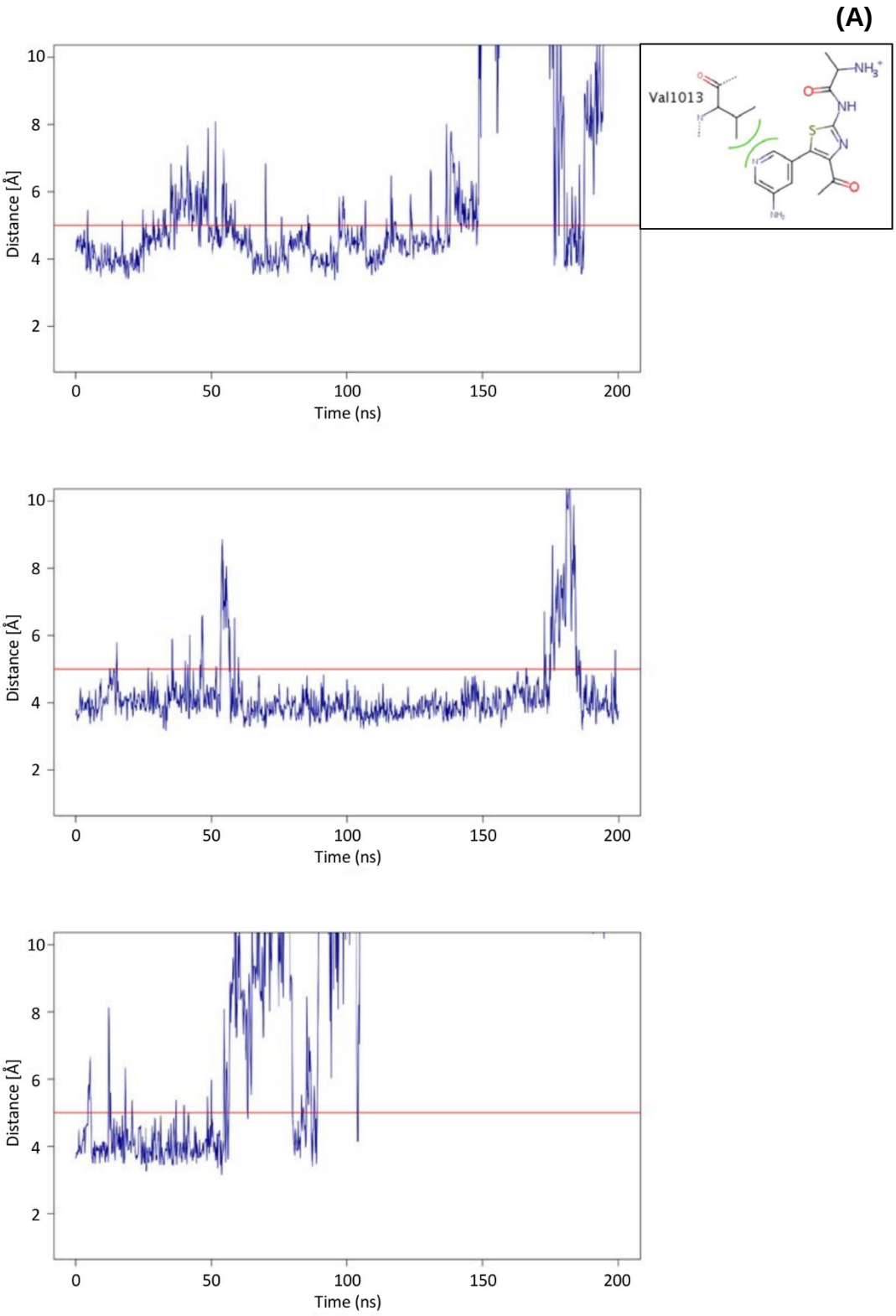
Supporting Figure 6. Initial docked poses involved in the molecular dynamics simulations of the complex of ATAD2 with compound **8**.



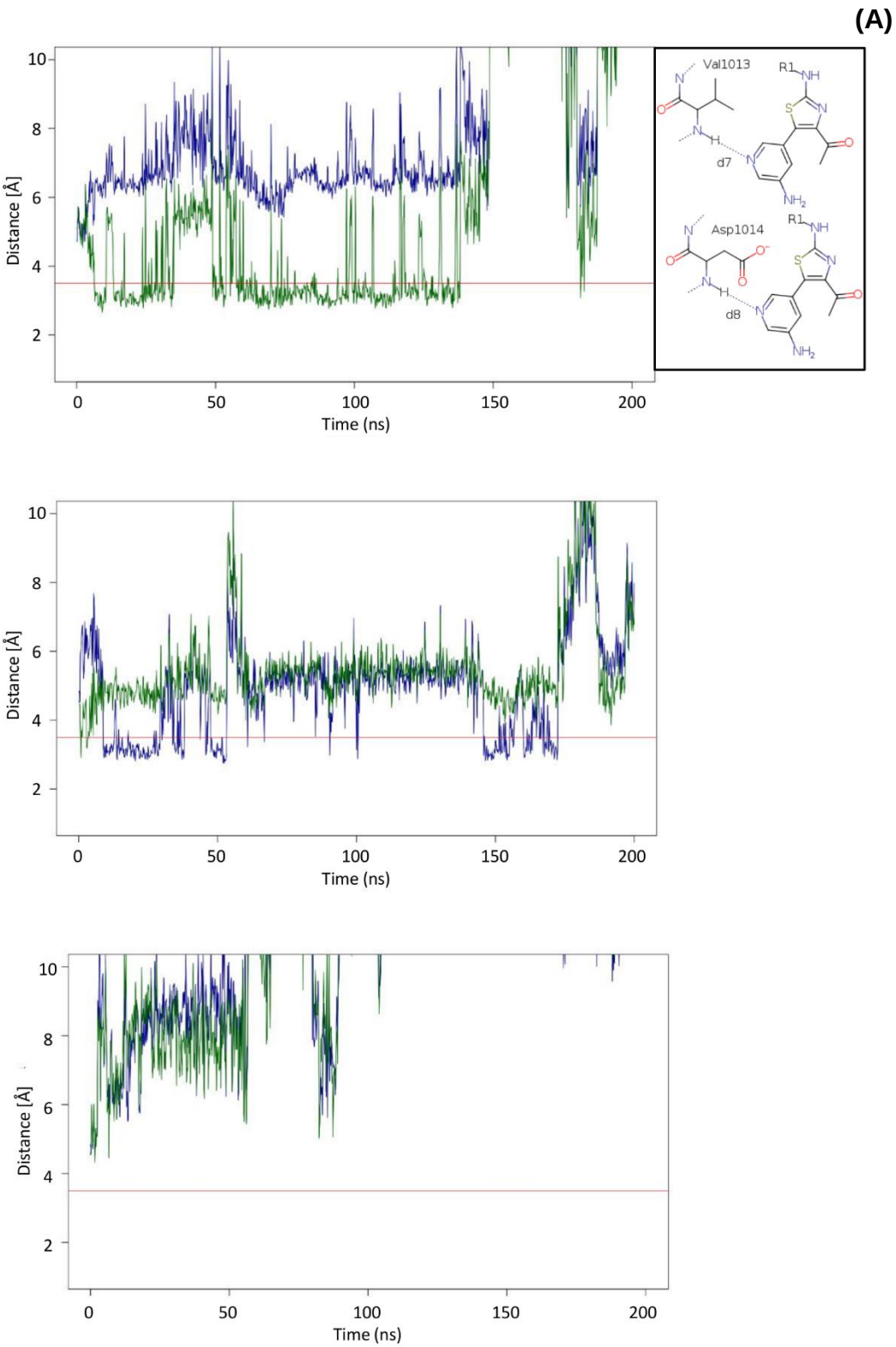
Supporting Figure 7. KAc mimic interactions within the binding pocket. The distances d1 (blue line), d2 (green line) and d3 (red line) between heavy atoms of compound **8** and the conserved asparagine residue in ATAD2 are represented over 200 ns (1000 frames) for the three different trajectories **A**, **B** and **C**. H-bonding was considered for distance values below 3.5Å (horizontal red line)



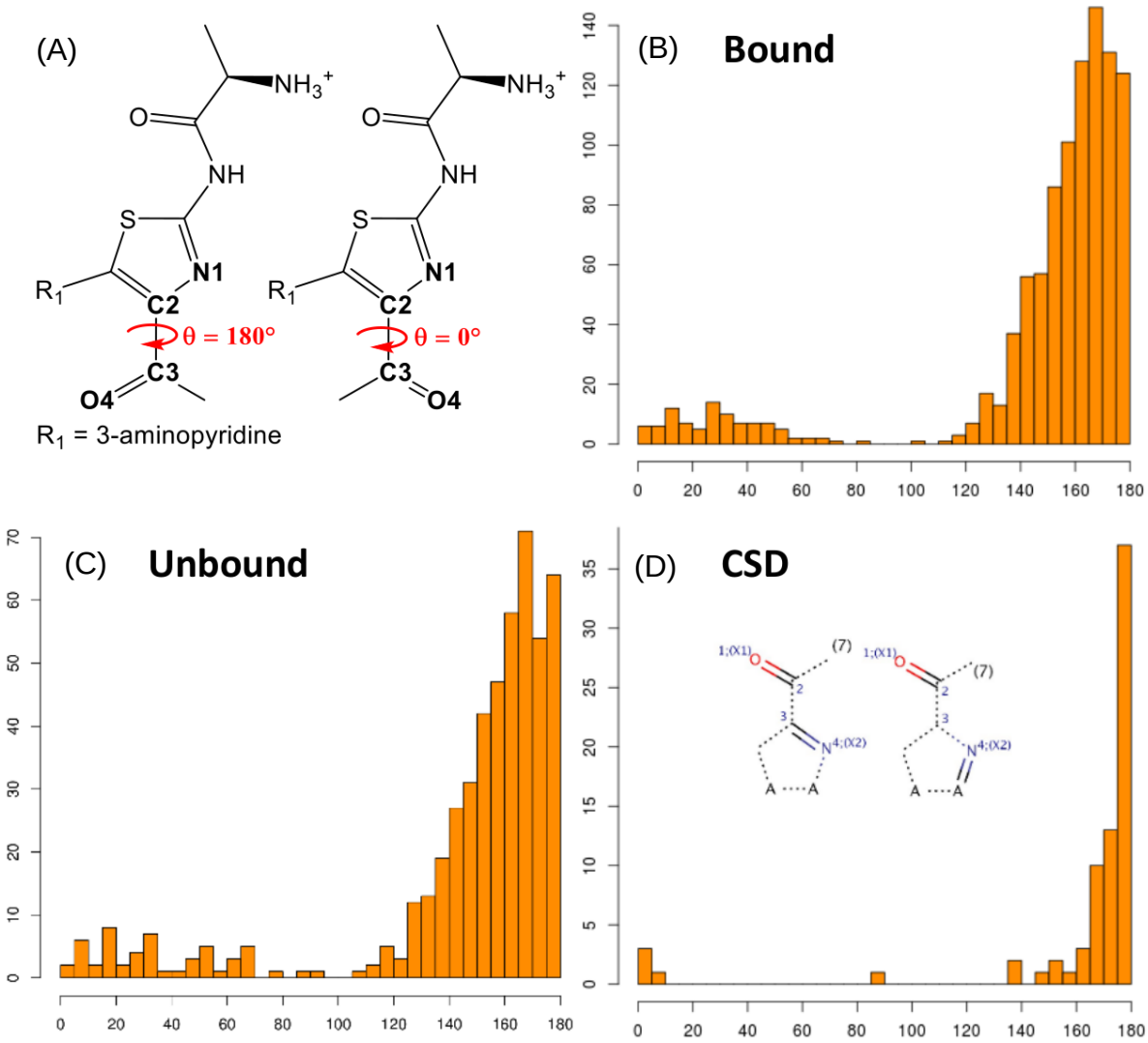
Supporting Figure 8. Salt bridge between compound **8**'s primary amino and Asp1071's carboxylate. The minimum of the distances d4 and d5 is presented for the three different trajectories **A**, **B** and **C**. Salt-bridge interaction was considered for distance values below 3.2Å (horizontal red line).



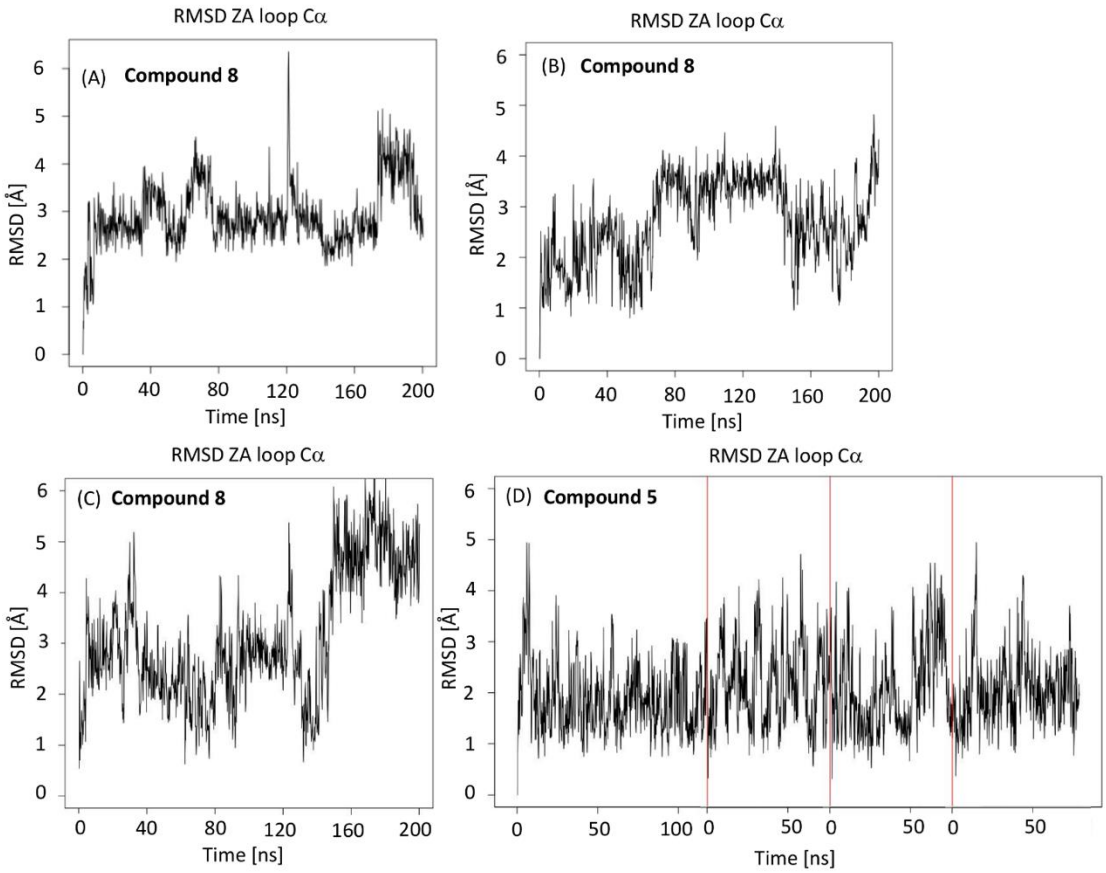
Supporting Figure 9. Lipophilic interactions between the pyridine ring of **8** and the isopropyl residue of Val1013. The minimum of the distances between any of the pyridine ring atoms and any of the isopropyl carbons is represented (blue line) for the three different trajectories **A**, **B** and **C.** Van der Waals contact was considered for distance values below 5 Å (horizontal red line).



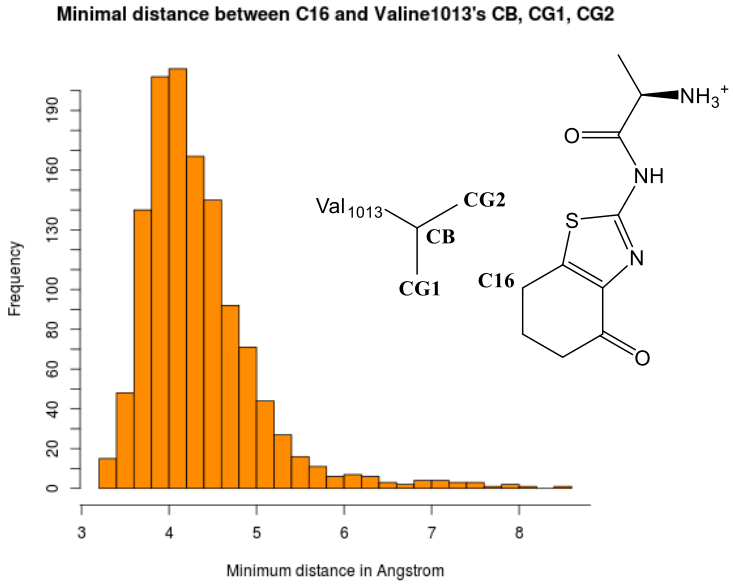
Supporting Figure 10. H-bonding between the pyridine's N of compound **8** and the N-H of Val1013 (green line) or the N-H of Asp1014 (blue line) for the three different trajectories **A**, **B** and **C**. H-bonding was considered for distance values below 3.5 Å (horizontal red line).



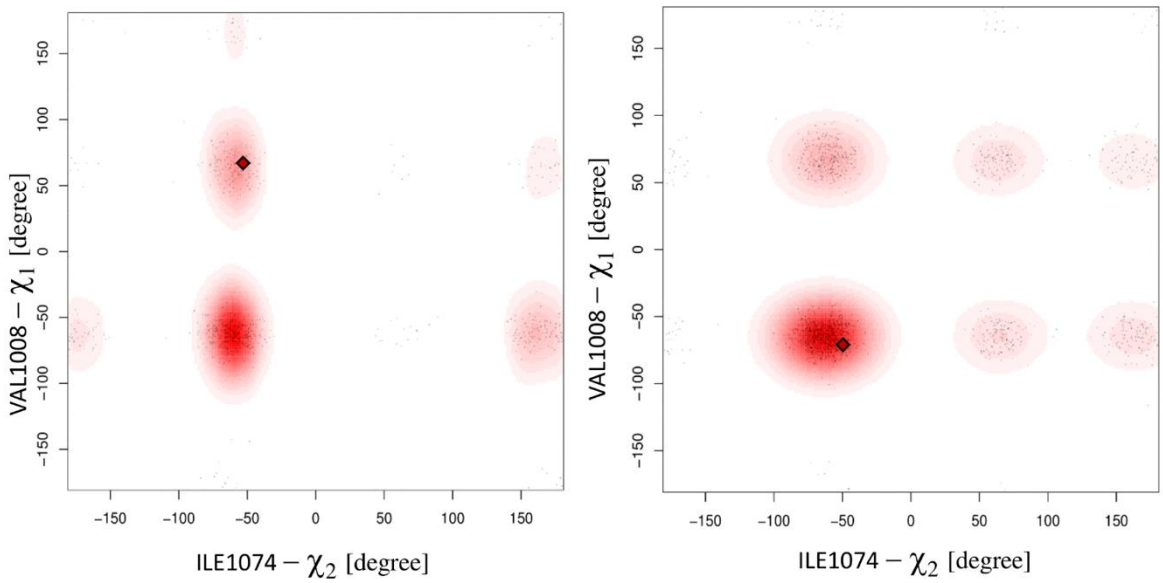
Supporting Figure 11. (A) *Trans*- and *cis*-conformers of compound **8** with regard to the N1-C2-C3-O4 dihedral. (B) Distribution of the absolute values of the N1-C2-C3-O4 dihedral angle for compound **8** in complex with ATAD2 along trajectory **A**; (C) distribution in MD simulations of compound **8** freely solvated in a TIP3P-water cubix box or (D) distribution within a set of 75 crystallographic structures retrieved from the Cambridge Structural Database (CSD). The two molecular patterns were used for the substructure search (with the carbon (7) not being in a ring).



Supporting Figure 12. (A,B,C) RMSD of the Cα atoms of residues Lys1011 to Pro1015 in Å with the initial equilibrated pose as reference for MD trajectories **A**,**B** and **C** of the ATAD2-compound **8** complex; (D) for all four MD trajectories of the ATAD2-compound **5** complex.



Supporting Figure 13. Distribution of minimum distance (in Å) from C16 of **5** to CB, CG1 and CG2 of Val1013. The lipophilic 6-membered ring mostly remains at vdW distance from the Valine1013 hereby stabilizing the ZA loop.

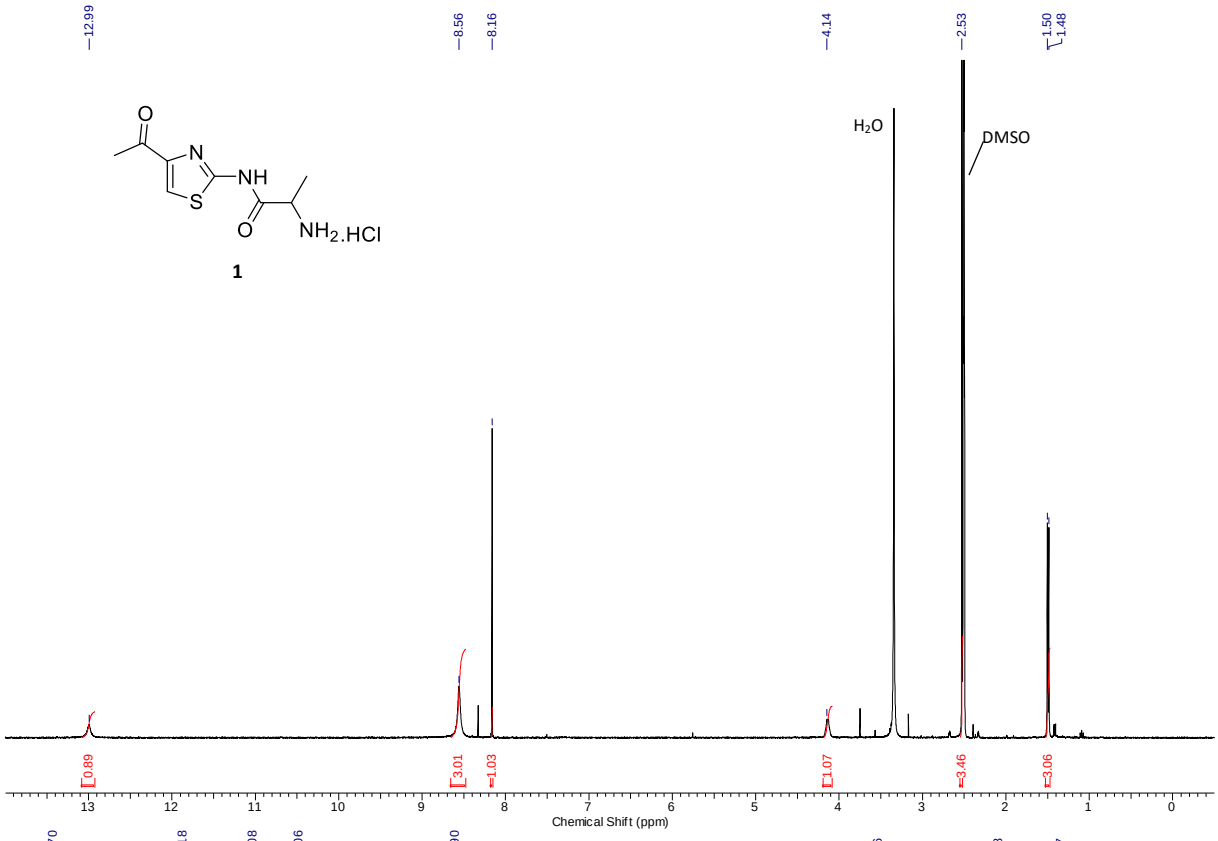


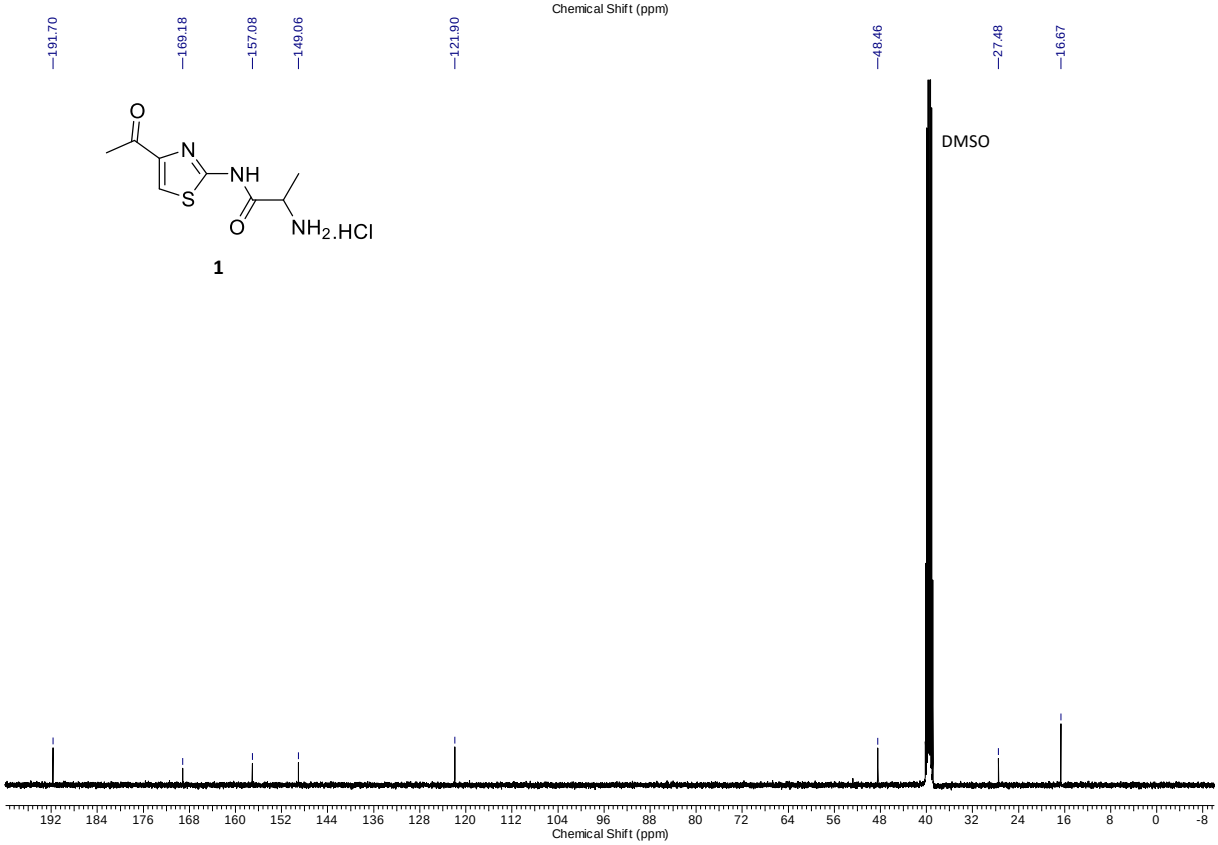
Supporting Figure 14. Two-dimensional histogram (kernel density plot) for the dihedral angles χ_2 of Ile1074 and χ_1 of Val1008 for the MD simulations of compound **8** (A) and **5** (B). The dihedral values χ_2 of Ile1074 and χ_1 of Val1008 in the crystal structure are indicated (diamonds).

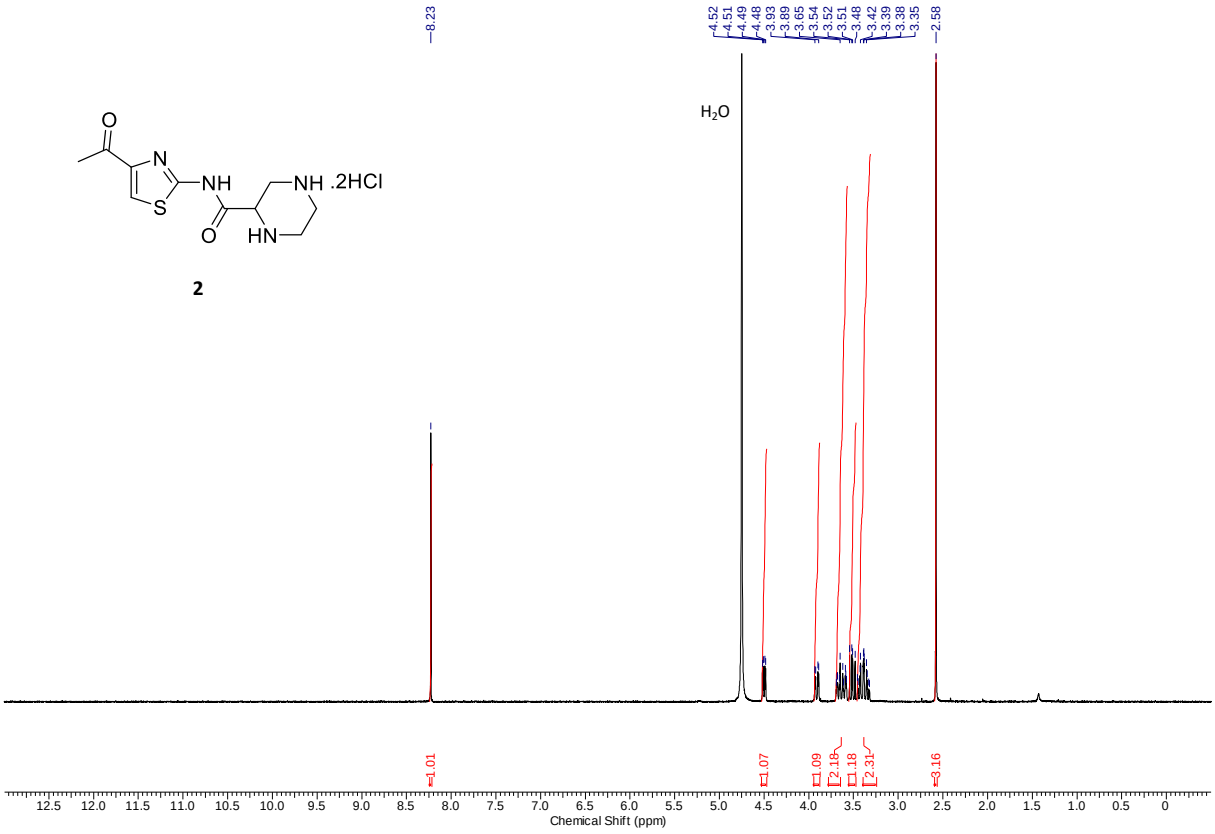
atad2.mpg

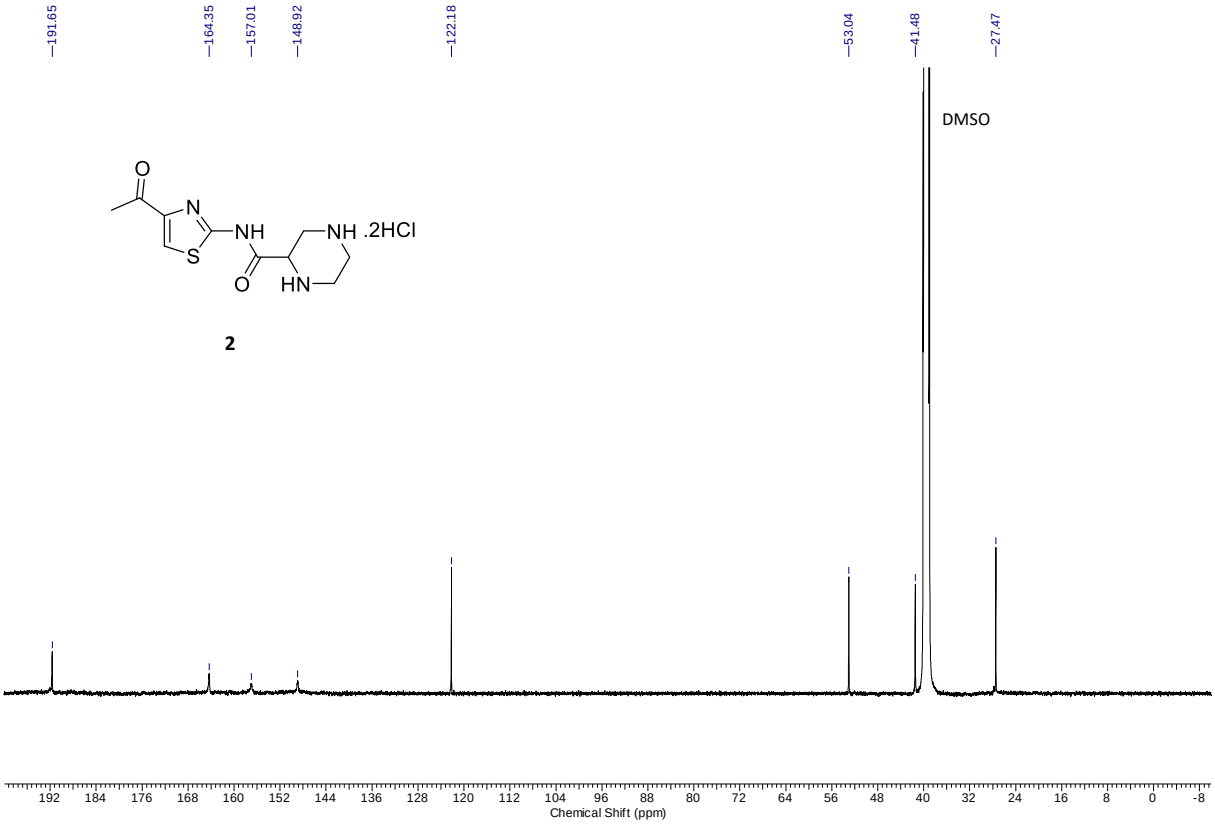
Supporting MD simulation movie. Short movie of compound **1** with ATAD2 from a MD simulation with explicit solvent. The movie shows 20 ns of a 40 ns run. Reading with VLC is prefered.

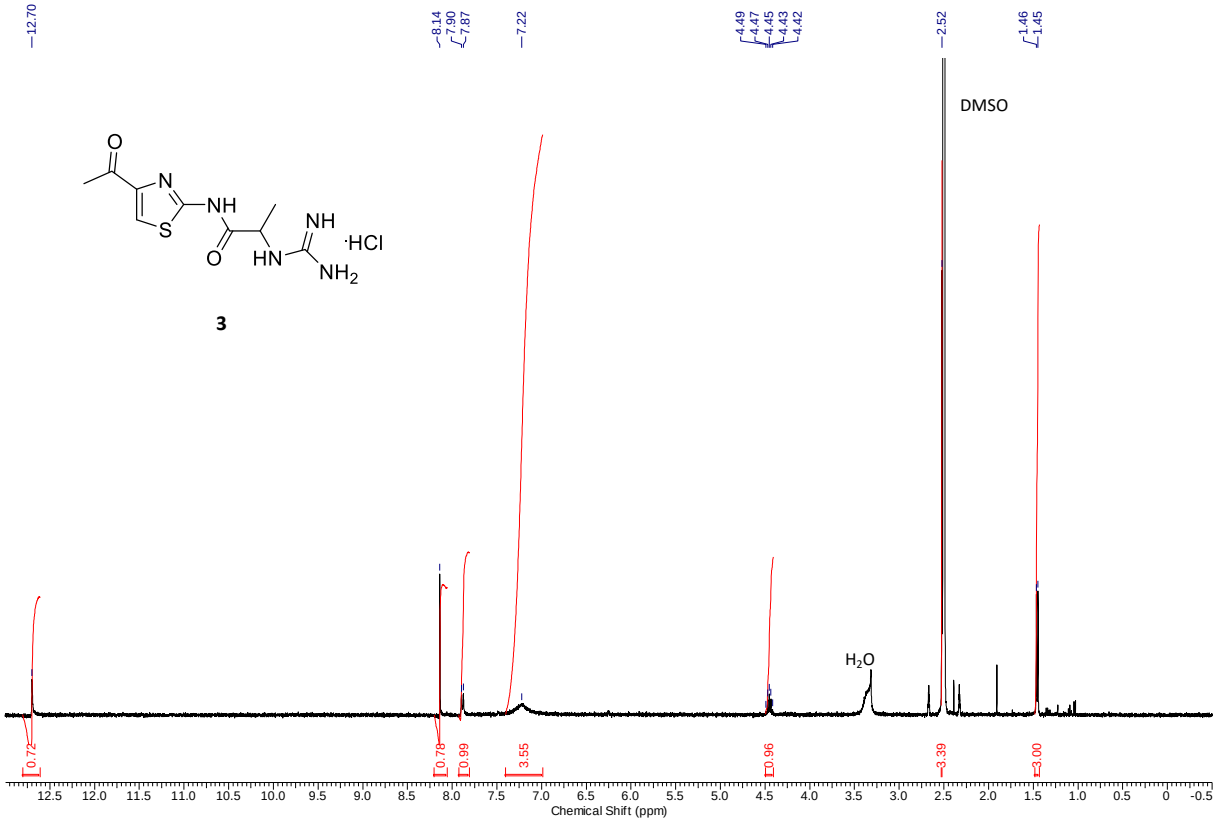
4. NMR traces of final compounds

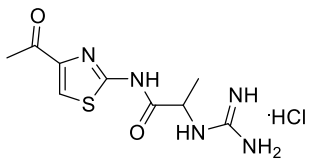





8.23
4.52
4.51
4.49
4.48
3.93
3.89
3.65
3.54
3.52
3.51
3.48
3.42
3.39
3.38
3.35
2.58
H2O
O
N
NH
NH .2HCl
S
O
HN
2
1.01
1.07
1.09
2.18
1.18
2.31
3.16
Chemical Shift (ppm)


191.65
164.35
157.01
148.92
122.18
53.04
41.48
27.47
DMSO
O
N
NH
NH .2HCl
S
O
HN
2
Chemical Shift (ppm)


12.70
8.14
7.90
7.87
7.22
4.49
4.47
4.45
4.43
4.42
2.52
1.46
1.45
DMSO
H2O
0.72
0.78
0.99
3.55
0.96
3.39
3.00
Chemical Shift (ppm)

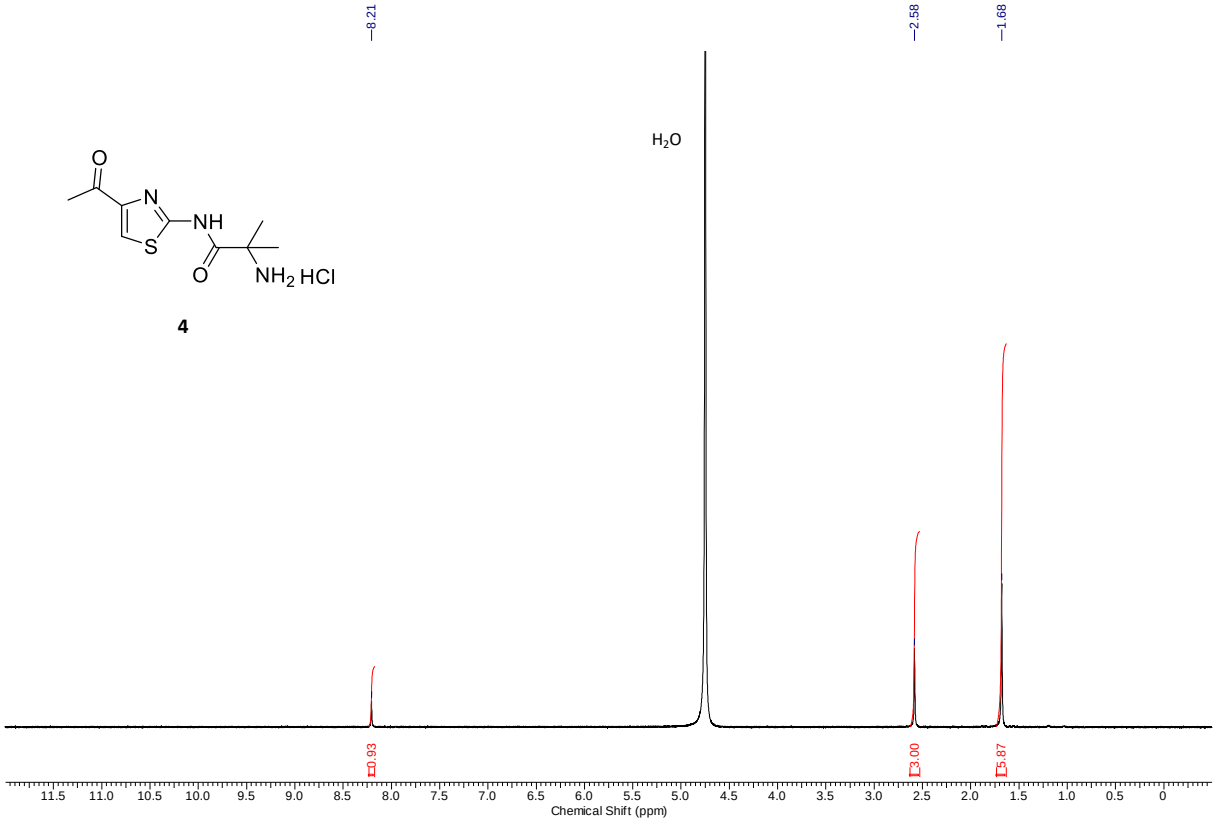

·HCl

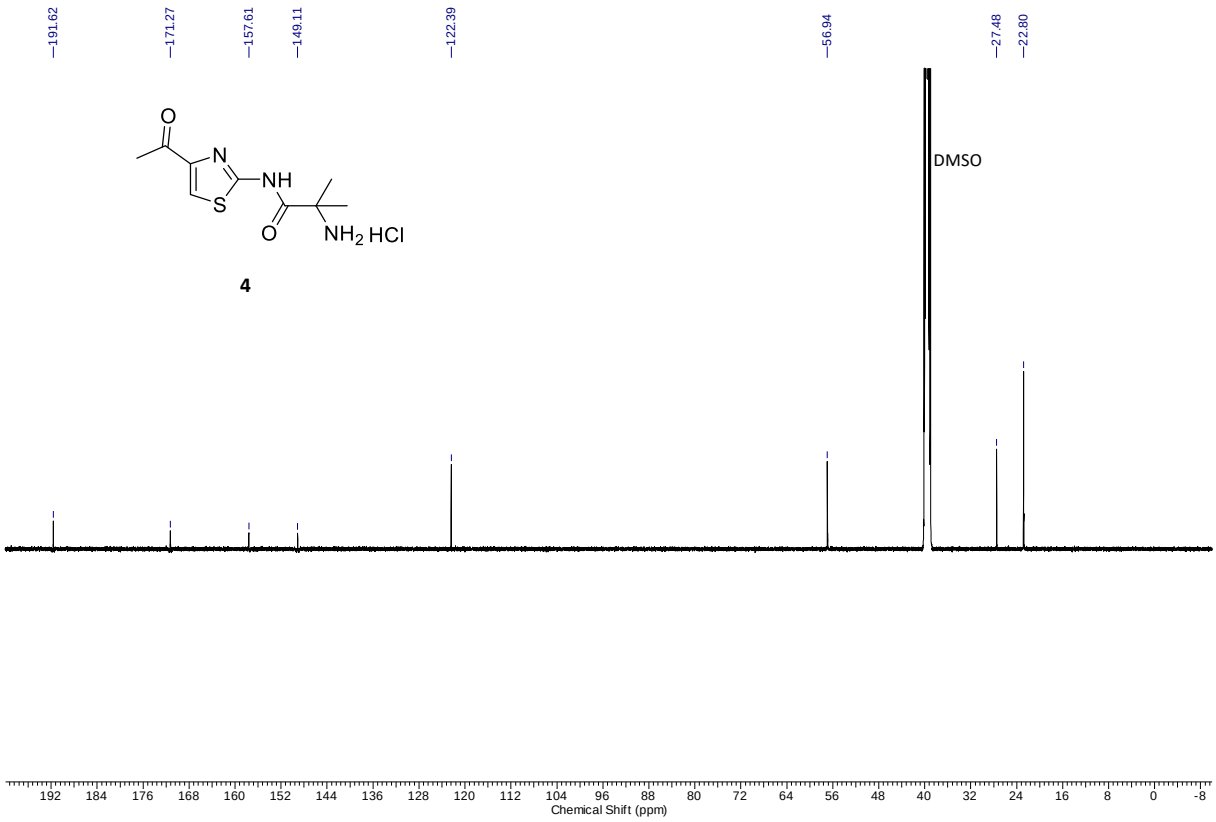
3

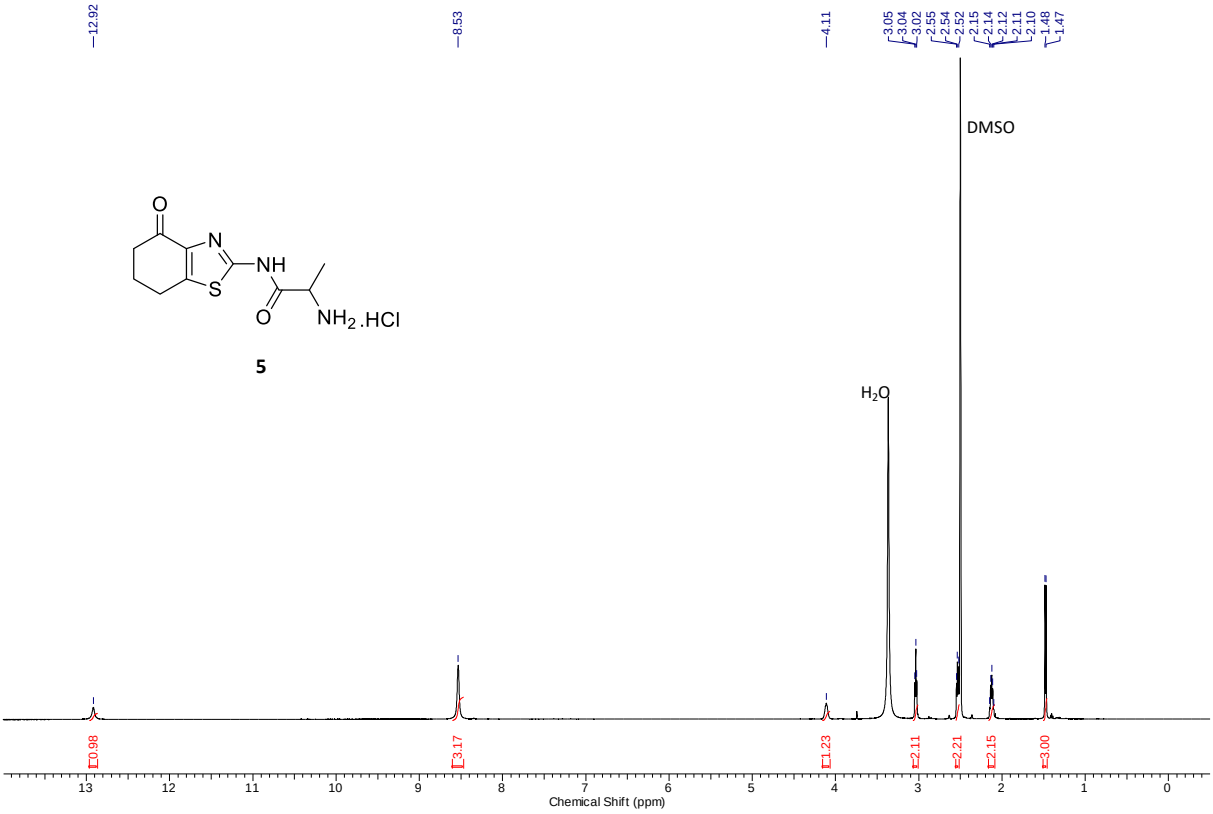
191.69
170.35
157.60
156.78
148.99
121.66
50.00
27.43
18.15
DMSO
Chemical Shift (ppm)

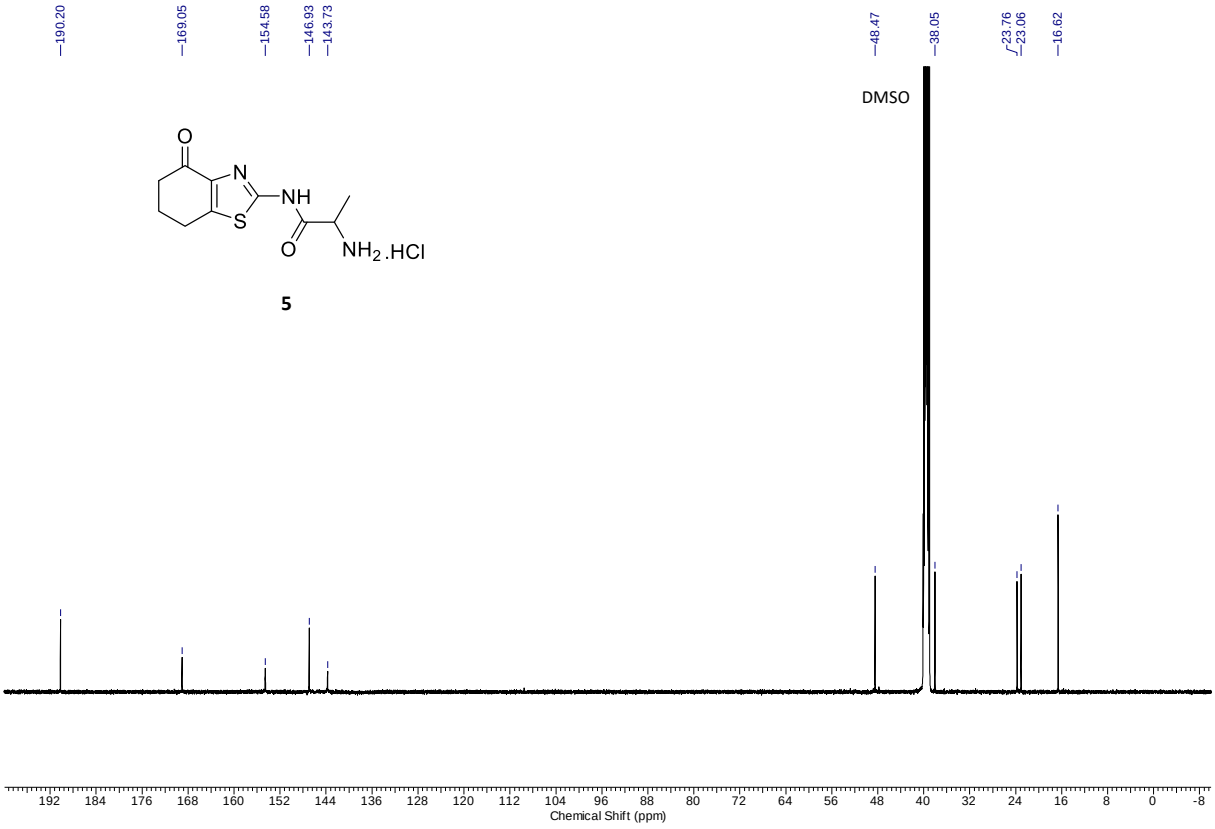
·HCl

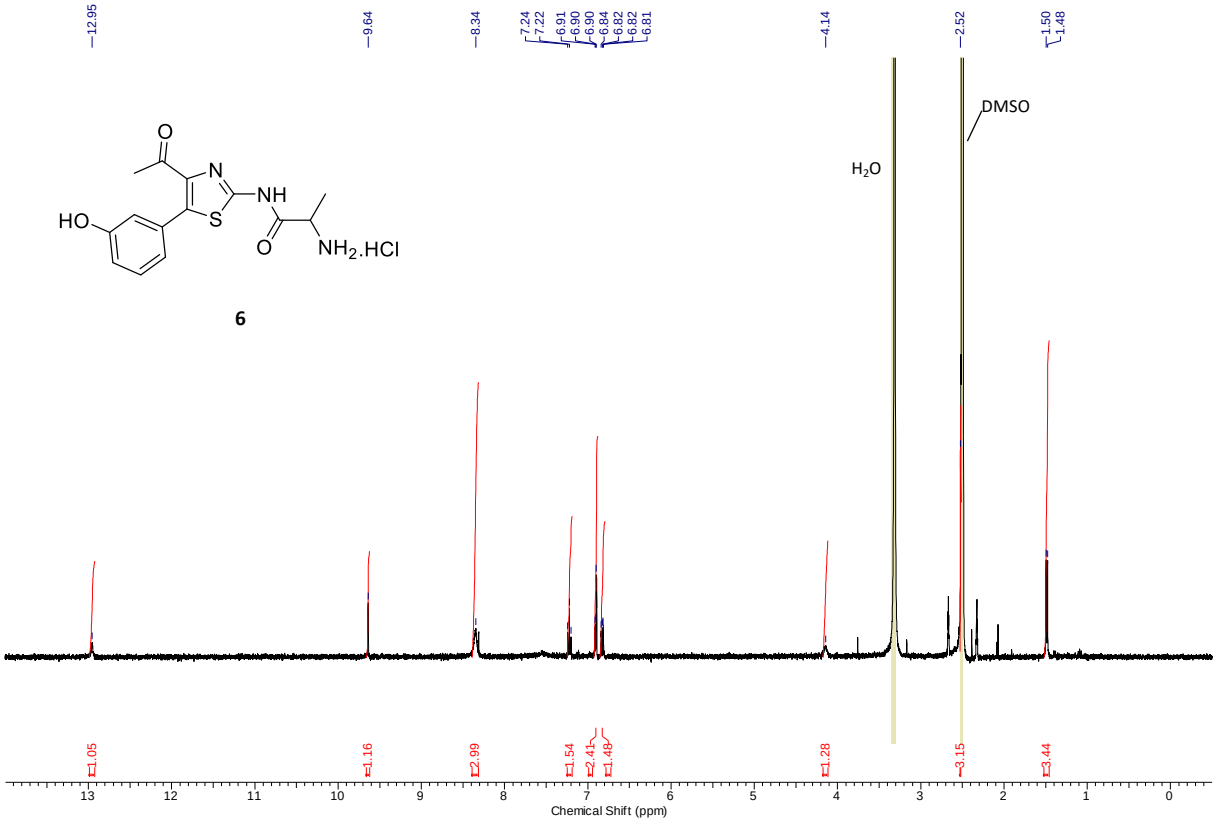
3


8.21
2.58
1.68
H2O
O
N
NH
S
O
NH2 HCl
4
0.93
3.00
5.87
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)

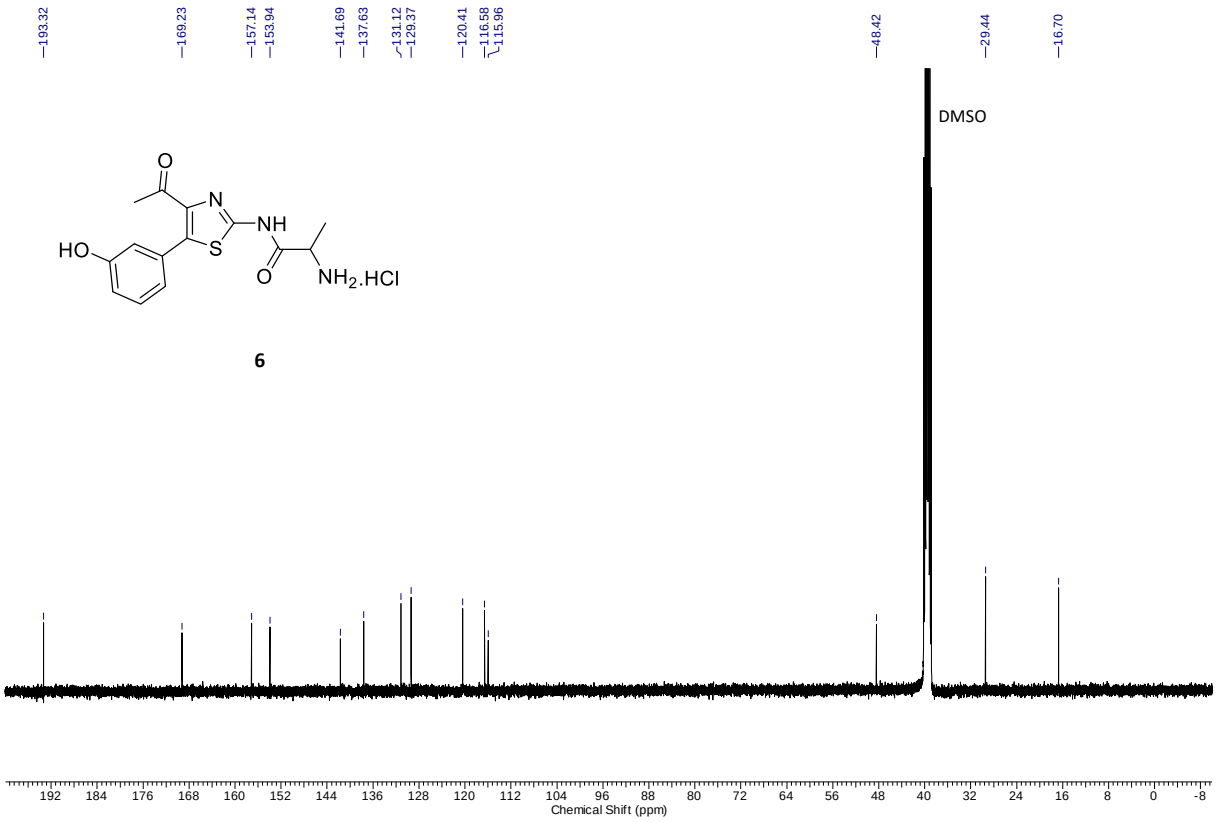

191.62
171.27
157.61
149.11
122.39
56.94
27.48
22.80
DMSO
O
N
NH
S
O
NH2 HCl
4
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0 -8
Chemical Shift (ppm)


12.92
8.53
4.11
3.05
3.04
3.02
2.55
2.54
2.52
2.15
2.14
2.12
2.11
2.10
1.48
1.47
DMSO
H2O
O
N
NH
S
O
NH2.HCl
5
0.98
3.17
1.23
2.11
2.21
2.15
3.00
Chemical Shift (ppm)

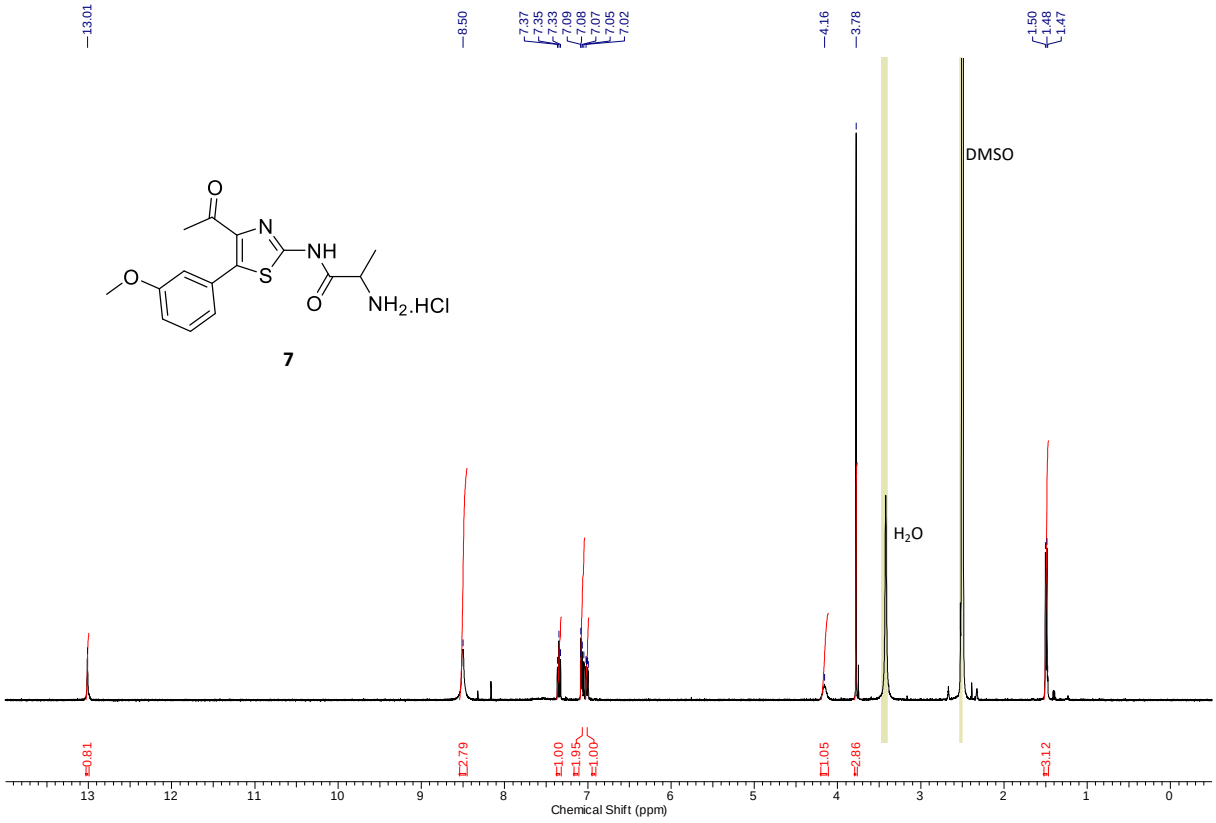

190.20
169.05
154.58
146.93
143.73
48.47
38.05
23.76
23.06
16.62
DMSO
O
N
NH
S
O
NH2.HCl
5
Chemical Shift (ppm)

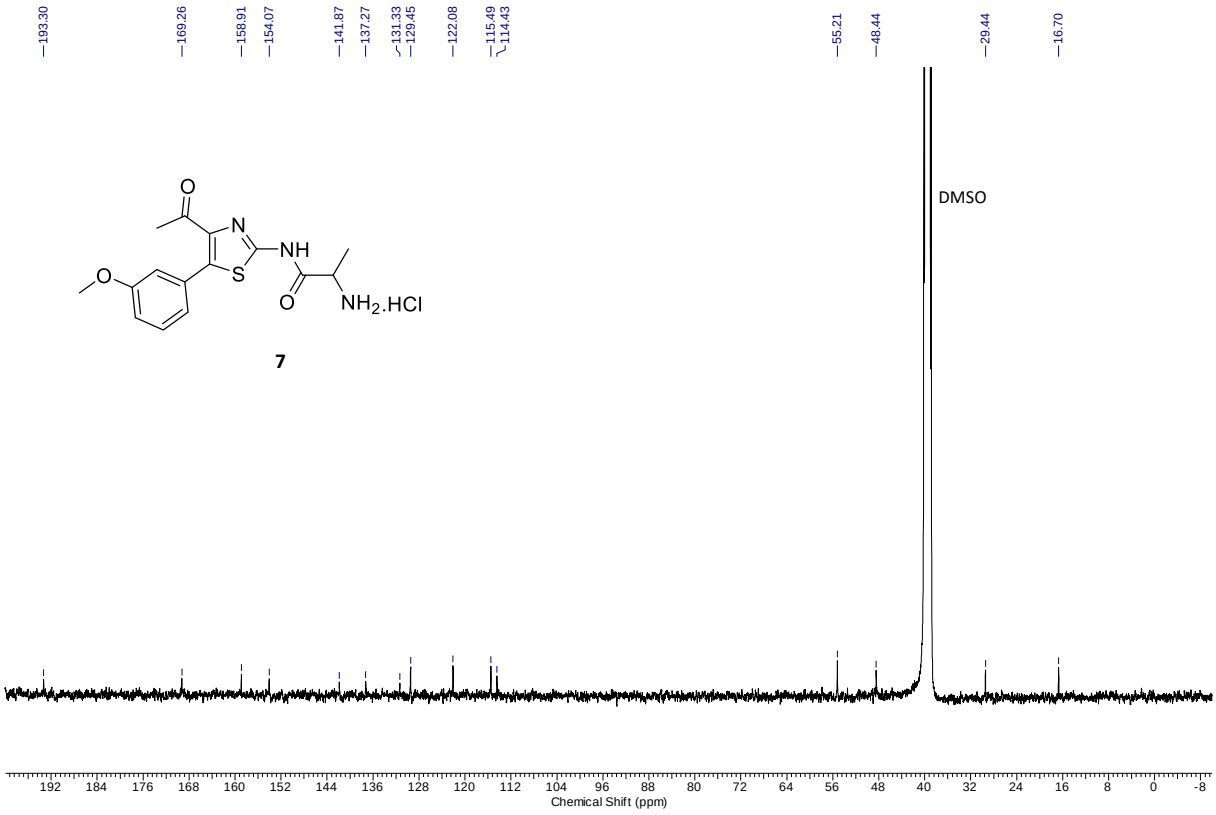

12.95
9.64
8.34
7.24
7.22
6.91
6.90
6.90
6.84
6.82
6.82
6.81
4.14
2.52
1.50
1.48
DMSO
H2O
HO
O
N
NH
S
O
NH2.HCl
1.05
1.16
2.99
1.54
2.41
1.49
1.28
3.15
3.44
Chemical Shift (ppm)

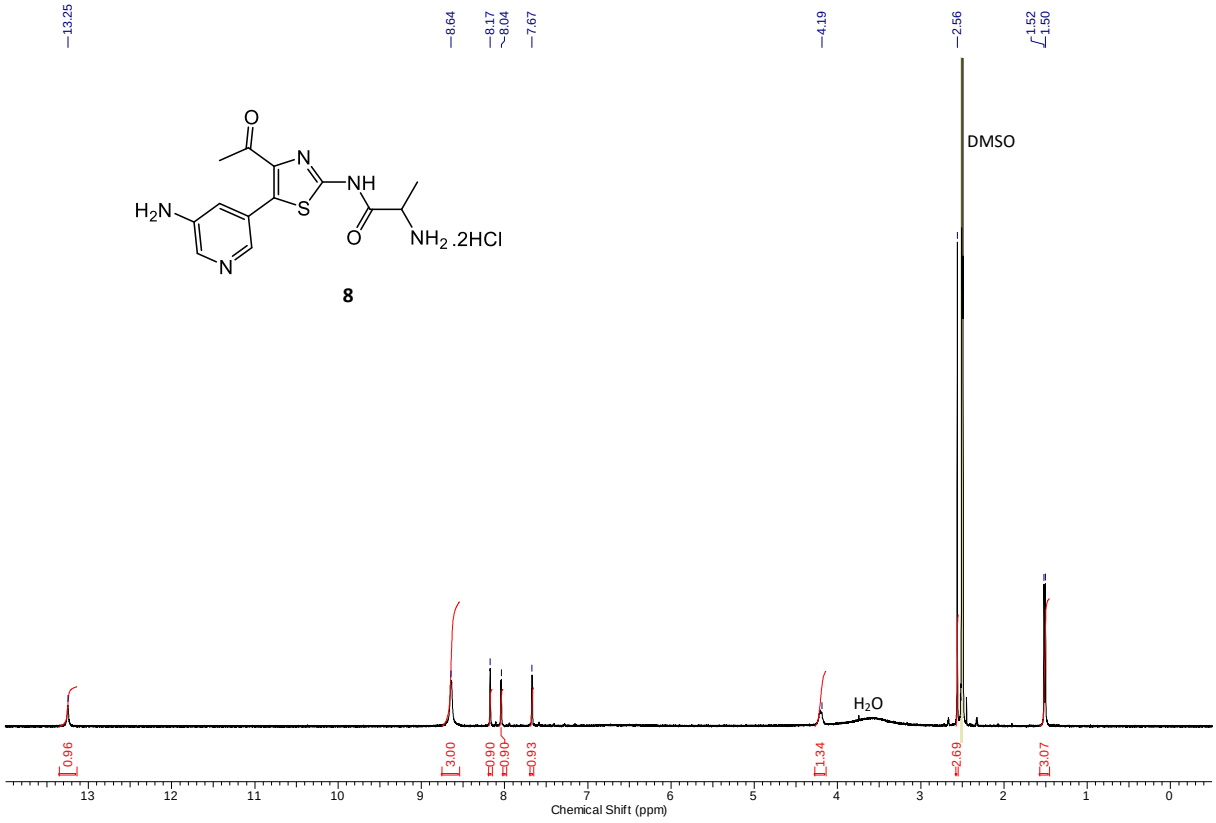
6

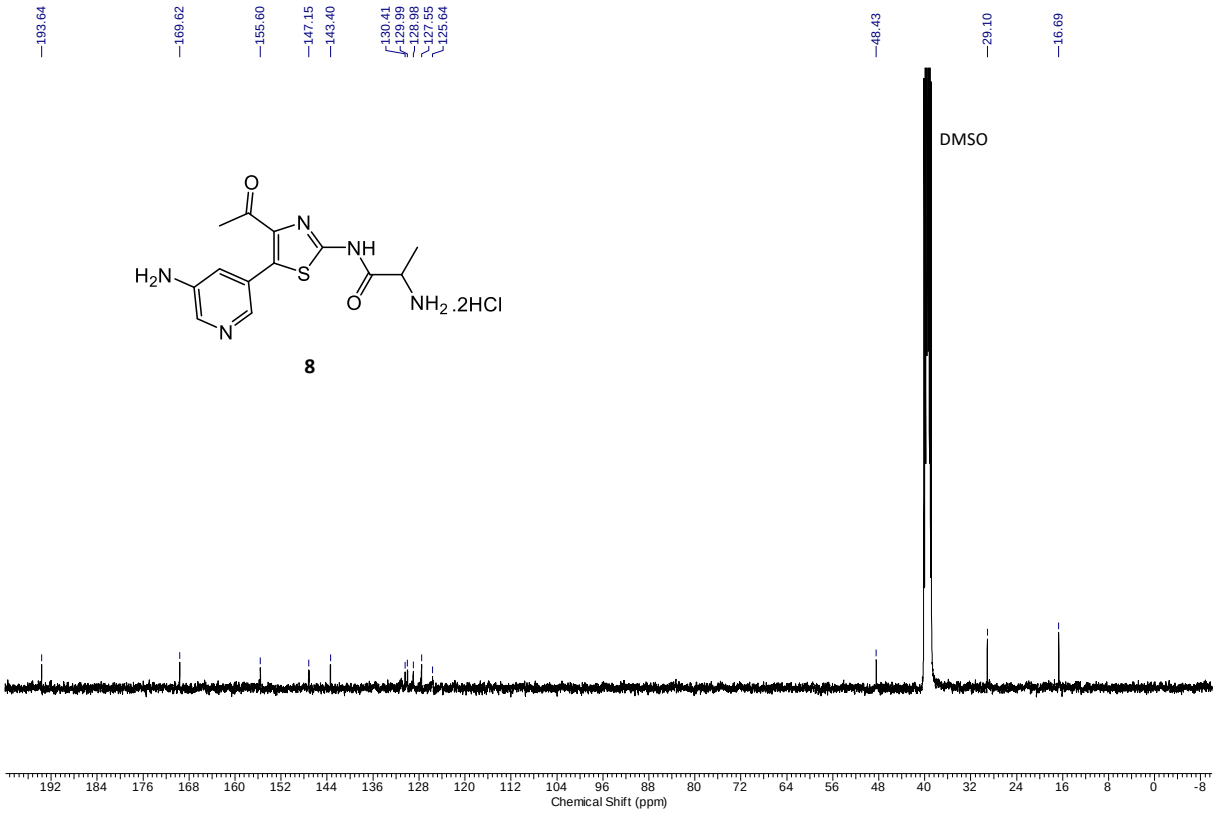

193.32
169.23
157.14
153.94
141.69
137.63
131.12
129.37
120.41
116.58
115.96
48.42
29.44
16.70
DMSO
HO
O
N
NH
S
O
NH2.HCl
Chemical Shift (ppm)

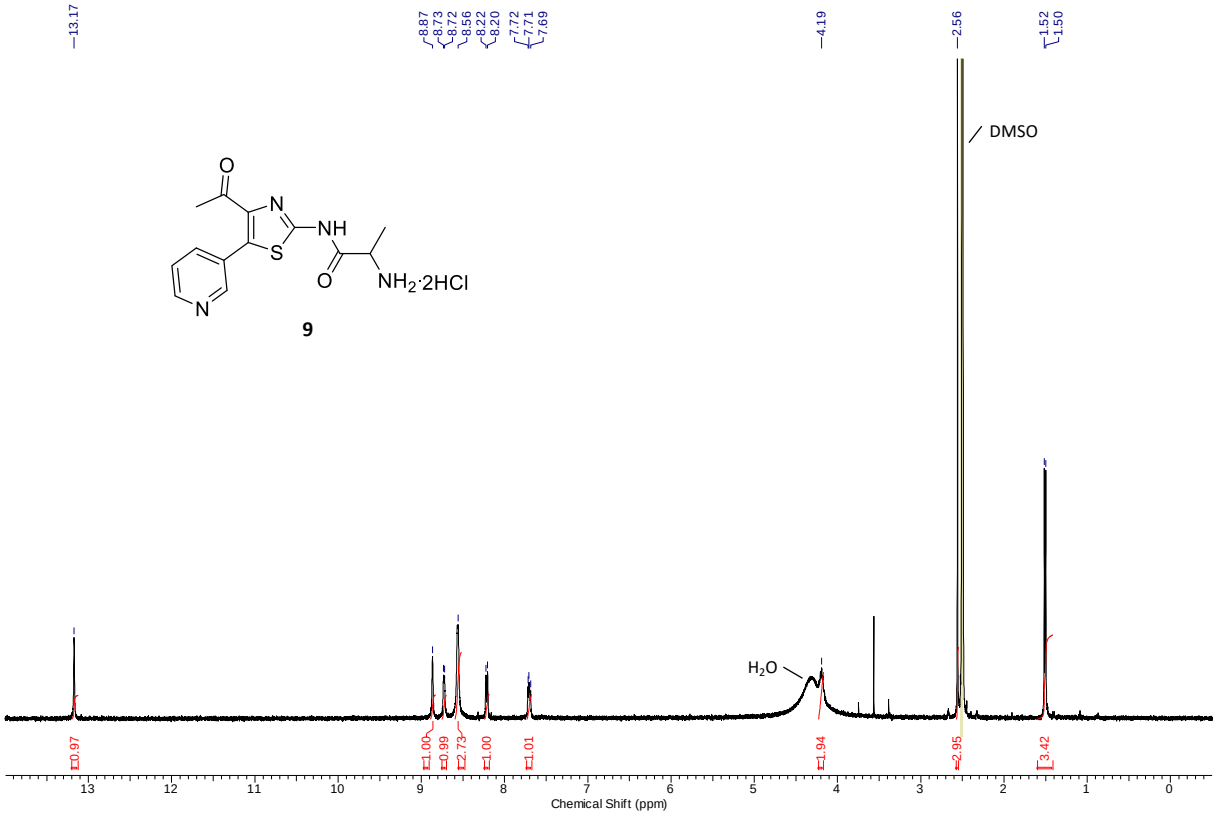
6

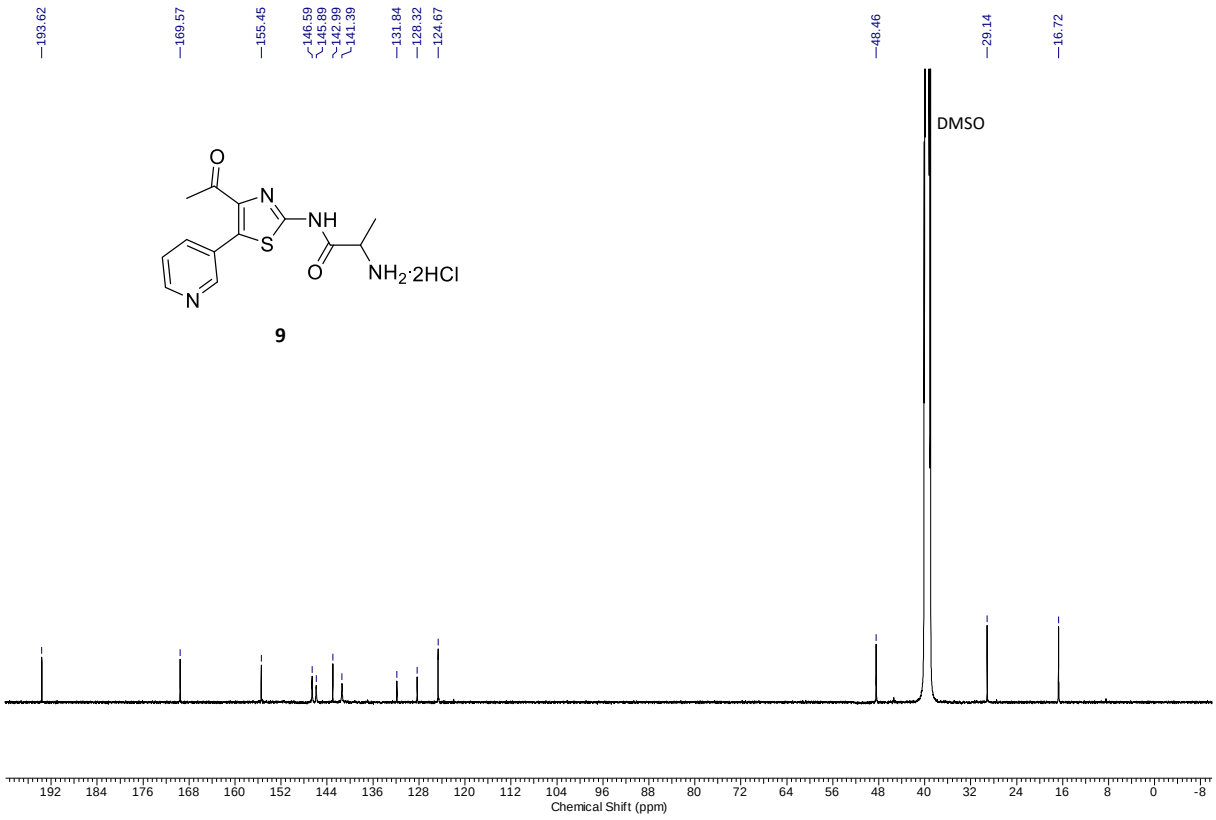

13.01
8.50
7.37
7.35
7.33
7.09
7.08
7.07
7.05
7.02
4.16
3.78
1.50
1.48
1.47
DMSO
H2O
O
N
NH
S
O
NH2.HCl
7
0.81
2.79
1.00
1.95
1.00
1.05
2.86
3.12
Chemical Shift (ppm)

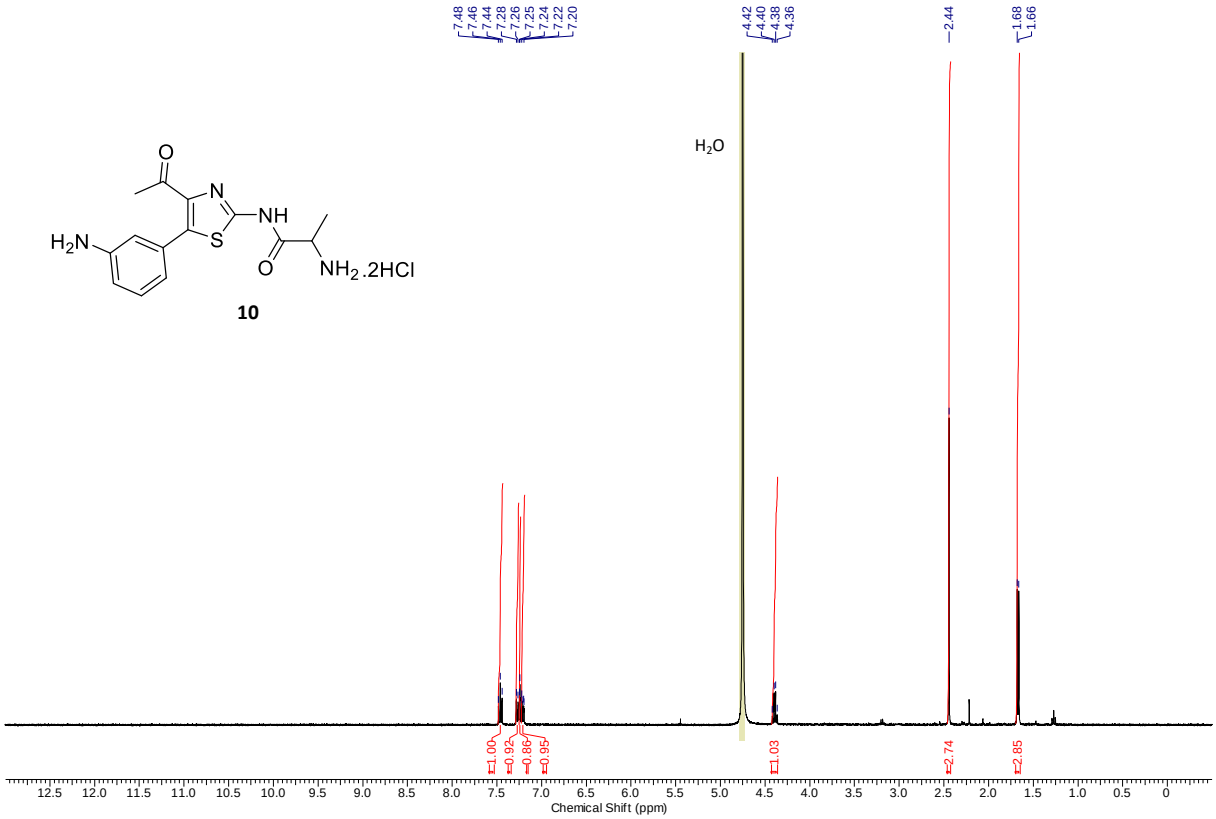

193.30
169.26
158.91
154.07
141.87
137.27
131.33
129.45
122.08
115.49
114.43
55.21
48.44
29.44
16.70
DMSO
O
N
NH
S
O
NH2.HCl
7
Chemical Shift (ppm)

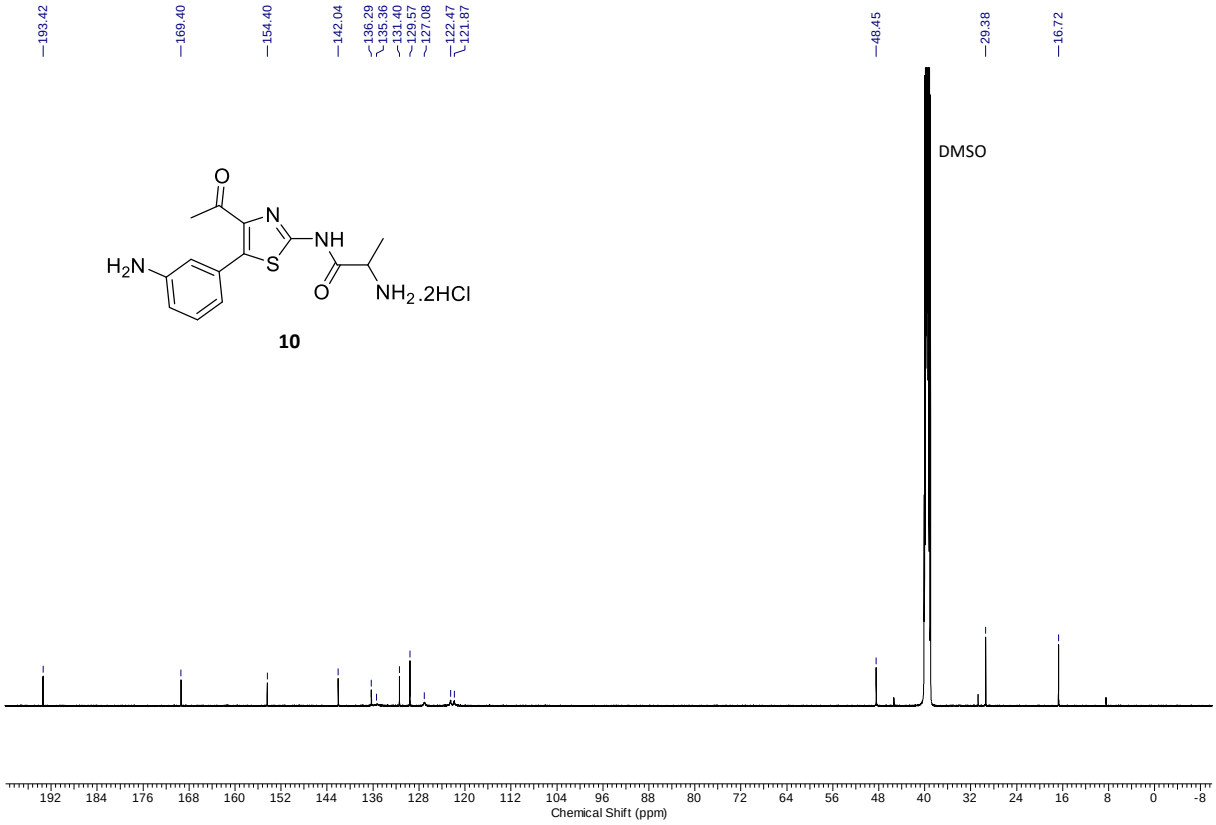

13.25
8.64
8.17
8.04
7.67
4.19
2.56
1.52
1.50
DMSO
H2N
N
S
NH
O
O
NH2 .2HCl
N
8
H2O
0.96
3.00
0.90
0.90
0.93
1.34
2.69
3.07
Chemical Shift (ppm)

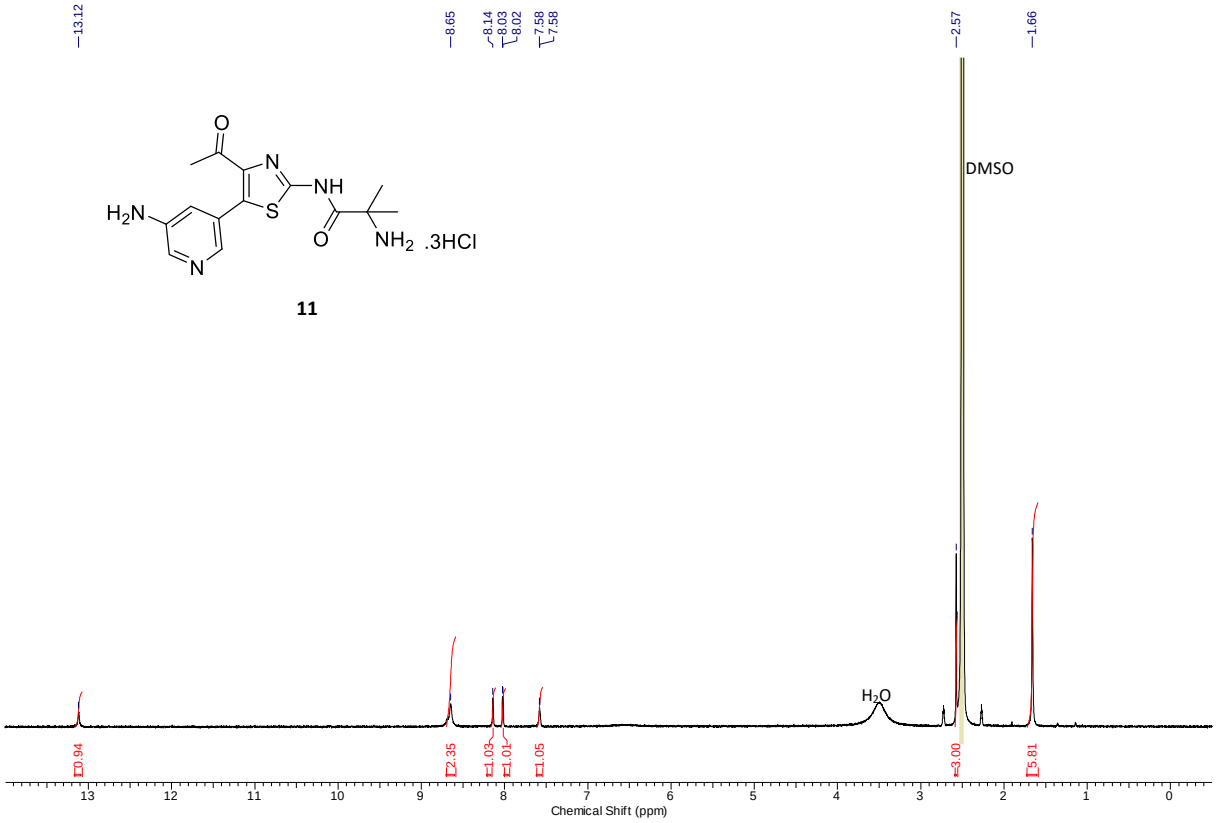

193.64
169.62
155.60
147.15
143.40
130.41
129.99
128.98
127.55
125.64
48.43
29.10
16.69
DMSO
H2N
N
S
NH
O
O
NH2 .2HCl
N
8
Chemical Shift (ppm)

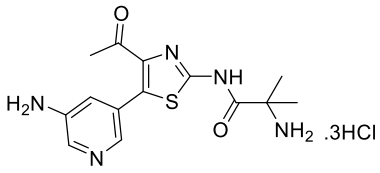

13.17
8.87
8.73
8.72
8.56
8.22
8.20
7.72
7.71
7.69
4.19
2.56
1.52
1.50
DMSO
H2O
O
N
NH
S
O
NH2·2HCl
N
9
0.97
1.00
0.99
2.73
1.00
1.01
1.94
2.95
3.42
Chemical Shift (ppm)


193.62
169.57
155.45
146.59
145.89
142.99
141.39
131.84
128.32
124.67
48.46
29.14
16.72
DMSO
O
N
NH
S
O
NH2·2HCl
N
9
Chemical Shift (ppm)


7.48
7.46
7.44
7.28
7.26
7.25
7.24
7.22
7.20
4.42
4.40
4.38
4.36
2.44
1.68
1.66
H2O
O
N
NH
H2N
S
O
NH2.2HCl
10
1.00
0.92
0.86
0.95
1.03
2.74
2.85
Chemical Shift (ppm)


193.42
169.40
154.40
142.04
136.29
135.36
131.40
129.57
127.08
122.47
121.87
48.45
29.38
16.72
DMSO
O
N
NH
H2N
S
O
NH2.2HCl
10
Chemical Shift (ppm)


13.12
8.65
8.14
8.03
8.02
7.58
7.58
2.57
1.66
DMSO
H2O
0.94
2.35
1.03
1.01
1.05
3.00
3.81
Chemical Shift (ppm)

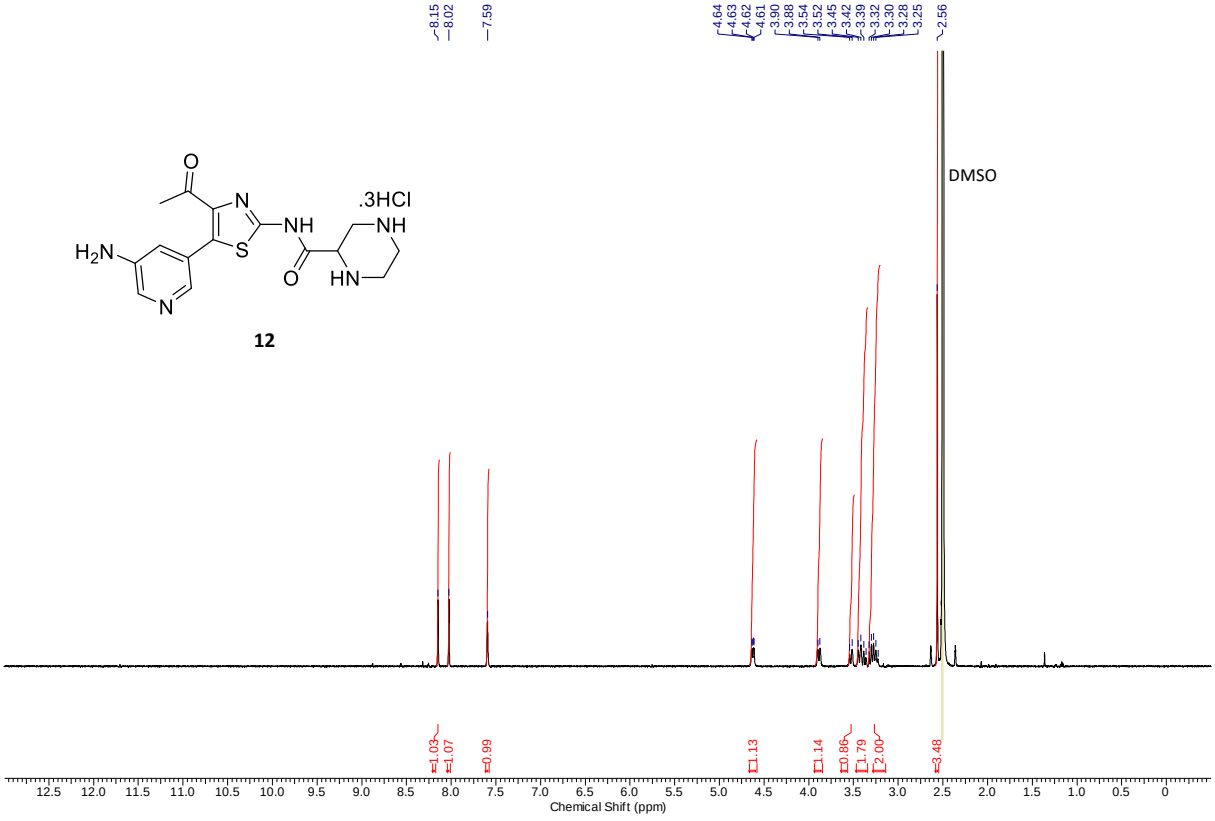

O
N
NH
S
H2N
O
NH2 .3HCl
N

11

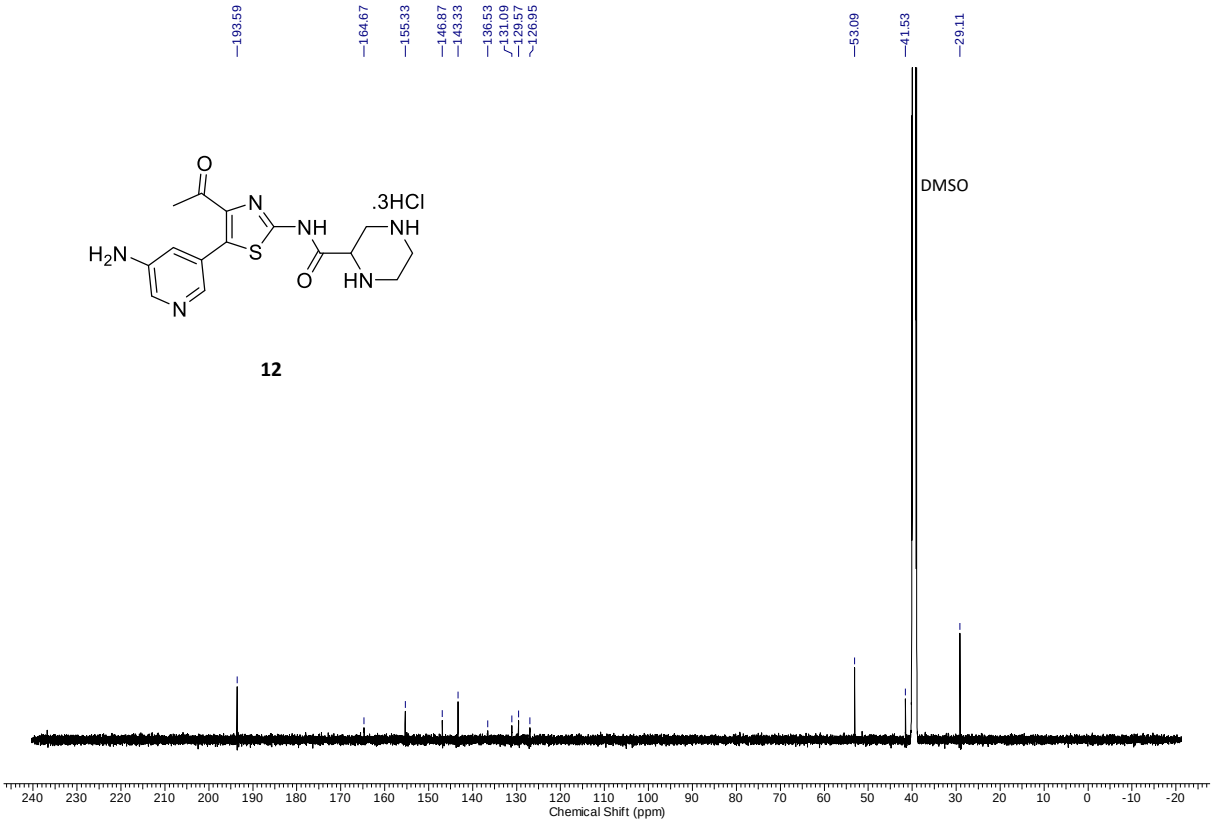
193.66
171.62
156.13
147.10
143.47
130.64
129.94
129.09
127.43
125.85
56.98
29.15
22.78
DMSO
Chemical Shift (ppm)

O
N
NH
S
H2N
O
NH2 .3HCl
N

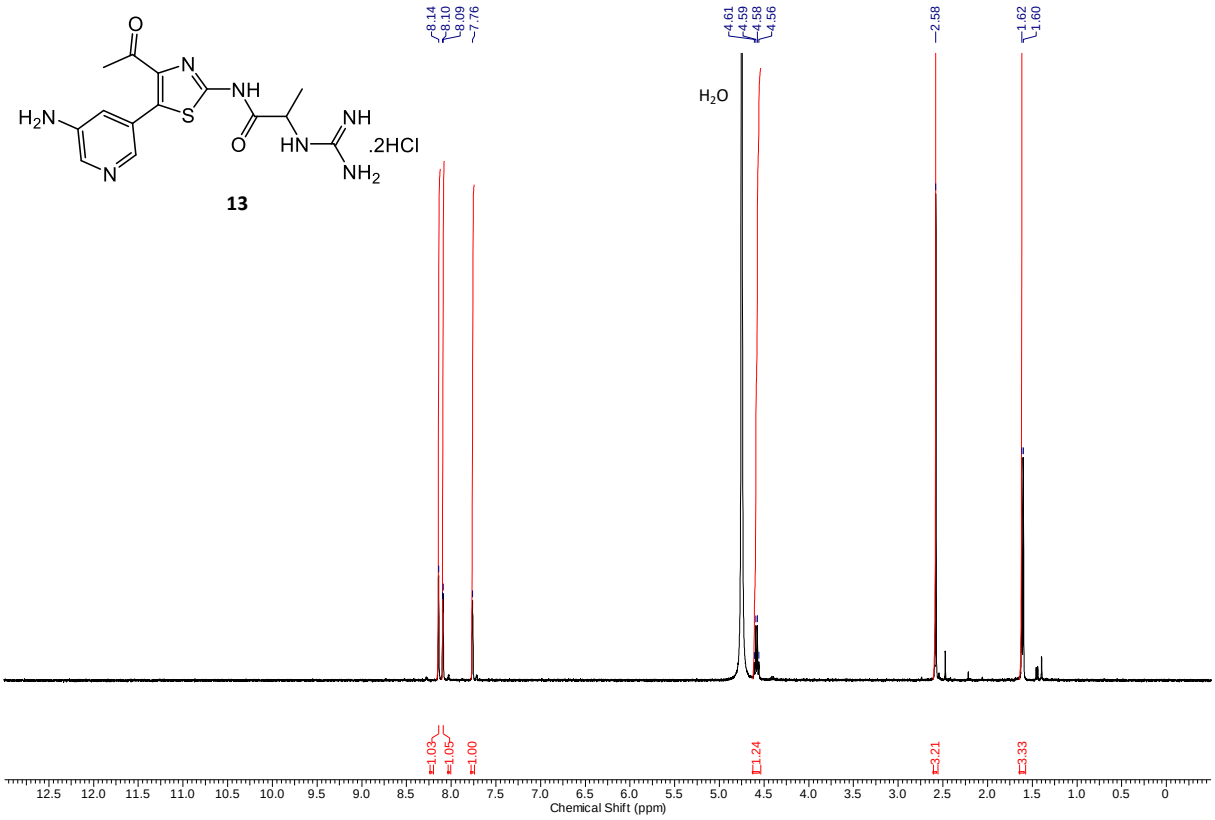
11


8.15
8.02
7.59
4.64
4.63
4.62
4.61
3.90
3.88
3.54
3.52
3.45
3.42
3.39
3.32
3.30
3.28
3.25
2.56
DMSO
O
N
NH
.3HCl
NH
H_2N
S
O
HN
N
1.03
1.07
0.99
1.13
1.14
0.86
1.79
2.00
3.48
Chemical Shift (ppm)

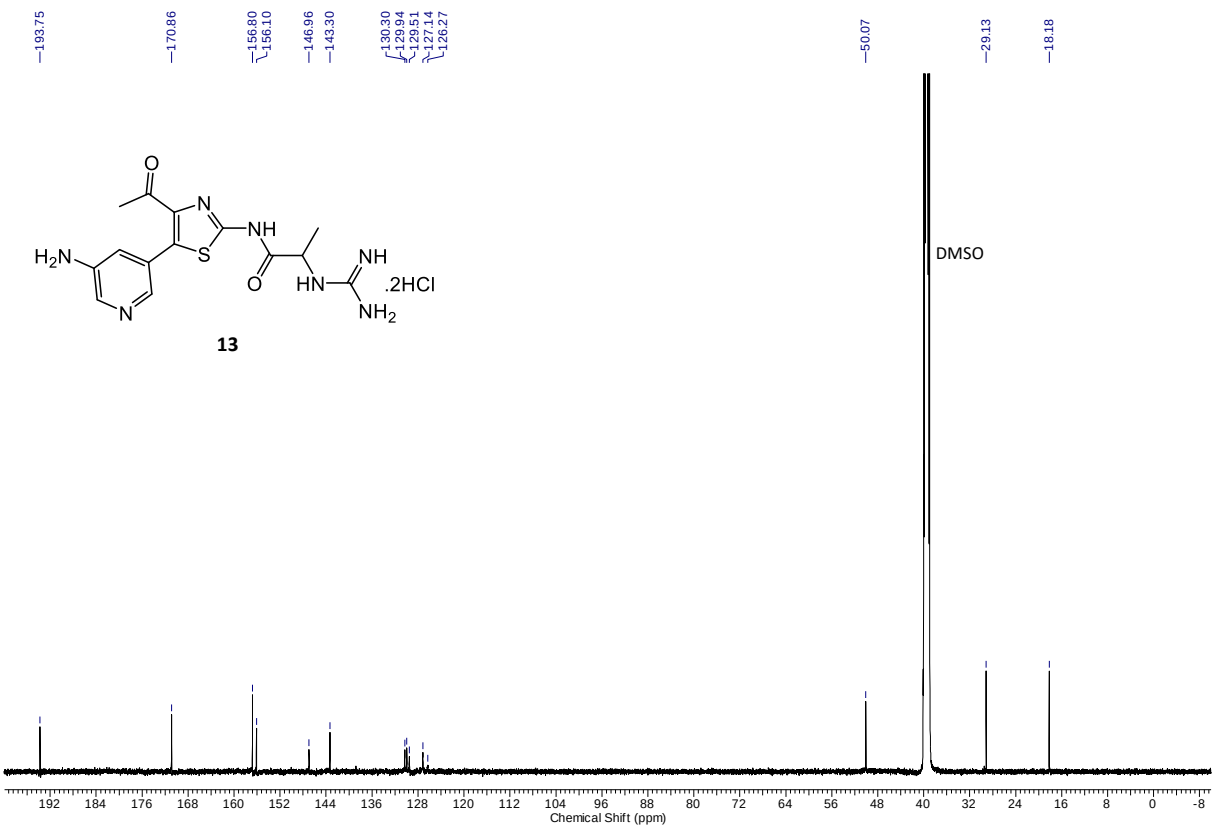
12


193.59
164.67
155.33
146.87
143.33
136.53
131.09
129.57
126.95
53.09
41.53
29.11
DMSO
O
N
NH
.3HCl
NH
H_2N
S
O
HN
N
Chemical Shift (ppm)

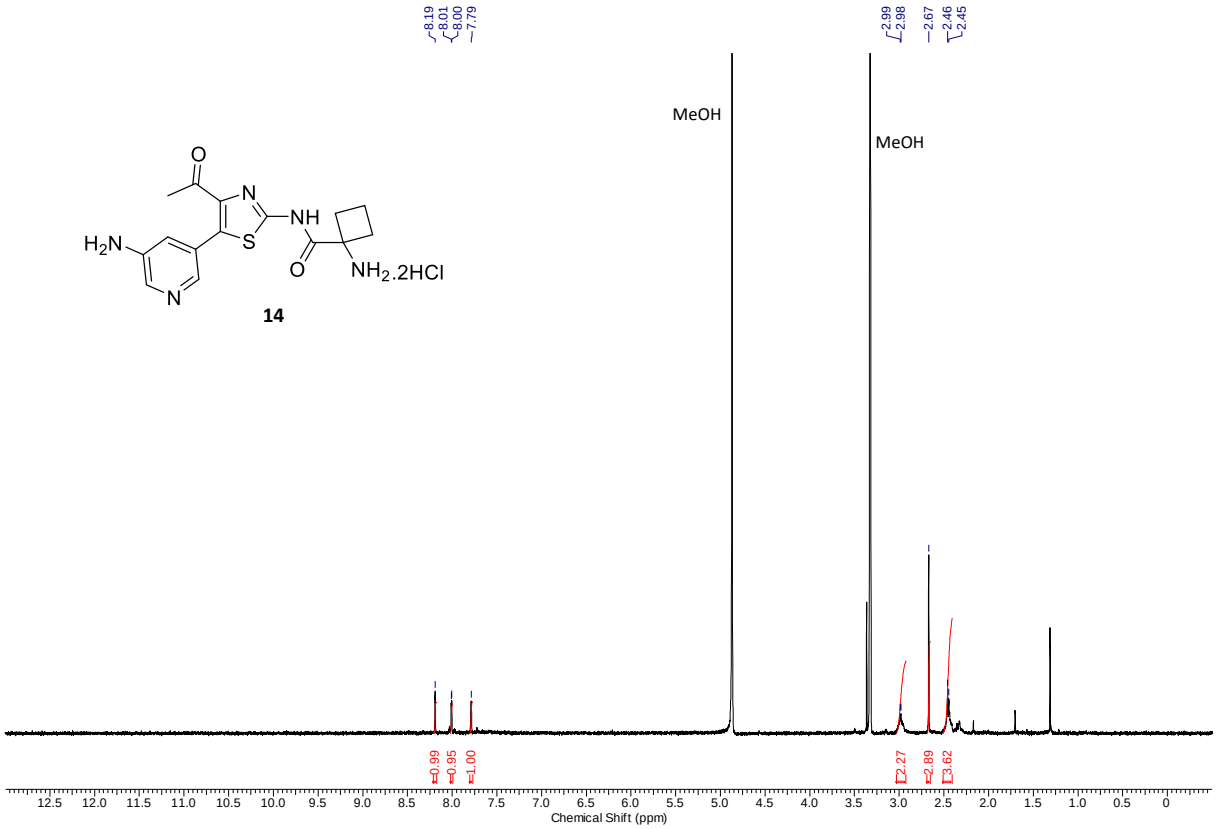
12

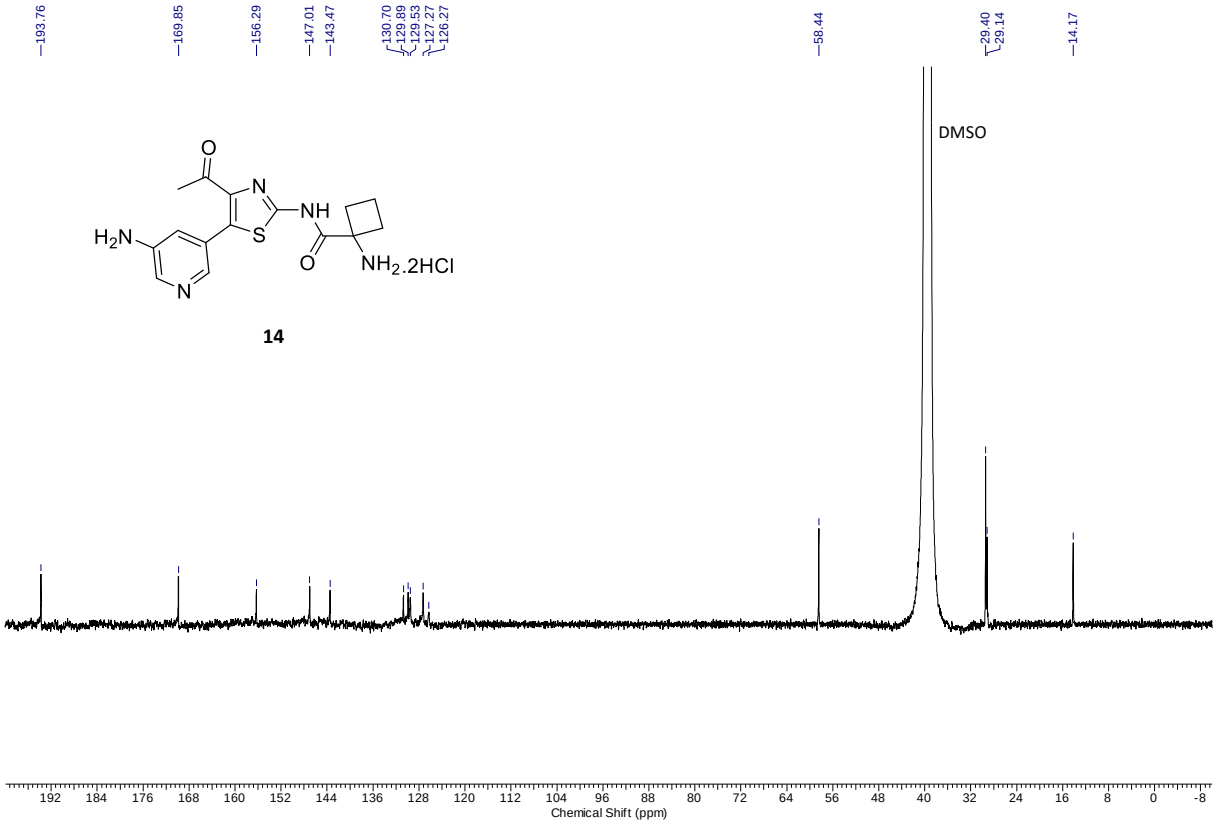

8.14
8.10
8.09
7.76
4.61
4.59
4.58
4.56
2.58
1.62
1.60
H2O
1.03
1.05
1.00
1.24
3.21
3.33
Chemical Shift (ppm)

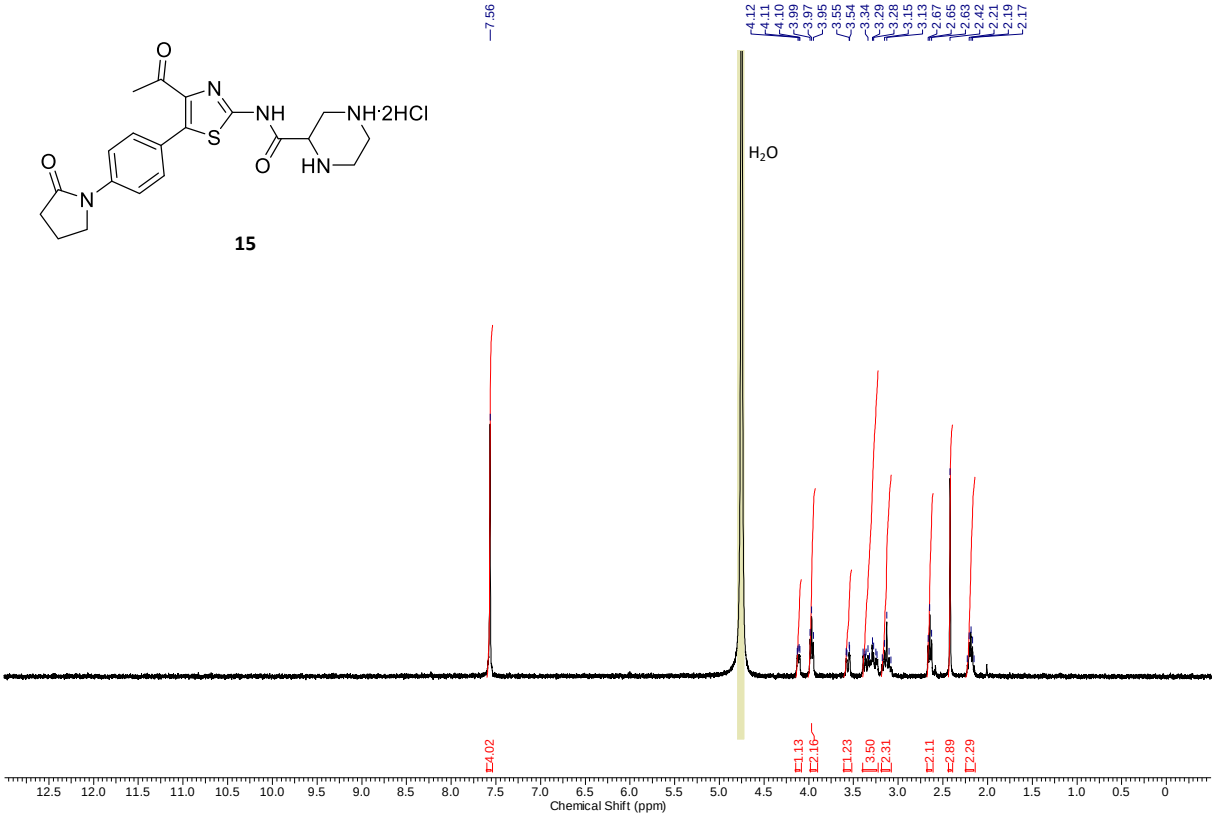
13

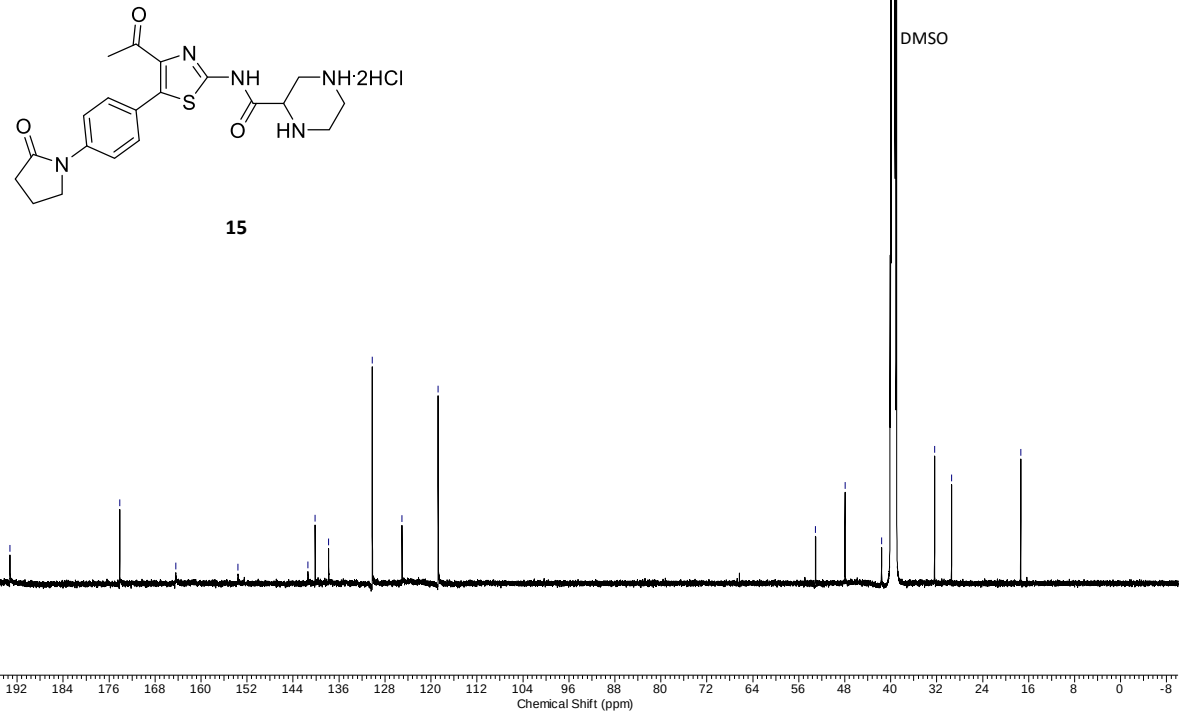

193.75
170.86
156.80
156.10
146.96
143.30
130.30
129.94
129.51
127.14
126.27
50.07
29.13
18.18
DMSO
Chemical Shift (ppm)

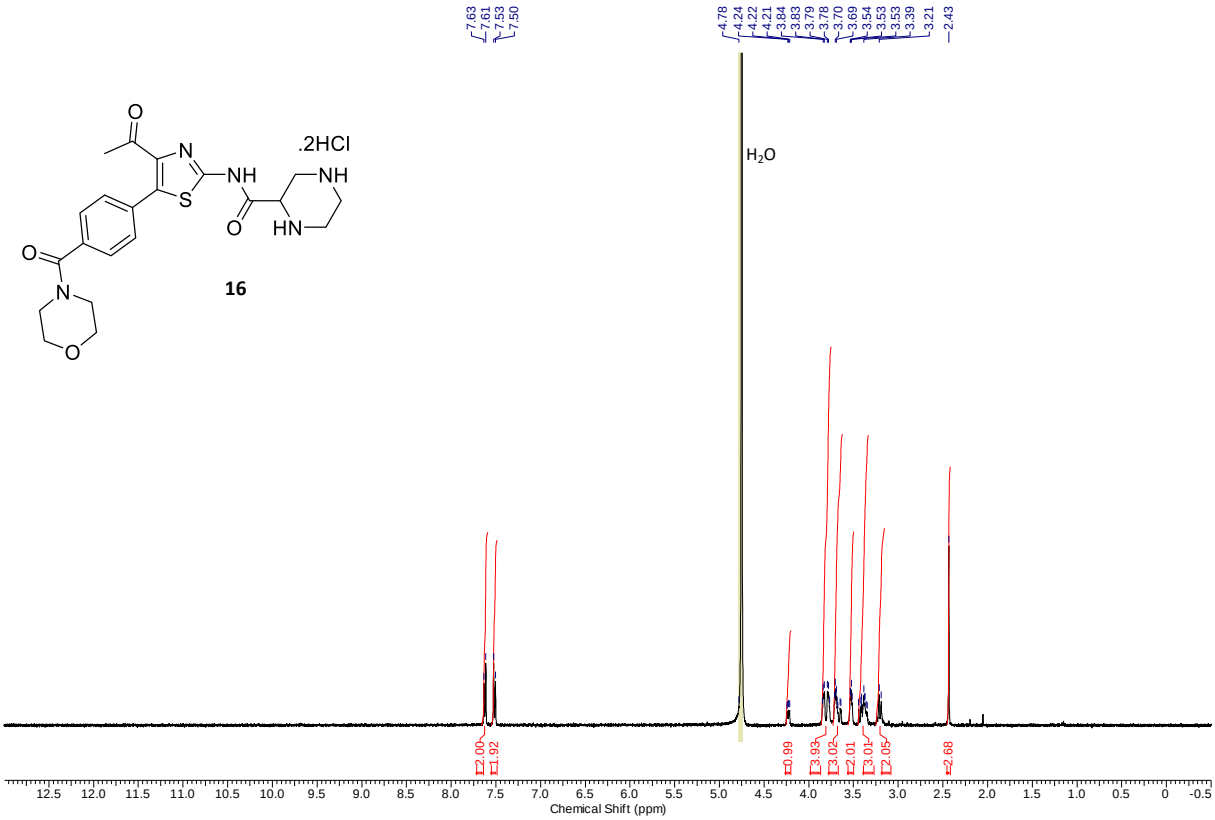
13

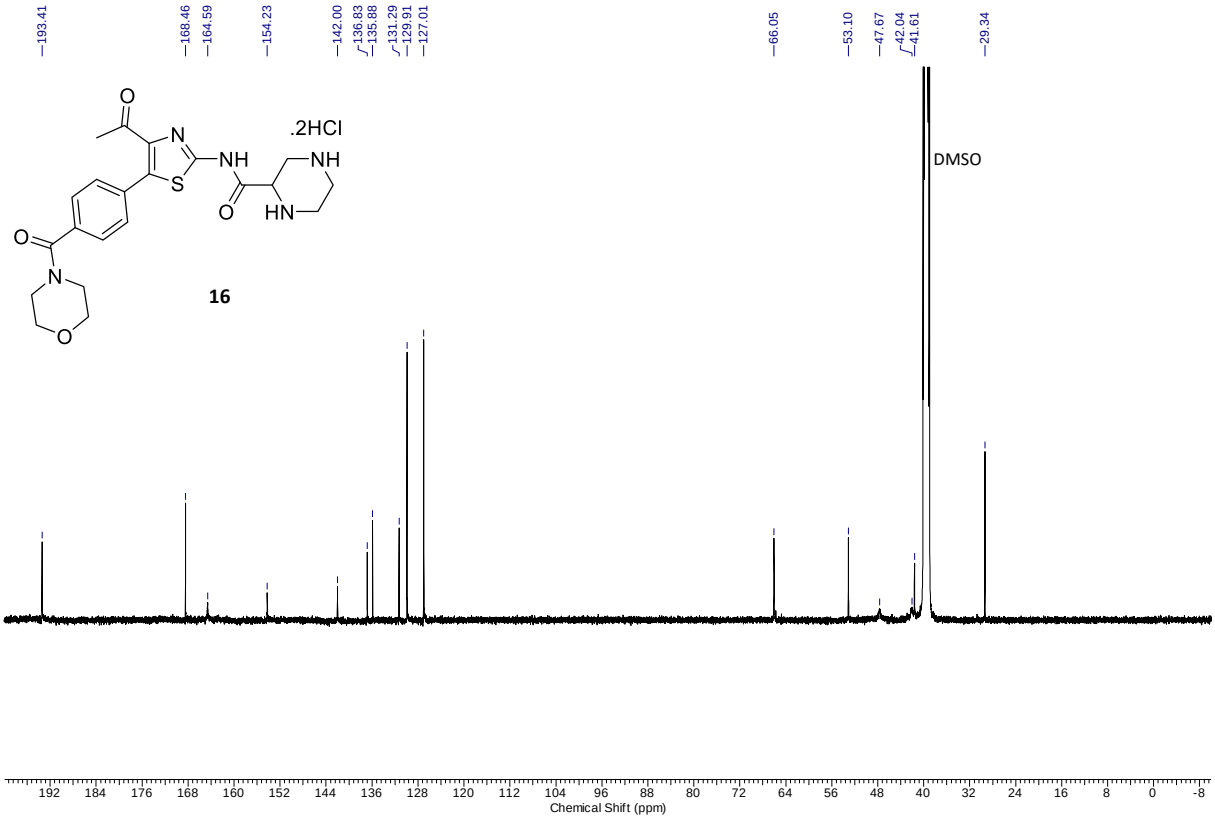

8.19
8.01
8.00
7.79
2.99
2.98
2.67
2.46
2.45
MeOH
MeOH
O
N
NH
S
H_2N
O
$NH_2.2HCl$
N
14
0.99
0.95
1.00
2.27
2.89
3.62
Chemical Shift (ppm)

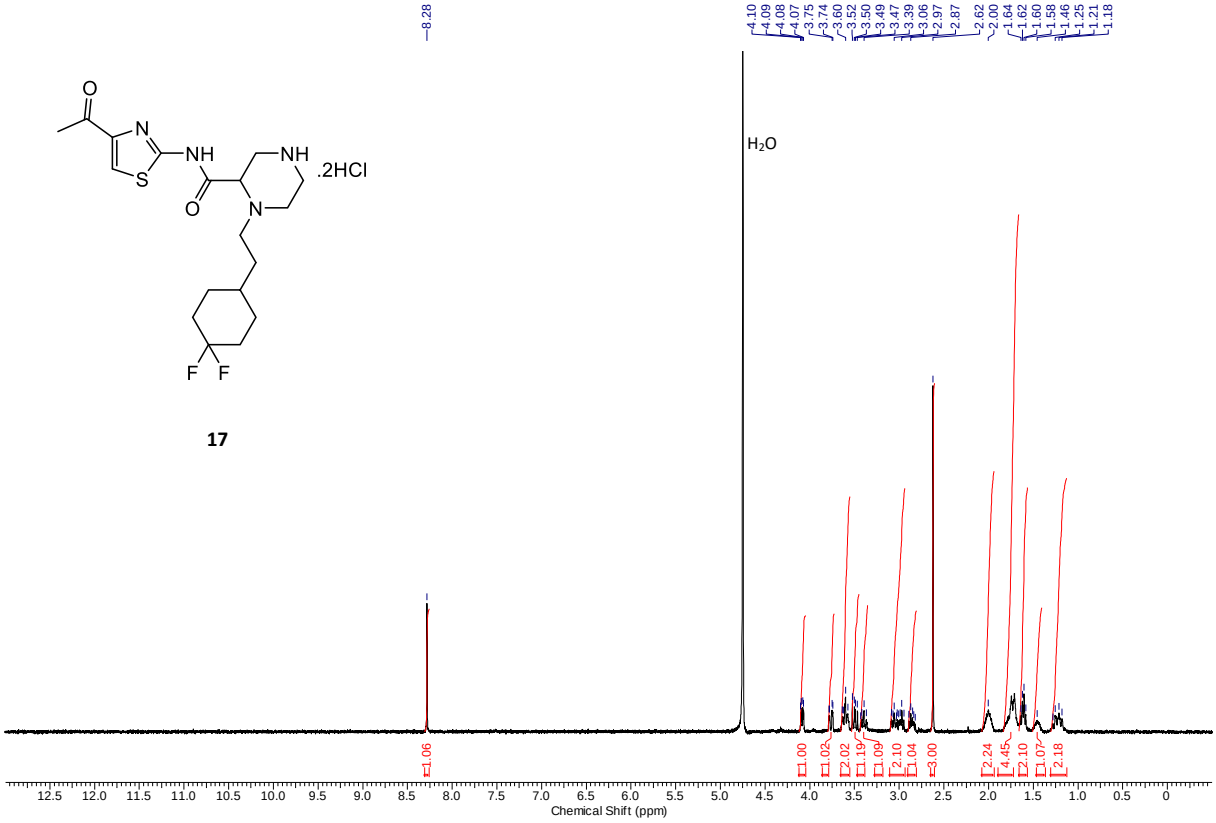

193.76
169.85
156.29
147.01
143.47
130.70
129.89
129.53
127.27
126.27
58.44
29.40
29.14
14.17
DMSO
O
N
NH
S
H_2N
O
$NH_2.2HCl$
N
14
Chemical Shift (ppm)


7.56
4.12
4.11
4.10
3.99
3.97
3.95
3.55
3.54
3.34
3.29
3.28
3.15
3.13
2.67
2.65
2.63
2.42
2.21
2.19
2.17
H2O
4.02
1.13
2.16
1.23
3.50
2.31
2.11
2.89
2.29
Chemical Shift (ppm)

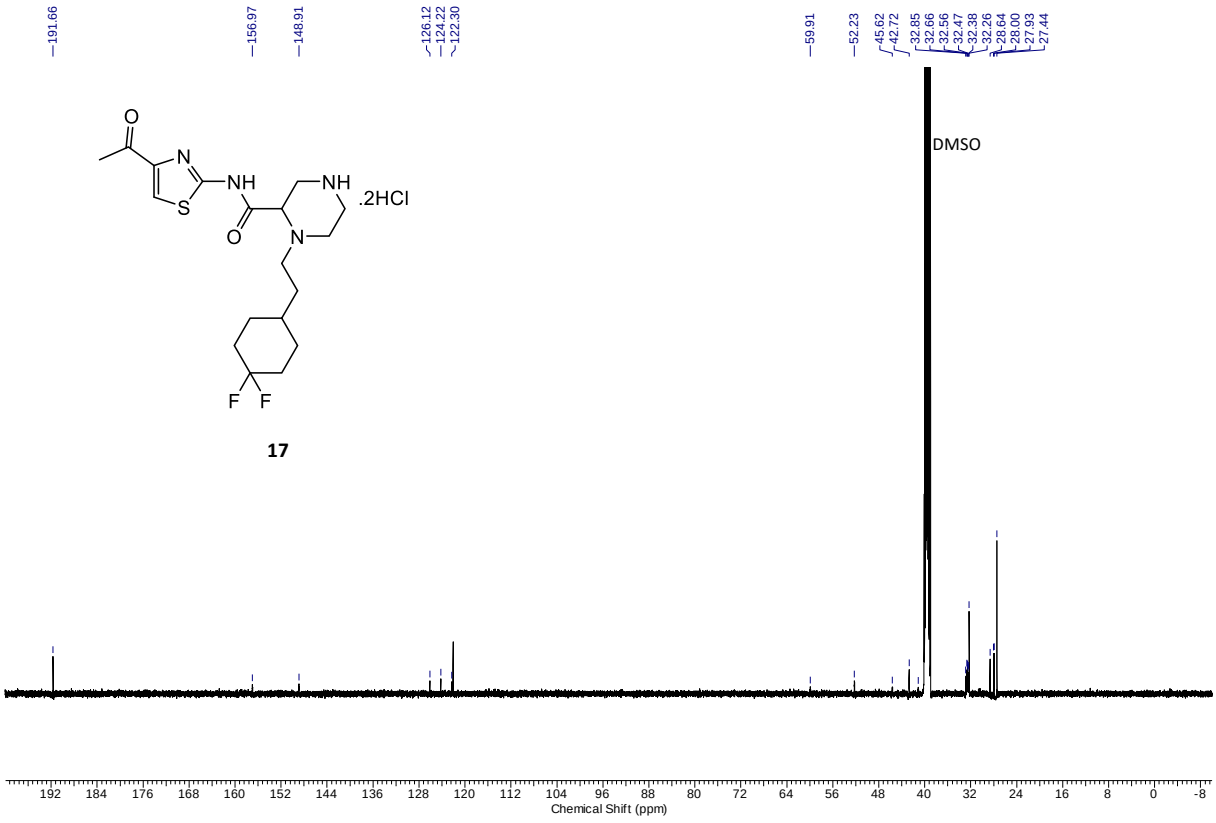

193.27
174.16
164.41
153.56
141.42
140.14
137.80
130.22
125.04
118.74
53.08
47.96
41.58
32.37
29.42
17.37
DMSO
Chemical Shift (ppm)


7.63
7.61
7.53
7.50
4.78
4.24
4.22
4.21
3.84
3.83
3.79
3.78
3.70
3.69
3.54
3.53
3.53
3.39
3.21
2.43
H2O
.2HCl
16
2.00
1.92
0.99
3.93
3.02
2.01
3.01
2.05
2.68
Chemical Shift (ppm)

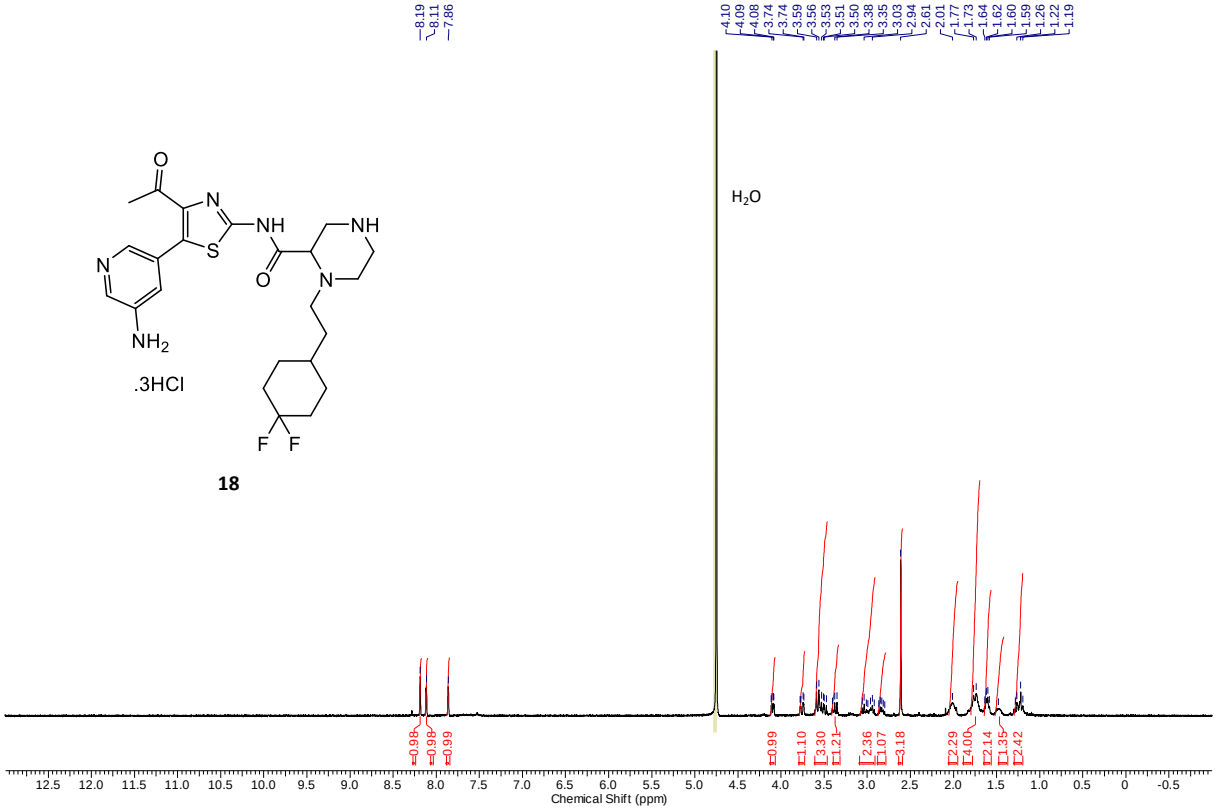

193.41
168.46
164.59
154.23
142.00
136.83
135.88
131.29
129.91
127.01
66.05
53.10
47.67
42.04
41.61
29.34
DMSO
.2HCl
16
Chemical Shift (ppm)

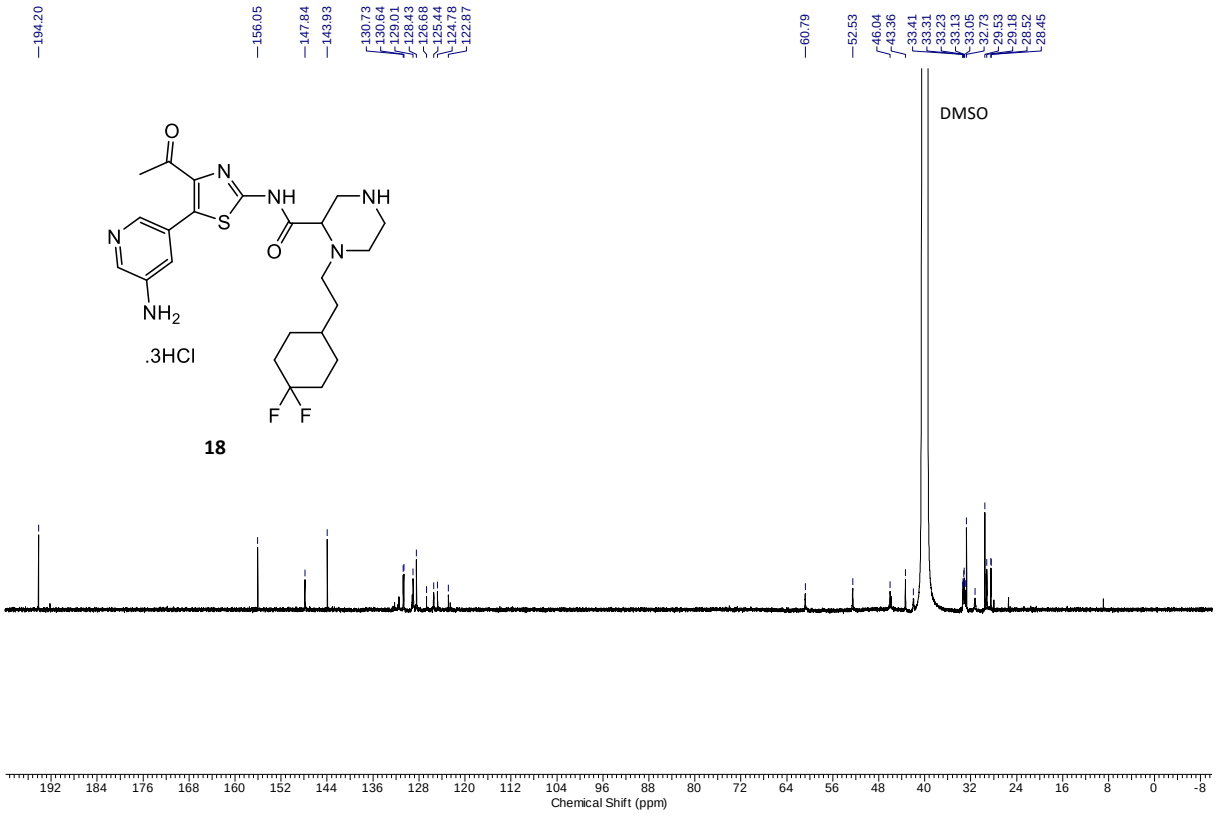

8.28
4.10
4.09
4.08
4.07
3.75
3.74
3.60
3.52
3.50
3.49
3.47
3.39
3.06
2.97
2.87
2.62
2.00
1.64
1.62
1.60
1.58
1.46
1.25
1.21
1.18
H2O
.2HCl
1.06
1.00
1.02
2.02
1.19
1.09
2.10
1.04
3.00
2.24
4.45
2.10
1.07
2.18
Chemical Shift (ppm)

17

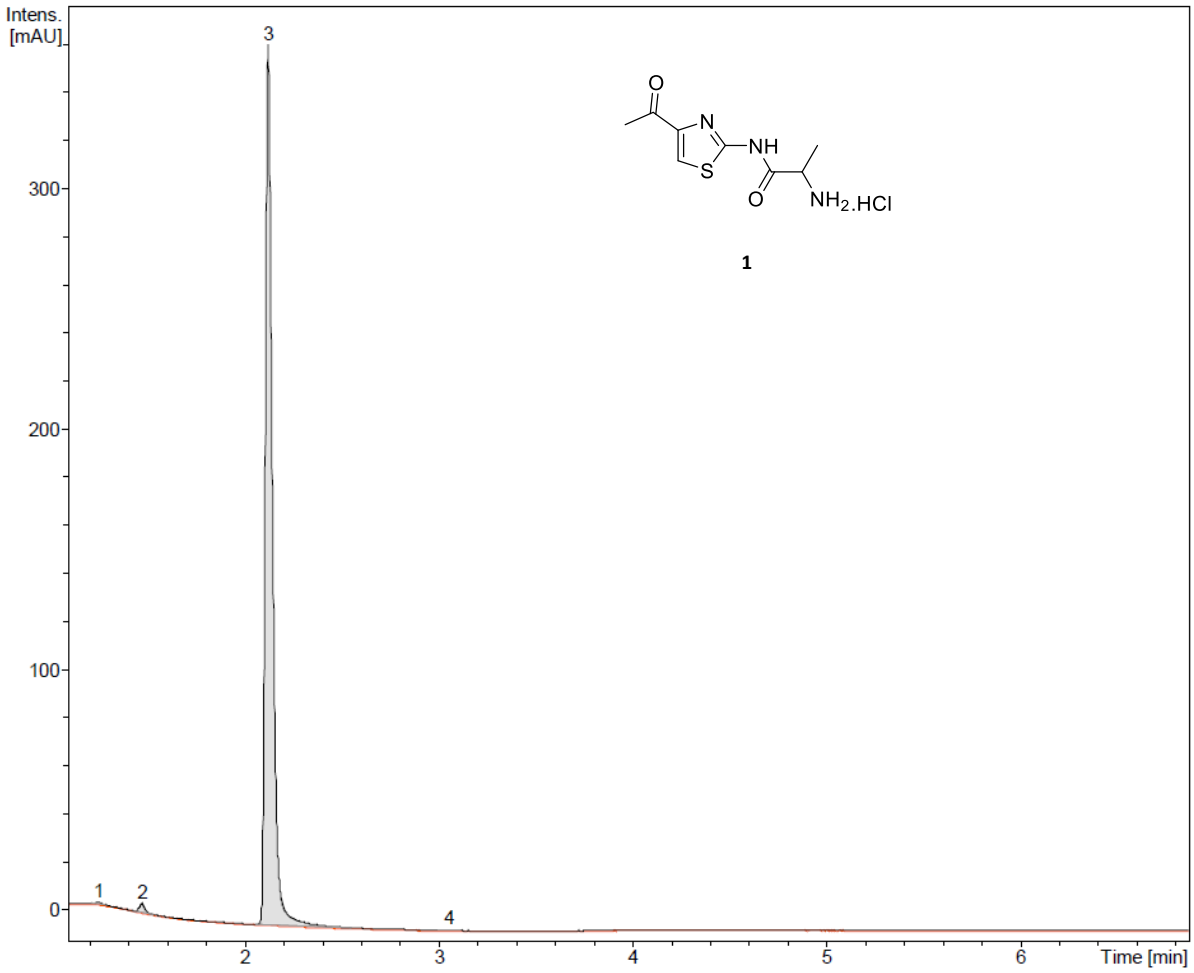

191.66
156.97
148.91
126.12
124.22
122.30
59.91
52.23
45.62
42.72
32.85
32.66
32.56
32.47
32.38
32.26
28.64
28.00
27.93
27.44
DMSO
.2HCl
Chemical Shift (ppm)

17


8.19
8.11
7.86
4.10
4.09
4.08
3.74
3.74
3.59
3.56
3.53
3.51
3.50
3.38
3.35
3.03
2.94
2.61
2.01
1.77
1.73
1.64
1.62
1.60
1.59
1.26
1.22
1.19
H2O
O
N
NH
NH
S
N
O
N
NH2
.3HCl
F F
18
0.99
0.98
0.99
1.10
3.30
1.21
2.36
1.07
3.18
2.29
4.00
2.14
1.39
2.42
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5
Chemical Shift (ppm)


194.20
156.05
147.84
143.93
130.73
130.64
129.01
128.43
126.68
125.44
124.78
122.87
60.79
52.53
46.04
43.36
33.41
33.31
33.23
33.13
33.05
32.73
29.53
29.18
28.52
28.45
DMSO
O
N
NH
NH
S
N
O
N
NH2
.3HCl
F F
18
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0 -8
Chemical Shift (ppm)

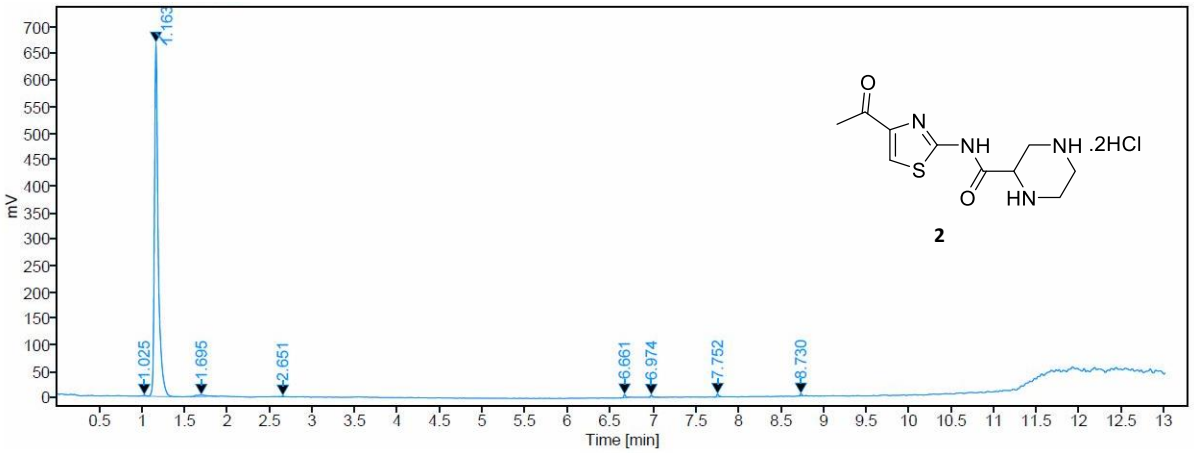
5. HPLC and ELSD traces of final compounds



1

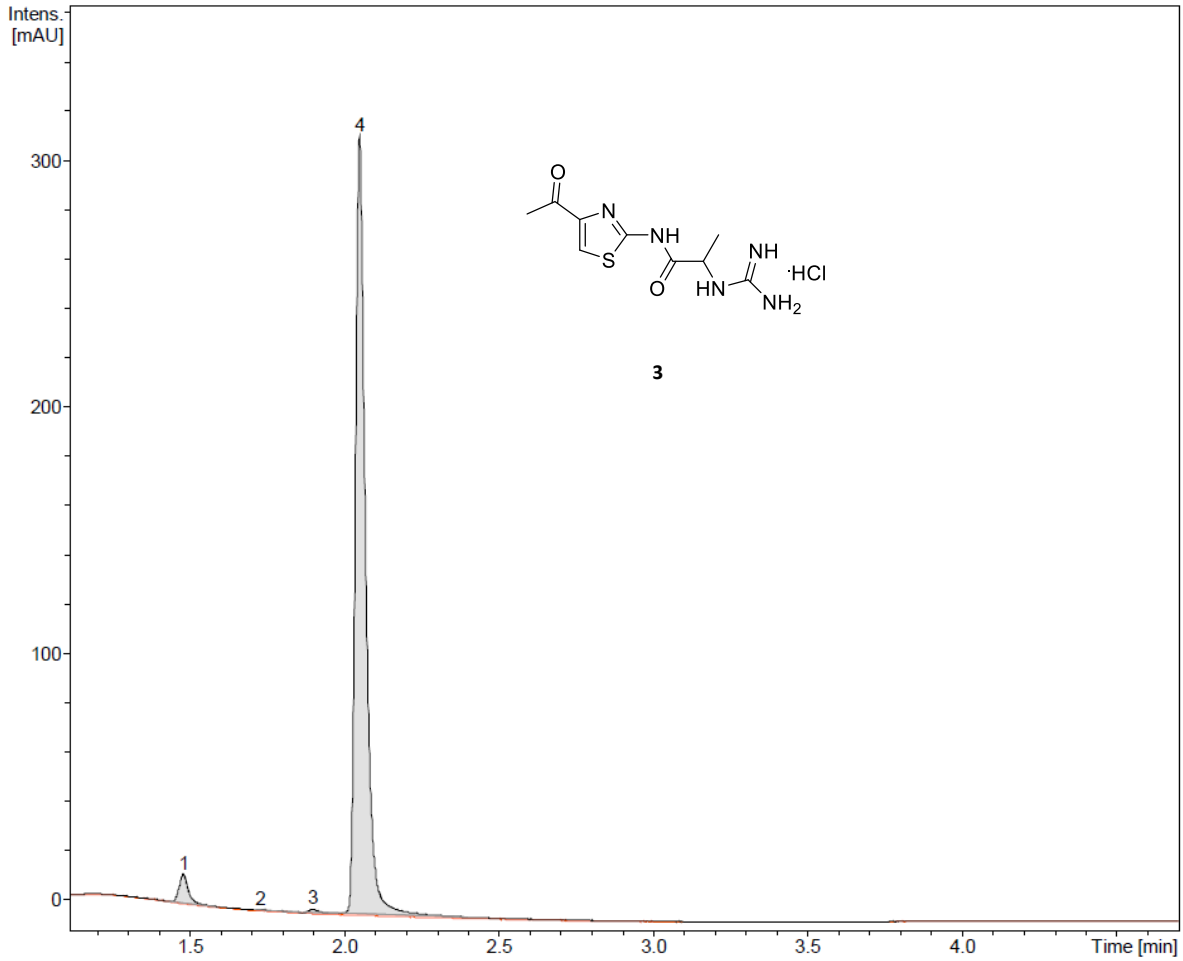
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.2	1.6776	0.18	Manual	UV Chromatogram, 254 nm
2	1.5	7.3129	0.77	Manual	UV Chromatogram, 254 nm
3	2.1	934.9105	98.98	Manual	UV Chromatogram, 254 nm
4	3.1	0.6616	0.07	Manual	UV Chromatogram, 254 nm



Purity measurement: Method 4

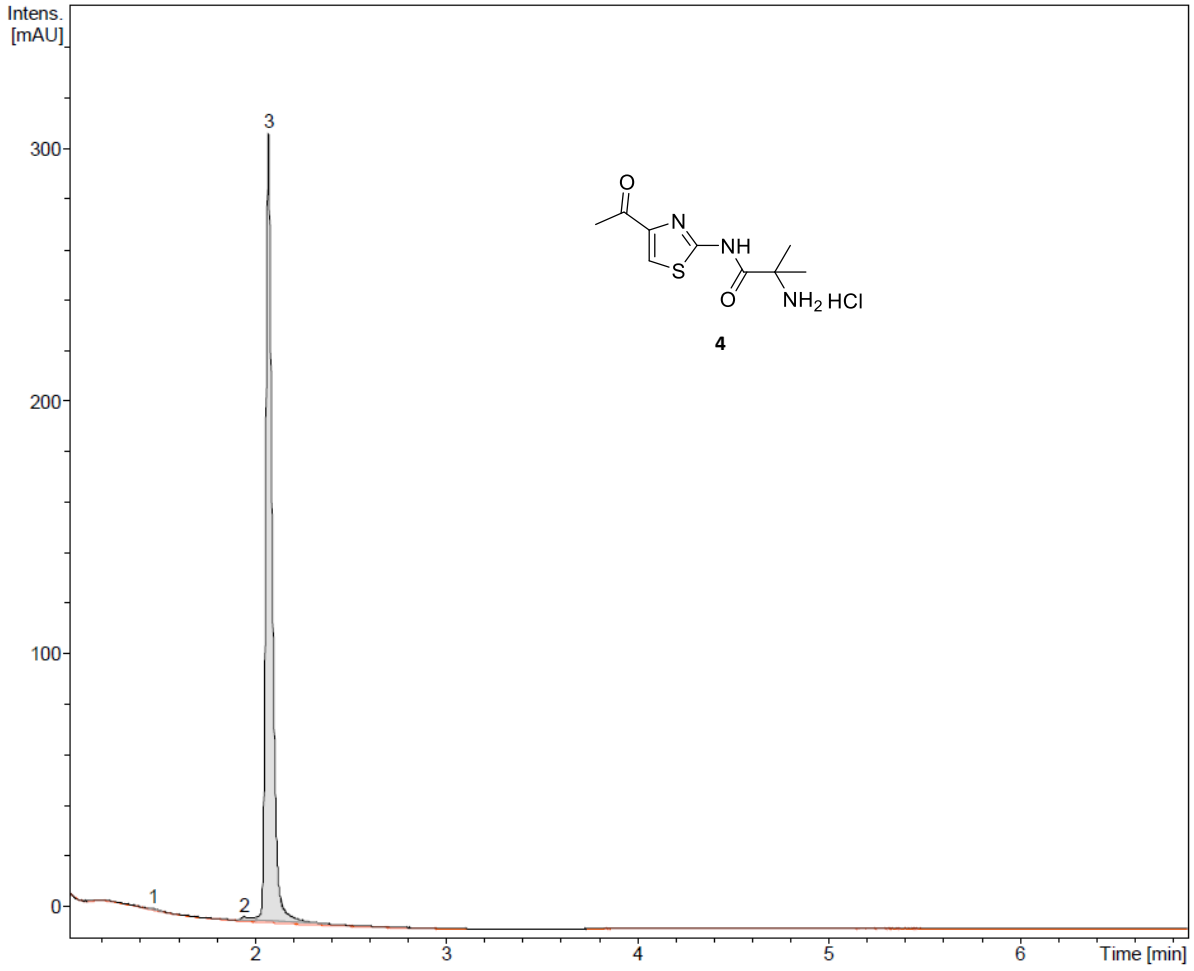
RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1.025	6.75	0.32	Manual	ELSD Chromatogram
1.163	2058.76	96.93	Manual	ELSD Chromatogram
1.695	25.49	1.20	Manual	ELSD Chromatogram
2.651	2.50	0.12	Manual	ELSD Chromatogram
6.661	8.66	0.41	Manual	ELSD Chromatogram
6.974	6.81	0.32	Manual	ELSD Chromatogram
7.752	9.66	0.45	Manual	ELSD Chromatogram
8.730	5.31	0.25	Manual	ELSD Chromatogram



3

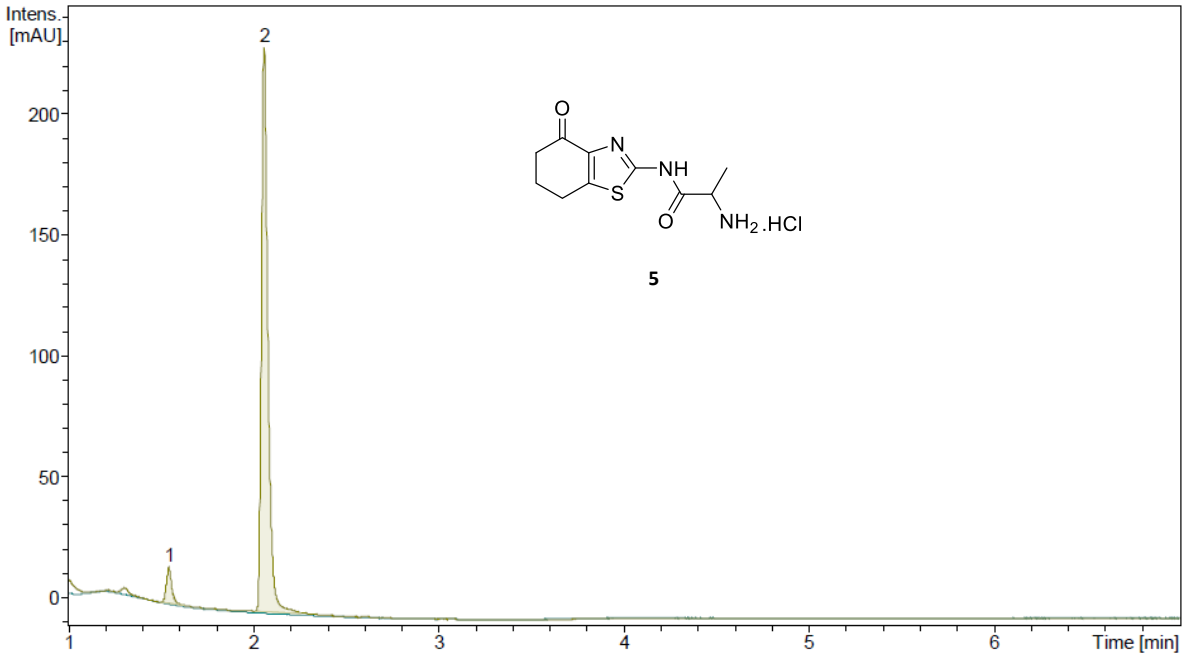
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.5	24.1811	3.22	Manual	UV Chromatogram, 254 nm
2	1.7	1.1567	0.15	Manual	UV Chromatogram, 254 nm
3	1.9	2.6641	0.35	Manual	UV Chromatogram, 254 nm
4	2.1	723.9791	96.28	Manual	UV Chromatogram, 254 nm



Purity measurement: Method 1

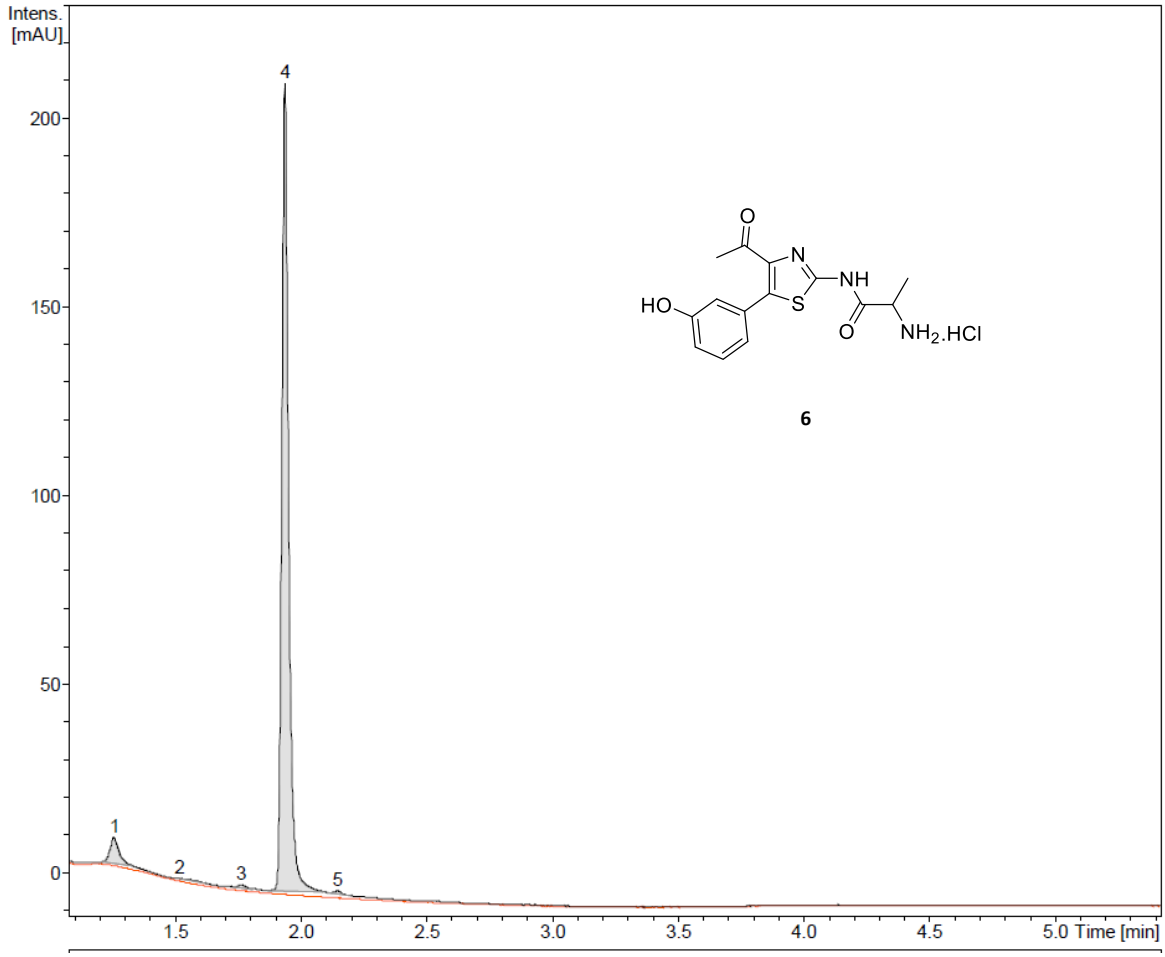
#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.5	1.0380	0.14	Manual	UV Chromatogram, 254 nm
2	1.9	5.4547	0.73	Manual	UV Chromatogram, 254 nm
3	2.1	736.9258	99.13	Manual	UV Chromatogram, 254 nm



5

Purity measurement: Method 1

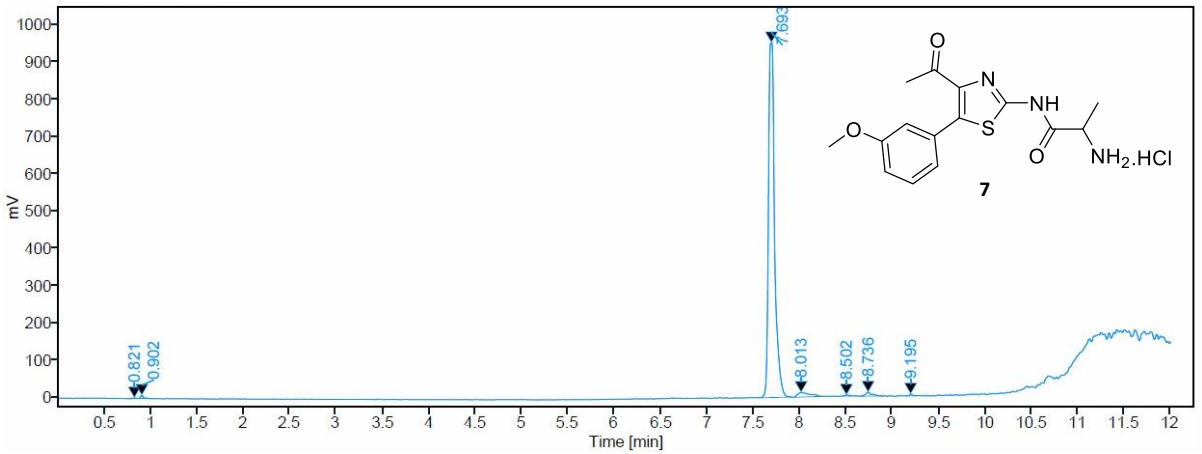
#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.5	29.651	4.99	Chromatogram	UV Chromatogram, 254 nm
2	2.1	564.629	95.01	Chromatogram	UV Chromatogram, 254 nm



6

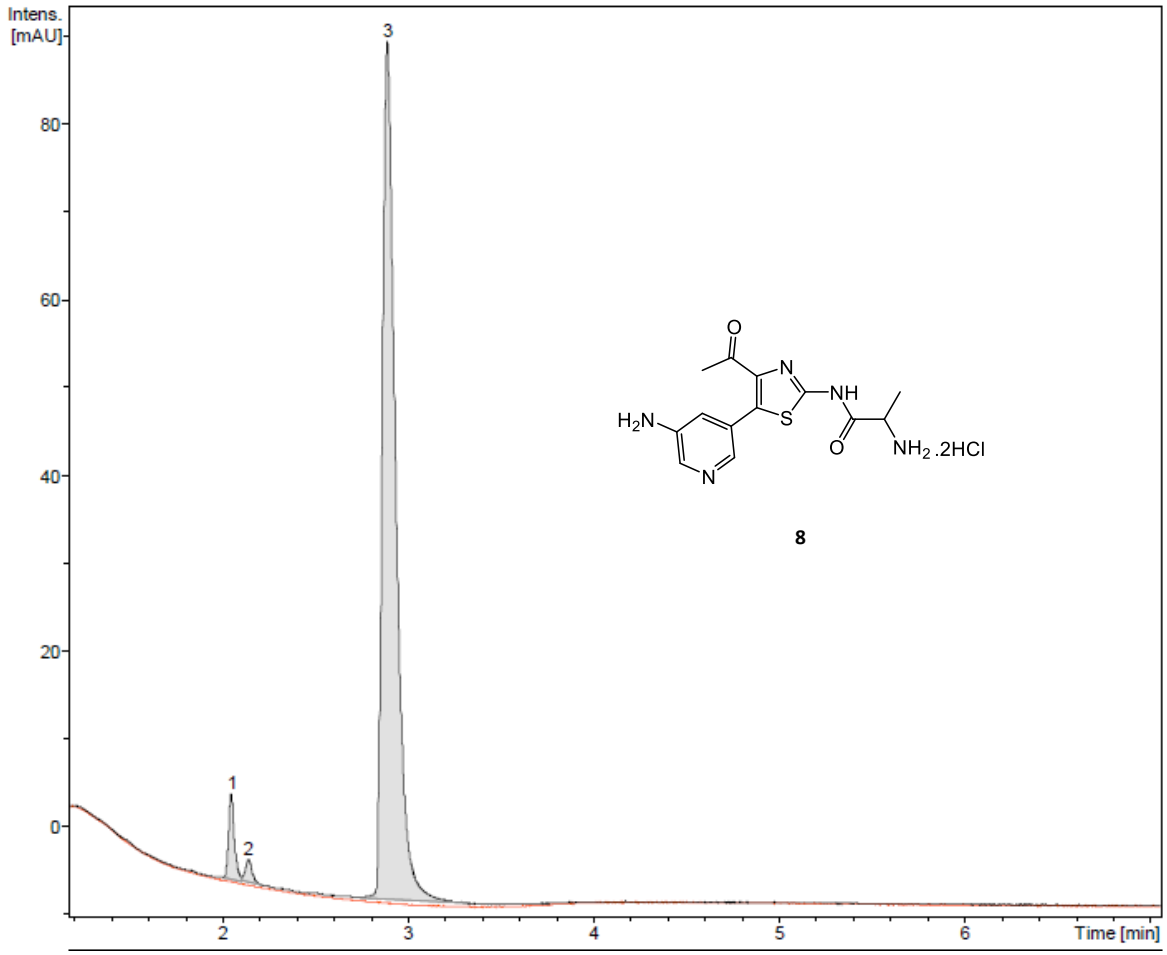
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.3	16.6755	3.96	Manual	UV Chromatogram, 254 nm
2	1.5	1.6060	0.38	Manual	UV Chromatogram, 254 nm
3	1.8	1.8905	0.45	Manual	UV Chromatogram, 254 nm
4	1.9	398.9413	94.82	Manual	UV Chromatogram, 254 nm
5	2.1	1.6165	0.38	Manual	UV Chromatogram, 254 nm



Purity measurement: Method 2

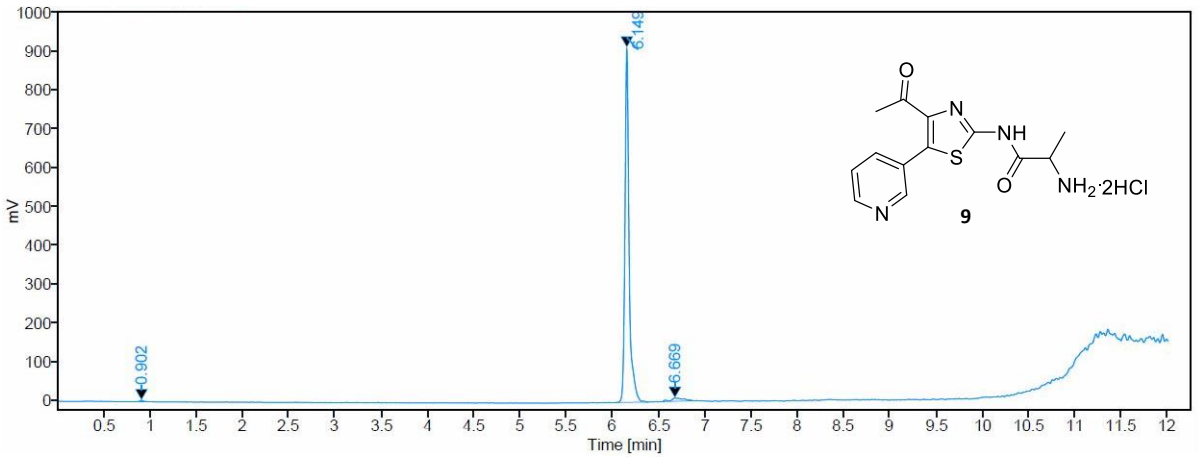
RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
0.821	3.18	0.07	Manual	ELSD Chromatogram
0.902	20.84	0.45	Manual	ELSD Chromatogram
7.693	4408.48	95.46	Manual	ELSD Chromatogram
8.013	113.43	2.46	Manual	ELSD Chromatogram
8.502	8.59	0.19	Manual	ELSD Chromatogram
8.736	53.71	1.16	Manual	ELSD Chromatogram
9.195	9.97	0.22	Manual	ELSD Chromatogram



8

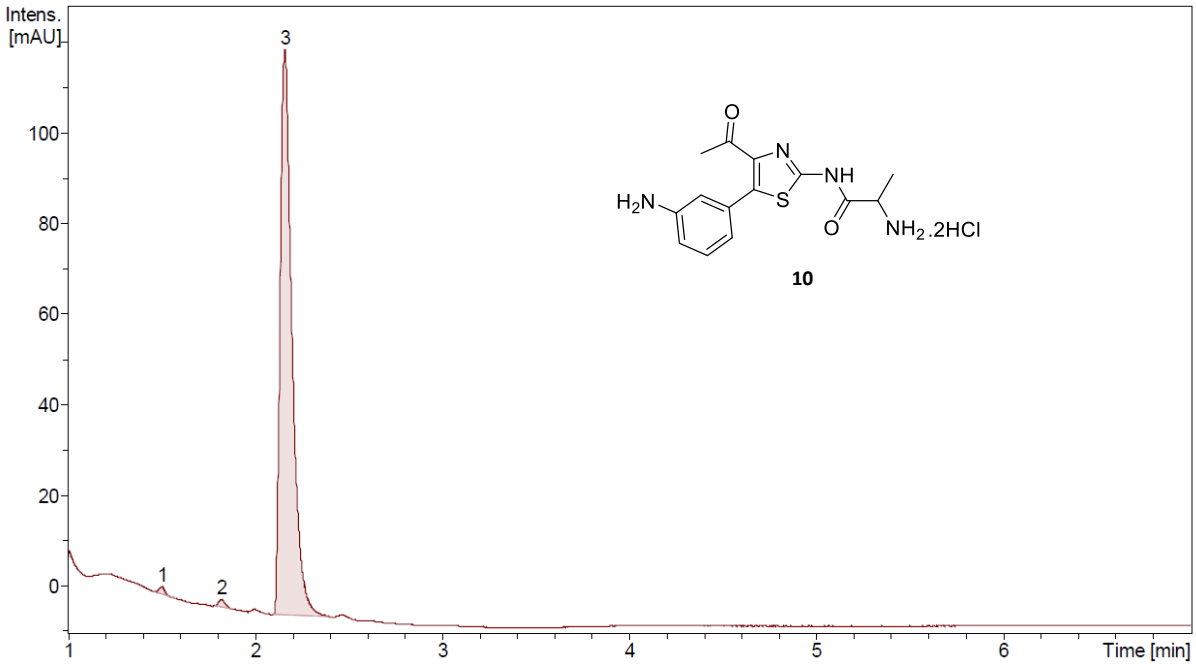
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	2.1	20.7469	4.02	Manual	UV Chromatogram, 254 nm
2	2.1	6.6104	1.28	Manual	UV Chromatogram, 254 nm
3	2.9	488.6316	94.70	Manual	UV Chromatogram, 254 nm



Purity measurement: Method 2

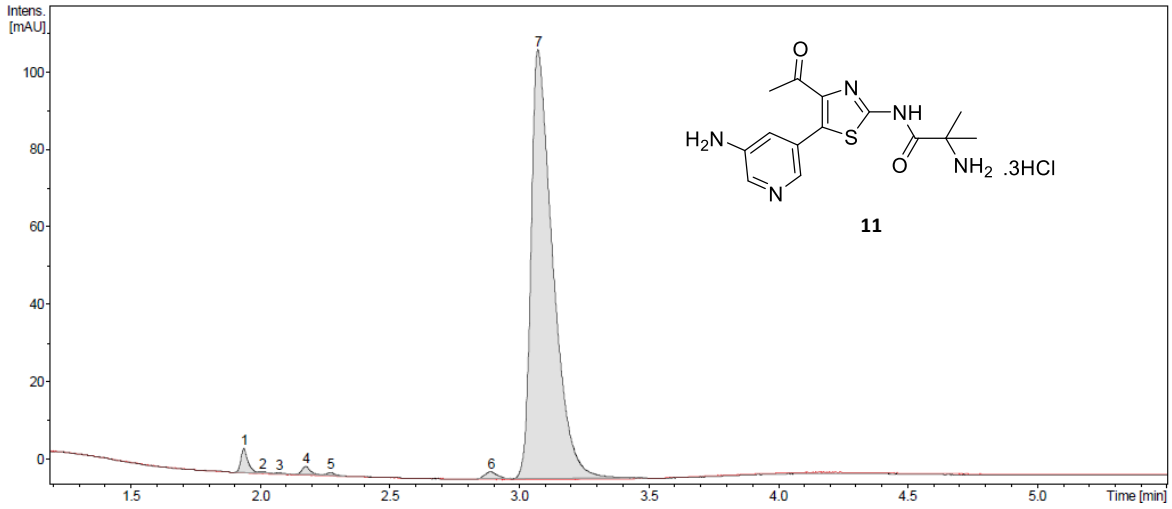
RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
0.902	9.65	0.33	Manual	ELSD Chromatogram
6.149	2855.64	96.92	Manual	ELSD Chromatogram
6.669	80.96	2.75	Manual	ELSD Chromatogram



10

Purity measurement: Method 1

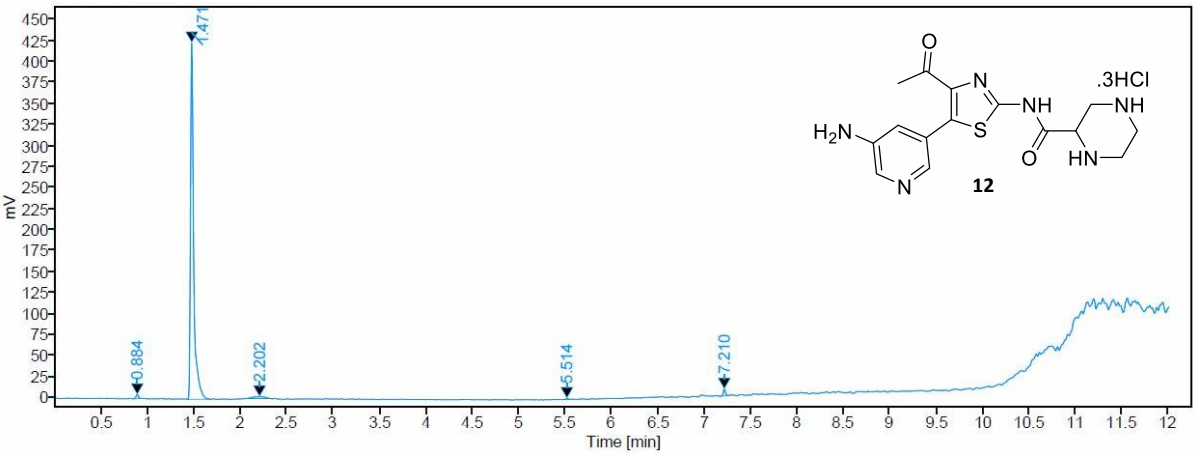
#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.5	3.1153	0.56	Chromatogram	UV Chromatogram, 254 nm
2	1.8	4.1067	0.74	Chromatogram	UV Chromatogram, 254 nm
3	2.2	546.8333	98.70	Chromatogram	UV Chromatogram, 254 nm



11

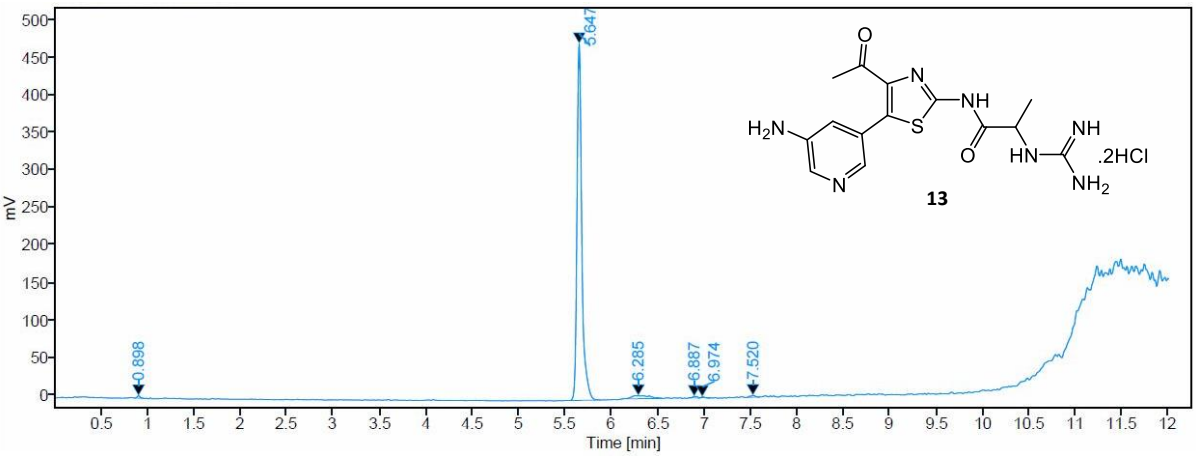
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.9	11.8841	1.377	Manual	UV Chromatogram, 254 nm
2	2.0	0.9066	0.14	Manual	UV Chromatogram, 254 nm
3	2.1	0.4734	0.07	Manual	UV Chromatogram, 254 nm
4	2.2	4.6269	0.69	Manual	UV Chromatogram, 254 nm
5	2.3	1.5767	0.24	Manual	UV Chromatogram, 254 nm
6	2.9	6.1761	0.92	Manual	UV Chromatogram, 254 nm
7	3.1	644.3562	96.17	Manual	UV Chromatogram, 254 nm



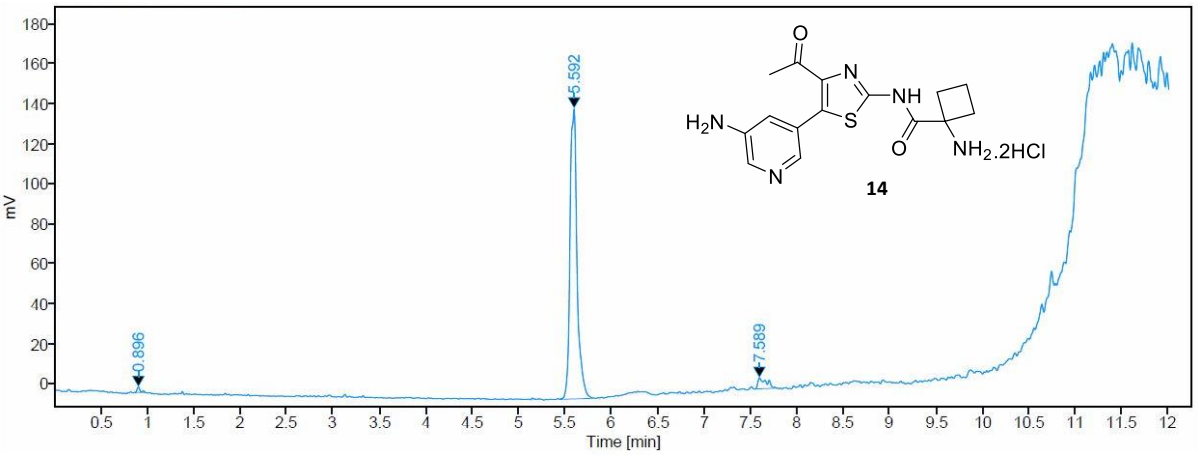
Purity measurement: Method 2

RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
0.884	8.64	0.82	Manual	ELSD Chromatogram
1.471	1003.54	95.71	Manual	ELSD Chromatogram
2.202	21.15	2.02	Manual	ELSD Chromatogram
5.514	1.92	0.18	Manual	ELSD Chromatogram
7.210	13.28	1.27	Manual	ELSD Chromatogram



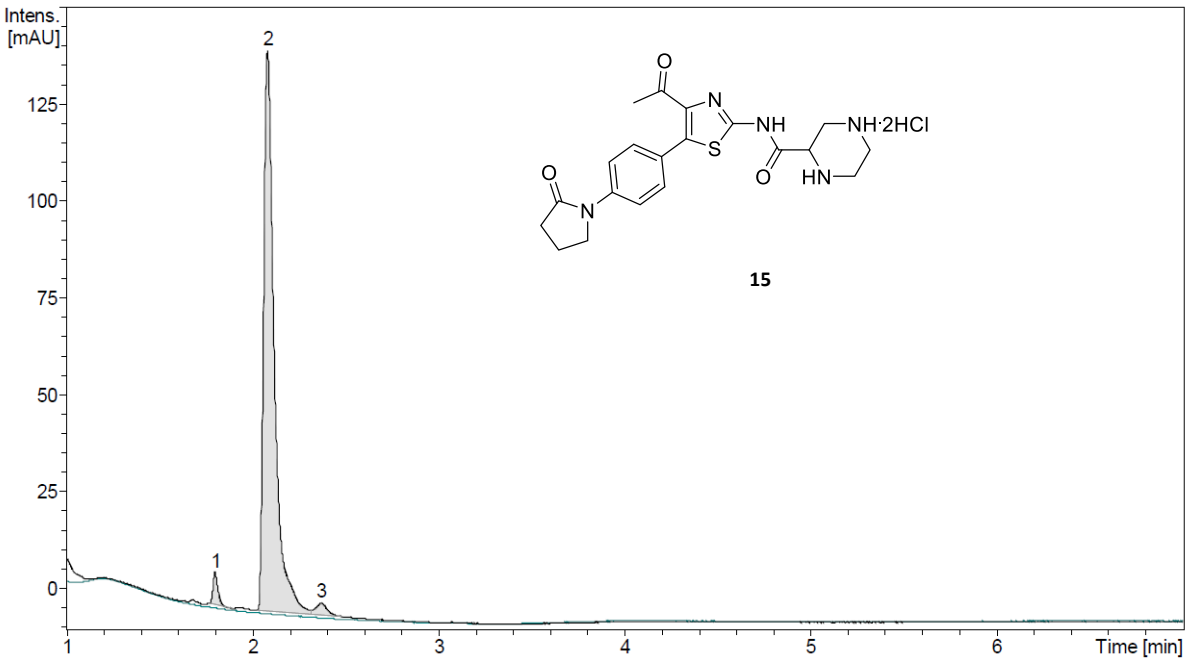
Purity measurement: Method 2

RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
0.898	8.02	0.45	Manual	ELSD Chromatogram
5.647	1714.11	95.33	Manual	ELSD Chromatogram
6.285	55.85	3.11	Manual	ELSD Chromatogram
6.887	7.11	0.40	Manual	ELSD Chromatogram
6.974	3.97	0.22	Manual	ELSD Chromatogram
7.520	9.00	0.50	Manual	ELSD Chromatogram



Purity measurement: Method 2

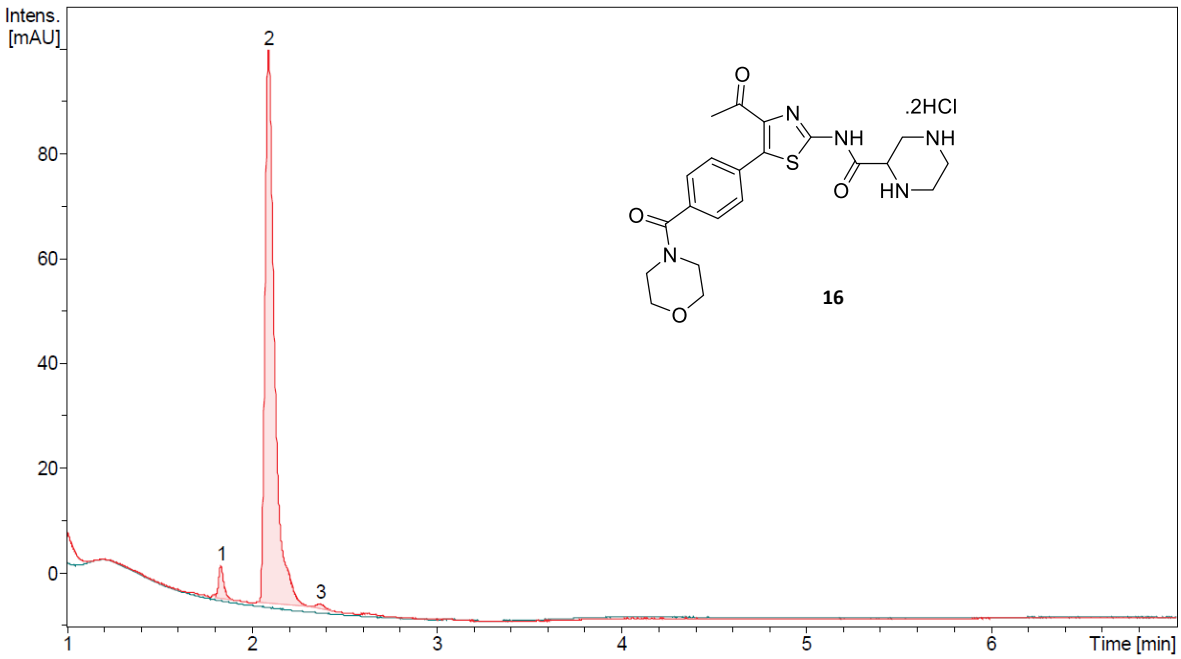
RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
0.896	6.49	0.81	Manual	ELSD Chromatogram
5.592	765.01	94.84	Manual	ELSD Chromatogram
7.589	35.10	4.35	Manual	ELSD Chromatogram



15

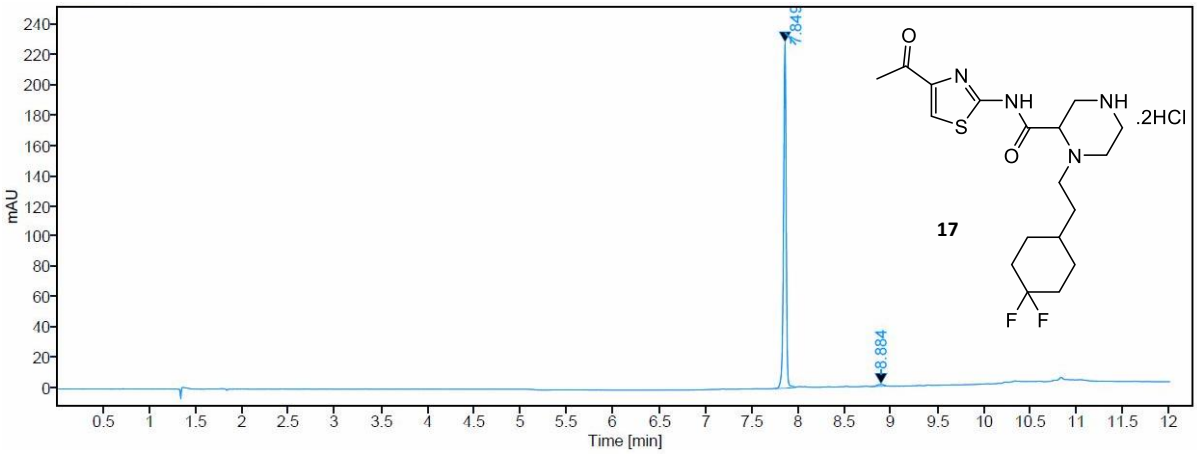
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.8	14.509	2.42	Chromatogram	UV Chromatogram, 254 nm
2	2.1	572.012	95.32	Chromatogram	UV Chromatogram, 254 nm
3	2.4	13.572	2.26	Chromatogram	UV Chromatogram, 254 nm



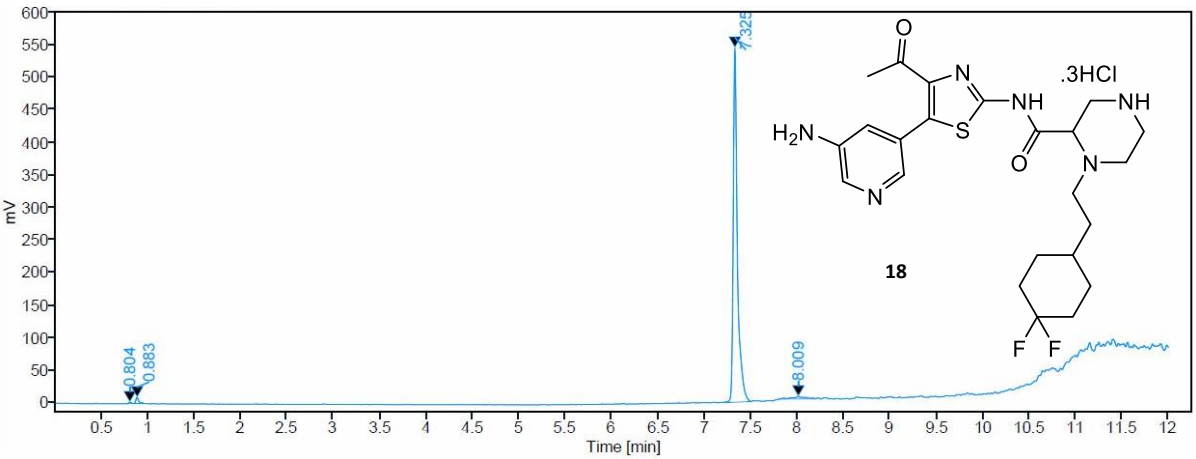
Purity measurement: Method 1

#	RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
1	1.8	12.0124	3.03	Chromatogram	UV Chromatogram, 254 nm
2	2.1	381.9789	96.28	Chromatogram	UV Chromatogram, 254 nm
3	2.4	2.7528	0.69	Chromatogram	UV Chromatogram, 254 nm



Purity measurement: Method 3

RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
7.849	443.06	98.61	Manual	UV Chromatogram, 254 nm
8.884	6.25	1.39	Manual	UV Chromatogram, 254 nm



Purity measurement: Method 2

RT [min]	Area	Area Frac. %	Int. Type	Chromatogram
0.804	4.23	0.24	Manual	ELSD Chromatogram
0.883	19.75	1.13	Manual	ELSD Chromatogram
7.325	1689.89	96.34	Manual	ELSD Chromatogram
8.009	40.20	2.29	Manual	ELSD Chromatogram