Exploring the Chemical Space of Benzothiazole-Based DNA Gyrase B Inhibitors

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1. EXPERIMENTAL SECTION

1.1. Determination of Inhibitory Activities on *E. coli* and *S. aureus* DNA Gyrase and Topoisomerase IV

The assay for the determination of IC_{50} values (Inspiralis) was performed on black streptavidin-coated 96-well microtiter plates (Thermo Scientific Pierce). The assay was performed according to previously described protocols.[1]

1.2. Determination of Antibacterial Activity

Clinical control strains of *Enterococcus faecalis* (Gram-positive, ATCC 29212), *Escherichia coli* (Gram-negative, ATCC 25922), *Pseudomonas aeruginosa* (Gram-negative, ATCC 27853), and *Staphylococcus aureus* (Gram-positive, ATCC 29213) were obtained from Microbiologics Inc. (St. Cloud, Minnesota, USA). Bacterial cultures were initiated on cation-adjusted MH (Mueller Hinton) agar (Becton Dickinson, Franklin Lakes, NJ, USA) slants and, prior to the assays, suspensions were prepared into cation-adjusted MH broth (Becton Dickinson, Franklin Lakes, NJ, USA) and incubated at 37°C for 16–20 h at 100 rpm. Antimicrobial assays were performed by the broth microdilution method in 96-well plate format according to the CLSI guidelines.[2] Briefly, bacterial suspension was diluted with MH broth to obtain a final inoculum of 5×10^5 CFU/mL in the assay. An equal volume of bacterial suspension and test compound solution diluted into assay media were mixed together on the plate and incubated for 24 h at 37°C. Absorbance values measured at 620 nm were used for evaluating the antimicrobial effects of test compounds by comparing to untreated controls and expressed as percentage inhibition of growth. Ciprofloxacin was used as positive control on every assay plate. MIC experiments against *E. coli* were carried out by using 8 different concentrations in the range of 6.25-200 µM (n = 3). Compounds were additionally assayed against *E. faecalis*, *P. aeruginosa* and *S. aureus* at a final concentration of 50 µM (n = 3).

2. Crystallography

2.1. Protein purification and crystallization

E. coli GyrB24 protein, purified as described previously,[3] was a gift from Sara Henderson (John Innes Centre).

Crystals were grown using vapour diffusion using GyrB24 at 10.6 mg/ml in the presence of 1 mM inhibitor. Screening of crystallisation conditions was performed using commercially available (Molecular Dimensions) and in-house screens. Drops containing 0.3 µl protein solution and 0.3 µl of precipitant were set up using an Oryx 8 liquid handling robot (Douglas Instruments) and equilibrated against 50 µl reservoir solution at a constant temperature of 19˚C. Crystals suitable for harvesting grew from a precipitant containing 0.1 M MIB buffer (malonate, imidazole, borate) pH 4.0; 25% (w/v)

PEG 1500. These grew to full size over a period of four weeks and were harvested for data collection in precipitant supplemented with 25% (v/v) ethylene glycol using LithoLoops (Molecular Dimensions). The harvested crystals were then flash-cooled in liquid nitrogen for transport to Diamond Light Source (Oxfordshire, UK).

2.2. X-ray data collection and structure solution

Pre-cooled crystals were robotically transferred to the goniostat on beamline I04 at Diamond Light Source (Oxford, UK) before X-ray diffraction data were collected at 100 K at a wavelength of 0.9795 Å. A total of 3600 x 0.1° images were recorded using an Eiger2 XE 16M pixel array detector (Dectris). The data were integrated and scaled using DIALS[4] via the XIA2 expert system[5] and merged using AIMLESS[6] to a maximum resolution of 1.6 Å; the resultant data statistics are shown in Table 1. All successive data processing was carried out using programs in the CCP4 suite via the CCP4i2 graphical user interface.[7] The GyrB24-16a structure was solved via molecular replacement in PHASER[8] using a previously solved *E. coli* GyrB24 inhibitor complex as the template (6F86).[9] Following refinement with REFMAC5[10] the density for **16a** could be clearly seen. The **16a** compound was drawn using AceDRG[11] and built into this density. Following multiple iterations of model building using COOT[12], and refinement through REFMAC5, the final model was validated using MOLPROBITY[13], PDB-REDO[14] and the PDB-validation server (https://validate-rcsb-2.wwpdb.org). The refinement and validation statistics are summarized in Table 1. Omit mF_{obs}-DF_{calc} difference electron density for the bound ligand was calculated using phases from the final model without the ligand after the application of small random shifts to the atomic coordinates, re-setting temperature factors, and re-refining to convergence. Figure 2 was prepared using CCP4mg.[15]

Table 1 - Summary of X-ray data and model parameters for EcGyrB24 with 16a

Data set	EcGyrB24 – 16a
Data collection	
Diamond Light Source beamline	I04 DLS, UK
Wavelength (Å)	0.9795
Detector	Eiger2 XE 16M
Resolution range (Å)	41.18-1.60 (1.63-1.60)
Space Group	$P2_12_12_1$
Cell parameters (Å)	a = 41.18, b = 67.14, c = 68.97
Total no. of measured intensities	324943 (9895)
Unique reflections	25674 (1062)
Multiplicity	12.7 (9.3)
Mean $I/\sigma(I)$	7.2 (1.8)

Completeness (%)	98.9 (86.0)
R_{merge}[a]	0.190 (1.160)
R_{meas}[b]	0.202 (1.234)
$CC_{½}$[c]	0.993 (0.778)
Wilson *B* value (Å^2)	16.1
Refinement	
Resolution range (Å)	35.38–1.60 (1.64-1.60)
Reflections: working/free[d]	24258/1346 (1543/95)
R_{work}/R_{free}	0.182/0.214 (0.260/0.272)
Ramachandran plot: favoured/allowed/disallowed[f] (%)	98.45/1.03/0.52
R.m.s. bond distance deviation (Å)	0.010
R.m.s. bond angle deviation (º)	1.59
No. of protein residues: ranges	196: 4-99, 117-218
No. of molecules: water/ethylene glycol/16a	196/2/1
Mean *B* factors: protein/ligands/waters (Å^2)	21.5/27.2/31.5
RSCC score for 16a[g]	0.97
PDB accession code	6YD9

Values in parentheses are for the outer resolution shell.

[a] $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$.

[b] $R_{meas} = \sum_{hkl} [N/(N-1)]^{1/2} \times \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th observation of reflection *hkl*, $\langle I(hkl)\rangle$ is the weighted average intensity for all observations *i* of reflection *hkl* and *N* is the number of observations of reflection *hkl*.

[c] $CC_{½}$ is the correlation coefficient between symmetry equivalent intensities from random halves of the dataset.

[d] The data set was split into "working" and "free" sets consisting of 95 and 5% of the data respectively. The free set was not used for refinement.

[e] The R-factors R_{work} and R_{free} are calculated as follows: $R = \sum(|F_{obs} - F_{calc}|)/\sum|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively.

[f] From MolProbity.[27]

[g] From the PDB validation server.

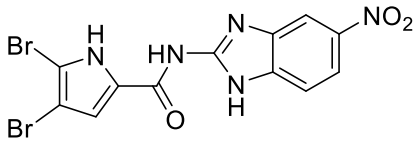
3. Chemistry

3.1. General Procedures

Chemicals were obtained from Apollo Scientific (Stockport, UK), Sigma-Aldrich (St. Louis, MO, USA) and Acros Organics (Geel, Belgium), and used without further purification. Analytical TLC was performed on silica gel Merck 60 F254 plates (0.25 mm), using visualization with UV light and spray reagents. Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). High resolution mass spectra were obtained using a Exactive™ Plus Orbitrap Mass Spectrometer (Thermo Fischer Scientific, Waltham, MA, USA) or low resolution mass spectra on ADVION expression CMS^L mass spectrometer (Advion, Ithaca, USA). HPLC analyses were performed on an Agilent Technologies 1100 instrument (Agilent Technologies, Santa Clara, CA, USA) with a G1365B UV-Vis detector, a G1316A thermostat and a G1313A autosampler using a Phenomenex Luna 5-µm C18 column (4.6 × 150 mm or 4.6 × 250 mm, Phenomenex, Torrance, CA, USA) and a flow rate of 1.0 mL/min. The mobile phase consisted of trifluoroacetic acid (0.1% in water) as solvent A and methanol or acetonitrile as solvent B. Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA, USA) in DMSO-d_6 or $CDCl_3$ solutions, with TMS as the internal standard. The purity of the tested compounds was established to be ≥95% (λ = 254 nm).

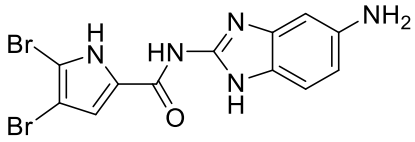
3.2. Synthetic Procedures

3.2.1. Synthesis of 4,5-dibromo-*N*-(6-nitro-1*H*-benzo[*d*]imidazol-2-yl)-1*H*-pyrrole-2-carboxamide (**2**)



6-nitro-1*H*-benzo[d]imidazol-2-amine (**1,** 0.534 g, 3 mmol) was dissolved in DMF with 1.1 eq of 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (1.22 g, 3.3 mmol) and Na_2CO_3 (0.35 g, 3.3 mmol). Reaction mixture was stirred at 80 °C for 16h. After the reaction was complete, the solvent was removed under reduced pressure and the crude product was dissolved in 50 mL of EtOAc and washed with water (50 mL), $NaHCO_3$ (50 mL), citric acid (50 mL) and brine (50 mL). Organic phase was dried over Na_2SO_4, filtered and evaporated to obtain **2** as beige solid (0.90 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.52 (s, 1H, Ar-H), 7.63 (bs, 1H, Ar-H), 8.08 (dd, 1H, *J* = 8.8, 2.3 Hz, Ar-H), 8.37 (bd, 1H, *J* = 29.5 Hz, Ar-H), 12.06 (bs, 1H, NH), 12.74 (d, 1H, *J* = 18.2 Hz, NH), 13.14 (s, 1H, NH).

3.2.2. Synthesis of *N*-(6-amino-1*H*-benzo[*d*]imidazol-2-yl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**3**)

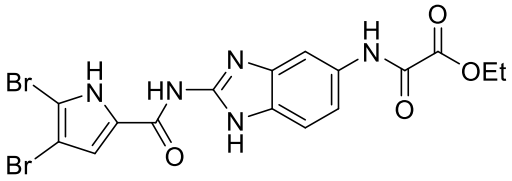


Compound **2** (0.44 g, 1.02 mmol) was dissolved in ethanol (50 mL) and reduced with tin chloride dihydrate (1.16 g, 5.12 mmol) under reflux overnight. Solvent was removed under reduced pressure and crude solid was dissolved in ethyl acetate (50 mL) and washed with 1M sodium hydroxide (50 mL). Organic phase was further dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Product was additionally purified via column chromatography using DCM:MeOH 7:1 as mobile phase to obtain brown solid **3** (0.190 g, 47%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.92 (s, 2H, NH_2), 6.44 (dd, 1H, *J* = 8.4, 2.1 Hz, Ar-H-5), 6.63 (d, 1H *J* = 2.1 Hz, Ar-H-7), 7.05 (s, 1H, pyrrole-CH), 7.08 (dd, 1H, *J* = 8.4 Hz, Ar-H-4), 10.63-13.12 (m, 3H, 3 × NH).

3.2.3. General procedure for the synthesis of the compounds **4a** and **4b**

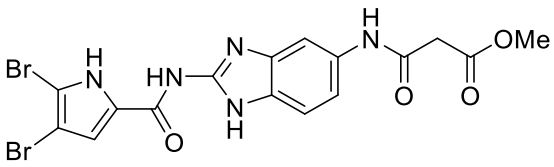
A solution of **3** (0.10 g, 0.35 mmol) and triethylamine (0.06 mL, 0.42 mmol) was dissolved in 1,4-dioxane (10 mL) and cooled at 0 °C. Ethyl oxalyl chloride (0.047 mL, 0.42 mmol) or methyl malonyl chloride (0.045 mL, 0.42 mmol) was added dropwise. Reaction mixture was stirred for 12 hours at room temperature and then quenched with saturated $NaHCO_3$ solution. The solvent was removed under reduced pressure. Crude solid was washed with saturated $NaHCO_3$ solution (20 mL), 10% citric acid solution (20 mL) and water (20 mL). Product was filtered and dried to obtain solid product.

3.2.3.1. Ethyl 2-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)benzo[*d*]imidazol-6-yl)amino)-2-oxoacetate (**4a**)



Beige solid, (0.160 g, 91%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.33 (t, 3H, *J* = 7.1 Hz, CH_3), 4.32 (q, 2H, *J* = 7.1 Hz, CH_2), 7.35 (s, 1H, Ar-H), 7.41 (s, 2H, 2 × Ar-H), 8.00 (s, 1H, Ar-H), 10.74 (s, 1H, NH), 12.11 (s, 2H, 2 × NH), 12.98 (s, 1H, NH).

3.2.3.2. Methyl 3-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)-1*H*-benzo[*d*]imidazol-6-yl)amino)-3-oxopropanoate (**4b**)

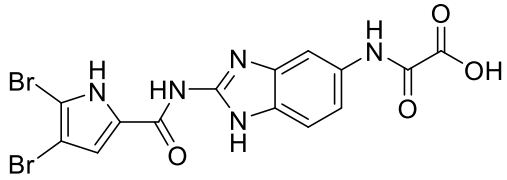


Brown solid, (0.15 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.48 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 7.19 (dd, *J* = 8.5, 2.1 Hz, 1H, Ar-H-5), 7.32 (s, 1H, pyrrole-CH), 7.37 (d, *J* = 8.5 Hz, 1H, Ar-H-4), 7.91 (d, *J* = 2.4 Hz, 1H, Ar-H-7), 10.16 (s, 1H, NH), 12.06 (s, 2H, 2 × NH), 12.95 (s, 1H, NH).

3.2.4. General procedure for the synthesis of the compounds **5a** and **5b**.

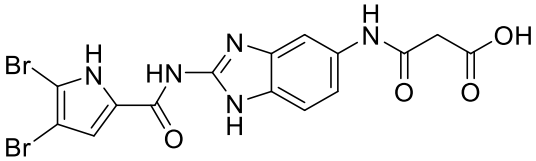
Compound **4a** or **4b** (0.1 mmol) was dissolved in 1,4-dioxane (15 mL) and hydrolysed with 1M NaOH (0.3 mmol) at 40 °C for 6h. Solvent was removed under reduced pressure. Aqueous solution of the crude product (10 mL) was extracted with 10 mL of EtOAc to remove impurities, water phase was then acidified with 1M hydrochloric acid to pH~4 and formed precipitate was filtered to obtain a solid product.

3.2.4.1. 2-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)-1*H*-benzo[*d*]imidazol-6-yl)amino)-2-oxoacetic acid (**5a**)



Beige solid (0.035 g, 72%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.74 – 7.52 (m, 3H, 3 × Ar-H), 8.28 (s, 1H, Pyrr-CH), 10.92 (s, 1H, NHCOCOOH), 13.35 (bs, 3H, 2 × NH (2-aminoimidazol) and COOH).

3.2.4.2. 3-((2-(4,5-dibromo-1*H*-pyrrole-2-carboxamido)-1*H*-benzo[*d*]imidazol-6-yl)amino)-3-oxo-propanoic acid (**5b)**



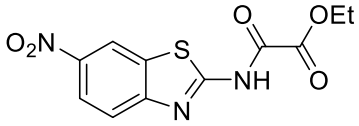
Brown solid (0.019 g, 20%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.20 (dd, 1H, *J* = 8.6, 2.0 Hz, Ar-H^5), 7.32 (s, 1H, pyrrole-CH), 7.39 (d, 1H, *J* = 8.6, Ar-H^4), 7.94 (d, 1H, *J* = 2.0 Hz, Ar-H^7), 10.12 (s, 1H, NHCOCH$_2$), 12.24 (bs, 2H) and 12.98 (bs, 1H) (Pyrr-NH, 2 × NH (2-aminoimidazol) and COOH).

3.2.5. General procedure for the synthesis of the compounds **7a, 7b** and **7c**

A solution of 6-nitrobenzo[*d*]thiazol-2-amine (**6**, 1.95 g, 10 mmol) and triethylamine (2.09 mL, 15 mmol) in 1,4-dioxane (100 mL) was cooled at 0 °C. Ethyl oxalyl chloride (1.68 mL, 15 mmol) or methyl malonyl chloride (1.61 mL, 15 mmol) or acetyl chloride (1.61 mL, 15 mmol) was added dropwise over 10 minutes. Reaction mixture was stirred for 2 hours at room temperature, and quenched with saturated $NaHCO_3$ solution. The solvent was removed under reduced pressure. Crude solid was dissolved in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (20 mL), 10% citric acid solution (20 mL), water (20 mL) and brine (20 mL). Product precipitated between

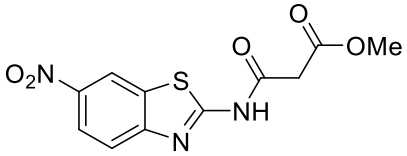
organic and water phases and was filtered off. Organic phase was further dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Both fractions were combined.

.2.5.1. Ethyl 2-((6-nitrobenzo[*d*]thiazol-2-yl)amino)-2-oxoacetate (**7a**)



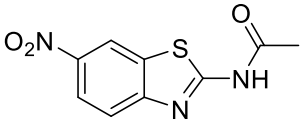
Yellow solid; (2.53 g, 86%); mp 225-228 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.34 (t, 3H, *J* = 7.1 Hz, $\underline{CH_3}CH_2$), 4.34 (q, 2H, *J* = 7.1 Hz, $CH_3\underline{CH_2}$), 7.97 (d, 1H, *J* = 8.9 Hz, Ar-H^4), 8.31 (dd, 1H, *J* = 8.9 Hz, *J* = 2.4 Hz, Ar-H^5), 9.12 (dd, 1H, *J* = 2.4 Hz, Ar-H^7), 13.64 (s br, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 13.71, 62.85, 119.19, 120.96, 121.83, 132.27, 143.30, 152.86, 157.43, 158.48, 163.03.

3.2.5.2. Methyl 3-((6-nitrobenzo[*d*]thiazol-2-yl)amino)-3-oxopropanoate (**7b)**



Yellow solid; (1.78 g, 61%); mp 199-202 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.68 (s, 3H, OCH_3), 3.82 (s, 2H, OCH_2CO), 7.93 (d, 1H, *J* = 8.9 Hz, Ar-H^4), 8.30 (dd, 1H, *J* = 8.9 Hz, *J* = 2.4 Hz, Ar-H^5), 9.09 (d, 1H, *J* = 2.4 Hz, Ar-H^7), 13.19 (s br, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 42.21, 52.27, 119.11, 120.75, 121.77, 132.13, 143.06, 153.27, 163.04, 165.95, 167.16.

3.2.5.3. *N*-(6-nitrobenzo[*d*]thiazol-2-yl)acetamide (**7c**)

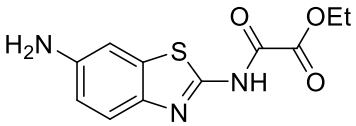


Yellow solid; (0.64 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.14 (s, 3H, CH_3), 7.89 (d, 1H, *J* = 8.9 Hz, Ar-H^4), 8.28 (dd, 1H, *J* = 8.9 Hz, *J* = 2.4 Hz, Ar-H^5), 9.04 (d, 1H, *J* = 2.4 Hz, Ar-H^7), 12.76 (s br, 1H, CONH).

3.2.6. General procedure for the synthesis of the compounds **8a**, **8b** and **8c.**

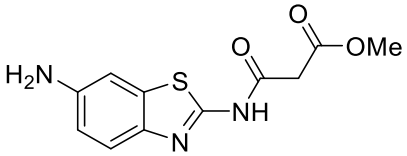
Compound **7a**, compound **7b** or compound **7c** (0.295 g, 1 mmol) was dissolved in ethanol (methanol for **8b**) (20 mL), and catalytic hydrogenation was performed in presence of 10% of Pd/C (0.029 g) at room temperature and atmospheric pressure (H_2) for 16h. Reaction mixture was filtered and solvent was removed under reduced pressure, to obtain crude product that was used without further purification.

3.2.6.1. Ethyl 2-((6-aminobenzo[*d*]thiazol-2-yl)amino)-2-oxoacetate (**8a**)



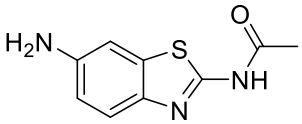
Brown solid; (0.283 g, 96%); mp 147-150 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.31 (t, 3H, J = 7.1 Hz, $CH_2\underline{CH_3}$), 4.31 (q, 2H, J = 7.1 Hz, $\underline{CH_2}CH_3$), 5.34 (s br, 2H, NH_2), 6.76 (dd, 1H, J = 8.6 Hz, J = 2.1 Hz, Ar-H^5), 7.05 (d, 1H, J = 2.1 Hz, Ar-H^7), 7.47 (d, 1H, J = 8.6 Hz, Ar-H^4), 12.82 (s br, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 13.74, 62.51, 103.94, 114.88, 120.88, 132.99, 138.48, 146.31, 152.84, 156.77, 159.41.

3.2.6.2. Methyl 3-((6-aminobenzo[*d*]thiazol-2-yl)amino)-3-oxopropanoate (**8b**)



Brown solid; (0.289 g, 98%); mp 162-165 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.62 (s, 2H, $COCH_2CO$), 3.67 (s, 3H, OCH_3), 5.24 (s br, 2H, NH_2), 6.72 (dd, 1H, J = 8.6 Hz, J = 2.1 Hz, Ar-H^5), 7.01 (d, 1H, J = 2.1 Hz, Ar-H^7), 7.42 (d, 1H, J = 8.6 Hz, Ar-H^4), 12.23 (s br, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6, δ); 42.10, 52.14, 103.97, 114.49, 120.99, 132.92, 139.43, 145.91, 152.51, 164.41, 167.51.

3.2.6.3. *N*-(6-aminbenzo[*d*]thiazol-2-yl)acetamide (**8c**)

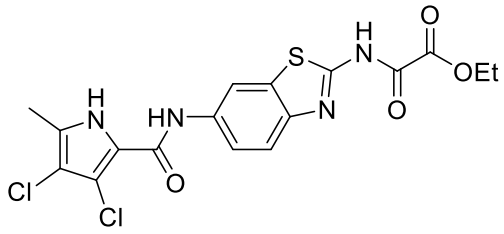


Brown solid; (0.391 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.14 (s, 3H, CH_3), 5.51 (br s, 2H, NH_2), 6.69 (dd, 1H, J = 8.6 Hz, J = 2.3 Hz, Ar-H^5), 6.99 (d, 1H, J = 2.3 Hz, Ar-H^7), 7.39 (d, 1H, J = 8.6 Hz, Ar-H^4), 11.99 (s br, 1H, CONH).

3.2.7. General procedure for the synthesis of the compounds **9a**, **9b** and **9c**

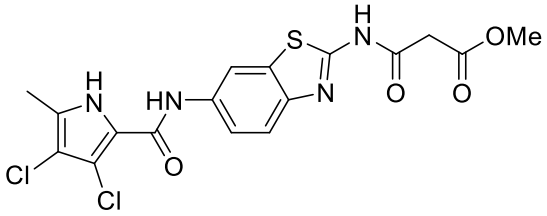
3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (0.223 g, 1.15 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (20 mL), oxalyl chloride (0.49 mL, 5.75 mmol, 5.0 eq.) was added and the reaction mixture was stirred at room temperature for 8h. The solvent was removed under reduced pressure. Crude solid was dissolved together with compound **8a** (0.426 g, 1.61 mmol, 1.4 eq.) or **8b** (0.446 g, 1.68 mmol, 1.4 eq.) or **8c** in dichloromethane (25 mL) and pyridine (2.5 mL) and stirred for 12h at room temperature. Formed precipitate was filtered and recrystallized from chloroform, to obtain **9a** (0.20 g), **9b** (0.22 g) or **9c**.

3.2.7.1. Ethyl 2-((6-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-2-yl)amino)-2-oxoacetate (**9a**)



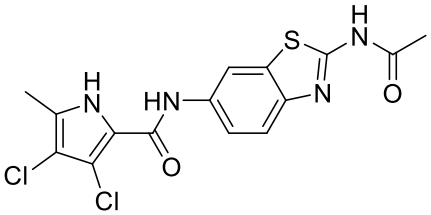
Brown solid, (0.20 g, 40%); mp 243-245 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.34 (t, 3H, *J* = 7.1 Hz, $CH_2C\underline{H}_3$), 2.25 (s, 3H, pyrrole-CH_3), 4.34 (q, 2H, *J* = 7.1 Hz, $C\underline{H}_2CH_3$), 7.69 (dd, 1H, *J* = 8.8, 2.1 Hz, Ar-H^5), 7.79 (d, 1H, *J* = 8.8 Hz, Ar-H^4), 8.42 (d, 1H, *J* = 2.1 Hz, Ar-H^7), 9.68 (s, 1H, NH), 12.25 (s, 1H, NH), 13.21 (s, 1H, NH). HRMS for $C_{17}H_{13}O_4N_4Cl_2S$ ([M-H]$^-$): calculated 439.0040, found 439.0041.

3.2.7.2. Methyl 3-((6-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-2-yl)amino)-3-oxopropanoate (**9b**)



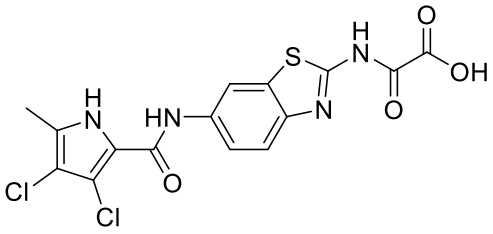
Brown solid, (0.200 g, 43%); mp 268-270 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.24 (s, 3H, pyrrole-CH_3), 3.69 (s, 5H, $C\underline{H}_2COOC\underline{H}_3$), 7.66 (dd, 1H, *J*=8.8, 2.1 Hz, Ar-H^5), 7.74 (d, 1H, *J*=8.7 Hz, Ar-H^4), 8.37 (d, 1H, *J*=2.1 Hz, Ar-H^7), 9.65 (s, 1H, NH), 12.27 (s, 1H, NH), 12.55 (s, 1H, NH). HRMS for $C_{17}H_{13}O_4N_4Cl_2S$ ([M-H]$^-$): calculated 439.0040, found 439.0040

3.2.7.3 *N*-(2-acetamidobenzo[*d*]thiazol-6-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (**9c**)



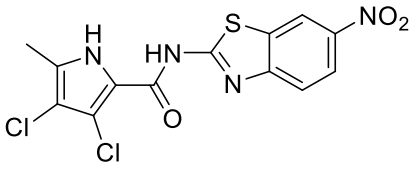
Using 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylic acid (0.353 g, 1.82 mmol), oxalyl chloride (0.77 mL, 9.1 mmol), **8c** (0.377 g, 1.82 mmol). Pink solid, (0.441 g, 63%), ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.20 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 7.63 (dd, 1H, *J*=8.8, 2.2 Hz, Ar-H^5), 7.71 (d, 1H, *J*=8.7 Hz, Ar-H^4), 8.33 (d, 1H, *J*=2.1 Hz, Ar-H^7), 9.56 (s, 1H, NH), 12.20 (s, 1H, NH), 12.32 (s, 1H, NH). HRMS for $C_{15}H_{13}Cl_2N_4O_2S$ ([M+H]$^+$): calculated 383.0131, found 383.0117.

3.2.8. Synthesis of 2-((6-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-2-yl)amino)-2-oxoacetic acid (**10**)



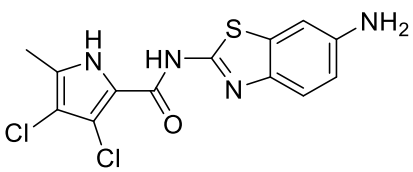
Compound **9a** (0.050 g, 0.11 mmol) was dissolved in 1,4-dioxane (15 mL) and hydrolysed with 2M NaOH (0.22 mmol) at 50 °C for 24h. Solvent was removed under reduced pressure. Water solution of the product (10 mL) was acidified with 1M hydrochloric acid to pH~4 and formed precipitate was filtered to obtain purple solid (0.035 g, 75%). mp 225-227 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.25 (s, 3H, CH_3), 7.66 (dd, 1H, *J*=8.8, 2.1 Hz, Ar-H^5), 7.75 (d, 1H, *J*=8.7 Hz, Ar-H^4), 8.38 (d, 1H, *J*=2.0 Hz, Ar-H^7), 9.67 (s, 1H, NHCO), 12.30 (brs, 3H*, Pyrr-NH, NHCO and COOH). HRMS for $C_{15}H_9O_4N_4Cl_2S$ ([M-H]$^-$): calculated 410.9727, found 410.9729. *signal integrates less than 3 due to interchangeable nature of protons

3.2.9. Synthesis of 3,4-dichloro-5-methyl-*N*-(6-nitrobenzo[d]thiazol-2-yl)-1*H*-pyrrole-2-carboxamide (**11**)



Compound **6** (1 g, 5.12 mmol) was dissolved in toluene and coupled with 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carbonyl chloride (0.543 g, 2.56 mmol) prepared in situ according to general procedure ***3.2.70.*** Reaction mixture was stirred under reflux overnight. Precipitate was filtered and recrystallized from methanol to obtain grey solid **11** (0.78 g, 82%). mp 235-237 °C, ^{1}H NMR (400 MHz, DMSO-d$_6$, δ): 2.28 (s, 3H, CH_3), 7.89 (d, 1H, *J* = 8.6 Hz, Ar-H^4), 8.30 (dd, 1H, *J* = 8.6, 2.3 Hz, Ar-H^5), 9.06 (d, 1H, *J* = 2.3 Hz, Ar-H^7), 12.14 (bs, 1H,) and 12.38 (bs, 1H), CONH and pyrrole-NH.

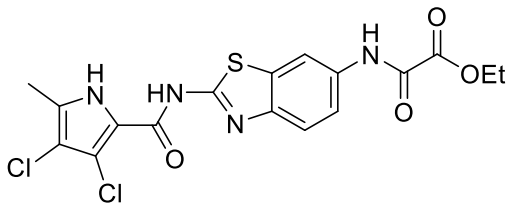
3.2.10. Synthesis of *N*-(6-aminobenzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (**12**)



Compound **11** (0.5 g, 1.34 mmol) was dissolved in ethanol (50 mL) and reduced with tin chloride dihydrate (1.52 g, 6.73 mmol) under reflux overnight. Solvent was removed under reduced pressure and crude solid was dissolved in ethyl acetate (50 mL) and extracted with 1M sodium hydroxide (50 mL). Organic phase was further dried over Na_2SO_4, filtered and the solvent was removed under

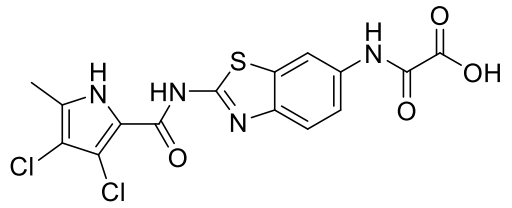
reduced pressure to obtain **12** as brown solid (0.297 g, 65%). mp 235-237 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.26 (s, 3H, CH_3), 5.22 (bs, 2H, NH_2), 6.73 (dd, 1H, *J* = 8.6, 2.2 Hz, Ar-H^5), 7.02 (d, 1H, *J* = 2.1 Hz, Ar-H^7), 7.41 (d, 1H, *J* = 8.6 Hz, Ar-H^4), 11.40 (bs, 1H,) and 12.27 (bs, 1H), CONH and pyrrole-NH.

3.2.11. Synthesis of ethyl 2-((2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*] thiazol-6-yl)amino)-2-oxoacetate (**13**)



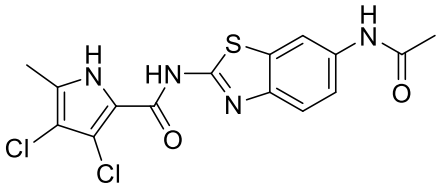
A solution of **12** (0.20 g, 0.59 mmol) and triethylamine (0.09 mL, 0.65 mmol) in 1,4-dioxane (20 mL) wascooled at 0 °C. Ethyl oxalyl chloride (0.07 mL, 0.65 mmol) was added dropwise. Reaction mixture was stirred for 8 hours at room temperature and then quenched with saturated $NaHCO_3$ solution. The solvent was removed under reduced pressure. Crude solid was washed with saturated $NaHCO_3$ solution (20 mL), 10% citric acid solution (20 mL) and water (20 mL). Product was filtered and dried to obtain **13** as beige solid (0.210 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.33 (t, 3H, *J*=7.1 Hz, $CH_2C\underline{H_3}$), 2.28 (s, 3H, pyrrole-CH_3), 4.33 (q, 2H, *J*=7.1 Hz, $C\underline{H_2}CH_3$), 7.68 – 7.80 (m, 2H, 2 × Ar-H), 8.40 (s, 1H, Ar-H), 11.02 (s, 1H, NH), 12.12 (s, 1H, NH), 13.16 (s, 1H, NH). HRMS for $C_{17}H_{13}O_4N_4Cl_2S$ ([M-H]$^-$): calculated 439.0040, found 439.0042.

3.2.12. Synthesis of 2-((2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-6-yl)amino)-2-oxoacetic acid (**14**)



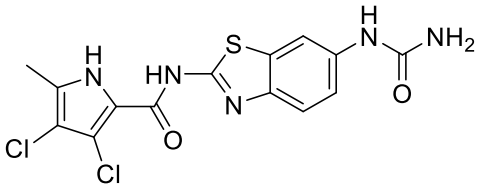
Compound **13** (0.11 g, 0.25 mmol) was dissolved in 1,4-dioxane (15 mL) and hydrolyzed with 1M NaOH (0.75 mmol) at room temperature for 12h. Solvent was removed under reduced pressure. Water solution of the product (10 mL) was acidified with 1M hydrochloric acid to pH~4 and formed precipitate was filtered to obtain beige solid (0.060 g, 58%). Yield; mp 247-249 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.27 (s, 3H, CH_3), 7.71 (d, 1H, *J*=8.8 Hz, Ar-H-4), 7.79 (dd, 1H, *J*=8.8, 2.1 Hz, Ar-H-5), 8.43 (d, 1H, *J*=2.1 Hz, Ar-H-7), 10.83 (s, 1H, NHCO), 12.39 (brs, 3H*, Pyr-NH, NHCO and COOH). * signal integrates less than 3 due to interchangeable nature of protons.

3.2.13. Synthesis of *N*-(6-acetamidobenzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (**15a**)



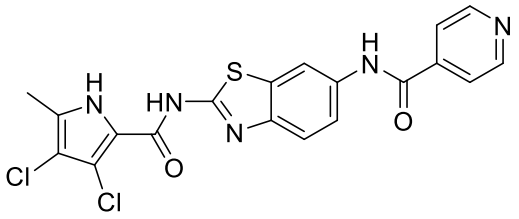
N-(6-aminobenzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (**12**) (170 mg, 0.5 mmol, 1eq) was dispersed in anh. DCM (7 mL) under argon, acetanhydride (0.28 mL, 6 eq) and then triethylamine (0.19 mL, 4 eq) was added dropwise. Reaction mixture was stirred at r.t. for 6 h. After the reaction was completed (TLC 9:1; DCM: MeOH; R_f = 0.4), reaction was quenched by adding 1M aq. HCl. DCM was evaporated at reduced pressure and the residue was dispersed in 1M aq. HCl (60mL/ mmol) in ultrasound bath and stirred for 1 h at r.t. The resulting precipitate was filtered out, washed with water and acetone to give the product as beige-grey solid (115 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.31 (s, 1H), 11.64 (s, 1H), 10.11 (s, 1H), 8.30 (d, *J* = 2.0 Hz, 1H), 7.66 (d, *J* = 8.8 Hz, 1H), 7.50 (dd, *J* = 8.7, 2.1 Hz, 1H), 2.26 (s, 3H), 2.07 (s, 3H) . HRMS for $C_{15}H_{11}O_2N_4Cl_2S$ ([M-H]$^-$): calculated 380.9985, found 380.9956.

3.2.14. Synthesis of 3,4-dichloro-5-methyl-*N*-(6-ureidobenzo[*d*]thiazol-2-yl)-1*H*-pyrrole-2-carboxamide **(15b)**



N-(6-aminobenzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (**12**) (1 eq) and 1,1'-carbonyldiimidazole (1.2 eq) were dissolved in anh. DMF (30mL/ mmol) under argon and the reaction mixture was stirred at r.t. for 3 h. Ammonia gas was bubbled into the reaction for 5 mins, the reaction vessel was sealed with septum and the reaction mixture was stirred at 50°C overnight. 25% aq. ammonia was added and the reaction was stirred at 50°C under the septum for 2 h and then at 60°C with opened vessel for 1 h (to let the ammonia gas escape). Reaction mixture was cooled down to r.t., acidified by dropwise addition of 1M aq. HCl and diluted with water. Resulting precipitate was filtered out, washed with water and acetone to give the product (TLC 9:1; DCM: MeOH; R_f = 0.4) as light brown solid (62%). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.26 (s, 3H), 5.90 (s, 2H), 7.33 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 8.11 (d, *J* = 2.2 Hz, 1H), 8.69 (s, 1H), 11.57 (s, 1H), 12.30 (s, 1H). HRMS for $C_{14}H_{10}O_2N_5Cl_2S$ ([M-H]$^-$): calculated 381.9938, found 381.9939.

3.2.15. Synthesis of *N*-(2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)isonicotinamide (**15c**)

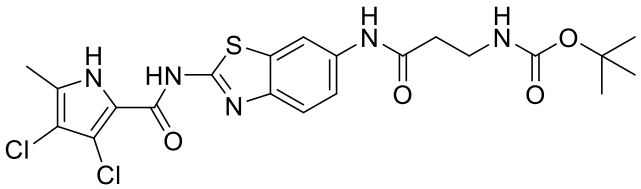


Nicotinic acid (36 mg, 0.29 mmol) was dissolved in DMF (5 mL) at 0 °C with EDC-HCl (67 mg, 0.35 mmol), NMM (64 µL, 0.58 mmol), and HOBt (58 mg, 0.38 mmol). After 15 minutes, **12** (100 mg, 0.29 mmol) was added and the reaction mixture was stirred at rt for 12h. Solvent was removed under reduced pressure, the residue suspended in 10 % citric acid and the precipitate was filtered off. Crude solid was further recrystallized from methanol to provide product **15c** as brown solid (50 mg, 38 %). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ): 2.28 (s, 3H, CH_3), 7.60 (ddd, 1H, *J* = 8.0, 4.8, 0.9 Hz, pyridine-H), 7.76 (m, 2H, 2×Ar-H), 8.33 (ddd, 1H, *J* = 8.0, 2.3, 1.7 Hz, pyridine-H), 8.48 (s, 1H, Ar-H), 8.79 (dd, 1H, *J* = 4.8, 1.6 Hz, pyridine-H), 9.15 (dd, 1H, *J* = 2.4, 0.9 Hz, pyridine-H), 10.63 (s, 1H, CONH), 11.71 (s, 1H, CONH or pyrrole-NH), 12.33 (s, 1H, CONH or pyrrole-NH). HRMS for $C_{19}H_{14}O_2N_5Cl_2S$ ([M+H]$^+$): calculated 446.0240, found 446.0234.

3.2.16. General procedure for the synthesis of the compounds **15d** and **15e**

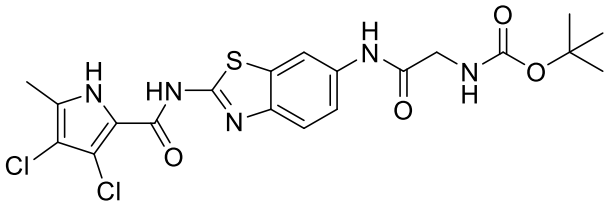
Boc-β-alanine or Boc-glycine (0.38 mmol) was dissolved in DMF (5 mL) at 0 °C and EDC-HCl (88 mg, 0.46 mmol), NMM (84 µL, 0.76 mmol), and HOBt (76 mg, 0.49 mmol) were added. After 15 minutes, **12** (130 mg, 0.38 mmol) was added and the reaction mixture was stirred at rt for 12 h. Solvent was removed under reduced pressure, the residue was suspended in 10 % citric acid and filtered. The obtained precipitate which was further recrystallized from methanol to provide product

3.2.16.1. *tert*-Butyl (3-((2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-6-yl)amino)-3-oxopropyl)carbamate (**15d**)



Brown solid (53 mg, 27 %). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ): 1.38 (s, 9H, *tert*-butyl), 2.26 (s, 3H, CH_3), 3.24 (q, *J* = 6.8 Hz, 2H, CH_2), 6.90 (t, 1H, *J* = 5.6 Hz, NHBoc), 7.52 (dd, 1H, *J* = 8.8, 2.1 Hz, Ar-H-5), 7.66 (d, 1H, *J* = 8.7 Hz, Ar-H-4), 8.32 (s, 1H, Ar-H-7), 10.12 (s, 1H), 11.65 (s, 1H), 12.31 (s, 1H); (2 × CONH and pyrrole-NH, the second CH_2 signal is covered with DMSO signal). HRMS for $C_{21}H_{24}O_4N_5Cl_2S$ ([M+H]$^+$): calculated 512.0921, found 512.0911.

3.2.16.2. *tert*-Butyl (2-((2-(3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamido)benzo[*d*]thiazol-6-yl)amino)-2-oxoethyl)carbamate (**15e**)

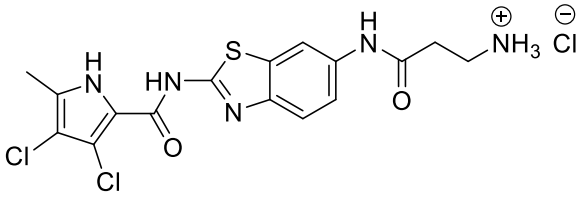


Brown solid (86 mg, 45 %).[1]H NMR (400 MHz, DMSO-d_6, δ): 1.41 (s, 9H, *tert*-butyl), 2.27 (s, 3H, CH_3), 3.75 (d, *J* = 6.1 Hz, 2H, CH_2), 7.10 (t, 1H, *J* = 6.1 Hz, NHBoc), 7.54 (dd, 1H, *J* = 8.7, 2.0 Hz, Ar-H-5), 7.69 (d, 1H, *J* = 8.7 Hz, Ar-H-4), 8.31 (s, 1H, Ar-H-7), 10.11 (s, 1H), 11.66 (s, 1H), 12.32 (s, 1H), (2 × CONH and pyrrole-NH). HRMS for $C_{20}H_{22}O_4N_5Cl_2S$ ([M+H]$^+$): calculated 498.0764, found 498.0758.

3.2.17. General procedure for the synthesis of the compounds **16a** and **16b**

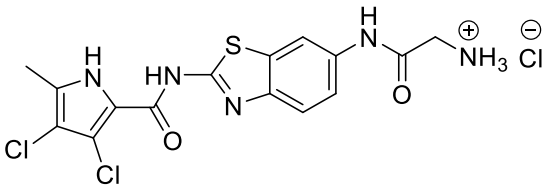
15d or **15e** (0.1 mmol) was dissolved in dioxane (4 mL) and treated with 4M HCl in dioxane (3 mL). A grey precipitate formed after 5 h and was filtered off under the reduced pressure to give product.

3.2.17.1. *N*-(6-(3-aminopropanamido)benzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide hydrochloride (**16a**)



White solid (35 mg, 100 %). [1]H NMR (400 MHz, DMSO-d_6, δ):, δ): 2.27 (s, 3H, CH_3), 2.79 (t, 2H, *J* = 6.8 Hz, NHCOC$\underline{H_2}$), 3.10 (sext, 2H, *J* = 6.3 Hz, C$\underline{H_2}$$NH_3^+$), 7.58 (dd, 1H, *J* = 8.8, 2.1 Hz, Ar-H-5), 7.69 (d, 1H, *J* = 8.7 Hz, Ar-H-4), 8.03 (s, 3H, NH_3^+), 8.34 (d, 1H, *J* = 2.0 Hz, Ar-H-7), 10.53 (s, 1H, N$\underline{H}$COCH_2), 12.67 (s, 1H, pyrrole-NH), signal for NHCO-pyrrole not visible. [13]C NMR (100 MHz, DMSO-d_6, δ): 11.0, 33.2, 34.9, 109.7, 111.5, 115.0, 117.3, 118.6, 119.8, 120.2, 129.6, 131.6, 168.3, three signals not visible. HRMS for $C_{16}H_{16}O_2N_5Cl_2S$ ([M+H]$^+$): calculated 412.0396, found 412.0389.

3.2.17.2. *N*-(6-(2-aminoacetamido)benzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide hydrochloride (**16b**)

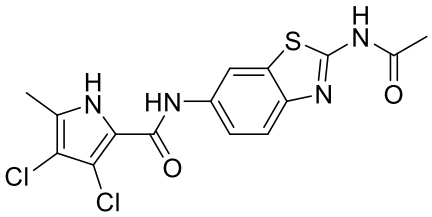


White solid (33 mg, 100 %). [1]H NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H, CH_3), 3.83 (q, 2H, *J* = 5.6 Hz, NHCOC$\underline{H_2}$), 7.60 (dd, 1H, *J* = 8.8, 2.1 Hz, Ar-H-5), 7.74 (d, 1H, *J* = 8.5 Hz, Ar-H-4), 8.21 (s,

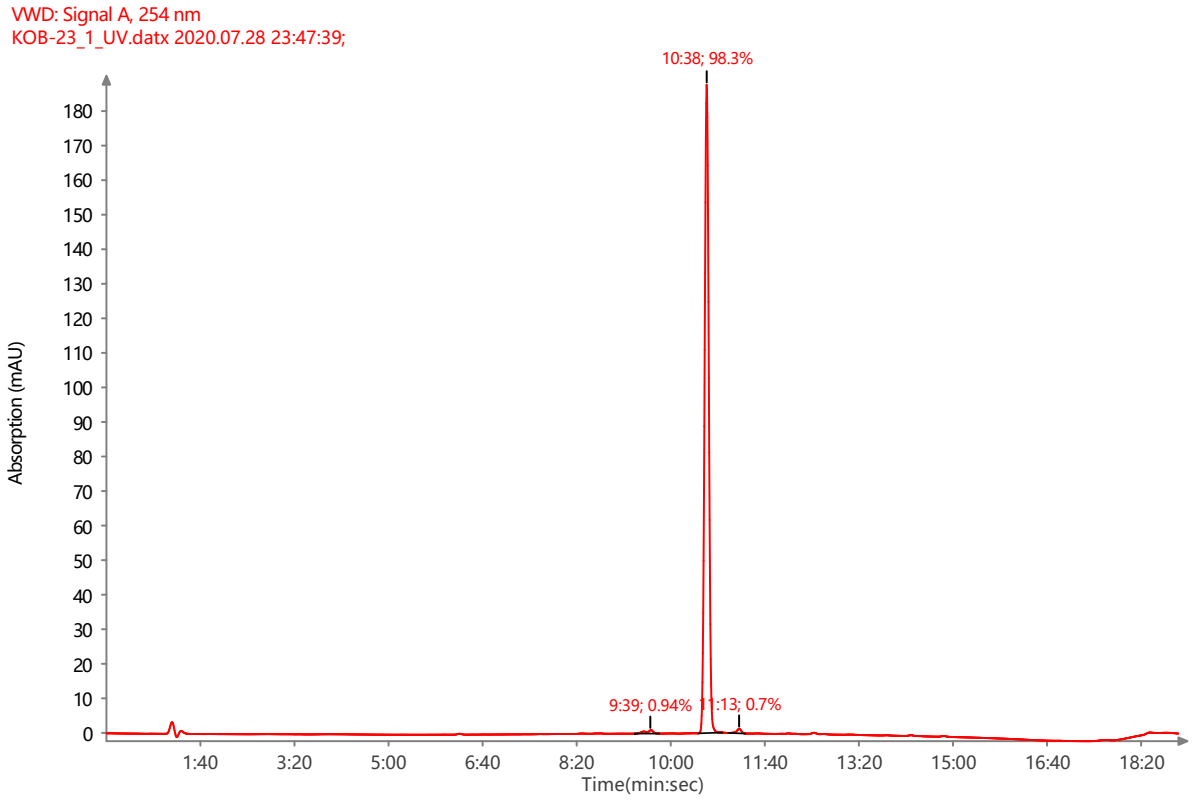
3H, NH_3^+), 8.31 (s, 1H, Ar-H-7), 10.79 (s, 1H, N<u>H</u>$COCH_2$), 12.77 (s, 1H), 12.53 (brs, 2H, pyrrole-NH and NHCO). HRMS for $C_{15}H_{14}O_2N_5Cl_2S$ ([M+H]+): calculated 398.0240, found 398.0236.

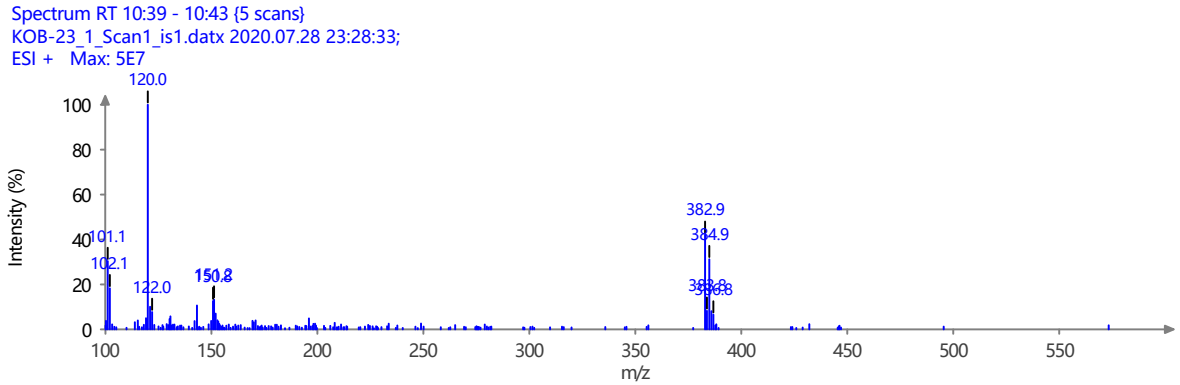
4. Representative analytical data

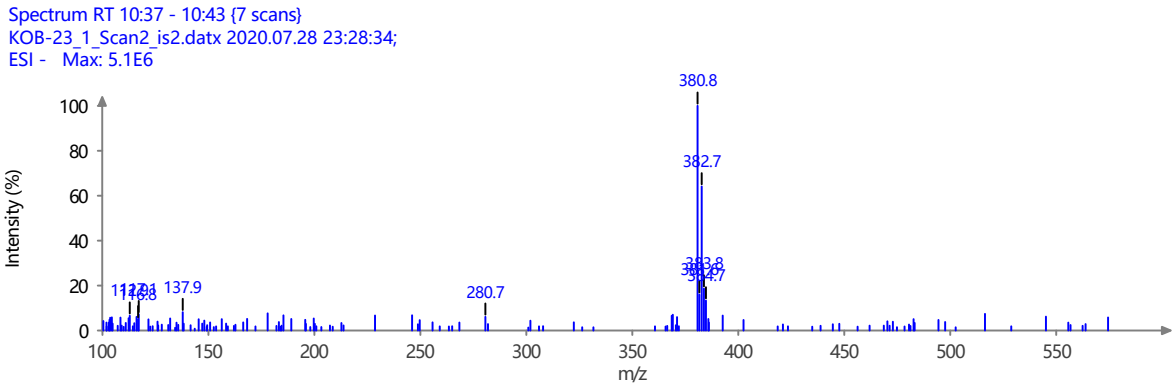
N-(2-acetamidobenzo[*d*]thiazol-6-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide (**9c**)



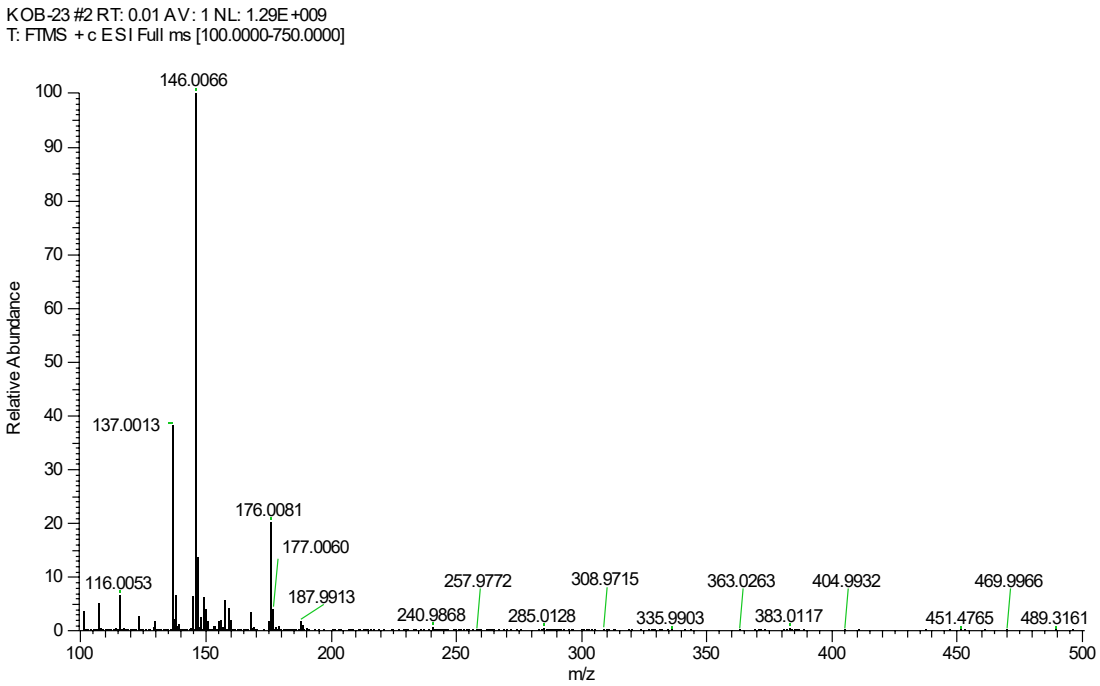
HPLC-UV/VIS-MS, λ = 254 nm, m/z (ESI) for **9c**

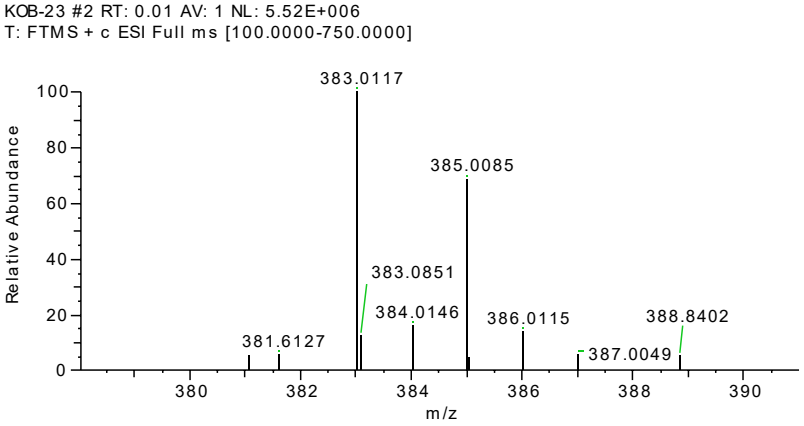




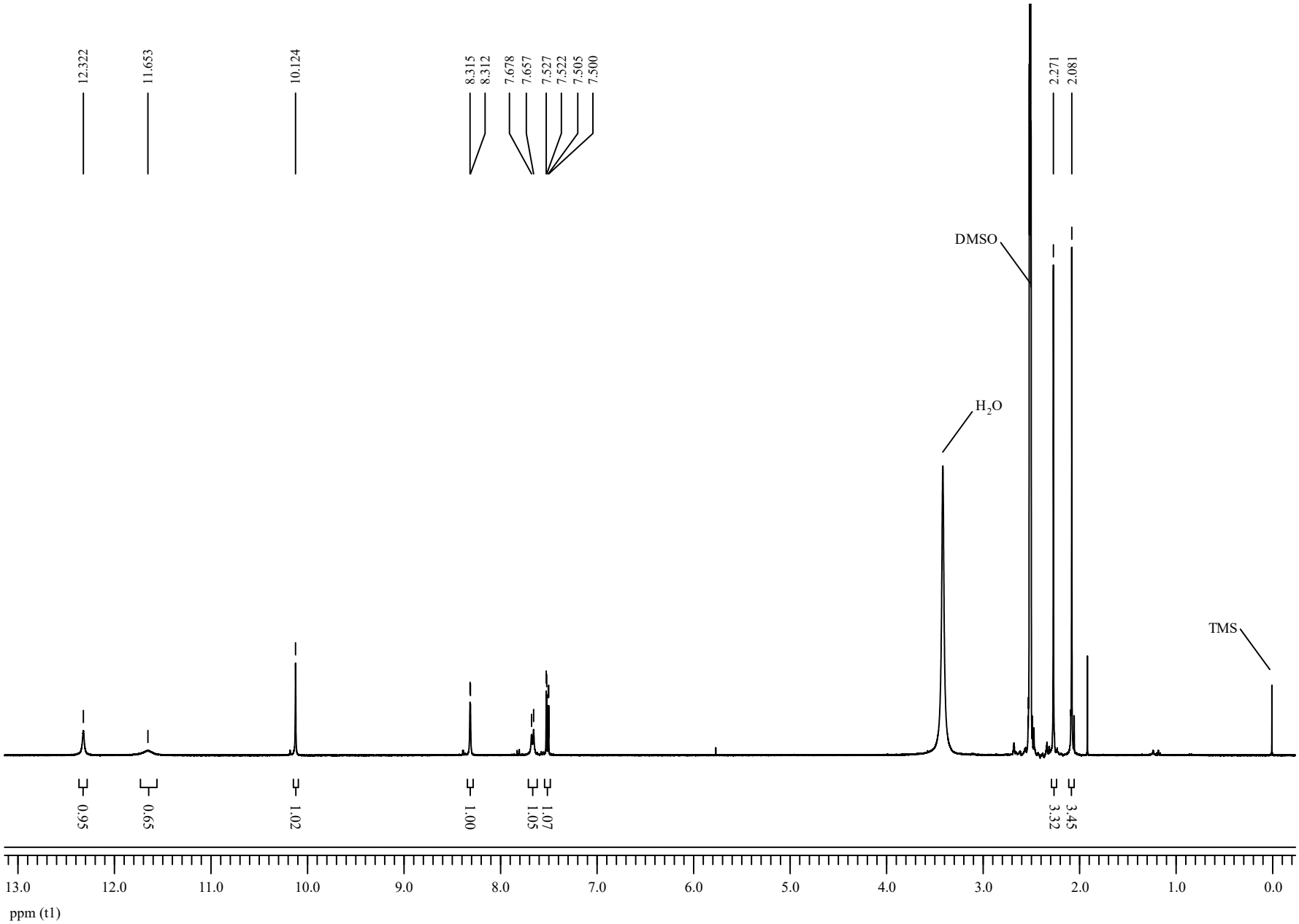


HRMS (ESI+) for **9c**

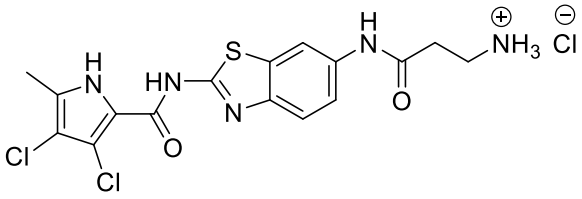




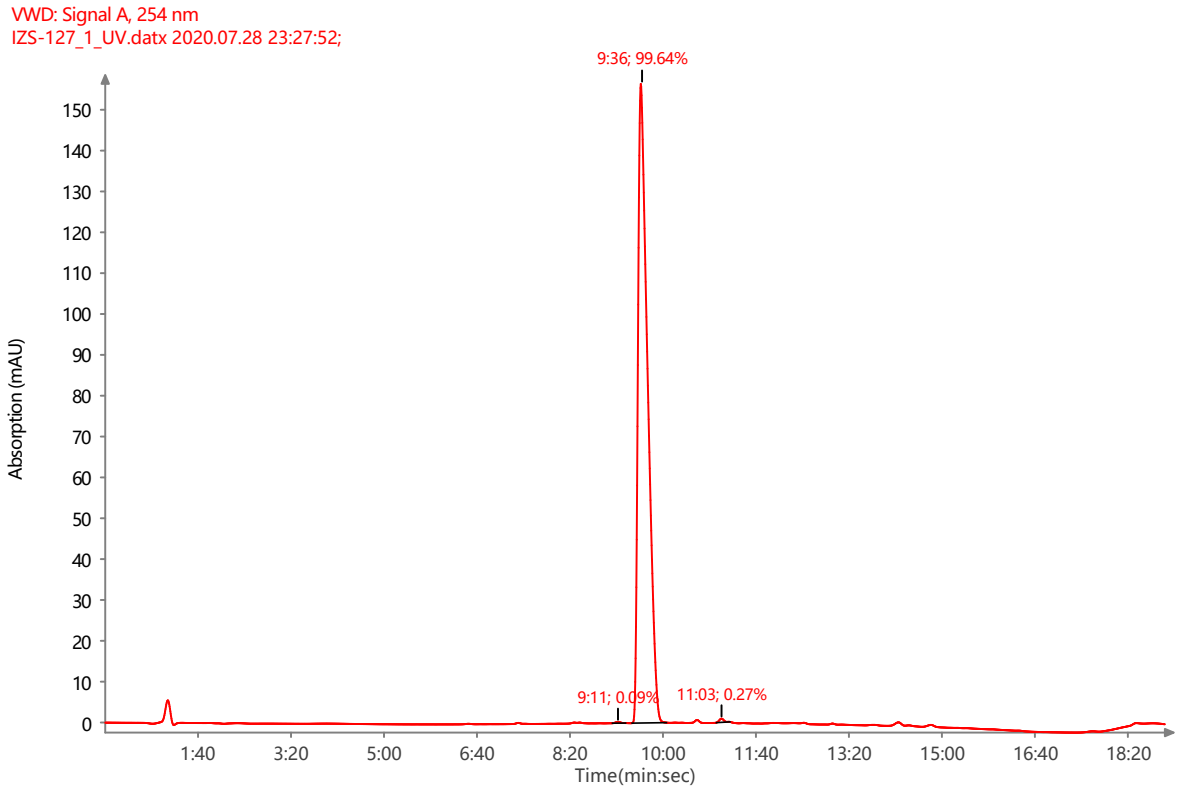
^{1}H NMR (400 MHz, DMSO-d_6) for **9c**

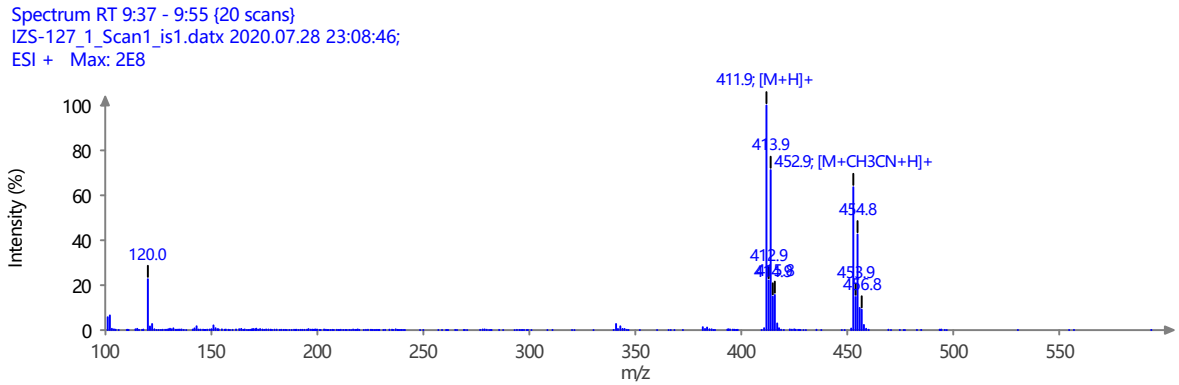


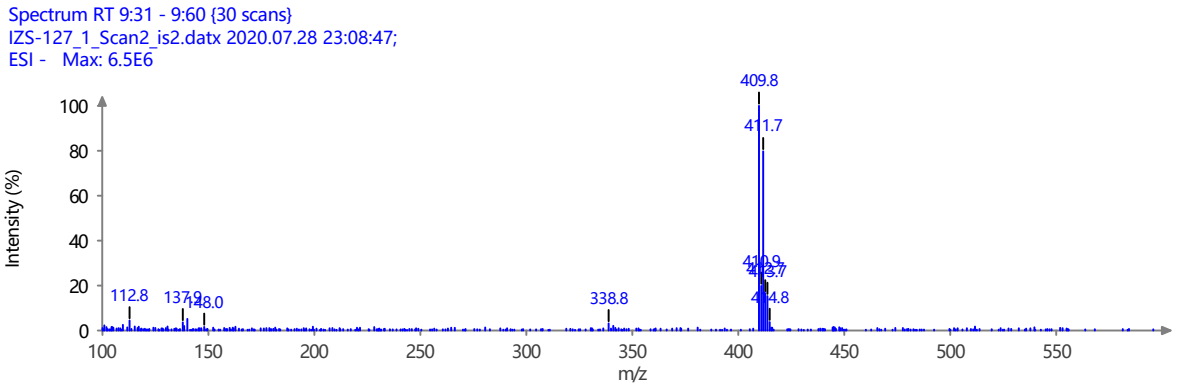
N-(6-(3-aminopropanamido)benzo[*d*]thiazol-2-yl)-3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxamide hydrochloride (**16a**)



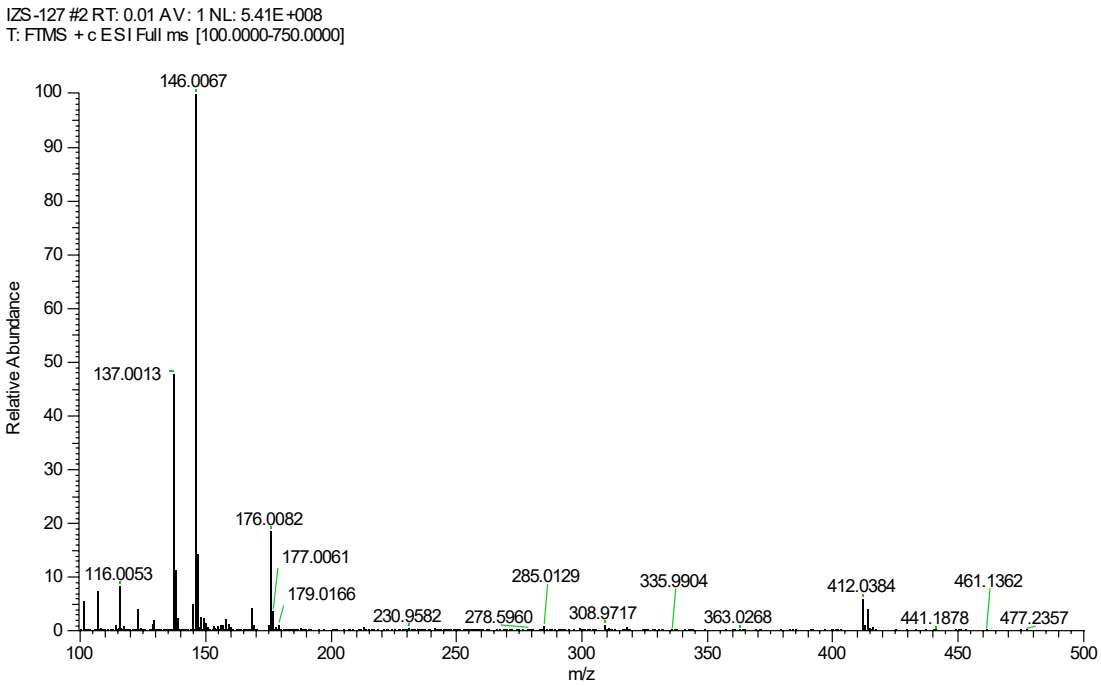
HPLC-UV/VIS-MS, λ = 254 nm, m/z (ESI) for **16a**

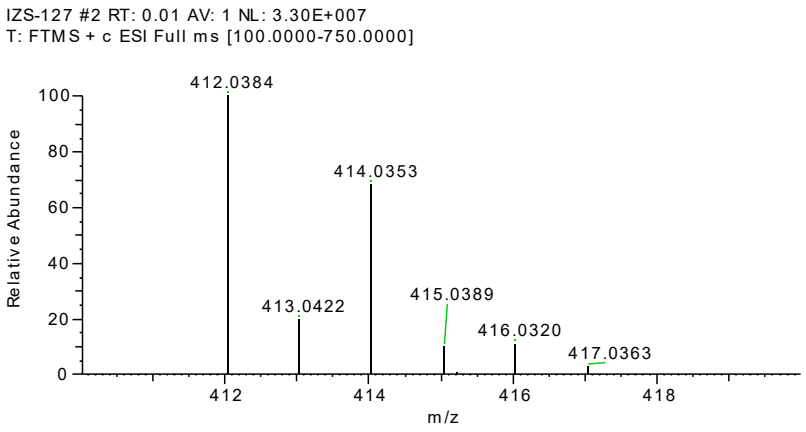




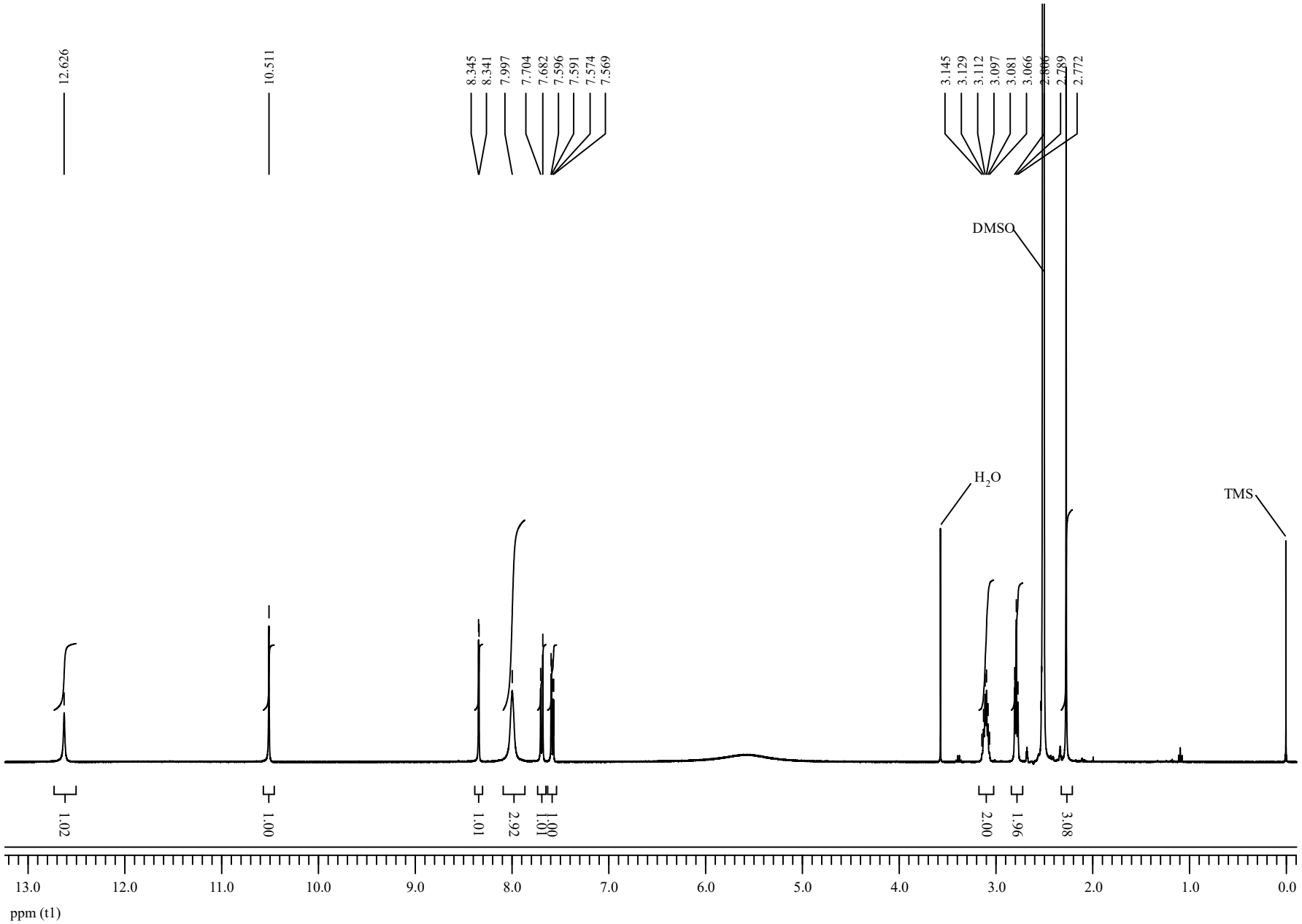


HRMS (ESI+) for **16a**





^{1}H NMR (400 MHz, DMSO-d_6) for **16a**



5. References

(1) M. Gjorgjieva, T. Tomašič, M. Barančokova, S. Katsamakas, J. Ilaš, P. Tammela, L.P. Mašič, D. Kikelj, Discovery of Benzothiazole Scaffold-Based DNA Gyrase B Inhibitors, Med Chem, 59 (2016) 8941-8954.

(2) CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2012

(3) Lewis, R.J., Singh, O.M., Smith, C.V., Maxwell, A., Skarzynski, T., Wonacott, A.J. and Wigley, D.B. (1994) Crystallization of inhibitor complexes of an N-terminal 24 kDa fragment of the DNA gyrase B protein. J Mol Biol, 241, 128-130

(4) Winter, G., Waterman, D. G., Parkhurst, J. M., Brewster, A. S., Gildea, R. J., Gerstel, M., Fuentes-Montero, L., Vollmar, M., Michels-Clark, T., Young, I. D., Sauter, N. K. & Evans, G. (2018). DIALS: implementation and evaluation of a new integration package. Acta Crystallogr D Struct Biol 74, 85-97.

(5) Winter, G. (2010). Xia2: an expert system for macromolecular crystallography data reduction. J. Appl. Crystallogr. 43, 186-190.

(6) Evans, P. R., and Murshudov, G. N. (2013) How good are my data and what is the resolution? Acta Crystallogr. D Biol. Crystallogr. 69, 1204-1214

(7) Potterton, L., Agirre, J., Ballard, C., Cowtan, K., Dodson, E., Evans, P. R., Jenkins, H. T., Keegan, R., Krissinel, E., Stevenson, K., Lebedev, A., McNicholas, S. J., Nicholls, R. A., Noble, M., Pannu, N. S., Roth, C., Sheldrick, G., Skubak, P., Turkenburg, J., Uski, V., von Delft, F., Waterman, D., Wilson, K., Winn, M. & Wojdyr, M. (2018). CCP4i2: the new graphical user interface to the CCP4 program suite. Acta Crystallogr D Struct Biol 74, 68-84.

(8) McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). Phaser crystallographic software. J. Appl. Crystallogr. 40, 658-674.

(9) Narramore, S., Stevenson, C. E. M., Maxwell, A., Lawson, D. M. & Fishwick, C. W. G. (2019). New insights into the binding mode of pyridine-3-carboxamide inhibitors of E. coli DNA gyrase. Bioorg Med Chem 27, 3546-3550.

(10) Murshudov, G. N., Skubak, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). REFMAC5 for the refinement of macromolecular crystal structures. Acta Crystallogr D Biol Crystallogr 67, 355-367.

(11) Long, F., Nicholls, R. A., Emsley, P., Graaeulis, S., Merkys, A., Vaitkus, A. & Murshudov, G. N. (2017). AceDRG: a stereochemical description generator for ligands. Acta Crystallogr D Struct Biol 73, 112-122.

(12) Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126-2132

(13) Davis, I. W., Leaver-Fay, A., Chen, V. B., Block, J. N., Kapral, G. J., Wang, X., Murray, L. W., Arendall, W. B., 3rd, Snoeyink, J., Richardson, J. S., and Richardson, D. C. (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. 35, W375-383

(14) Joosten, R. P., Long, F., Murshudov, G. N. & Perrakis, A. (2014). The PDB_REDO server for macromolecular structure model optimization. IUCrJ 1, 213-220.

(15) McNicholas, S., Potterton, E., Wilson, K. S., and Noble, M. E. (2011) Presenting your structures: the CCP4mg molecular-graphics software. Acta Crystallogr. D Biol. Crystallogr. 67, 386-394.