SUPPLEMENTARY INFORMATION

Exploration of a Benzothiophene Scaffold for Use as Adjuvants with β-Lactam Antibiotics against Methicillin-Resistant *Staphylococcus aureus*

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MATERIALS AND METHODS

Bacterial strains, media, and culture conditions

Bacterial strains MRSA ATCC BAA-1556 and MRSA ATCC 43300 were obtained from ATCC. MRSA AH-1263, MRSA AH 2087 (Δ*vraRS*), MRSA AH 2090 (Δ*kdpDE*), and MRSA AH 5929 (*mecA*:Tn mutant) were obtained from the Horswill lab at the University of Colorado Anschutz Medical Campus. MSSA 29213 was obtained from ATCC. CRB bacterial strain was kindly gifted by Dr. Som S. Chatterjee, University of Maryland, USA. Bacterial stocks were stored in 25% glycerol and maintained at -80 °C. Prior to use, colonies were grown on tryptic soy agar (Fisher Bioreagents Agar, catalog #251879). Cation adjusted Mueller Hinton broth (CAMHB) (catalog #212322) was purchased from BD Diagnostics. Mueller Hinton broth (MHB) (catalog #DF0757-17-6) was purchased from Difco, Fisherscientific USA. Tryptic soy broth (TSB) was purchased from Sigma-Aldrich (catalog no. 22092). Oxacillin sodium salt monohydrate (catalog #O0353) was purchased from TCI Chemicals and dissolved in sterile Milli-Q water. Ceftobiprole was purchased from Medchemexpress (catalog #HY-112579) and dissolved in DMSO. Penicillin G sodium salt was purchased from Sigma-Aldrich (catalog no. 018K06011) and dissolved in sterile Milli-Q water. Cefoxitin sodium salt was purchased from Thermo Scientific (catalog no. 455280010) and dissolved in sterile Milli-Q water.

Broth microdilution method for MIC determination

Bacteria were cultured for six hours in CAMHB and sub-cultured to 5 x 10^5 CFU/mL in CAMHB. Subcultures were aliquoted (1 mL) into culture tubes and then dosed with compound from 25 mM, 50 mM or 100 mM stock in DMSO to a concentration of 200 μM. Samples were then aliquoted (200 μL) into the first row of wells of a 96-well microtiter plate in which wells 2-11 were prefilled with 100 μL of subculture. From row 1, 100 μL was withdrawn and transferred to row 2, mixed 5-8 times. This procedure was used to serially dilute the rest of the rows through 10. Row 12 was filled with 100 μL of CAMHB. The microtiter plate was then covered in Press ‘n Seal and incubated statically at 37 °C. After 16 hours, the OD_{600} was measured, and MIC calculated as 90% inhibition of bacterial growth compared to the untreated control.

Broth microdilution method for antibiotic potentiation

Bacteria were cultured for six hours in CAMHB and sub-cultured to 5 x 10^5 CFU/mL in CAMHB. Subcultures were aliquoted (4 mL) into culture tubes and then dosed with compound from 4 mM, 10 mM, 25 mM, 50 mM or 100 mM stock in DMSO to the desired concentration. Compounds were tested at 30% MIC or at 60 μM if the MIC was above 200 μM, from this, 1 mL was aliquoted into a second tube and dosed with antibiotic at the highest concentration to be tested. Bacteria treated with antibiotic alone served as a control. Samples from the secondary culture tubes were then aliquoted (200 μL) into the first row of wells of a 96-well microtiter plate. Rows 2-11 were filled (100 μL) with the remaining 3 mL of bacterial subculture containing the adjuvant. Row 12 was filled (100 μL) with uninoculated media. From row 1, 100 μL was transferred to row 2 and mixed 5-8 times before being transferred to row 3. This procedure was used to serially dilute the rest of the rows through 10. The microtiter plate was then covered in Press ‘n Seal and incubated statically at 37 °C. After 16 hours, the OD_{600} was measured, and MIC calculated as 90% inhibition of bacterial growth compared to the untreated control.

Fractional Inhibitory Concentration (FIC) Assay

MIC testing in a checkerboard design to determine whether **NDM-335** potentiates ceftobiprole towards the ceftobiprole susceptible MRSA strain ATCC BAA-1556. Briefly, $5x10^5$ CFU/mL of bacterial cells (10 µL) were added to individual wells of a microtiter plate containing 88 µL of Muller-Hinton Broth media. Each row of the 96 well plate was supplemented with increasing concentrations of the **NDM-335** (0, 3.12, 6.25, 12.5, 25, 50, 100, and 200 µM), and each column was supplemented with increasing concentrations of ceftobiprole (0, 0.125, 0.25, 0.5, 1.0, 2, 4, 8, 16, 32, and 64 µg/mL). Plates were incubated at 37 °C for 16 - 20 hours, and the lowest concentration of each **NDM-335** or ceftobiprole that inhibited bacterial growth alone or in combination was determined by naked eye. In absence of **NDM-335**, the ceftobiprole MIC was recorded as 1 mg/mL. In the presence of 3.12 µM and 6.25 µM **NDM-335** the MIC shifts to 0.5 µg/mL. All other concentrations of **NDM-335** (12.5-200 mM) elicited a four-fold shift to 0.25 µg/mL.[1]

Growth Curve

MRSA ATCC BAA-1556 was cultured overnight in CAMHB and sub-cultured to 5 X 10^5 CFU/mL in fresh CAMHB. The subculture was then transferred to culture tubes in 3 mL aliquots, which were then dosed with NDM-427 and oxacillin. One aliquot was not dosed and served as a control. Samples were dosed with 7.5 µM NDM-427 and 2 µg/mL oxacillin, 15 µM NDM-427 and 4 µg/mL oxacillin, and 30 µM and 8 µg/mL oxacillin. All samples were incubated at 37 °C with shaking at 200 rpm. At 2, 4, 6, 8, and 24-hour time points, 100 µL was taken from each sample and serially diluted 10-fold in fresh CAMHB up to seven times. From the diluted culture, 100 µL was plated on Tryptic Soy agar and incubated at 37 °C overnight. The total number of bacterial colonies on each plate was determined using a SphereFlash colony counter (NEUTEC Group Inc.).

*Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) of MRSA AH-1263 after treatment with **NDM-335***

The effect of **NDM-335** on *mecA, graR*, and *graS* transcripts of MRSA AH-1263 cells was measured using real-time quantitative PCR (RT-*q*PCR). Briefly, overnight cultures of MRSA AH-1263 were grown overnight in Muller-Hinton Broth media. Overnight grown cells were diluted (1:100 dilution) in fresh media and sub-cultured to a final OD 600 nm of 0.2 at 37 °C. Each bacterial cell culture was treated with 0, or 6.2 or 12.5 or 25, or 50 µM of **NDM-335** for 1 hour at 37 °C, and cells were collected by centrifugation at 3800 g for 10 minutes. Total bacterial RNA was isolated from cells using the Qiagen RNeasy kit, according to the manufacturer's recommendations for prokaryotic RNA isolation (Qiagen, Germantown, MD). For *q*RT-PCR, 2 µg of RNA of each sample was treated with 2 units of DNase I (New England Biolabs, Ipswich, MA, USA) for 1 h at 37 °C and re-purified using Qiagen RNeasy kits, following the manufacturer's instructions. The quantity of RNA was measured using the Thermo Scientific NanoDrop OneC UV-Vis Spectrophotometer (Thermo Scientific, East Lyme, CT), and 300 ng of RNA was converted to cDNA using qScript cDNA Supermix (Quantabio, Beverly, MA). cDNA was amplified using PerfeCTa SYBR Green FastMix (Quantabio, Beverly, MA), and fluorescence was read on the Bio-Rad (Hercules, CA, USA) CFX 96 Connect Real-Time PCR System. Transcript levels were compared to the internal 16S rRNA control (ΔΔCt) and expressed as fold change compared to the control samples. The following primer pairs were used in this assay for qRT-PCR: 16S rRNA forward TAACCTACCTATAAGACTGGGATAA; 16S rRNA reverse

GCTTTCACATCAGACTTAAAAA; *mecA* forward GTACTGCTATCCACCCTCAAAC; *mecA* reverse TTCTTCGTTACTCATGCCATACA; *graS* forward ACGTCAACGGCTAACAGAAA; *graS* reverse GACGTGACTTGCAGGTGAATA; *graR* forward GAGAGAAGTTTCCAACGTACCA an *graR* reverse TCTGCGCCAAGTTCCATAC.

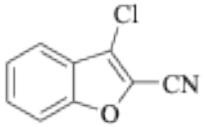
GENERAL CHEMISTRY EXPERIMENTAL

All reagents for chemical synthesis were purchased from commercial sources and used as received without further purification. All reactions were conducted under an atmosphere of nitrogen using anhydrous solvents unless otherwise specified. Thin layer chromatography (TLC) was performed using 250 μM Silica Gel 60 F_{254} pre-coated plates (EMD Chemicals Inc.). Flash chromatography was performed using 230–400 Mesh 60Å Silica Gel from Sorbent Technologies. All NMR spectra were recorded at 25 °C on Bruker AVANCE III HD spectrometers. Chemical shift values (δ) are reported in parts per million (ppm) relative to the respective NMR solvent; coupling constants (*J*) are in hertz (Hz). Abbreviations are s = singlet; d = doublet; dd = doublet of doublets; t = triplet; dt = doublet of triplets; tt = triplet of triplets; m = multiplet. NMR solvents were obtained from Cambridge Isotope Labs and used as is. High-resolution mass spectrometry (HRMS) measurements were determined by electrospray ionization (ESI) on a LC/ESI-QTOF-MS system (Thermo Scientific UltiMate 3000 UHPLC system coupled to a Bruker micrOTOF-Q II quadrupole time-of-flight mass spectrometer, ESI-QqTOF-MS) at the Mass Spectrometry and Proteomics Facility at the University of Notre Dame. Infrared spectra were recorded on a Bruker Alpha II FT-IR spectrometer (ν_{max} in cm^{-1}). UV-Vis measurements were conducted on a Thermo Spectronic Genesys 10 UV-Vis scanning spectrophotometer and reported as the wavelength of maximum absorbance (λ_{max} in nm). All compounds were characterized and biologically tested at ≥95% purity, as determined by LC-MS analysis on a Advion AVANT Expression Model L LC/CMS (compact mass spectrometer) system (Kinetex Polar C18 column, 2.6 μm particle size, 50 x 2.1 mm) or a Thermo Scientific UltiMate 3000 UHPLC system.

General Synthetic Procedure for EDC Coupling

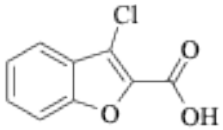
An oven-dried round-bottom flask under N_2 was charged with the appropriate carboxylic acid (207 mg, 1.1 eq.), dimethyl amino pyridine (54.1 mg, 0.5 eq.) and 3-(((ethylimino)methylene)amino)-*N,N*-dimethylpropan-1-amine hydrochloride (EDC) (510 mg, 3 eq.). The flask was put under vacuum and purged with argon, then anhydrous DCM was added (5 mL), and the reaction mixture stirred for 30 minutes at room temperature. The appropriate aniline (200 mg, 1 eq.) was then added to the reaction mixture, and it was allowed to stir overnight. The resulting mixture was diluted with chloroform, washed with brine (3 X 10 mL), and dried with $MgSO_4$. The crude material was purified via flash chromatography using 9:1 $CHCl_3$:CH_3OH to obtain pure product

KNOWN COMPOUND CHARACTERIZATION



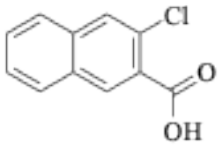
3-Chlorobenzofuran-2-carbonitrile

$POCl_3$ (0.278 mL, 2.98 mmol) was added dropwise to a stirred solution of benzofuran-3(2H)-one (200 mg, 1.49 mmol) in dry DMF (10 mL) at 0 °C. It was stirred at ambient temperature for 1 h, again cooled to 0 °C, and then a solution of $NH_2OH{\cdot}HCl$ (207 mg, 2.98 mmol) in DMF (5 mL) was added dropwise to this solution. The reaction mixture was stirred at 50 °C for 1 h and poured into ice-cold water (90 mL). The formed precipitate was collected by filtration, washed with water (3 × 20 mL), and dried under vacuum to provide the title compound in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 7.9 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.38 (ddd, J = 8.0, 6.7, 1.4 Hz, 1H). Spectral data matches published values.[2]



3-Chlorobenzofuran-2-carboxylic acid

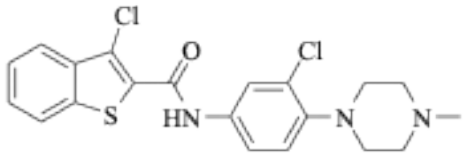
A mixture of 3-chlorobenzofuran-2-carboxylic acid (265 mg, 1.49 mmol) and KOH (837 mg, 14.9 mmol) in 1:1 CH_3OH/H_2O (2 mL) was stirred at 100 °C for 16 h. After cooling to RT, the mixture was adjusted to pH 7 using 3 N HCl. The mixture was concentrated to provide the product as a white solid (41 mg, 14% yield). ^{1}H NMR (400 MHz, DMSO-d_6). δ 14.01 (s, 1H), 7.79 (d, J = 6.4 Hz, 1H), 7.77 (d, J = 4.9 Hz, 1H), 7.66 – 7.60 (m, 1H), 7.48 (t, J = 7.6 Hz, 1H). Spectral data matches published values by Enamine Ltd.



3-Chloro-2-naphthoic acid

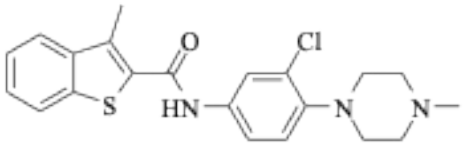
A solution of sodium nitrite (208 mg, 3.01 mmol) in water (2 mL) was added drop wise to a cooled (0 °C) solution of 3-amino-2-naphthoic acid (512 mg, 2.74 mmol) in acetonitrile (16 mL) and 3 M HCl (10 mL). The reaction was stirred at 0 °C for 1 h and a solution of copper(l) chloride (1.62 g, 16.4 mmol) in 2 M aq. HCl (5 mL) was added dropwise to the cooled mixture. The solution was stirred at 0 °C for 10 min, then heated to 50 °C for 45 min. The resulting mixture was allowed to cool to RT and poured onto cold water. The mixture was extracted three times with ethyl acetate, and the combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford 3-chloro-2-naphthoic acid as a brown solid (95 mg, 17% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.92 – 7.83 (m, 2H), 7.74 (d, J = 8.2 Hz, 1H), 7.57 (t, J = 7.5 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H). Spectral data matches published values.[3]

NOVEL COMPOUND CHARACTERIZATION



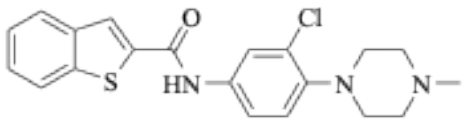
3-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)benzo[b]thiophene-2-carboxamide (NDM-335)

Using the general procedure for EDC-mediated coupling, 3-chlorobenzo[b]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-335**). **NDM-335** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 49.5% yield (92 mg, 220 μmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.17 (dt, J = 7.0, 3.5 Hz, 1H), 7.97 – 7.92 (m, 2H), 7.64 (ddt, J = 10.1, 7.1, 3.2 Hz, 3H), 7.26 (d, J = 8.8 Hz, 1H), 3.50 (d, J = 11.1 Hz, 2H), 3.37 (s, 2H), 3.17 (m, 4H), 2.82 (d, J = 4.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 159.4, 146.0, 137.1, 136.1, 134.5, 132.4, 128.1, 127.9, 126.7, 124.0, 123.0, 122.3, 121.5, 120.2, 55.2, 51.3, 46.2. HRMS (ESI) m/z calculated for $C_{20}H_{20}Cl_2N_3OS$ $[M + H]^+$: 420.0699, found 420.0697. LC trace: 100%. UV (λ_{max} nm): 306 nm. IR ν_{max} (cm^{-1}): 3396, 3015, 2923, 2362, 1650.



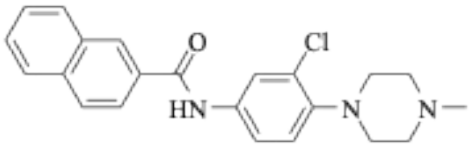
N-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzo[b]thiophene-2-carboxamide (NDM-343)

Using the general procedure for EDC-mediated coupling, 3-methylbenzo[b]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-343**). **NDM-343** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 19% yield (68 mg, 0.17 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.07 – 8.01 (m, 1H), 7.97 – 7.90 (m, 1H), 7.87 (d, J = 2.4 Hz, 1H), 7.61 (dd, J = 8.8, 2.5 Hz, 1H), 7.54 – 7.48 (m, 2H), 7.18 (d, J = 8.8 Hz, 1H), 2.95 (s, 4H), 2.61 (s, 3H), 2.50 (dt, J = 3.6, 1.8 Hz, 4H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.0, 145.6, 140.1, 138.8, 135.0, 134.6, 132.3, 127.9, 127.0, 125.4, 123.8, 123.3, 122.4, 121.3, 120.3, 55.3, 51.4, 46.3, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.3, 13.5. HRMS (ESI) m/z calculated for $C_{21}H_{23}ClN_3OS$ $[M + H]^+$: 400.1245, found 400.1247. LC trace: 100%. UV (λ_{max} nm): 308 nm. IR ν_{max} (cm^{-1}): 3246, 2360, 1643.



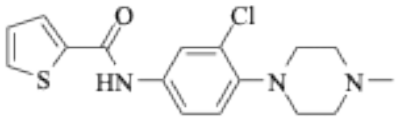
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)benzo[b]thiophene-2-carboxamide (NDM-400)**

Using the general procedure for EDC-mediated coupling, benzo[b]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-400**). **NDM-400** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 74.9% yield (256 mg, 663 μmol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (s, 1H), 7.95 (dd, *J* = 6.8, 2.5 Hz, 2H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.62 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.53 – 7.42 (m, 2H), 7.18 (d, *J* = 8.7 Hz, 1H), 3.10 (s, 4H), 2.69 (s, 4H), 2.40 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.6, 145.5, 141.0, 140.2, 139.6, 134.9, 127.9, 127.1, 126.3, 125.9, 125.6, 123.4, 122.3, 121.4, 120.2, 55.3, 51.4, 46.2. HRMS (ESI) m/z calculated for $C_{20}H_{21}lN_3OS$ $[M + H]^+$: 386.1088, found 386.1079. LC trace: 100%. UV (λ_{max} nm): 310 nm. IR ν_{max} (cm^{-1}): 2920, 2362, 1643.



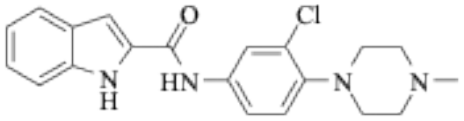
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)-2-naphthamide (NDM-365)**

Using the general procedure for EDC-mediated coupling, 2-naphthoic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-365**). **NDM-365** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 44% yield (74 mg, 0.19 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.58 (s, 1H), 8.12 – 8.05 (m, 2H), 8.05 – 7.99 (m, 3H), 7.77 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.69 – 7.61 (m, 2H), 7.28 (d, *J* = 8.8 Hz, 1H), 3.52 (s, 2H), 3.40 (s, 2H), 3.22 (s, 2H), 3.04 (s, 2H), 2.87 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.3, 143.5, 139.1, 135.5, 131.4, 129.0, 128.7, 127.6, 122.6, 120.8, 120.1, 53.8, 42.3. HRMS (ESI) m/z calculated for $C_{22}H_{23}ClN_3O$ $[M + H]^+$: 380.1524, found 380.1530. LC trace: 100%. UV (λ_{max} nm): 308 nm. IR ν_{max} (cm^{-1}): 3267, 2363, 1647.



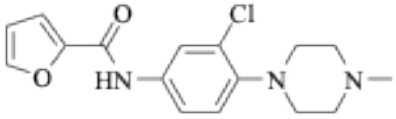
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)thiophene-2-carboxamide (NDM-366)**

Using the general procedure for EDC-mediated coupling, thiophene-2-carboxylic acid was reacted with 4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-366**). **NDM-366** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a gray solid in 89.4% yield (266 mg, 792 μmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 8.11 (d, *J* = 3.1 Hz, 1H), 7.97 (d, *J* = 2.3 Hz, 1H), 7.87 (d, *J* = 5.0 Hz, 1H), 7.70 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.27 – 7.18 (m, 2H), 3.48 (s, 4H), 3.14 (s, 4H), 2.81 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.3, 143.5, 139.1, 135.5, 131.4, 129.0, 128.7, 127.6, 122.6, 120.8, 120.1, 53.8, 48.4, 42.23. HRMS (ESI) m/z calculated for $C_{16}H_{19}ClN_3OS$ $[M + H]^+$: 336.0932, found 336.0926. LC trace: 100%. UV (λ_{max} nm): 308 nm. IR ν_{max} (cm^{-1}): 2926, 2360, 1646.



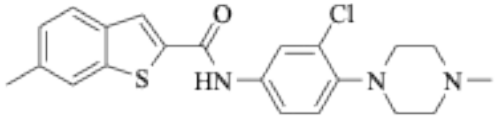
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)-1H-indole-2-carboxamide (NDM-406)**

Using the general procedure for EDC-mediated coupling, 1H-indole-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-406**). **NDM-406** was dissolved in CH3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a tan solid in 39% yield (63 mg, 0.17 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, *J* = 2.4 Hz, 1H), 7.71 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.31 (s, 1H), 7.30 – 7.21 (m, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 3.64 (d, *J* = 12.3 Hz, 2H), 3.55 (d, *J* = 13.8 Hz, 2H), 3.40 (d, *J* = 10.0 Hz, 2H), 3.16 – 3.07 (m, 2H), 3.02 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.8, 143.2, 137.3, 135.9, 130.6, 128.7, 127.6, 124.1, 122.3, 121.6, 120.8, 119.9, 119.8, 111.7, 103.9, 53.8, 48.5, 42.3. HRMS (ESI) m/z calculated for $C_{20}H_{22}ClN_4O$ $[M + H]^+$: 369.1477, found 369.1472. LC trace: 100%. UV (λ_{max} nm): 308 nm. IR ν_{max} (cm^{-1}): 3290, 2360, 1650.



***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)furan-2-carboxamide (NDM-407)**

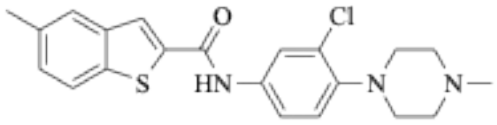
Using the general procedure for EDC-mediated coupling, furan-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-407**). **NDM-407** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 35% yield (50 mg, 0.16 mmol). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.05 (s, 1H), 7.68 (d, *J* = 2.4 Hz, 1H), 7.45 (s, 1H), 7.37 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.10 (d, *J* = 3.3 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.48 (dd, *J* = 3.4, 1.7 Hz, 1H), 2.94 (s, 4H), 2.48 (s, 4H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 157.4, 147.3, 145.5, 143.5, 135.2, 128.7, 122.6, 120.7, 120.1, 115.1, 111.9, 53.8, 48.4, 42.3. HRMS (ESI) m/z calculated for $C_{16}H_{19}ClN_3O_2$ $[M + H]^+$: 320.1160, found 320.1169. LC trace: 100%. UV (λ_{max} nm): 304 nm. IR ν_{max} (cm^{-1}): 2926, 2360, 1696.



***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)-6-methylbenzo[b]thiophene-2-carboxamide (NDM-408)**

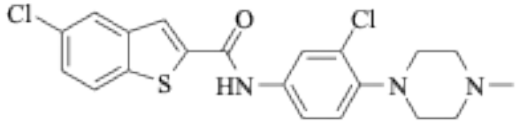
Using the general procedure for EDC-mediated coupling, 6-methylbenzo[b]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-408**). **NDM-408** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 59.3% yield (105 mg, 263 μmol). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.86 (s, 1H), 7.83 – 7.78 (m, 2H), 7.73 (s, 1H), 7.50 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 3.08 (s, 4H), 2.61 (s, 4H), 2.53 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.8, 143.5, 141.8, 137.7, 137.2, 137.1, 135.6,

128.7, 126.6, 125.6, 124.7, 122.6, 121.8, 120.8, 120.0, 53.8, 48.4, 42.3, 20.4. HRMS (ESI) m/z calculated for $C_{21}H_{23}ClN_3OS$ $[M + H]^+$: 400.1245, found 400.1242. LC trace: 100%. UV (λ_{max} nm): 310 nm. IR ν_{max} (cm^{-1}): 1650, 2362, 3443.



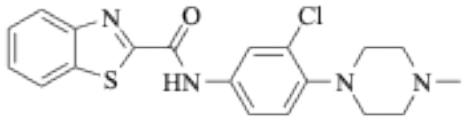
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)-5-methylbenzo[b]thiophene-2-carboxamide (NDM-427)**

Using the general procedure for EDC-mediated coupling, 5-methylbenzo[b]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-427**). **NDM-427** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 45.1% yield (159.7 mg, 399 μmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.25 (s, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.80 (s, 1H), 7.66 (dd, J = 8.8, 2.4 Hz, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.19 (d, J = 8.8 Hz, 1H), 2.96 (s, 4H), 2.52 – 2.48 (m, 4H), 2.45 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.7, 145.5, 140.3, 139.9, 138.3, 135.0, 134.9, 128.8, 127.8, 126.0, 125.5, 123.0, 122.4, 121.3, 120.2, 55.3, 51.4, 46.2, 21.4. HRMS (ESI) m/z calculated for $C_{21}H_{23}ClN_3OS$ $[M + H]^+$: 400.1245, found 400.1234. LC trace: 100%. UV (λ_{max} nm): 302. IR ν_{max} (cm^{-1}): 2923, 2850, 2359, 1643.



5-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)benzo[b]thiophene-2-carboxamide (NDM-419)

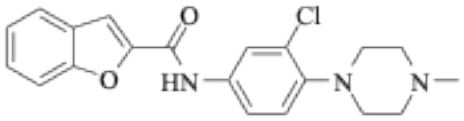
Using the general procedure for EDC-mediated coupling, 5-chlorobenzo[b]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-419**). **NDM-419** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 84.3% yield (157 mg, 373 μmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.31 – 8.27 (m, 1H), 8.14 (d, J = 2.1 Hz, 1H), 8.11 (d, J = 8.7 Hz, 1H), 7.91 (d, J = 2.5 Hz, 1H), 7.66 (dd, J = 8.8, 2.5 Hz, 1H), 7.53 (dd, J = 8.7, 2.1 Hz, 1H), 7.19 (d, J = 8.8 Hz, 1H), 2.96 (s, 4H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.3, 145.67, 142.5, 140.8, 139.4, 134.7, 130.56, 127.8, 127.1, 125.5, 125.2, 125.1, 122.4, 121.4, 120.3, 55.3, 51.3, 49.1, 46.3. HRMS (ESI) m/z calculated for $C_{20}H_{20}Cl_2N_3OS$ $[M + H]^+$: 420.0699, found 420.0704. LC trace: 100%. UV (λ_{max} nm): 304 nm. IR ν_{max} (cm^{-1}): 2926, 2376, 1646.



***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)benzo[d]thiazole-2-carboxamide (NDM-428)**

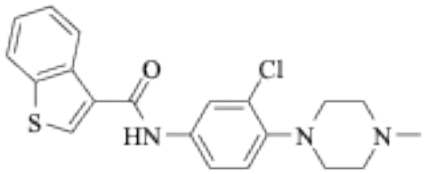
Using the general procedure for EDC-mediated coupling, benzo[d]thiazole-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash

chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-428**). **NDM-428** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a yellow solid in 67.1% yield (115 mg, 297 µmol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (d, *J* = 8.1 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 2.4 Hz, 1H), 7.80 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.60 (t, *J* = 7.2 Hz, 1 H), 7.27 (d, *J* = 8.8 Hz, 1H), 3.65 (d, *J* = 12.2 Hz, 2H), 3.57 (d, *J* = 13.5 Hz, 2H), 3.43 – 3.36 (m, 2H), 3.14 (t, *J* = 11.6 Hz, 2H), 3.02 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 158.4, 153.1, 144.0, 134.8, 128.8, 127.0, 126.9, 124.2, 122.5, 122.3, 120.9, 120.0, 53.8, 48.4, 42.3. HRMS (ESI) m/z calculated for $C_{19}H_{20}ClN_4OS$ [M + H] $^+$: 387.1041, found 387.1051. LC trace: 100%. UV (λ_{max} nm): 294 nm. IR ν_{max} (cm^{-1}): 3353, 2362, 1677.



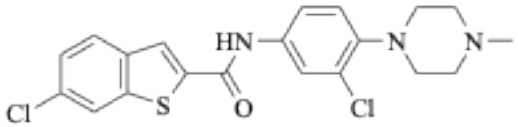
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)benzofuran-2-carboxamide (NDM-511)**

Using the general procedure for EDC-mediated coupling, benzofuran-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-511**). **NDM-511** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 51.5% yield (211 mg, 571 µmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 7.97 (d, *J* = 2.4 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 0.7 Hz, 1H), 7.74 – 7.69 (m, 2H), 7.55 – 7.49 (m, 1H), 7.40 – 7.35 (m, 1H), 7.18 (d, *J* = 8.8 Hz, 1H), 2.96 (s, 3H), 2.50 (dt, *J* = 3.5, 1.8 Hz, 7H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.0, 154.91, 149.0, 145.6, 134.6, 127.8, 127.7, 127.6, 124.4, 123.4, 122.6, 121.3, 120.5, 112.4, 111.3, 55.3, 51.3, 46.3. HRMS (ESI) m/z calculated for $C_{20}H_{21}ClN_3O_2$ $[M + H]^+$: 370.1317, found 370.1316. LC trace: 100%. UV (λ_{max} nm): 306 nm. IR ν_{max} (cm^{-1}): 3569, 2356, 1667.



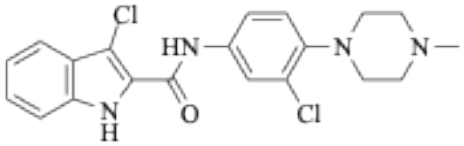
***N*-(3-Chloro-4-(4-methylpiperazin-1-yl)phenyl)benzo[b]thiophene-3-carboxamide (NDM-547)**

Using the general procedure for EDC-mediated coupling, benzo[b]thiophene-3-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/DCM) to afford the amide (**NDM-547**). **NDM-547** was dissolved in CH_3OH(10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 34.2% yield (117 mg, 303 µmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.55 (s, 1H), 8.43 – 8.40 (m, 1H), 8.11 – 8.07 (m, 1H), 7.95 (d, *J* = 2.4 Hz, 1H), 7.66 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.47 (m, 2H), 7.18 (d, *J* = 8.7 Hz, 1H), 3.39 (s, 5H), 2.96 (dd, *J* = 6.1, 3.3 Hz, 4H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.2, 145.2, 139.9, 137.5, 135.5, 132.4, 131.3, 127.9, 125.5, 125.5, 124.8, 123.4, 122.2, 121.3, 120.1, 55.3, 51.4, 46.2. HRMS (ESI) m/z calculated for $C_{20}H_{21}ClN_3OS$ $[M + H]^+$: 386.1088, found 386.1079. LC trace: 100%. UV (λ_{max} nm): 290. IR ν_{max} (cm^{-1}): 3257, 2926, 2356, 1660.



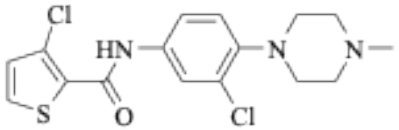
6-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)benzo[*b*]thiophene-2-carboxamide (NDM-703)

Using the general procedure for EDC-mediated coupling, 6-chlorobenzo[*b*]thiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/chloroform) to afford the amide (**NDM-703**). **NDM-703** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 4.37% yield (12.2 mg, 29.0 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.77 – 7.75 (m, 2H), 7.64 – 7.59 (m, 2H), 7.40 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.27 (dd, *J* = 8.6, 1.8 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 2.98 (s, 4H), 2.55 (s, 4H), 2.18 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.5, 143.7, 142.2, 138.3, 135.8, 128.0, 127.2, 126.1, 126.0, 122.8, 122.6, 122.3, 121.6, 120.2, 79.7, 79.4, 79.1, 53.2, 48.4, 42.6, 14.1 HRMS (ESI) m/z calculated for $C_{20}H_{20}Cl_2N_3OS$ $[M + H]^+$: 420.0699, found: 420.0696. LC trace: 100%. UV (λ_{max} nm): 302 nm. IR ν_{max} (cm^{-1}): 3443, 2361, 1650.



3-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)-1*H*-indole-2-carboxamide (NDM-704)

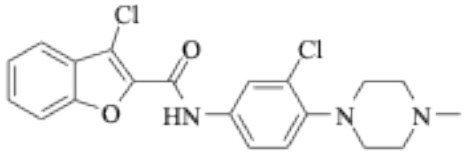
Using the general procedure for EDC-mediated coupling, 3-chloroindole-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/chloroform) to afford the amide (**NDM-704)**. **NDM-704** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 45.4% yield (81.1 mg, 201 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.70 (s, 1H), 8.62 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 2.5 Hz, 1H), 7.62 (t, *J* = 7.9 Hz, 2H), 7.48 – 7.44 (m, 2H), 7.32 (t, *J* = 7.0 Hz, 1H), 7.02 (d, *J* = 8.7 Hz, 1H), 3.02 (s, 4H), 2.57 (s, 4H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 143.7, 128.1, 125.6, 125.3, 122.0, 121.6, 121.3, 120.0, 119.0, 113.2, 79.7, 79.3, 79.0, 53.3, 48.7, 48.4, 48.0, 47.8, 47.6, 47.4, 42.5. HRMS (ESI) m/z calculated for $C_{20}H_{20}Cl_2N_4O$ $[M + H]^+$: 403.1087, found: 403.1082. LC trace: 100%. UV (λ_{max} nm): 304 nm. IR ν_{max} (cm^{-1}): 3288, 2376, 1642.



3-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)thiophene-2-carboxamide (NDM-702)

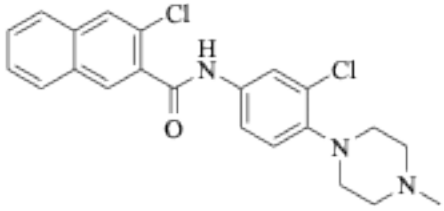
Using the general procedure for EDC-mediated coupling, 3-chlorothiophene-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/chloroform) to afford the amide (**NDM-702**). **NDM-702** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 34% yield (56 mg, 0.15 mmol). ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.59 (s, 1H), 7.64 (d, *J* = 2.5 Hz, 1H), 7.45 (d, *J* = 5.3 Hz, 1H), 7.40 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.00 – 6.93 (m, 2H), 3.00 (s, 4H), 2.54 (s, 4H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.2, 144.0 135.5, 131.6, 130.2, 129.5, 127.9, 125.4, 122.2, 121.7, 120.3, 53.1, 48.4, 42.5. HRMS (ESI) m/z calculated for $C_{16}H_{17}Cl_2N_3OS$ $[M + H]^+$: 370.0542, found.: 370.0559. LC trace: 96.44%. UV (λ_{max} nm): 296 nm. IR ν_{max} (cm^{-1}): 2940, 2370, 1656.



3-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)benzofuran-2-carboxamide (NDM-713)

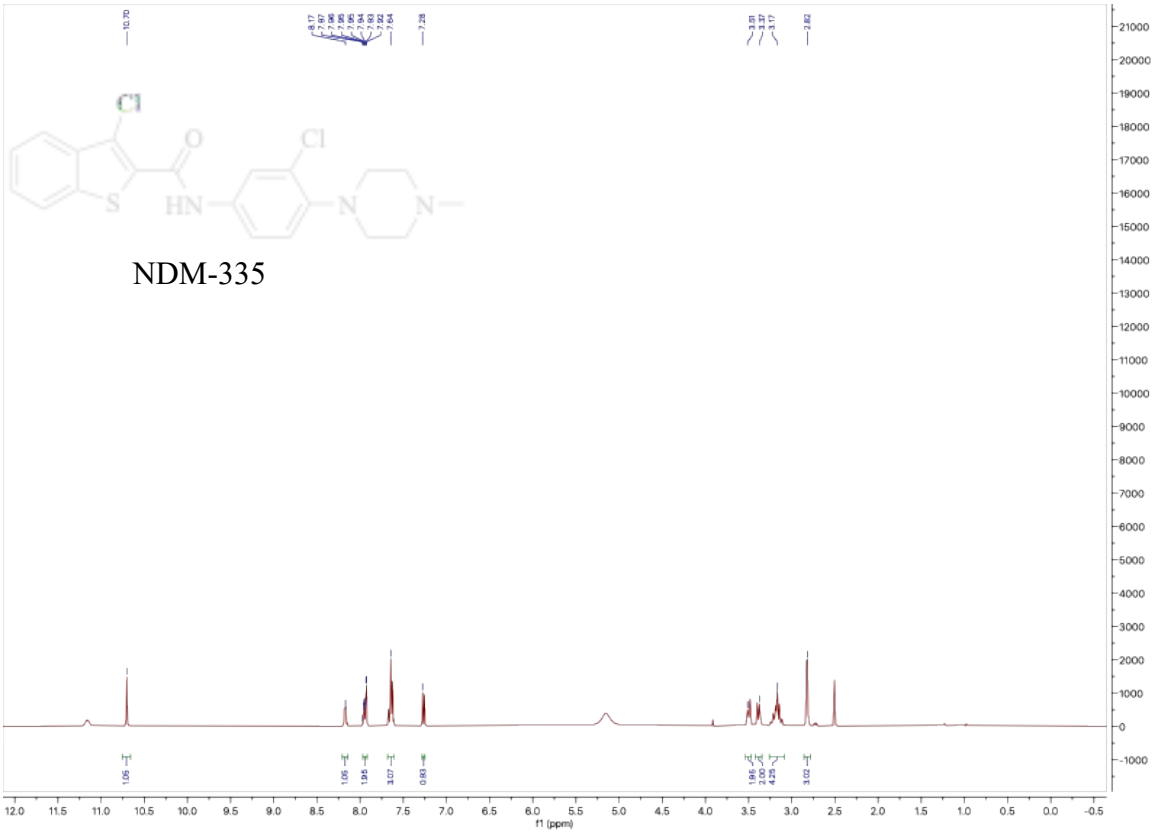
Using the general procedure for EDC-mediated coupling, 3-chlorobenzofuran-2-carboxylic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/chloroform) to afford the amide (**NDM-713**). **NDM-713** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 40.0% yield (29.5 mg, 73.0 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 6.5 Hz, 1H), 7.77 (d, *J* = 2.5 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.51 – 7.45 (m, 3H), 7.34 (ddd, *J* = 8.0, 6.5, 1.7 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 3.02 (s, 4H), 2.57 (s, 4H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d^6) δ 156.1, 152.5, 144.1, 142.3, 135.2, 129.4, 127.8, 126.6, 125.3, 122.7, 121.6, 120.7, 120.6, 116.5, 113.0, 53.1, 48.4, 42.5. HRMS (ESI) m/z calculated for $C_{20}H_{19}Cl_2N_3O_2$ $[M + H]^+$: 404.0927, found: 404.0900. LC trace: 100%. UV (λ_{max} nm): 296 nm. IR ν_{max} (cm^{-1}): 2926, 2366, 1697.

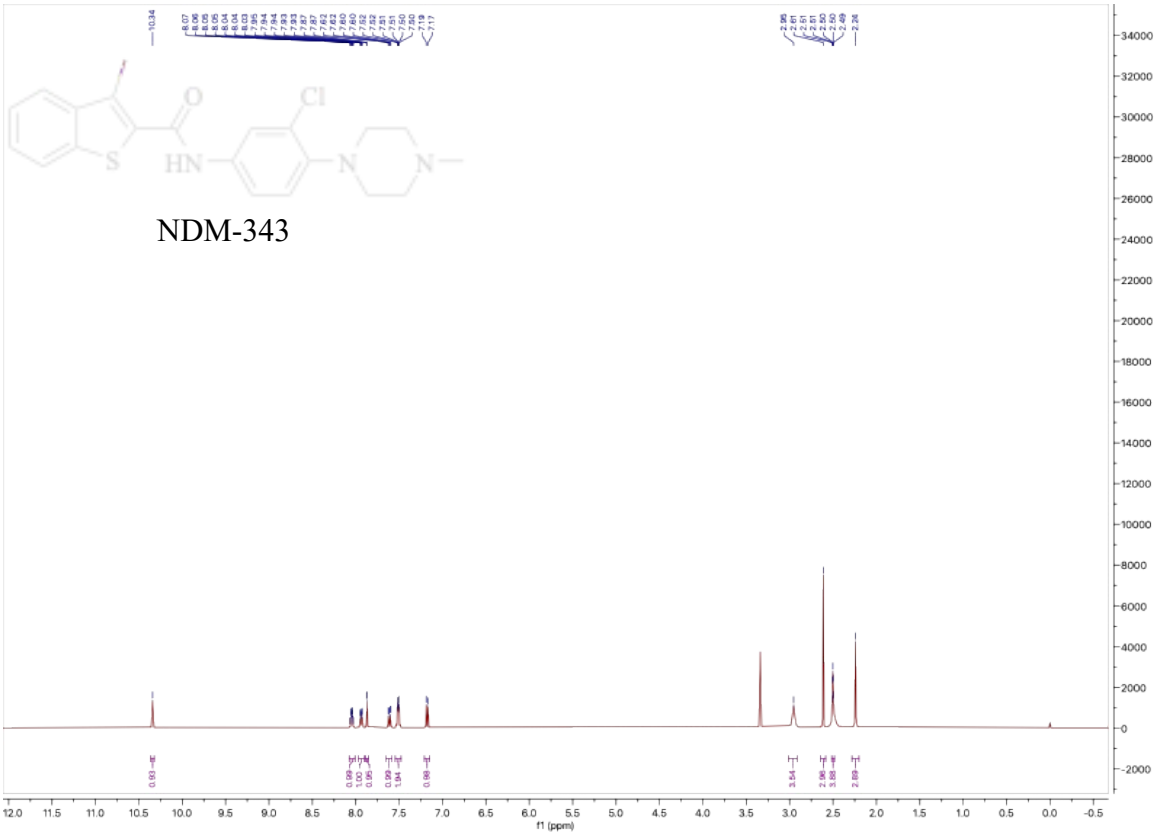


3-Chloro-*N*-(3-chloro-4-(4-methylpiperazin-1-yl)phenyl)-2-naphthamide (NDM-712)

Using the general procedure for EDC-mediated coupling, 3-chloro-2-napthoic acid was reacted with 3-chloro-4-(4-methylpiperazin-1-yl)aniline. The crude mixture was purified by flash chromatography (10% CH_3OH/chloroform) to afford the amide (**NDM-712**). **NDM-712** was dissolved in CH_3OH (10 mL) and spiked with 12 M HCl (0.5 mL), and solvent was removed to provide the HCl salt as a white solid in 31.0% yield (54.6 mg, 132 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 8.06 (s, 1H), 7.76 – 7.61 (m, 4H), 7.52 – 7.37 (m, 3H), 6.94 (d, *J* = 8.7 Hz, 1H), 2.98 (s, 4H), 2.54 (s, 4H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d^6) δ 165.4, 143.8, 136.1, 134.5, 134.1, 131.3, 129.1, 128.7, 128.7, 128.5, 128.0, 127.8, 127.6, 127.5, 121.8, 121.6, 119.7, 53.2, 48.5, 42.6. HRMS (ESI) m/z calculated for $C_{22}H_{21}Cl_2N_3O$ $[M + H]^+$: 414.1134, found: 414.1133. LC trace: 100%. UV (λ_{max} nm): 286 nm. IR ν_{max} (cm^{-1}): 3270, 2363, 1660.

NMR of Novel Compounds

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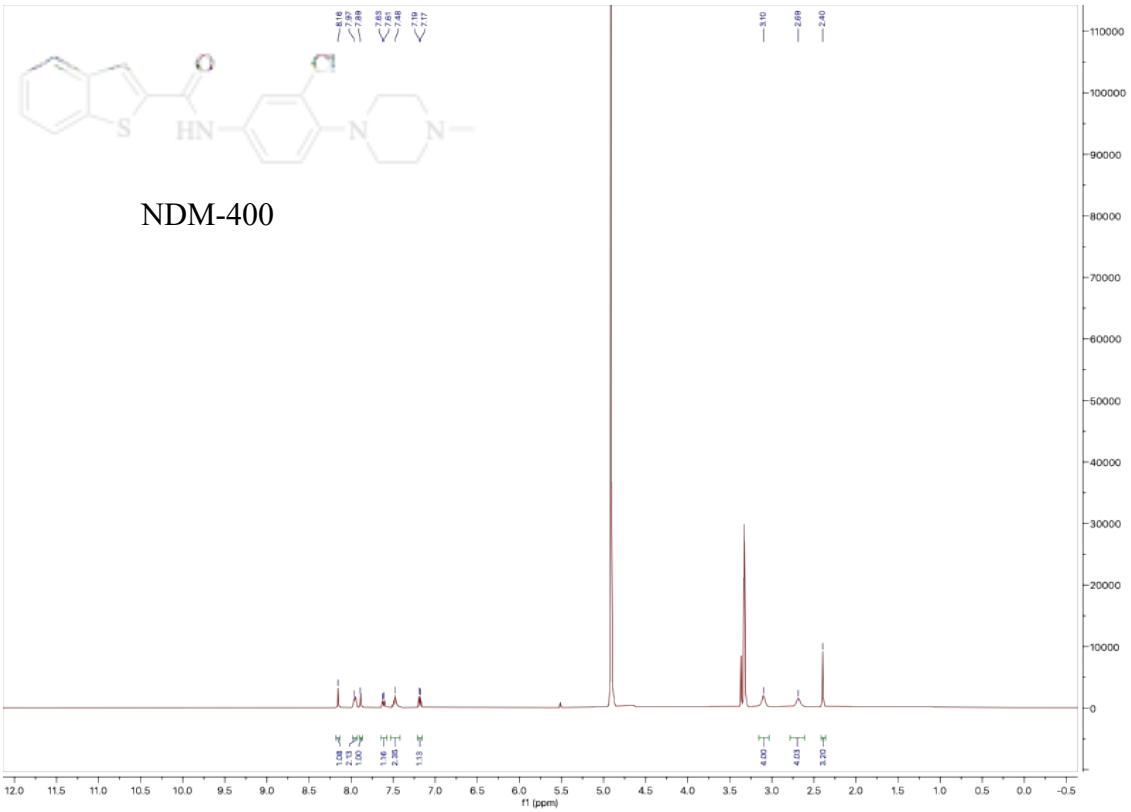
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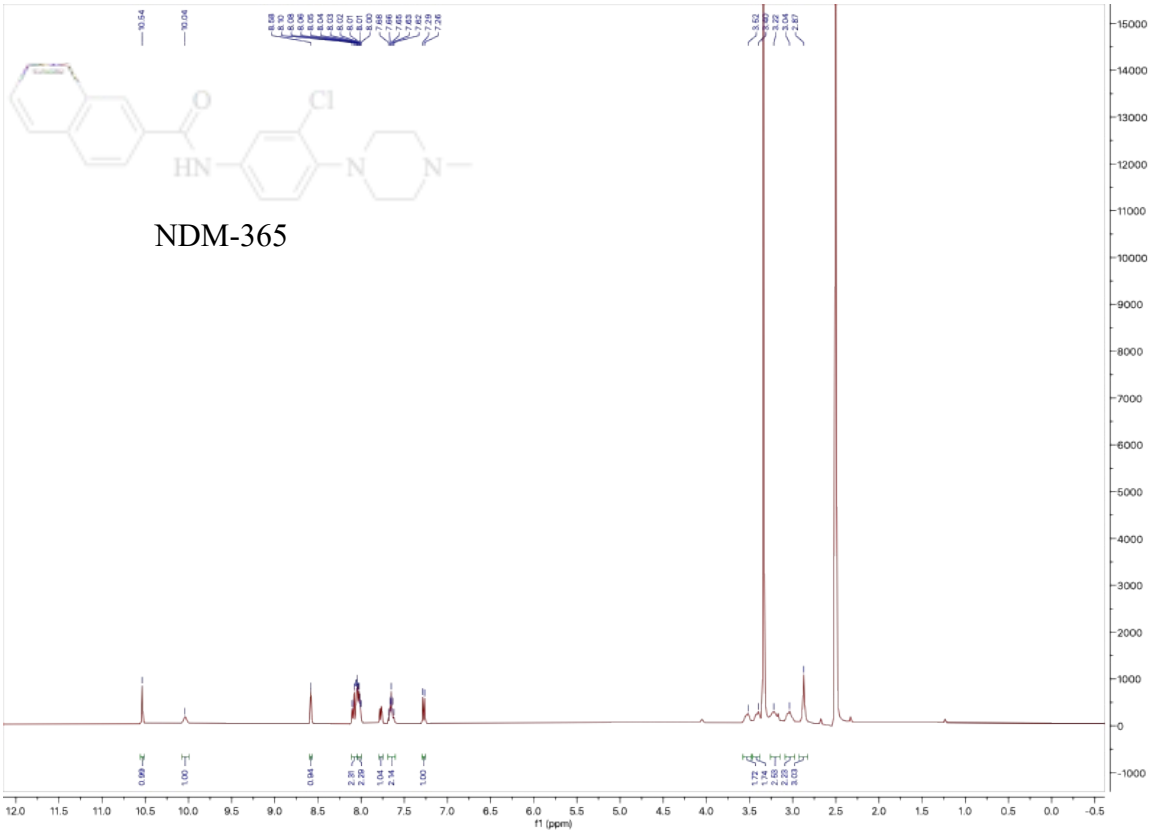
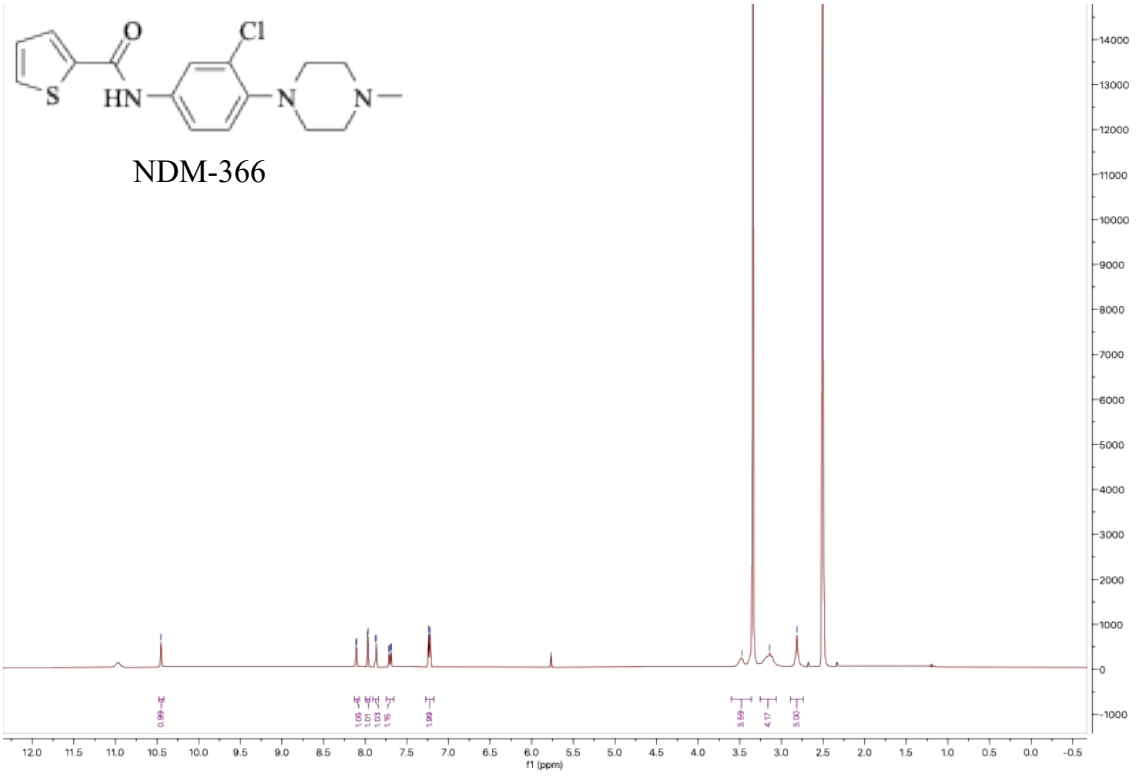
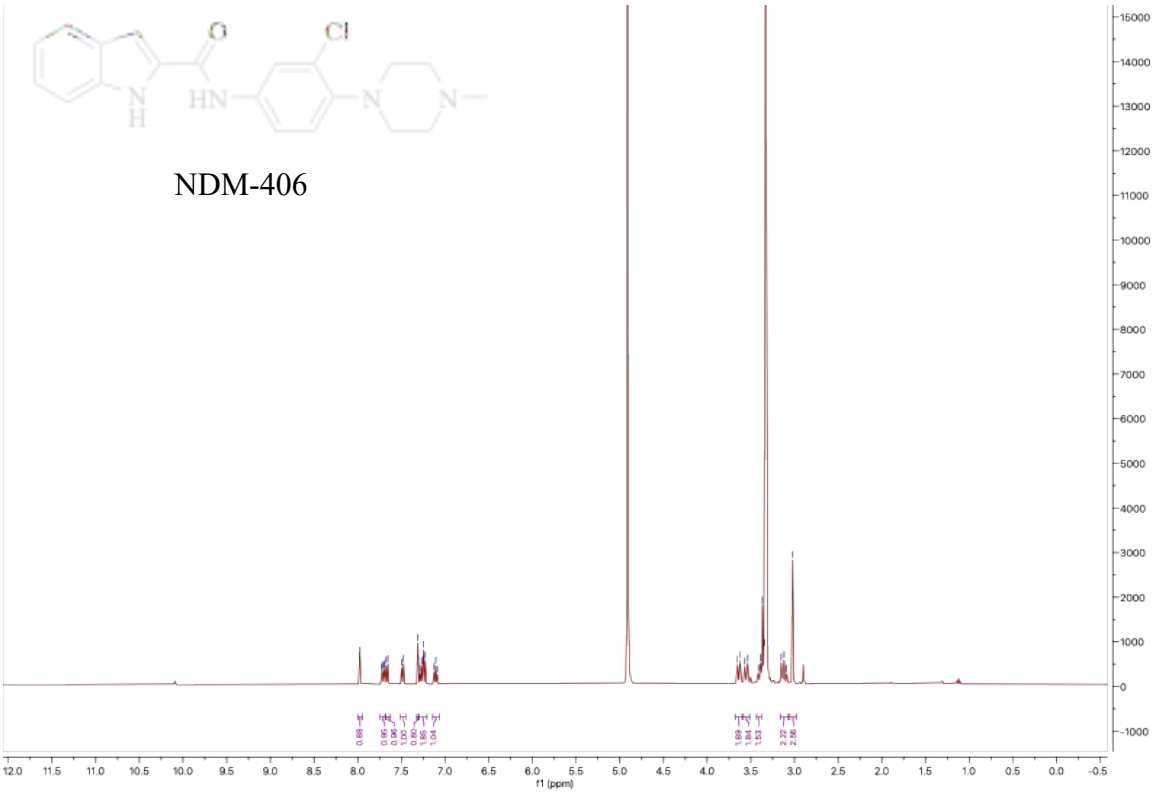


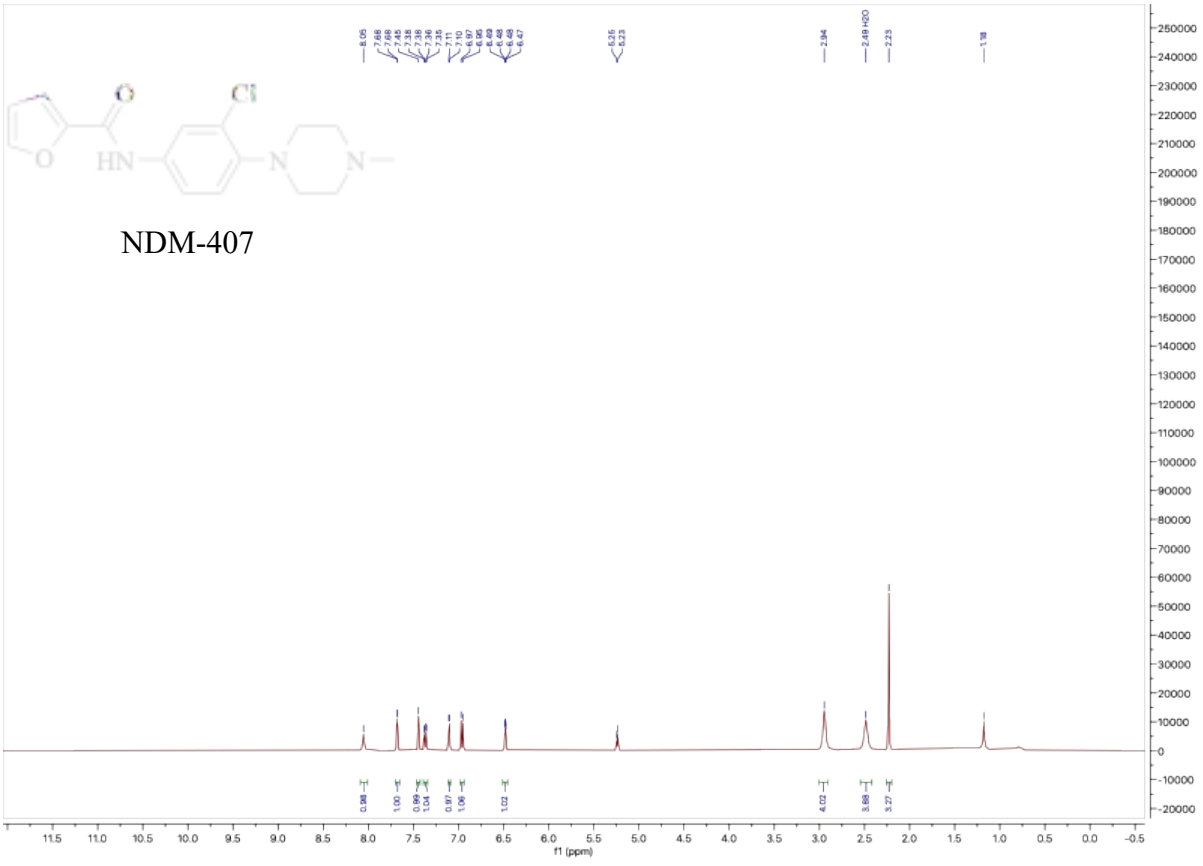
NDM-365



NDM-366



NDM-406



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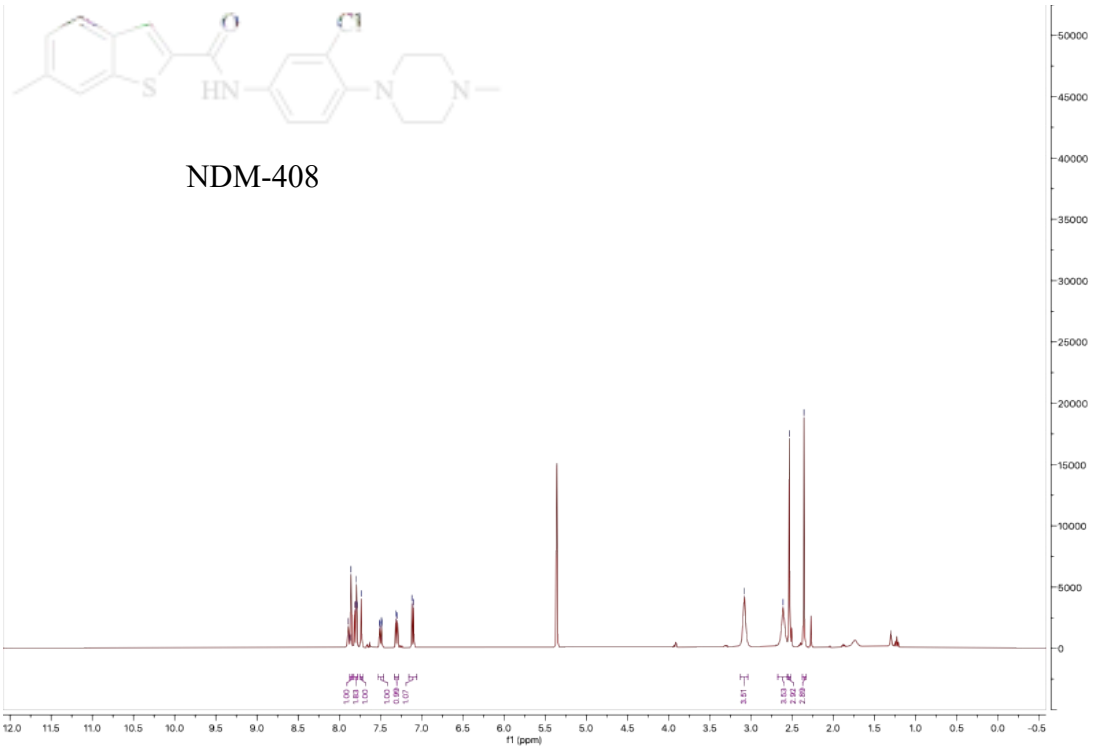

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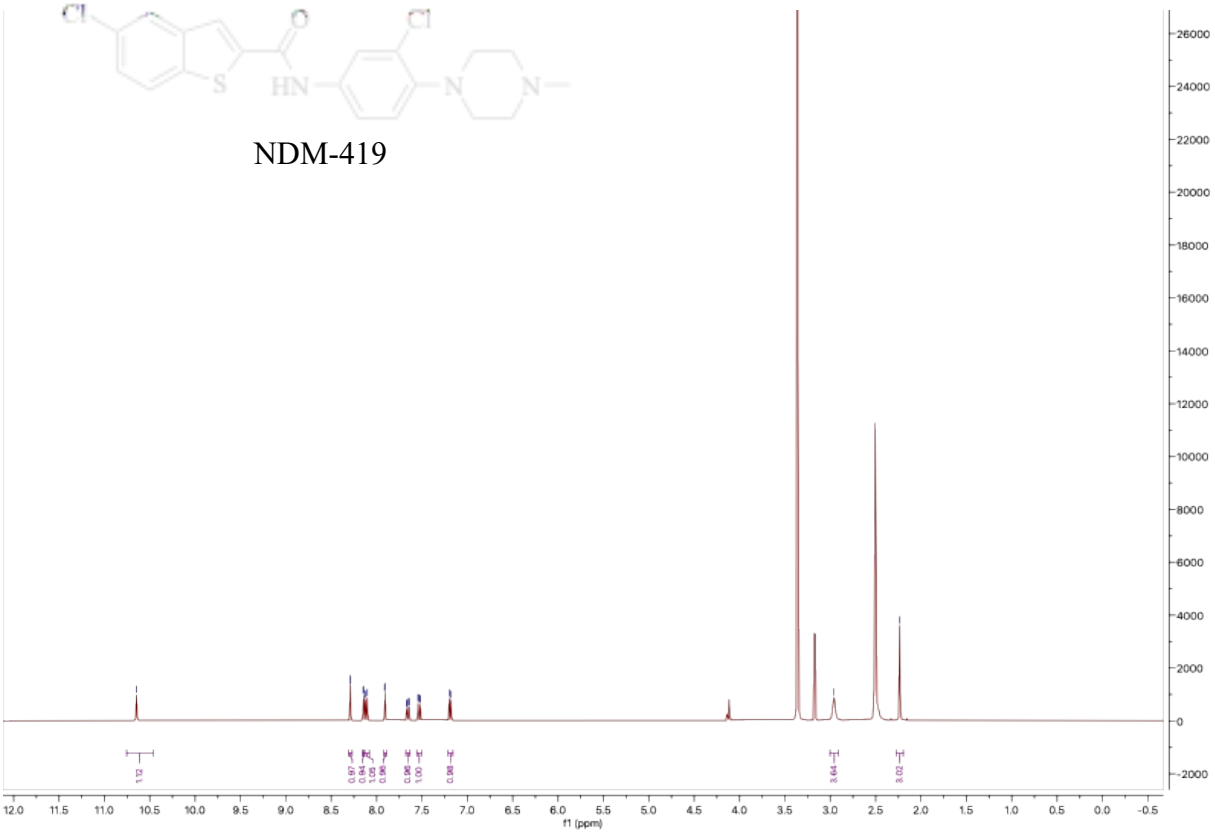
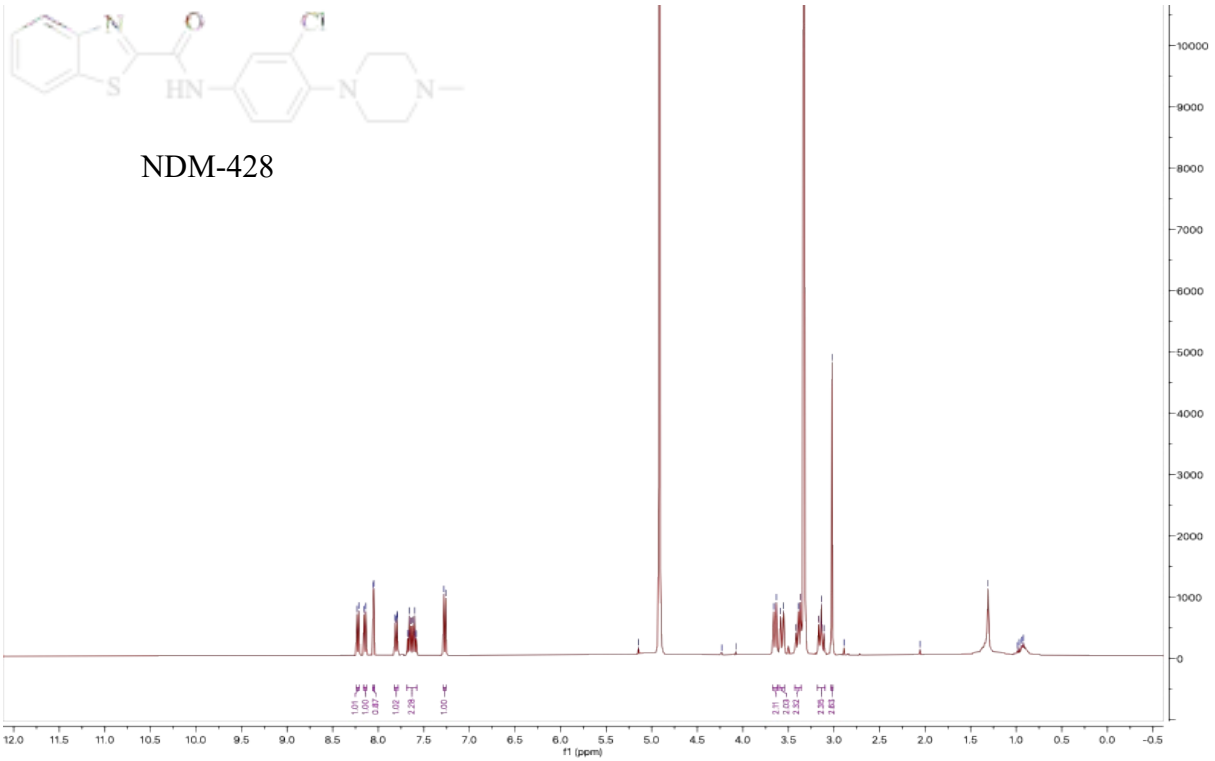


NDM-419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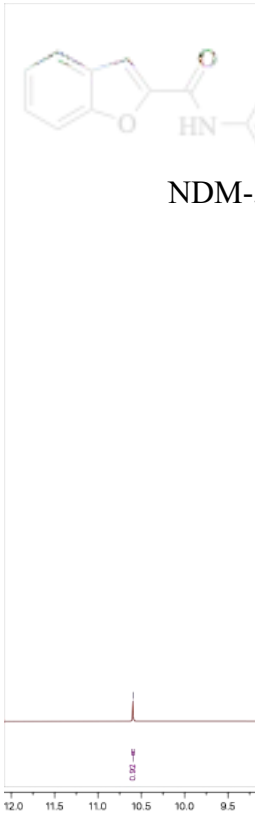
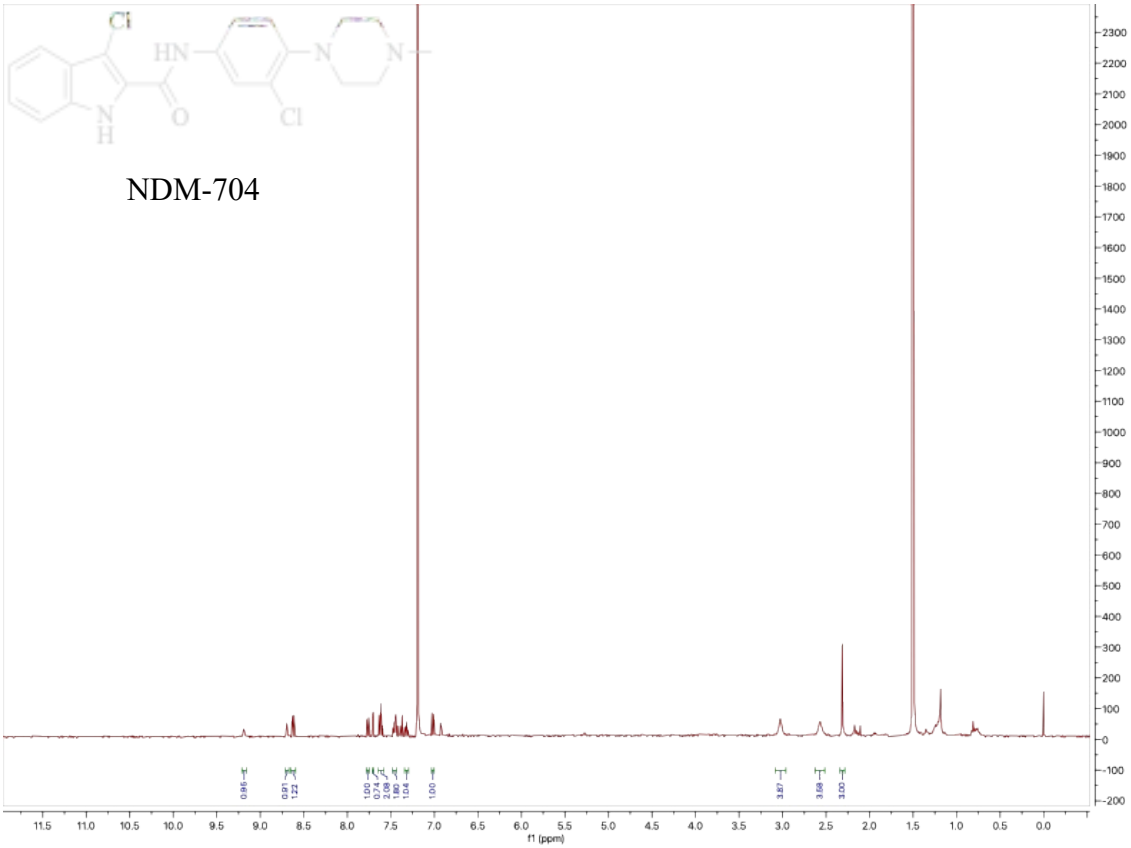

NDM-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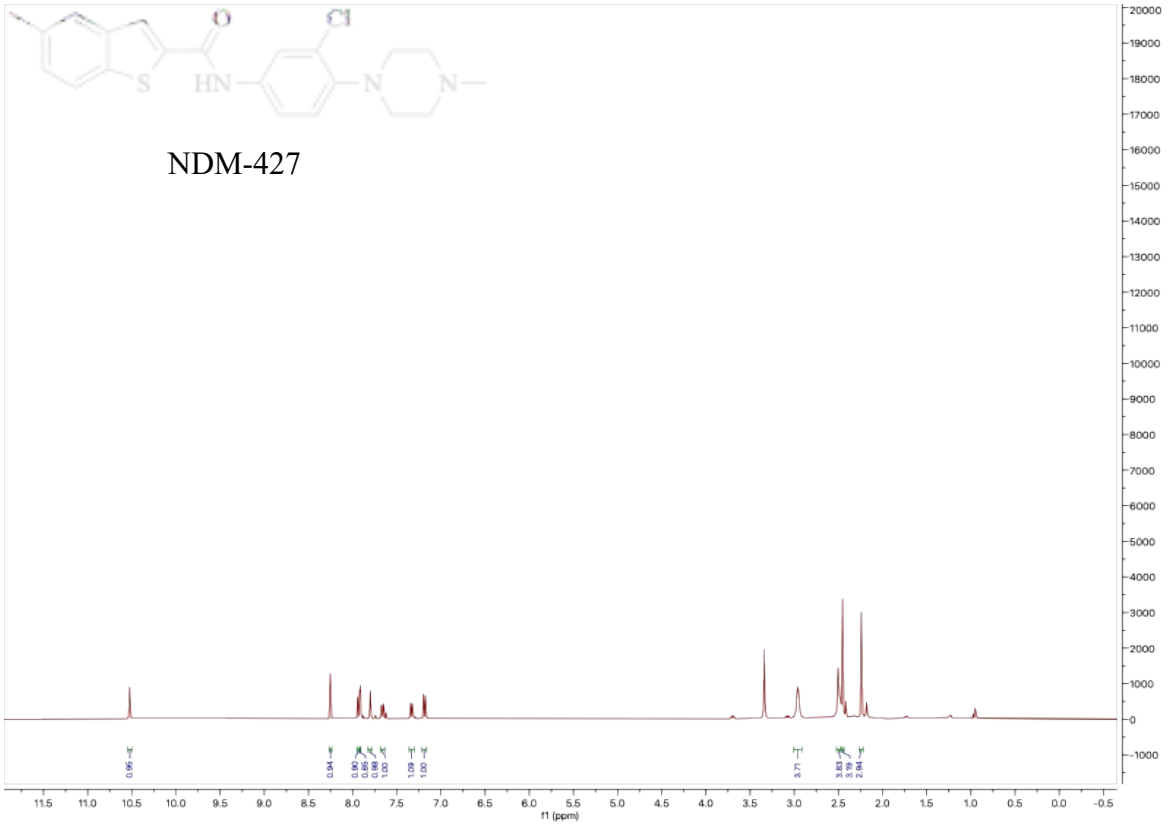
f1 (ppm)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HN
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9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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5.0
4.5
4.0
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2.0
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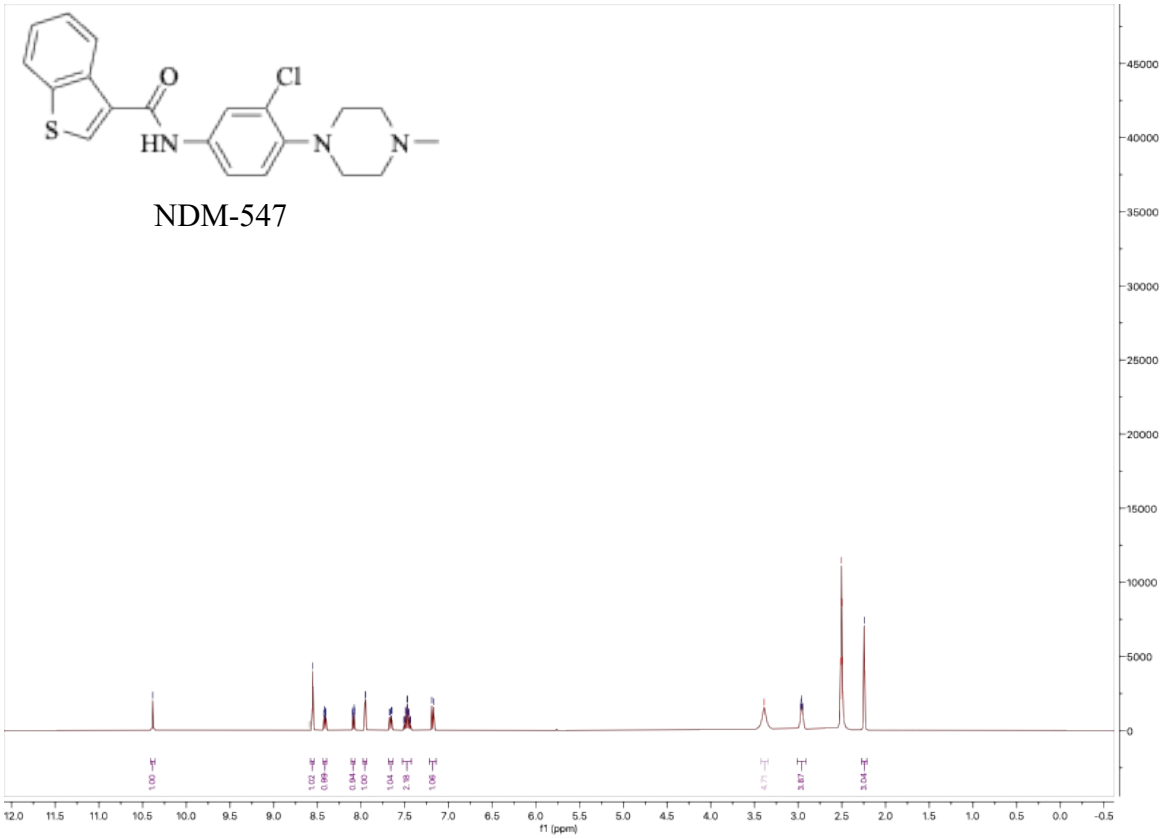
0.0
-0.5
f1 (ppm)

NDM-427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HN
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5000
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11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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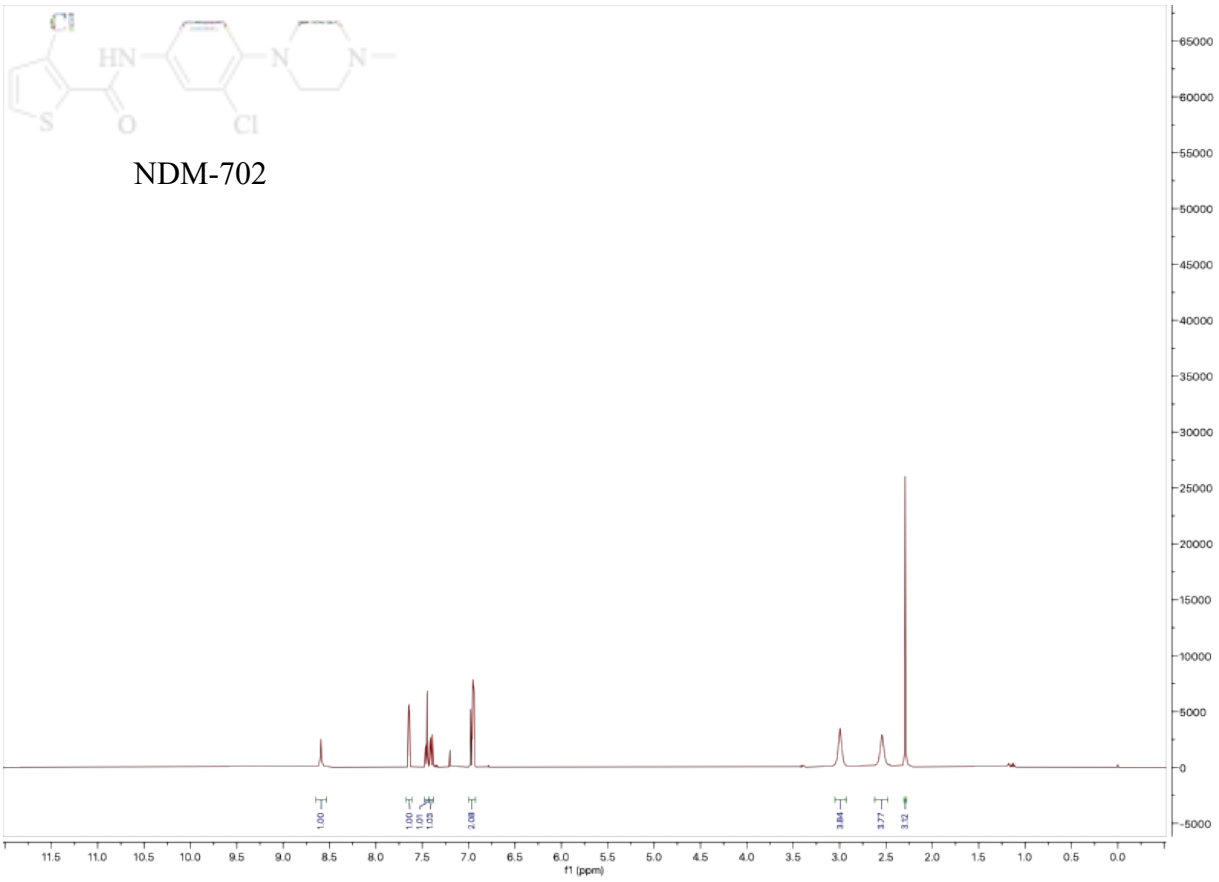
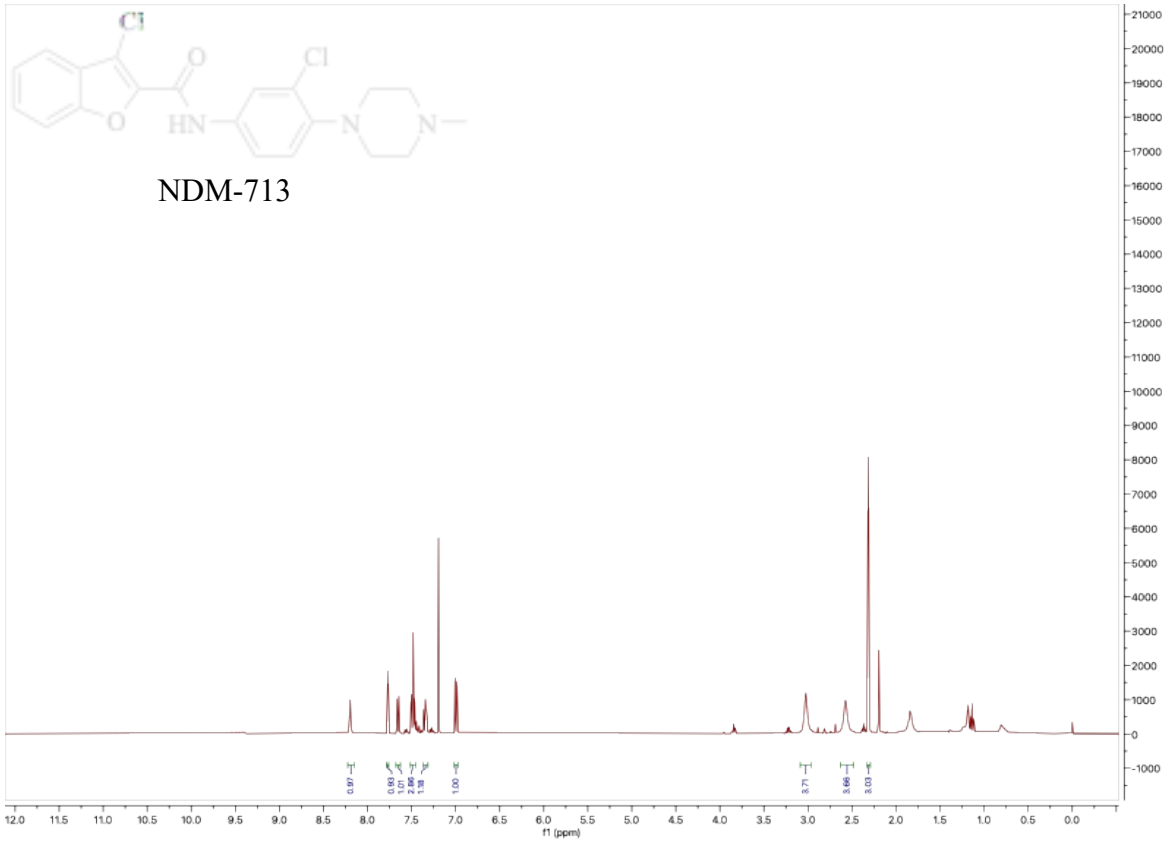
f1 (ppm)

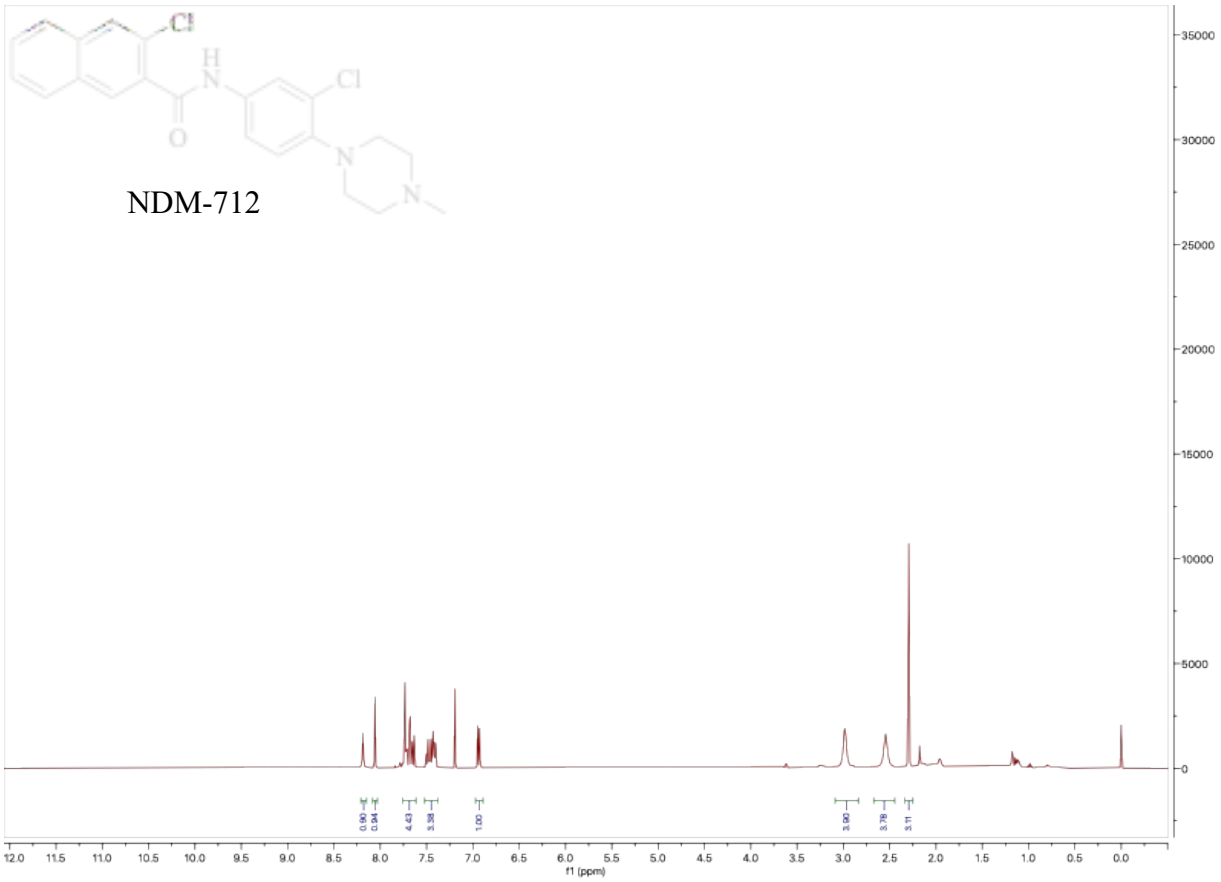
NDM-547



NDM-702



NDM-713


NDM-712
f1 (ppm)

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X-RAY CRYSTALLOGRAPHIC DATA FOR NDM-335

DISCUSSION

The compound crystallizes as colorless, needle-like crystals. There are four molecules of the protonated compound and associated chloride anion in the unit cell of the primitive, centrosymmetric, monoclinic space group $P2_1/c$.

The structure of the compound is as anticipated (see Figures). There are no unusual bond distances or angles within the molecule. The amide hydrogen atoms bonded to N1 and N3 were located from a difference Fourier map and refined freely. The hydrogen bonded to N1 forms a hydrogen bond to the carbonyl oxygen, O1, of a neighboring molecule. This results in a one-dimensional chain of hydrogen-bonded molecules parallel to the *b*-axis (see Table of Hydrogen Bonds and Figures for details). N3 forms a hydrogen bond to the counteranion chloride, Cl3.

ACKNOWLEDGMENT
Funding for instrumentation used in this study was supported by NSF MRI Award CHE-2214606.

CRYSTAL SUMMARY
Crystal data for $C_{20}H_{20}Cl_3N_3OS$; M_r = 456.80; Monoclinic; space group $P2_1/c$; a = 20.4070(7) Å; b = 11.1705(4) Å; c = 9.3384(3) Å; α = 90°; β = 98.4500(10)°; γ = 90°; V = 2105.64(12) Å^3; Z = 4; T = 120(2) K; λ(Cu-Kα) = 1.54178 Å; μ(Cu-Kα) = 5.001 mm^{-1}; d_{calc} = 1.441 g.cm^{-3}; 81758 reflections collected; 4304 unique (R_{int} = 0.0571); giving R_1 = 0.0263, wR_2 = 0.0699 for 4184 data with [I>2σ(I)] and R_1 = 0.0269, wR_2 = 0.0703 for all 4304 data. Residual electron density (e$^-$.Å^{-3}) max/min: 0.666/-0.304.

An arbitrary sphere of data was collected on a colorless needle-like crystal, having approximate dimensions of 0.338 × 0.064 × 0.043 mm, on a Bruker Venture diffractometer equipped with a Bruker PHOTON-III detector using a combination of ω- and φ-scans of 0.5°.[4] Data were corrected for absorption and polarization effects and analyzed for space group determination.[5] The structure was solved by dual-space methods and expanded routinely.[6] The model was refined by full-matrix least-squares analysis of F^2 against all reflections.[7] All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Unless otherwise noted, hydrogen atoms were included in calculated positions. Atomic displacement parameters for the hydrogens were tied to the equivalent isotropic displacement parameter of the atom to which they are bonded (U_{iso}(H) = 1.5U_{eq}(C) for methyl, 1.2U_{eq}(C) for all others).

Table 1. Crystal data and structure refinement for stefaniak_7974147.

Identification code	Stefaniak_7974147
Empirical formula	$C_{20}H_{20}Cl_3N_3OS$
Formula weight	456.80
Temperature (K)	120(2)
Wavelength (Å)	1.54178
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a (Å)	20.4070(7)
b (Å)	11.1705(4)
c (Å)	9.3384(3)
α (°)	90
β (°)	98.4500(10)
γ (°)	90
Volume (Å^3)	2105.64(12)
Z	4
Density (calculated, g.cm^{-3})	1.441
Absorption coefficient (μ, mm^{-1})	5.001
F(000)	944
Crystal color, habit	colorless, needle
Crystal size (mm^3)	0.338 × 0.064 × 0.043
θ range for data collection (°)	2.189 to 74.621
Index ranges	$-25 \leq h \leq 25$, $-13 \leq k \leq 13$, $-11 \leq l \leq 11$
Reflections collected	81758
Independent reflections	4304 [R_{int} = 0.0571]
Completeness to θ = 67.679°	100.0 %
Absorption correction	Numerical
Max. and min. transmission	0.7317 and 0.3199
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4304 / 0 / 262
Goodness-of-fit on F^2	1.033
Final R indices [I>2σ(I)]	R_1 = 0.0263, wR_2 = 0.0699
R indices (all data)	R_1 = 0.0269, wR_2 = 0.0703
Extinction coefficient	n/a
Largest diff. peak and hole (e$^-$.Å^{-3})	0.666 and -0.304

Table 2. Atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for stefaniak_7974147. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Cl(1)	0.51642(2)	1.02371(3)	0.27860(4)	0.031(1)
Cl(2)	0.17518(2)	0.72663(3)	0.11883(4)	0.029(1)
S(1)	0.55111(2)	0.71310(3)	0.54234(4)	0.022(1)
O(1)	0.41518(5)	0.81960(10)	0.23108(10)	0.023(1)
N(1)	0.40402(6)	0.71371(10)	0.43440(12)	0.019(1)
N(2)	0.14781(6)	0.51214(11)	0.29857(11)	0.020(1)
N(3)	0.03875(6)	0.34811(11)	0.24108(12)	0.021(1)
C(1)	0.50715(7)	0.81041(12)	0.41732(13)	0.019(1)
C(2)	0.54377(7)	0.90782(13)	0.39296(14)	0.022(1)
C(3)	0.60876(7)	0.90995(13)	0.47684(15)	0.025(1)
C(4)	0.65910(9)	0.99694(16)	0.48159(19)	0.036(1)
C(5)	0.71780(9)	0.97786(18)	0.5729(2)	0.043(1)
C(6)	0.72759(8)	0.87504(19)	0.6595(2)	0.043(1)
C(7)	0.67894(8)	0.78882(17)	0.65705(19)	0.036(1)
C(8)	0.61932(7)	0.80787(14)	0.56441(16)	0.025(1)
C(9)	0.43804(7)	0.78281(12)	0.35195(13)	0.018(1)
C(10)	0.33999(7)	0.66533(12)	0.39433(13)	0.018(1)
C(11)	0.29354(7)	0.71338(12)	0.28477(14)	0.020(1)
C(12)	0.23147(7)	0.66067(13)	0.25509(13)	0.020(1)
C(13)	0.21289(7)	0.56062(12)	0.33043(13)	0.019(1)
C(14)	0.26055(7)	0.51538(13)	0.43990(14)	0.021(1)
C(15)	0.32283(7)	0.56602(12)	0.47182(14)	0.021(1)
C(16)	0.13639(7)	0.44377(14)	0.16272(14)	0.023(1)
C(17)	0.06301(7)	0.41822(13)	0.12363(14)	0.023(1)
C(18)	0.05280(7)	0.41529(13)	0.38046(14)	0.023(1)
C(19)	0.12638(7)	0.44129(13)	0.41502(14)	0.023(1)
C(20)	-0.03238(7)	0.31361(14)	0.20408(17)	0.028(1)
Cl(3)	0.09457(2)	0.10283(3)	0.26766(3)	0.022(1)
H(1N)	0.4210(9)	0.6982(16)	0.519(2)	0.026(4)
H(3N)	0.0604(9)	0.2814(18)	0.252(2)	0.031(5)
H(4)	0.65279	1.06694	0.42343	0.043
H(5)	0.75223	1.03555	0.57707	0.052
H(6)	0.76854	0.86447	0.72117	0.052
H(7)	0.68563	0.71925	0.71589	0.043
H(11A)	0.30428	0.78116	0.23132	0.023
H(14)	0.24984	0.44791	0.49392	0.026
H(15)	0.35411	0.53308	0.54686	0.025
H(16A)	0.16136	0.36754	0.17431	0.027
H(16B)	0.15222	0.49023	0.08426	0.027
H(17A)	0.03831	0.49459	0.10917	0.028

H(17B)	0.05507	0.37249	0.03191	0.028
H(18A)	0.03845	0.36731	0.45942	0.027
H(18B)	0.02767	0.49139	0.37276	0.027
H(19A)	0.13559	0.48583	0.50744	0.027
H(19B)	0.15140	0.36510	0.42610	0.027
H(20A)	-0.04509	0.26273	0.28095	0.042
H(20B)	-0.03884	0.26959	0.11231	0.042
H(20C)	-0.05991	0.38587	0.19468	0.042

Table 3. Anisotropic displacement parameters ($Å^2$) for stefaniak_7974147.
The anisotropic displacement factor exponent takes the form:
$-2\pi^2[h^2a^{*2}U_{11} + ... + 2hka^*b^*U_{12}]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Cl(1)	0.0547(2)	0.0192(2)	0.0221(2)	0.0035(1)	0.0139(2)	-0.0010(2)
Cl(2)	0.0222(2)	0.0345(2)	0.0277(2)	0.0139(1)	-0.0025(1)	-0.0001(1)
S(1)	0.0218(2)	0.0228(2)	0.0195(2)	0.0033(1)	-0.0001(1)	-0.0022(1)
O(1)	0.0263(5)	0.0324(5)	0.0112(4)	0.0039(4)	0.0029(4)	0.0031(4)
N(1)	0.0231(6)	0.0237(6)	0.0089(5)	0.0005(4)	-0.0011(4)	-0.0020(4)
N(2)	0.0232(6)	0.0246(6)	0.0108(5)	-0.0003(4)	0.0029(4)	-0.0028(5)
N(3)	0.0208(6)	0.0208(6)	0.0201(5)	-0.0003(5)	0.0030(4)	0.0004(5)
C(1)	0.0253(7)	0.0204(6)	0.0104(6)	-0.0011(5)	0.0037(5)	-0.0001(5)
C(2)	0.0309(7)	0.0226(7)	0.0150(6)	-0.0023(5)	0.0099(5)	-0.0015(6)
C(3)	0.0284(7)	0.0274(7)	0.0226(7)	-0.0080(6)	0.0126(6)	-0.0058(6)
C(4)	0.0397(9)	0.0336(8)	0.0384(9)	-0.0125(7)	0.0223(7)	-0.0121(7)
C(5)	0.0308(9)	0.0492(11)	0.0541(11)	-0.0240(9)	0.0200(8)	-0.0182(8)
C(6)	0.0223(8)	0.0572(12)	0.0495(10)	-0.0207(9)	0.0038(7)	-0.0061(8)
C(7)	0.0242(8)	0.0438(10)	0.0373(9)	-0.0085(7)	0.0008(6)	-0.0001(7)
C(8)	0.0213(7)	0.0301(7)	0.0251(7)	-0.0078(6)	0.0055(5)	-0.0039(6)
C(9)	0.0234(6)	0.0193(6)	0.0113(6)	-0.0025(5)	0.0032(5)	0.0024(5)
C(10)	0.0225(6)	0.0201(6)	0.0113(5)	-0.0034(5)	0.0023(5)	-0.0003(5)
C(11)	0.0238(7)	0.0208(7)	0.0143(6)	0.0014(5)	0.0030(5)	0.0001(5)
C(12)	0.0217(6)	0.0236(7)	0.0134(6)	0.0014(5)	0.0014(5)	0.0034(5)
C(13)	0.0227(6)	0.0211(6)	0.0121(6)	-0.0024(5)	0.0035(5)	-0.0003(5)
C(14)	0.0286(7)	0.0206(7)	0.0143(6)	0.0016(5)	0.0017(5)	-0.0020(5)
C(15)	0.0275(7)	0.0217(7)	0.0129(6)	0.0006(5)	-0.0019(5)	-0.0005(5)
C(16)	0.0252(7)	0.0294(7)	0.0133(6)	-0.0030(5)	0.0039(5)	-0.0036(6)
C(17)	0.0261(7)	0.0279(7)	0.0148(6)	0.0003(5)	0.0016(5)	-0.0030(6)
C(18)	0.0267(7)	0.0260(7)	0.0166(6)	-0.0004(5)	0.0069(5)	-0.0018(6)
C(19)	0.0269(7)	0.0271(7)	0.0137(6)	0.0014(5)	0.0038(5)	-0.0034(6)
C(20)	0.0211(7)	0.0283(7)	0.0340(8)	-0.0023(6)	0.0035(6)	-0.0014(6)
Cl(3)	0.0252(2)	0.0228(2)	0.0182(2)	-0.0009(1)	0.0040(1)	0.0010(1)

Table 4. Bond lengths [Å] for stefaniak_7974147.

atom-atom	distance	atom-atom	distance
Cl(1)-C(2)	1.7184(15)	Cl(2)-C(12)	1.7469(13)
S(1)-C(8)	1.7368(15)	S(1)-C(1)	1.7439(13)
O(1)-C(9)	1.2271(16)	N(1)-C(9)	1.3519(18)
N(1)-C(10)	1.4127(17)	N(1)-H(1N)	0.834(19)
N(2)-C(13)	1.4246(17)	N(2)-C(19)	1.4629(17)
N(2)-C(16)	1.4697(16)	N(3)-C(17)	1.4903(18)
N(3)-C(20)	1.4923(17)	N(3)-C(18)	1.4933(17)
N(3)-H(3N)	0.86(2)	C(1)-C(2)	1.358(2)
C(1)-C(9)	1.4845(18)	C(2)-C(3)	1.438(2)
C(3)-C(8)	1.401(2)	C(3)-C(4)	1.410(2)
C(4)-C(5)	1.381(3)	C(4)-H(4)	0.9500
C(5)-C(6)	1.402(3)	C(5)-H(5)	0.9500
C(6)-C(7)	1.381(3)	C(6)-H(6)	0.9500
C(7)-C(8)	1.402(2)	C(7)-H(7)	0.9500
C(10)-C(11)	1.3958(18)	C(10)-C(15)	1.3970(19)
C(11)-C(12)	1.3874(19)	C(11)-H(11A)	0.9500
C(12)-C(13)	1.4022(19)	C(13)-C(14)	1.3978(19)
C(14)-C(15)	1.383(2)	C(14)-H(14)	0.9500
C(15)-H(15)	0.9500	C(16)-C(17)	1.5155(19)
C(16)-H(16A)	0.9900	C(16)-H(16B)	0.9900
C(17)-H(17A)	0.9900	C(17)-H(17B)	0.9900
C(18)-C(19)	1.5169(19)	C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900	C(19)-H(19A)	0.9900
C(19)-H(19B)	0.9900	C(20)-H(20A)	0.9800
C(20)-H(20B)	0.9800	C(20)-H(20C)	0.9800

Symmetry transformations used to generate equivalent atoms:

Table 5. Bond angles [°] for stefaniak_7974147.

atom-atom-atom	angle	atom-atom-atom	angle
C(8)-S(1)-C(1)	91.37(7)	C(9)-N(1)-C(10)	126.86(11)
C(9)-N(1)-H(1N)	118.8(12)	C(10)-N(1)-H(1N)	114.3(12)
C(13)-N(2)-C(19)	114.94(10)	C(13)-N(2)-C(16)	113.73(10)
C(19)-N(2)-C(16)	109.49(11)	C(17)-N(3)-C(20)	112.48(11)
C(17)-N(3)-C(18)	109.59(11)	C(20)-N(3)-C(18)	112.78(11)
C(17)-N(3)-H(3N)	108.6(13)	C(20)-N(3)-H(3N)	105.6(13)
C(18)-N(3)-H(3N)	107.6(13)	C(2)-C(1)-C(9)	127.62(13)
C(2)-C(1)-S(1)	111.72(10)	C(9)-C(1)-S(1)	120.66(10)
C(1)-C(2)-C(3)	114.09(13)	C(1)-C(2)-Cl(1)	124.84(11)
C(3)-C(2)-Cl(1)	121.03(11)	C(8)-C(3)-C(4)	119.58(15)
C(8)-C(3)-C(2)	110.79(13)	C(4)-C(3)-C(2)	129.64(15)
C(5)-C(4)-C(3)	118.36(17)	C(5)-C(4)-H(4)	120.8
C(3)-C(4)-H(4)	120.8	C(4)-C(5)-C(6)	121.31(16)
C(4)-C(5)-H(5)	119.3	C(6)-C(5)-H(5)	119.3
C(7)-C(6)-C(5)	121.37(17)	C(7)-C(6)-H(6)	119.3
C(5)-C(6)-H(6)	119.3	C(6)-C(7)-C(8)	117.46(17)
C(6)-C(7)-H(7)	121.3	C(8)-C(7)-H(7)	121.3
C(3)-C(8)-C(7)	121.93(15)	C(3)-C(8)-S(1)	112.02(11)
C(7)-C(8)-S(1)	126.06(13)	O(1)-C(9)-N(1)	123.88(13)
O(1)-C(9)-C(1)	121.23(12)	N(1)-C(9)-C(1)	114.88(11)
C(11)-C(10)-C(15)	119.35(12)	C(11)-C(10)-N(1)	123.31(12)
C(15)-C(10)-N(1)	117.33(12)	C(12)-C(11)-C(10)	119.01(12)
C(12)-C(11)-H(11A)	120.5	C(10)-C(11)-H(11A)	120.5
C(11)-C(12)-C(13)	122.97(12)	C(11)-C(12)-Cl(2)	116.73(10)
C(13)-C(12)-Cl(2)	120.29(10)	C(14)-C(13)-C(12)	116.41(12)
C(14)-C(13)-N(2)	122.84(12)	C(12)-C(13)-N(2)	120.70(12)
C(15)-C(14)-C(13)	121.89(13)	C(15)-C(14)-H(14)	119.1
C(13)-C(14)-H(14)	119.1	C(14)-C(15)-C(10)	120.37(12)
C(14)-C(15)-H(15)	119.8	C(10)-C(15)-H(15)	119.8
N(2)-C(16)-C(17)	109.36(11)	N(2)-C(16)-H(16A)	109.8
C(17)-C(16)-H(16A)	109.8	N(2)-C(16)-H(16B)	109.8
C(17)-C(16)-H(16B)	109.8	H(16A)-C(16)-H(16B)	108.3
N(3)-C(17)-C(16)	110.19(11)	N(3)-C(17)-H(17A)	109.6
C(16)-C(17)-H(17A)	109.6	N(3)-C(17)-H(17B)	109.6
C(16)-C(17)-H(17B)	109.6	H(17A)-C(17)-H(17B)	108.1
N(3)-C(18)-C(19)	109.81(11)	N(3)-C(18)-H(18A)	109.7
C(19)-C(18)-H(18A)	109.7	N(3)-C(18)-H(18B)	109.7
C(19)-C(18)-H(18B)	109.7	H(18A)-C(18)-H(18B)	108.2
N(2)-C(19)-C(18)	109.89(11)	N(2)-C(19)-H(19A)	109.7
C(18)-C(19)-H(19A)	109.7	N(2)-C(19)-H(19B)	109.7
C(18)-C(19)-H(19B)	109.7	H(19A)-C(19)-H(19B)	108.2
N(3)-C(20)-H(20A)	109.5	N(3)-C(20)-H(20B)	109.5

H(20A)-C(20)-H(20B)	109.5	N(3)-C(20)-H(20C)	109.5
H(20A)-C(20)-H(20C)	109.5	H(20B)-C(20)-H(20C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 6. Torsion angles [°] for stefaniak_7974147.

atom-atom-atom-atom	angle	atom-atom-atom-atom	angle
C(8)-S(1)-C(1)-C(2)	1.25(11)	C(8)-S(1)-C(1)-C(9)	-178.36(11)
C(9)-C(1)-C(2)-C(3)	178.21(12)	S(1)-C(1)-C(2)-C(3)	-1.36(15)
C(9)-C(1)-C(2)-Cl(1)	0.6(2)	S(1)-C(1)-C(2)-Cl(1)	-179.00(8)
C(1)-C(2)-C(3)-C(8)	0.74(17)	Cl(1)-C(2)-C(3)-C(8)	178.48(10)
C(1)-C(2)-C(3)-C(4)	-178.71(14)	Cl(1)-C(2)-C(3)-C(4)	-1.0(2)
C(8)-C(3)-C(4)-C(5)	0.3(2)	C(2)-C(3)-C(4)-C(5)	179.75(15)
C(3)-C(4)-C(5)-C(6)	-0.2(2)	C(4)-C(5)-C(6)-C(7)	0.0(3)
C(5)-C(6)-C(7)-C(8)	0.1(3)	C(4)-C(3)-C(8)-C(7)	-0.3(2)
C(2)-C(3)-C(8)-C(7)	-179.81(14)	C(4)-C(3)-C(8)-S(1)	179.74(11)
C(2)-C(3)-C(8)-S(1)	0.22(15)	C(6)-C(7)-C(8)-C(3)	0.1(2)
C(6)-C(7)-C(8)-S(1)	-179.96(13)	C(1)-S(1)-C(8)-C(3)	-0.82(11)
C(1)-S(1)-C(8)-C(7)	179.22(14)	C(10)-N(1)-C(9)-O(1)	4.9(2)
C(10)-N(1)-C(9)-C(1)	-174.00(12)	C(2)-C(1)-C(9)-O(1)	28.6(2)
S(1)-C(1)-C(9)-O(1)	-151.88(11)	C(2)-C(1)-C(9)-N(1)	-152.49(13)
S(1)-C(1)-C(9)-N(1)	27.05(16)	C(9)-N(1)-C(10)-C(11)	-22.8(2)
C(9)-N(1)-C(10)-C(15)	158.61(13)	C(15)-C(10)-C(11)-C(12)	-0.34(19)
N(1)-C(10)-C(11)-C(12)	-178.86(12)	C(10)-C(11)-C(12)-C(13)	0.0(2)
C(10)-C(11)-C(12)-Cl(2)	178.95(10)	C(11)-C(12)-C(13)-C(14)	0.3(2)
Cl(2)-C(12)-C(13)-C(14)	-178.57(10)	C(11)-C(12)-C(13)-N(2)	178.10(12)
Cl(2)-C(12)-C(13)-N(2)	-0.80(17)	C(19)-N(2)-C(13)-C(14)	19.93(18)
C(16)-N(2)-C(13)-C(14)	-107.41(14)	C(19)-N(2)-C(13)-C(12)	-157.70(12)
C(16)-N(2)-C(13)-C(12)	74.97(16)	C(12)-C(13)-C(14)-C(15)	-0.4(2)
N(2)-C(13)-C(14)-C(15)	-178.08(12)	C(13)-C(14)-C(15)-C(10)	0.0(2)
C(11)-C(10)-C(15)-C(14)	0.3(2)	N(1)-C(10)-C(15)-C(14)	178.91(12)
C(13)-N(2)-C(16)-C(17)	-168.52(11)	C(19)-N(2)-C(16)-C(17)	61.37(15)
C(20)-N(3)-C(17)-C(16)	-176.32(12)	C(18)-N(3)-C(17)-C(16)	57.37(15)
N(2)-C(16)-C(17)-N(3)	-59.54(15)	C(17)-N(3)-C(18)-C(19)	-56.96(15)
C(20)-N(3)-C(18)-C(19)	176.91(12)	C(13)-N(2)-C(19)-C(18)	169.05(11)
C(16)-N(2)-C(19)-C(18)	-61.50(14)	N(3)-C(18)-C(19)-N(2)	59.33(15)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for stefaniak_7974147 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1N)...O(1)#1	0.834(19)	2.010(19)	2.7714(14)	151.4(17)
N(3)-H(3N)...Cl(3)	0.86(2)	2.11(2)	2.9636(13)	168.6(17)

Symmetry transformations used to generate equivalent atoms:
#1 x,-y+3/2,z+1/2