Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Potent trifluoromethoxy, trifluoromethylsulfonyl, trifluoromethylthio and pentafluorosulfanyl containing (1,3,4-oxadiazol-2-yl)benzamides against drug-resistant Gram-positive bacteria

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Supplementary Information:

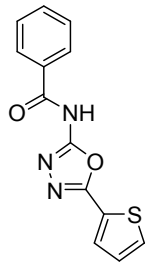
I. Chemistry:

General Considerations: unless noted otherwise, all reagents and solvents were purchased from commercial sources and used as received. The ^{1}H and ^{13}C NMR spectra were obtained in DMSO-d_6 as solvent using a 500 MHz or 800 MHz spectrometer with Me_4Si as an internal standard. Chemical shifts are reported in parts per million (δ) and are calibrated using residual non-deuterated solvent as an internal reference. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, or combinations thereof. High resolution mass spectra (HRMS) were obtained using electron spray ionization (ESI) technique and as TOF mass analyzer. New compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS data.

General Procedure for the Synthesis of Compounds 1-17.

A 20 mL screw caped vial, charged with the corresponding acid (0.5 mmol), amine (0.5 mmol), BOP reagent (1.4 mmol) and diisopropylethylamine (13 mmol) in DMF solvent (3 mL) was stirred at room temperature for 16 h. After completion, the reaction mixture was concentrated under reduced pressure, followed by flash column chromatography (hexanes:ethyl acetate 80:20 to 60:40) give the desired product.

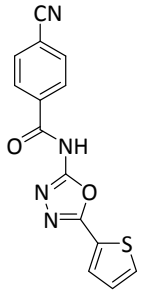
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (1):



1

Off-white solid (80 mg, 36%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.13 (s, 1H), 8.01 (d, J = 7.3 Hz, 2H), 7.92 (dd, J = 5.0, 1.2 Hz, 1H), 7.76 (dd, J = 3.7, 1.2 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 7.55 (t, J = 7.7 Hz, 2H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.8, 165.7, 157.9, 133.4, 132.7, 131.6, 130.2, 129.2, 129.1, 128.8, 124.8. HRMS (ESI) m/z calcd for $C_{13}H_{10}N_3O_2S$ $[M + H]^+$ 272.0494, found 272.0496.

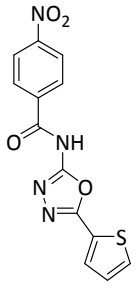
4-Cyano-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (2):



2

Off-white solid (75 mg, 37%).[1]H NMR (500 MHz, DMSO-d_6) δ 8.15 (d, J = 8.0 Hz, 2H), 8.03 (d, J = 8.1 Hz, 2H), 7.93 (dd, J = 5.0, 1.2 Hz, 1H), 7.76 (dd, J = 3.7, 1.2 Hz, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (201 MHz, DMSO-d_6) δ 164.8, 157.7, 137.0, 133.0, 131.6, 130.3, 129.5, 129.1, 124.5, 118.5, 115.4. HRMS (ESI) m/z calcd for $C_{14}H_9N_4O_2S$ $[M + H]^+$ 297.0440, found 297.0440.

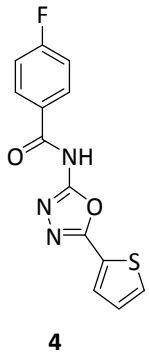
4-Nitro-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (3):



3

Off-white solid (82 mg, 43%).[1]H NMR (500 MHz, DMSO-d_6) δ 8.37 (d, J = 8.8 Hz, 2H), 8.24 (d, J = 8.5 Hz, 2H), 7.94 (dd, J = 5.0, 1.2 Hz, 1H), 7.77 (d, J = 3.7 Hz, 1H), 7.29 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (126 MHz, DMSO-d_6) δ 163.8, 162.8, 158.0, 150.3, 138.7, 131.8, 130.4, 129.2, 127.7, 124.6, 124.1. HRMS (ESI) m/z calcd for $C_{13}H_9N_4O_4S$ $[M + H]^+$ 317.0339, found 317.0339.

4-Fluoro-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (4):

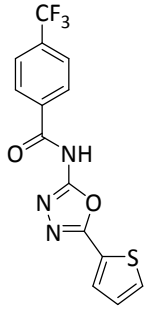


4

Off-white solid (63 mg, 31%). [1]H NMR (500 MHz, DMSO-d_6) δ 8.12 – 8.07 (m, 2H), 7.92 (dd, J = 5.0, 1.2 Hz, 1H), 7.75 (dd, J = 3.7, 1.3 Hz, 1H), 7.39 (t, J = 8.8 Hz, 2H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C

NMR (126 MHz, DMSO-d_6) δ 166.3 (d, J = 252 Hz), 164.4, 157.9, 131.8, 131.7, 131.6, 130.2, 129.2, 124.7, 116.3, 116.1. HRMS (ESI) m/z calcd for $C_{13}H_9FN_3O_2S$ $[M + H]^+$ 290.0400, found 290.0401.

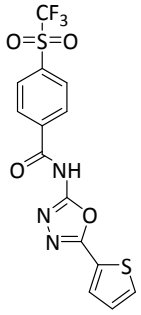
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)benzamide (5):



5

Off-white solid (55 mg, 31%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, J = 8.0 Hz, 2H), 7.97 – 7.90 (m, 3H), 7.76 (dd, J = 3.7, 1.2 Hz, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.2, 157.9, 157.5, 136.9, 132.9 (q, J = 31.5 Hz), 131.7, 130.3, 129.7, 129.20, 126.07, 125.31, 124.62 (q, J = 273.4 Hz). HRMS (ESI) m/z calcd for $C_{14}H_9F_3N_3O_2S$ $[M + H]^+$ 340.0364, found 340.0362.

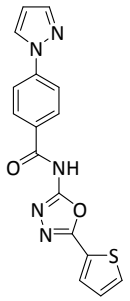
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-4 (trifluoromethyl)sulfonyl)benzamide (6):



6

Off-white solid (40 mg, 25%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 (d, J = 8.2 Hz, 2H), 8.32 (d, J = 8.2 Hz, 2H), 7.94 (dd, J = 5.0, 1.2 Hz, 1H), 7.82 – 7.75 (m, 1H), 7.29 (dd, J = 5.0, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.7, 158.3, 156.9, 141.7, 133.0, 131.8, 131.6, 130.9, 130.4, 129.2, 124.5, 123.7 (q, J = 326.3 Hz). HRMS (ESI) m/z calcd for $C_{14}H_9F_3N_3O_4S_2$ $[M + H]^+$ 403.9980, found 403.9981.

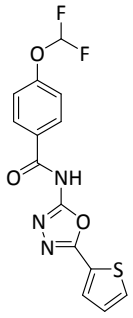
4-(1H-Pyrazol-1-yl)-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (7):



7

Off-white solid (57 mg, 32%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.67 (d, J = 2.6 Hz, 1H), 8.17 (d, J = 8.5 Hz, 2H), 8.05 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 5.0 Hz, 1H), 7.84 (s, 1H), 7.77 (d, J = 3.7 Hz, 1H), 7.30 (t, J = 4.4 Hz, 1H), 6.62 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.3, 158.1, 157.8, 143.1, 142.5, 131.6, 130.5, 130.1, 129.2, 128.8, 124.8, 118.3, 109.2. HRMS (ESI) m/z calcd for $C_{16}H_{12}N_5O_2S$ $[M + H]^+$ 338.0706, found 338.0709.

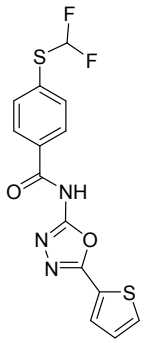
4-(Difluoromethoxy)-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (8):



8

Off-white solid (33 mg, 18%).^{1}H NMR (500 MHz, DMSO-d_6) δ 12.17 (s, 1H), 8.11 – 8.06 (m, 2H), 7.92 (dd, J = 5.0, 1.2 Hz, 1H), 7.75 (dd, J = 3.8, 1.2 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.29 – 7.26 (m, 1H). ^{13}C NMR (201 MHz, DMSO-d_6) δ 164.9, 157.9, 154.8, 131.6, 131.2, 130.2, 129.3, 129.2, 124.7, 118.4, 116.4 (t, J = 258.3 Hz). HRMS (ESI) m/z calcd for $C_{14}H_{10}F_2N_3O_3S$ $[M + H]^+$ 338.0408, found 338.0406.

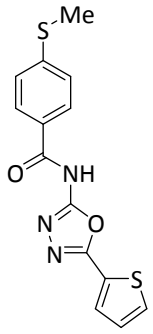
4-((Difluoromethyl)thio)-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (9):



9

Off-white solid (53 mg, 30%).[1]H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, J = 8.5 Hz, 2H), 8.10 (d, J = 8.9 Hz, 2H), 7.93 (dd, J = 5.0, 1.2 Hz, 1H), 7.76 (dd, J = 3.7, 1.2 Hz, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (201 MHz, DMSO-d_6) δ 165.0, 157.6, 157.2, 133.2, 131.4, 131.0, 129.6, 129.2, 128.6, 124.2, 120.7 (t, J = 273.4 Hz). HRMS (ESI) m/z calcd for $C_{13}H_9F_5N_3O_2S_2$ $[M + H]^+$ 398.0053, found 398.0051.

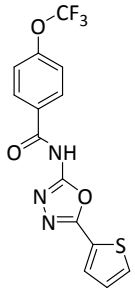
4-(Methylthio)-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (14):



10

Off-white solid (37 mg, 20%).[1]H NMR (500 MHz, DMSO-d_6) δ 7.93 (dq, J = 12.6, 7.6 Hz, 3H), 7.75 (t, J = 6.8 Hz, 1H), 7.42 – 7.34 (m, 2H), 7.29 – 7.25 (m, 1H), 2.53 (d, J = 11.9 Hz, 3H). [13]C NMR (126 MHz, DMSO-d_6) δ 164.8, 158.0, 145.7, 131.6, 130.1, 129.3, 129.2, 128.4, 125.3, 124.8, 14.4. HRMS (ESI) m/z calcd for $C_{14}H_{12}N_3O_2S_2$ $[M + H]^+$ 318.0371, found 318.0371.

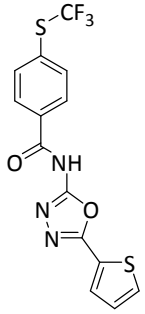
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-4-(trifluoromethoxy)benzamide (11):



11

Off-white solid (30 mg, 17%).[1]H NMR (500 MHz, DMSO-d_6) δ 12.28 (s, 1H), 8.14 (d, J = 8.4 Hz, 2H), 7.93 (dd, J = 5.0, 1.2 Hz, 1H), 7.76 (d, J = 3.7 Hz, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (126 MHz, DMSO-d_6) δ 164.9, 157.9, 157.6, 151.8, 132.0, 131.6, 131.3, 130.2, 129.2, 124.7, 121.4 (q, J = 258.3 Hz), 121.2. HRMS (ESI) m/z calcd for $C_{14}H_{10}F_3N_3O_3S$ $[M + H]^+$ 356.0313, found 356.0311.

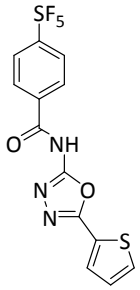
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (12):



12

Off-white solid (46 mg, 28%). [1]H NMR (500 MHz, DMSO-d_6) δ 8.12 (d, J = 8.1 Hz, 2H), 7.92 (d, J = 5.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 3.7 Hz, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (126 MHz, DMSO-d_6) δ 165.3, 157.9, 157.5, 136.2, 135.6, 131.7, 131.1 (q, J = 308.7 Hz), 130.2, 130.1, 129.2, 128.5, 124.7. HRMS (ESI) m/z calcd for $C_{14}H_9F_3N_3O_2S_2$ $[M + H]^+$ 372.0084, found 372.0082.

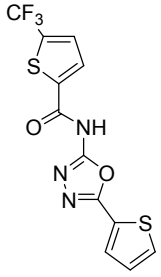
4-(Pentafluoro- sulfanyl)-*N*-(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl)benzamide (13):



13

Off-white solid (56 mg, 35%).[1]H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, J = 8.5 Hz, 2H), 8.10 (d, J = 8.9 Hz, 2H), 7.93 (dd, J = 5.0, 1.2 Hz, 1H), 7.76 (dd, J = 3.7, 1.2 Hz, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (126 MHz, DMSO-d_6) δ 165.0, 158.0, 157.3, 155.7, 136.8, 131.7, 130.3, 130.0, 129.2, 126.7, 124.6. HRMS (ESI) m/z calcd for $C_{13}H_9F_5N_3O_2S_2$ $[M + H]^+$ 398.0053, found 398.0051.

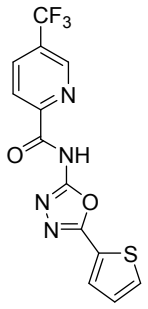
N-5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-5-(trifluoromethyl)thiophene-2-carboxamide (14):



14

Off-white solid (62 mg, 35%). [1]H NMR (500 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.93 (dd, J = 5.0, 1.2 Hz, 1H), 7.84 – 7.81 (m, 1H), 7.80 – 7.72 (m, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). [13]C NMR (201 MHz, DMSO-d_6) δ 161.0, 157.9, 143.2, 134.9 (q, J = 39 Hz), 131.8, 131.5, 131.3, 131.2, 130.4, 129.1, 124.4, 123.0 (q, J = 269.6 Hz). HRMS (ESI) m/z calcd for $C_{12}H_7F_3N_3O_2S_2$ $[M + H]^+$ 345.9926, found 345.9924.

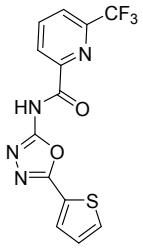
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-5-(trifluoromethyl)picolinamide (15):



15

Off-white solid (33 mg, 19%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.35 (s, 1H), 9.14 (d, J = 2.4 Hz, 1H), 8.52 (dd, J = 8.3, 2.3 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 7.94 (dd, J = 5.0, 1.3 Hz, 1H), 7.77 (dd, J = 3.7, 1.3 Hz, 1H), 7.29 (dd, J = 5.0, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.6, 158.6, 157.0, 152.0, 146.2, 136.5, 131.9, 130.4, 129.2, 128.8 (q, J = 32.7 Hz), 124.8 (q, J = 274.6 Hz), 124.6, 124.1. HRMS (ESI) m/z calcd for $C_{13}H_8F_3N_4O_2S$ $[M + H]^+$ 341.0315, found 341.0315.

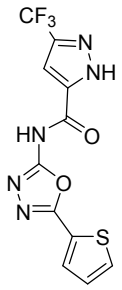
N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-6-(trifluoromethyl)picolinamide (16):



16

Off-white solid (47 mg, 27%).^{1}H NMR (500 MHz, DMSO-d_6) δ 9.41 (s, 1H), 9.20 (s, 1H), 8.75 (s, 1H), 7.96 – 7.91 (m, 1H), 7.76 (d, J = 2.5 Hz, 1H), 7.29 – 7.26 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.1, 157.8, 157.0, 153.6, 149.8, 133.8, 131.8, 130.3, 129.2, 129.1, 125.5 (q, J = 32.7 Hz), 124.8 (q, J = 274.6 Hz), 124.5. HRMS (ESI) m/z calcd for $C_{13}H_8F_3N_4O_2S$ $[M + H]^+$ 341.0315, found 341.0314.

N-(5-(Thiophen-2-yl)-1,3,4-oxadiazol-2-yl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (17):



17

Off-white solid (52 mg, 28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 – 7.92 (m, 1H), 7.75 (dd, J = 3.7, 1.2 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 157.1, 142.1 (q, J = 36.5 Hz), 131.8, 130.3, 129.2, 127.7, 122.7 (q, J = 269.6 Hz), 118.4, 106.1. HRMS (ESI) m/z calcd for $C_{11}H_7F_3N_5O_2S$ $[M + H]^+$ 330.0267, found 330.0266.

II. Biological Analysis of Analogs

Experimental Procedures:

Bacterial strains, media, cell lines and reagents
Clinical isolates used in this study (**Table 1S**) were obtained from the Biodefense and Emerging Infections Research Resources Repository (BEI Resources) and the American Type Culture Collection (ATCC). Cation-adjusted Mueller Hinton broth, tryptic soy broth (TSB) and tryptic soy agar (TSA) were purchased from Becton, Dickinson and Company (Cockeysville, MD, USA). Human colorectal adenocarcinoma epithelial cells (Caco-2) (ATCC HTB-37), and murine macrophage cells (J774) (ATCC TIB-67) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and phosphate-buffered saline (PBS) was purchased from Corning (Manassas, VA, USA). Single donor human red blood cells (RBCs) were purchased from Innovative Research (MI, USA). Linezolid (Chem-Impex International, Wood Dale, IL, USA) and vancomycin hydrochloride (Gold Biotechnology, St. Louis, MO, USA) were purchased from commercial sources.

Screening of compounds for antibacterial activity against *S. aureus*
An overnight culture of *S. aureus* was diluted in Cation-adjusted Mueller–Hinton broth (CAMHB) and further incubated to reach the early exponential phase. Bacterial aliquots were subsequently incubated with compounds at 16 μg/mL at 37°C for 24h. Afterwards, the OD_{600} was measured and percent normalized OD_{600} was obtained by using the equation:

$$\%Normalized\ OD_{600} = \left(\frac{X - X_o}{X_T - X_o}\right) \times 100$$

Where for a given compound, X is the OD_{600} of culture with the compound, X_o is that of media only and X_T is the OD_{600} of the DMSO control.

Determination of the MICs and MBCs against clinically-important Gram-positive bacteria
The broth microdilution method was utilized to test the antibacterial activity of the new (1,3,4-oxadiazol-2-yl)benzamides against a panel of clinically-important Gram-positive bacteria according to the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI)[1]. Bacterial strains were grown aerobically overnight on tryptic soy agar (TSA) plates at 37° C. Afterwards, a bacterial solution equivalent to 0.5 McFarland standard was prepared and diluted in CAMHB to achieve a bacterial concentration of about 5×10^5 CFU/mL and seeded in 96-well plates. Compounds and control drugs were added in the first row of the 96-well plates and serially diluted along the plates. Plates were then incubated aerobically at 37° C for 18-20 hours. MICs reported are the minimum concentration of the compounds and control drugs that completely inhibited the visual growth of bacteria. The minimum bactericidal concentration (MBC) was tested by spotting 4 μL from wells with no growth onto TSA plates. Plates were incubated at 37 ºC for at least 18 hours before recording the MBC. The MBC was categorized as the lowest concentration that reduced bacterial growth by 99.9%.

***In vitro* cytotoxicity analysis of compounds 6, 11, 12 and 13 against human colorectal and murine macrophage cells.**

Compounds **6, 11, 12** and **13** were assayed for potential cytotoxicity against a human colorectal adenocarcinoma (Caco-2) cell line, and murine macrophage (J774) cells, as described previously[2,3]. Briefly, tested compounds were incubated with caco-2 cells for 2 hours, and with J774 cells for 24 hours. Then, cells were incubated with MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) reagent for 4 hours before measuring absorbance values (OD_{490}).

Hemolysis assay

Hemolysis assay was performed following the procedure described previously[4]. Briefly, single donor human red blood cells (RBCs) (Innovative Research, MI, USA) suspension was prepared at the concentration of 5% v/v in phosphate buffered saline (PBS). Afterwards, compounds (in triplicate) were serially diluted in PBS and incubated with RBCs suspension at 37°C for 60 minutes. For the negative and positive controls, PBS and Triton-X were used respectively. Afterwards, the mixture was centrifuged at 4,000 rpm for 5 min. The supernatant was aliquoted into 96-well plates and absorbance at 540 nm was read. Percent hemolysis was obtained using the following equation:

$$Percent\ hemolysis = 100 \times \frac{(Absorbance\ of\ sample - Absorbance\ of\ negative\ control)}{(Absorbance\ of\ positive\ control - Absorbance\ of\ negative\ control)}$$

Time-kill kinetics analysis

The time-kill analysis was performed as previously described[5]. MRSA ATCC 33592 cells in logarithmic growth phase were diluted to 2.92×10^6 colony-forming units per mL (CFU/mL) and exposed to concentrations equivalent to 3 × MIC (in triplicate) of compounds **6**, **11**, **12**, or **13**, linezolid or vancomycin in tryptic soy broth (TSB). Aliquots (100 µL) were collected from each treatment after 0, 2, 4, 8, 12, and 24 hours of incubation at 37 °C and subsequently serially diluted in PBS. Bacteria were then transferred to TSA plates and incubated at 37 °C for 18-20 hours before viable CFU/mL was determined.

Intracellular infection of J774 cells with MRSA and treatment with compounds 6 and 12.

The ability of compounds **6, 12** and vancomycin (at 8 and 16 µg/mL) to reduce the burden of intracellular MRSA USA400 inside murine macrophage (J774) cells was evaluated as described earlier[6,7]. In brief, murine macrophage cells (J774) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with CO_2 (5%). J774 cells were exposed to MRSA USA400 cells at a multiplicity of infection of approximately 10:1. After 1 hour of infection, J774 cells were washed with gentamicin to kill extracellular MRSA. The compounds or vancomycin (at 8 and 16 µg/mL) were subsequently added to each well (in triplicate). Control cells received DMSO at a concentration equal to that in drug-treated cell samples. After 24 hours incubation at 37°C with 5% CO_2, the test agents were removed.

J774 cells were washed and subsequently lysed using 0.1% Triton-X. The solution was serially diluted in PBS and transferred to TSA plates in order to determine viable MRSA CFU inside the J774 cells. Plates were incubated at 37ºC for 18-22 hour before counting viable CFU/mL. Statistical significance was assessed with two-way ANOVA, with post hoc Dunnet's multiple comparisons test ($P < 0.05$), utilizing GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA).

Synergistic interactions of (1,3,4-oxadiazol-2-yl)benzamides with standard antibiotics

Fractional inhibitory concentration index (ΣFICI) was calculated for each interaction. The checkerboard assay[8,9] was used to determine antibiotics-compounds interactions against MRSA ATCC 33592. Four antibiotics (methicillin, vancomycin, linezolid and daptomycin) were tested in combination with compounds **6, 11, 12** and **13.** The ΣFICI was calculated for each combination as follows:

$$FICI_{compound} = \text{MIC of } \mathbf{6}, \mathbf{11}, \mathbf{12}, \text{ or } \mathbf{13} \text{ in combination/MIC of } \mathbf{6}, \mathbf{11}, \mathbf{12}, \text{ or } \mathbf{13} \text{ alone}$$

$$FICI_{antibiotic} = \text{MIC of antibiotic in combination/MIC of antibiotic alone}$$

The cumulative FICI (∑FICI) was then calculated as:

$$\sum FICI = FICI_{compound} + FICI_{antibiotic}$$

Interactions where the ΣFICI was $\leqq 0.5$ were categorized as synergistic (SYN). An ΣFICI of >0.5-1.25 was categorized as additive (ADD). ΣFICI of >1.25-4 was considered as indifference (IND), while ΣFICI values of > 4 were categorized as antagonistic[10].

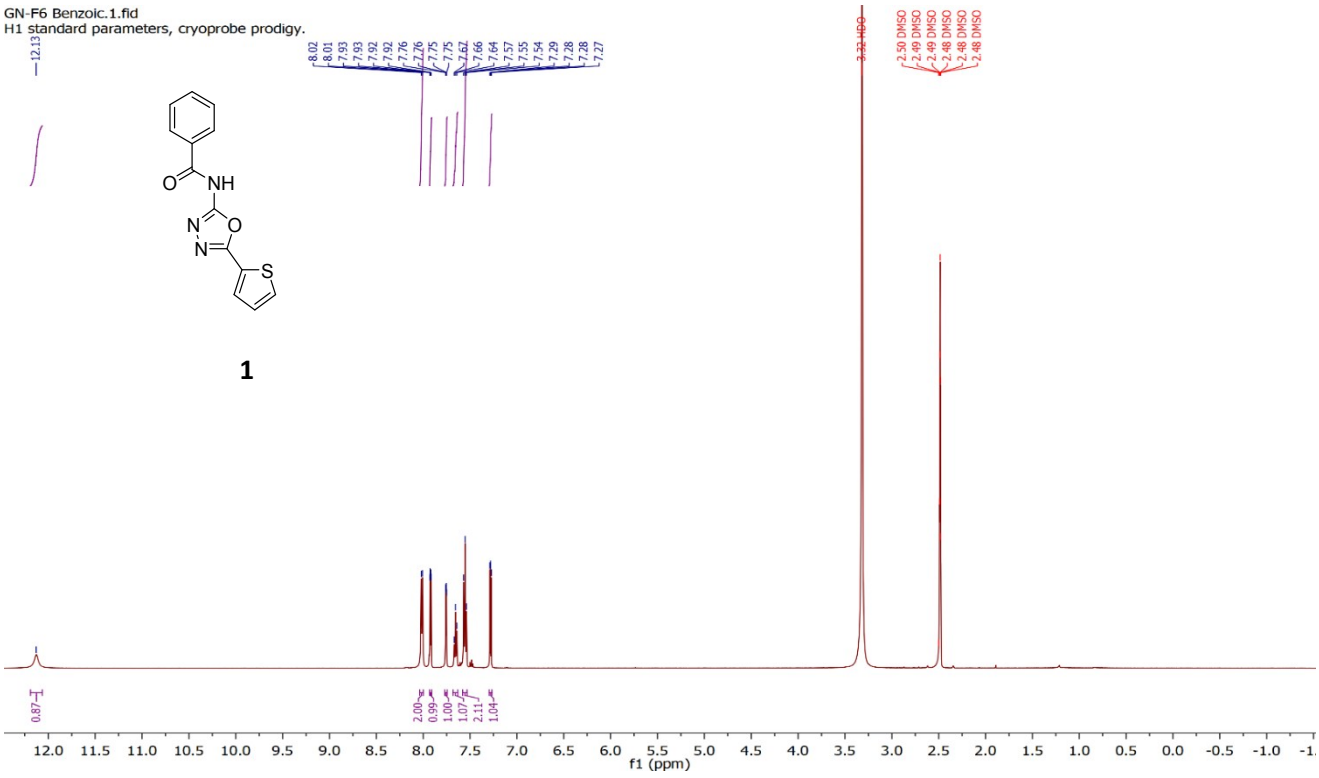
Table 1S. Bacterial isolates used in this study

Bacterial isolates	**Isolation**	**Description**
Staphylococcus aureus ATCC 25923	Clinical isolate	Quality control strain for media testing and susceptibility testing.
Staphylococcus aureus ATCC 33592	Blood	Used in drug discovery and emerging infectious disease research. Gentamicin-resistant. Methicillin-resistant.
Escherichia coli ATCC 25404	_____	Host for transducing phage P1. Wild-type. Non-lysogenic
Pseudomonas aeruginosa ATCC 27853	Blood culture	Quality control strain for media testing and susceptibility testing.
Acinetobacter baumannii ATCC 19606	Urine	Quality control strain for media testing and food testing.

Staphylococcus aureus ATCC 6538	Human lesion	Quality control strain for media testing, food testing, drug discovery and susceptibility testing.
Staphylococcus aureus NRS 107	Isolated in 1991 during an outbreak of mupirocin-resistant *S. aureus* on a dermatology ward of a university hospital in Connecticut, USA.	Resistant to mupirocin, rifampicin and novobiocin.
MRSA NRS 119	Isolated in 2001 from an 85-year-old male with dialysis-associated peritonitis in Massachusetts, USA.	Linezolid, tedizolid and methicillin -resistant
MRSA NRS382 (USA100)	Bloodstream sample in Ohio, USA	Resistant to erythromycin, clindamycin and levofloxacin. Endemic in many U.S. hospitals
MRSA NRS383 (USA200)	Bloodstream sample in North Carolina, USA	Resistant to erythromycin, clindamycin and gentamicin. The second most common health care-associated pulsed-field type in the U.S.
MRSA NRS384 (USA300)	Wound in Mississippi, USA	Community-acquired MRSA strain. Resistant to erythromycin and tetracycline.
MRSA NRS123 (USA400)	Human abscess in Michigan, 2004.	Community-associated MRSA strain.
MRSA NRS 385 (USA500)	Bloodstream sample in Connecticut, USA.	Hospital-acquired MRSA strain. Resistant to erythromycin, clindamycin, trimethoprim/sulfamethoxazole, levofloxacin, gentamicin and tetracycline.
MRSA NRS 386 (USA700)	Bloodstream sample in Louisiana, USA.	Associated with infections in both community and healthcare settings. Resistant to erythromycin.
MRSA NRS 387 (USA800)	Wound in Washington, USA	Resistant to β-lactams and fluoroquinolones.
MRSA NRS 483 (USA1000)	From a wound during a 1993-1994 MRSA outbreak among high school wrestlers and the surrounding	Associated with sporadic outbreaks of community-acquired infections. Resistant to erythromycin.

	community in Vermont, USA.	
MRSA NRS 484 (USA1100)	From wound in Alaska, USA in 1996.	Associated with community-acquired infections. Resistant to β-lactams
VRSA 10	Isolated in 2009 from a plantar foot wound of a 53-year-old female in Michigan, USA.	Resistant to erythromycin and spectinomycin. Positive for *mec* and *vanA*. Negative for *vanB*.
VRSA 12	Isolated from a foot wound	Positive for *vanA*
Staphylococcus epidermidis NRS101	From septicemic patient with colonized intravascular catheters, Tennessee, USA	Resistant to methicillin, erythromycin, kanamycin, gentamicin, clindamycin and trimethoprim.
Streptococcus pneumoniae ATCC 51916	Cerebrospinal fluid of 13-month-old girl, Tennessee	Cephalosporin-resistant. Representative strain of the Pneumococcal Molecular Epidemiology Network.
Streptococcus pneumoniae ATCC 700677	Human patient, Czechoslovakia, 1987	Resistant to methicillin, erythromycin, penicillin, and tetracycline.
Enterococcus faecalis ATCC 51299	Peritoneal fluid, Missouri, USA.	Resistant to vancomycin. Sensitive to Teicoplanin. Positive for *vanB*
Enterococcus faecium ATCC 700221	Human feces, Connecticut	Resistant to Vancomycin and Teicoplanin. Positive for *vanA*
Listeria monocytogenes ATCC 19111	Poultry, England	Control strain for media testing, enteric research and food testing.

^{1}H and ^{13}C NMR Spectra:



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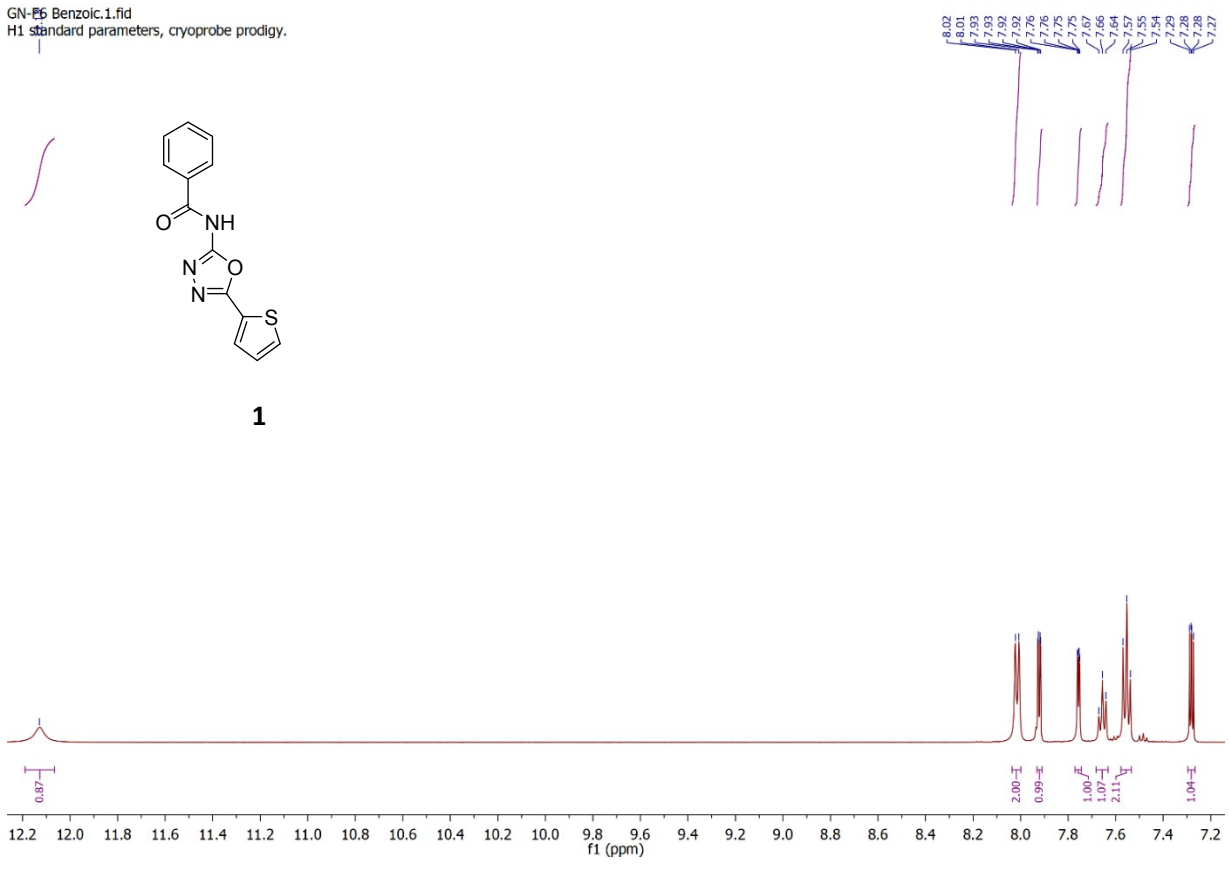

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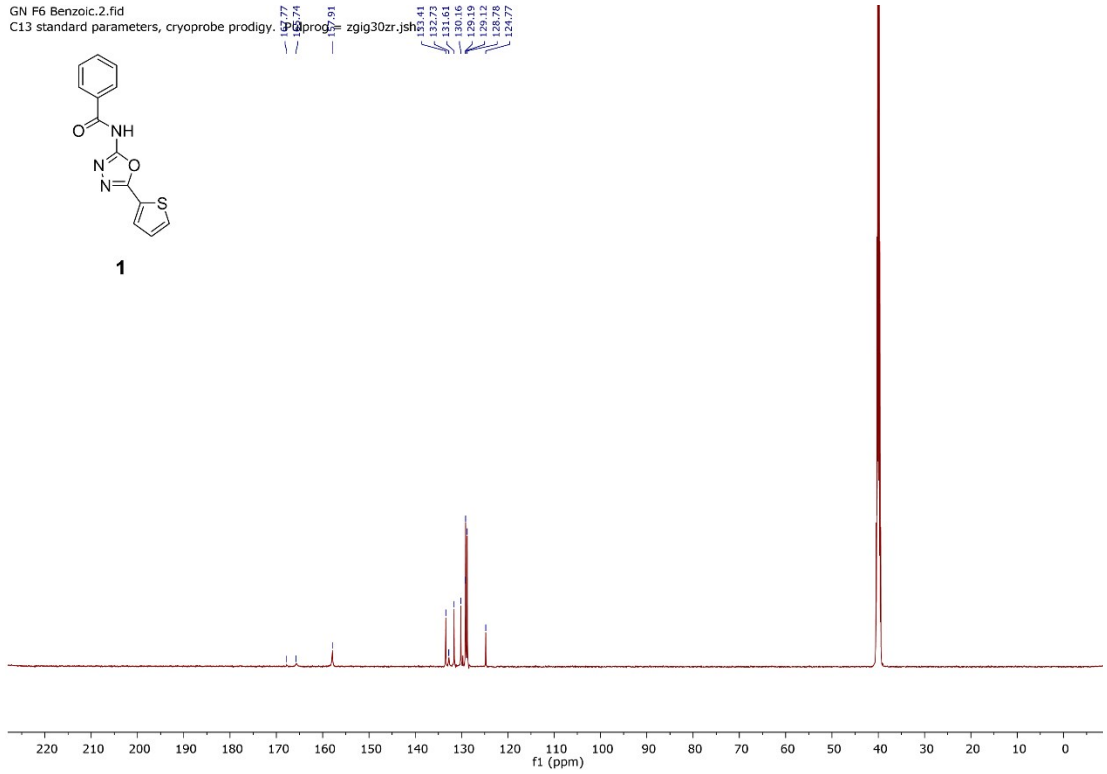
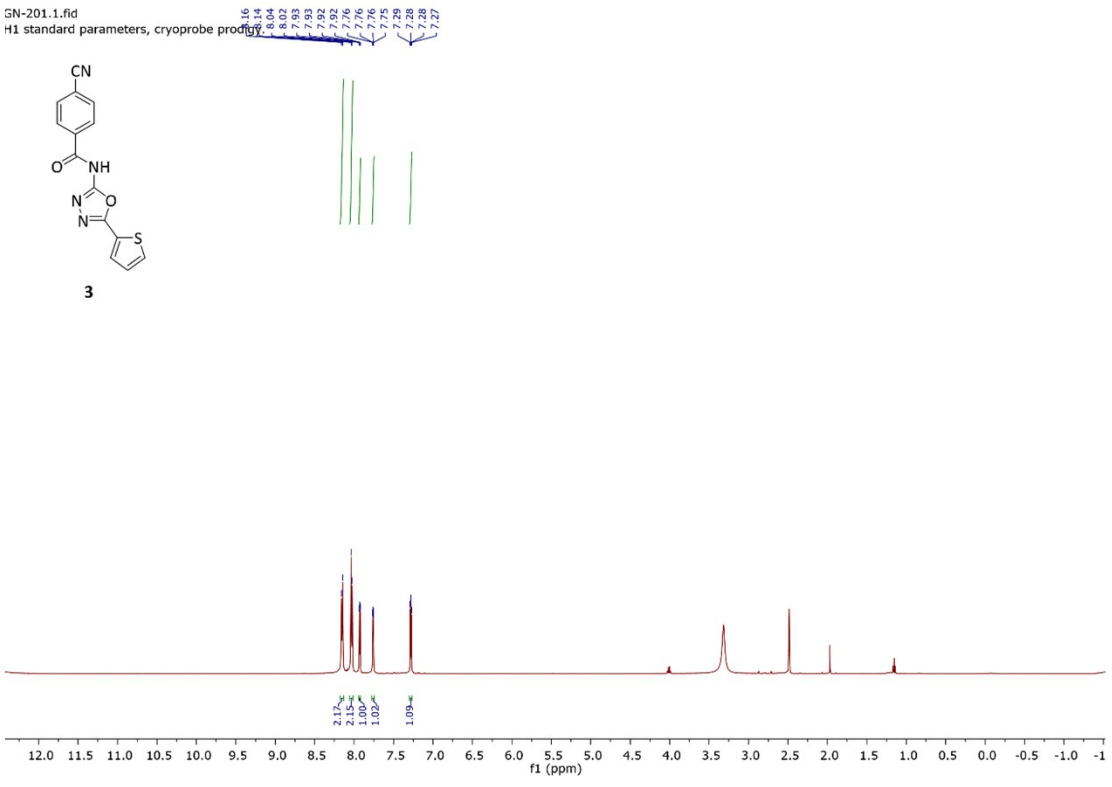

GN F6 Benzoic.2.fid
C13 standard parameters, cryoprobe prodigy. PULprog = zgig30zr.jsh
177.77
174.74
157.91
135.41
132.73
131.61
130.16
129.19
129.12
128.78
124.77
O
NH
N
O
N
S
1
f1 (ppm)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8.14
8.04
8.02
7.93
7.92
7.92
7.92
7.76
7.76
7.76
7.76
7.75
7.29
7.28
7.28
7.27
CN
O
NH
N
O
N
S
3
2.17
2.15
1.00
1.02
1.09
f1 (ppm)

GN-201.1.fid
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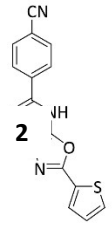

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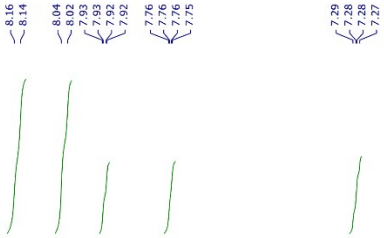

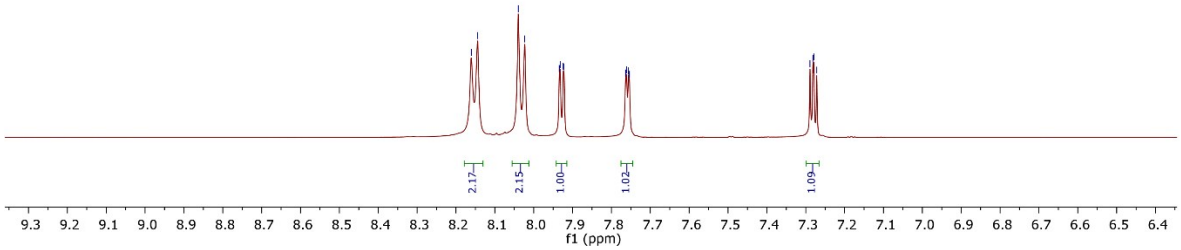

GN-201-800.2.fid
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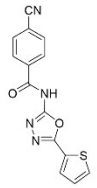
Use "abs13c" to flatten spectrum baseline.



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GN-202.1.fid
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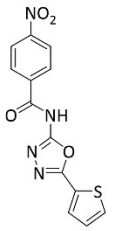

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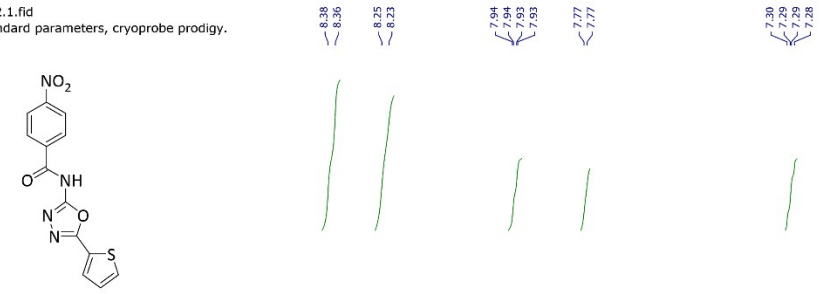

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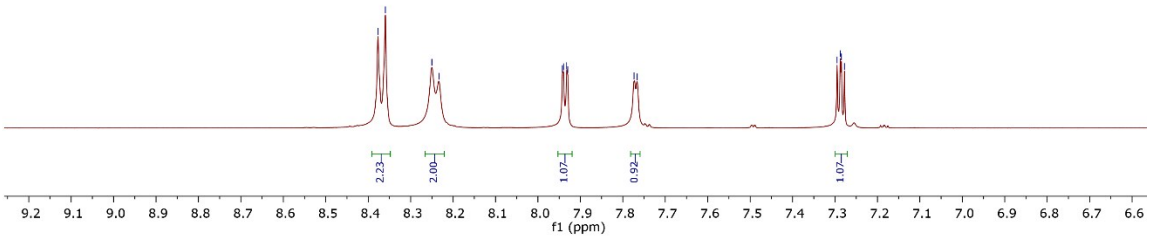

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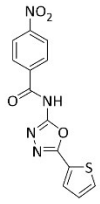

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GN-222.1.fid
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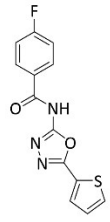

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GN-222.1.fid
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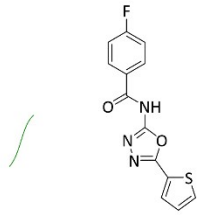

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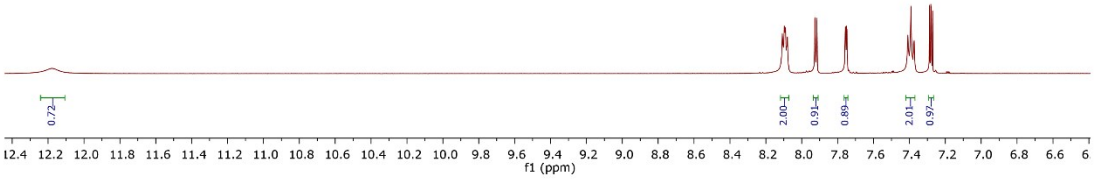

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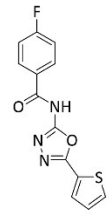
C13 standard parameters, cryoprobe prodigy. pulprog = zgig30zr.jsh.



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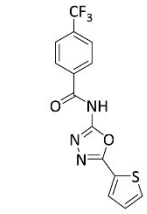
GN-152.1.fid
H1 standard parameters, cryoprobe prodigy.



5

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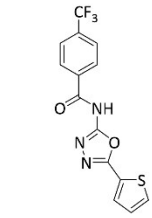
H1 standard parameters, cryoprobe prodigy.



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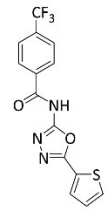

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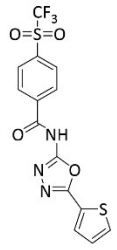
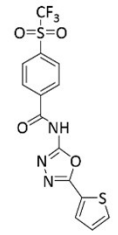


6

GN-103.1.fid
H1 standard parameters, cryoprobe prodigy.



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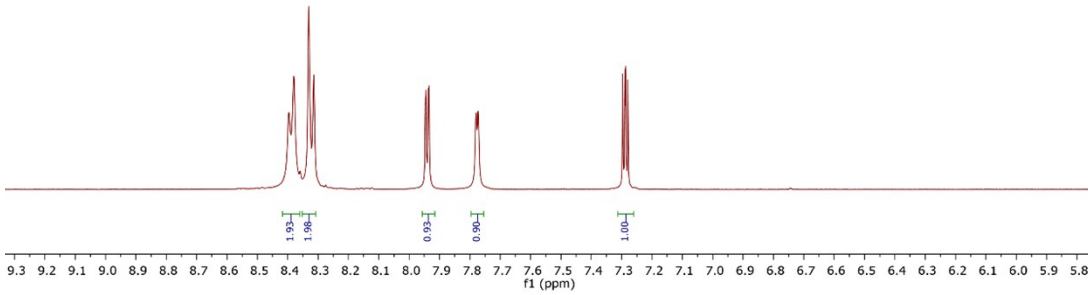
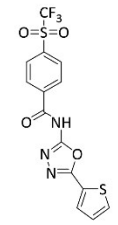


GN-103.2.fid
C13 standard parameters, cryoprobe prodigy. PULprog: zgig30zr.jjd



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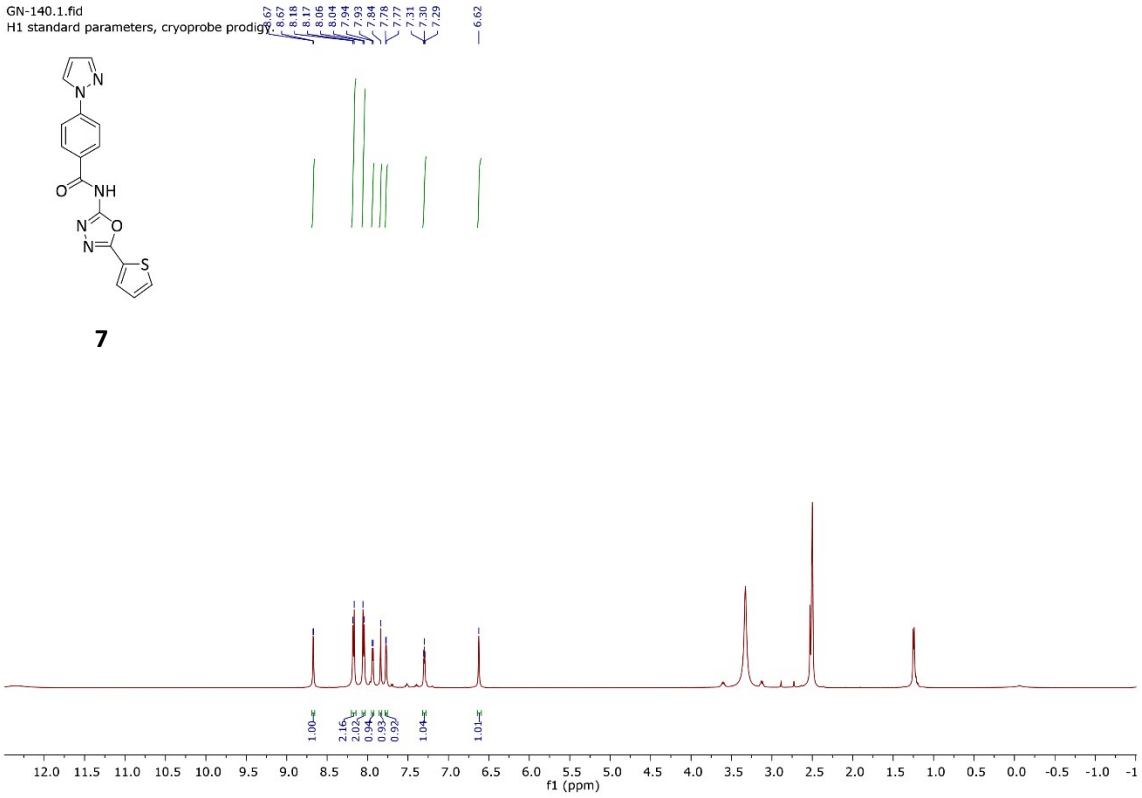
GN-140.1.fid
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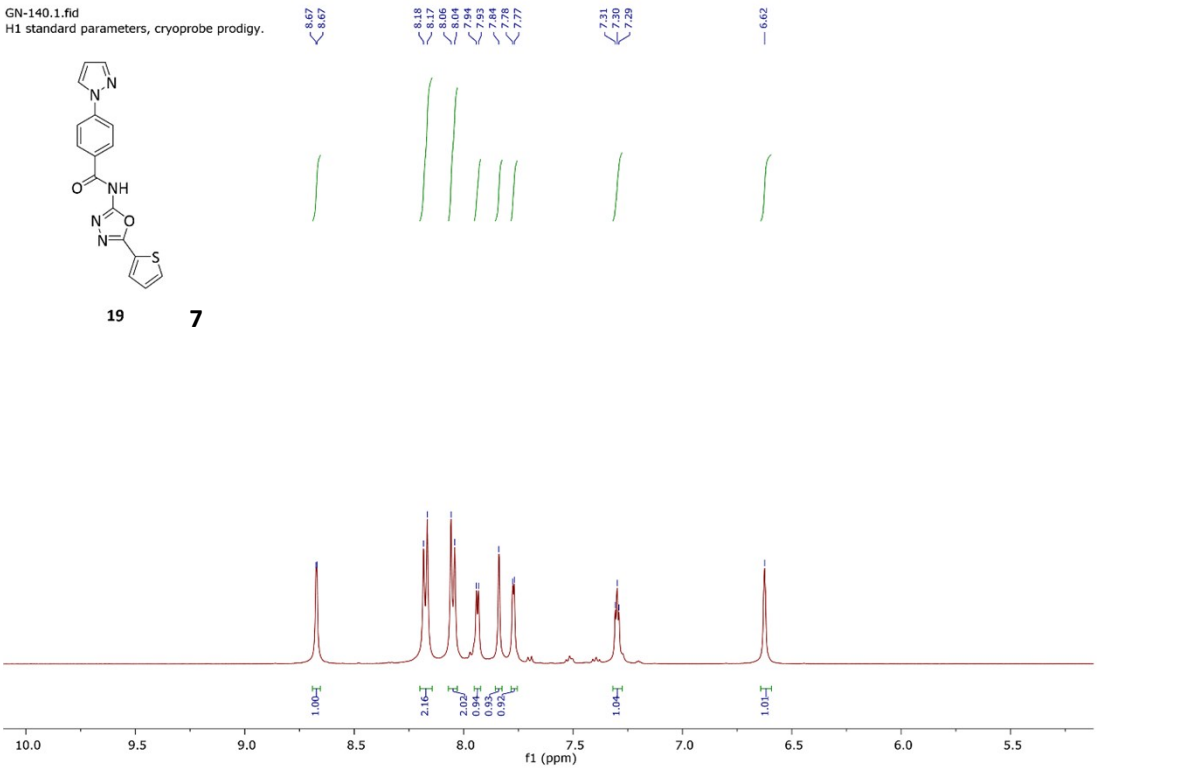
8.67
8.67
8.18
8.17
8.06
8.04
7.94
7.93
7.84
7.78
7.77
7.31
7.30
7.29
6.62
NH
O
N
O
S
1.00
2.16
2.02
0.94
0.93
0.92
1.04
1.01
f1 (ppm)

7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8.67
8.18
8.17
8.06
8.04
7.94
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7.84
7.78
7.77
7.31
7.30
7.29
6.62
NH
O
N
O
S
1.00
2.16
2.02
0.94
0.93
0.92
1.04
1.01
f1 (ppm)

19 **7**

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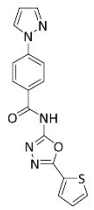
C13 standard parameters, cryoprobe prodigy. 2dlprodbb zgig30ze1sh.



7

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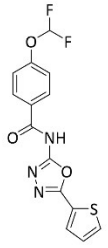
H1 standard parameters, cryoprobe prodigy.



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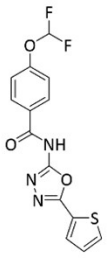

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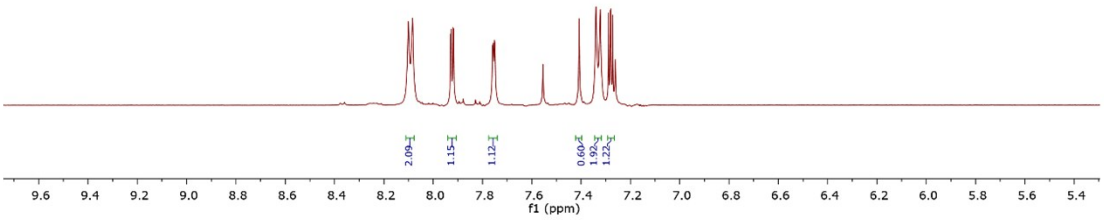

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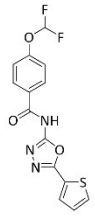
C13 standard parameters, cryoprobe prodigy. Pulprog=zgig30zr.jsh.



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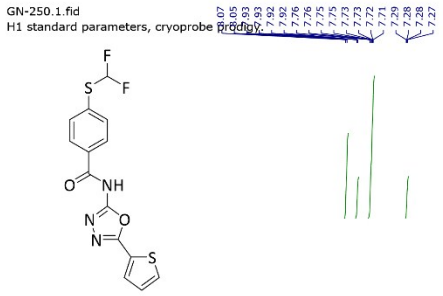
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8.05
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7.92
7.92
7.76
7.75
7.73
7.73
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7.29
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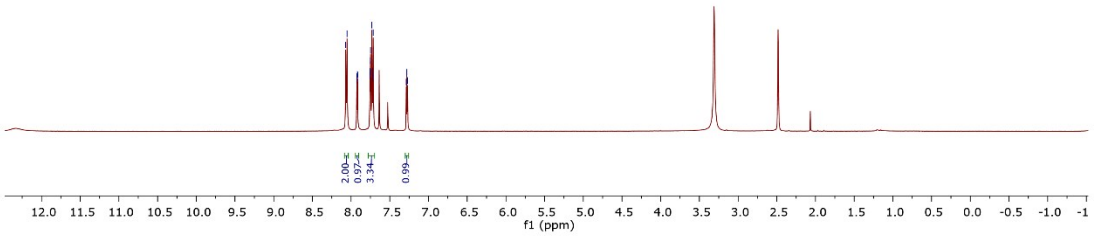
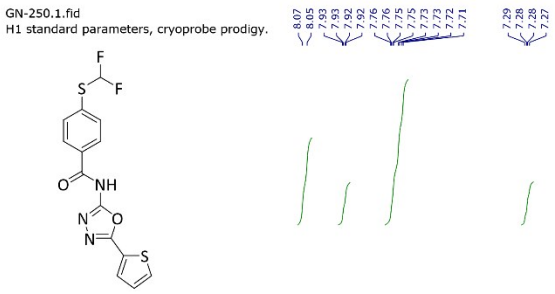
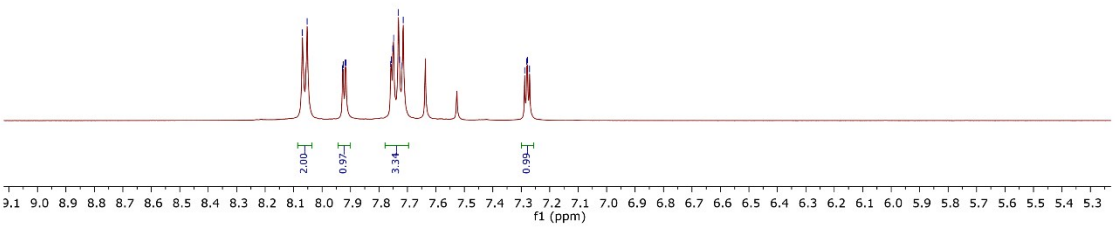
F
S
F
O
NH
N
O
N
S

9

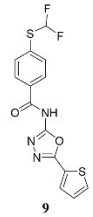

2.00
0.97
3.34
0.99
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1
f1 (ppm)


GN-250.1.fid
H1 standard parameters, cryoprobe prodigy.
8.07
8.05
7.93
7.92
7.92
7.76
7.75
7.73
7.73
7.72
7.71
7.29
7.28
7.27
F
S
F
O
NH
N
O
N
S

9

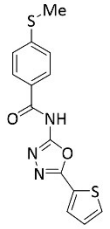

2.00
0.97
3.34
0.99
9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3
f1 (ppm)

GN-250-800.4.fid
C13 standard parameters, cryoprobe.
Use "abs13c" to flatten spectrum baseline.
Run at 318K.



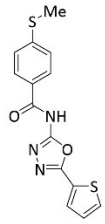
9

GN-251.1.fid
H1 standard parameters, cryoprobe prodigy.

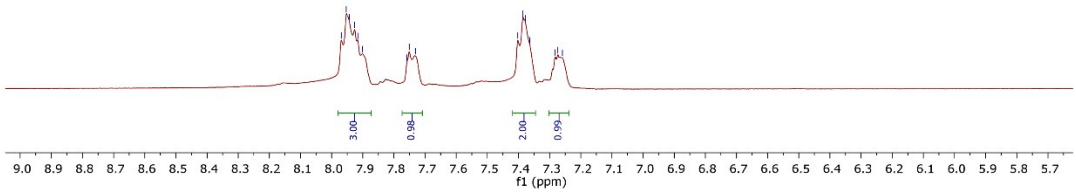


10

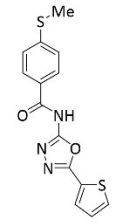
GN-251.1.fid
H1 standard parameters, cryoprobe prodigy.



10

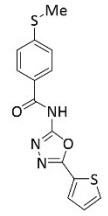


GN-251.1.fid
H1 standard parameters, cryoprobe prodigy.



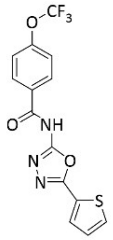
10

GN-251.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog = zgig30dr.jsh.



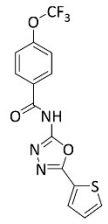
10

GN-145.1.fid
H1 standard parameters, cryoprobe prodigy.

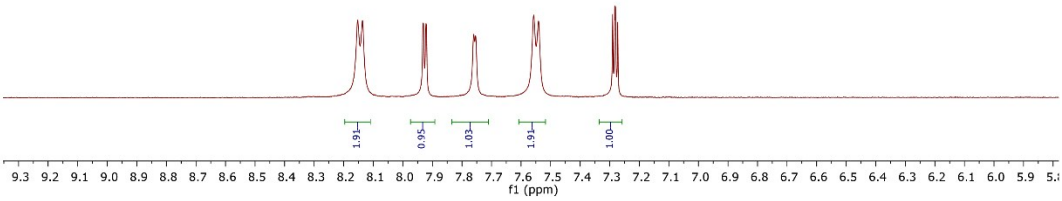


12 **11**

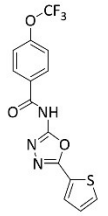
GN-145.1.fid
H1 standard parameters, cryoprobe prodigy.



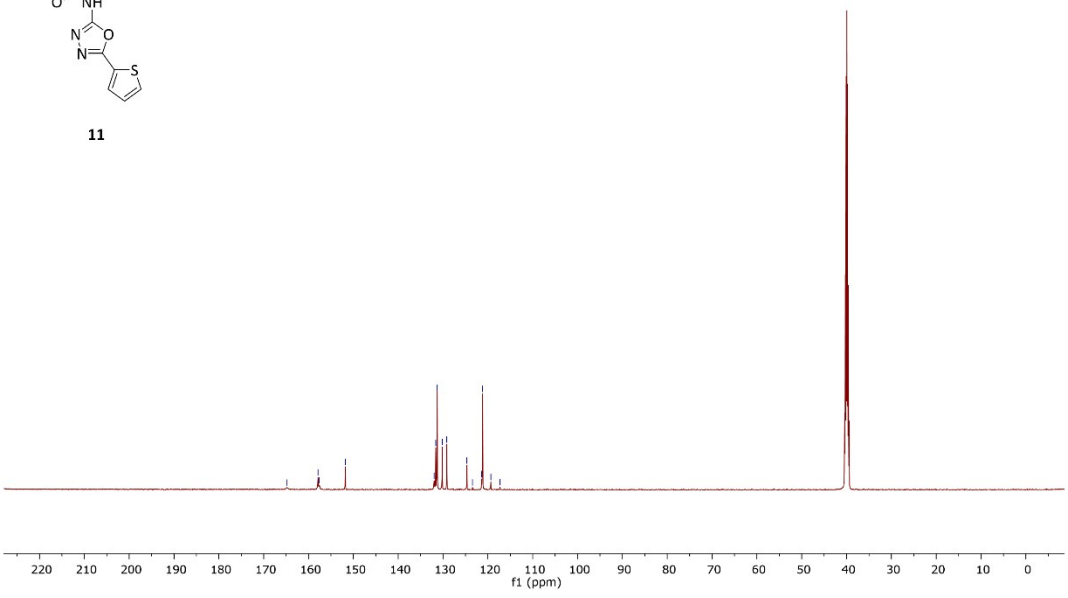
11



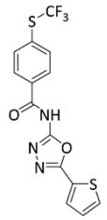
GN-145.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgig30zr.jsh



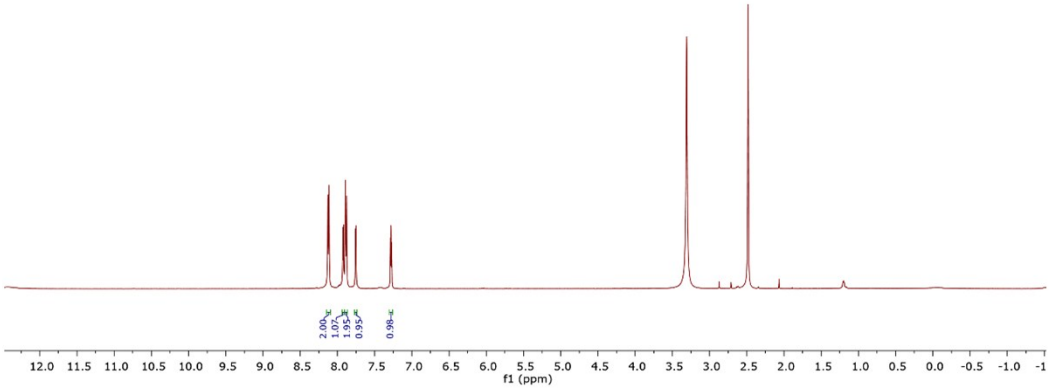
11



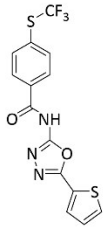
GN-148.1.fid
H1 standard parameters, cryoprobe prodigy.



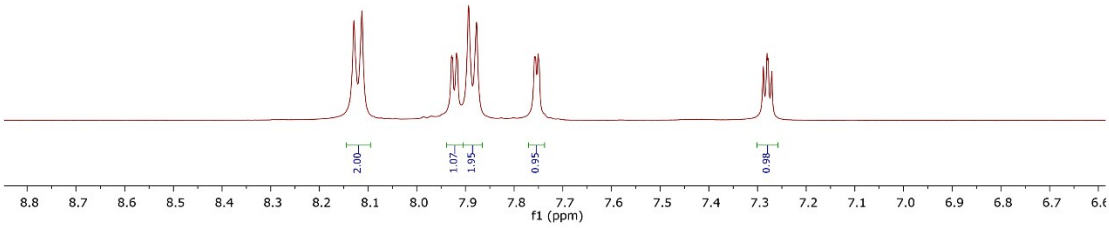
12



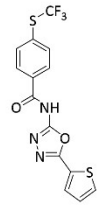
GN-148.1.fid
H1 standard parameters, cryoprobe prodigy.



12

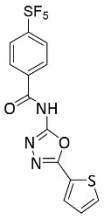


GN-148.2.fid
C13 standard parameters, cryoprobe prodigy. PUprog: zgig30zr.jsp

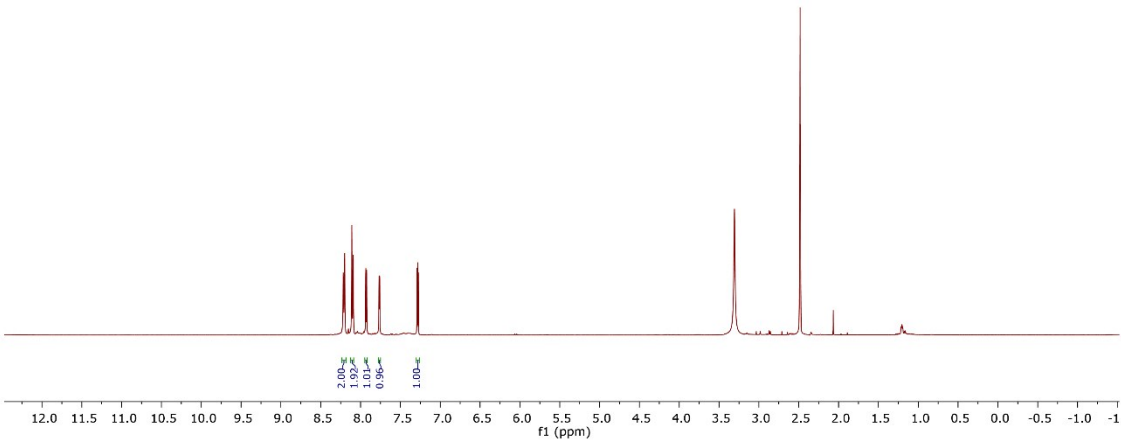


12

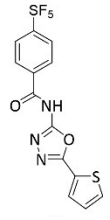
GN-144.1.fid
H1 standard parameters, cryoprobe prodigy.



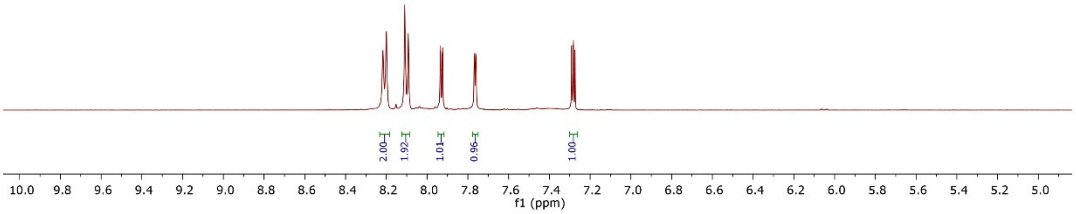
13



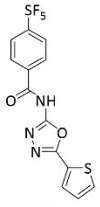
GN-144.1.fid
H1 standard parameters, cryoprobe prodigy.



13

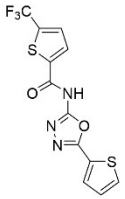


GN-144.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog: zgpg30zr.jsh



13

GN-170.1.fid
H1 standard parameters, cryoprobe prodigy.



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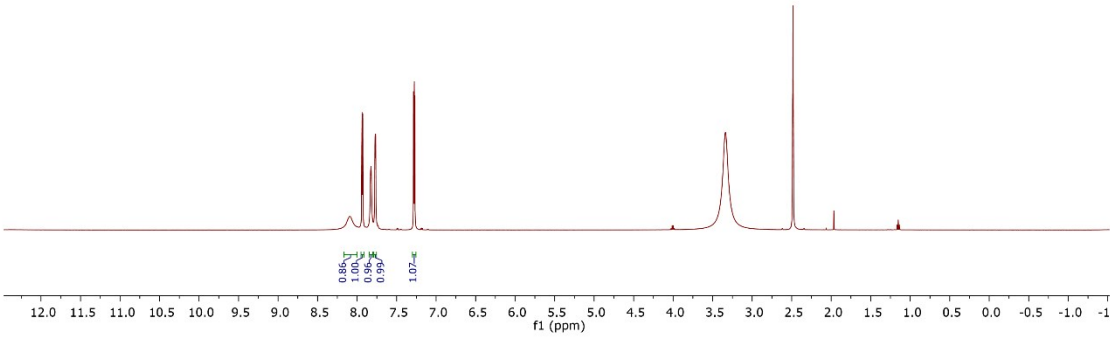
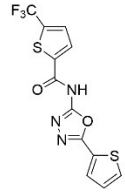


GN-170.1.fid
H1 standard parameters, cryoprobe prodigy.



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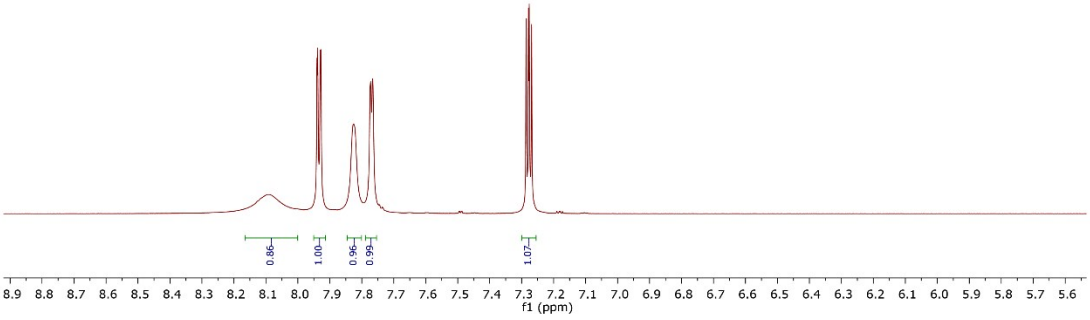
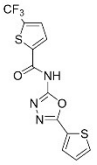
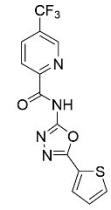


GN-170.2.fid
C13 standard parameters, cryoprobe.
Use "abs13c" to flatten spectrum baseline.



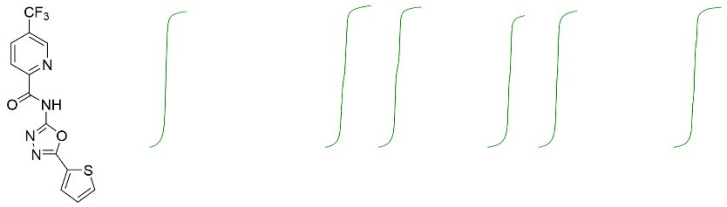
14

GN-172.1.fid
H1 standard parameters, cryoprobe prodigy.

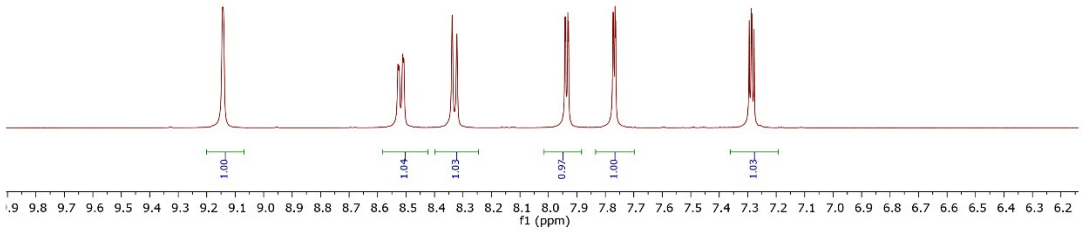


15

GN-172.1.fid
H1 standard parameters, cryoprobe prodigy.

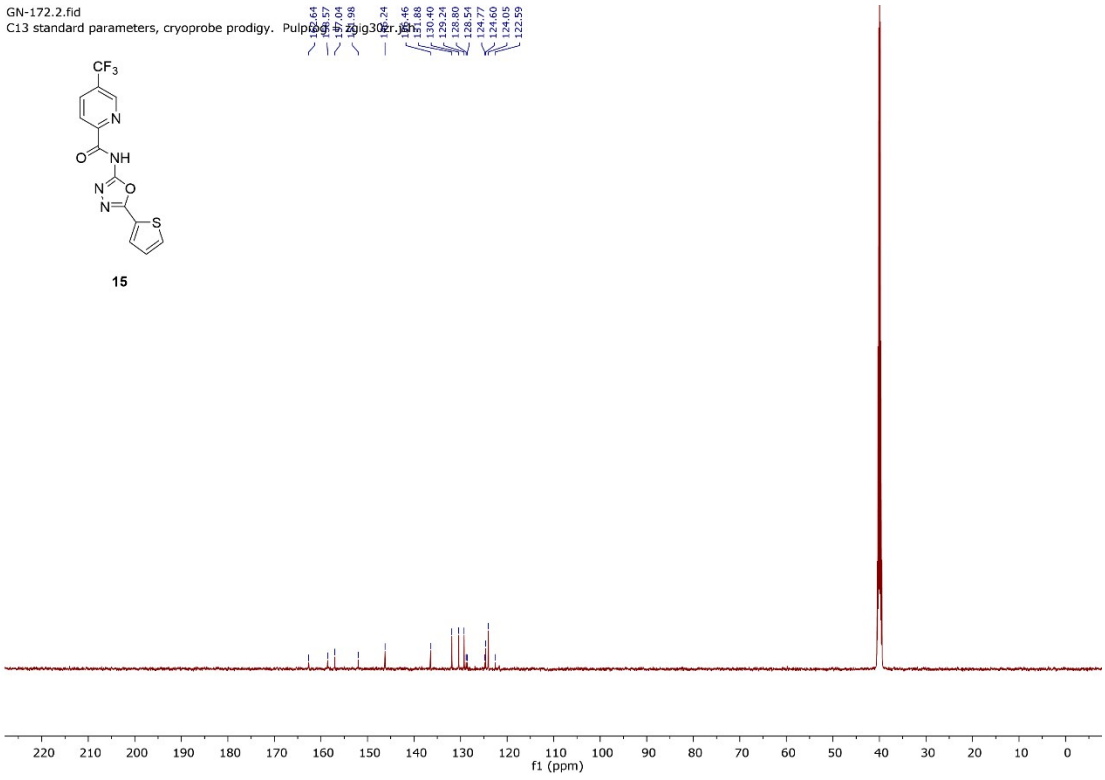


15

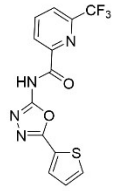


GN-172.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgig30 ns 1

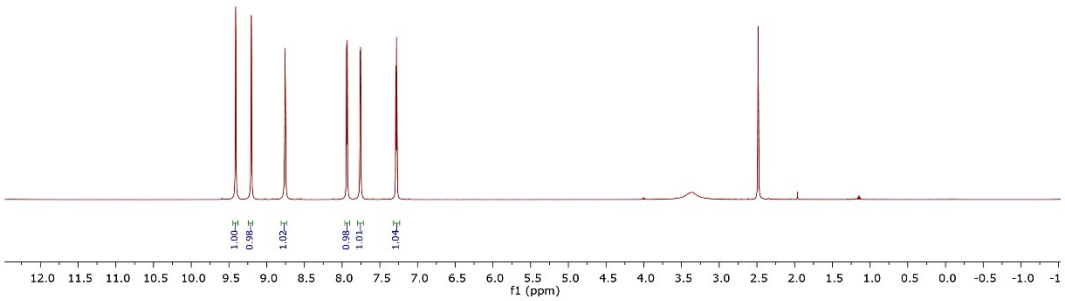
15



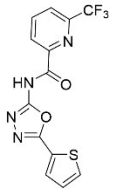
GN-184.1.fid
H1 standard parameters, cryoprobe prodigy.



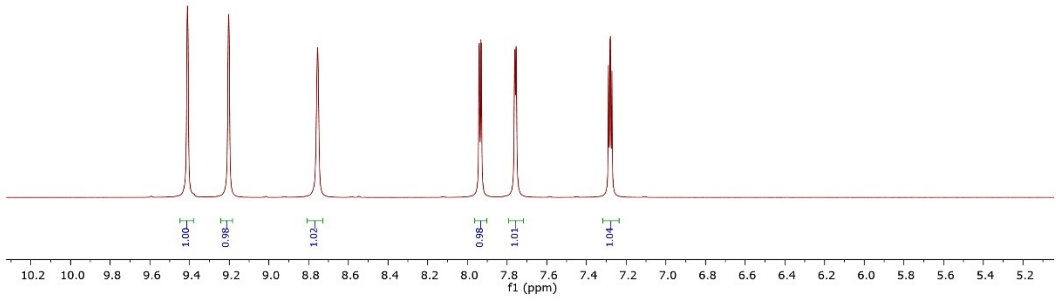
16



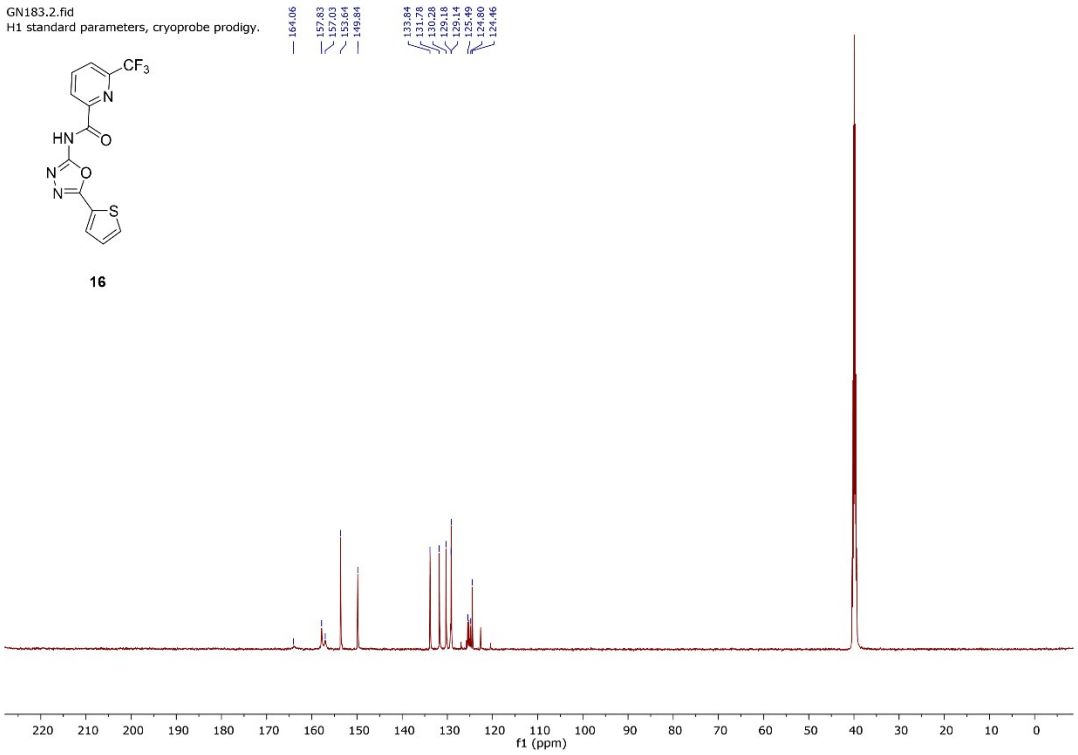
GN-184.1.fid
H1 standard parameters, cryoprobe prodigy.



16

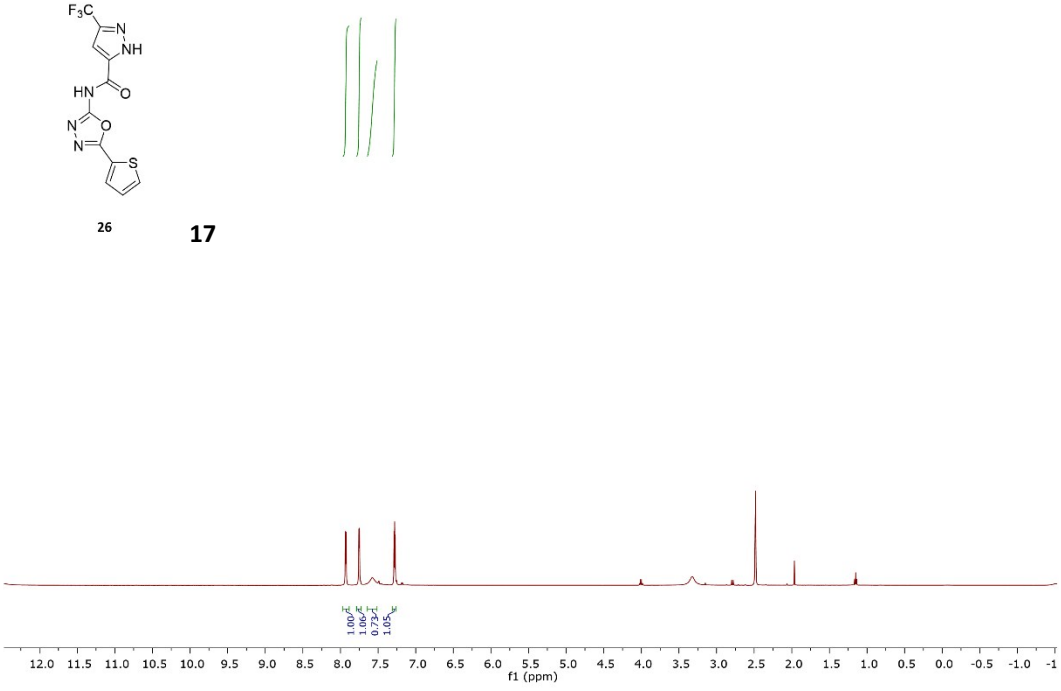


GN183.2.fid
H1 standard parameters, cryoprobe prodigy.



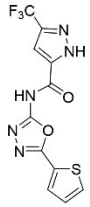
16

GN-183.1.fid
H1 standard parameters, cryoprobe prodigy.



26 **17**

GN-183.1.fid
H1 standard parameters, cryoprobe prodigy.



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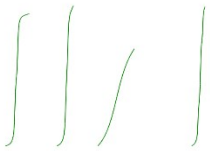
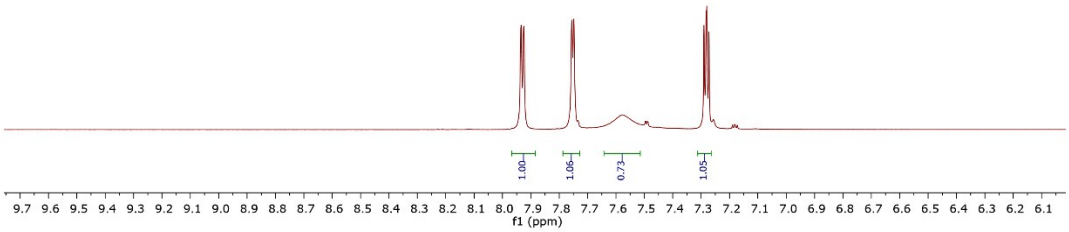
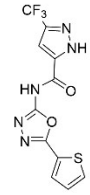




GN-183.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog : zgig30zgdc.



17

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