

Synthesis of unprotected thienyl sulfonamides and its activities against Carbapenem-resistant *Klebsiella pneumoniae*, docking studies and ADMET analysis

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1. Identification and isolation of bacterial strain

Ethical approval from the Ethical Review Board of Government College University, Faisalabad, and informed parental consent, compliant with the Declaration of Helsinki, preceded the collection of a 3 mL blood sample from a 5-year-old pediatric patient suspected of septicemia in tertiary care. This blood was added to a BACTEC/Alert Pediatric vial. Using the BACTEC/Alert automated system (BD, UK), the vial was incubated at 37 °C for a maximum of 5 days. Upon positivity, subculture occurred on blood agar and MacConkey agar (Oxoid, UK), with overnight aerobic incubation at 37 °C. The resultant isolate underwent primary identification via colony characteristics, followed by biochemical confirmation via a Gram-negative card and the VITEK 2 compact system (Biomérieux, France).

1.1. Sequence Typing of *K. pneumoniae*

The selected housekeeping genes for *K. pneumoniae* sequence typing included RNA polymerase β -subunit (rpoB), glyceraldehyde-3-phosphate dehydrogenase (gapA), translation initiation factor 2 (infB), malate dehydrogenase (mdh), phosphoglucose isomerase (pgi), phosphorene E (phoE), and periplasmic energy transducer (tonB). Following agarose gel extraction via QIAquick Kit (Qiagen, Germany), amplicons were outsourced for DNA sequencing to Eurofins Scientific (London). The obtained sequencing data were then analyzed using the *K. pneumoniae* MLST database available at <http://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>.

1.2. Antibigram of the Isolate

Antimicrobial susceptibility was assessed using the VITEK 2 compact automated system (BioMérieux, France) to derive MIC values for relevant antibiotics. MICs are quantified in $\mu\text{g/mL}$ units. The antimicrobials tested were ampicillin/sulbactam, piperacillin, levofloxacin, cefepime, aztreonam, ceftriaxone, amikacin, minocycline, meropenem, moxifloxacin, tetracycline, colistin, and chloramphenicol. Susceptibility was interpreted according to the following CLSI 2021 recommendations ¹.

1.3. Phenotypic Detection of Carbapenemase Enzyme

Carbapenemase activity was assessed via the Modified Hodge Test (MHT) ². For the Modified Hodge Test (MHT), bacterial isolates were streaked radially on Mueller-Hinton agar (MHA) plates. A meropenem disc was placed centrally, and plates were incubated at 35 ± 2 °C for 18-24 hours. Carbapenemase production was confirmed by a characteristic cloverleaf-shaped indentation of bacterial growth toward the meropenem disc. Metallo- β -lactamase (MBL) production was detected using a double-disc synergy test. A confluent lawn of *K. pneumoniae* was prepared on MHA. Discs containing imipenem and meropenem were placed 20 mm apart, with 10 μL of 0.5 M EDTA applied directly to each carbapenem disc. A positive MBL result was defined as a ≥ 5 mm increase in the zone of inhibition around the EDTA-supplemented disc compared to the carbapenem disc alone ³.

1.4. Phenotypic Detection of Metallo- β -Lactamase (MBL) Enzyme

MBL-producing bacteria were identified using the double-disc synergy method, incorporating EDTA. Briefly, the isolates were spread onto Mueller-Hinton agar (MHA) plates, and two discs containing imipenem and meropenem were positioned 24 mm apart. Subsequently, 10 μL of a 0.5M EDTA solution was applied to each carbapenem disc. A positive result was indicated if the EDTA discs displayed a zone of inhibition greater than 5 mm compared to the non-EDTA discs ³.

1.5. Molecular Characterization of *bla*_{NDM}

The extraction of DNA from bacterial culture was carried out using a DNA kit for bacterial cells (TIANamp, Beijing, China). PCR reaction was performed using the gene-specific primers F1 (5'-ATGGAATTGCCCAATATTATGCAC-3') and R1 (5'-TCAGCGCAGCTTGTCTGGC-3') for the presence of blaNDM. The gene sequences were confirmed, and subsequently, the NDM variant was sequenced.

2. MIC against CRKP

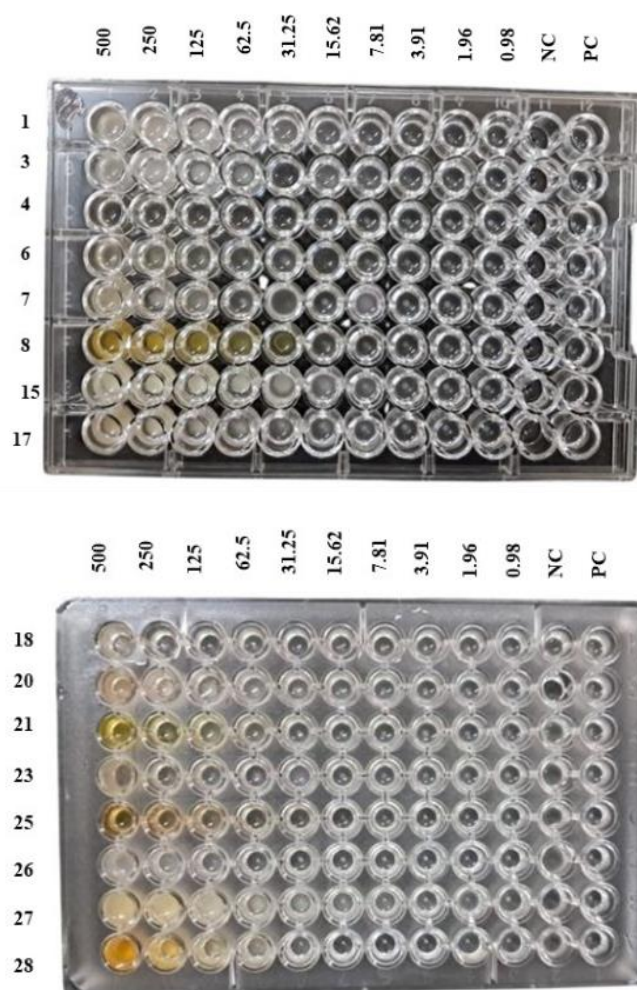


Figure S1- Minimum Inhibitory Concentration of Different Compounds Against CRKP.

3. Spectroscopic data:

5-(Isoquinolin-5-yl)thienyl-2-sulfonamide (**3a**). Yellow solid (273 mg, 94%). M.P. 236–237 °C; ^1H NMR (500 MHz, acetone- d_6) δ 9.40 (s, 1H), 8.61–8.59 (m, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 5.5 Hz, 1H), 7.92 (dd, J = 7.5, 1.5 Hz, 1H), 7.80–7.73 (m, 2H), 7.41–7.39 (m, 1H), 7.06 (br s, 2H); ^{13}C NMR (125.8 MHz, acetone- d_6) δ 154.0, 147.0, 145.7, 145.3, 134.5, 133.2, 131.7, 130.6, 130.1, 129.9, 128.8, 127.9, 118.2.

5-(3-Cyanophenyl)thienyl-2-sulfonamide (**3b**). Yellow solid (232 mg, 88%). M.P. 168–170 °C; ^1H NMR (500 MHz, DMSO- d_6) δ 8.27 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.82 (br s, 2H), 7.67 (t, J = 8.0 Hz, 1H), 7.59 (d, J = 4.0 Hz, 1H), 7.00 (d, J = 3.5 Hz, 1H); ^{13}C NMR (125.8 MHz, DMSO- d_6) δ 145.6, 145.1, 133.6, 132.2, 131.0, 130.6, 130.5, 129.2, 125.4, 118.3, 112.5.

5-(3-Hydroxyphenyl)thienyl-2-sulfonamide (**3c**). Yellow solid (240 mg, 94%). M.P. 176–178 °C; ¹H NMR (500 MHz, acetone-d₆) δ 7.56 (dd, J = 3.5, 1.5 Hz, 1H), 7.38 (dd, J = 4.0, 1.5 Hz, 1H), 7.31–7.24 (m, 2H), 7.20–7.14 (m, 3H), 6.89 (d, J = 8.0 Hz, 1H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 158.6, 149.7, 144.6, 134.8, 132.0, 131.0, 123.7, 118.0, 116.7, 113.3.

5-(2-Formylphenyl)thienyl-2-sulfonamide (**3d**). Yellow solid (246 mg, 92%). M.P. 177–178 °C; ¹H NMR (500 MHz, acetone-d₆) δ 10.18 (s, 1H), 7.99 (d, J = 7.5 Hz, 1H), 7.79–7.74 (m, 1H), 7.68–7.62 (m, 3H), 7.26 (dd, J = 7.8, 3.8 Hz, 1H), 7.01 (br s, 2H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 191.3, 147.7, 144.5, 136.7, 135.2, 134.7, 132.3, 131.4, 130.3, 130.2, 128.9.

5-(6-Formylbenzo[d][1,3]dioxol-5-yl)thienyl-2-sulfonamide (**3e**). Yellow solid (274 mg, 88%). M.P. 210–212 °C; ¹H NMR (500 MHz, acetone-d₆) δ 9.94 (s, 1H), 7.63 (dd, J = 4.0, 1.5 Hz, 1H), 7.36 (s, 1H), 7.63 (dd, J = 4.0, 1.5 Hz, 1H), 7.07 (s, 1H), 6.99 (br s, 2H), 6.24 (s, 2H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 189.4, 153.4, 150.2, 147.8, 144.3, 133.9, 131.4, 130.8, 130.4, 111.6, 106.9, 104.1.

5-(2-Nitropyridin-4-yl)thienyl-2-sulfonamide (**3f**). Yellow solid (223 mg, 78%). M.P. 202–203 °C; ¹H NMR (500 MHz, acetone-d₆) δ 9.02 (d, J = 1.5 Hz, 1H), 8.60–8.53 (m, 1H), 8.45–8.36 (m, 1H), 7.84 (d, J = 6.5 Hz, 1H), 7.71 (d, J = 6.5 Hz, 1H), 7.12 (br s, 2H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 157.1, 148.7, 146.5, 143.1, 137.8, 135.3, 132.4, 128.1, 119.5.

5-(4-(Methoxymethyl)phenyl)thienyl-2-sulfonamide (**3g**). Yellow solid (260 mg, 92%). M.P. 191–192 °C; ¹H NMR (500 MHz, acetone-d₆) δ 7.70–7.65 (m, 2H), 7.58 (d, J = 3.5 Hz, 1H), 7.46–7.39 (m, 3H), 6.89 (br s, 2H), 4.47 (s, 2H), 3.36 (s, 3H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 149.8, 145.0, 140.8, 133.1, 132.3, 129.2, 126.8, 124.0, 74.4, 58.3.

[2,3'-Bithienyl]-5-sulfonamide (**3h**). Brown solid (240 mg, 98%). M.P. 181–182 °C; ¹H NMR (500 MHz, acetone-d₆) δ 7.81–7.79 (m, 1H), 7.62 (dd, J = 5.0, 3.0 Hz, 1H), 5.54 (d, J = 4.0 Hz, 1H), 7.46 (dd, J = 5.0, 1.0 Hz, 1H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 144.8, 144.0, 135.1, 132.1, 128.4, 126.7, 124.0, 122.7.

N-(3-(5-Sulfamoylthiophen-2-yl)phenyl)acetamide (**3i**). Yellow solid (172 mg, 58%). M.P. 209–210 °C; ¹H NMR (500 MHz, acetone-d₆) δ 9.29 (s, 1H), 8.15 (s, 1H), 7.63–7.58 (m, 2H), 7.44–7.38 (m, 3H), 6.92 (br s, 2H), 2.13 (s, 3H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 169.3, 149.9, 145.1, 141.4, 134.3, 132.2, 130.5, 124.0, 121.5, 120.2, 117.3, 24.4.

5-(1-(Phenylsulfonyl)-3a,7a-dihydro-1H-indol-3-yl)thienyl-2-sulfonamide (**3j**). Yellow solid (326 mg, 78%). M.P. 246–248 °C; ¹H NMR (500 MHz, acetone-d₆) δ 8.20 (s, 1H), 8.13 (d, J = 7.0 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.70 (t, J = 6.5 Hz, 1H), 7.65 (d, J = 4.0 Hz, 1H), 7.63–7.59 (m, 2H), 7.53 (d, J = 4.0 Hz, 1H), 7.48 (t, J = 8.0 Hz, 1H), 7.43–7.39 (m, 1H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 144.7, 140.3, 138.4, 136.1, 135.4, 131.8, 130.5, 128.7, 127.9, 126.5, 125.6, 125.1, 121.1, 116.8, 114.6.

5'-Formyl-3'-methyl-[2,2'-bithienyl]-5-sulfonamide (**3k**). Yellow solid (224 mg, 78%). M.P. 188–189 °C; ¹H NMR (500 MHz, acetone-d₆) δ 9.92 (s, 1H), 7.85 (s, 1H), 7.64 (d, J = 7.0 Hz, 1H), 7.43 (d, J = 4.0 Hz, 1H), 7.02 (br s, 2H), 2.80 (s, 3H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 183.9, 147.5, 142.5, 141.7, 140.6, 139.1, 137.9, 131.9, 128.1, 15.8.

5-(4-(tert-Butyl)phenyl)thienyl-2-sulfonamide (**3l**). Yellow solid (280 mg, 95%). M.P. 188–190 °C; ¹H NMR (500 MHz, acetone-d₆) δ 7.68–7.50 (m, 5H), 7.42 (d, J = 4 Hz, 1H), 6.90 (br s, 2H), 1.36 (s, 9H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 152.8, 149.9, 144.4, 132.1, 130.9, 126.9, 126.5, 123.4, 35.1, 31.7, 31.3.

5-(Furan-3-yl)thienyl-2-sulfonamide (**3m**). Brown solid (179 mg, 78%). M.P. 186–187 °C; ¹H NMR (500 MHz, acetone-d₆) δ 8.04–8.01 (m, 1H), 7.67–7.63 (m, 1H), 7.53–7.49 (m, 1H), 7.23–7.19 (m, 1H), 6.87 (br s, 2H), 6.80 (s, 1H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 145.2, 143.5, 140.4, 131.7, 129.3, 124.0, 120.2, 109.6.

5-(2-(Benzyloxy)phenyl)thienyl-2-sulfonamide (**3n**). Yellow solid (235 mg, 68%). M.P. 262–264 °C; ¹H NMR (500 MHz, acetone-d₆) δ 7.89 (dd, J = 7.5, 1.5 Hz, 1H), 7.68–7.60 (m, 4H), 7.51–7.46 (m, 2H), 7.45–7.39 (m, 2H), 7.32 (d, J = 6.5 Hz, 1H), 7.17–7.11 (m, 1H), 6.90 (br s, 2H), 5.45 (s, 2H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 155.4, 145.3, 144.9, 137.6, 130.6, 129.3, 129.1, 128.8, 128.4, 125.4, 122.7, 122.1, 114.3, 71.1.

5-(2-Formyl-5-methoxyphenyl)thienyl-2-sulfonamide (**3o**). Yellow solid (268 mg, 90%). M.P. 208–209 °C; ¹H NMR (500 MHz, acetone-d₆) δ 10.06 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 3.5 Hz, 1H), 7.29 (d, J = 4.0 Hz, 1H), 7.23 (dd, J = 9.0, 2.5 Hz, 1H), 7.13 (d, J = 2.0 Hz, 1H), 7.03 (br s, 2H), 4.02 (s, 3H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 189.7, 164.7, 147.8, 144.5, 139.2, 131.4, 130.2, 130.2, 128.7, 116.9, 116.4, 59.5.

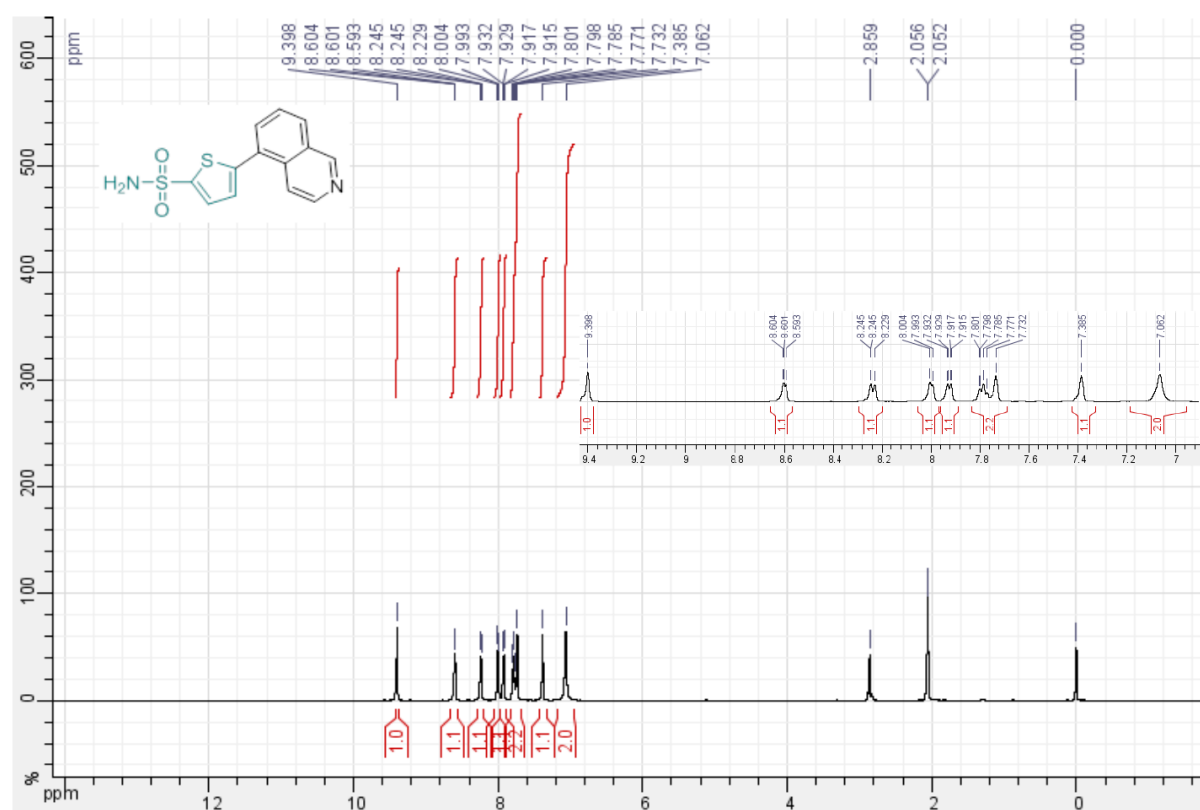
5-(5-Methylfuran-2-yl)thienyl-2-sulfonamide (**3p**). Brown solid (234 mg, 96%). M.P. 192–193 °C; ¹H NMR (500 MHz, acetone-d₆) δ 7.52 (d, J = 4.0 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.88 (br s, 2H), 6.71 (d, J = 3.5 Hz, 1H), 6.19–6.17 (m, 1H), 2.33 (s, 3H); ¹³C NMR (125.8 MHz, acetone-d₆) δ 153.6, 146.6, 142.8, 139.0, 131.7, 121.5, 109.1, 109.0, 13.1.

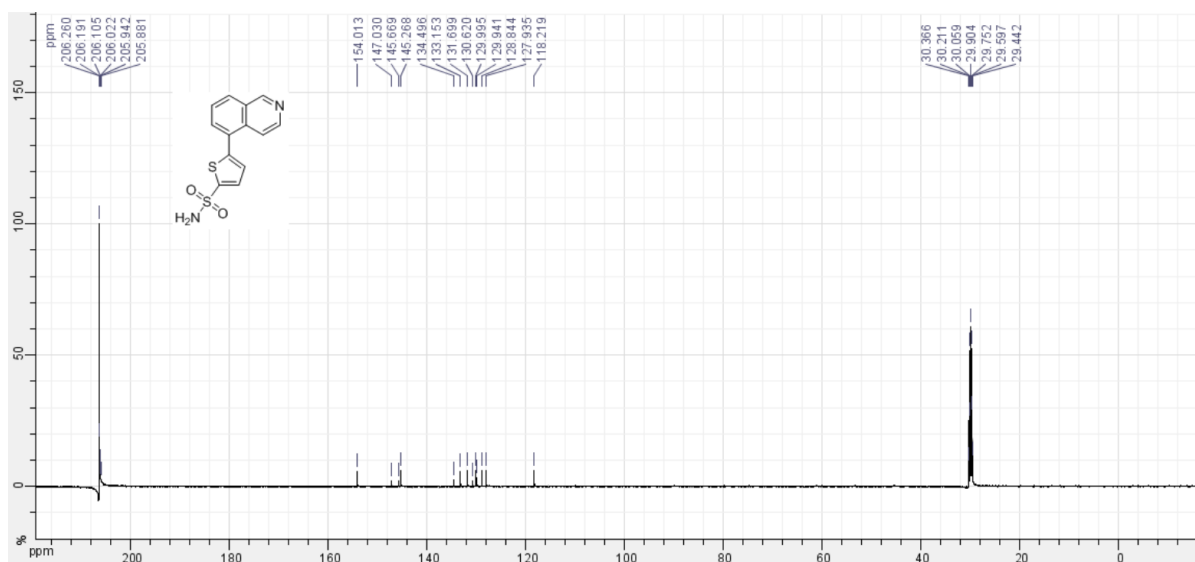
4. References:

1. I. Lewis and S. James, *Performance standards for antimicrobial susceptibility testing*, 33rd Edition, M100; 2023.
2. M. U. Qamar, T. R. Walsh, M. A. Toleman, J. M. Tyrrell, S. Saleem, A. Aboklaish and S. Jahan, *Future Microbiol.*, 2019, **14**, 691-704.
3. M. U. Qamar, S. Saleem, M. A. Toleman, M. Saqalein, M. Waseem, M. A. Nisar, M. Khurshid, Z. Taj and S. Jahan, *Future Microbiol.*, 2018, **13**, 13-26.

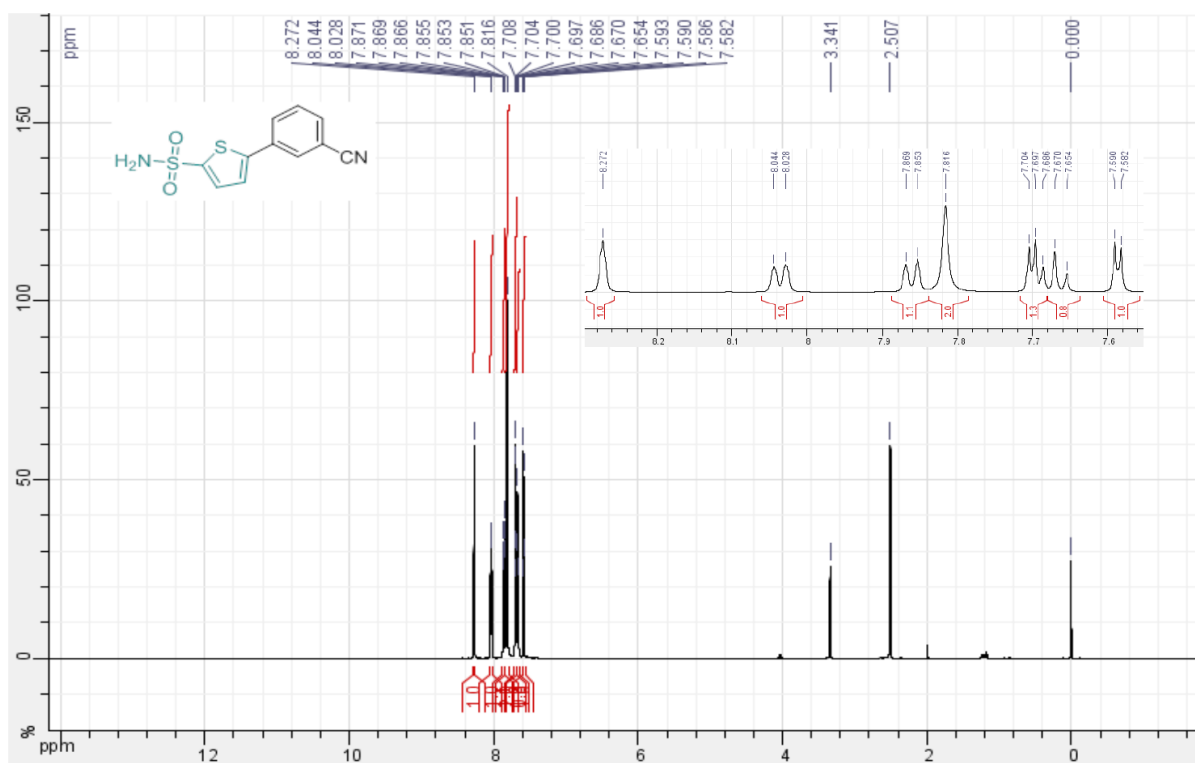
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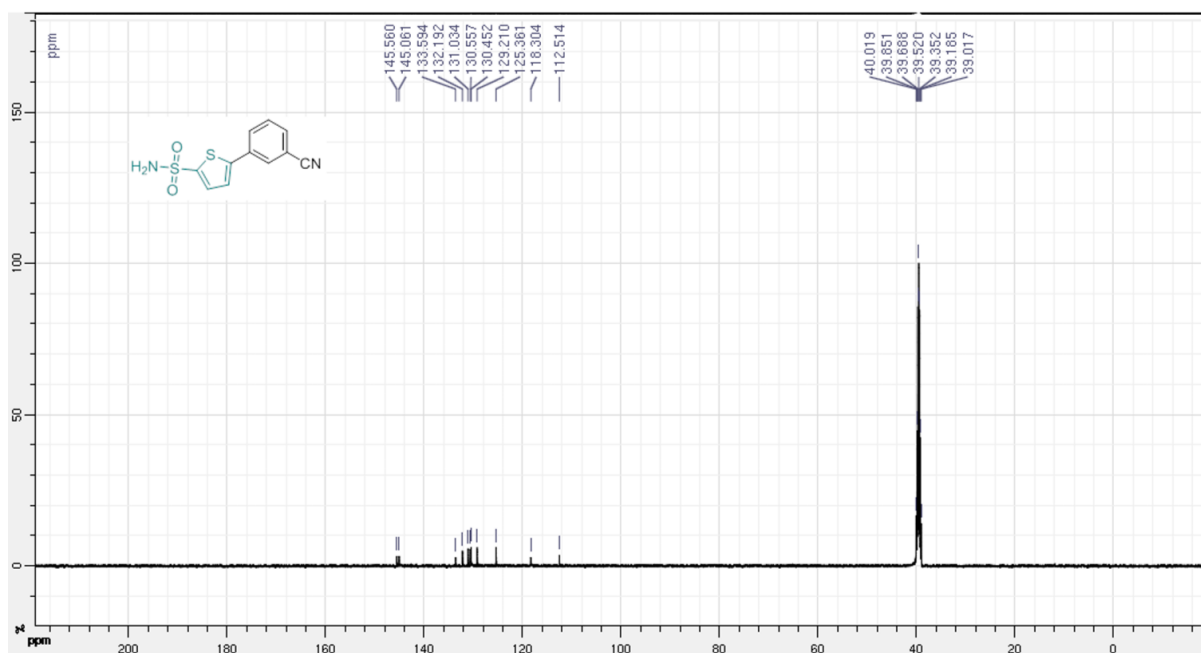
5-(Isoquinolin-5-yl)thienyl-2-sulfonamide (**3a**)



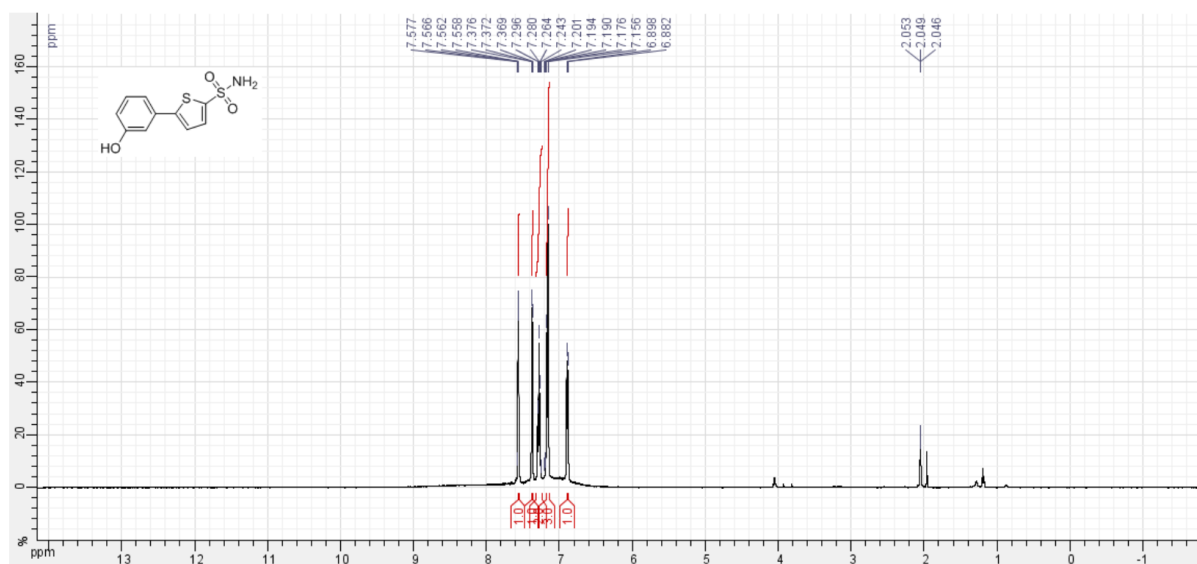


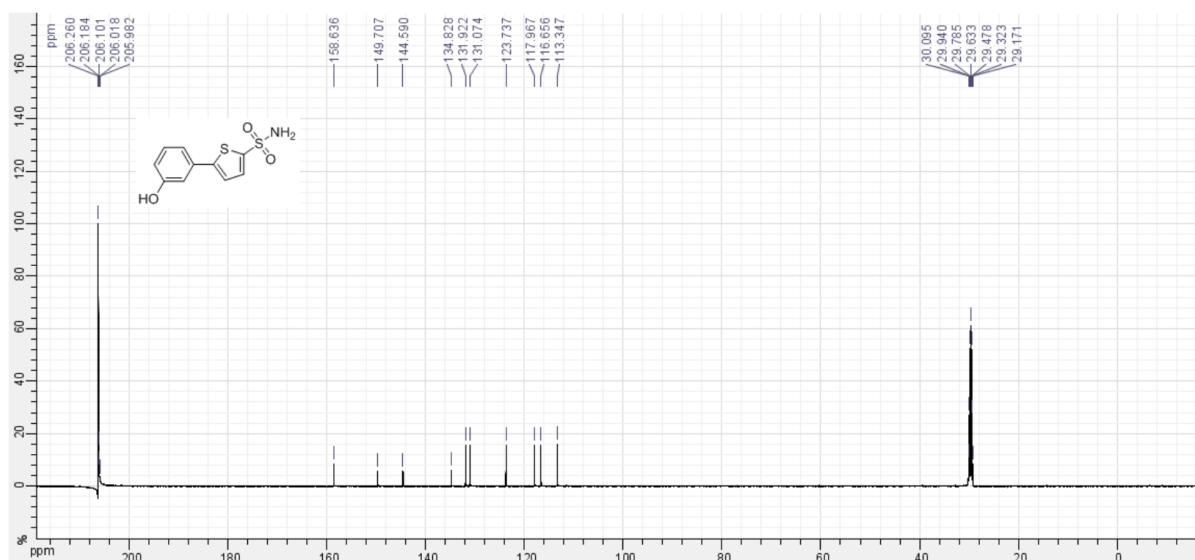
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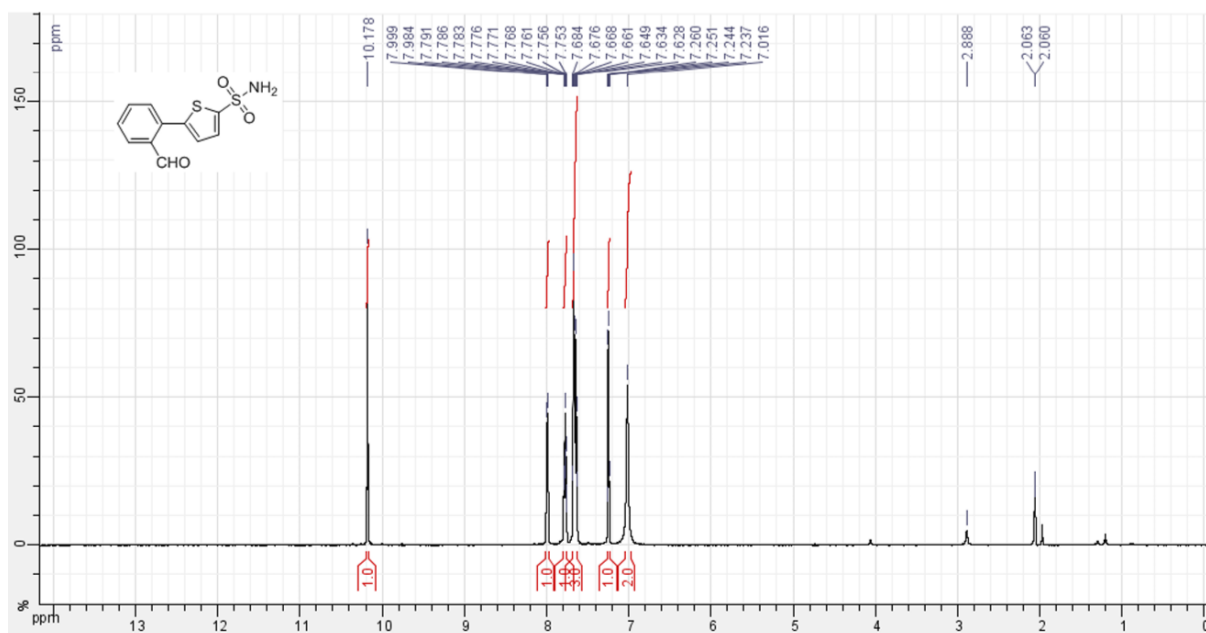


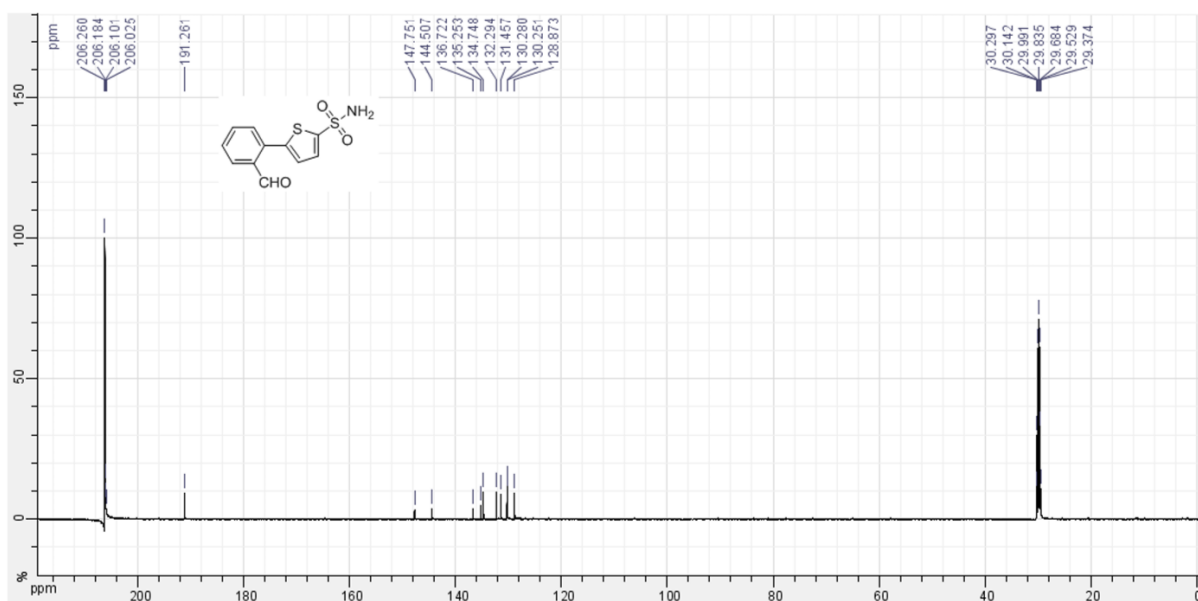
5-(3-Hydroxyphenyl)thiophene-2-sulfonamide (3c)



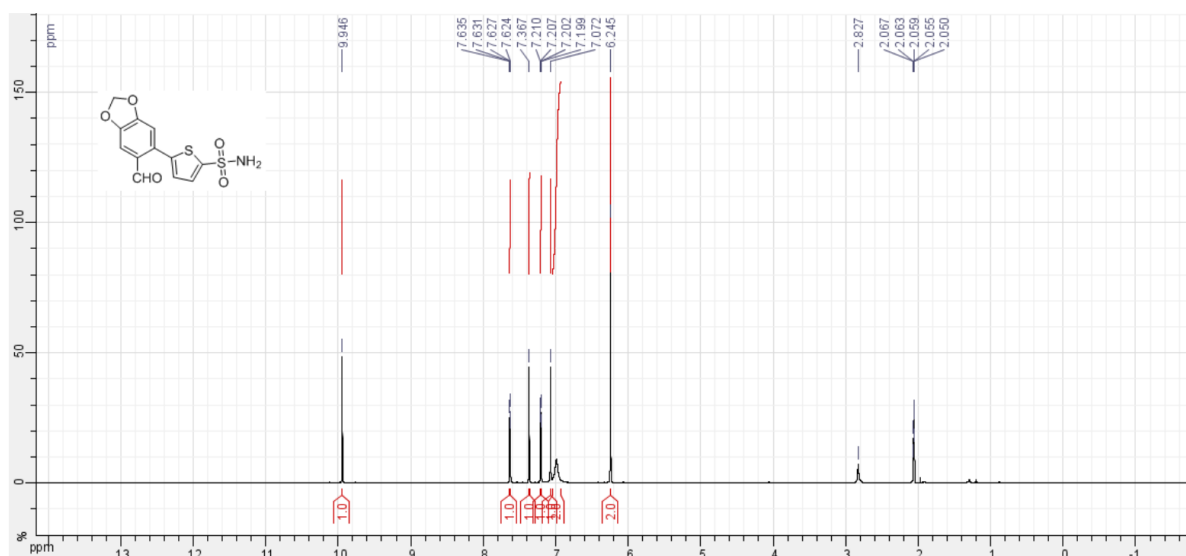


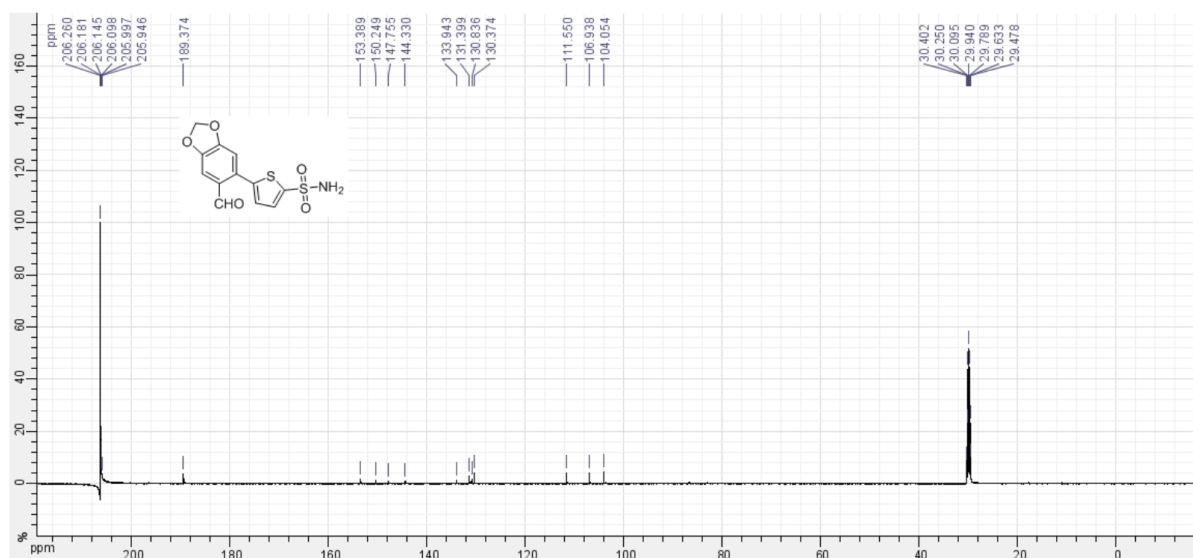
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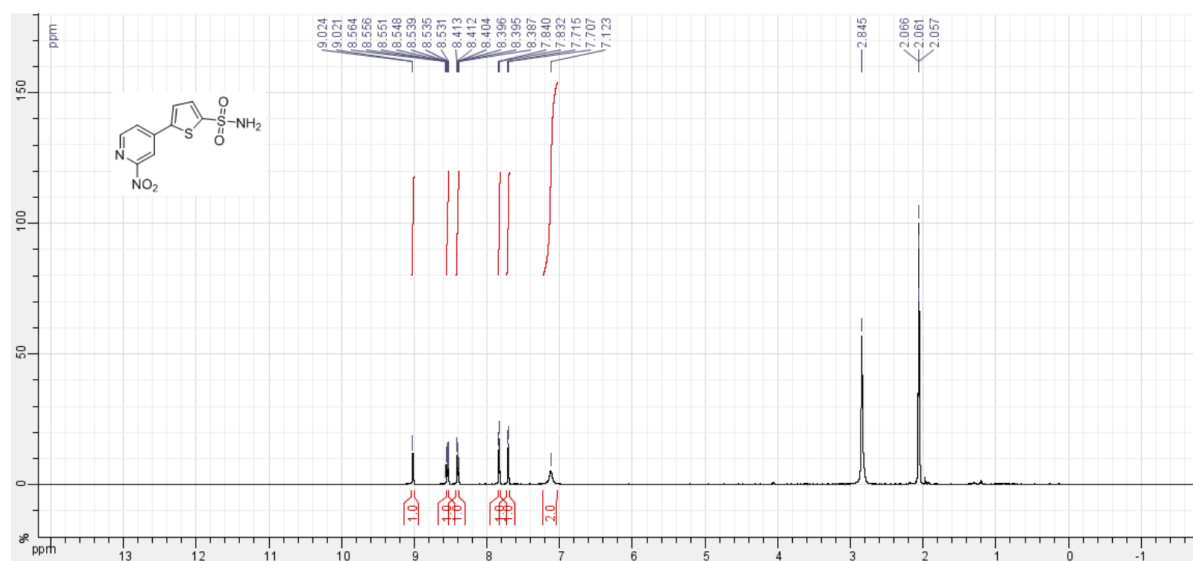


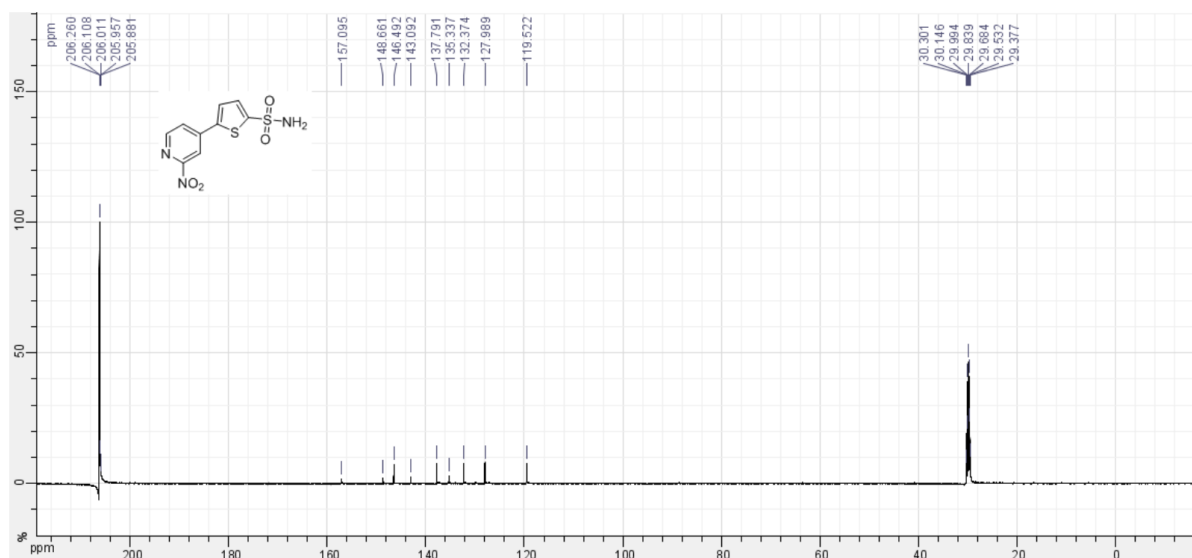
5-(6-Formylbenzo[d][1,3]dioxol-5-yl)thienyl-2-sulfonamide sulfonamide (3e)



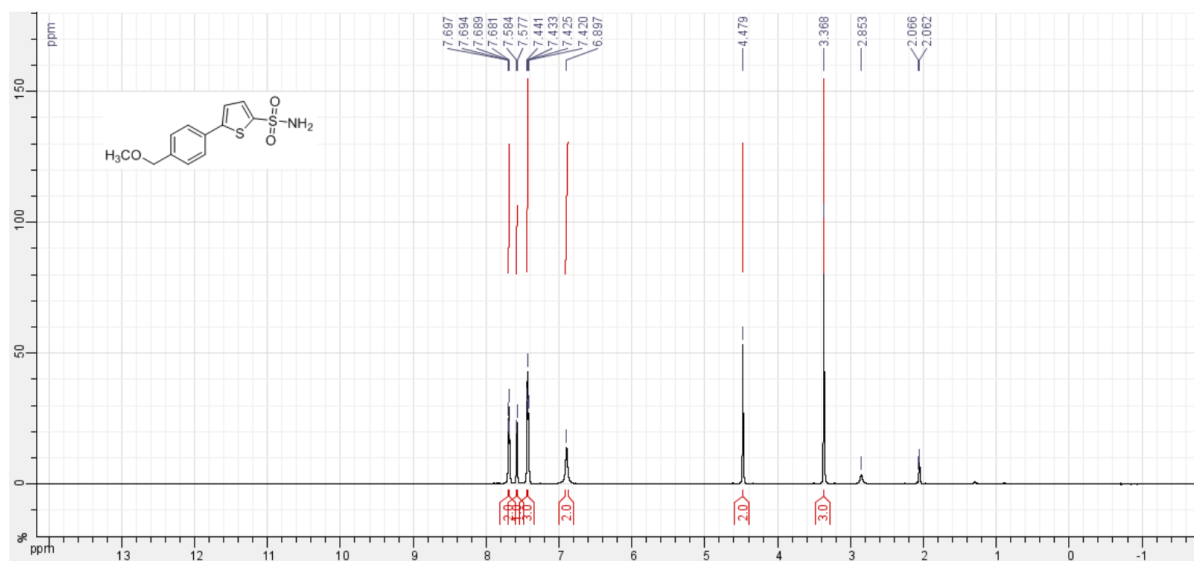


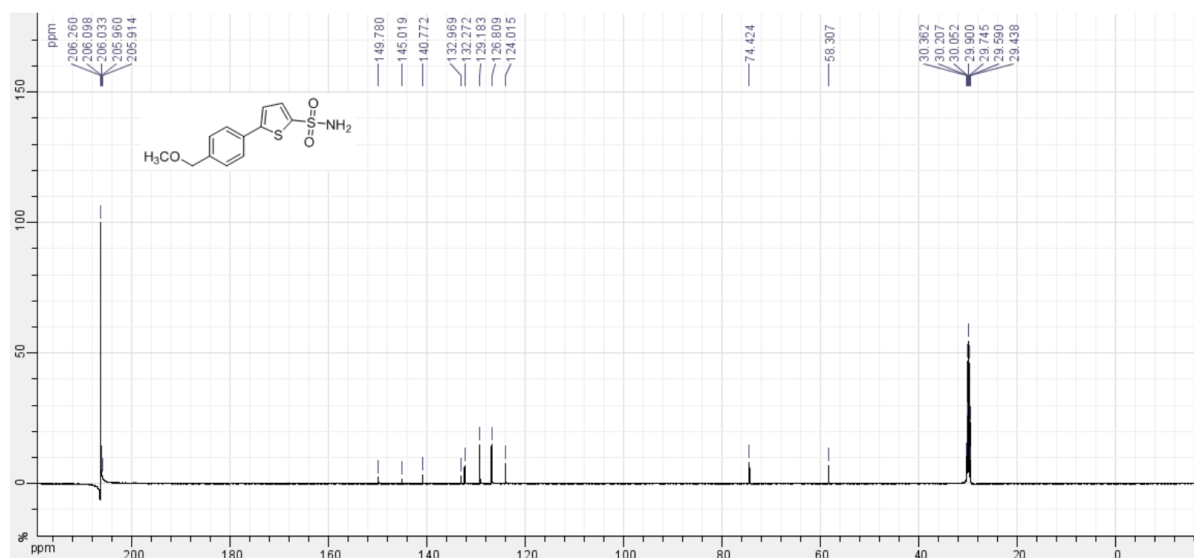
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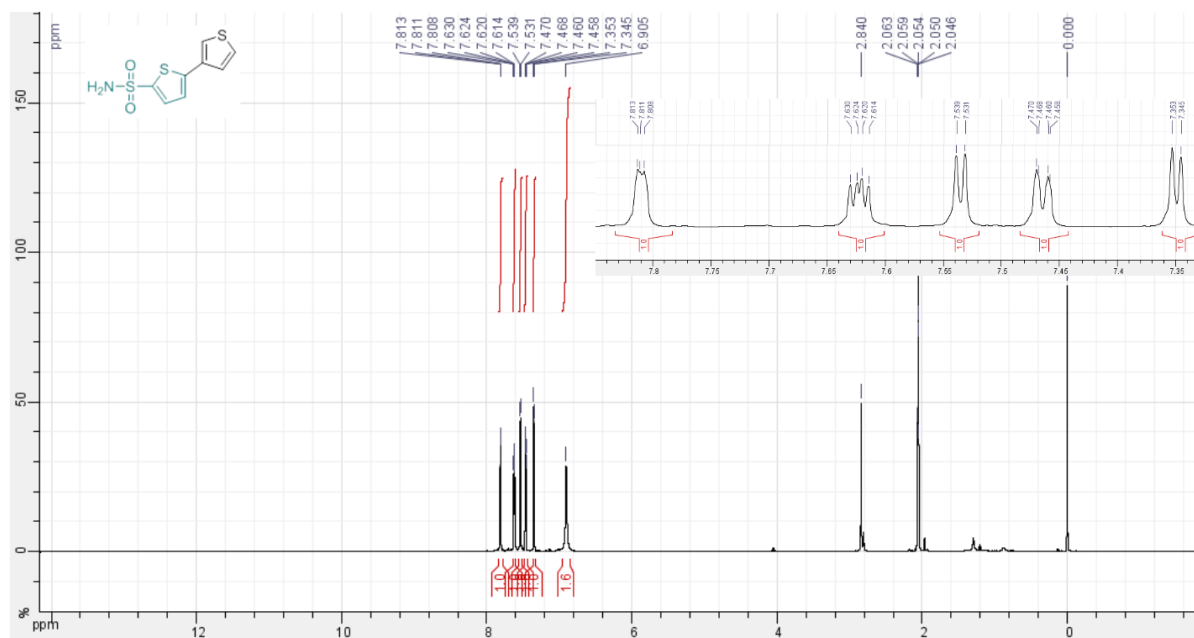


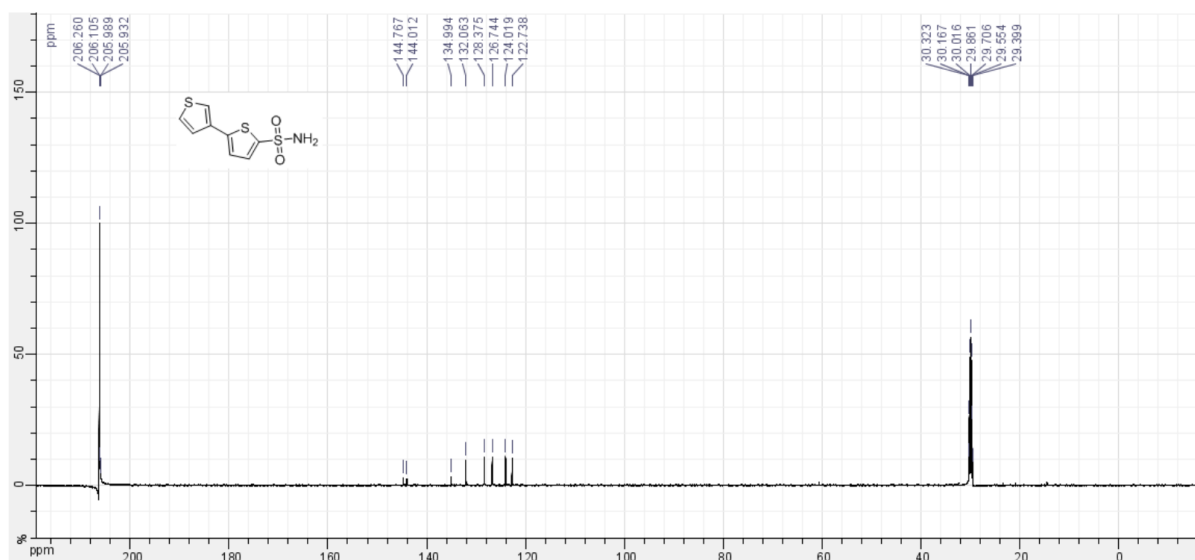
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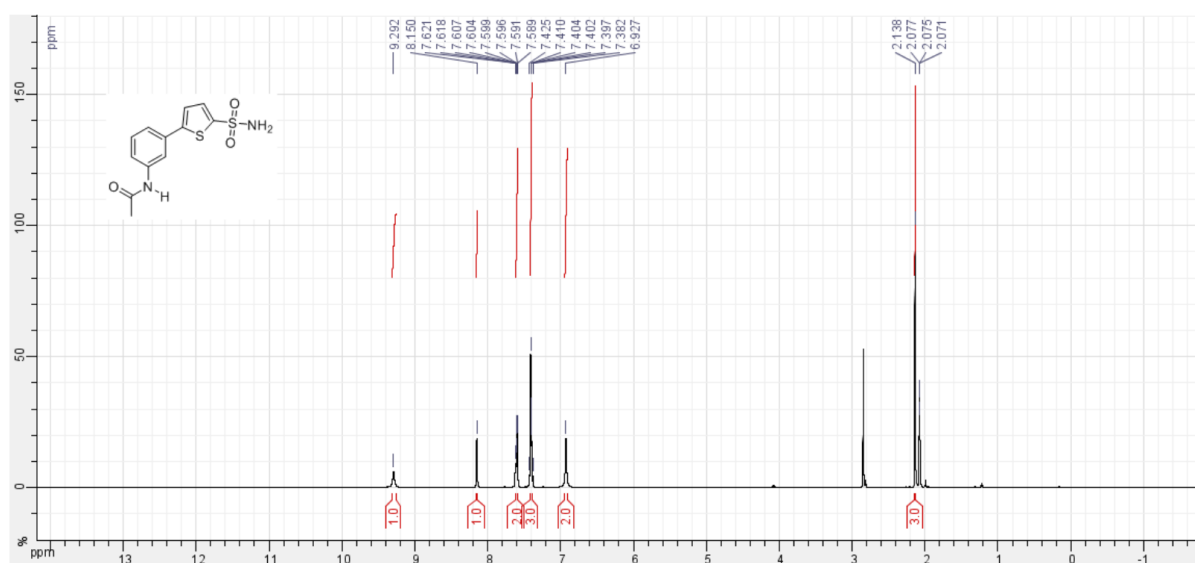


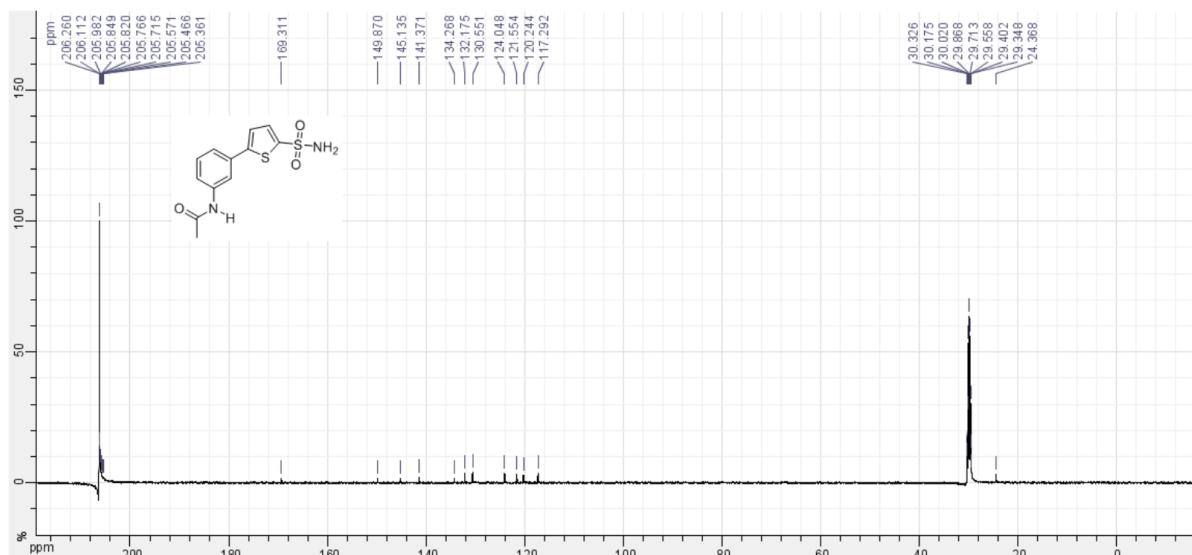
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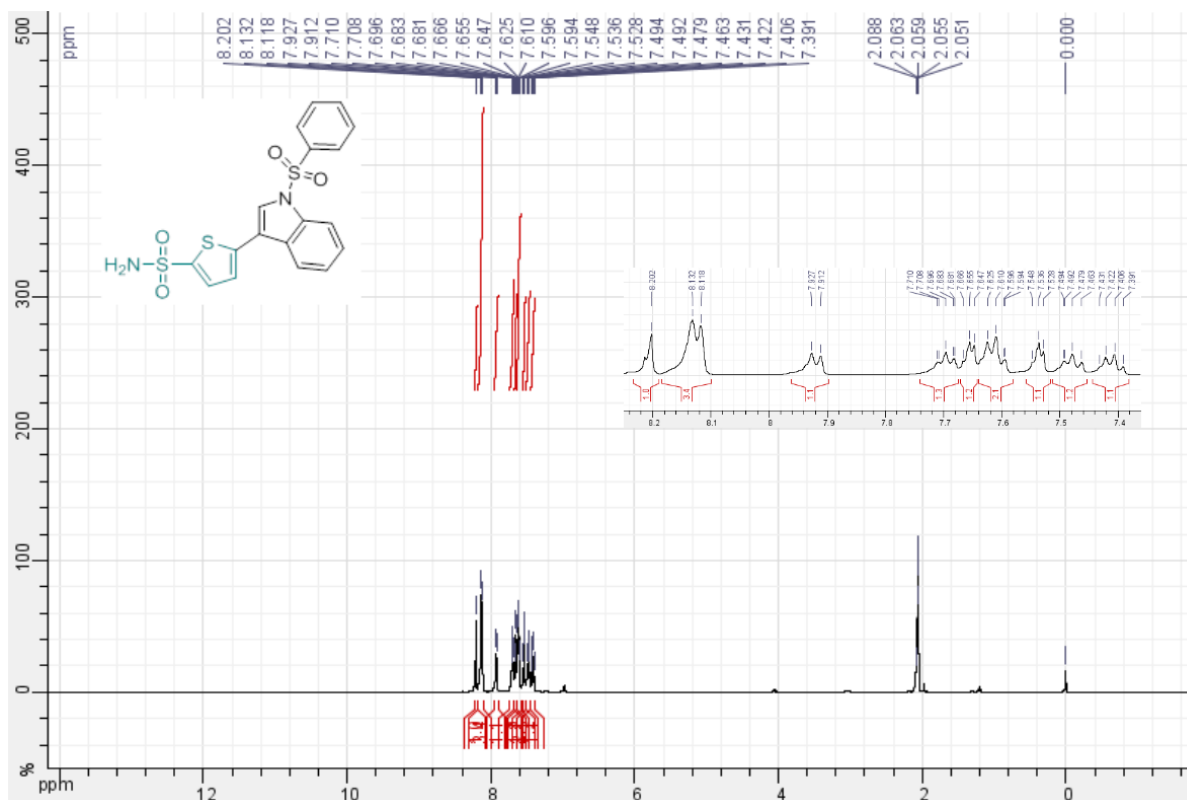


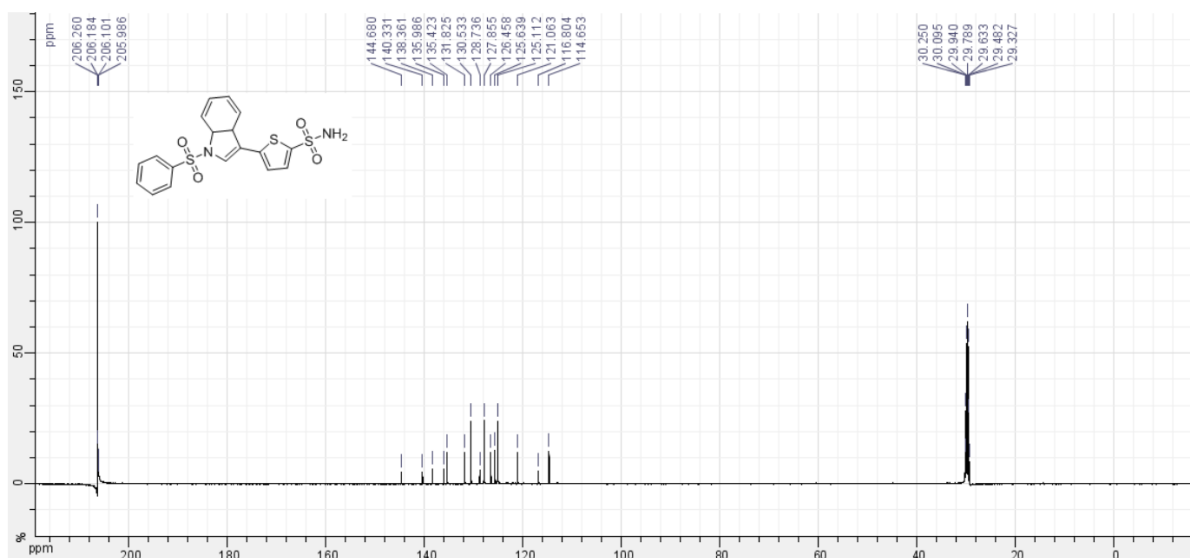
N-(3-(5-Sulfamoylthiophen-2-yl)phenyl)acetamide (**3i**)



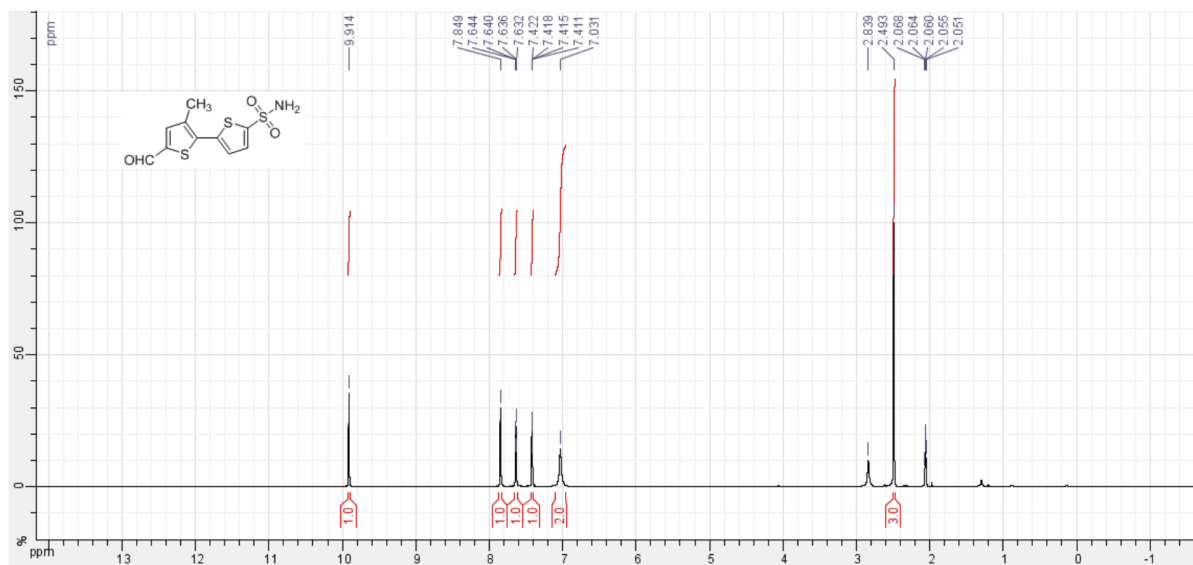


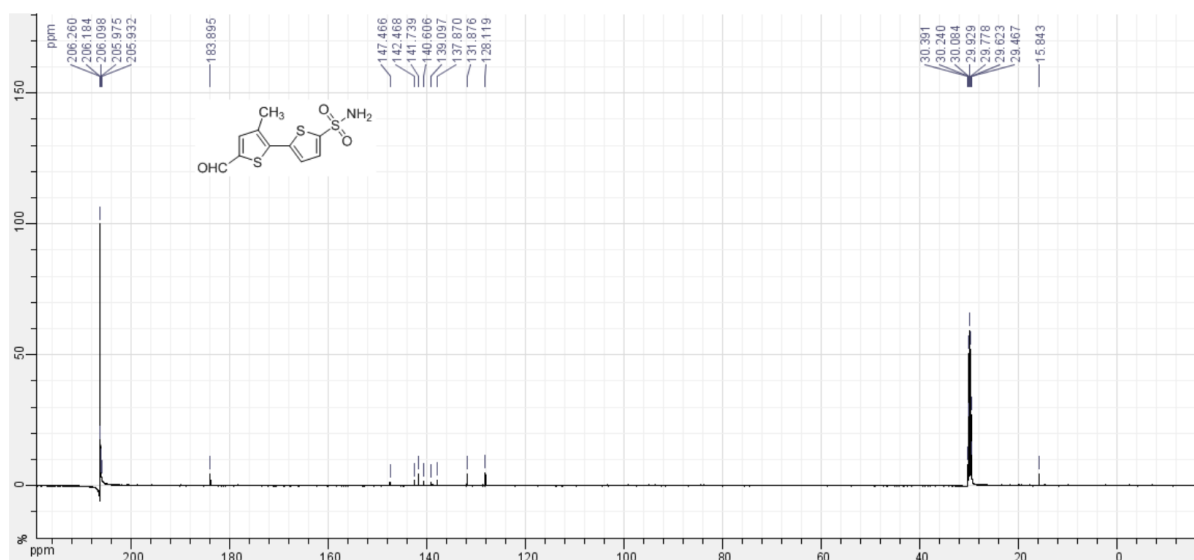
5-(1-(Phenylsulfonyl)-3a,7a-dihydro-1H-indol-3-yl)thienyl-2-sulfonamide (**3j**)



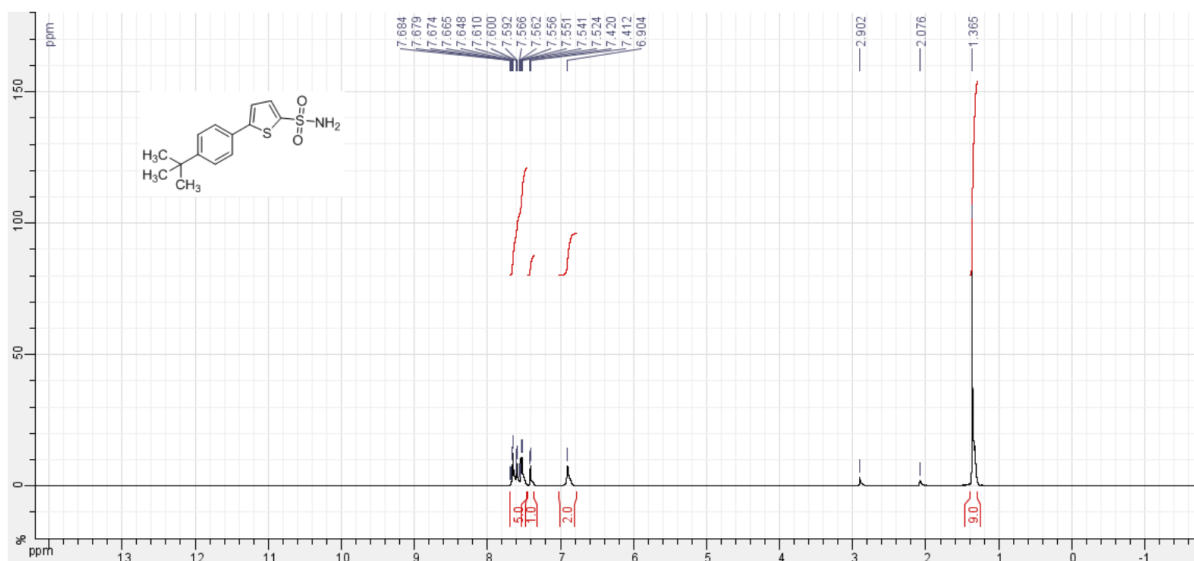


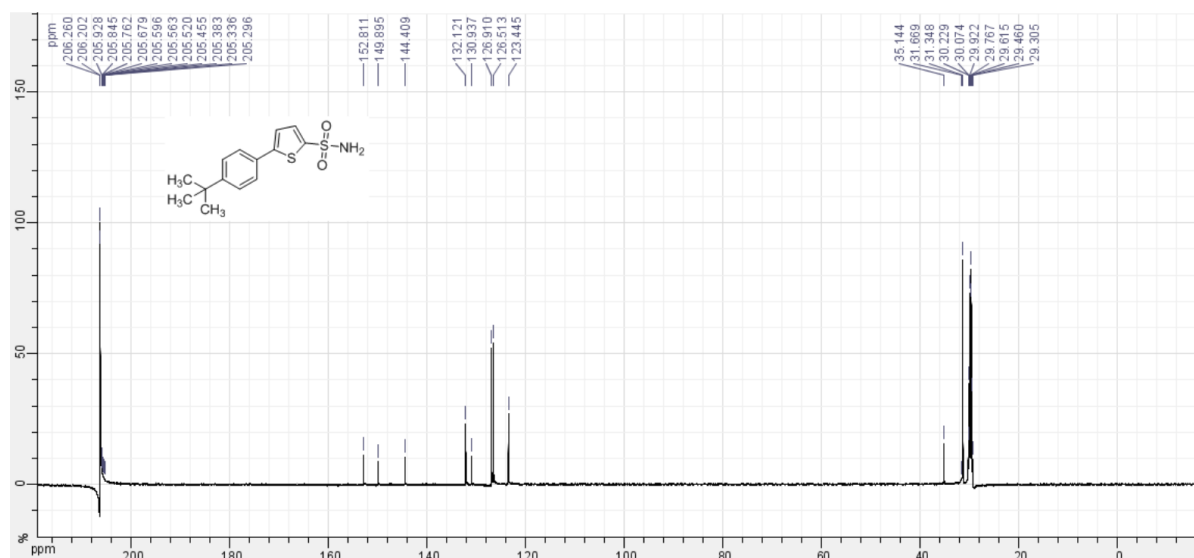
5' 3' [2,2' -Formyl-methyl-bithienyl]-5-sulfonamide (**3k**)



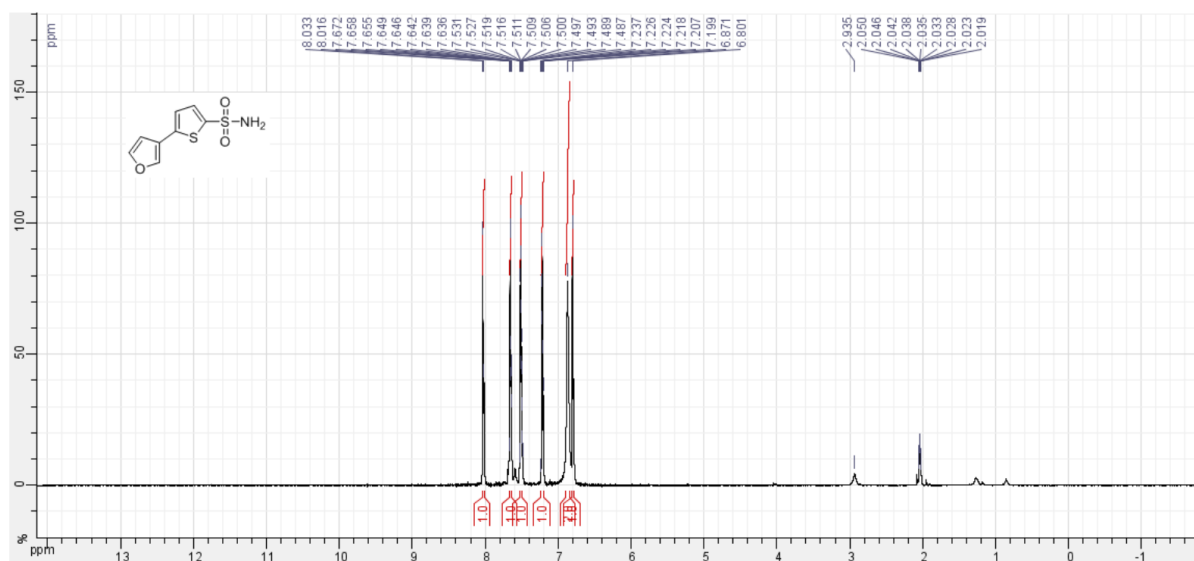


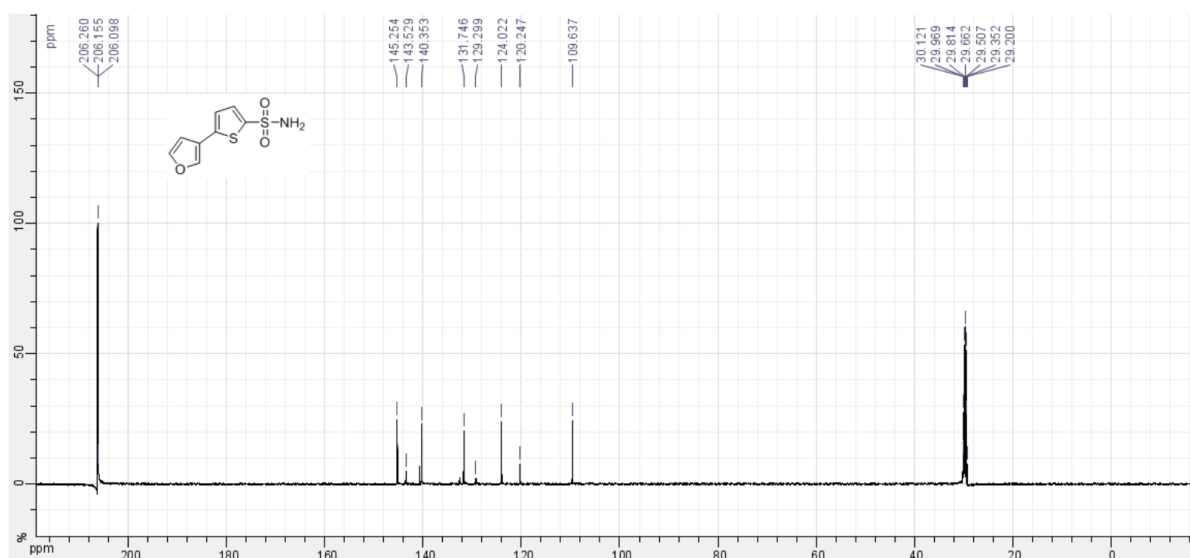
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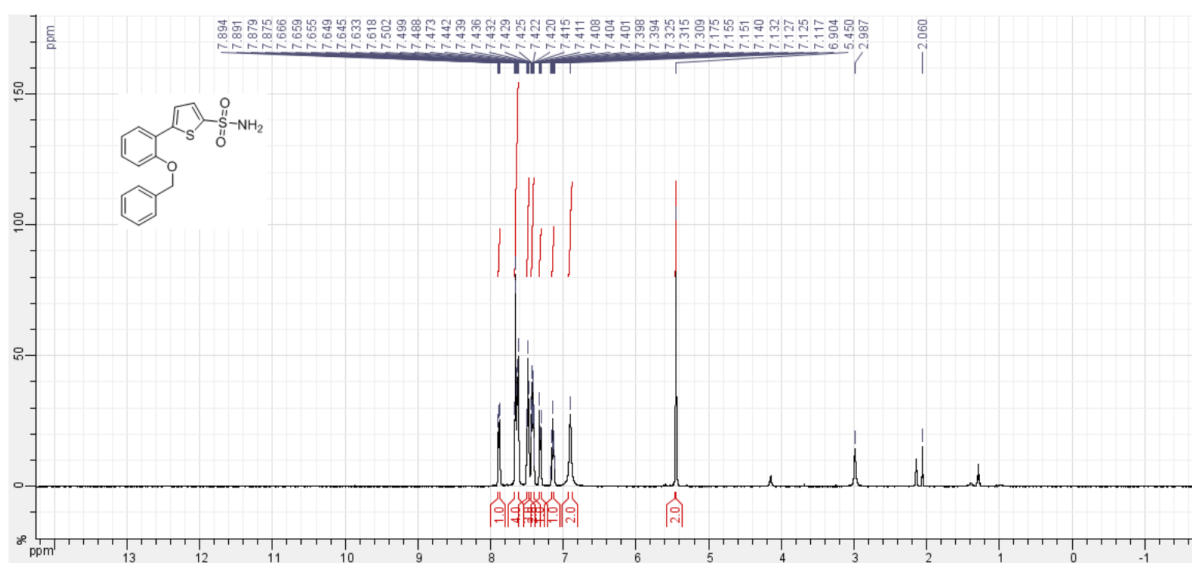


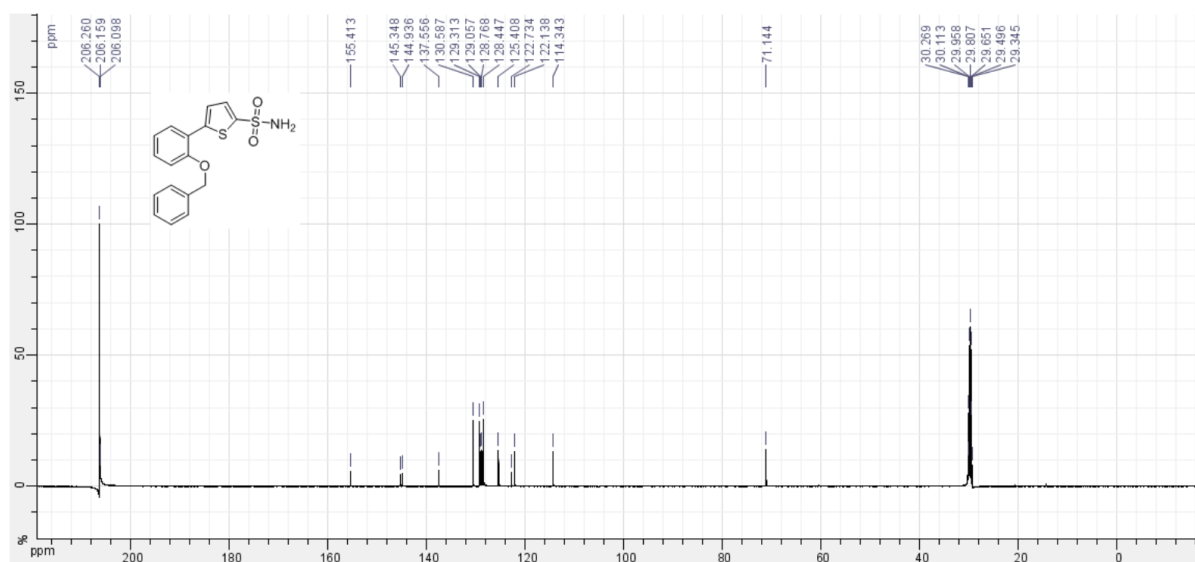
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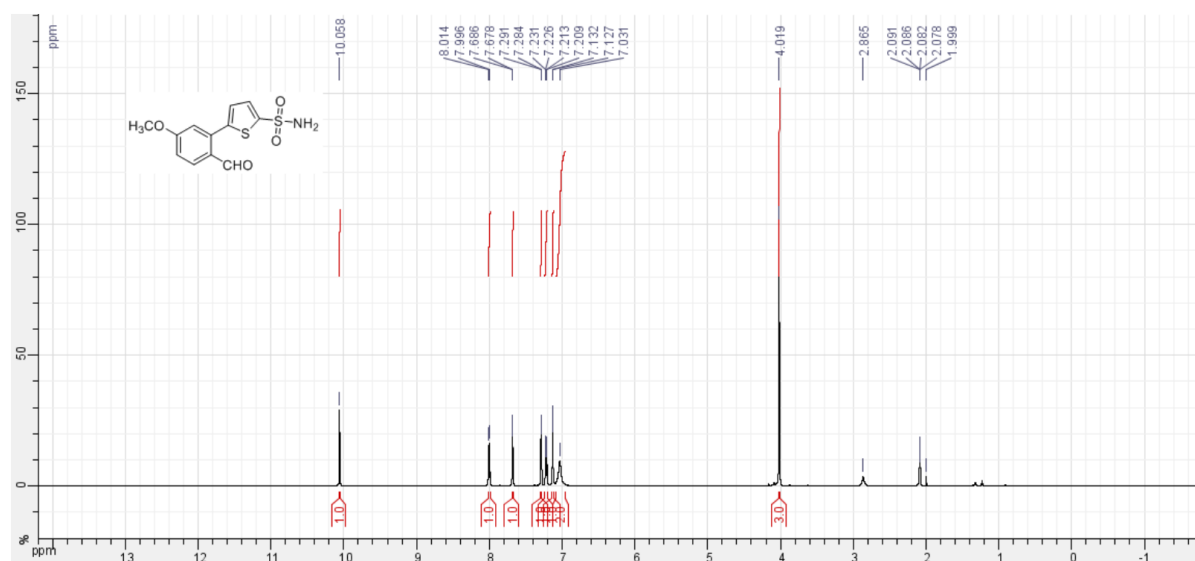


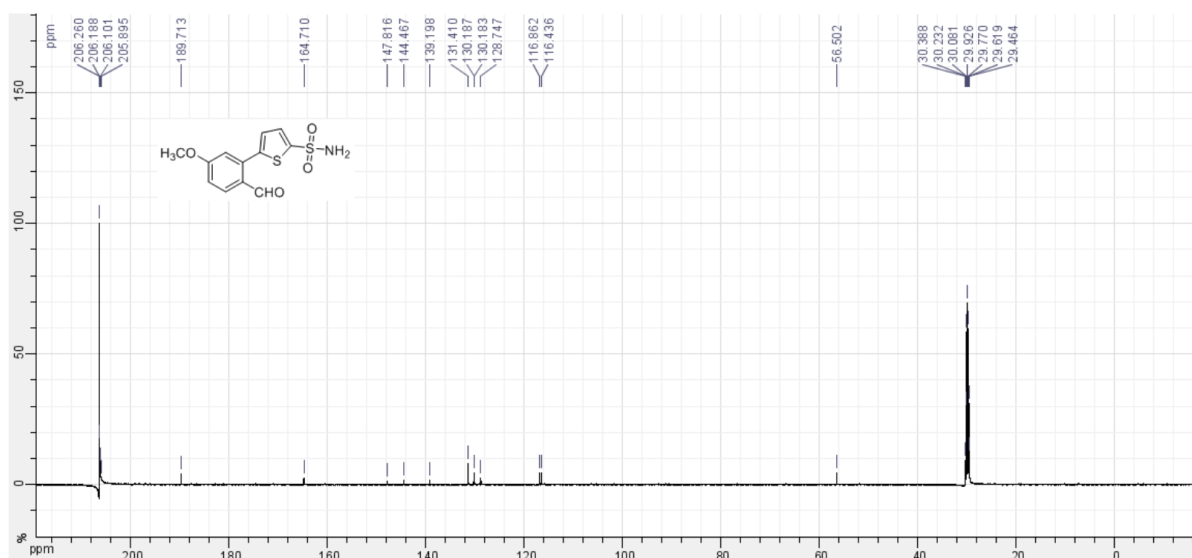
5-(2-(Benzyloxy)phenyl)thienyl-2-sulfonamide (**3n**)





5-(2-Formyl-5-methoxyphenyl)thienyl-2-sulfonamide (**3o**)





5-(5-Methylfuran-2-yl)thienyl-2-sulfonamide (**3p**)

