

Benzo[*d*]imidazole Anchored Oxadiazole Derivatives: Synthesis, Characterization, Biological Evaluation, *In Silico* docking and ADME-T analysis

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Antimicrobial activity

In vitro antimicrobial activity was examined for tested samples. The bacteria used in this study were Gram-positive bacterial strains (*Streptococcus pyogenes* ATCC 19615, *Staphylococcus epidermidis* ATCC 12228, and *Staphylococcus aureus* ATCC 29213), Gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, and *Pseudomonas aeruginosa* ATCC 27853). Microorganisms were provided by Microbiology laboratory of Global Hospital, Hyderabad. All strains were tested for purity by standard microbiological methods. The bacterial stock cultures were maintained on Mueller Hinton Agar (MHA) slants and stored at 4°C.

1.2. Disc-diffusion method

Antibacterial tests were carried out by disc diffusion method using 100 μ L of suspension containing 10^8 CFU/mL of bacteria, and 10^6 CFU/mL of yeast spread on MuellerHinton agar, Sabourand Dextrose Agar (SDA), and Potato Dextrose Agar (PDA) medium, respectively. The discs (6 mm in diameter) were impregnated with 15 μ L of each compound (100 mg/disc) at the concentration of 20 mg/mL and placed on the inoculated agar. DMSO impregnated discs were used as negative controls. Gemifloxacin (10 mg/disc), were used as positive reference standard to determine the sensitivity of one strain in each microbial species tested. The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against all the tested microorganisms. Each assay in this experiment was repeated thrice.

1.3. Microwell dilution assay

The minimal inhibition concentration (MIC) values were also studied for the bacterial strains which were determined as sensitive to in microwell dilution method. The inoculate of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Compounds **8a-8k** dissolved in 10% dimethylsulfoxide were first diluted to the highest concentration (100 mg/mL) to be tested, and then serial two fold dilutions were made in a concentration range from 10 to 100 mg/mL in 10 mL sterile test tubes containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 μ L of nutrient broth and 5 μ L of the inoculum. A 100 μ L **8a-8k** initially prepared at the concentration of 500 mg/mL was added into the first wells. Then, 100 μ L from their serial dilutions was transferred into six consecutive wells. Maxipime (BristoleMyers Squibb) at the concentration range of 100-10 mg/mL was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by plating 5 μ L samples from clear wells on nutrient agar medium. The compounds tested in this study was screened three times against each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms

2. Docking protocol

The in-silico molecular docking study was carried out using the Autodock 4.2. Protein preparation was carried out by AutodockTools. Ligand preparation and grid generation were carried out by Autodock 4.2. Receptor-ligand docking was carried out by Autodock.

2.1. Selection of target protein and preparation of protein target structure

The Crystal “Crystal Structure of *E. coli* Topoisomerase IV co-complexed with inhibitor”, whose PDB ID is 3FV5, & “Crystal Structure of Staph ParE 24kDa in complex with Novobiocin” whose PDB ID is 4URN was retrieved from the PDB (Protein Data Bank) and used for molecular docking. The molecular weight of the protein is 22321.15 kDa and 3FV5 seems to have a 2.00 Å resolution and one 190-amino-acid-residue protein of chain A. The molecular weight of the protein is 24759.73 kDa. AutodockTools (ADT) 1.5.6 was used to prepare the input files. Hetero atom i.e., Novobiocin have been removed completely, and also removed water molecules.

2.2. Selection and Preparation of ligand molecules

One synthesized molecule used in this study. The molecule was sketched in Tripos sybyl6.7 and minimized by using *Gasteiger-Hückel charges* and saved it in .mol2 format. All Ligands being uploaded individually in .mol2 format into the AutodockTools, and torsion angles were set and saved it in PDBQT format.

2.3. Molecular docking Analysis

The proteins and ligand were uploaded individually and removed water molecules and added all Hydrogens. The standard docking procedure was employed for a rigid protein and a flexible ligand whose torsion angles were identified. Grid dimensions selected as 60, 60, and 60 points and the amino acids which are present in the active site were selected and centred the in x, y, and z directions was built with a grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energetic map.

Lamarckian genetic algorithm method was employed for docking simulations. At the end of docking, the best poses were analyzed for hydrogen bonding calculations using Autodock 4.2, Ligplot plus and Biovia Discovery studio visualizer from the estimated free energy of ligand binding ($\Delta G_{\text{binding}}$, kcal/mol), the inhibition constant (K_i) for each ligand was calculated.

Supporting (1H/13C, IR and HRMS) Spectral Information

Commercial suppliers [Sigma Aldrich & Avra] provided all of the chemicals, reagents, and solvents, which were used exactly as is without any additional purification. Reactions were seen on TLC-MERCK pre-coated silica gel 60-F254 (0.5 mm) aluminum plates when exposed to UV light. The chemicals were purified using column chromatography with 60–120 mesh silica. Using tetramethyl silane (TMS) as the internal standard and samples prepared in either CDCl₃ or DMSO-d₆, ¹H and ¹³C NMR spectra were recorded using the Bruker Avance 500

MHz spectrometer. Chemical shifts in ppm are reported using TMS ($\delta 0.00$ for ^1H NMR and ^{13}C NMR) or NMR recording solvents like CDCl_3 ($\delta 7.26$ for ^1H NMR and 77.2 ^{13}C NMR) or $\text{DMSO}-d_6$ ($\delta 2.50$ for ^1H NMR and 39.5 for ^{13}C NMR). The spin multiplicities for ^1H NMR are s (singlet), brs (wide singlet), d (doublet), dd (double doublet), t (triplet), and m (multiplet). The unit of measurement for coupling constants is hertz (Hz). HRMS was recorded using electrospray ionization (ESI) procedures at 70 eV using an Agilent quadrupole-time-of-flight (QTOF) mass spectrometer 6540 series equipment. The Stuart R SMP30 device was used to measure the melting point.

Scheme 1. Synthesis of benzo[*d*]imidazole-substituted oxadiazole derivatives (**8a–8k**)

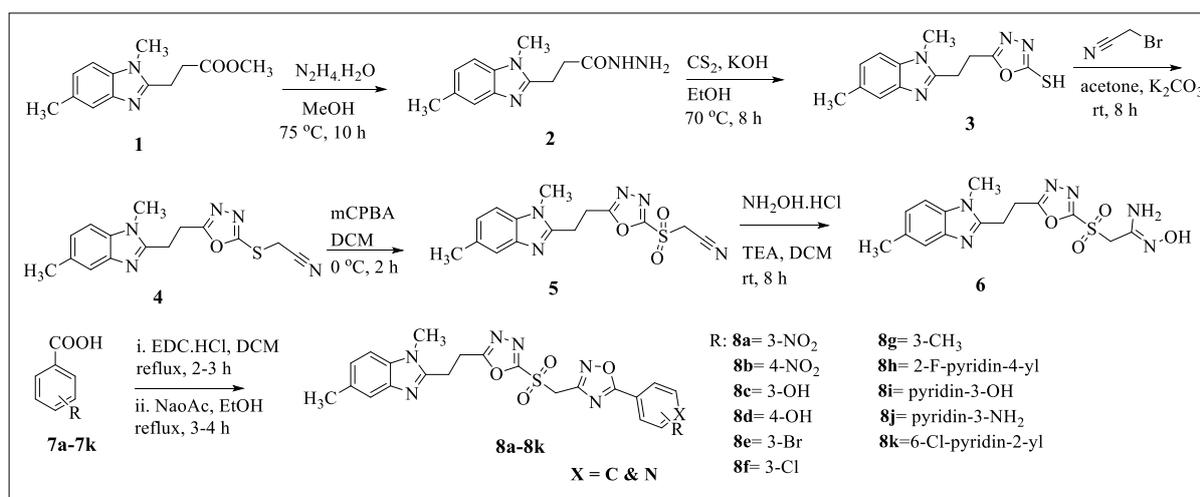
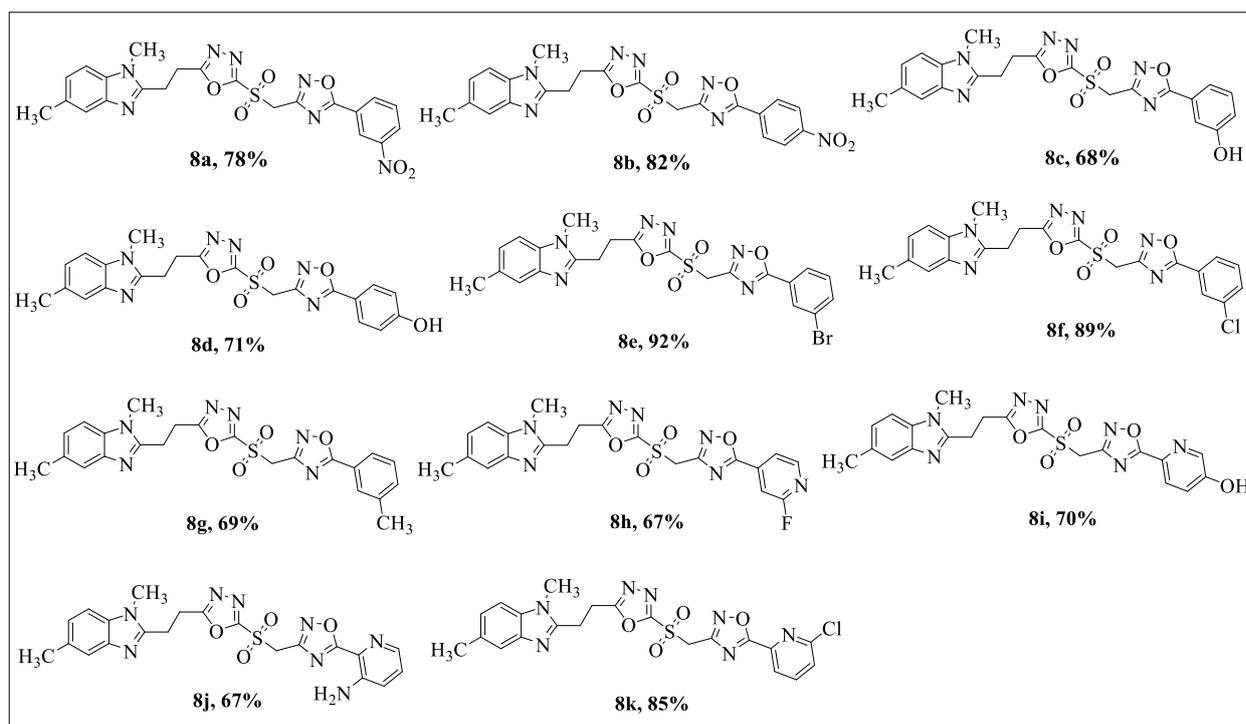


Chart 1. Oxadiazole scaffolds that have been synthesized and their yield percentages



Synthesis of 3-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)propane hydrazide (2)

10.77 mmol of ester (**1**, 2.5 g) was dissolved in 25 mL of methanol for the synthesis of hydrazide (**2**). 21.55 mmol of hydrazine hydrate (1.07 g) was then added dropwise to the reaction mixture and refluxed at 75 °C for around 10 hours while being continuously stirred. TLC was used to observe reaction completion (EtOAc: Hexane (2:8)). To acquire solid goods, surplus solvent was evaporated. The product was then cleaned with water to get rid of extra hydrazine hydrate, dried, and then recrystallized with ethanol to get a pure product.

3.3. Synthesis of 5-(2-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)ethyl)-1,3,4-oxadiazole-2-thiol (3)

Potassium hydroxide (18.9 mmol, 1.06 g) was added while stirring at room temperature after 9.48 mmol of hydrazide **2** (2.2 g) had been dissolved in 20 mL of ethanol. After 30 minutes, 18.9 mmol of carbon disulphide (1.44 g) was added to the reaction mixture, and for an additional 8 hours, the reaction was continually refluxed. TLC was used to track the progress and completion of the reaction using a 2:8 ethyl acetate-hexane solvent solution. Following completion, the mixture was quenched with crushed ice and neutralized with diluted HCl. The resultant residue was captured via filtering. This product was further purified by recrystallization in ethanol, yielding a 90% yield of chalcone **3** as a white solid.

3.4. Synthesis of 2-((5-(2-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)thio)acetonitrile (4)

2-Bromoacetonitrile (10.2 mmol) was added dropwise to the reaction mixture after 1,3,4-oxadiazole-2-thiol (**3**) (7.29 mmol) and K₂CO₃ (10.9 mmol) were combined in 25 mL of acetone and swirled for around 30 minutes. After that, the reaction was monitored for eight hours at room temperature. Upon completion of the reaction (monitored by TLC; EtOAc: Hexane (2:8)), the reaction mixture was cooled and placed in 100 mL of ice-cold water. After filtering out the precipitates, they were washed, dried, and ethanol-recrystallized.

3.5. Synthesis of 2-((5-(2-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)acetonitrile (5)

A solution of **4** (1.5 g, 4.80 mmol) in DCM (10 mL) was mixed with a solution of m-CPBA (1.24 g, 7.21 mmol) in 10 mL of DCM. After cooling to 0 °C and stirring the reaction for 30 minutes, the resultant liquid was stirred for two hours. After diluting the mixture with saturated NaHCO₃, DCM was used to extract it. To obtain crude sulfonyl compound, the combined DCM extracts were dried (MgSO₄), washed with 50 mL of brine solution, and then concentrated. Pure sulfonyl was obtained as a colorless oil by flash chromatography on silica gel (10:1 hexanes/EtOAc) in 85% of cases.

3.6. Synthesis of (Z)-2-((5-(2-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)-N'-hydroxy acetimidamide(6)

After dissolving compound **5** (1.3 g, 3.77 mmol) in 15 mL of dichloromethane, TEA (0.7 g, 6.80 mmol) was added and agitated for 10 minutes at 0 °C in an ice bath. The reaction mixture was stirred for eight hours at ambient temperature after the hydrazine hydrate was gradually added for thirty minutes. Following ethyl acetate extraction, quenching with saturated ammonium chloride solution, and washing with water (100 mL) and brine (25 mL) in that order, TLC was used to track the reaction's progress. 83% of **6** was obtained as an orange-red solid after being dried with anhydrous Na₂SO₄, concentrated, refined using a silica gel column, and eluted with EtOAc:hexane (2:8).

3.7. General procedure for the synthesis of 8a-8k

To make the EDC.HCl(1.02 g, 4.35mmol)reagentwas mixed with 10 mL of DCM and stirred for 10 minutes at 0 °C. After dissolving compound **6** (1.0 g, 2.64 mmol) in 10 mL of DCM and letting it cool to 0 °C, the resultant 3-nitrobenzoic acid (0.69 g, 3.99 mmol) is added dropwise. After that, the reaction is agitated at 65 °C for 2 hours. Then, the obtained acylated intermediatewasrefluxing with sodium acetate (0.97 g, 3.25 mmol) in the presence of ethanol for 3 h. After completion reaction solvent was distilled out then added 2N NaOH followed by extraction with ethyl acetate. Separated organic layer was distilled and into the crude was purified by column chromatography.This procedure was allowed for the production of final oxadiazole **8b–8k** with good yields (67–92%).

3.7.1 3-(((5-(2-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(3-nitrophenyl)-1,2,4-oxadiazole (**8a**)

Light brown solid; mp: 161–163 °C;IR (KBr, cm⁻¹): ν 3021.17 (=CH), 2900.87 (CH), 1595.08 (C=C), 1456.64 (CN), 1385.71 (NN), 1274.30 (CSC), 1159.24 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.20 (s, 3H, ArCH₃), 2.77 (t, *J* = 10.2 Hz, 2H, CH₂), 3.38 (t, *J* = 10.2 Hz, 2H, CH₂), 3.80 (s, 3H, NCH₃), 4.70 (s, 2H, CH₂), 7.56 (s, 1H, Ar-H), 7.67 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.71 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.76-7.86 (m, 2H, Ar-H), 8.01 (t, *J* = 10.0 Hz, 1H, Ar-H), 8.38 (d, *J* = 9.4 Hz, 1H, Ar-H), 9.15 (s, 1H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm:22.51, 30.10, 36.28, 59.82, 112.06, 125.07, 127.15, 128.02, 129.96, 131.51, 132.40, 132.81, 134.42, 135.04, 145.20, 152.87, 157.41, 164.28, 168.35, 177.24. *m/z*: 510.0491 [M+1]⁺. Found: C, 52.16; H, 3.88; N, 19.33; S, 6.38. C₂₂H₁₉N₇O₆S. Calculated, %: C, 51.86; H, 3.76; N, 19.24; S, 6.29.

3.7.2 3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (**8b**)

Light brown solid; mp: 153–155 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3166.01 (=CH), 2943.17, 2873.04 (CH), 1591.41 (C=C), 1453.31 (CN), 1370.54 (NN), 1268.93 (CSC), 1165.00 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.21 (s, 3H, ArCH₃), 3.10 (t, *J* = 10.2 Hz, 2H, CH₂), 3.70 (t, *J* = 10.2 Hz, 2H, CH₂), 4.12 (s, 3H, NCH₃), 4.90 (s, 2H, CH₂), 7.15 (s, 1H, Ar-H), 7.42 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.65 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.10 (d, *J* = 9.2 Hz, 2H, Ar-H), 8.61 (d, *J* = 9.2 Hz, 2H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 23.95, 30.61, 36.50, 59.91, 103.38, 112.65, 125.93, 127.91, 129.24, 132.14, 134.25, 134.69, 135.00, 137.06, 150.65, 154.82, 159.94, 164.60, 176.36. *m/z*: 510.0247 [M+1]⁺. Found: C, 51.86; H, 3.76; N, 19.24; S, 6.29. C₂₂H₁₉N₇O₆S. Calculated, %: C, 51.73; H, 3.65; N, 19.14; S, 6.18.

3.7.3 3-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)phenol (**8c**)

Off-white solid; mp: 190–192 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3416.51 (OH), 3096.53 (=CH), 2924.54, 2834.32 (CH), 1583.37 (C=C), 1447.54 (CN), 1364.21 (NN), 1258.46 (CSC), 1163.47 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.19 (s, 3H, ArCH₃), 2.72 (t, *J* = 10.2 Hz, 2H, CH₂), 3.30 (t, *J* = 10.2 Hz, 2H, CH₂), 4.01 (s, 3H, NCH₃), 4.78 (s, 2H, CH₂), 6.92 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 7.42–7.44 (m, 1H, Ar-H), 7.69 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.80 (t, *J* = 10.0 Hz, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 8.41 (d, *J* = 9.4 Hz, 1H, Ar-H), 9.68 (s, 1H, OH). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 24.30, 30.04, 36.53, 59.72, 112.85, 125.52, 127.51, 127.73, 128.35, 129.74, 129.98, 133.11, 134.35, 135.19, 137.00, 155.32, 164.50, 168.33, 173.18. *m/z*: 481.0573 [M+1]⁺. Found: C, 55.08; H, 4.31; N, 17.60; S, 6.79. C₂₂H₂₀N₆O₅S. Calculated, %: C, 54.99; H, 4.20; N, 17.49; S, 6.67.

3.7.4 4-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)phenol (**8d**)

Off-white solid; mp: 178–180 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3410.52 (OH), 3093.41 (=CH), 2841.52 (CH), 1575.01 (C=C), 1461.84 (CN), 1365.64 (NN), 1270.14 (CSC), 1157.71 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.76 (s, 3H, ArCH₃), 3.50 (t, *J* = 10.2 Hz, 2H, CH₂), 3.75 (t, *J* = 10.2 Hz, 2H, CH₂), 4.21 (s, 3H, NCH₃), 4.74 (s, 2H, CH₂), 6.75 (d, *J* = 9.2 Hz, 2H, Ar-H), 6.90 (s, 1H, Ar-H), 7.20 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.42 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.65 (d, *J* = 9.2 Hz, 2H, Ar-H), 9.49 (s, 1H, OH). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 21.82, 30.30, 36.13, 59.19, 104.61, 112.03, 112.96, 125.39, 127.10, 128.09, 129.60, 135.82, 137.16, 144.64, 155.41, 158.17, 164.88, 168.44. *m/z*: 481.9869 [M+1]⁺. Found: C, 54.99; H, 4.20; N, 17.49; S, 6.67. C₂₂H₂₀N₆O₅S. Calculated, %: C, 54.80; H, 4.10; N, 17.38; S, 6.56.

3.7.5 5-(3-bromophenyl)-3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazole (**8e**)

Reddish brown solid; mp: 192–194 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3198.22 (=CH), 2937.09, 2881.46 (CH), 1583.20 (C=C), 1454.06 (CN), 1227.58 (CSC), 1152.83 (COC), 781.92 (CBr). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.73 (s, 3H, ArCH₃), 3.05 (t, *J* = 10.2 Hz, 2H, CH₂), 3.76 (t, *J* = 10.2 Hz, 2H, CH₂), 4.09 (s, 3H, NCH₃), 4.65 (s, 2H, CH₂), 7.01 (t, *J* = 10.0 Hz, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 7.49 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.52 (m, 1H, Ar-H), 7.58 (m, 1H, Ar-H), 7.63 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.06 (d, *J* = 9.4 Hz, 1H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 25.30, 30.32, 35.63, 59.40, 112.75, 115.30, 126.97, 127.13, 127.42, 129.97, 131.80, 133.60, 134.56, 135.02, 145.08, 152.65, 155.11, 164.83, 168.92. *m/z*: 542.0561 [M+1]⁺, 543.0485 [M+2]⁺. Found: C, 48.68; H, 3.59; Br, 14.79; N, 15.55; S, 5.98. C₂₂H₁₉BrN₆O₄S. Calculated, %: C, 48.63; H, 3.52; Br, 14.70; N, 15.47; S, 5.90.

3.7.6 5-(3-chlorophenyl)-3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazole (**8f**)

Off-white solid, mp: 170–172 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3193.12 (=CH), 2972.68, 2841.39 (CH), 1594.27 (C=C), 1457.52 (CN), 1366.15 (NN), 1264.44 (CSC), 1168.13 (COC), 865.64 (CCl). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.12 (s, 3H, ArCH₃), 2.88 (t, *J* = 10.2 Hz, 2H, CH₂), 3.35 (t, *J* = 10.2 Hz, 2H, CH₂), 4.08 (s, 3H, NCH₃), 4.75 (s, 2H, CH₂), 6.81 (s, 1H, Ar-H), 7.31 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.62 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.80 (t, *J* = 10.0 Hz, 1H, Ar-H), 8.41 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.66 (s, 1H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 24.16, 32.50, 36.36, 59.24, 112.42, 114.37, 115.96, 116.10, 121.54, 122.95, 123.00, 126.91, 130.15, 131.74, 131.87, 143.31, 155.49, 158.74, 165.65, 169.18. *m/z*: 499.9658 [M+1]⁺, 501.9599 [M+3]⁺. Found: C, 52.99; H, 3.93; Cl, 7.19; N, 16.93; S, 6.52. C₂₂H₁₉ClN₆O₄S. Calculated, %: C, 52.96; H, 3.84; Cl, 7.11; N, 16.84; S, 6.43.

3.7.7.3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(*m*-tolyl)-1,2,4-oxadiazole (**8g**)

Gummy solid, mp: 150–152 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3137.43 (=CH), 2949.04, 2843.44 (CH), 1600.23 (C=C), 1463.94 (CN), 1366.11 (NN), 1233.35 (CSC), 1156.40 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.12 (s, 6H, ArCH₃), 2.92 (t, *J* = 10.2 Hz, 2H, CH₂), 3.47 (t, *J* = 10.2 Hz, 2H, CH₂), 4.05 (s, 3H, NCH₃), 4.73 (s, 2H, CH₂), 6.68 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 7.45 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.60–7.70 (m, 2H, Ar-H), 7.93 (s, 1H, Ar-H), 8.07 (d, *J* = 9.2 Hz, 2H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 22.03, 26.22, 33.69, 36.25, 60.60, 111.44, 114.81, 116.32, 118.75, 120.76, 128.67, 130.26, 130.65, 131.04, 131.72, 141.96, 144.97, 156.68, 158.65, 164.17, 168.71. *m/z*: 479.0746 [M+1]⁺. Found: C, 57.81; H, 4.70; N, 17.63; S, 6.81. C₂₃H₂₂N₆O₄S. Calculated, %: C, 57.73; H, 4.63; N, 17.56; S, 6.70.

3.7.8 3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(2-fluoropyridin-4-yl)-1,2,4-oxadiazole (**8h**)

Brownish solid, mp: 160–162 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3137.89 (=CH), 2919.84, 2864.55 (CH), 1595.13 (C=C), 1449.69 (CN), 1358.98 (NN), 1210.82 (CSC), 1081.21 (COC), 1020.33 (CF). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.08 (s, 3H, ArCH₃), 2.68 (t, *J* = 10.2 Hz, 2H, CH₂), 3.35 (t, *J* = 10.2 Hz, 2H, CH₂), 4.22 (s, 3H, NCH₃), 4.72 (s, 2H, CH₂), 7.06 (s, 1H, Ar-H), 7.30 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.70 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.85 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.53 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.72 (d, *J* = 9.4 Hz, 1H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 22.63, 32.16, 36.52, 59.19, 102.72, 112.81, 120.25, 125.37, 127.94, 129.52, 131.06, 135.41, 136.03, 137.46, 153.12, 159.53, 164.10, 168.86, 172.12. *m/z*: 484.0750[M+1]⁺. Found: C, 52.17; H, 3.75; F, 3.93; N, 20.28; S, 6.63. C₂₁H₁₈FN₇O₄S. Calculated, %: C, 52.06; H, 3.64; F, 3.83; N, 20.17; S, 6.52.

3.7.9 6-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)pyridin-3-ol (**8i**)

Brownish solid, mp: 196–198 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3366.00 (NH), 3076.36 (=CH), 2943.17, 2878.01 (CH), 1591.44 (C=C), 1433.21 (CN), 1370.54 (NN), 1268.94 (CSC), 1165.09 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.19 (s, 3H, ArCH₃), 2.73 (t, *J* = 10.2 Hz, 2H, CH₂), 3.30 (t, *J* = 10.2 Hz, 2H, CH₂), 3.58 (s, 3H, NCH₃), 4.54 (s, 2H, CH₂), 7.25 (s, 1H, Ar-H), 7.52 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.87 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.24 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.60 (d, *J* = 9.4 Hz, 1H, Ar-H), 8.82 (s, 1H, Ar-H), 10.35 (s, 1H, OH). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 24.35, 30.51, 36.54, 59.48, 112.77, 125.62, 127.15, 128.01, 129.95, 130.06, 135.54, 137.19, 139.70, 145.43, 150.35, 155.02, 164.73, 168.34. *m/z*: 482.1220[M+1]⁺. Found: C, 52.39; H, 3.98; N, 20.36; S, 6.66. C₂₁H₁₉N₇O₅S. Calculated, %: C, 52.30; H, 3.90; N, 20.25; S, 6.58.

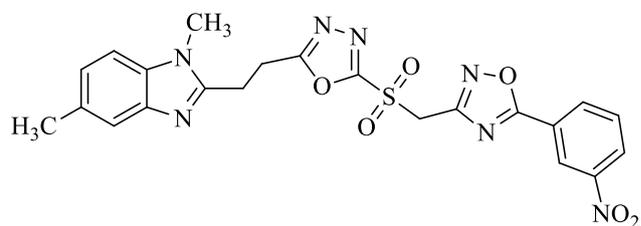
3.7.10 2-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)pyridin-3-amine (**8j**)

Brownish solid, mp: 183–185 °C; IR (KBr, cm⁻¹): $\tilde{\nu}$ 3235.42, 3059.39 (=CH), 2962.14 (CH), 1596.61 (C=C), 1467.08 (CN), 1360.50 (NN), 1265.92 (CSC), 1162.04 (COC). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm: 2.76 (s, 3H, ArCH₃), 3.17 (t, *J* = 10.2 Hz, 2H, CH₂), 3.55 (t, *J* = 10.2 Hz, 2H, CH₂), 4.18 (s, 3H, NCH₃), 4.81 (s, 2H, CH₂), 5.52 (s, 2H, NH₂), 7.22 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.45 (d, *J* = 9.4 Hz, 1H, Ar-H), 7.67 (m, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 8.10 (t, *J* = 10.0 Hz, 1H, Ar-H), 8.25 (d, *J* = 9.4 Hz, 1H, Ar-H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ ppm: 23.32, 33.87, 37.60, 59.25, 113.81, 116.67, 121.13, 127.11, 128.55, 129.70, 130.64, 130.89, 131.62, 137.37, 142.82, 144.16, 156.67, 158.84, 165.08, 168.85. *m/z*: 481.9604[M+1]⁺.

Found: C, 52.56; H, 4.32; N, 23.41; S, 6.78. $C_{21}H_{20}N_8O_4S$. Calculated, %: C, 52.49; H, 4.20; N, 23.32; S, 6.67.

3.7.11 5-(6-chloropyridin-2-yl)-3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazole (**8k**)

Off-white solid, mp: 180–182 °C; IR (KBr, cm^{-1}): ν 3138.57 (=CH), 2969.74 (CH), 2930.50, 1509.91 (C=C), 1466.53 (CN), 1398.19 (NN), 1268.60 (CSC), 1163.22 (COC), 860.12 (CCl). 1H NMR (500 MHz, DMSO- d_6) δ ppm: 2.57 (s, 3H, ArCH₃), 2.93 (t, $J = 10.2$ Hz, 2H, CH₂), 3.41 (t, $J = 10.2$ Hz, 2H, CH₂), 4.20 (s, 3H, NCH₃), 5.02 (s, 2H, CH₂), 6.67 (s, 1H, Ar-H), 6.93 (d, $J = 9.4$ Hz, 1H, Ar-H), 7.15-7.22 (m, 1H, Ar-H), 7.41 (d, $J = 9.4$ Hz, 1H, Ar-H), 8.02 (t, $J = 10.0$ Hz, 1H, Ar-H), 8.30 (d, $J = 9.4$ Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, DMSO- d_6) δ ppm: 24.97, 30.60, 36.11, 58.65, 112.68, 125.75, 127.12, 127.93, 128.17, 129.94, 130.12, 132.21, 135.15, 137.03, 152.97, 155.38, 164.51, 168.36, 173.65. m/z : 500.0751 [M+1]⁺, 501.0781 [M+2]⁺. Found: C, 50.45; H, 3.63; Cl, 7.09; N, 19.61; S, 6.41. $C_{21}H_{18}ClN_7O_4S$. Calculated, %: C, 50.34; H, 3.53; Cl, 7.01; N, 19.53; S, 6.32.



Compound 8a

3-(((5-(2-(1,5-dimethyl-1H-benzo[d]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(3-nitrophenyl)-1,2,4-oxadiazole

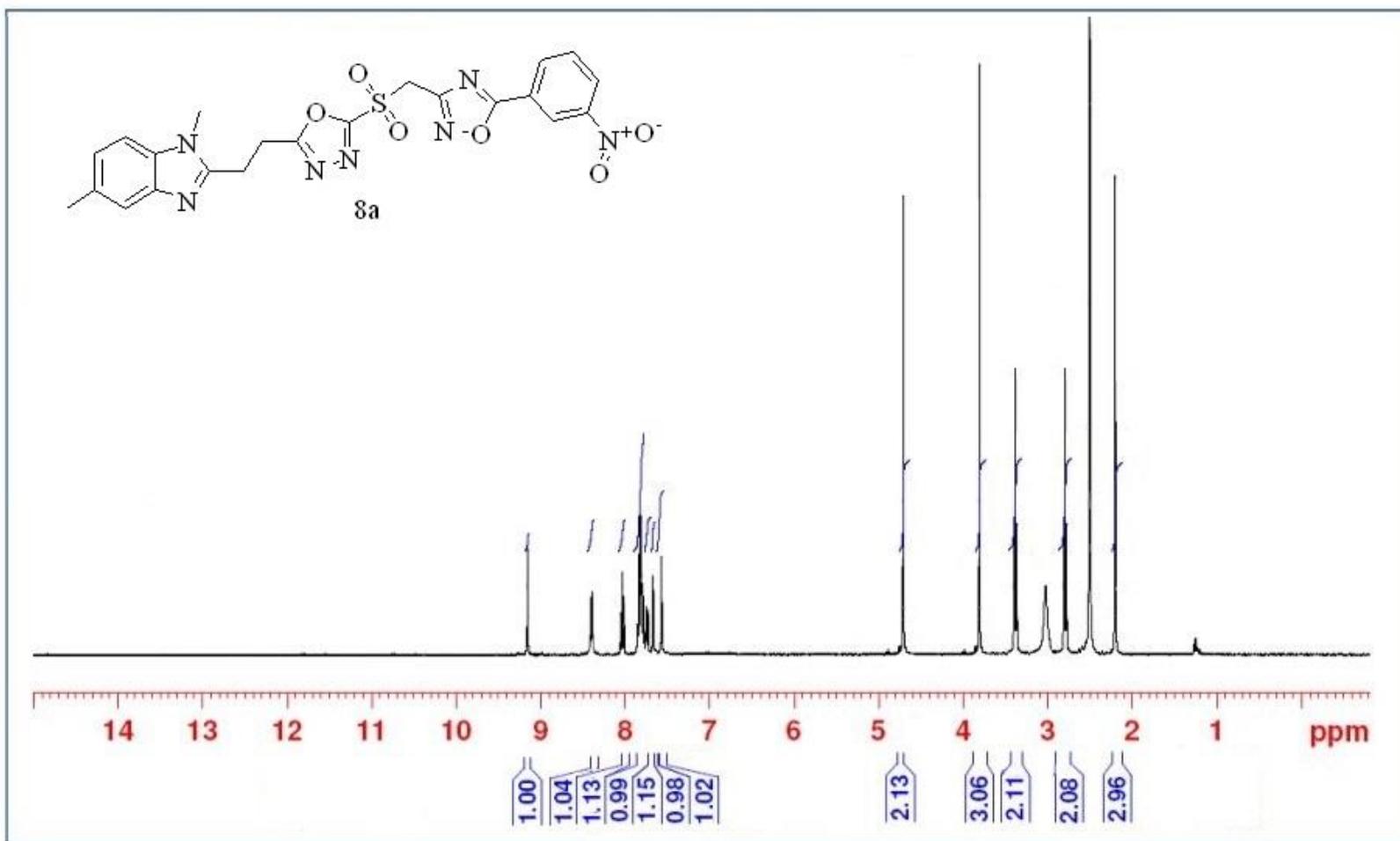
Chemical Formula: $C_{22}H_{19}N_7O_6S$

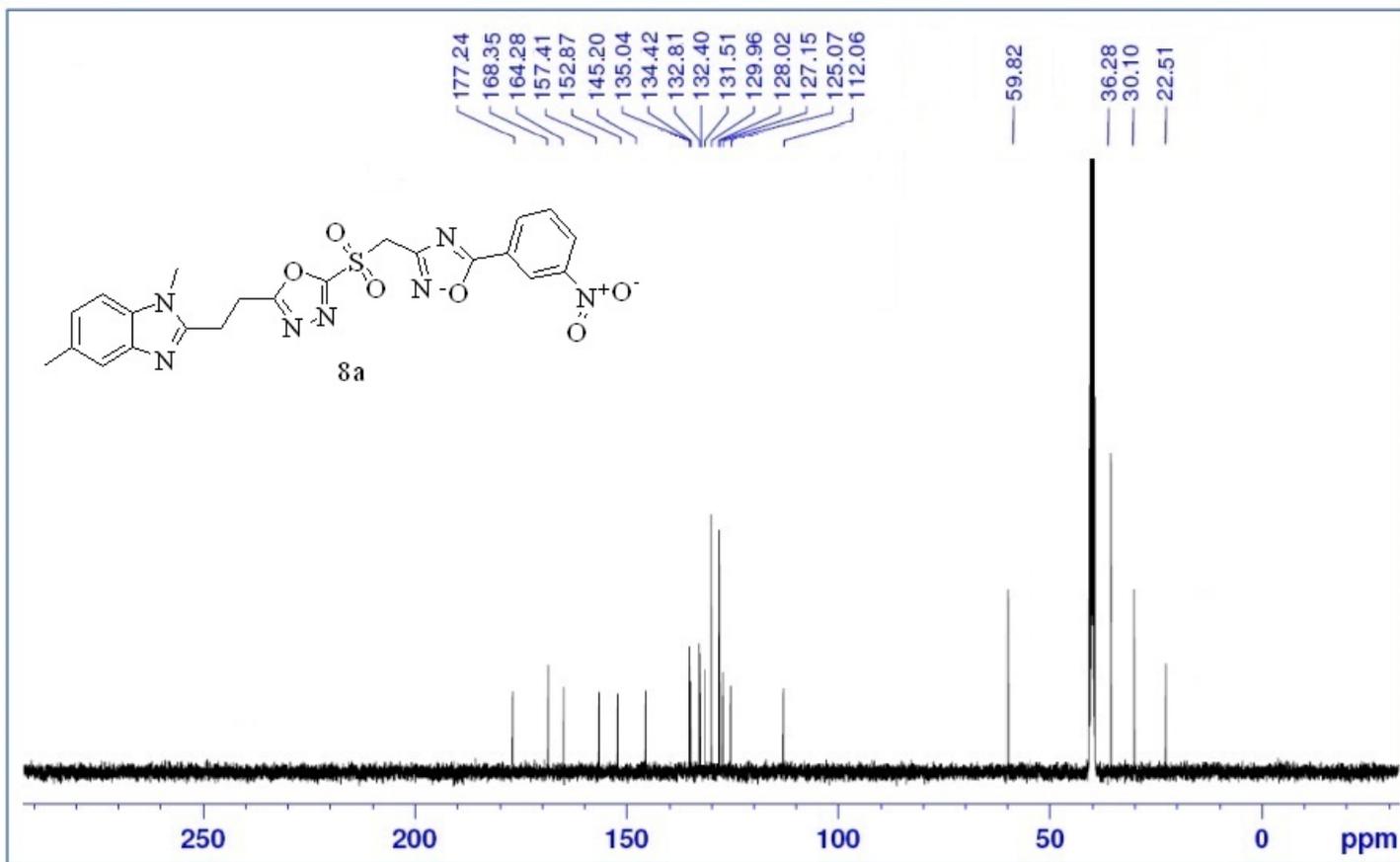
Exact Mass: 509.1118

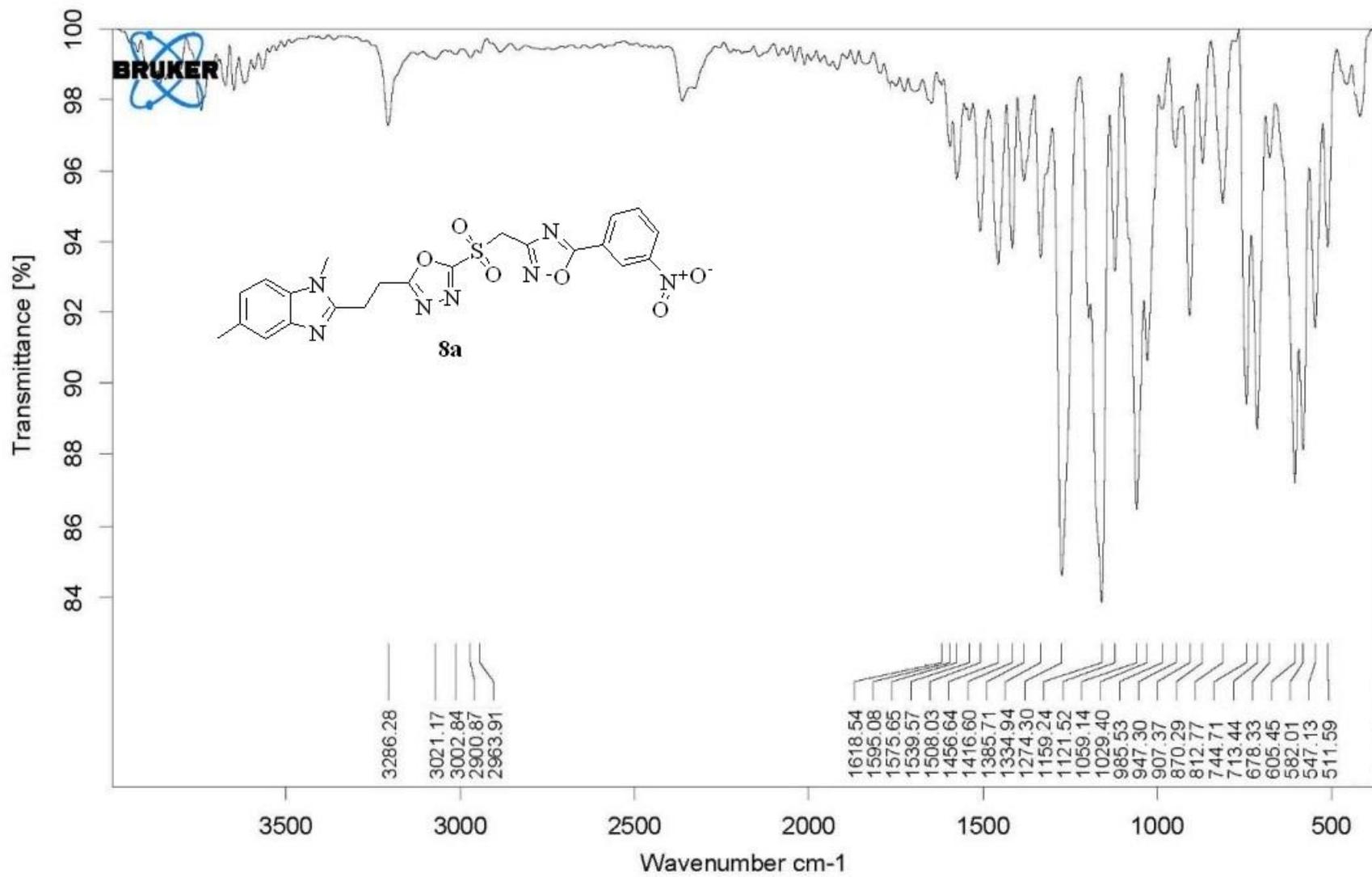
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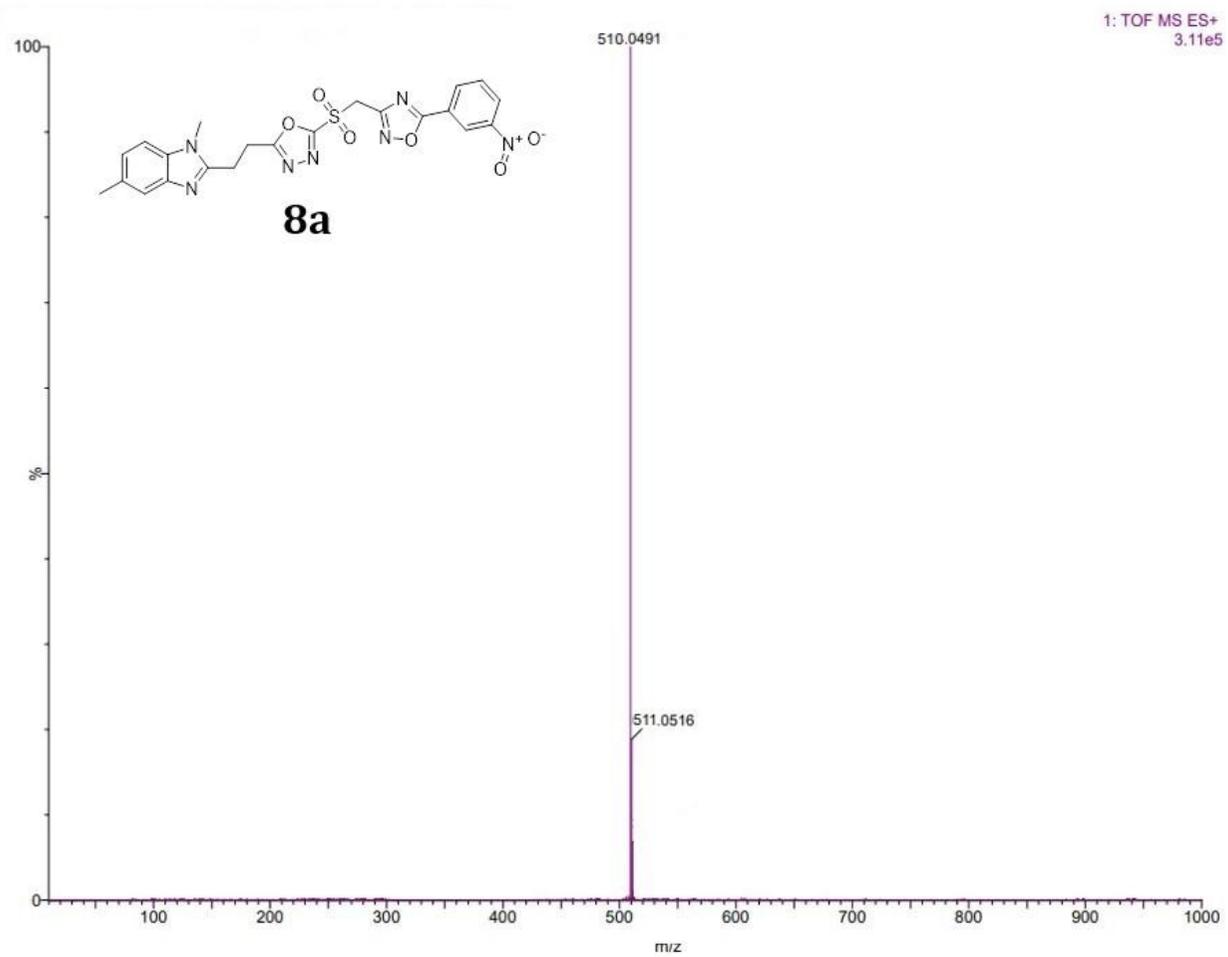
m/z: 509.1118 (100.0%), 510.1151 (23.8%), 511.1075 (4.5%), 511.1185 (2.7%), 510.1088 (2.6%), 511.1160 (1.2%), 512.1109 (1.1%)

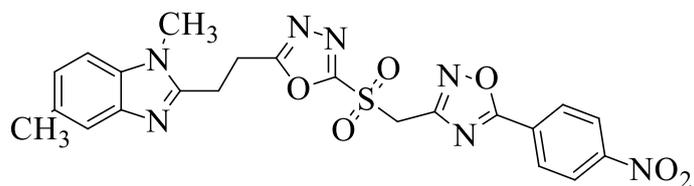
Elemental Analysis: C, 51.86; H, 3.76; N, 19.24; O, 18.84; S, 6.29











Compound 8b

3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(4-nitrophenyl)-1,2,4-oxadiazole

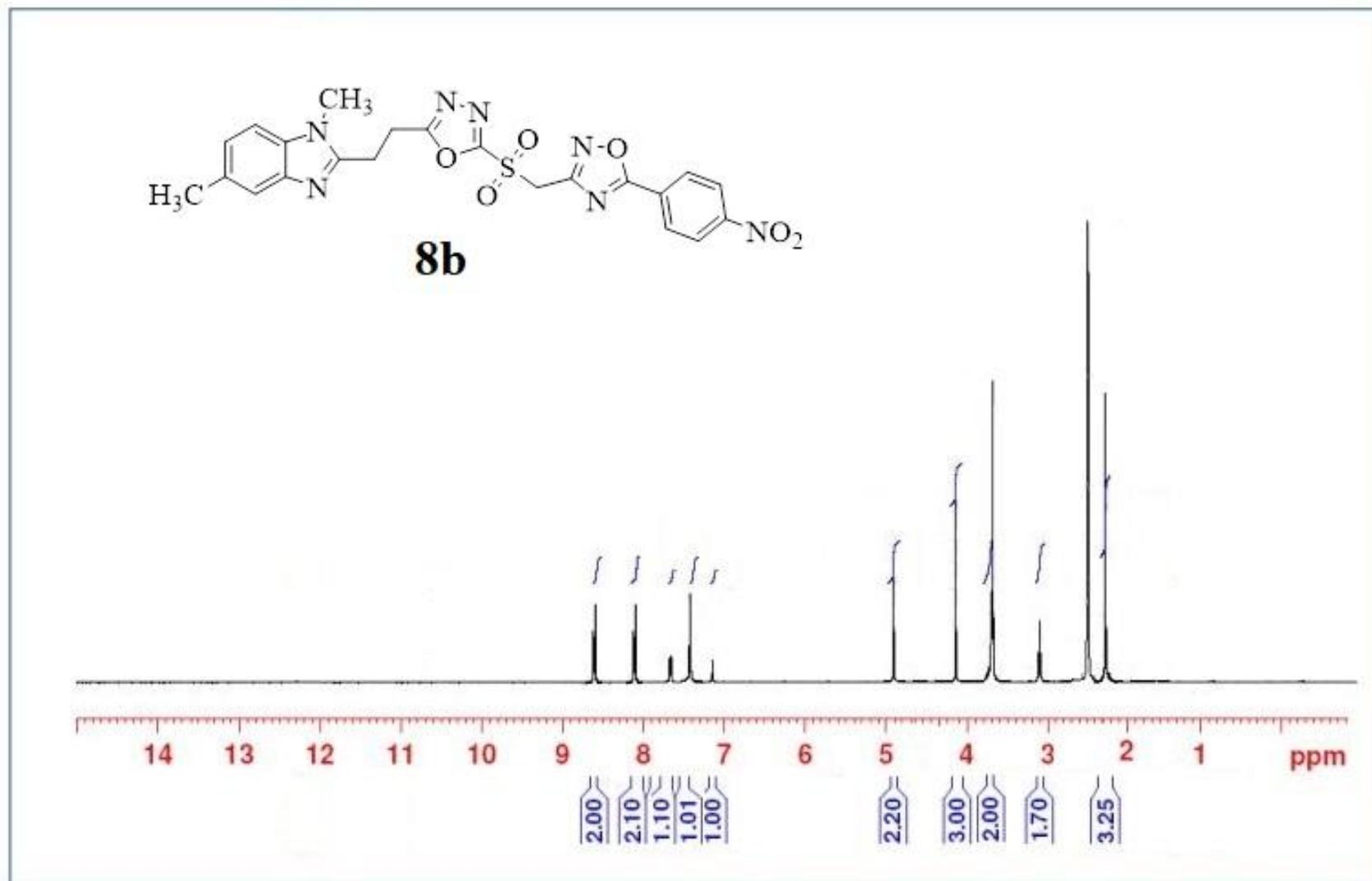
Chemical Formula: C₂₂H₁₉N₇O₆S

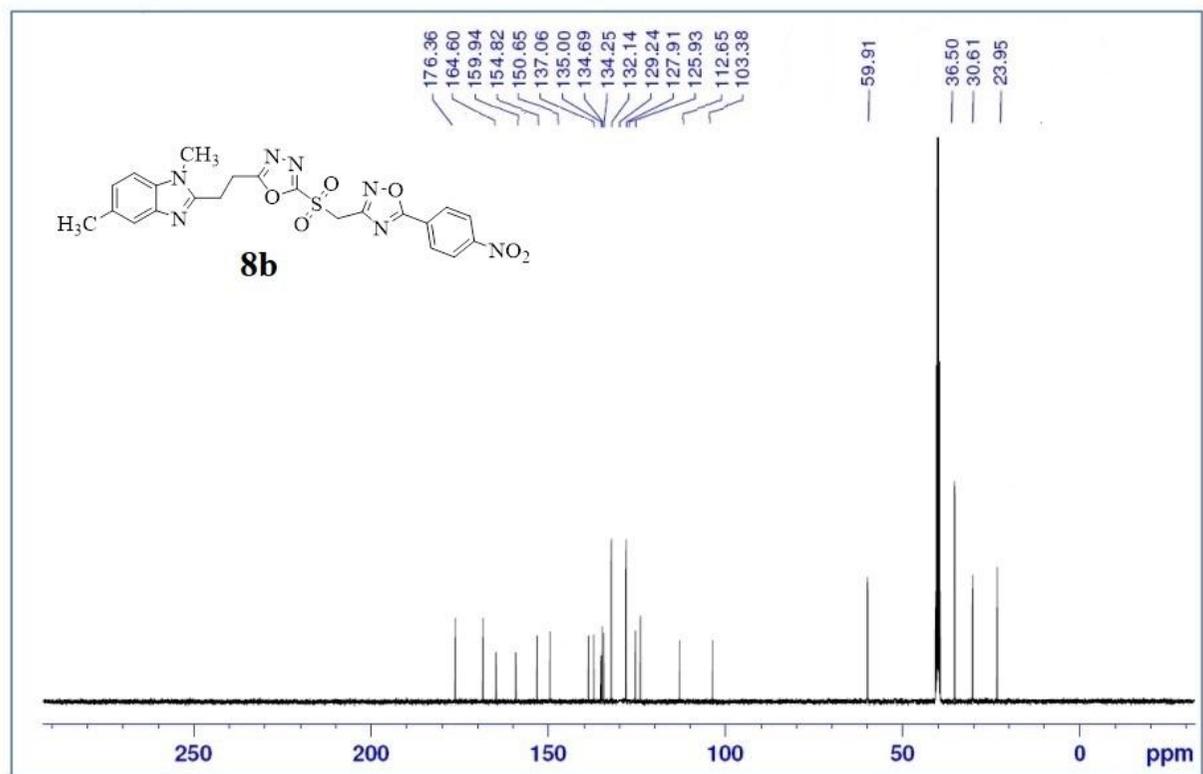
Exact Mass: 509.1118

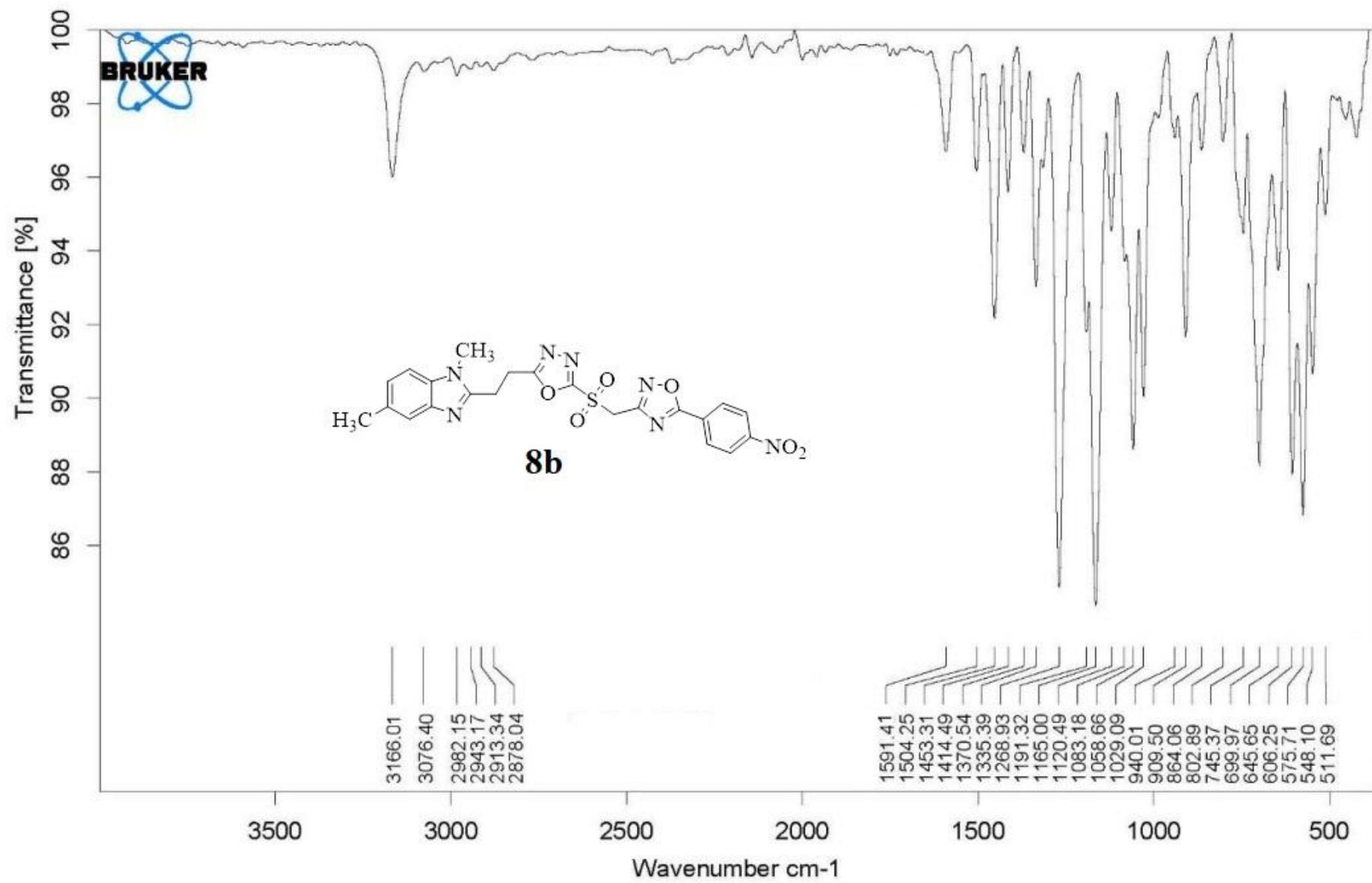
Molecular Weight: 509.4946

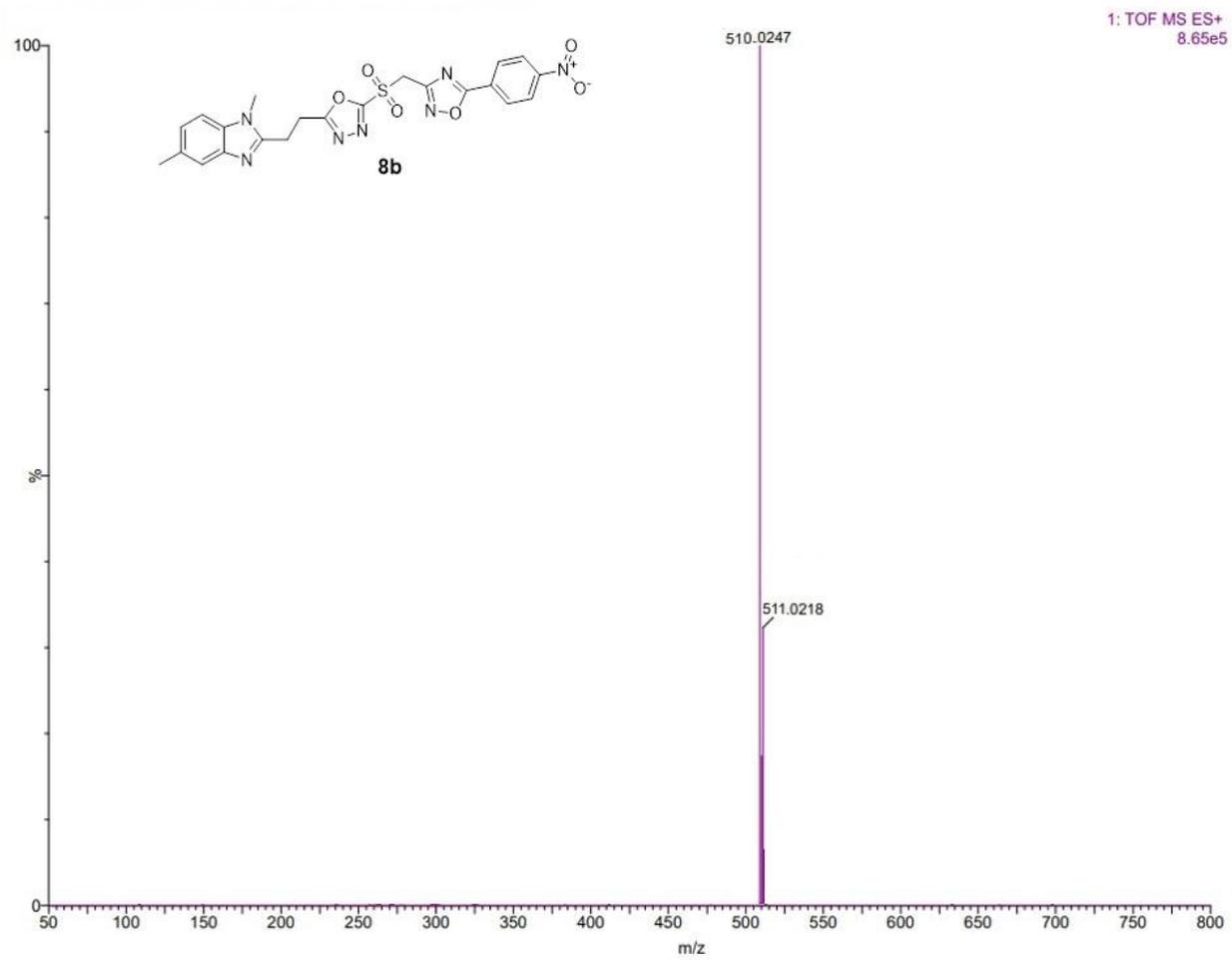
m/z: 509.1118 (100.0%), 510.1151 (23.8%), 511.1075 (4.5%), 511.1185 (2.7%),
510.1088 (2.6%), 511.1160 (1.2%), 512.1109 (1.1%)

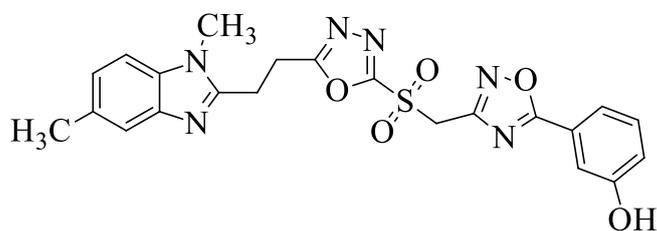
Elemental Analysis: C, 51.86; H, 3.76; N, 19.24; O, 18.84; S, 6.29











Compound 8c

3-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)phenol

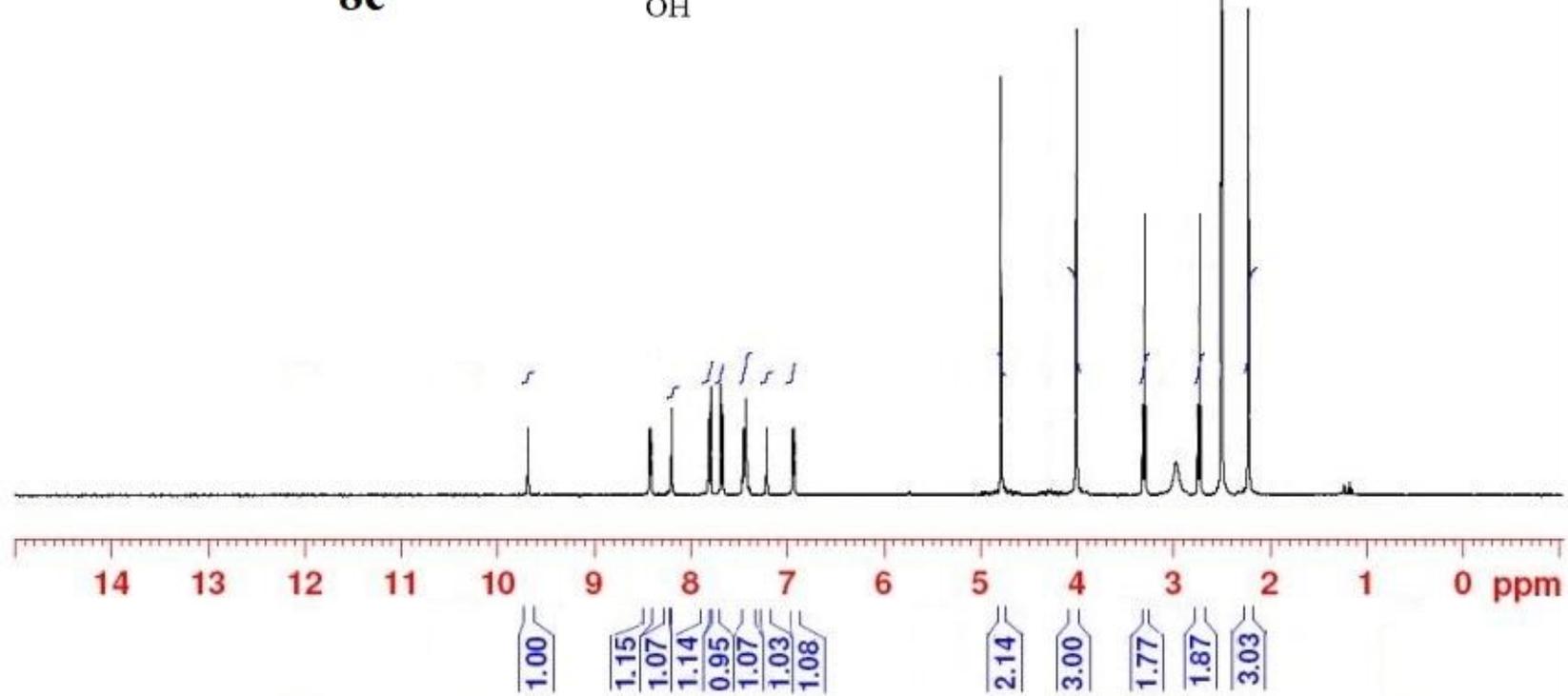
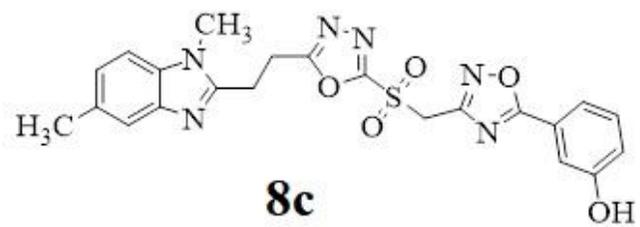
Chemical Formula: C₂₂H₂₀N₆O₅S

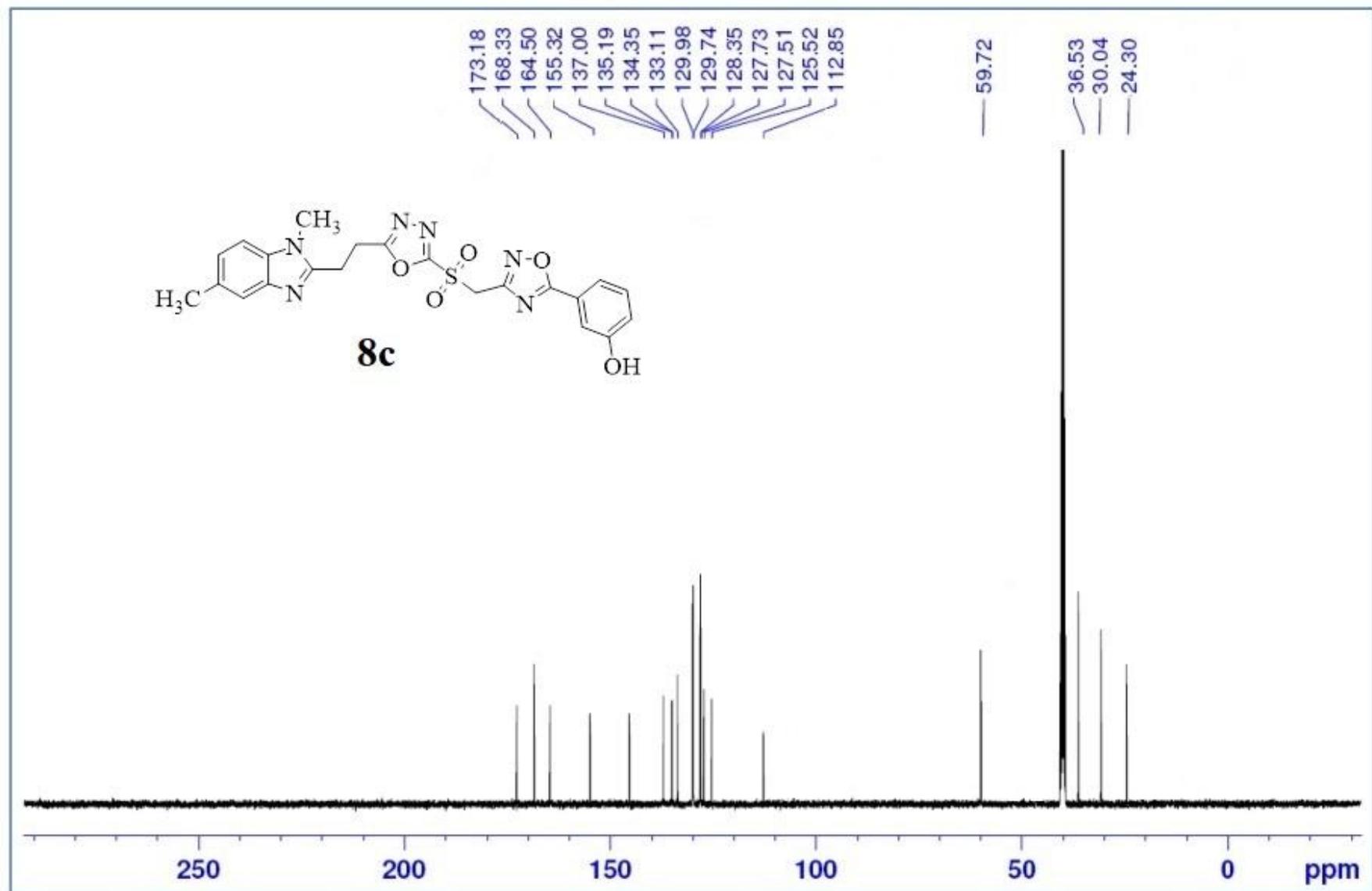
Exact Mass: 480.1216

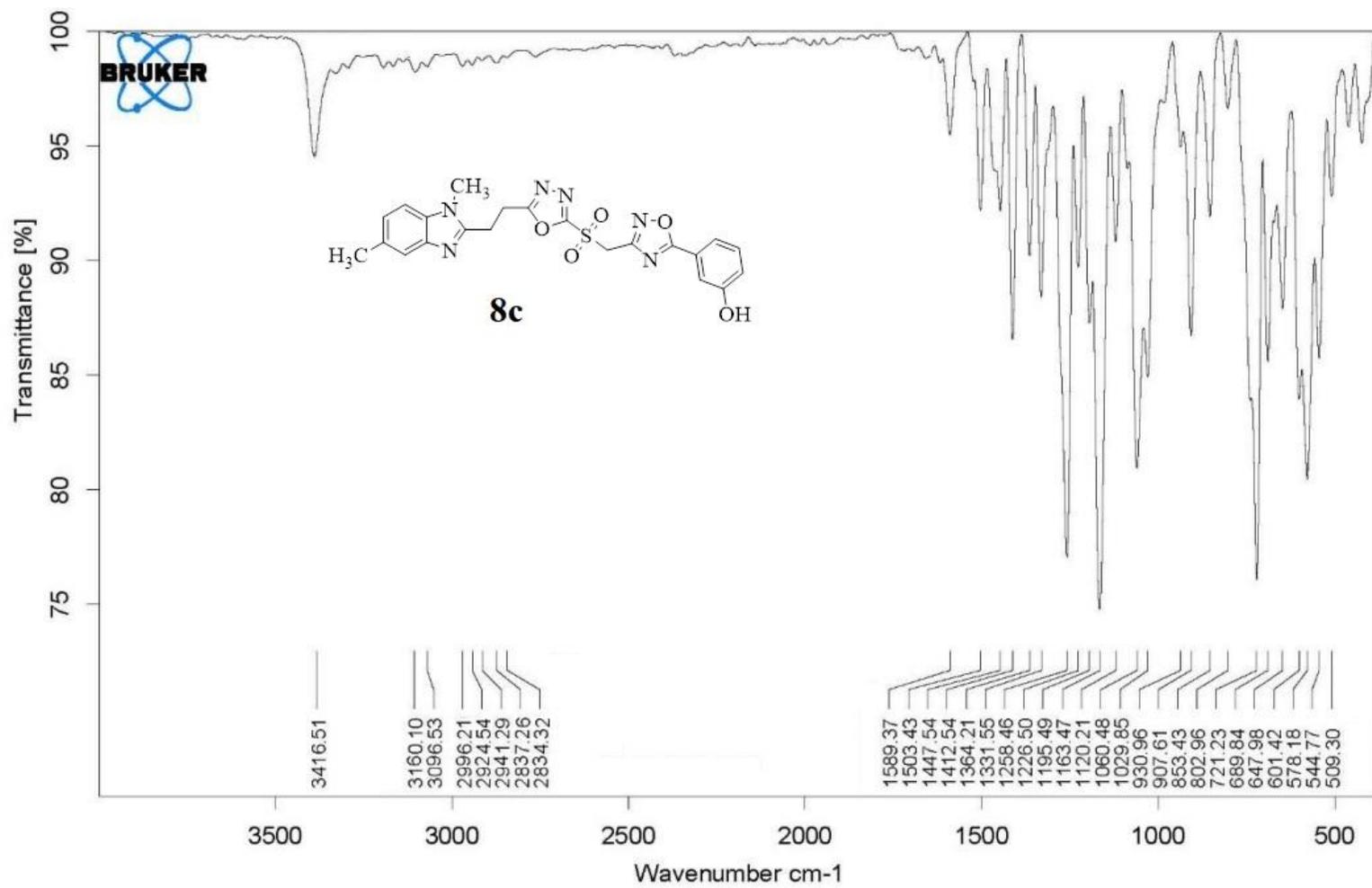
Molecular Weight: 480.4964

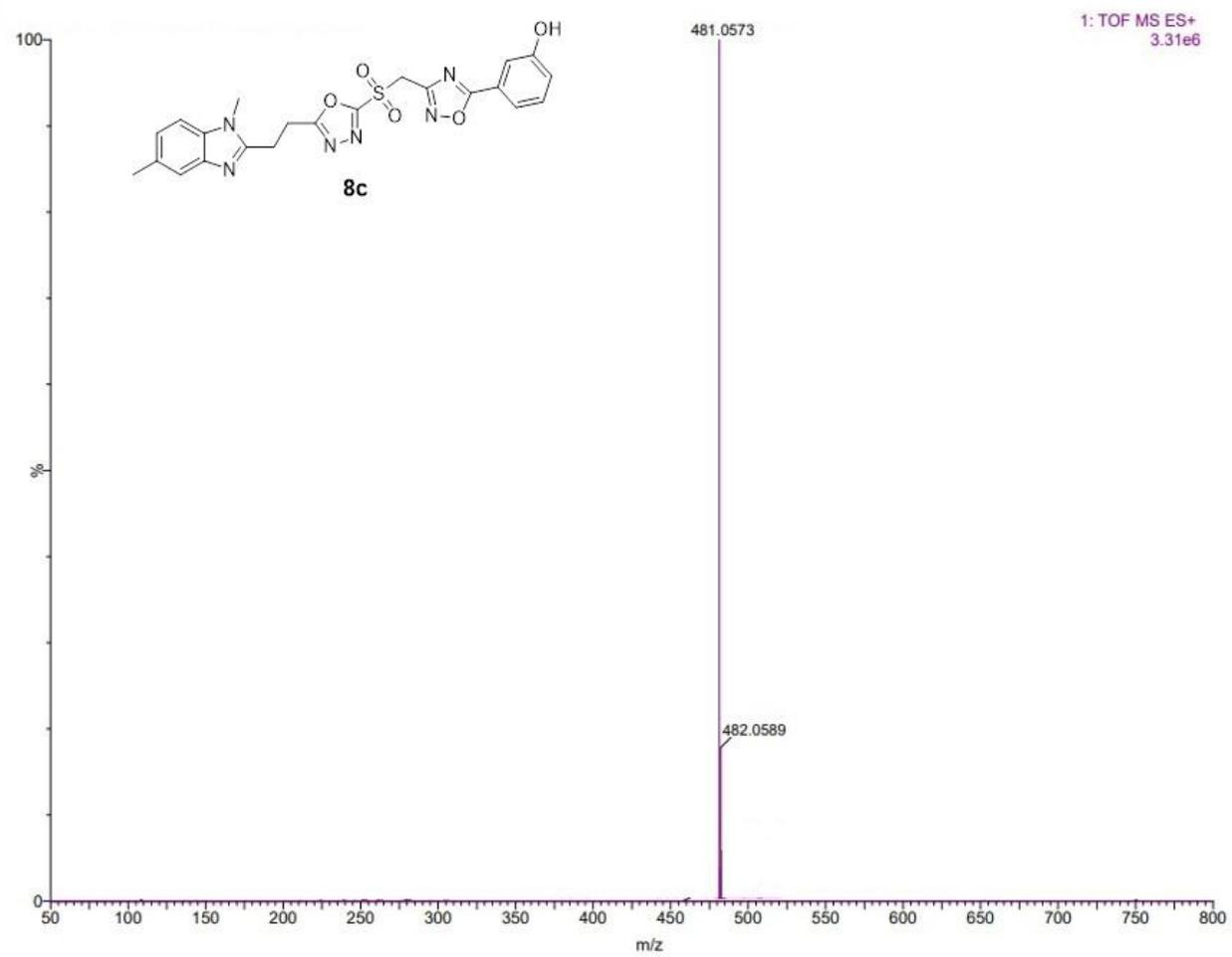
m/z: 480.1216 (100.0%), 481.1249 (23.8%), 482.1174 (4.5%), 482.1283 (2.7%), 481.1186 (2.2%), 483.1207 (1.1%), 482.1258 (1.0%)

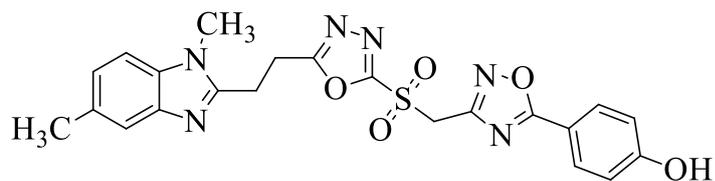
Elemental Analysis: C, 54.99; H, 4.20; N, 17.49; O, 16.65; S, 6.67











Compound 8d

4-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)phenol

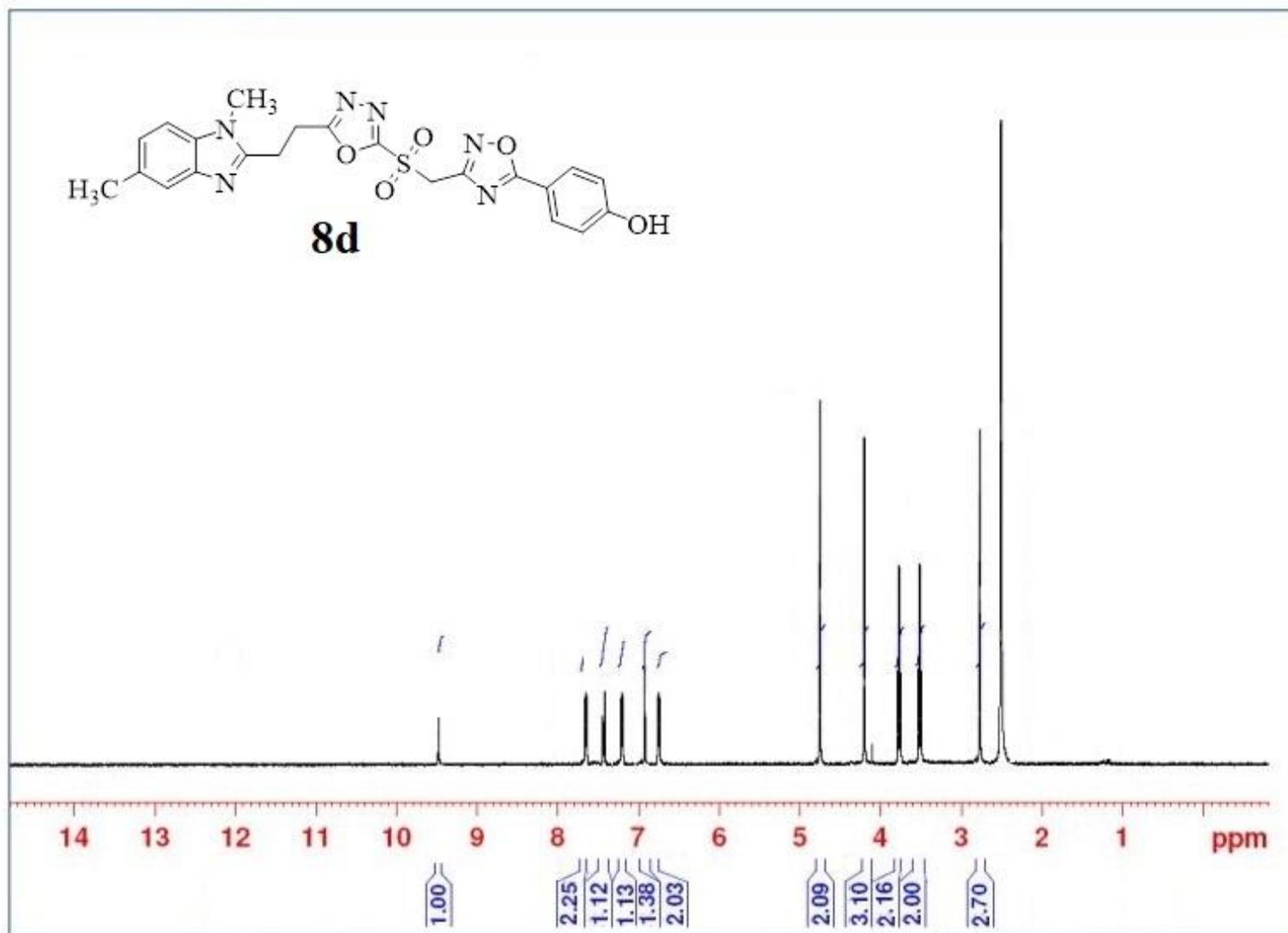
Chemical Formula: C₂₂H₂₀N₆O₅S

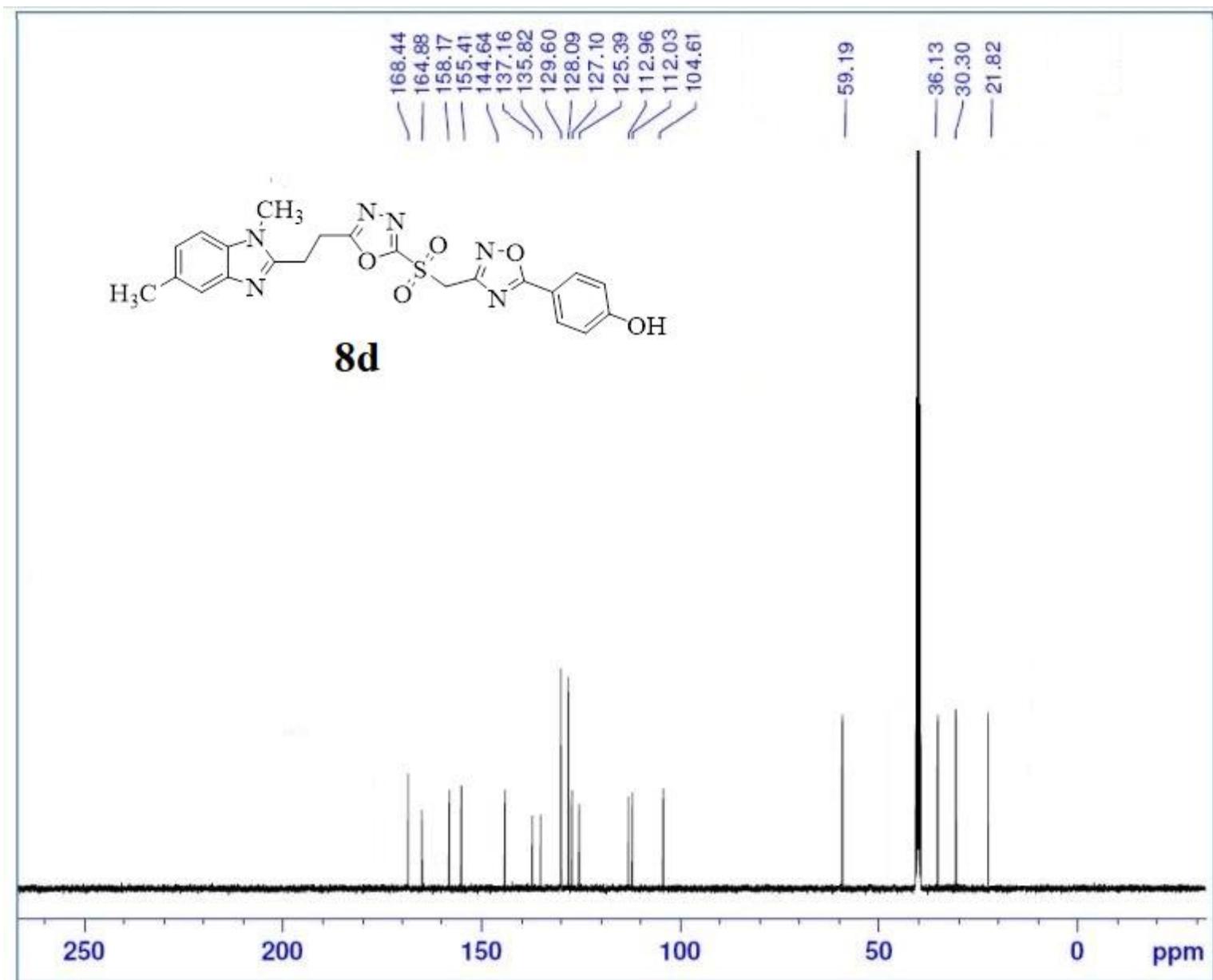
Exact Mass: 480.1216

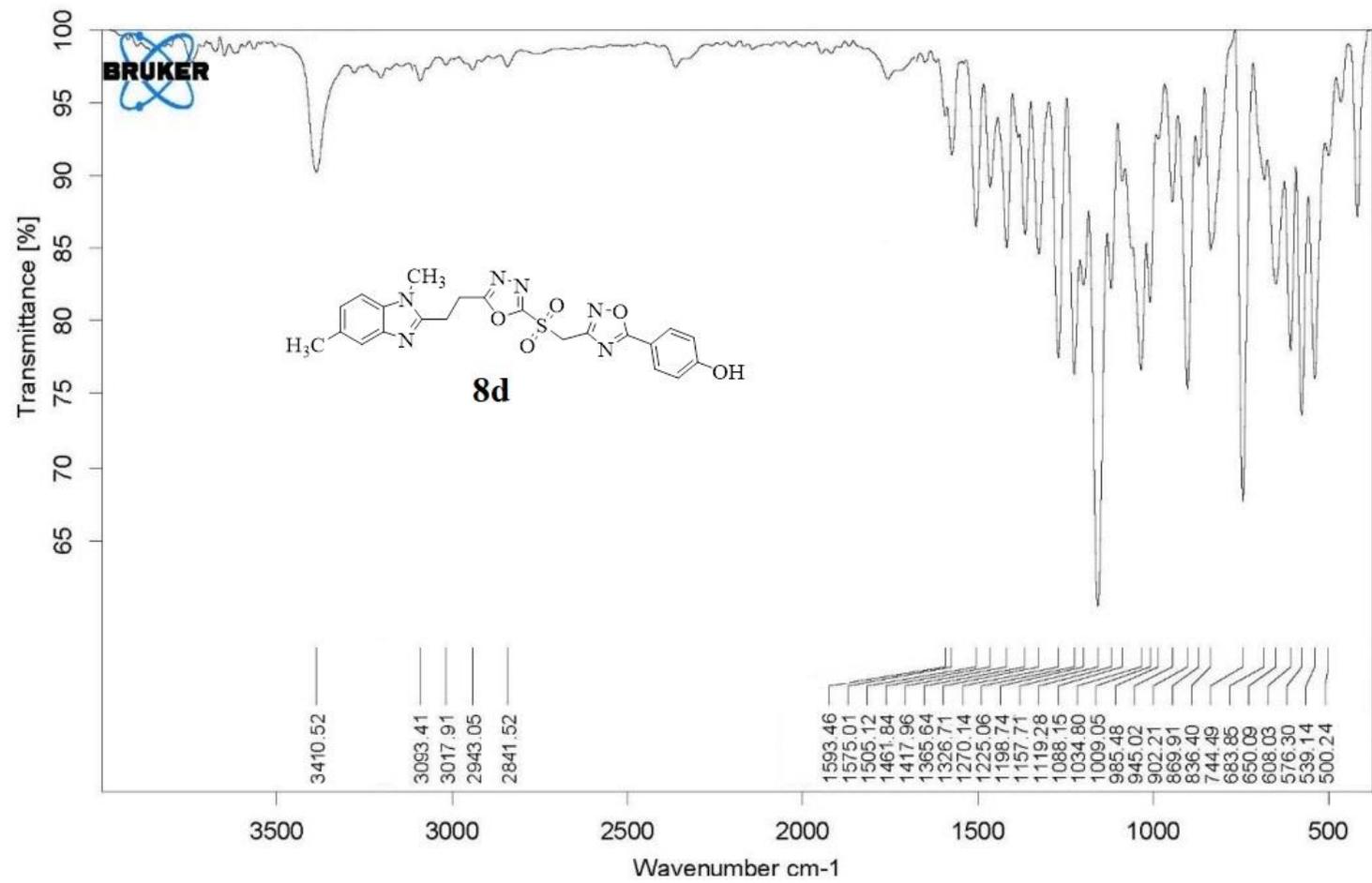
Molecular Weight: 480.4964

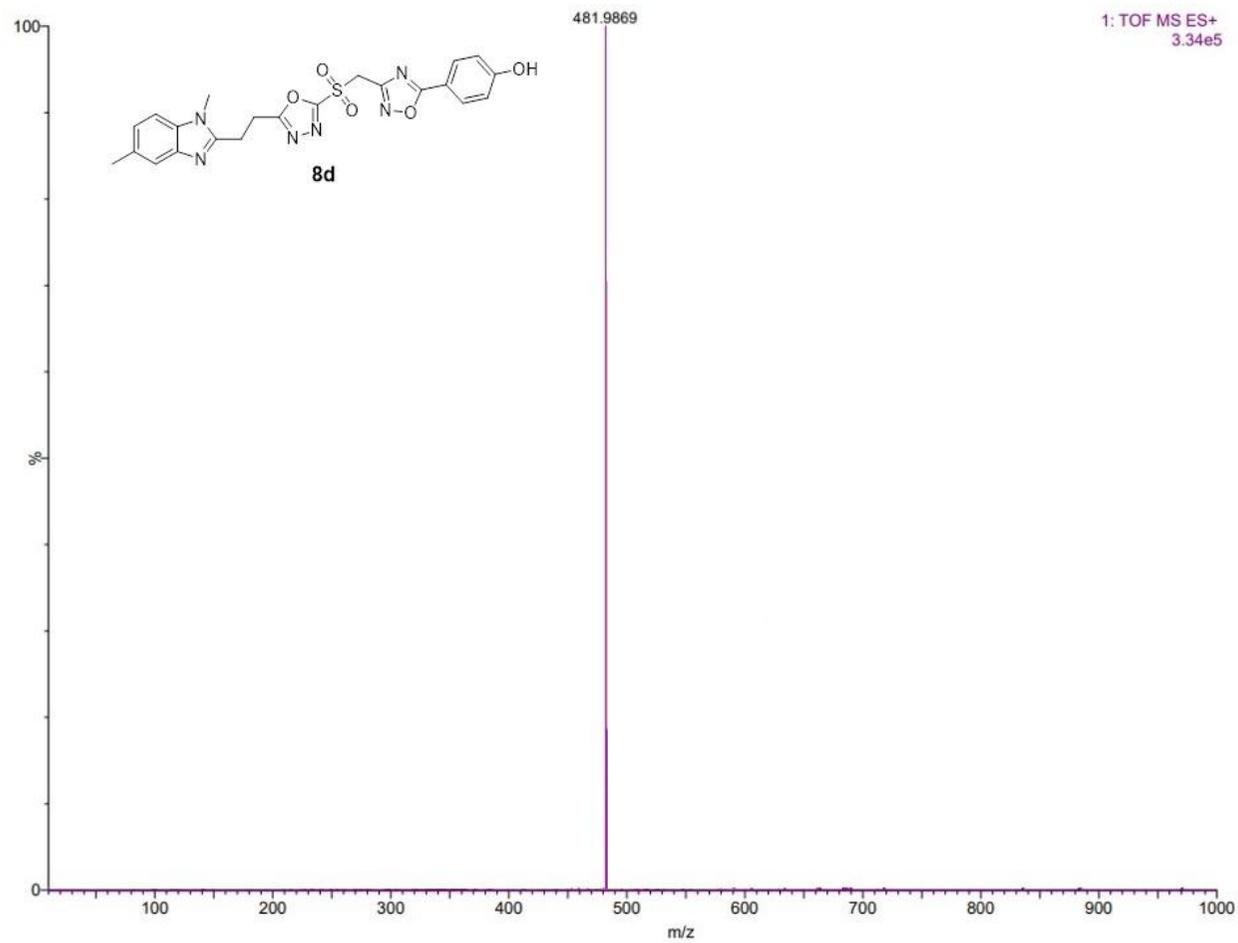
m/z: 480.1216 (100.0%), 481.1249 (23.8%), 482.1174 (4.5%), 482.1283 (2.7%), 481.1186 (2.2%), 483.1207 (1.1%), 482.1258 (1.0%)

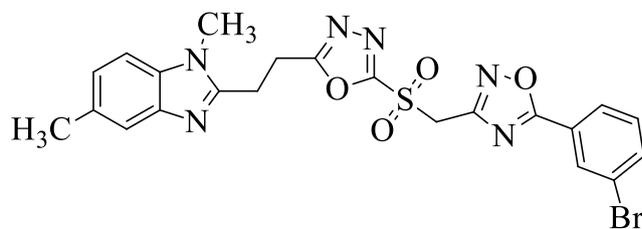
Elemental Analysis: C, 54.99; H, 4.20; N, 17.49; O, 16.65; S, 6.67











Compound 8e

5-(3-bromophenyl)-3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazole

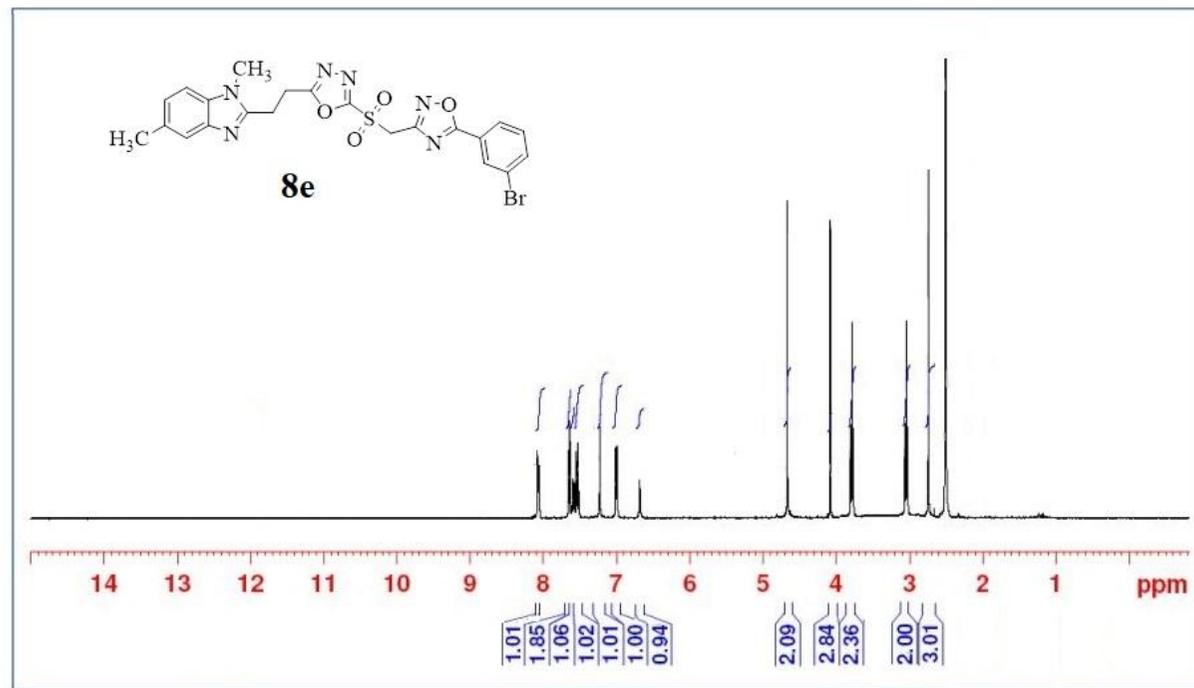
Chemical Formula: C₂₂H₁₉BrN₆O₄S

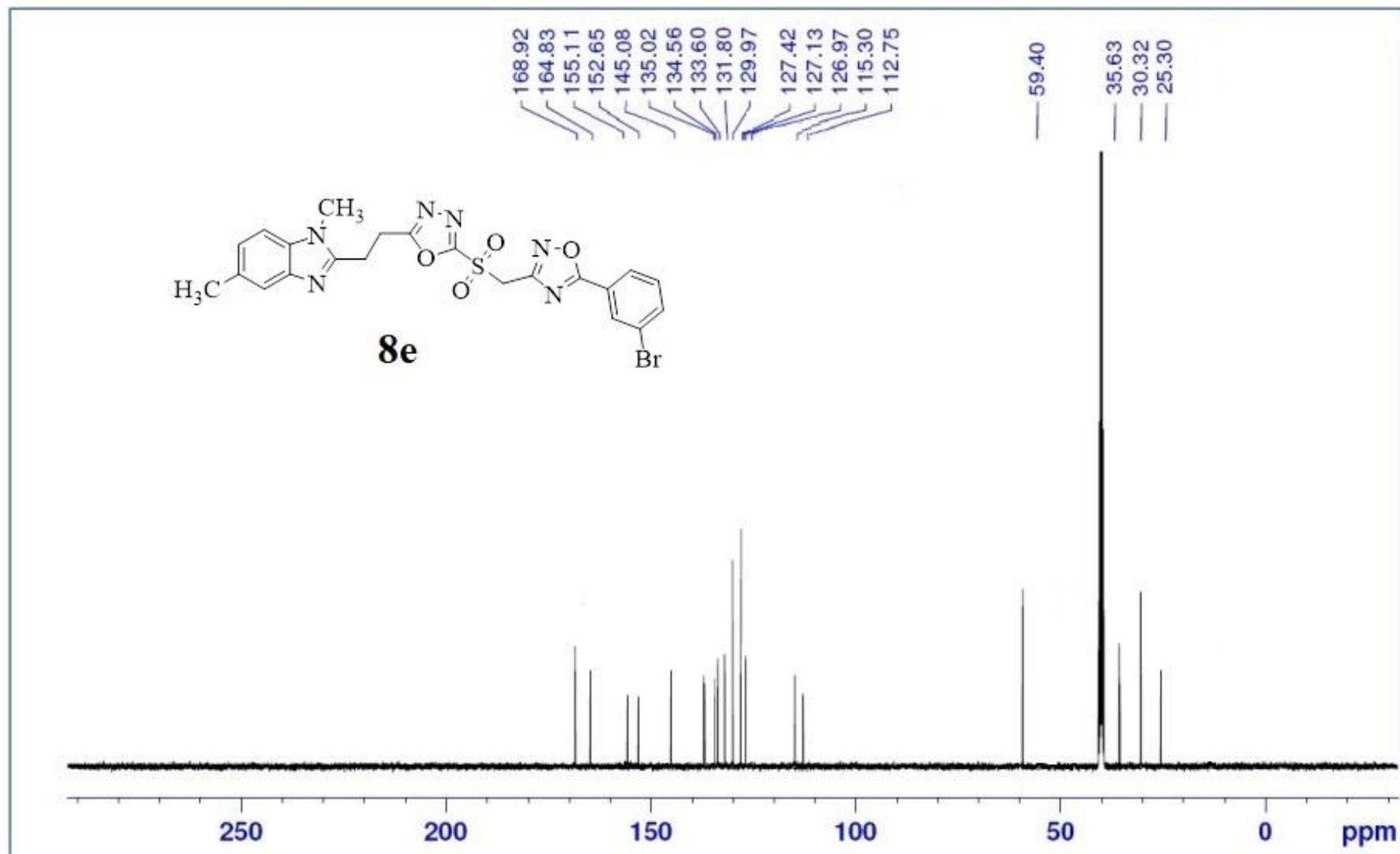
Exact Mass: 542.0372

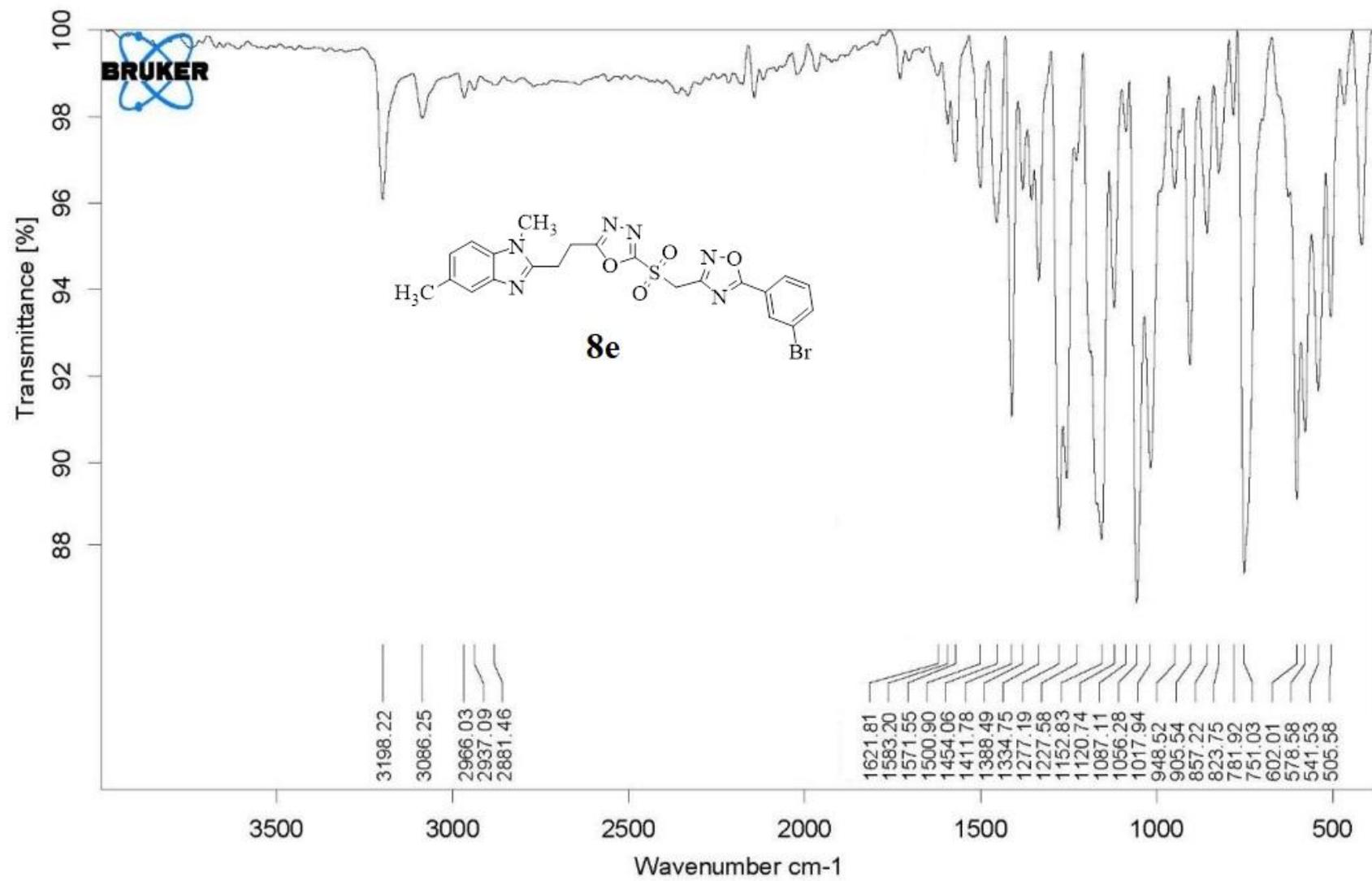
Molecular Weight: 543.3931

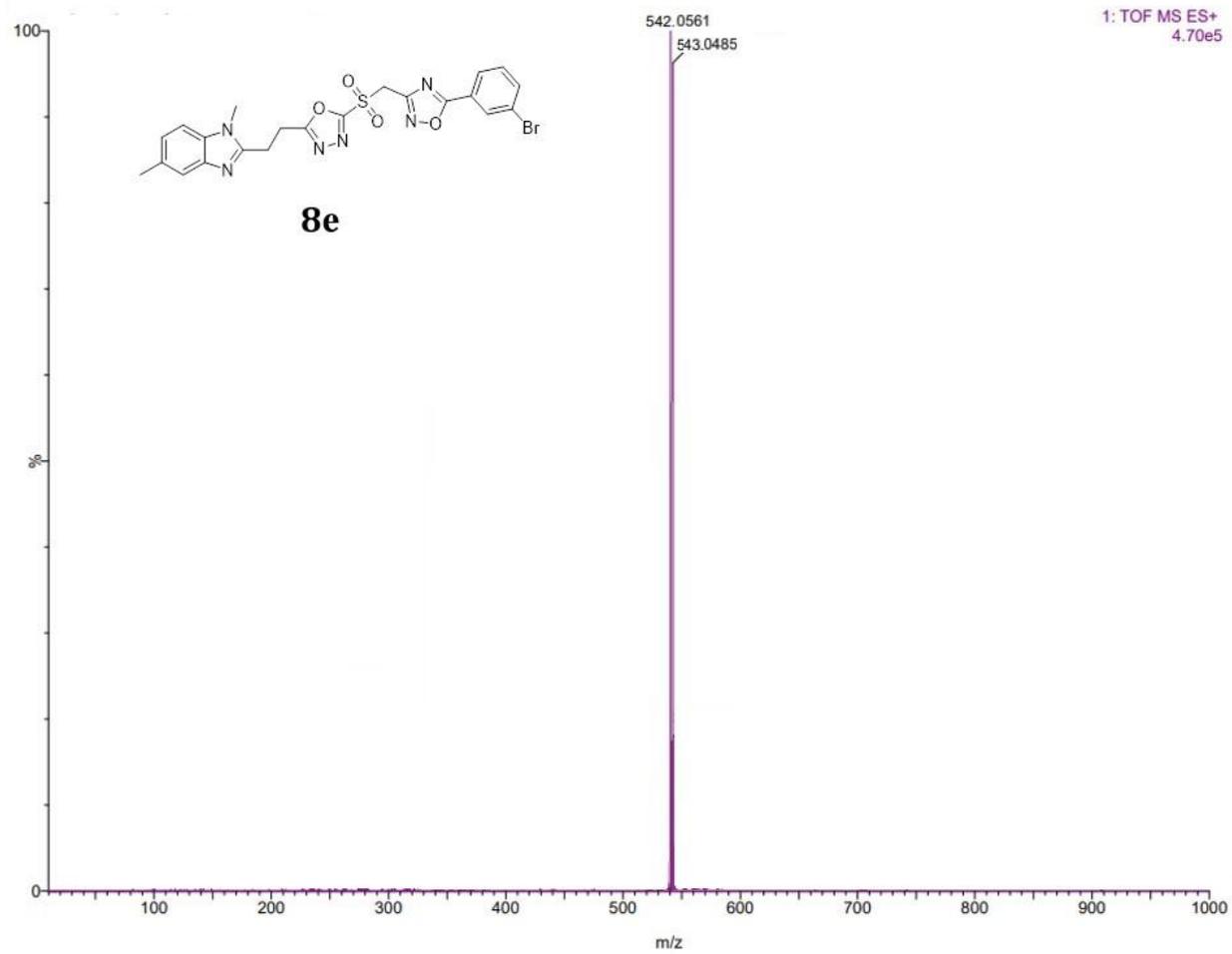
m/z: 542.0372 (100.0%), 544.0351 (97.3%), 543.0405 (23.8%), 545.0385 (23.2%), 544.0330 (4.5%), 546.0309 (4.4%), 544.0439 (2.7%), 546.0418 (2.6%), 543.0342 (2.2%), 545.0322 (2.2%), 545.0363 (1.1%), 547.0343 (1.0%)

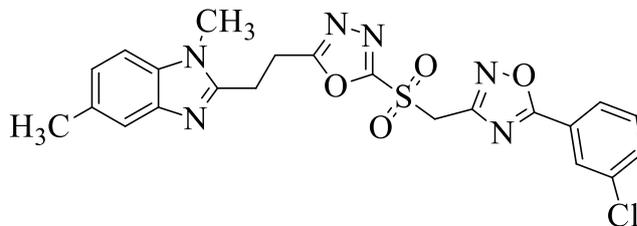
Elemental Analysis: C, 48.63; H, 3.52; Br, 14.70; N, 15.47; O, 11.78; S, 5.90











Compound 8f

5-(3-chlorophenyl)-3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazole

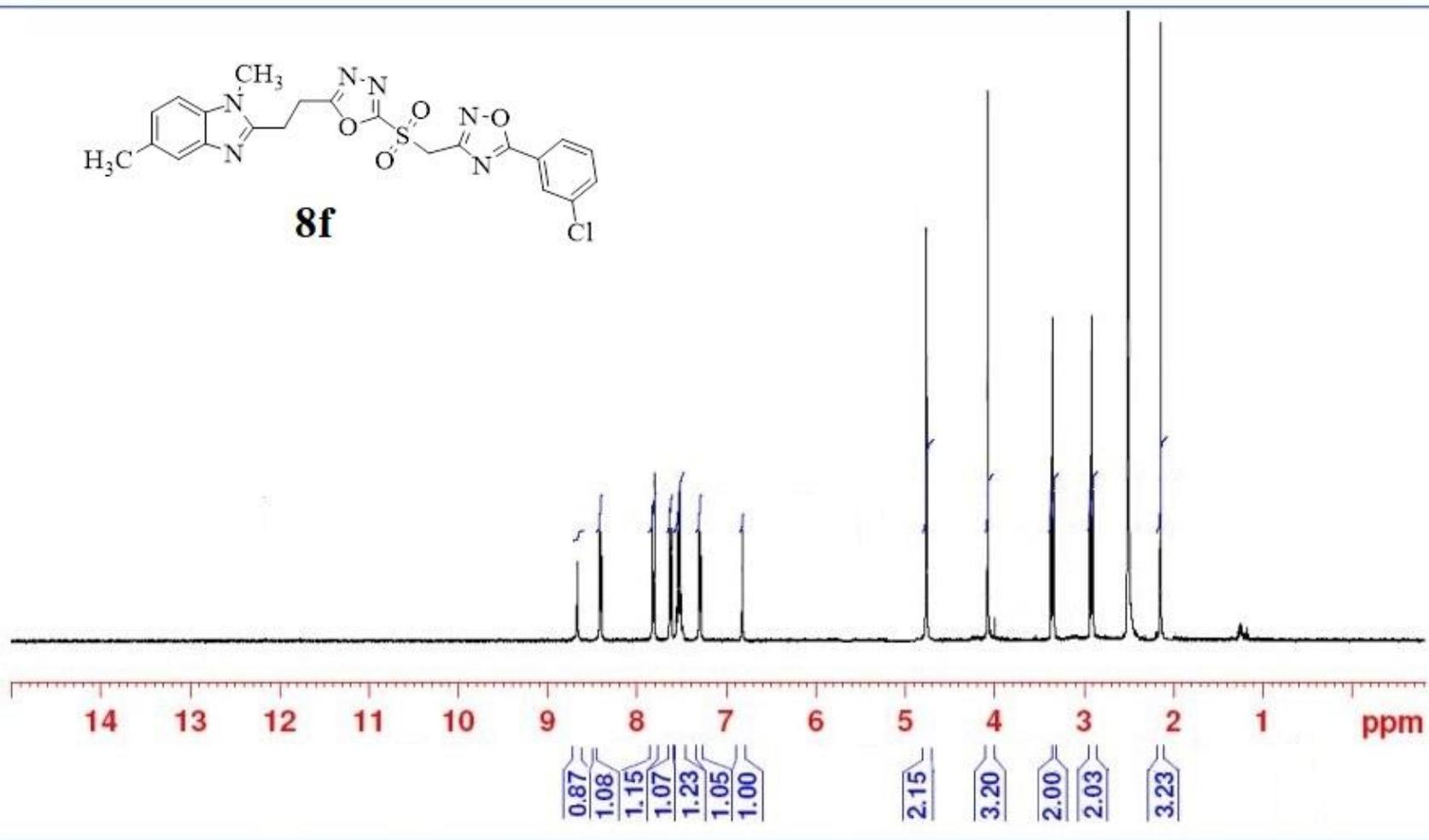
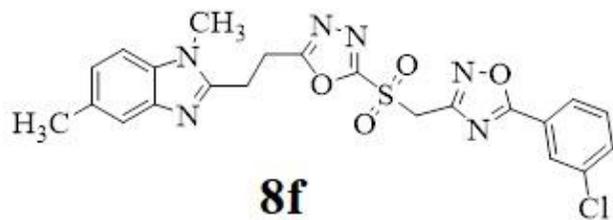
Chemical Formula: C₂₂H₁₉ClN₆O₄S

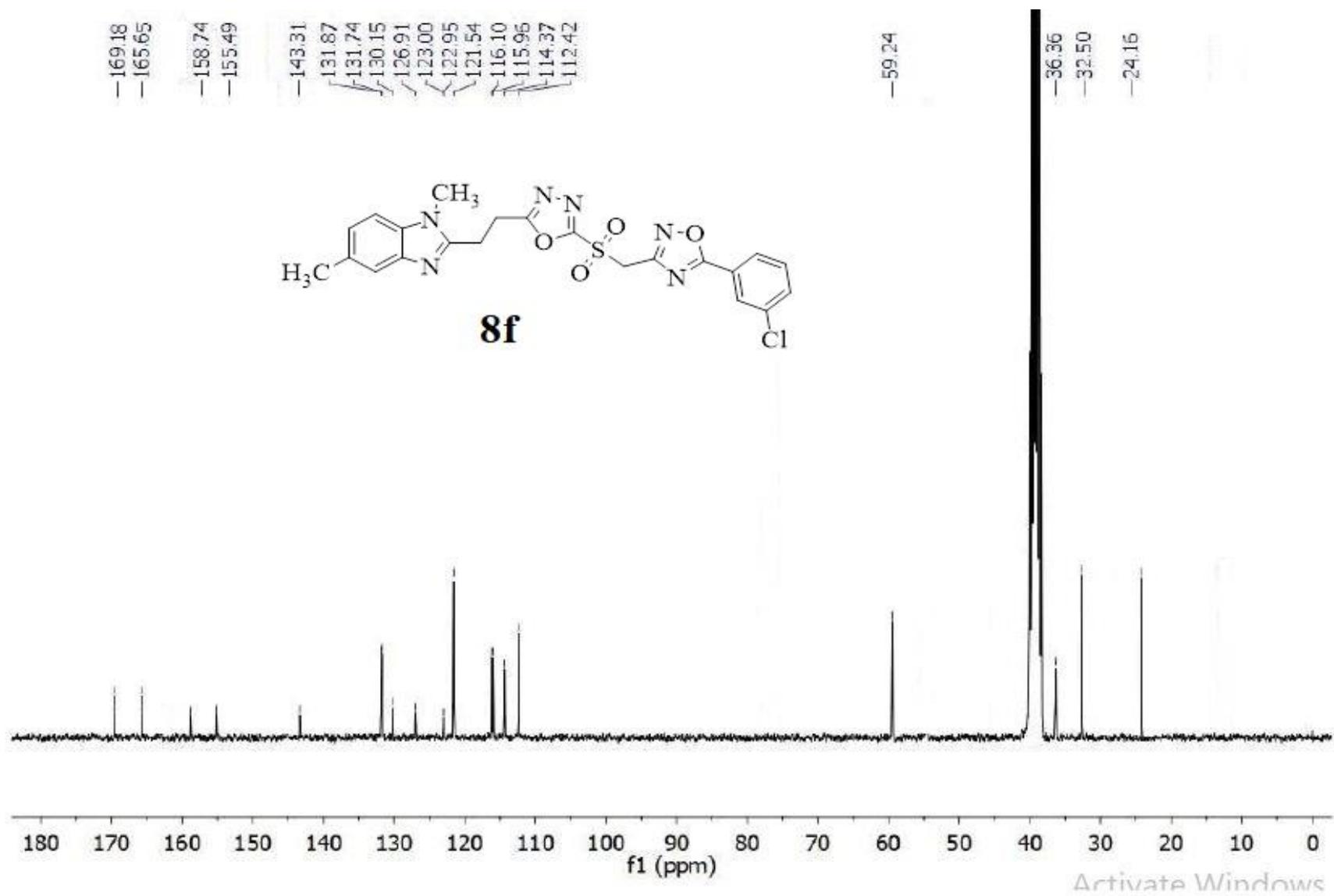
Exact Mass: 498.0877

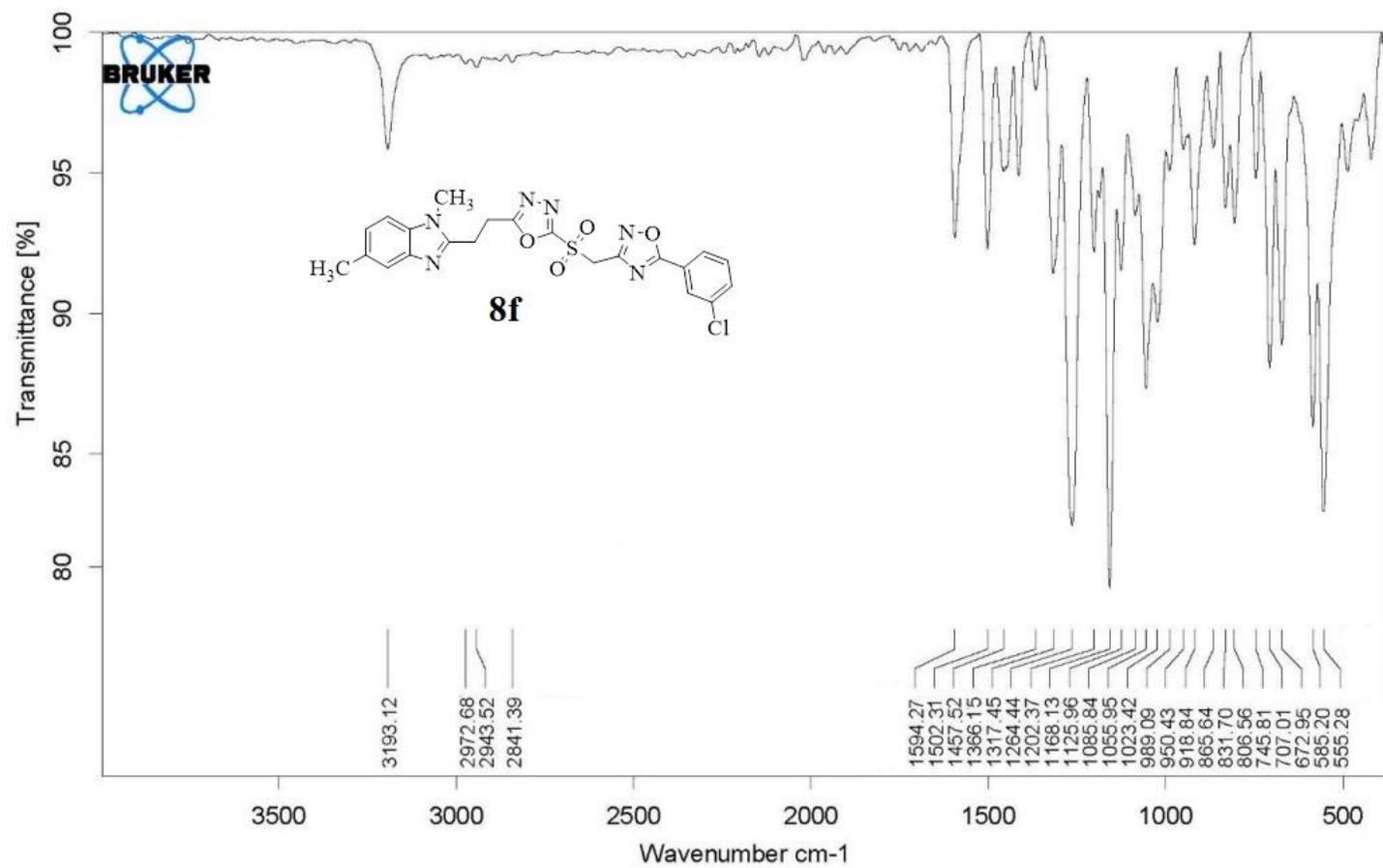
Molecular Weight: 498.9421

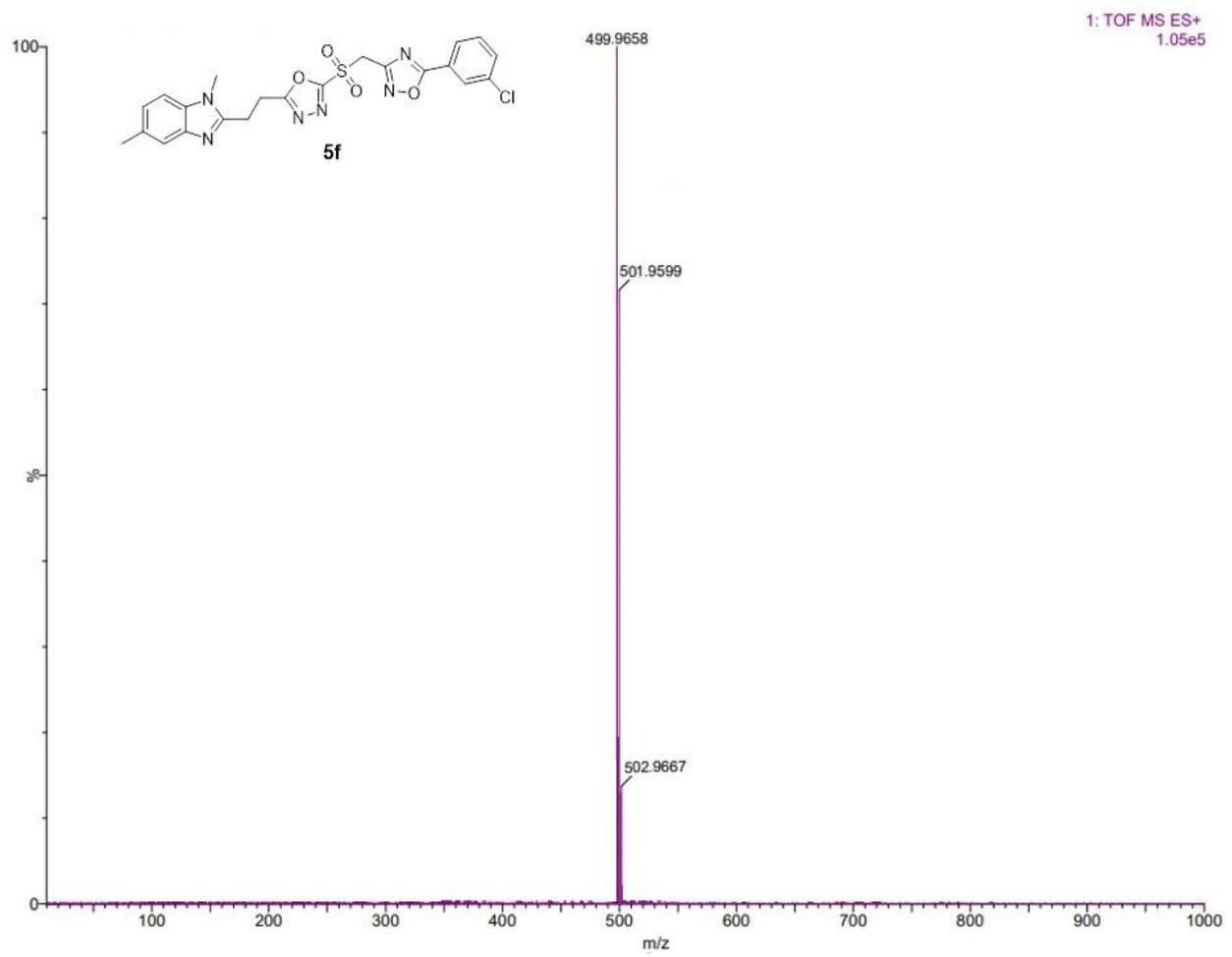
m/z: 498.0877 (100.0%), 500.0848 (32.0%), 499.0911 (23.8%), 501.0881 (7.6%), 500.0835 (4.5%), 500.0944 (2.7%), 499.0847 (2.2%), 502.0805 (1.4%), 501.0869 (1.1%)

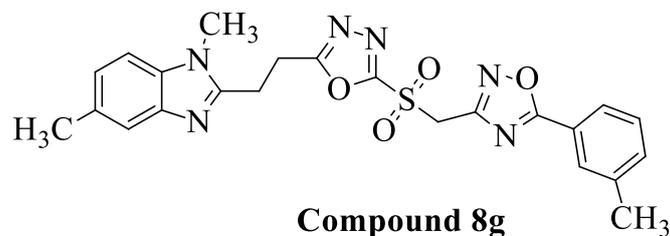
Elemental Analysis: C, 52.96; H, 3.84; Cl, 7.11; N, 16.84; O, 12.83; S, 6.43











3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(*m*-tolyl)-1,2,4-oxadiazole

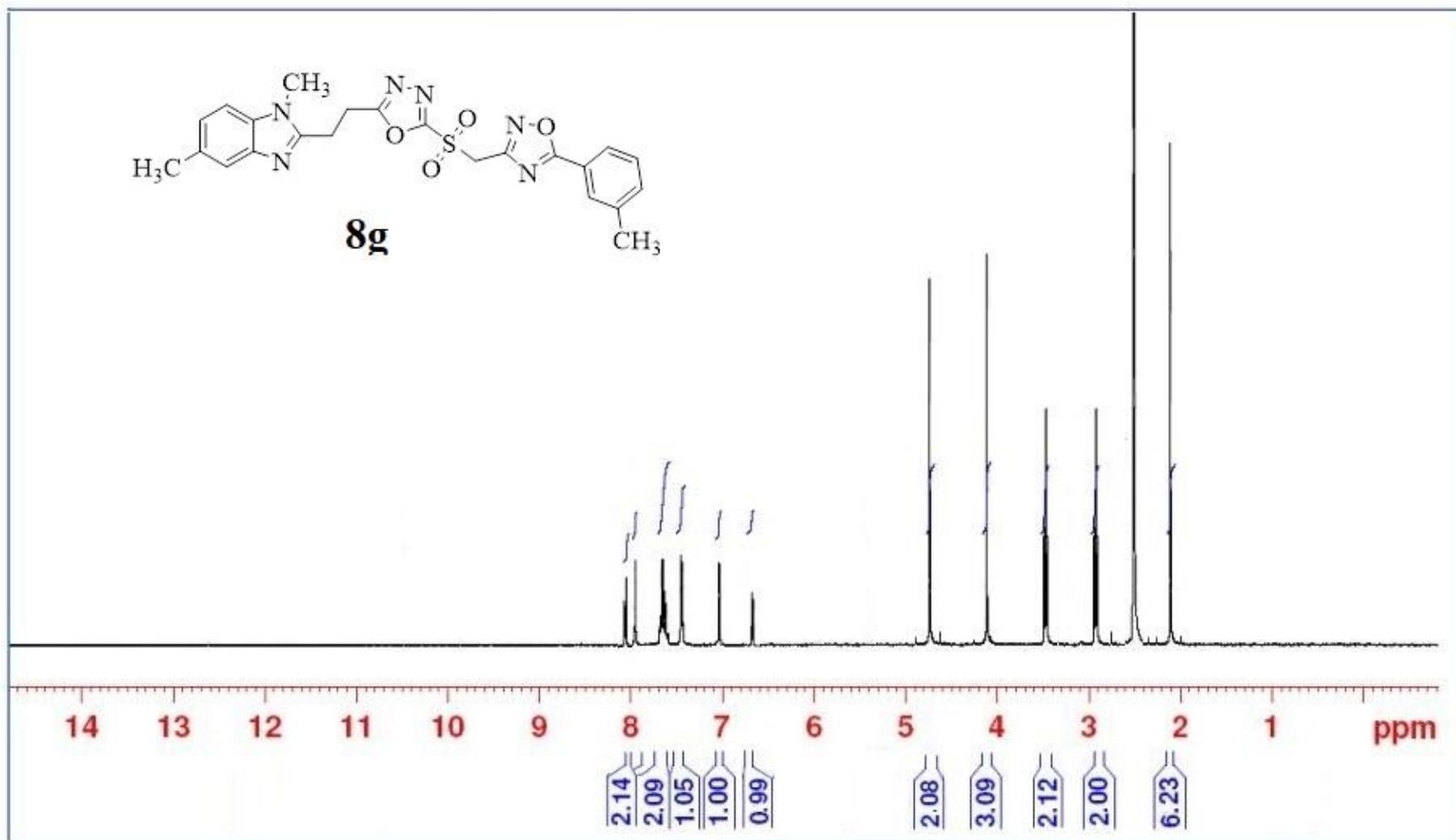
Chemical Formula: C₂₃H₂₂N₆O₄S

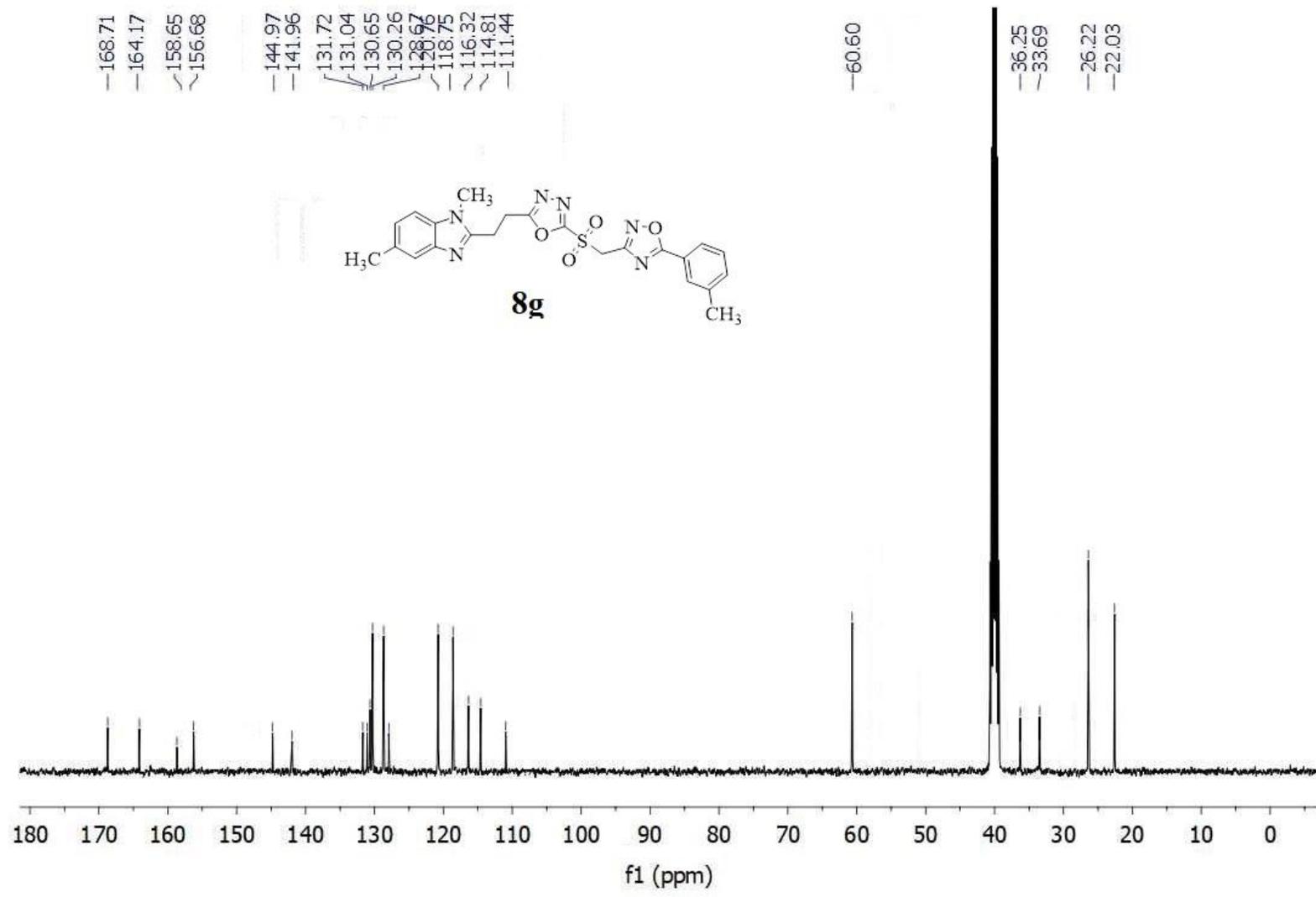
Exact Mass: 478.1423

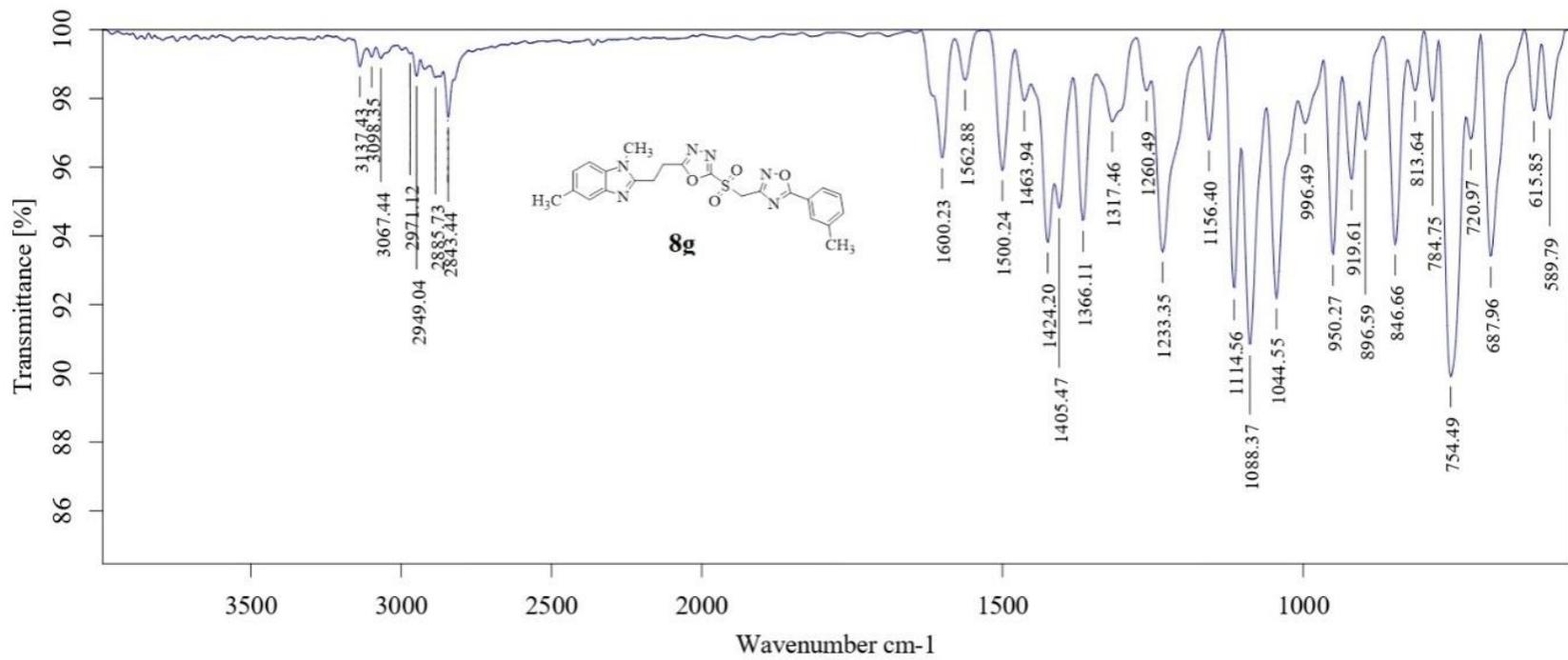
Molecular Weight: 478.5236

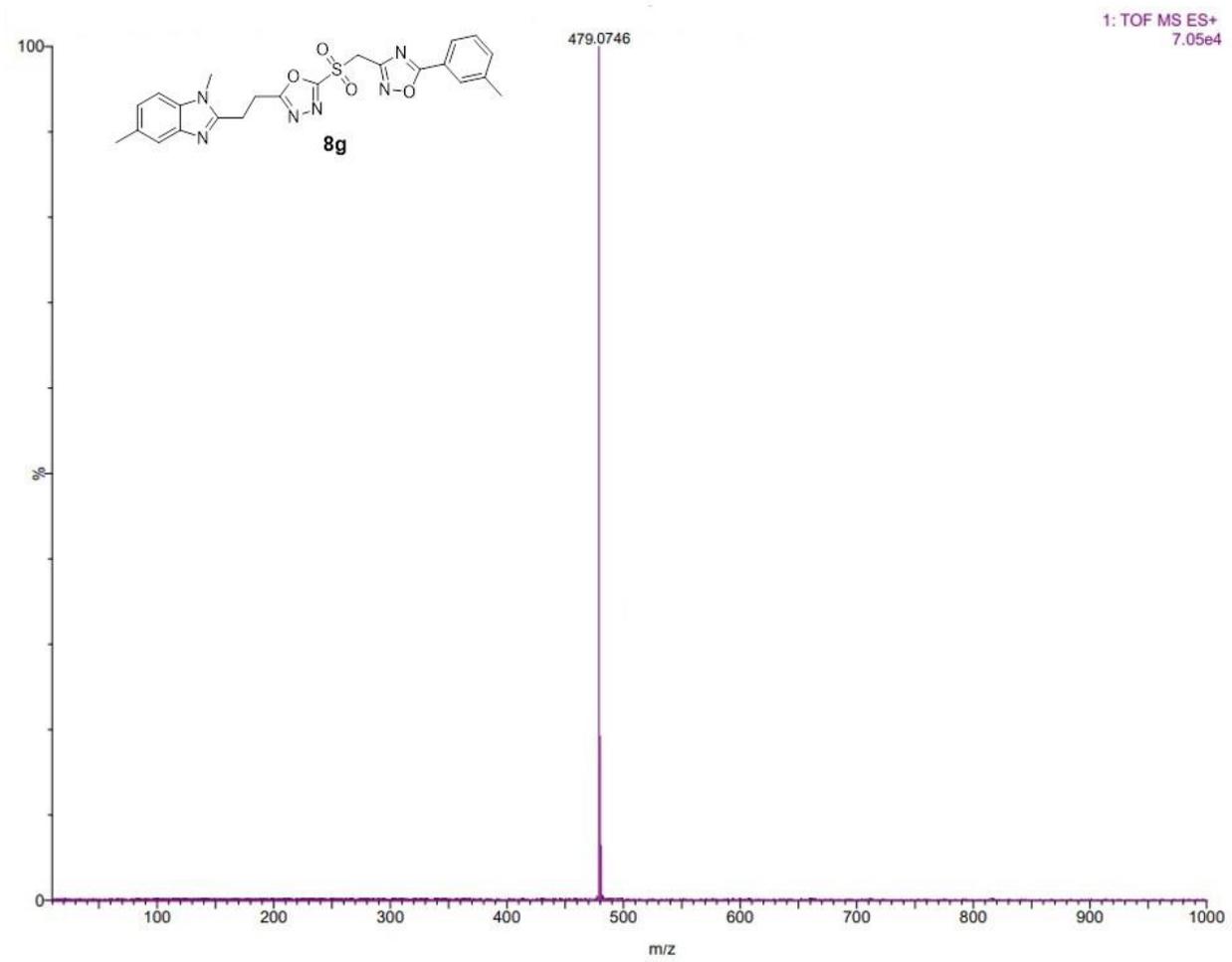
m/z: 478.1423 (100.0%), 479.1457 (24.9%), 480.1381 (4.5%), 480.1490 (3.0%),
479.1394 (2.2%), 481.1415 (1.1%)

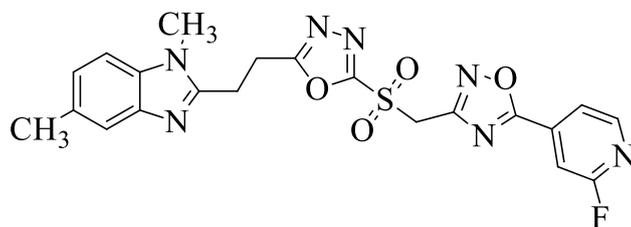
Elemental Analysis: C, 57.73; H, 4.63; N, 17.56; O, 13.37; S, 6.70











Compound 8h

3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-5-(2-fluoropyridin-4-yl)-1,2,4-oxadiazole

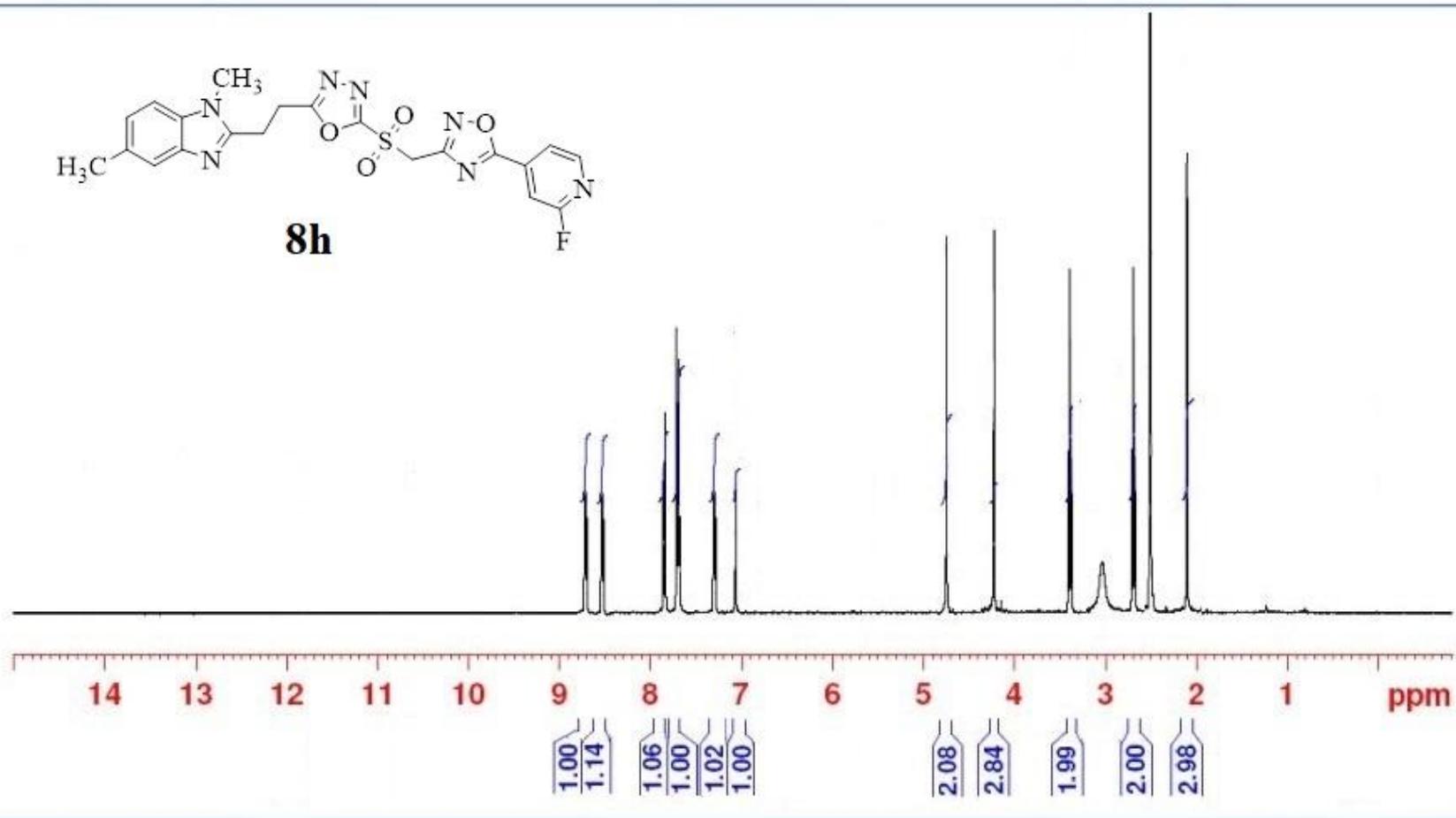
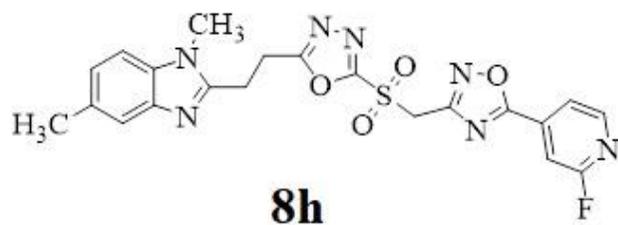
Chemical Formula: C₂₁H₁₈FN₇O₄S

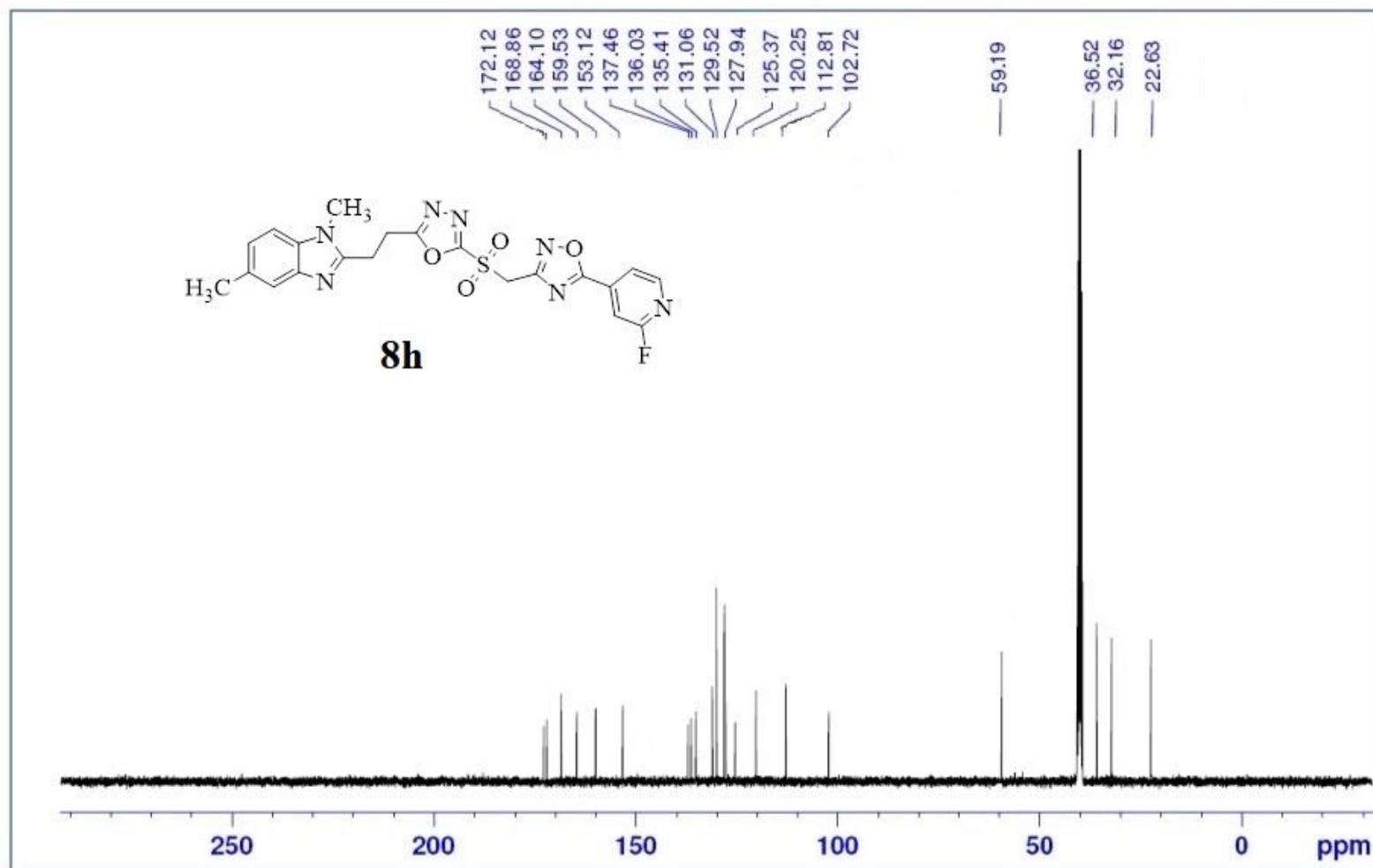
Exact Mass: 483.1125

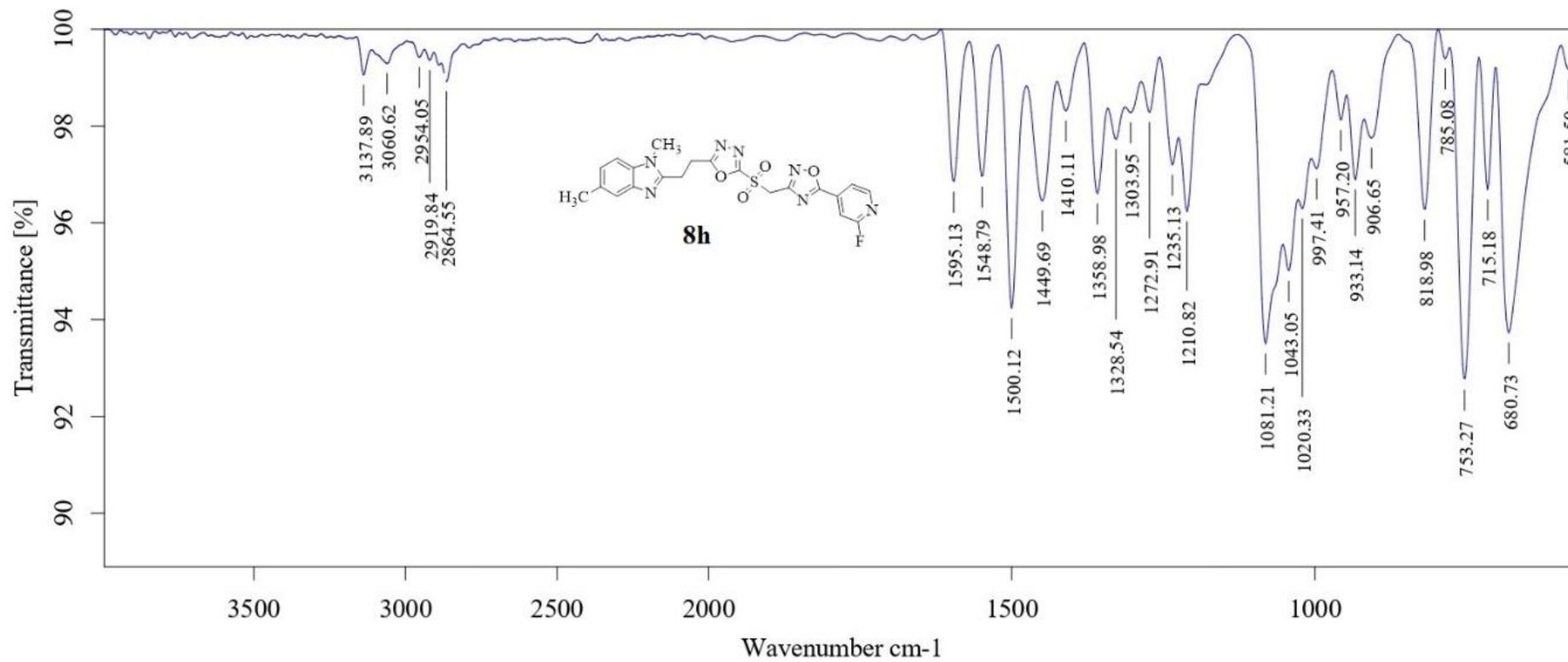
Molecular Weight: 483.4755

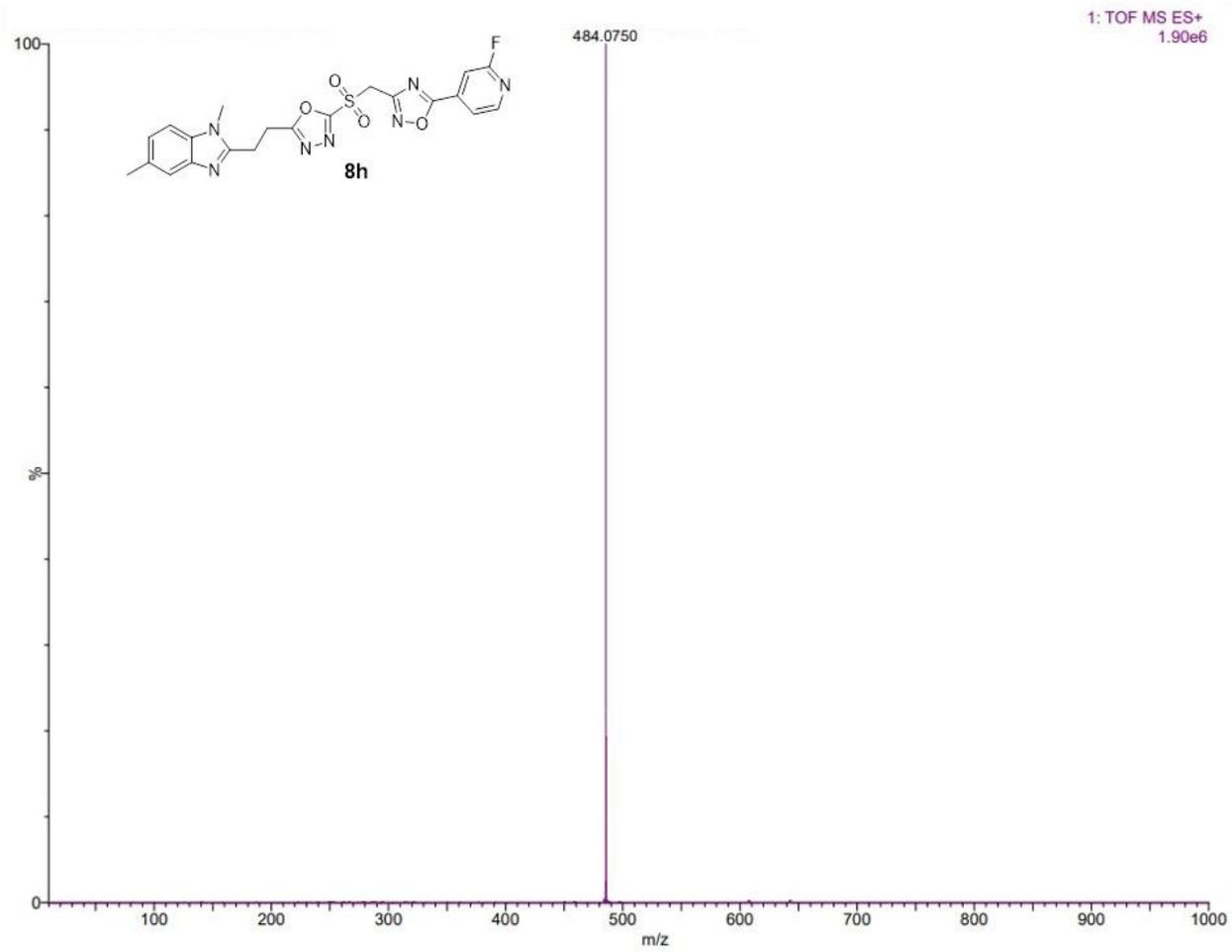
m/z: 483.1125 (100.0%), 484.1159 (22.7%), 485.1083 (4.5%), 484.1095 (2.6%), 485.1192 (2.5%), 486.1117 (1.0%)

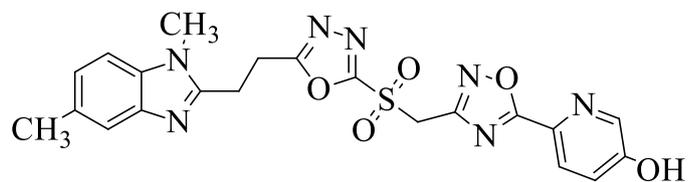
Elemental Analysis: C, 52.17; H, 3.75; F, 3.93; N, 20.28; O, 13.24; S, 6.63











Compound 8i

6-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)pyridin-3-ol

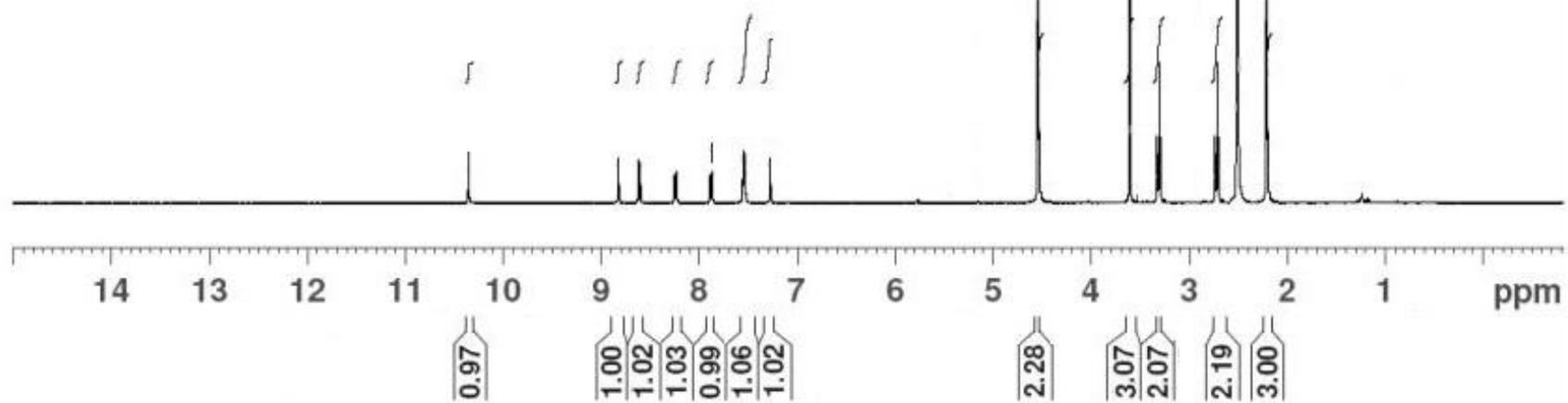
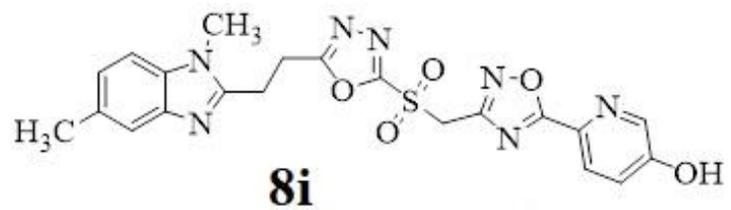
Chemical Formula: C₂₁H₁₉N₇O₅S

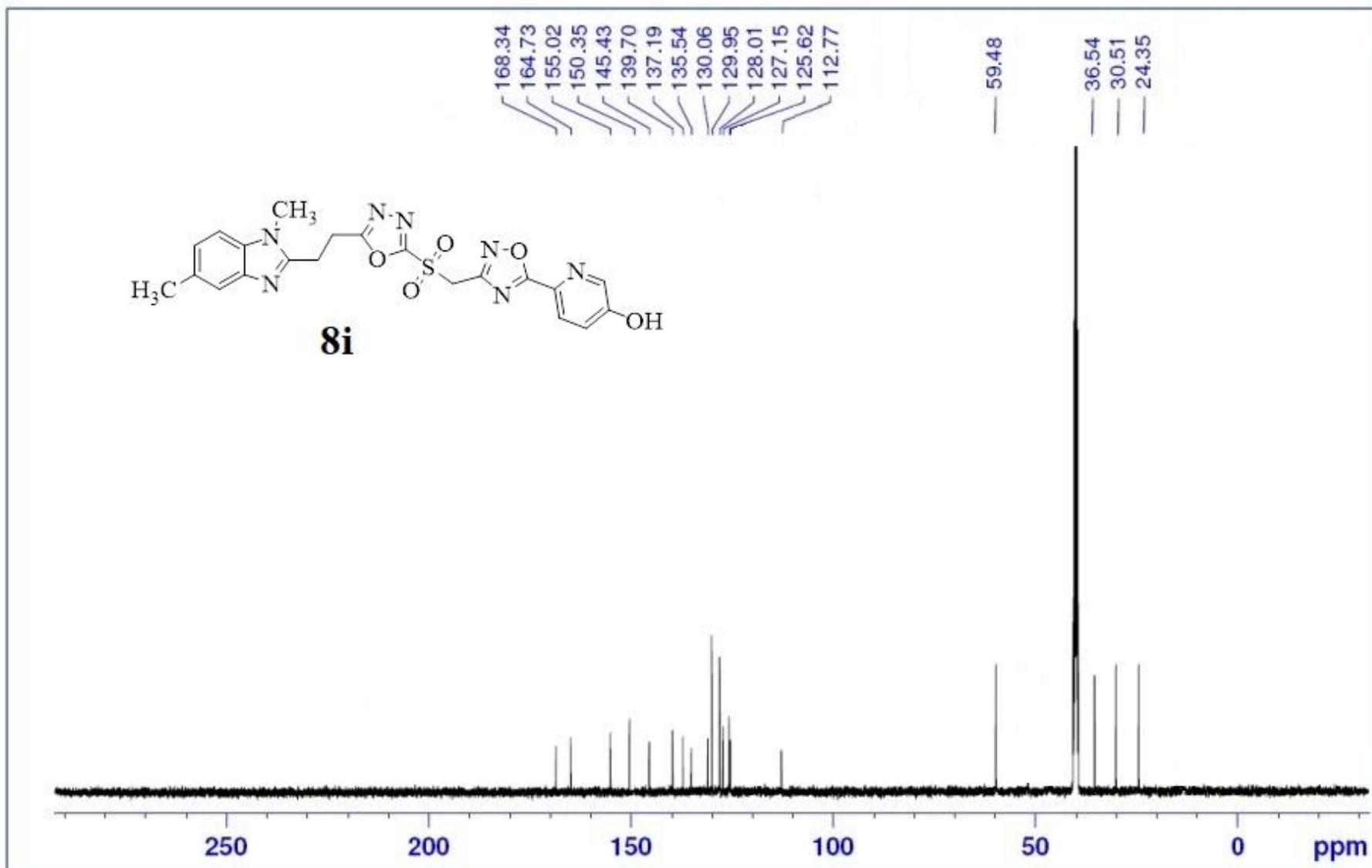
Exact Mass: 481.1168

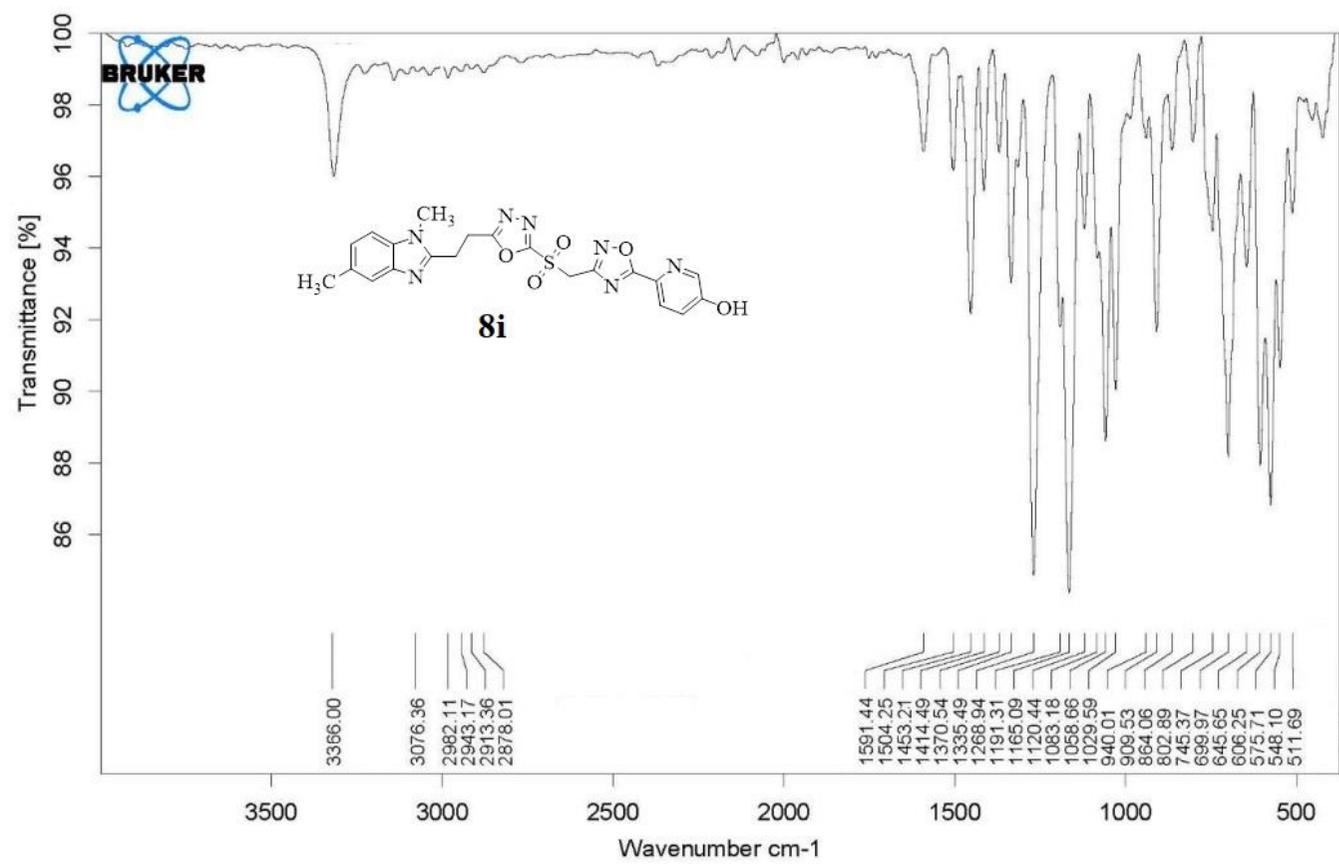
Molecular Weight: 481.4845

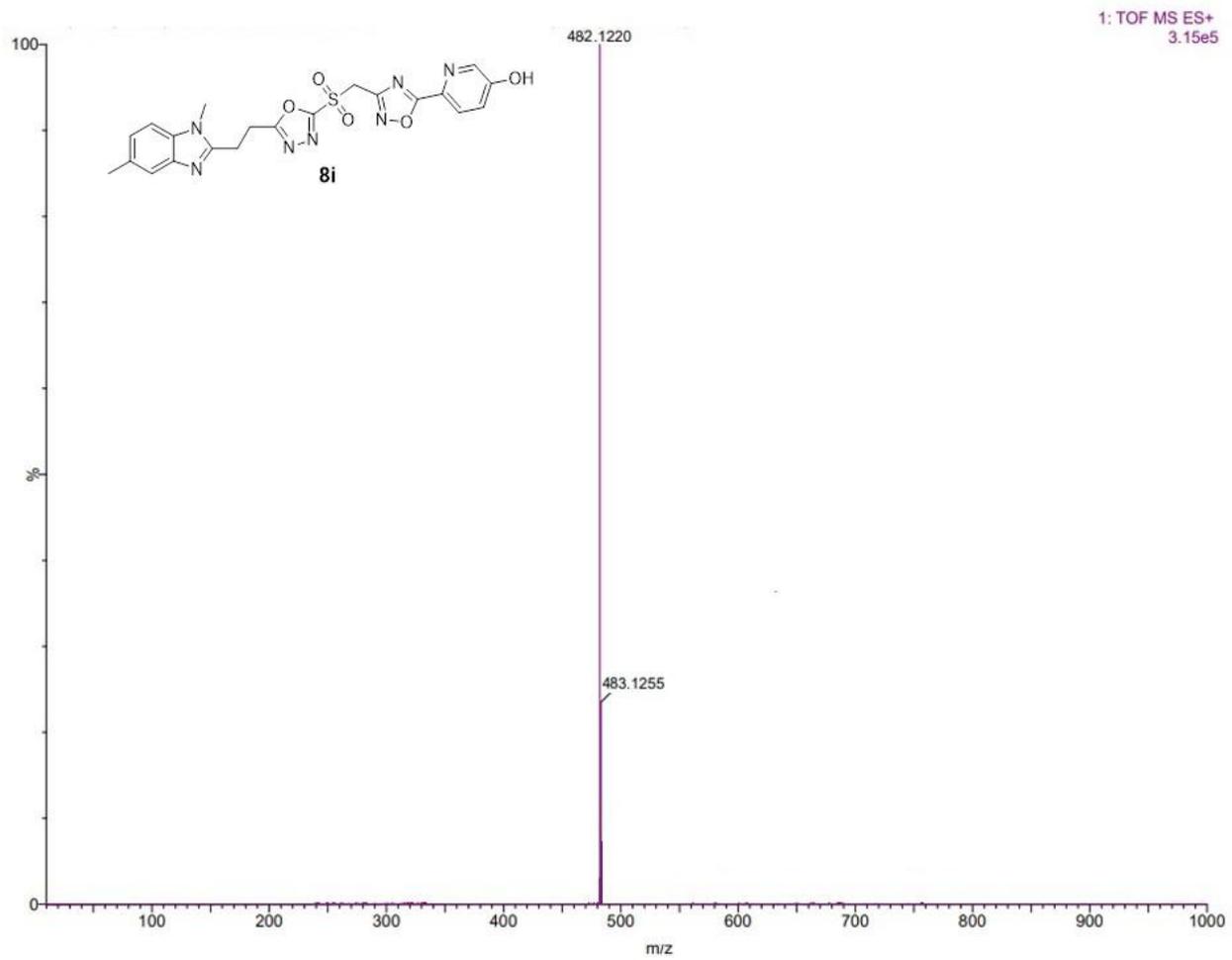
m/z: 481.1168 (100.0%), 482.1202 (22.7%), 483.1126 (4.5%), 482.1139 (2.6%), 483.1235 (2.5%), 483.1211 (1.0%), 484.1160 (1.0%)

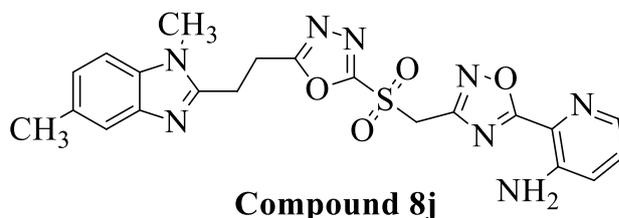
Elemental Analysis: C, 52.38; H, 3.98; N, 20.36; O, 16.61; S, 6.66











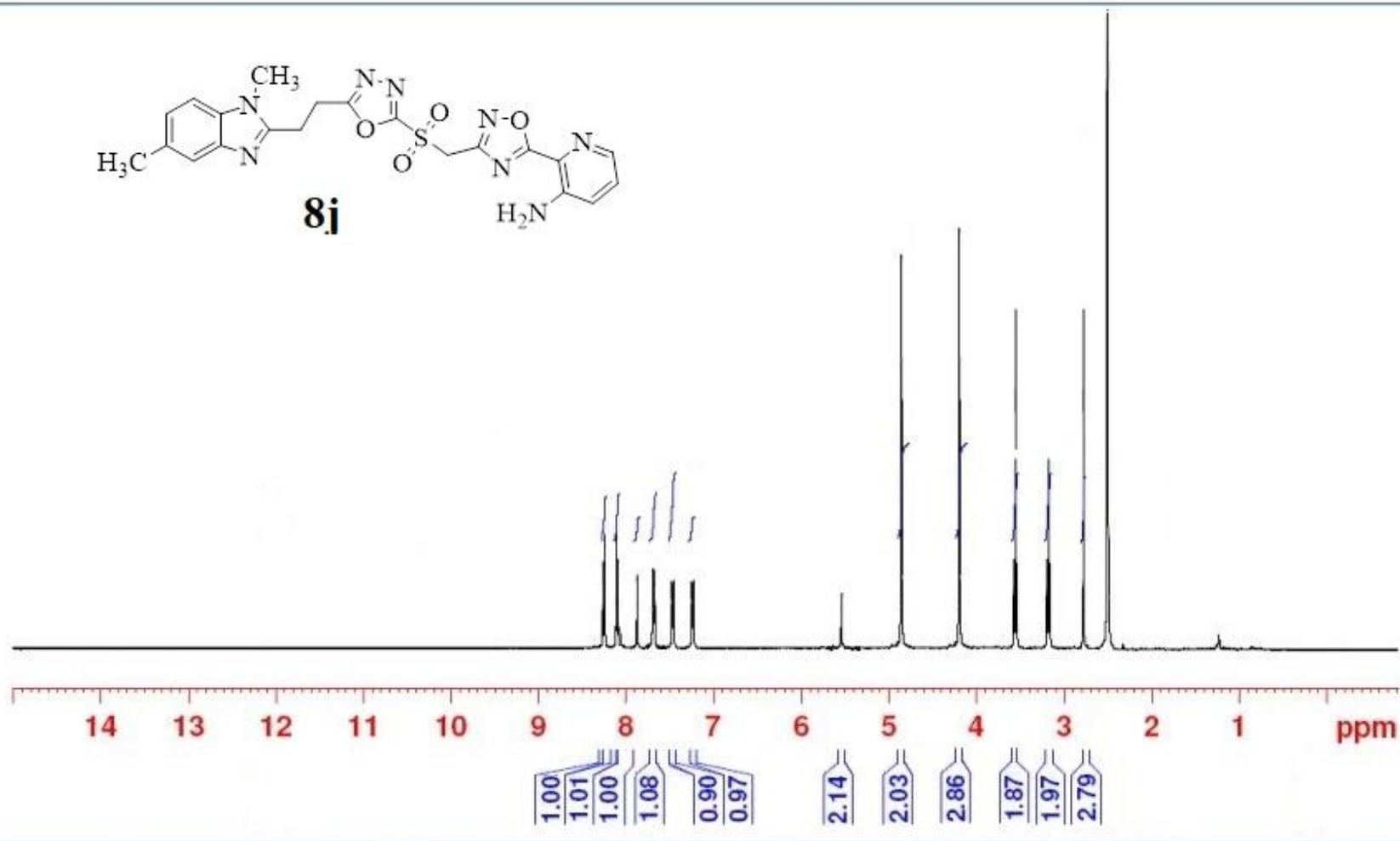
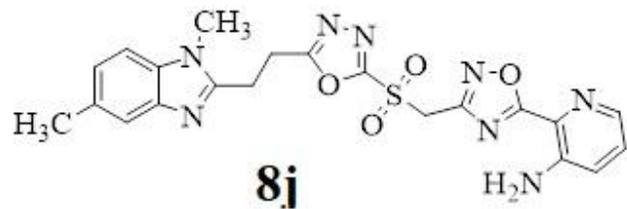
2-(3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazol-5-yl)pyridin-3-amine
 Chemical Formula: C₂₁H₂₀N₈O₄S

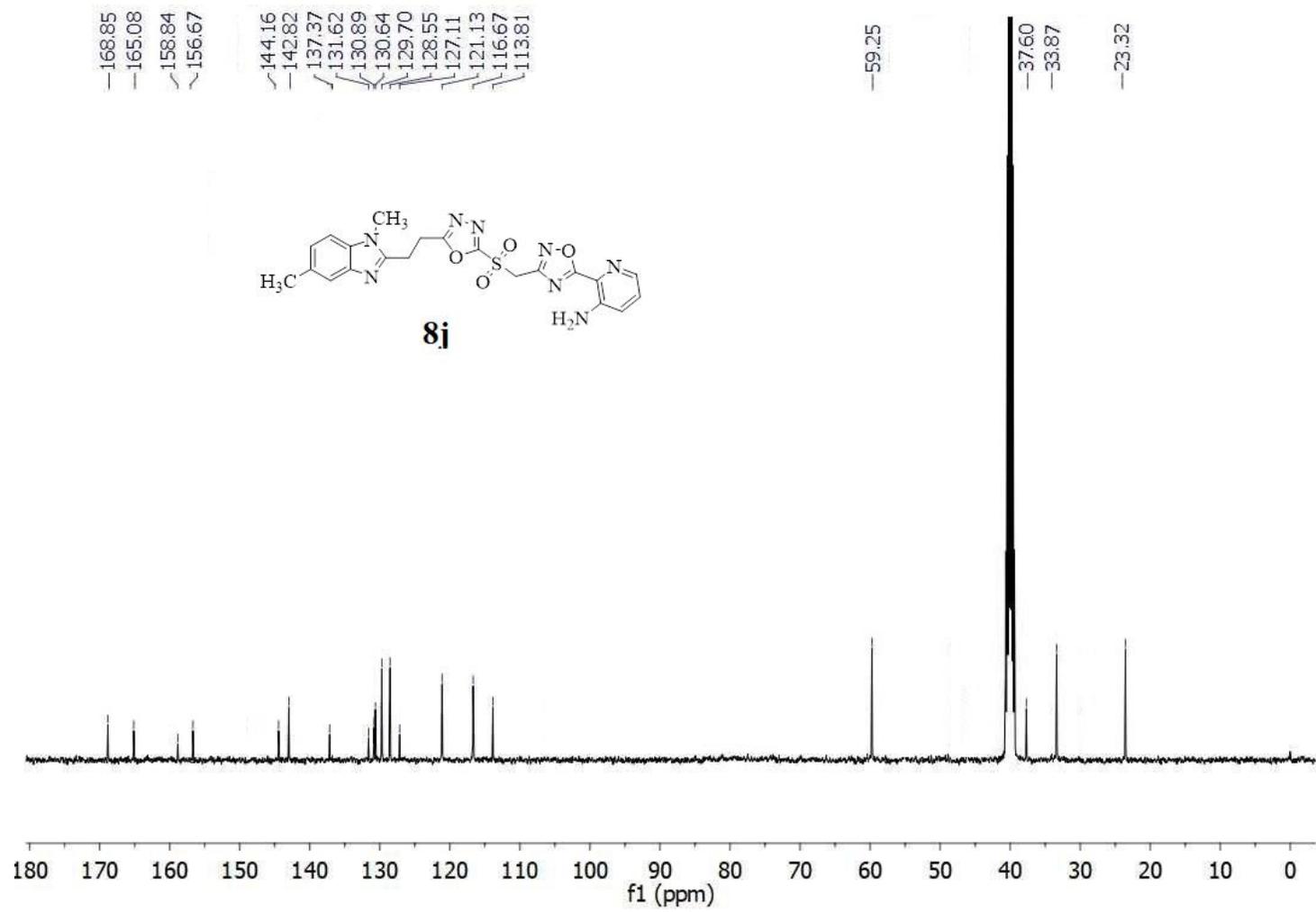
Exact Mass: 480.1328

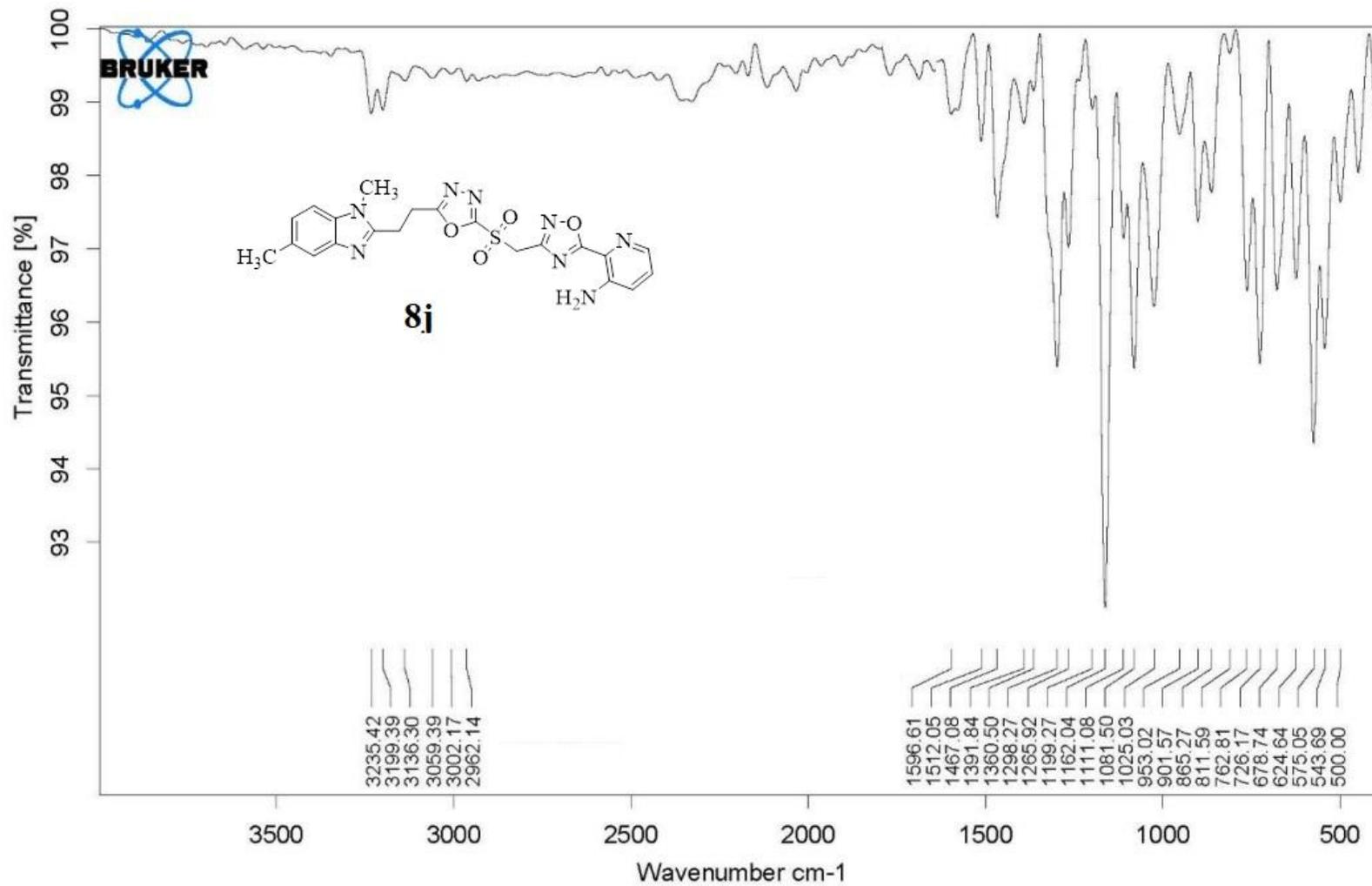
Molecular Weight: 480.4997

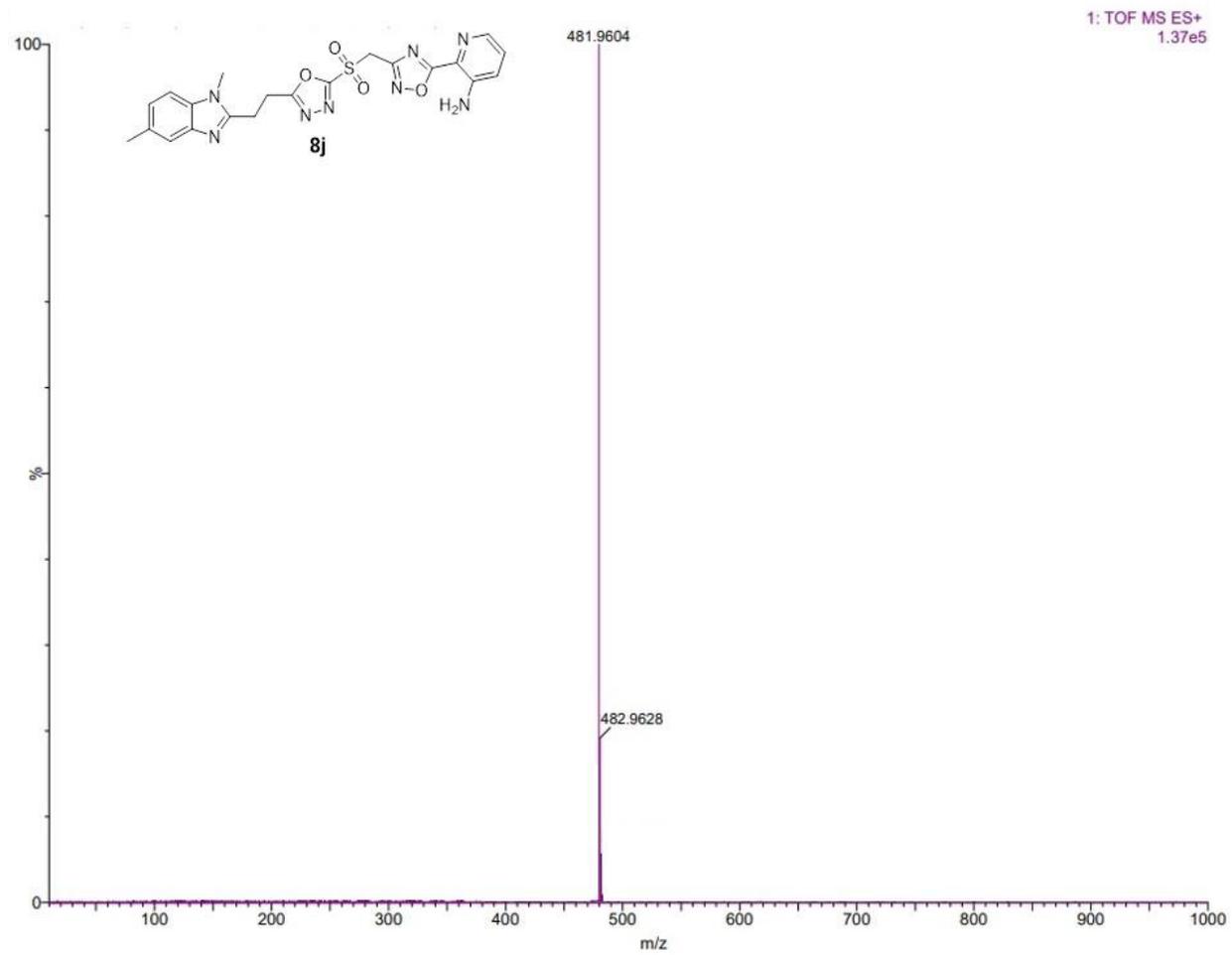
m/z: 480.1328 (100.0%), 481.1362 (22.7%), 482.1286 (4.5%), 481.1299 (3.0%), 482.1395 (2.5%), 483.1320 (1.0%)

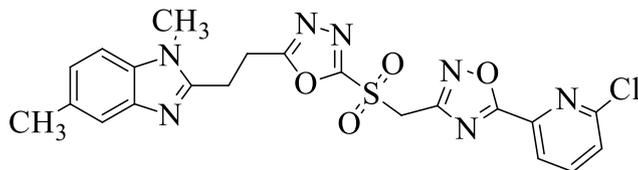
Elemental Analysis: C, 52.49; H, 4.20; N, 23.32; O, 13.32; S, 6.67











Compound 8k

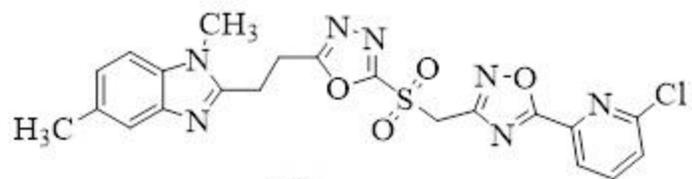
5-(6-chloropyridin-2-yl)-3-(((5-(2-(1,5-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)sulfonyl)methyl)-1,2,4-oxadiazole
 Chemical Formula: C₂₁H₁₈ClN₇O₄S

Exact Mass: 499.0830

Molecular Weight: 499.9301

m/z: 499.0830 (100.0%), 501.0800 (32.0%), 500.0863 (22.7%), 502.0834 (7.3%),
 501.0787 (4.5%), 500.0800 (2.6%), 501.0897 (2.5%), 503.0758 (1.4%), 502.0821 (1.0%)

Elemental Analysis: C, 50.45; H, 3.63; Cl, 7.09; N, 19.61; O, 12.80; S, 6.41



8k

