Supporting Information

Intramolecular oxidative deselenization of acylselenoureas: a facile synthesis of benzoxazole amides and carbonic anhydrase inhibitors

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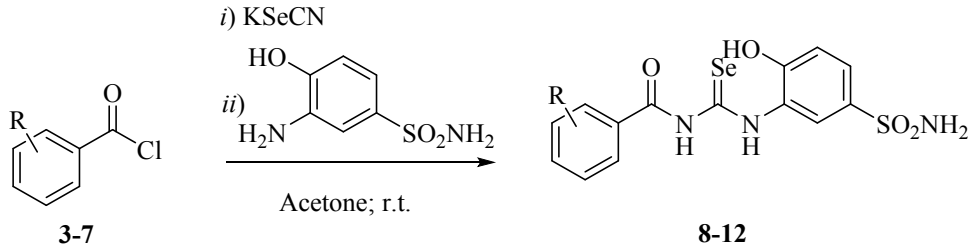
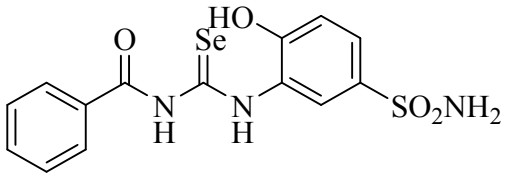
1.1 Chemistry. Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (1H-NMR, 13C-NMR, 19F NMR, 77Se-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (J) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double of doublets. The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase and ethyl acetate/n-hexane were used as eluents. Melting points (mp) were measured in open capillary tubes with a Gallenkamp MPD350.BM3.5 apparatus and are uncorrected.

The solvents used in MS measurements were acetone, acetonitrile (Chromasolv grade), purchased from Sigma-Aldrich (Milan - Italy), and mQ water 18 MΩ, obtained from Millipore's Simplicity system (Milan-Italy). The mass spectra were obtained using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped by Electrospray Source (ESI) operating in both positive and negative ions. The high resolution mass spectrometry analysis (HRMS) were performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electrospray ionization source (ESI). The analysis were carried out in positive ion mode observing the cluster of the protonated molecules $[M+H]^+$ using a proper dwell time acquisition to achieve 60,000 units of resolution at Full Width at Half Maximum (FWHM). The elemental composition of each compound was formulated on the basis of the measured accurate mass of the most intense signal of the considered cluster. They were accepted only results with an attribution error Delta less than 5 ppm and a not integer RDB (double bond/ring equivalents) value, in order to consider only the protonated species. Stock solutions of analytes were prepared in acetone at 1.0 mg mL^{-1} and stored at 4°C. Working solutions of each analyte were freshly prepared by diluting stock solutions in a mixture of mQ H_2O/ACN 1/1 (v/v) up to a concentration of 1.0 µg mL^{-1} The mass spectra of each analyte were acquired by introducing, via syringe pump at 10 µL min^{-1}, of the its working solution.

1.1.1 General procedure for the synthesis of compounds 8-12.[1] Potassium selenocyanate (0.14 g, 1.0 mmol) dissolved in acetone (5.0 mL) was treated with the appropriate acyl chloride 3-7 (1.0 mmol). The reaction mixture was stirred at r.t. for 15 min, followed by addition of 4-hydroxymetanilamide (1.0 mmol) and stirred for 40 min at the same temperature. After this time the mixture was quenched with H_2O and the formed precipitate was filtered-off and dried on air. The obtained products **8-12** were used as they are.



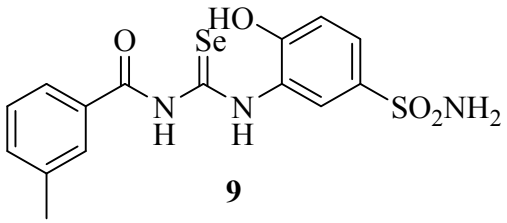
1.1.2 Synthesis of *N*-((2-hydroxy-5-sulfamoylphenyl)carbamoselenoyl)benzamide 8.



8

N-((2-Hydroxy-5-sulfamoylphenyl)carbamoselenoyl)benzamide **8** was obtained according to the above reported general procedure 1.1.1 using benzoyl chloride **3** (0.14 g) and potassium selenocyanate (1.0 eq). Yield 63%, 0.25 g; yellow solid, 1H-NMR (DMSO-d_6, 400 MHz): 13.39 (1H, s, N*H*, exchange with D_2O), 11.89 (1H, s, N*H*, exchange with D_2O), 11.17 (1H, s, O*H*, exchange with D_2O), 9.12 (1H, d, *J*=1.98), 8.02 (2H, d, *J*=7.78), 7.72 (1H, t, *J*=7.42), 7.66 (1H, dd, *J*=8.55, J^3=2.24), 7.59 (2H, t, *J*=7.71), 7.26 (2H, brs, NH_2, exchange with D_2O), 7.13 (1H, d, *J*=8.57); 13C-NMR (DMSO-d_6, 100 MHz): 180.1 (*C*=Se), 169.3 (*C*=O), 153.3 (*C*OH), 134.9, 134.2, 132.7, 129.7, 129.3, 127.1, 126.2, 123.0, 116.2; 77Se-NMR (DMSO-d_6, 76 MHz): 435; HRMS *m/z* $[M+H]^+$ calcd for $C_{14}H_{14}N_3O_4SSe$, 399.9865; found, 399.9872.

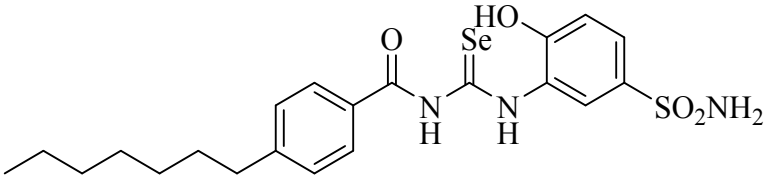
1.1.3 Synthesis of N-((2-Hydroxy-5-sulfamoylphenyl)carbamoselenoyl)-3-methylbenzamide 9.



9

N-((2-Hydroxy-5-sulfamoylphenyl)carbamoselenoyl)-3-methylbenzamide **9** was obtained according to the above reported general procedure **1.1.1** using 3-methylbenzoyl chloride **4** (0.15 g) and potassium selenocyanate (1.0 eq). Yield 77%, 0.32 g; yellow solid, 1H-NMR (DMSO-d_6, 400 MHz): 13.44 (1H, s, N*H*, exchange with D_2O), 11.82 (1H, s, N*H*, exchange with D_2O), 11.20 (1H, s, O*H*, exchange with D_2O), 9.16 (1H, d, *J*=1.43), 7.89 (1H, s), 7.83 (1H, d, *J*=7.51), 7.68 (1H, dd, *J*=8.50, J^3=1.90), 7.55-7.46 (2H, m), 7.29 (2H, brs, NH_2, exchange with D_2O), 7.15(1H, d, *J*=8.55), 3.48 (3H,s); 13C-NMR (DMSO-d_6, 100 MHz): 180.1 (*C*=Se), 169.3 (*C*=O), 153.3 (*C*OH), 138.8, 135.0, 134.9, 132.6, 130.2, 129.3, 127.2, 126.9, 126.3, 123.0, 116.3, 21.7; 77Se-NMR (DMSO-d_6, 76 MHz): 434; HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{16}N_3O_4SSe$, 414.0021; found, 414.0031.

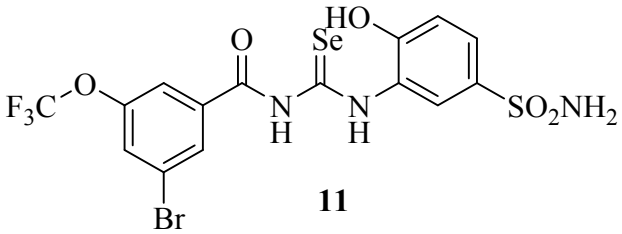
1.1.4 Synthesis of 4-n-Heptyl-N-((2-hydroxy-5-sulfamoylphenyl)carbamoselenoyl)benzamide 10.



10

4-*n*-Heptyl-*N*-((2-hydroxy-5-sulfamoylphenyl)carbamoselenoyl)benzamide **10** was obtained according to the above reported general procedure **1.1.1** using 4-*n*-heptylbenzoyl chloride **5** (0.24 g) and potassium selenocyanate (1.0 eq). Yield 91%, 0.45 g; yellow solid, ^{1}H-NMR (DMSO-d_6, 400 MHz): 13.46 (1H, s, N*H*, exchange with D_2O), 11.72 (1H, s, N*H*, exchange with D_2O), 11.22 (1H, s, O*H*, exchange with D_2O), 9.17 (1H, d, *J*=1.43), 7.96 (2H, d, *J*=8.15), 7.67 (1H, dd, *J*=8.51, J^3=1.89), 7.39 (2H, d, *J*=7.83), 7.26 (2H, brs, NH_2, exchange with D_2O), 7.14(1H, d, *J*=8.55), 2.68 (2H,t, *J*=7.27), 1.64 (2H, brs), 1.28-1.26 (8H, m), 0.87 (3H, t, *J*=6.73); ^{13}C-NMR (DMSO-d_6, 100 MHz): 180.0 (*C*=Se), 169.1 (*C*=O), 153.3 (*C*OH), 149.5, 135.0, 129.9, 129.4, 128.5, 127.3, 126.3, 122.9, 116.3, 36.1, 32.2, 31.5, 29.6, 29.5, 23.1, 14.9; ^{77}Se-NMR (DMSO-d_6, 76 MHz): 432; HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{28}N_3O_4SSe$, 498.0961; found, 498.0972.

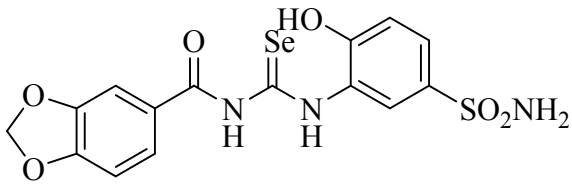
1.1.4 Synthesis of 3-Bromo-N-((2-hydroxy-5-sulfamoylphenyl)carbamoselenoyl)5(trifluoromethoxy)benzamide 11.



11

3-Bromo-*N*-((2-hydroxy-5-sulfamoylphenyl)carbamoselenoyl)-5-(trifluoromethoxy)benzamide **11** was obtained according to the above reported general procedure **1.1.1** using 3-bromo-5-(trifluoromethoxy)benzoyl chloride **6** (0.30 g) and potassium selenocyanate (1.0 eq). Yield 84%, 0.47 g; yellow solid, 1H-NMR (DMSO-d_6, 400 MHz): 13.19 (1H, s, N*H*, exchange with D_2O), 12.27 (1H, s, N*H*, exchange with D_2O), 11.24 (1H, s, O*H*, exchange with D_2O), 9.13 (1H, d, *J*=2.10), 8.25 (1H, t, *J*=1.40), 8.03 (1H, s), 7.97 (1H, s), 7.66 (1H, dd, *J*=8.55, J^3=2.26), 7.26 (2H, brs, NH_2, exchange with D_2O), 7.12 (1H,d, *J*=8.57); 13C-NMR (DMSO-d_6, 100 MHz): 179.8 (*C*=Se), 166.5 (*C*=O), 153.3 (*C*OH), 149.3, 136.7, 135.0, 131.9, 129.4, 127.1, 126.4, 123.0, 122.9, 121.7, 116.3; 19F-NMR (DMSO-d_6, 376 MHz): -56.86; HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}BrF_3N_3O_5SSe$, 561.8790; found, 561.8810.

1.1.5 Synthesis of *N*-((2-Hydroxy-5 sulfamoylphenyl)carbamoselenoyl)benzo[d][1,3]dioxole-5-carboxamide 12.



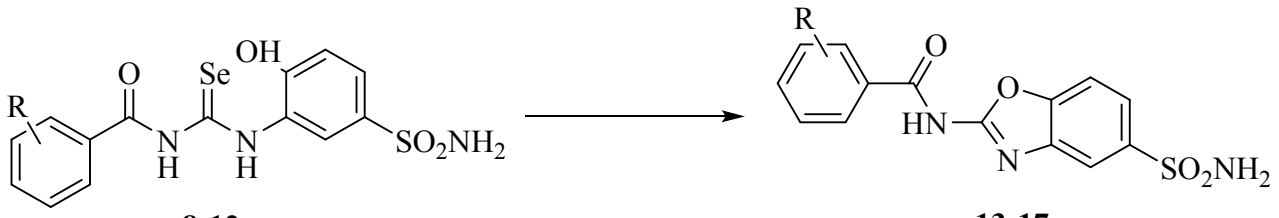
12

N-((2-Hydroxy-5-sulfamoylphenyl) carbamoselenoyl)benzo[d][1,3]dioxole-5-carboxamide **12** was obtained according to the above reported general procedure **1.1.1** using benzo[d][1,3]dioxole-5-carbonyl chloride **7** (0.18 g) and potassium selenocyanate (1.0 eq). Yield 79%, 0.35 g; yellow solid, 1H-NMR (DMSO-d_6, 400 MHz): 13.40 (1H, s, N*H*, exchange with D_2O), 11.66 (1H, s, N*H*, exchange with D_2O), 11.13 (1H, s, O*H*, exchange with D_2O), 9.11 (1H, d, *J*=1.82), 7.70-7.64 (2H, m), 7.58 (1H, d, *J*=1.43), 7.26 (2H, brs, NH_2, exchange with D_2O), 7.11 (2H, t, *J*=7.89), 6.20 (2H, s); 13C-NMR (DMSO-d_6, 100 MHz): 180.0 (*C*=Se), 168.1 (*C*=O), 153.3 (*C*OH), 152.6, 148.4, 134.9, 127.2, 126.2, 126.1, 126.0, 123.1, 116.3, 109.5, 108.9, 103.2; 77Se-NMR (DMSO-d_6, 76 MHz): 430; HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{14}N_3O_6SSe$, 443.9763; found, 443.9774.

1.1.6 General procedure for Synthesis Compounds 13-17.

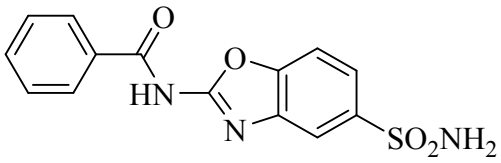
Method A: The appropriate acylselenourea **8-12** (0.5 mmol) was dissolved in a 1:1 biphasic mixture of acetonitrile and 3M NaOH aqueous solution, and was vigorously stirred overnight at r.t. The black solid residue (Se^0) was filtered-off and the reaction was quenched with a 3M HCl aqueous solution until a precipitate was formed, which was collected by filtration and dried on air and used as it is.

Method B: The appropriate acylselenourea **8-12** (0.5 mmol) was dissolved in pyridine (5.0 ml) and stirred onernight at r.t. The black solid residue (Se^0) was filtered-off and the reaction was quenched with 3M HCl aqueous solution until a precipitate was formed, which was collected by filtration and dried on air and used as it is.



8-12 **13-17**

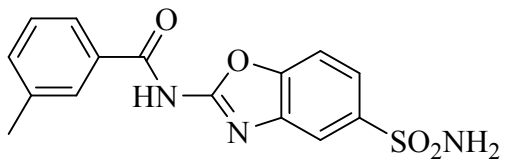
1.1.7 Synthesis of N-(5-Sulfamoylbenzo[d]oxazol-2-yl)benzamide 13.



13

N-(5-Sulfamoylbenzo[d]oxazol-2-yl)benzamide **13** was obtained according to the above reported general procedure **1.1.6** using acylselenourea **8** (0.20 g; 0.5 mmol). Yield 50%, 0.08 g; white solid, 1H-NMR (DMSO-d_6, 400 MHz): 12.32 (1H, s, N*H*, exchange with D_2O), 8.11-8.07 (3H, m), 7.90-7.83 (2H, m), 7.70 (1H, t, *J*=7.30), 7.60 (2H, t, *J*=7.57), 7.49 (2H, brs, NH_2, exchange with D_2O); 13C-NMR (DMSO-d_6, 100 MHz): 158.6, 150.2, 142.0, 133.8, 129.5, 129.4, 122.7, 116.7, 111.5; HRMS *m/z* $[M+H]^+$ calcd for $C_{14}H_{11}N_3O_4S$, 318.0543; found, 318.0550.

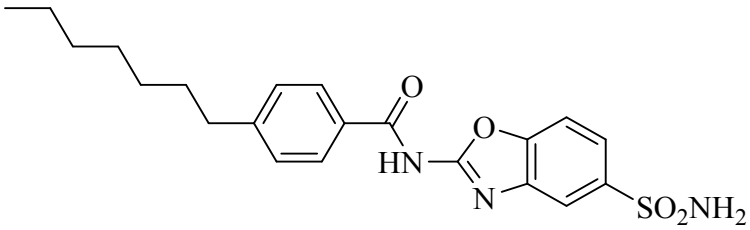
1.1.8 Synthesis of 3-Methyl-N-(5-sulfamoylbenzo[d]oxazol-2-yl)benzamide 14.



14

3-Methyl-*N*-(5-sulfamoylbenzo[d]oxazol-2-yl)benzamide **14** was obtained according to the above reported general procedure **1.1.6** using acylselenourea **9** (0.20 g; 0.5 mmol). Yield 35%, 0.06 g; white solid, 1H-NMR (DMSO-d_6, 400 MHz): 12.25 (1H, s, N*H*, exchange with D_2O), 8.07 (1H, s), 7.90-7.82 (4H, m), 7.53-7.48 (2H, m), 7.46 (2H, brs, NH_2, exchange with D_2O), 2.45 (3H, s); 13C-NMR (DMSO-d_6, 100 MHz): 165.8, 158.2, 150.4, 141.9, 138.8, 134.3, 133.7, 130.6, 129.8, 129.4, 126.5, 122.6, 116.8, 111.4, 21.8; HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{14}N_3O_4S$, 332.0699; found, 332.0706.

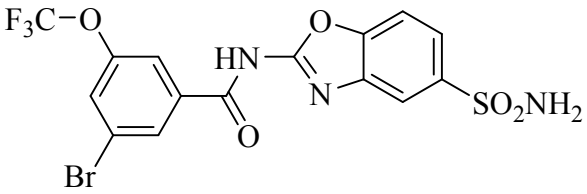
1.1.9 Synthesis of 4-Heptyl-N-(5-sulfamoylbenzo[d]oxazol-2-yl)benzamide 15.



15

4-Heptyl-*N*-(5-sulfamoylbenzo[d]oxazol-2-yl)benzamide **15** was obtained according to the above reported general procedure **1.1.6** using acylselenourea **10** (0.25 g, 0.5 mmol). Yield 29%, 0.06 g; white solid, 1H-NMR (DMSO-d_6, 400 MHz): 12.21 (1H, s, N*H*, exchange with D_2O), 8.08 (1H, s), 8.00 (2H, d, *J*=7.54), 7.90 (1H, d, *J*=8.44), 7.83 (1H, d, *J*=8.15), 7.46 (2H, brs, NH_2, exchange with D_2O), 7.42 (2H, d, *J*=7.82), 2.70 (2H, t, *J*=7.53), 1.64 (2H, t, *J*= 7.53), 1.33-1.29 (8H, m), 0.89 (3H, t, *J*=6.82); 13C-NMR (DMSO-d_6, 100 MHz): 165.5, 158.2, 150.4, 148.9, 141.9, 141.7, 130.9, 129.4, 122.6, 116.8, 111.4, 35.9, 32.1, 31.5, 29.5, 29.4, 22,9, 14.8; HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{26}N_3O_4S$, 416.1638; found, 416.1650.

1.1.10 Synthesis of 3-Bromo-N-(5-sulfamoylbenzo[d]oxazol-2-yl)-5-(trifluoromethoxy)benzamide 16.

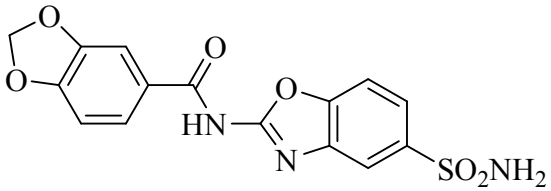


16

3-Bromo-*N*-(5-sulfamoylbenzo[d]oxazol-2-yl)-5-(trifluoromethoxy)benzamide **16** was obtained according to the above reported general procedure **1.1.6** using acylselenourea **11** (0.28 g, 0.5 mmol). Yield 36%, 0.09 g; white solid, 1H-NMR (DMSO-d_6, 400 MHz): 8.30 (1H, s), 8.14 (1H, s), 8.03 (1H, s), 7.78 (1H, s), 7.62 (1H, m), 7.53 (1H, d, *J*=7.86), 7.28 (2H, brs, NH_2, exchange with D_2O); 13C-NMR (DMSO-d_6, 100 MHz): 168.9 (*C*=O), 149.4, 144.6, 139.9, 131.9, 131.3, 126.4,

125.6, 122.5, 122.2, 121.1, 120.3, 119.6, 115.6, 109.3; 19F-NMR (DMSO-d_6, 376 MHz): -56.93; HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{10}BrF_3N_3O_5S$, 479.9471; found, 479.9490.

1.1.11 Synthesis of N-(5-Sulfamoylbenzo[d]oxazol-2-yl)benzo[d][1,3]dioxole-5-carboxamide 17.



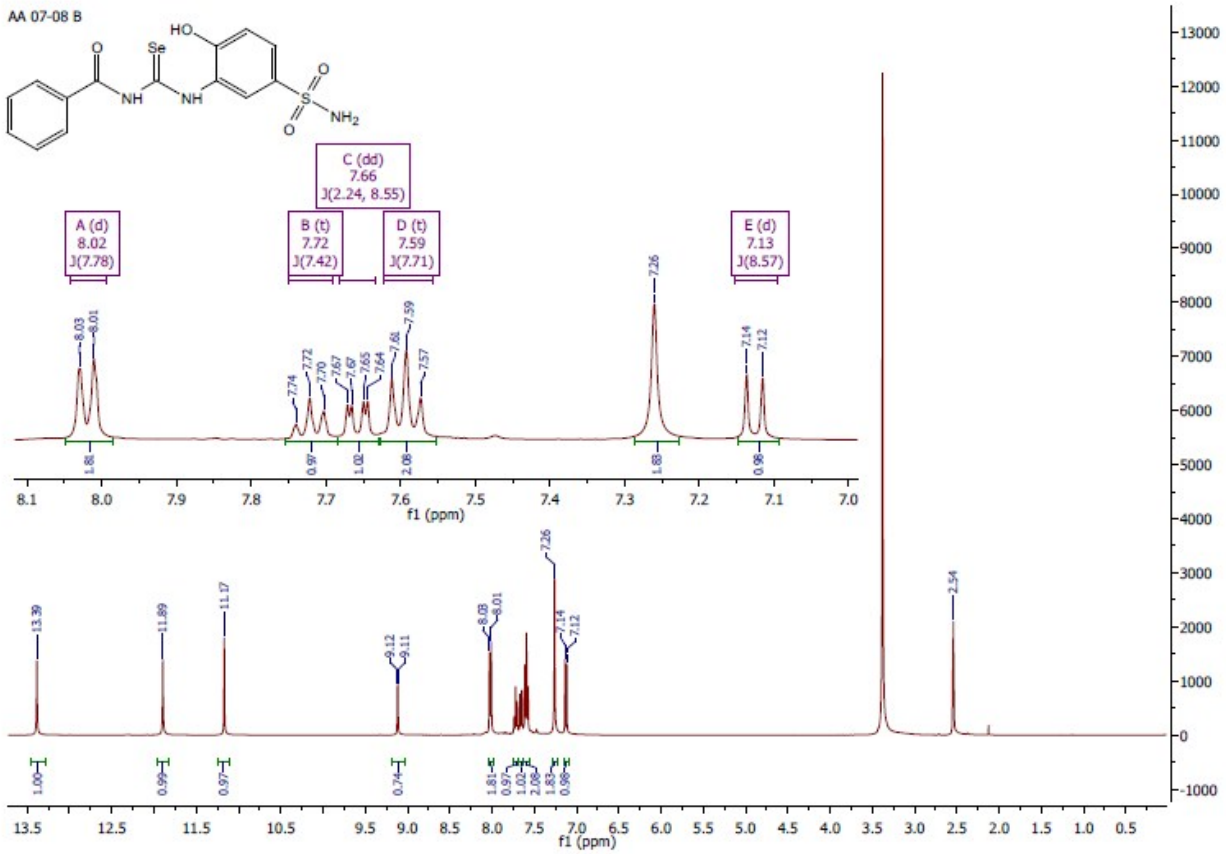
17

N-(5-Sulfamoylbenzo[d]oxazol-2-yl)benzo[d][1,3]dioxole-5-carboxamide **17** was obtained according to the above reported general procedure **1.1.6** using acylselenourea **12** (0.22 g, 0.5 mmol). Yield 70%, 0,13 g; white solid, 1H-NMR (DMSO-d_6, 400 MHz): 8.04 (1H, s),7.88-7.81 (2H, m), 7.73 (1H, d, *J*=7.97), 7.60 (1H, d, *J*=1.62), 7.47 (2H, brs, NH_2, exchange with D_2O), 7.11 (1H, d, *J*= 8.24), 6.19 (2H, s, CH_2); 13C-NMR (DMSO-d_6, 100 MHz): 167.5 (*C*=O), 152.1, 148.5, 141.9, 125.9, 125.6, 125.3, 122.6, 111.4, 109.7, 109.2, 109.1, 109.0, 108.6, 103.0; HRMS *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}N_3O_6S$, 362.0441; found, 362.0451.

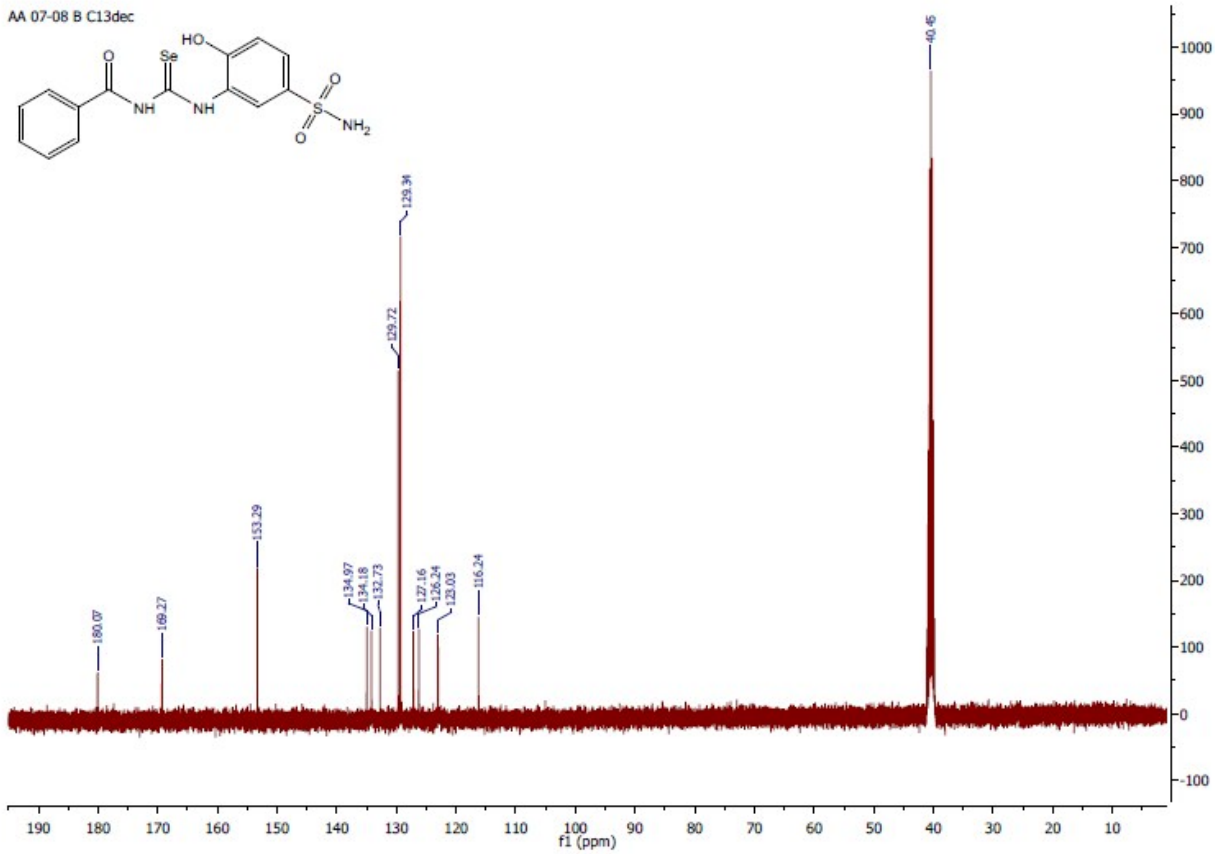
2.0 Protein X-ray Crystallography. hCA II was purified as previously described.[2] The protein was concentrated down to ~7 mg/mL and set up in 96 well SD-2 plates (Molecular Dimensions) using a Phoenix crystallization robot (ARI) with 250 nL of protein plus 250 nL of reservoir and incubated at 8 °C. The compound was added to the crystallization drop directly after crystals had formed. The reservoir condition consisted of 2.7 M ammonium sulfate with 0.1 M Tris buffer at pH 8.3. The crystals were cryo-protected by the addition of glycerol to the reservoir to 20% final concentration and addition of this to the crystals prior to cryo-cooling in liquid N2. Then, 360 frames of one degree oscillation were taken at the MX-1 beamline of the Australian Synchrotron. The data were indexed using XDS[3] and scaled using SCALA.[4] Molecular replacement was done using Phaser[5] using 4cq0 as the initial starting model. The model was manually rebuilt using Coot[6] and refined using Refmac[7]. The compound was placed in density using the program Afitt (OpenEye Scientific Software) and further refined using Refmac.

3.0 CA Inhibition. An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO2 hydration activity. Phenol red (at a concentration of 0.2 mM) has been used as a pH indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) as the buffer and 20 mM Na2SO4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO2 hydration reaction for a period of 10−100 s. The CO2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5−10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled− deionized water, and dilutions up to 0.01 nM were done thereafter with distilled−deionized water. Inhibitor and enzyme solutions were pre-incubated together for 15 min at room temperature prior to assay to allow for the formation of the E−I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3, as reported earlier,[8] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in house as reported earlier.[8]

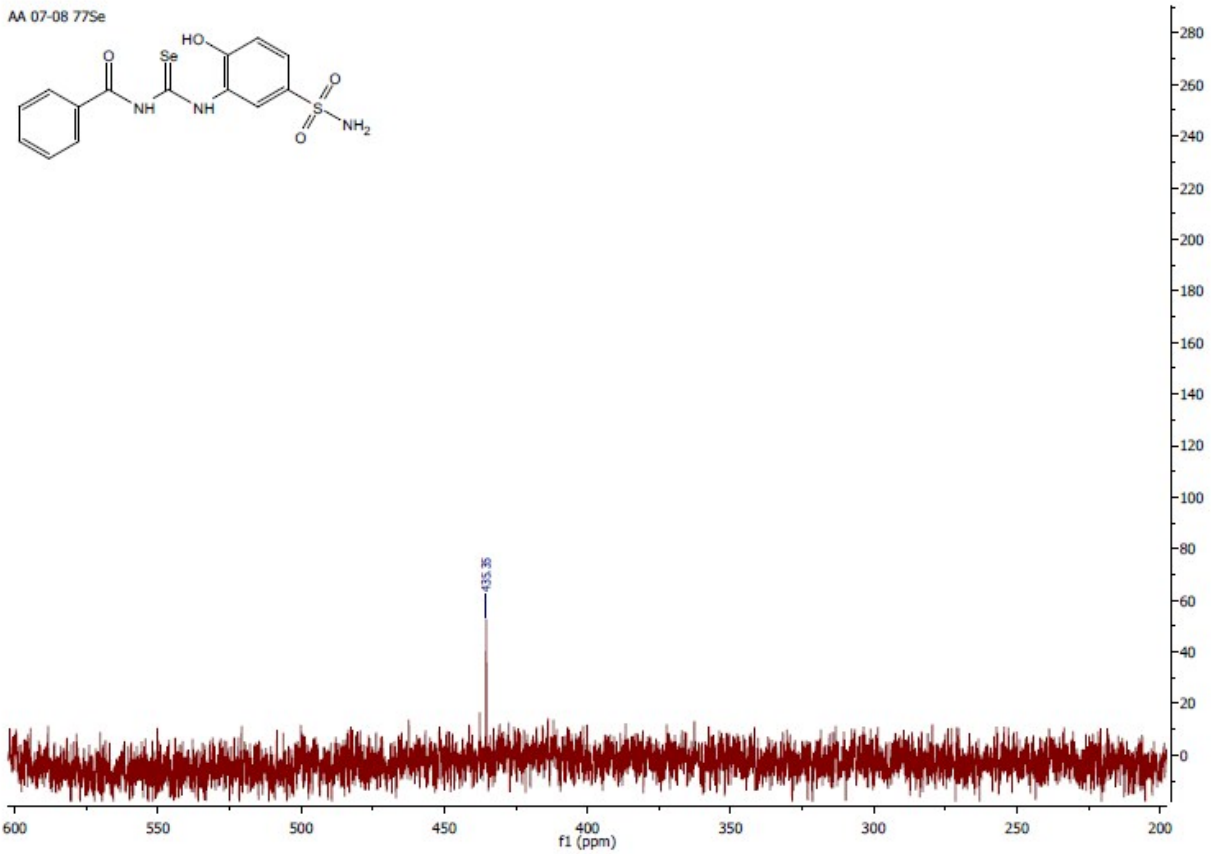
1H-NMR Compound 8



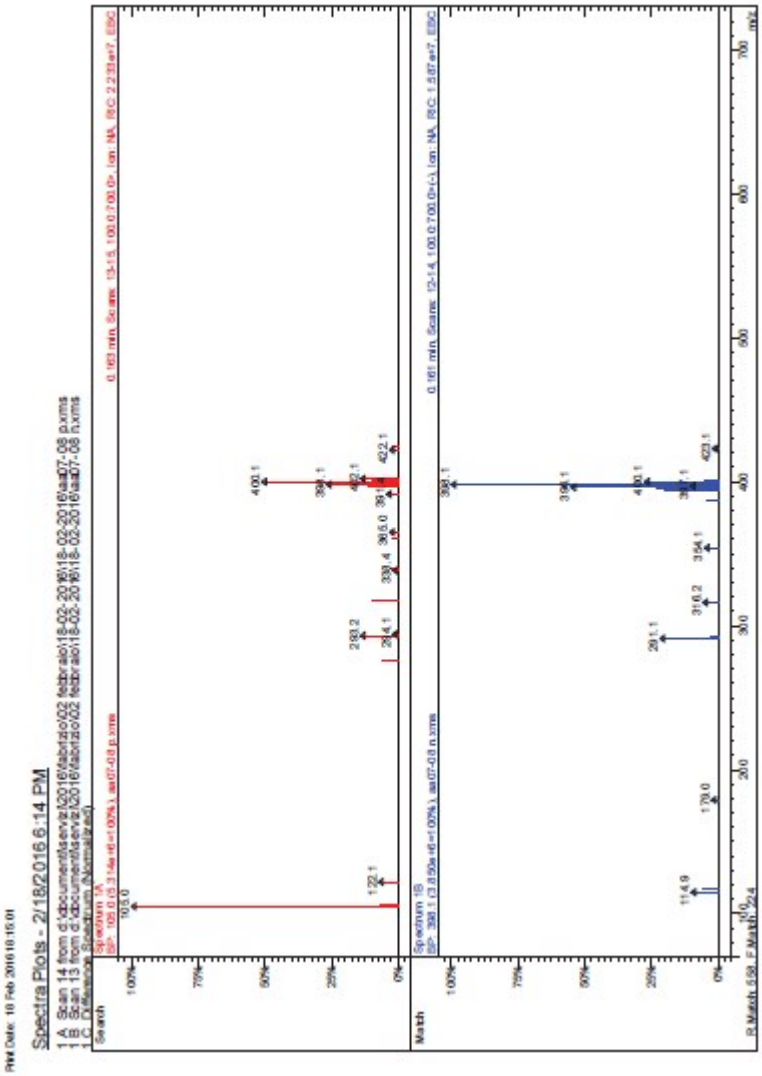
13C-NMR Compound 8



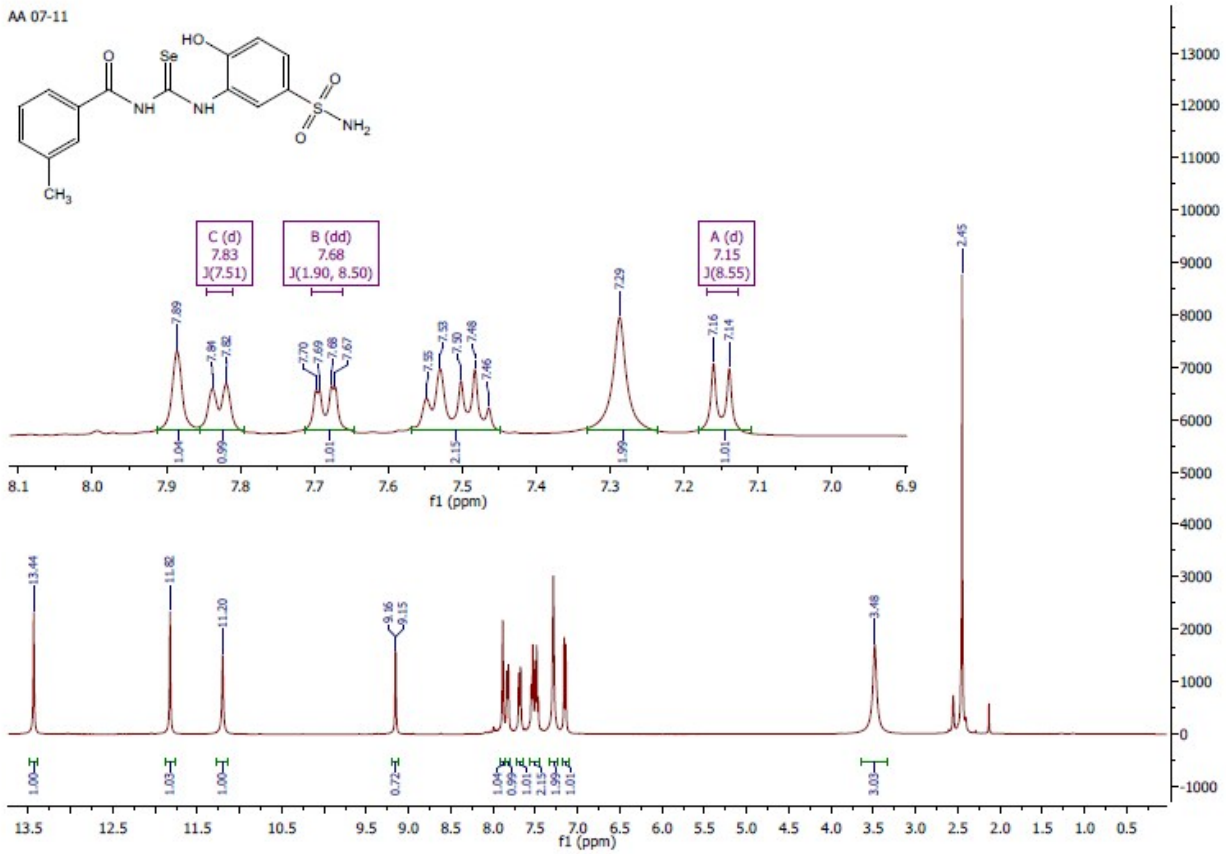
77Se-NMR Compound 8



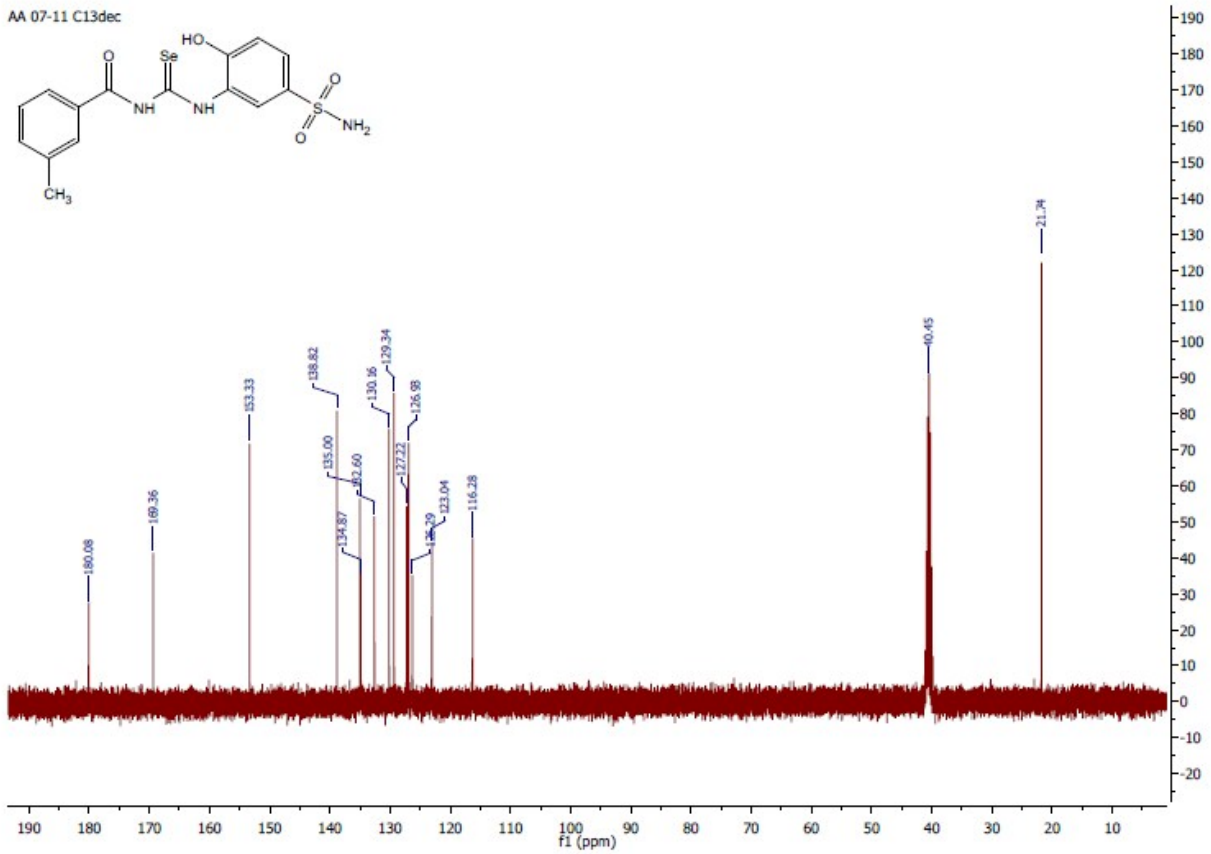
ESI-MS Compound 8



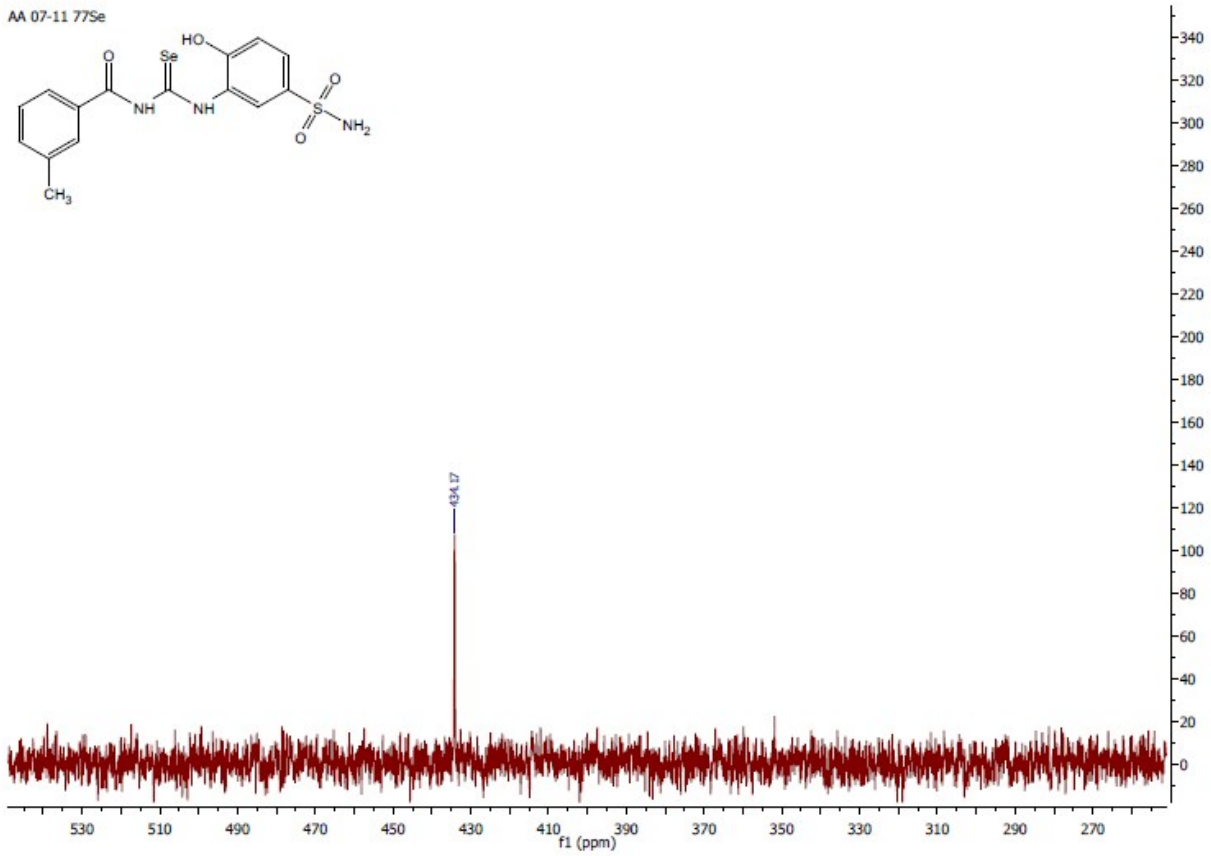
1H-NMR Compound 9



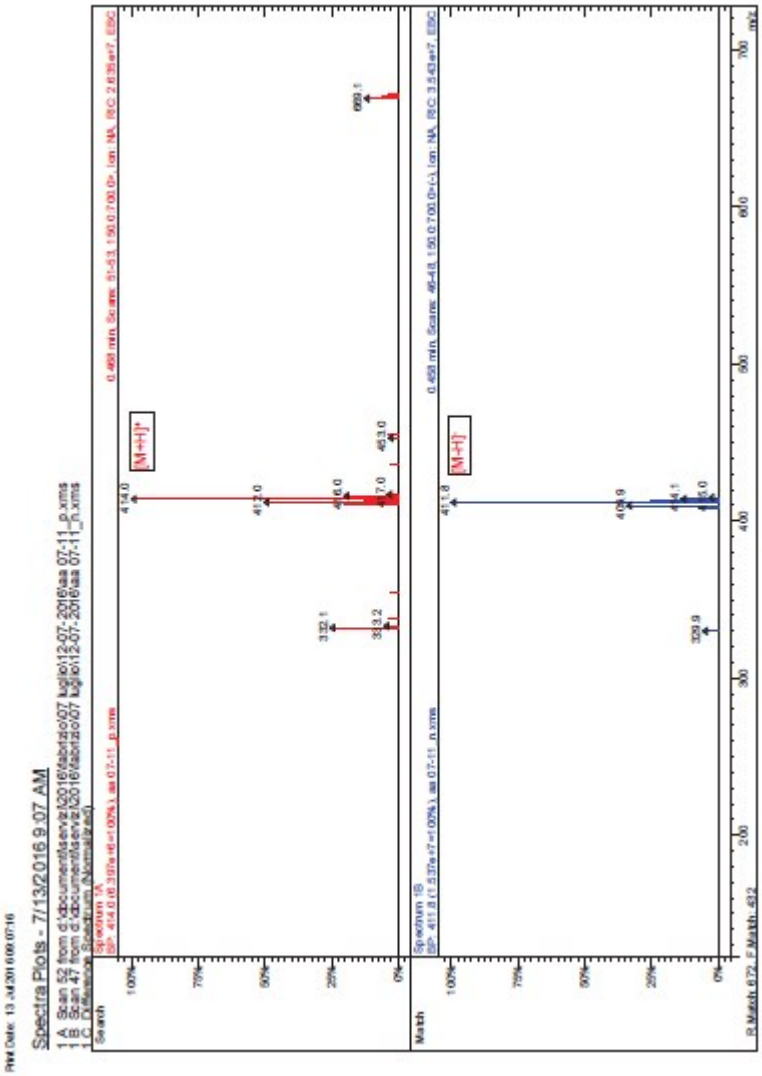
13C-NMR Compound 9



77Se-NMR Compound 9



ESI-MS Compound 9



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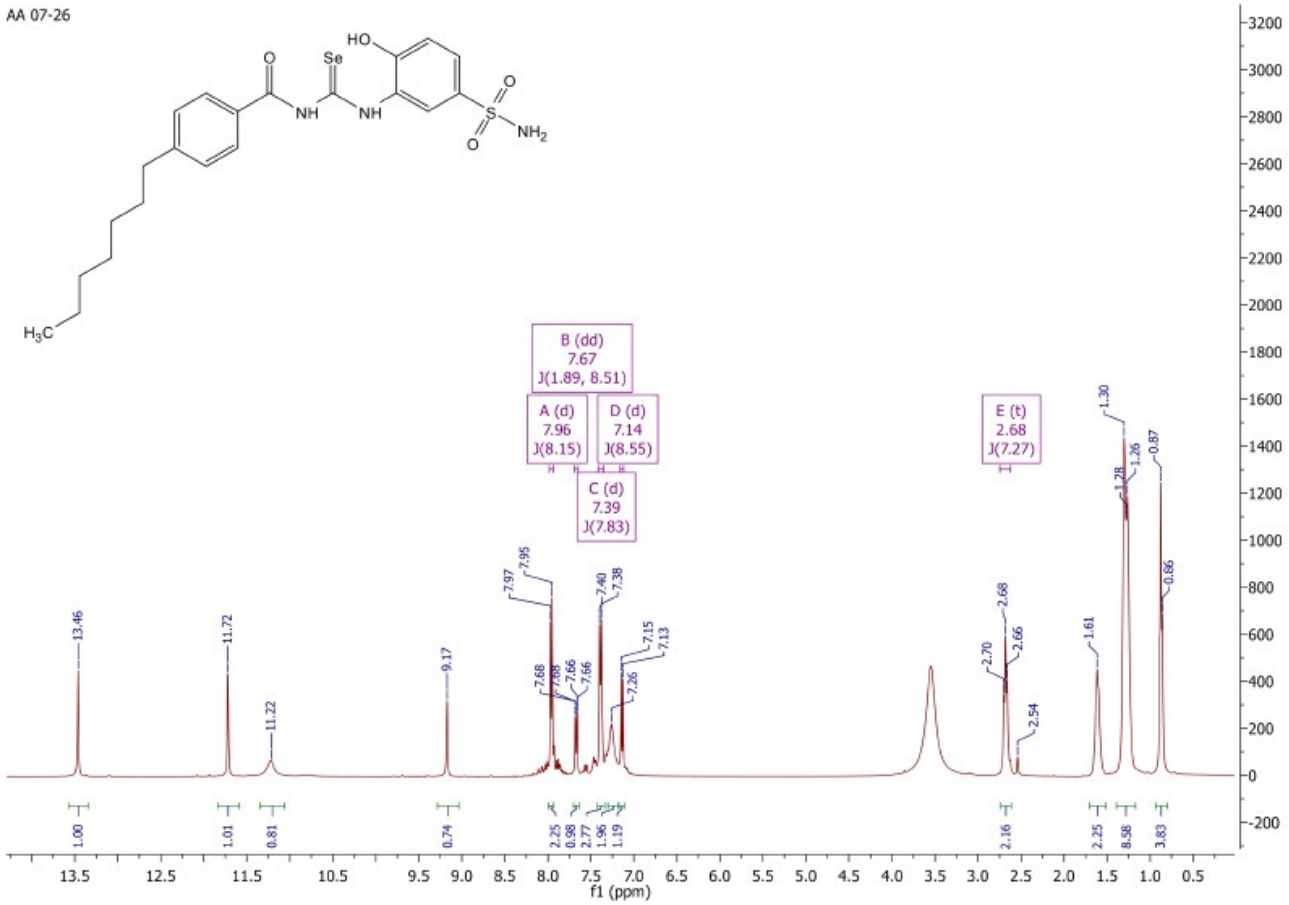
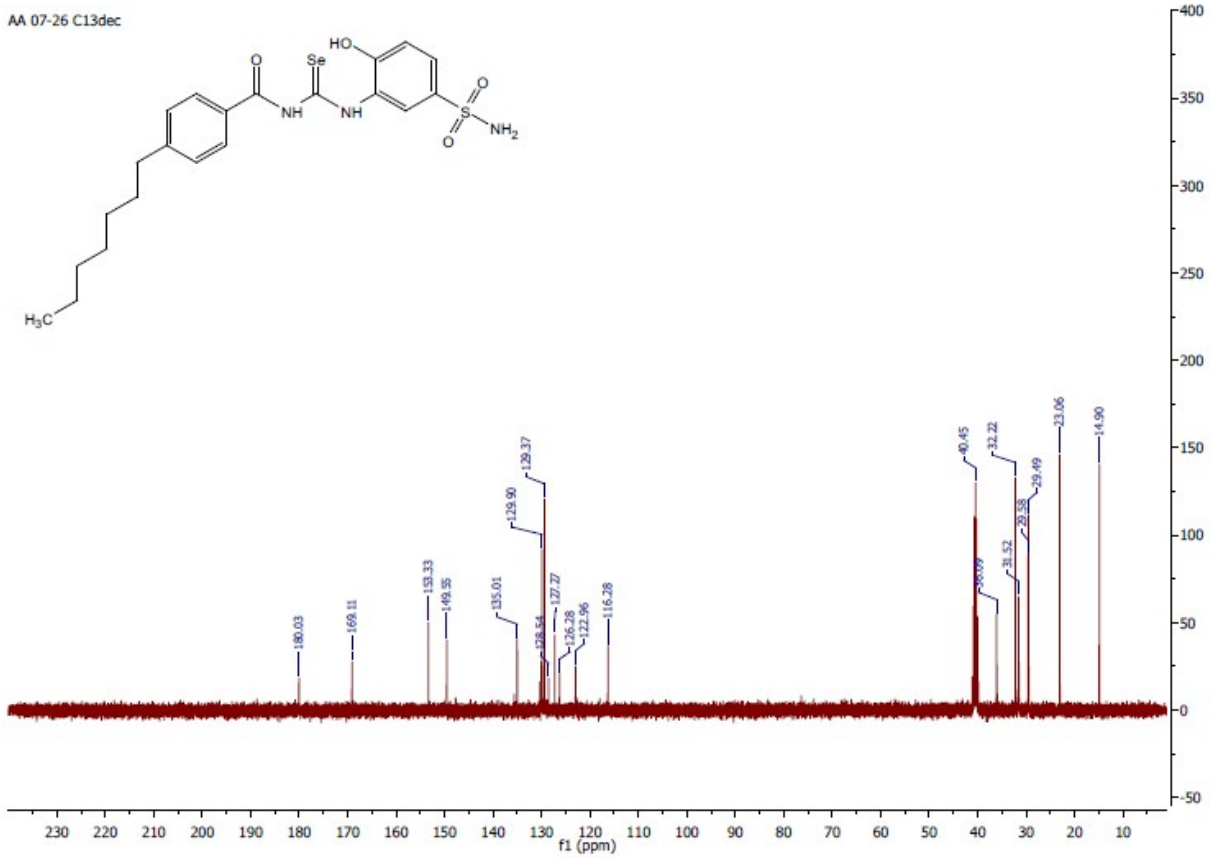


13C-NMR Compound 10



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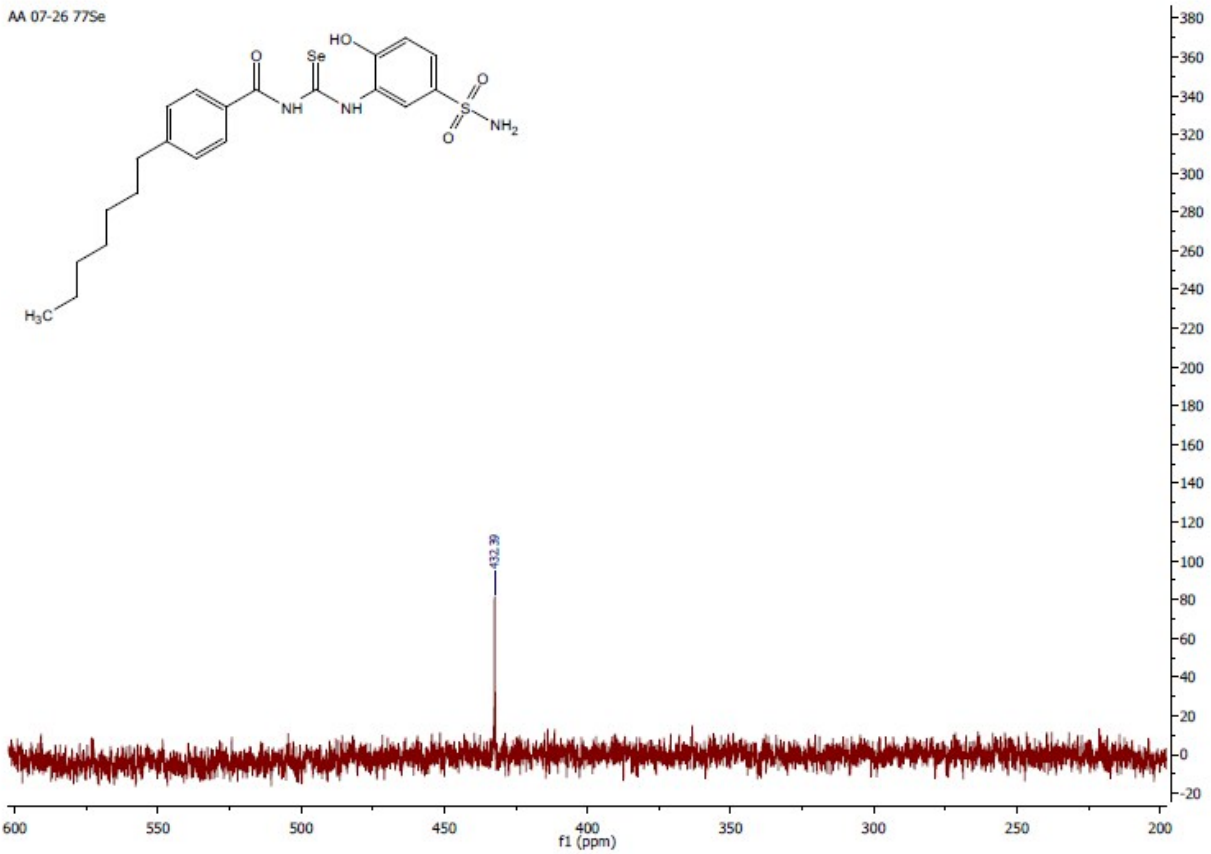

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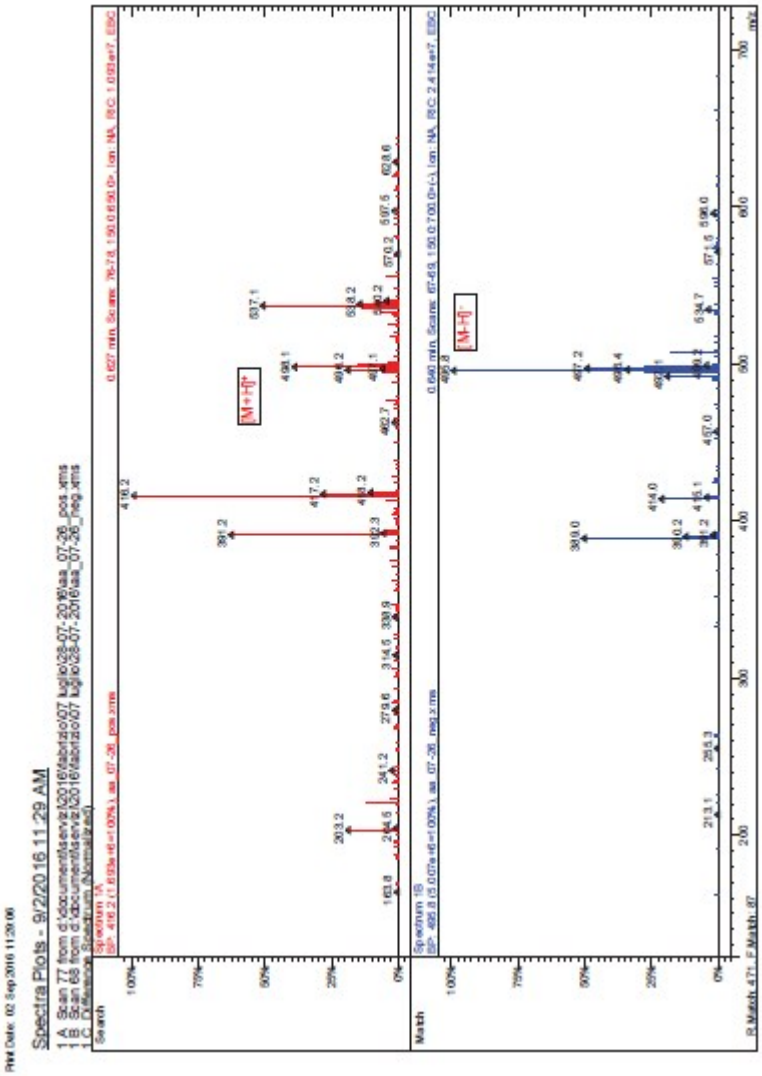
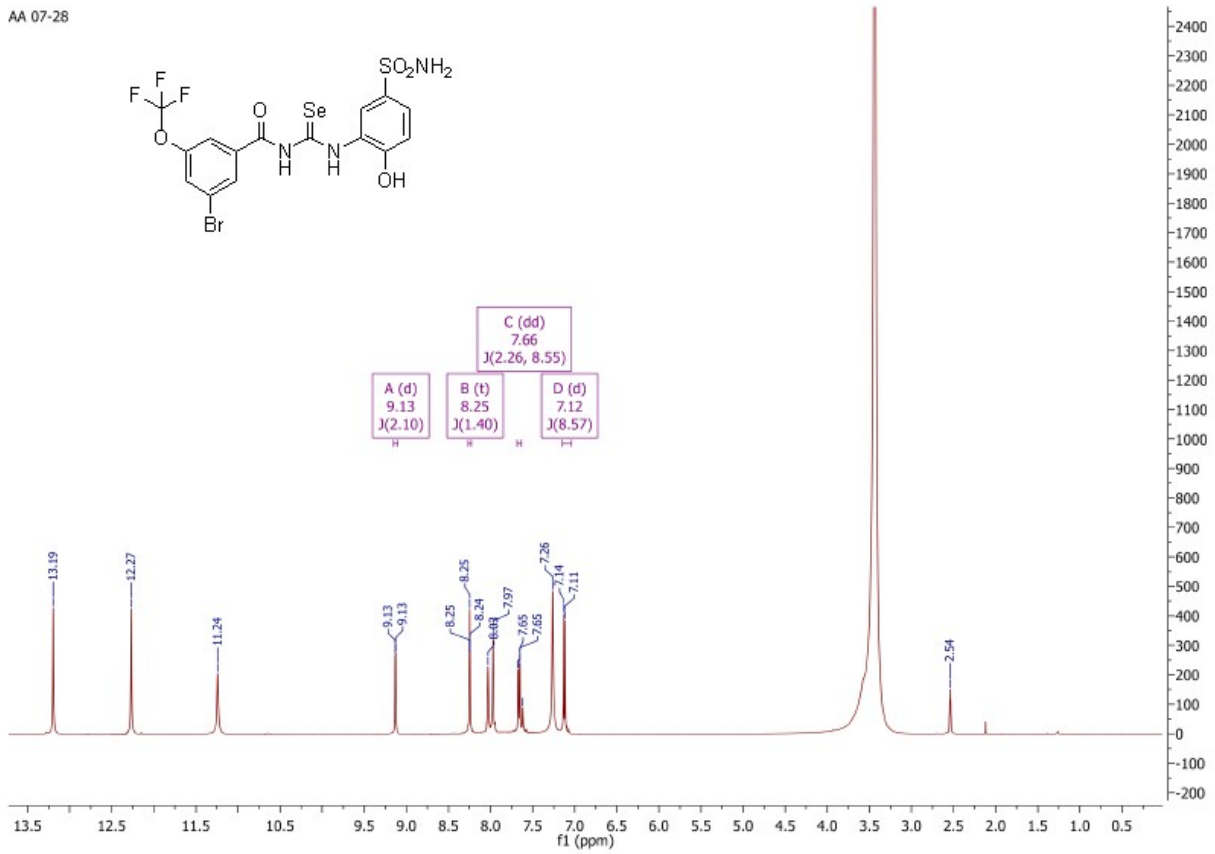


1H-NMR Compound 11



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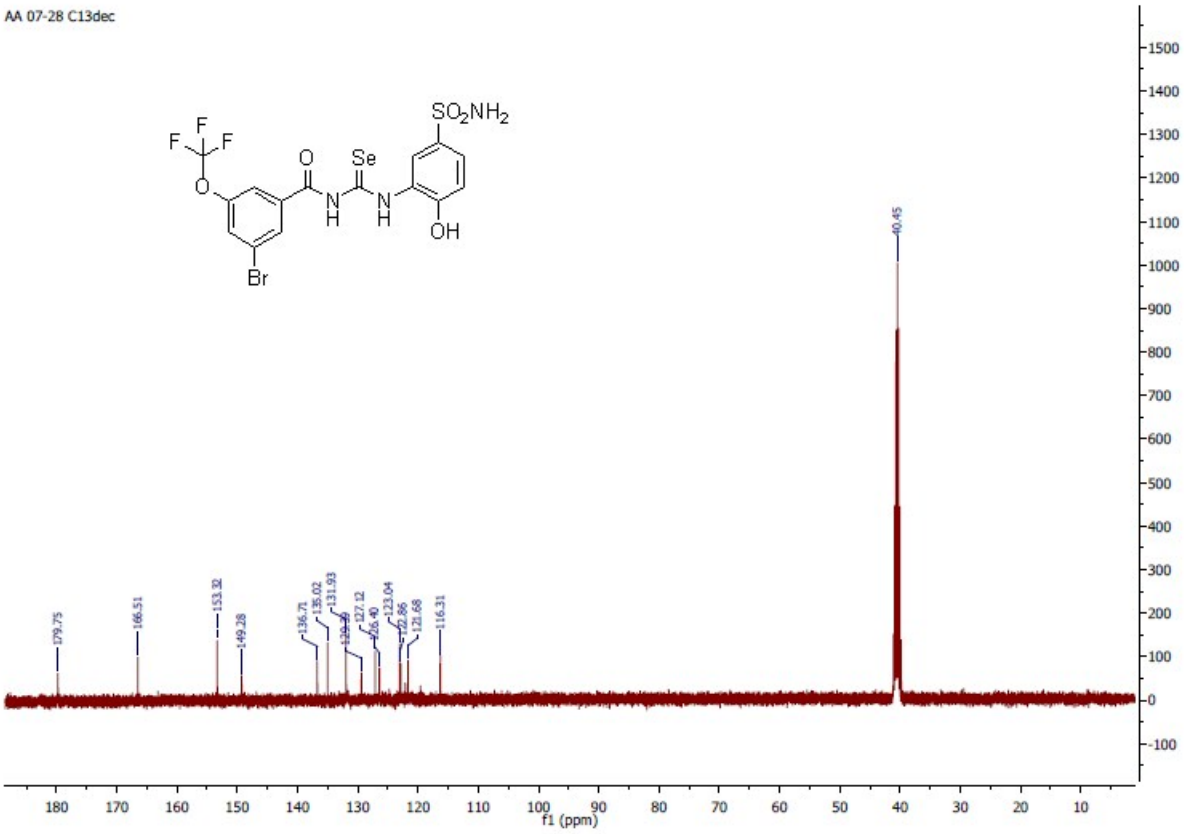
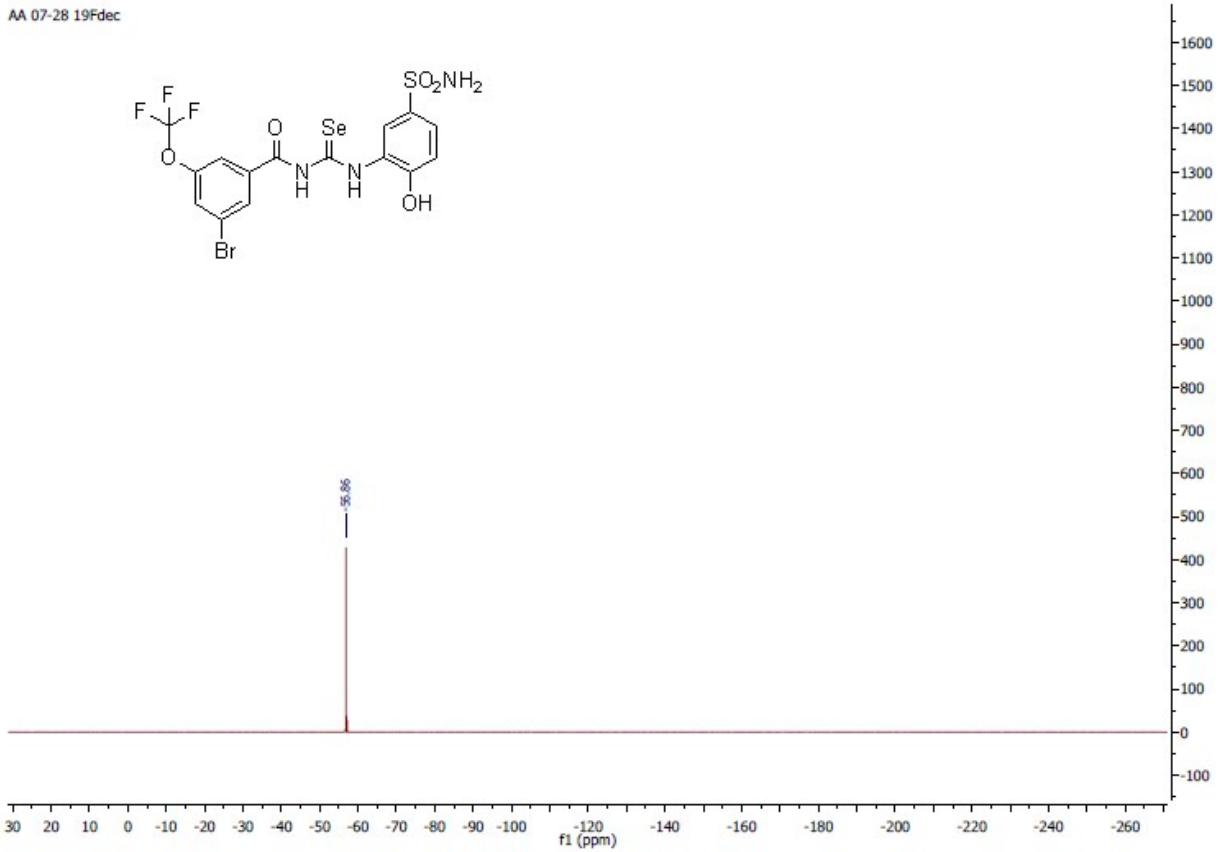


19F-NMR Compound 11



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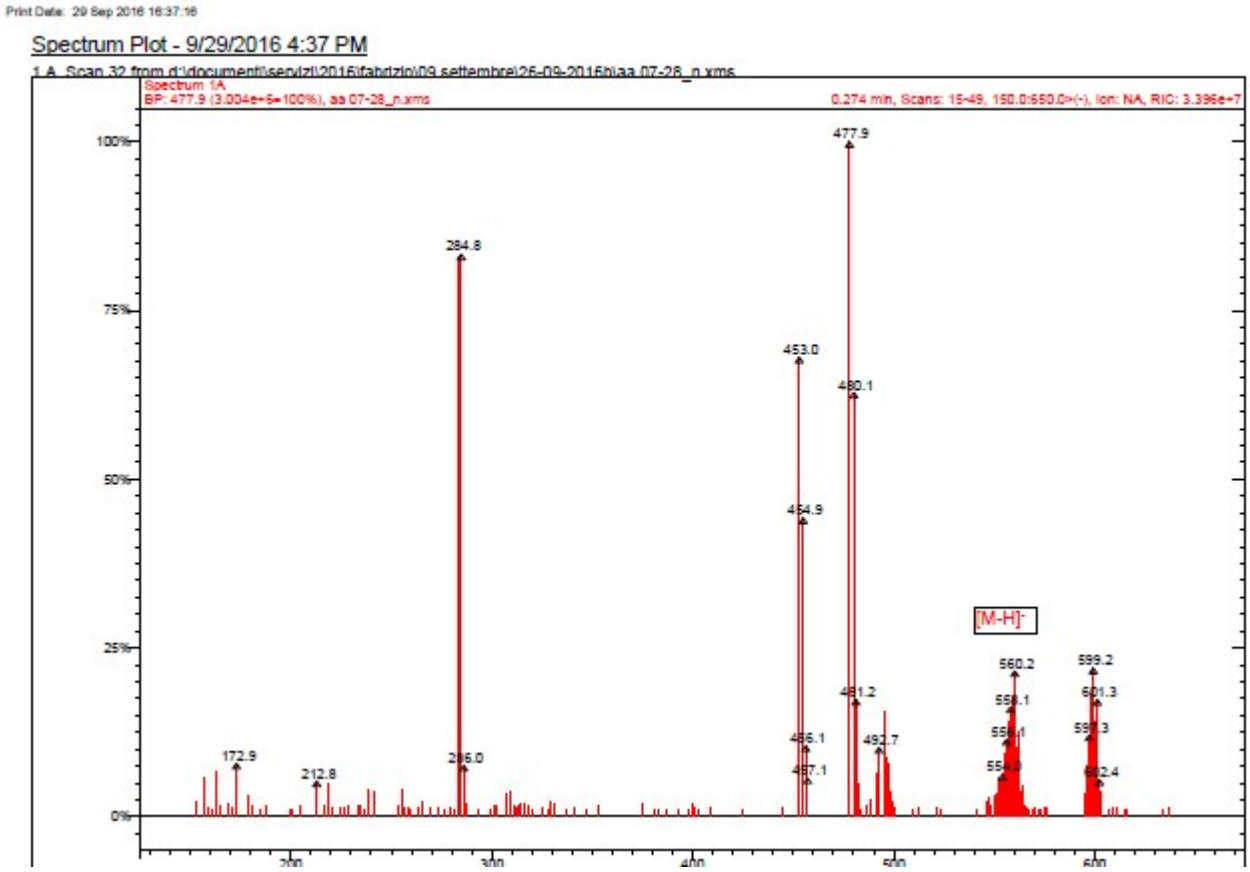
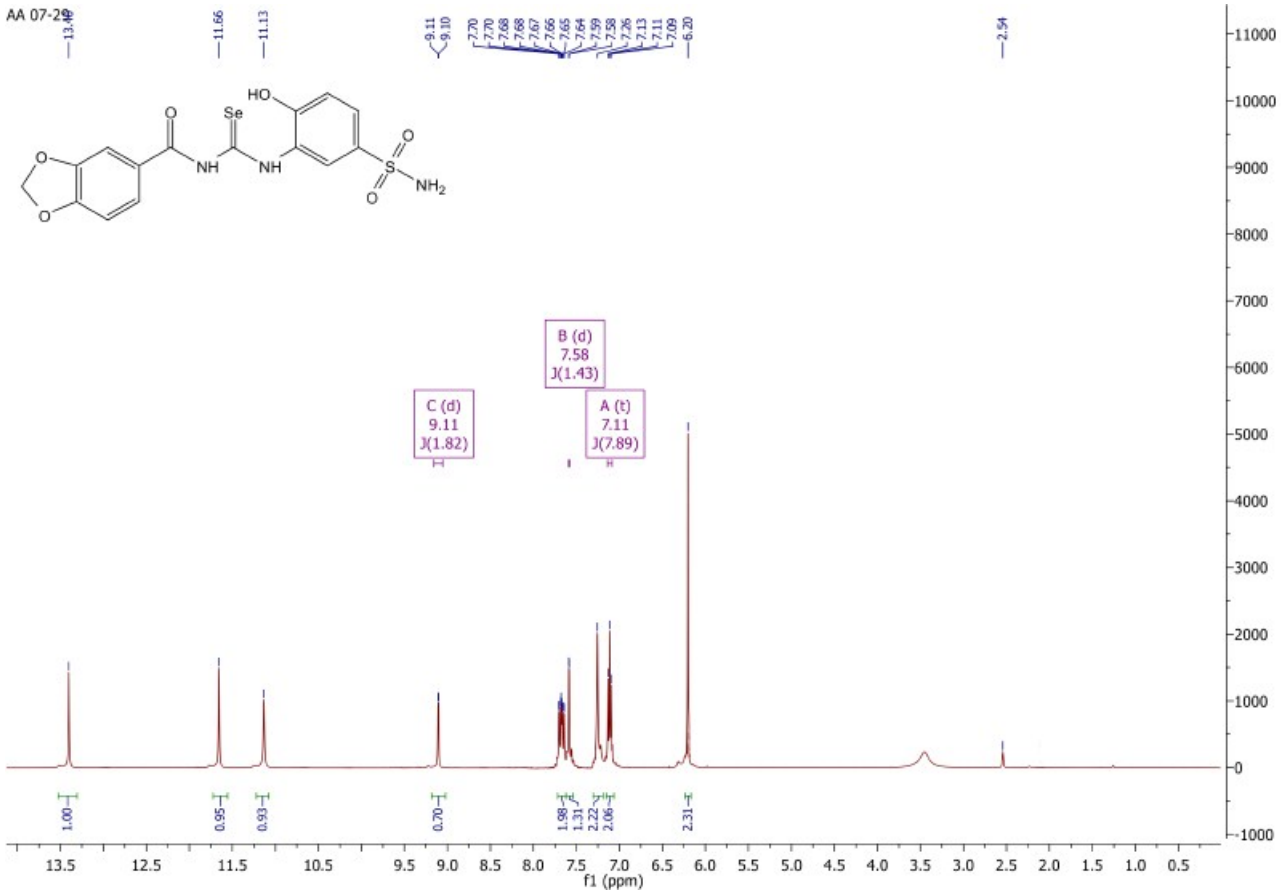
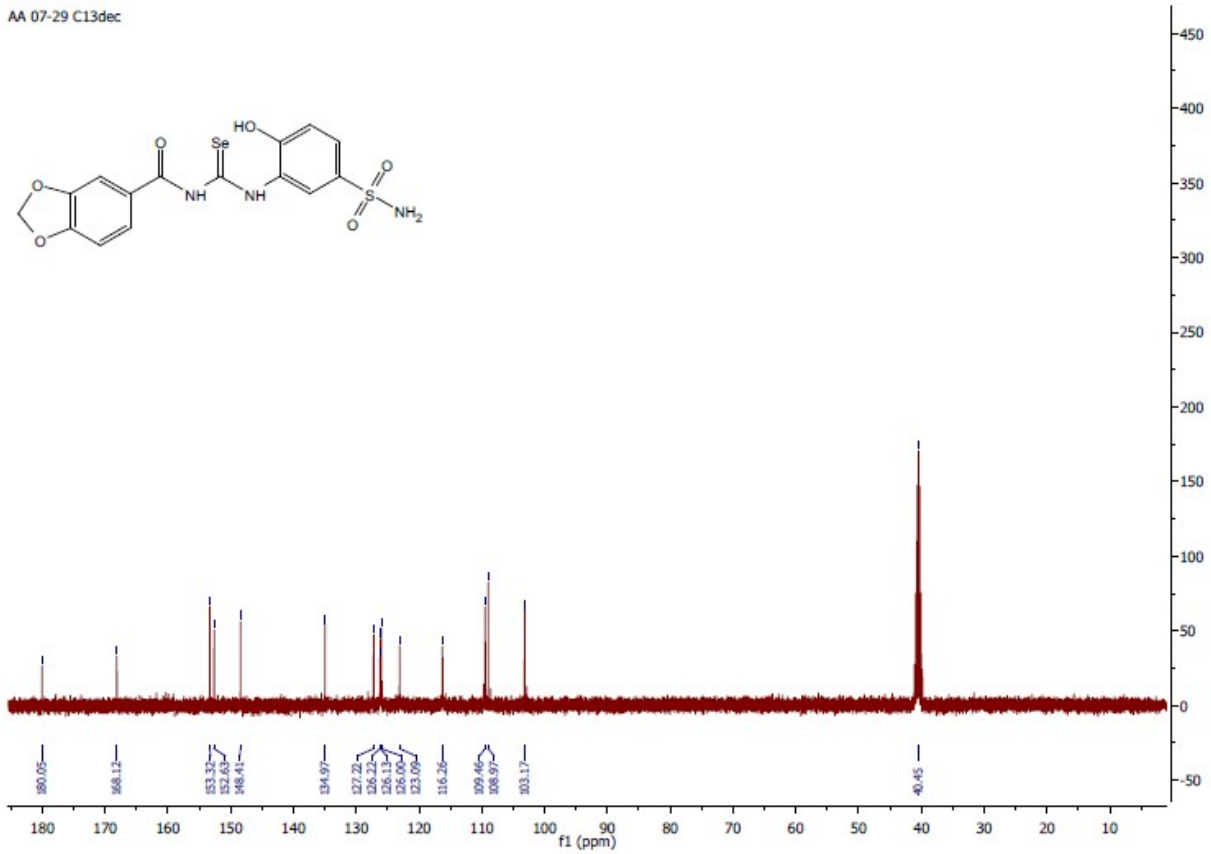


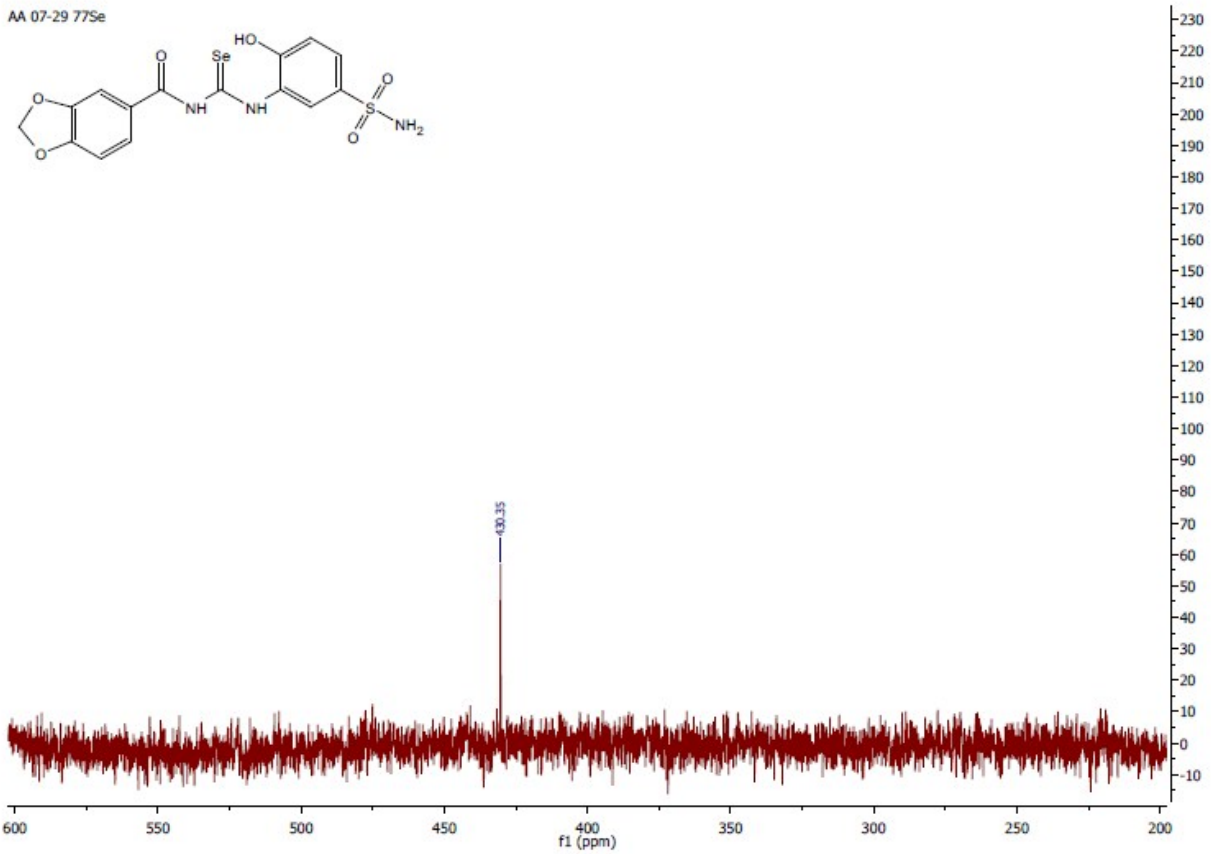
1H-NMR Compound 12



13C-NMR Compound 12



77Se-NMR Compound 12



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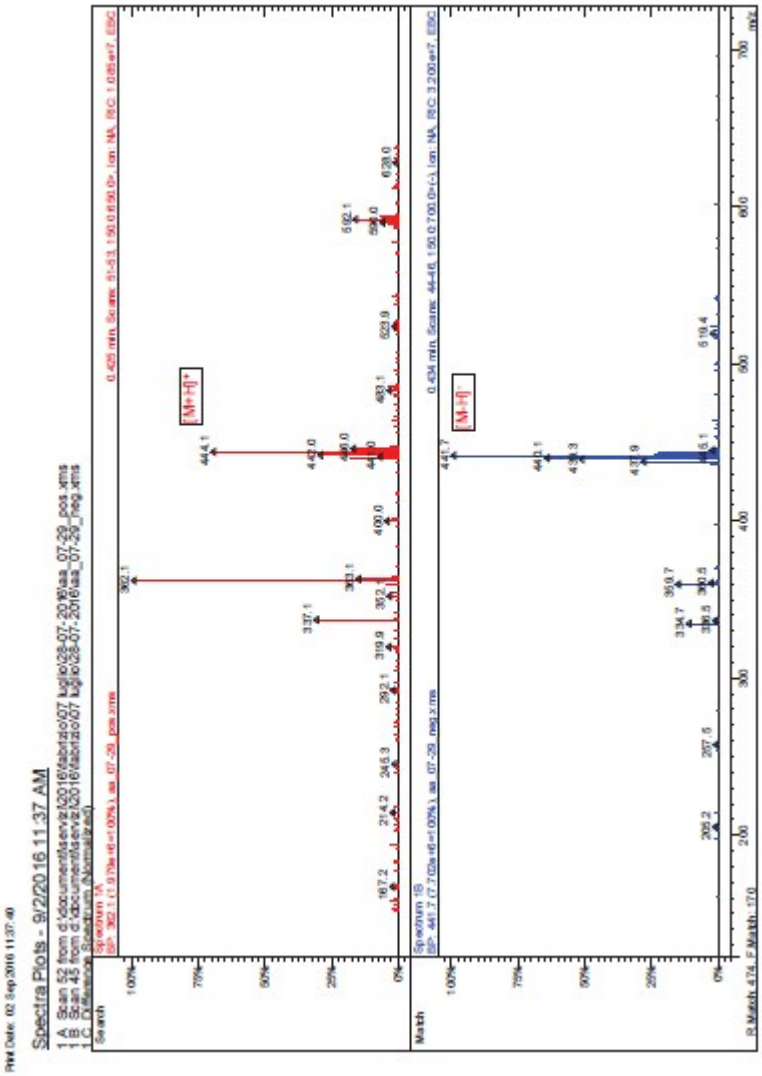
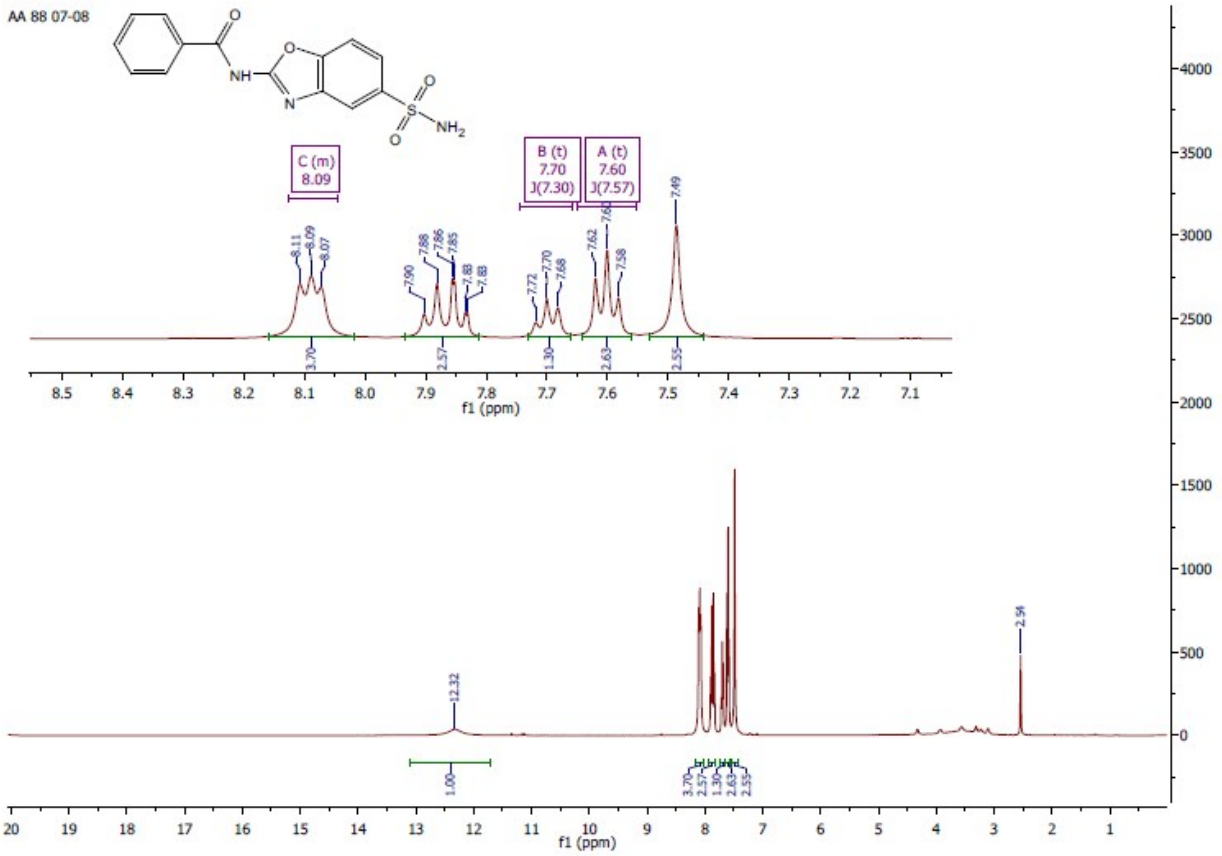


1H-NMR Compound 13



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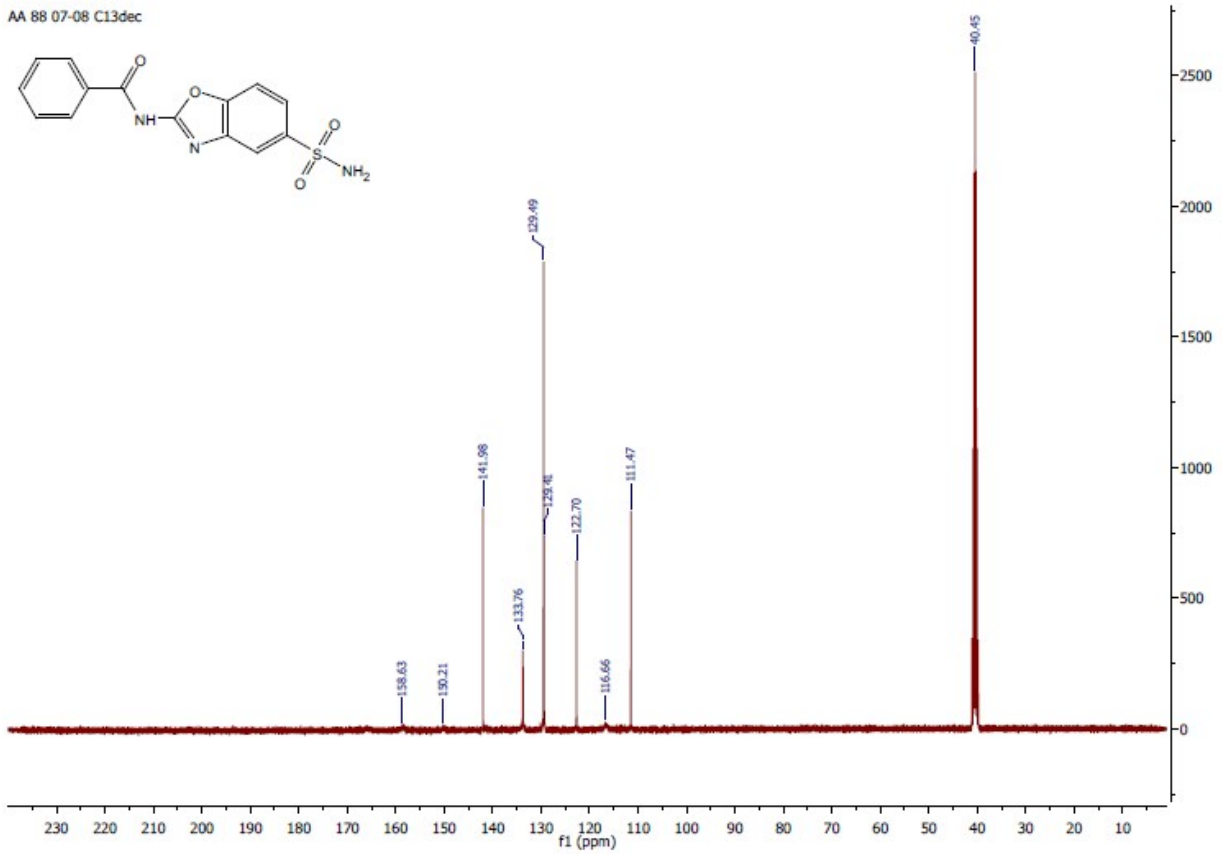

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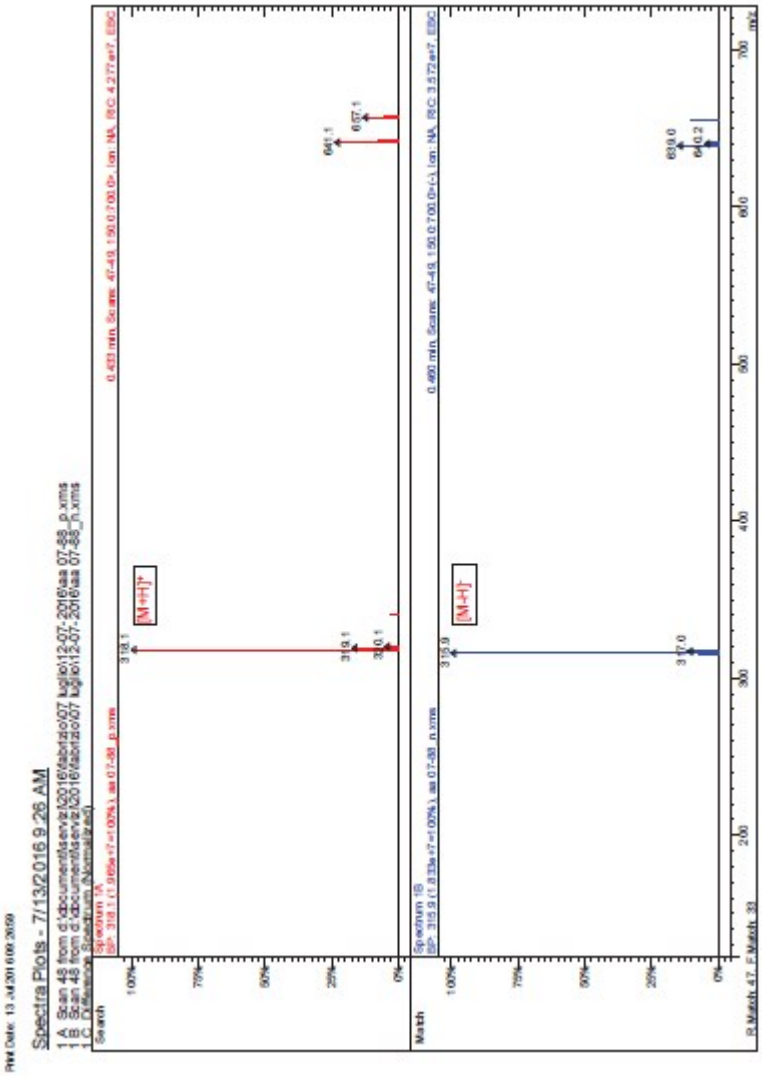

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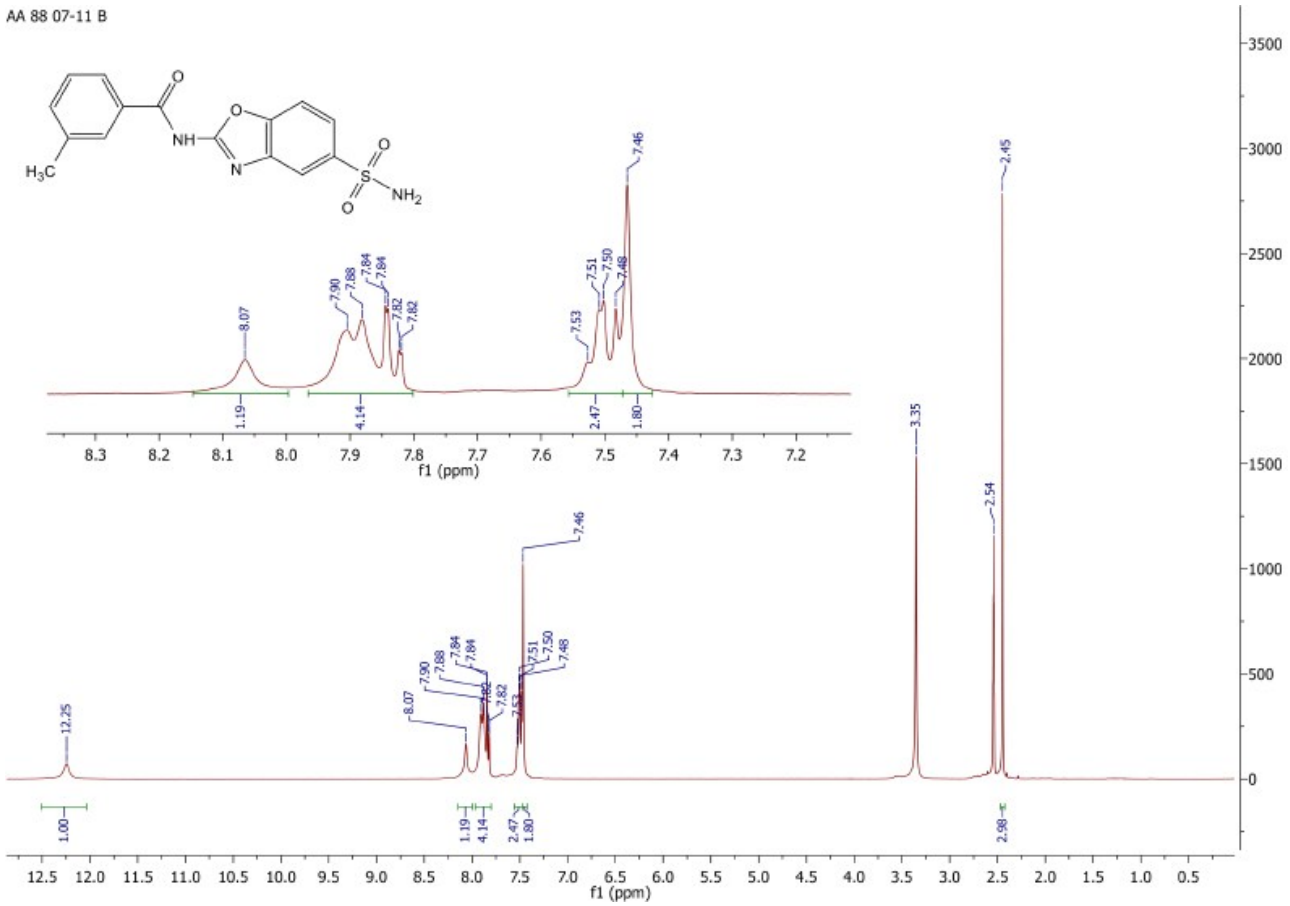
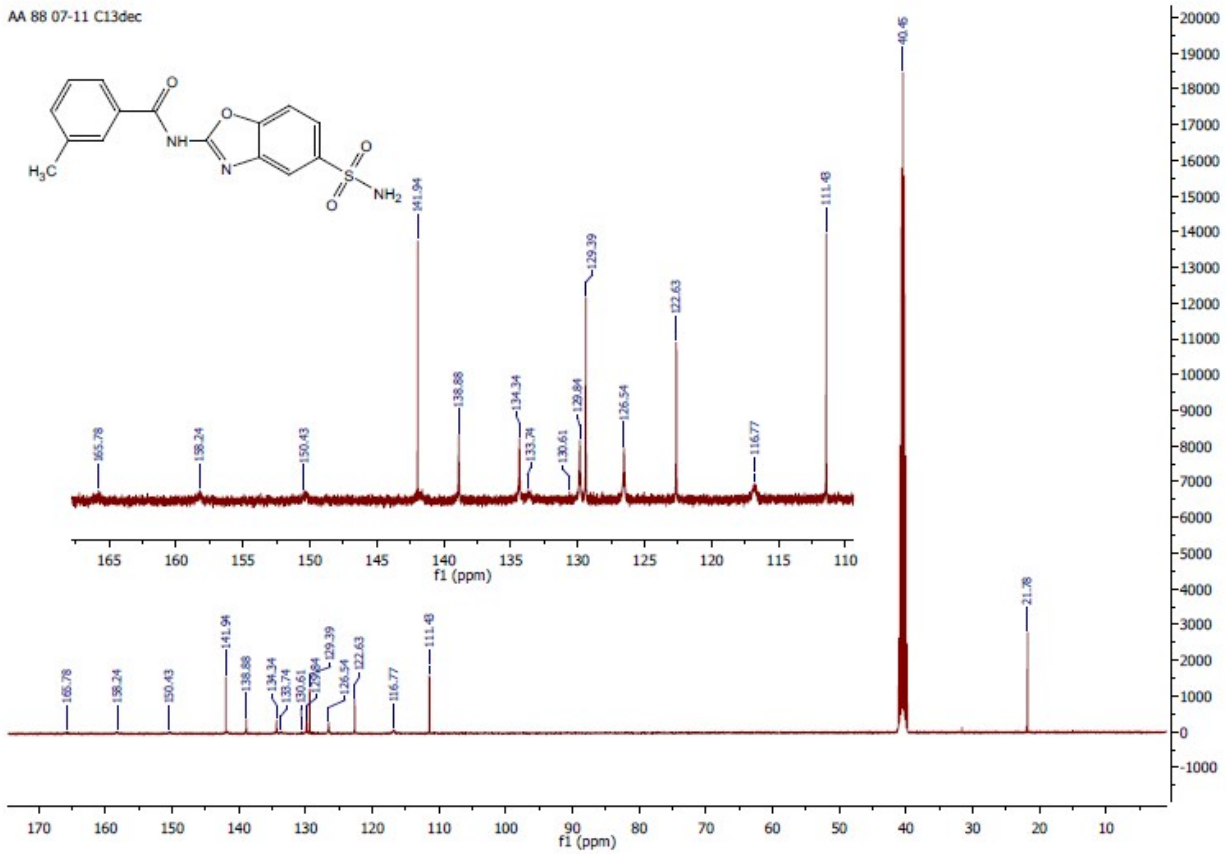


13C-NMR Compound 14



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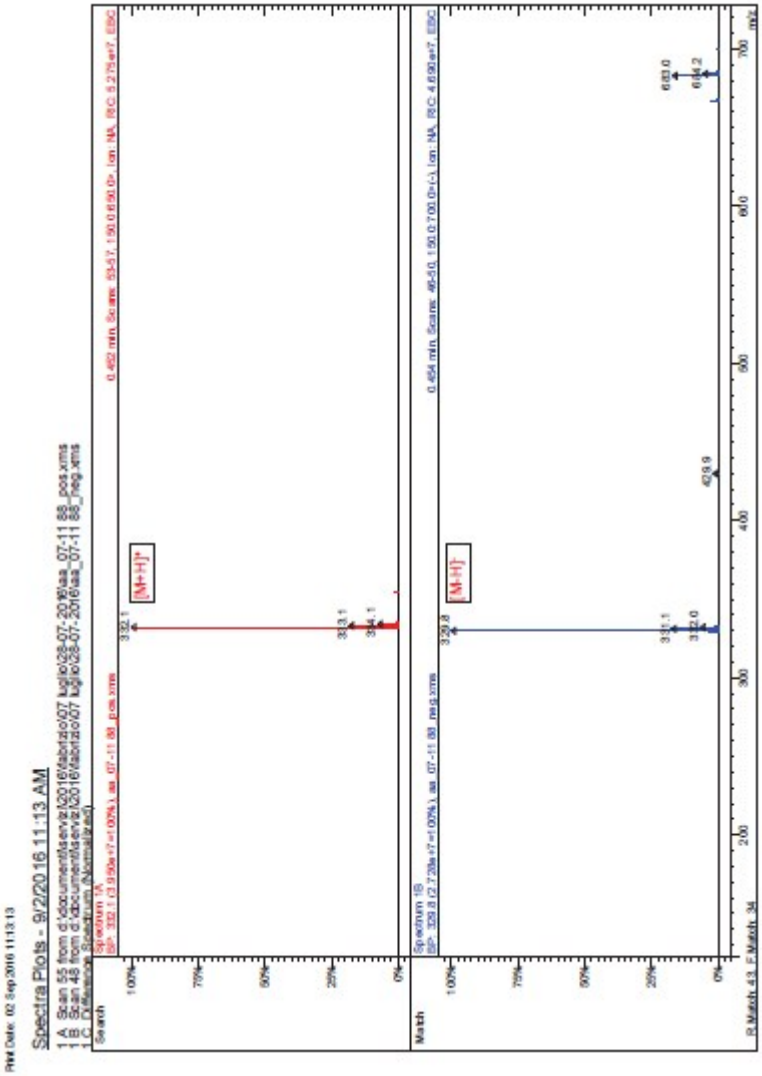

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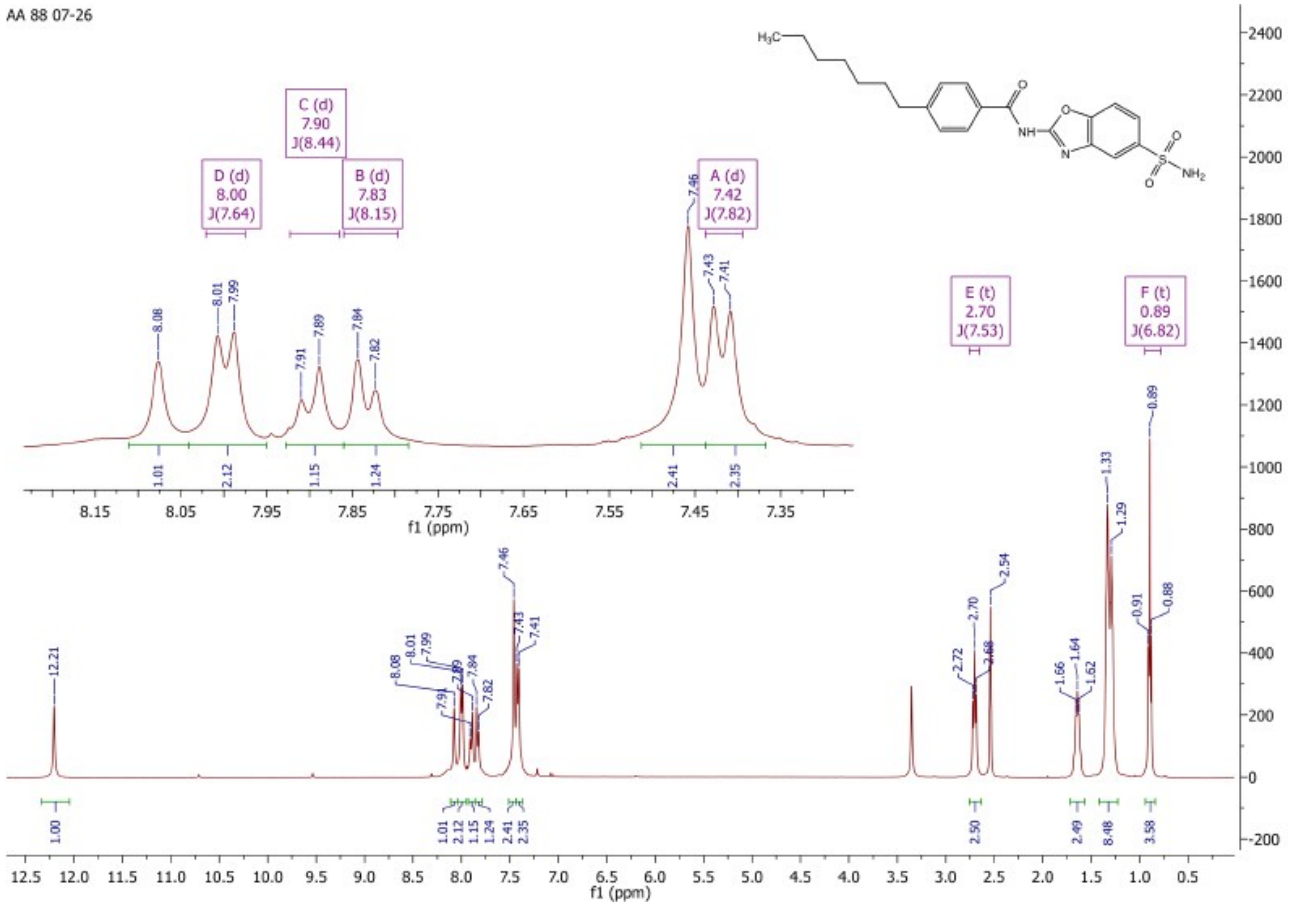

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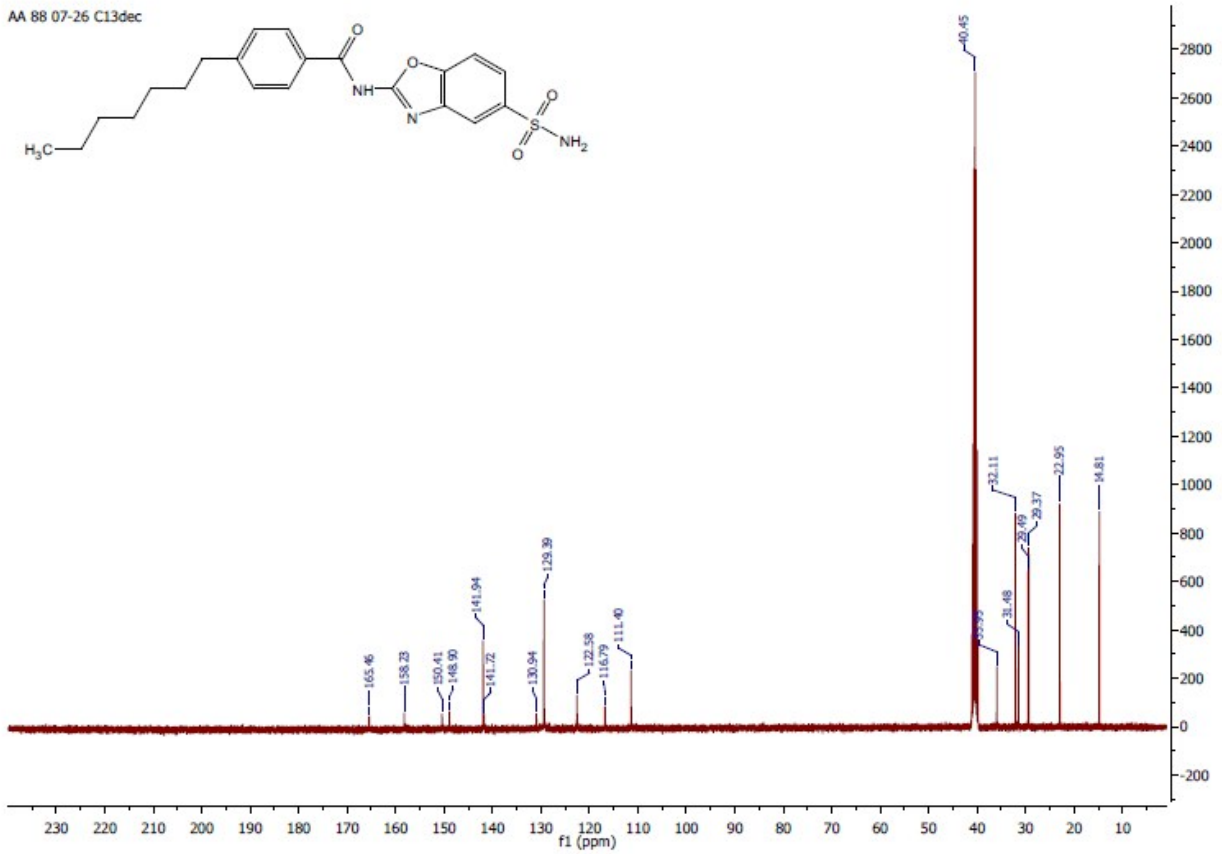

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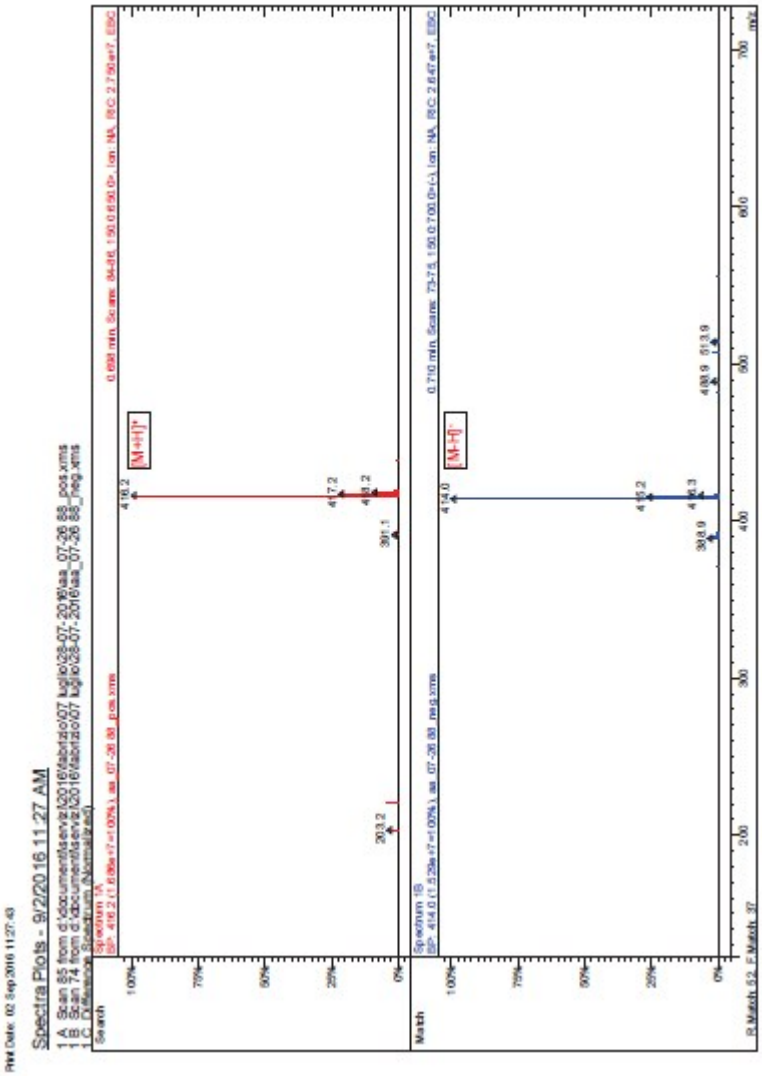
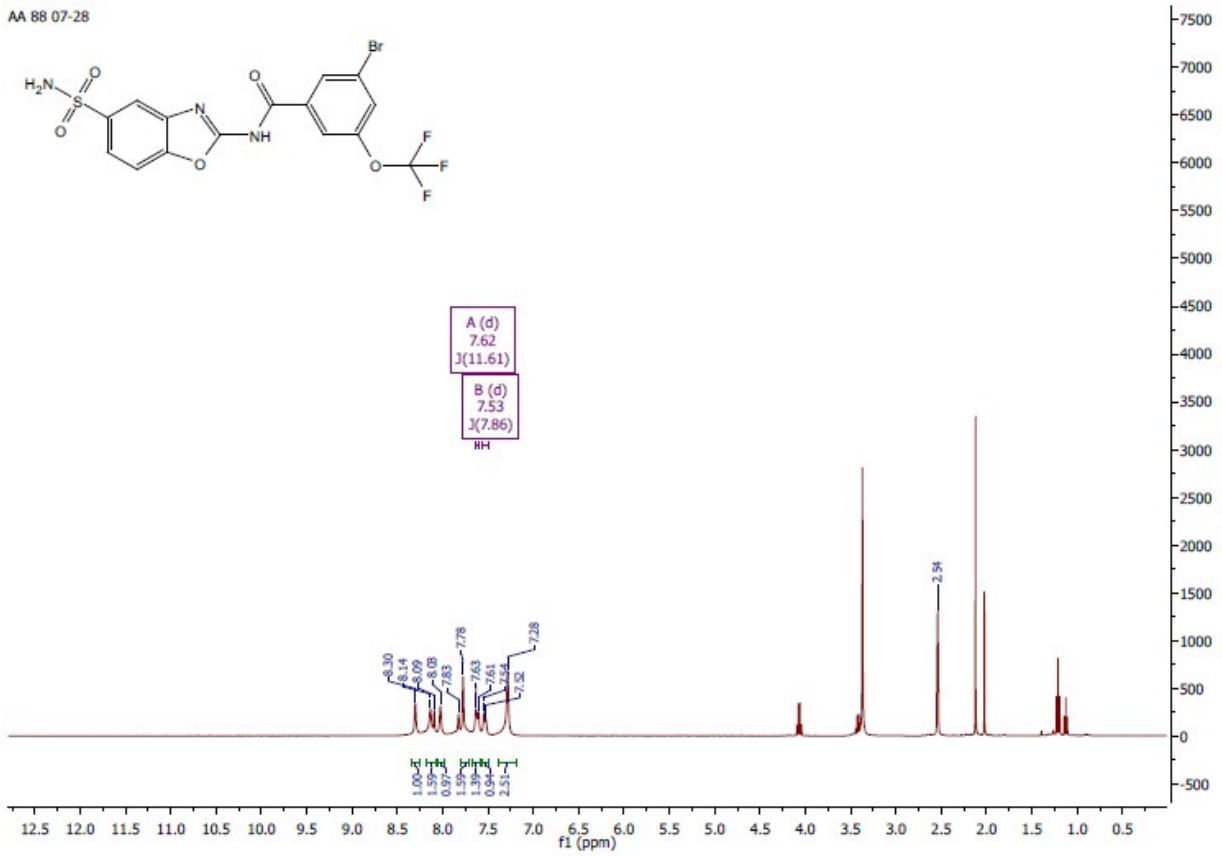
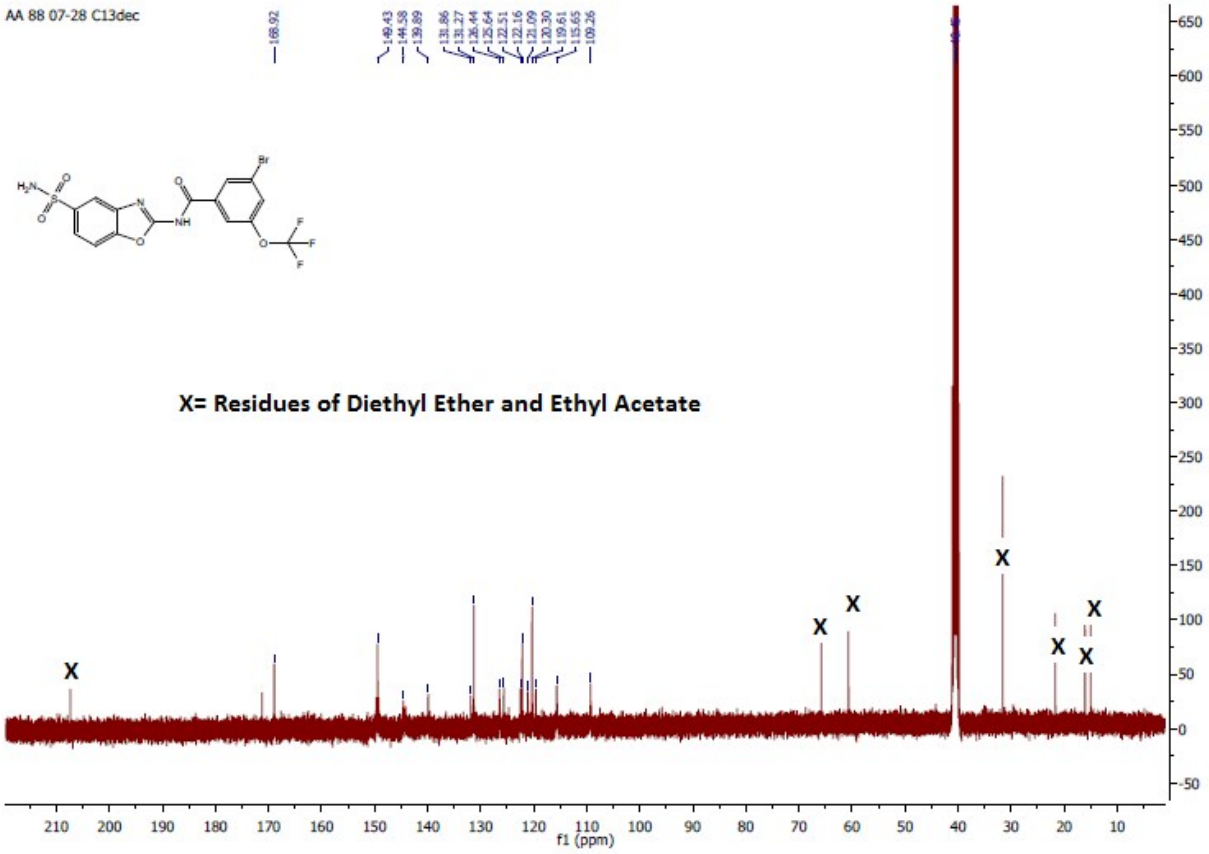


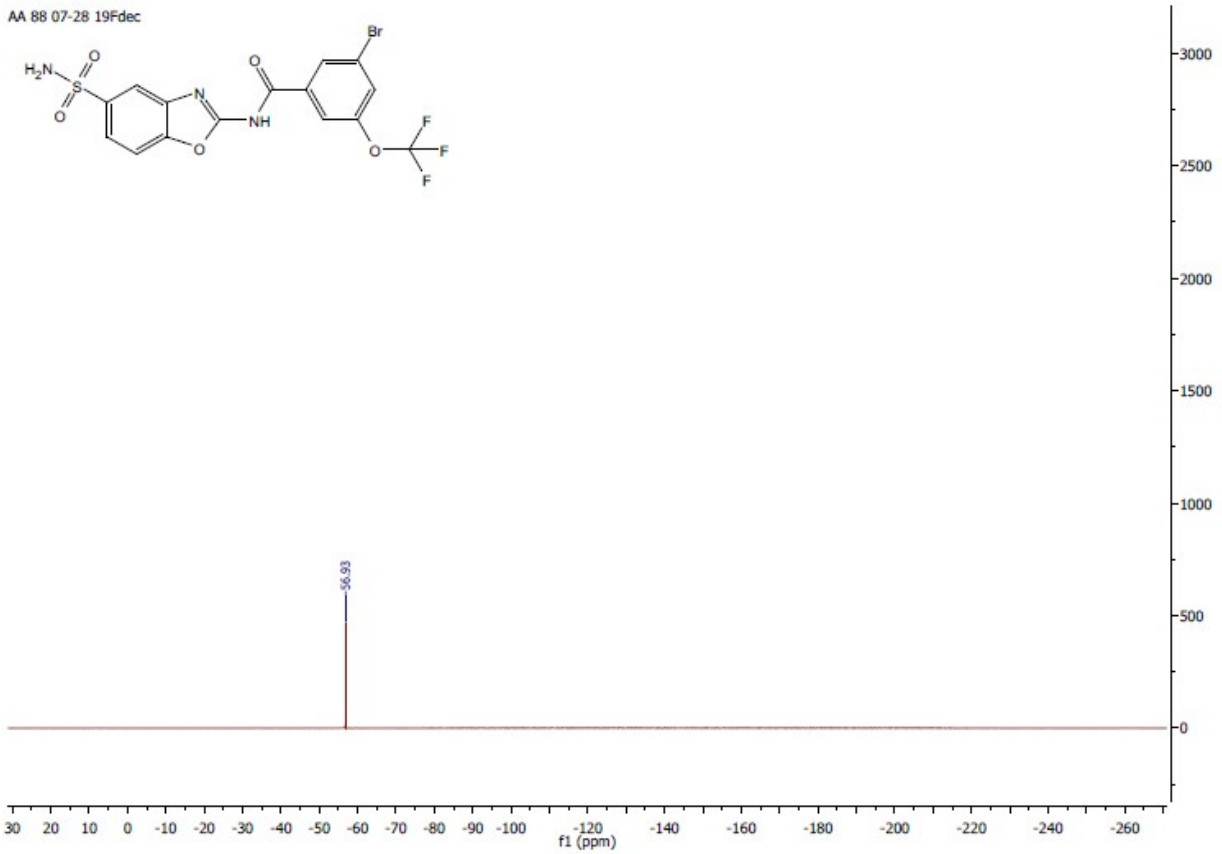
1H-NMR Compound 16



13C-NMR Compound 16



19F-NMR Compound 16



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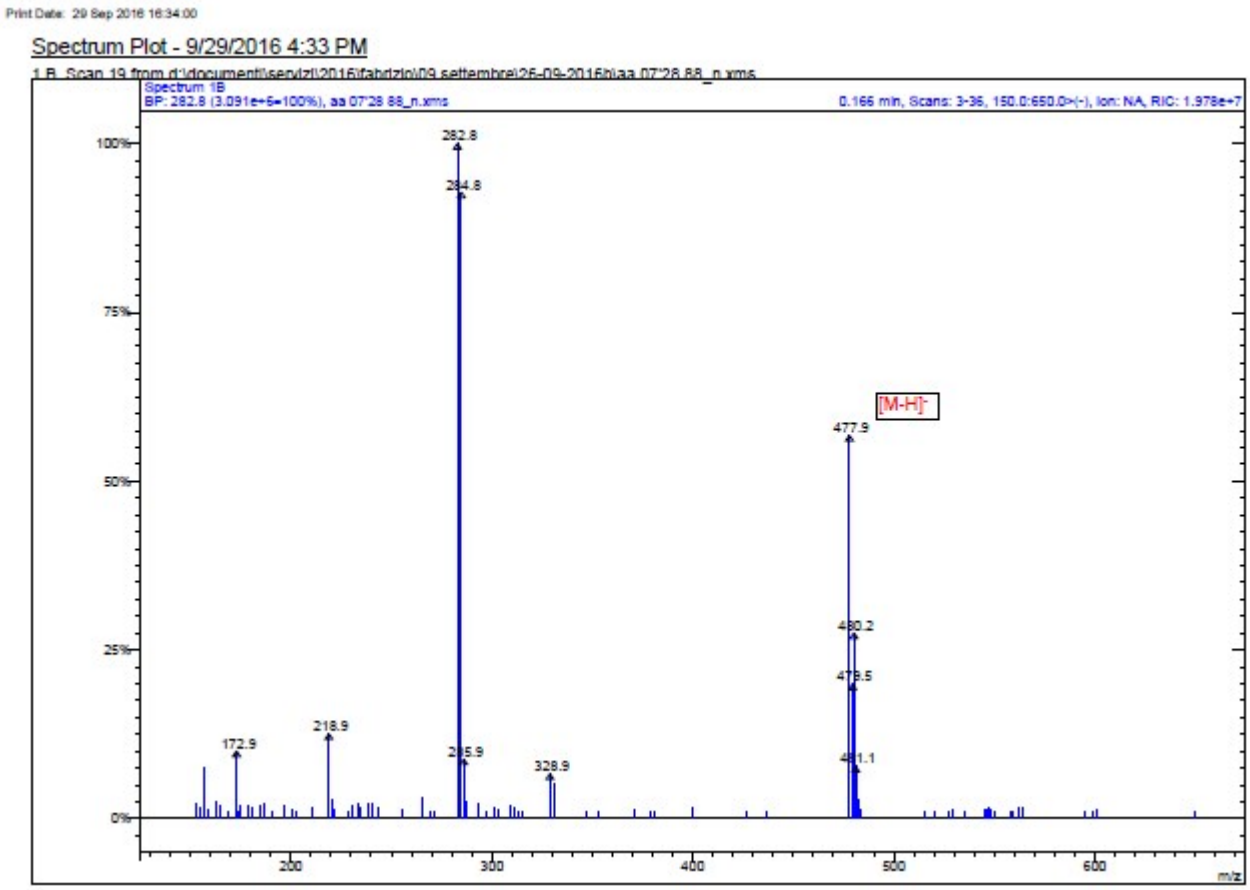

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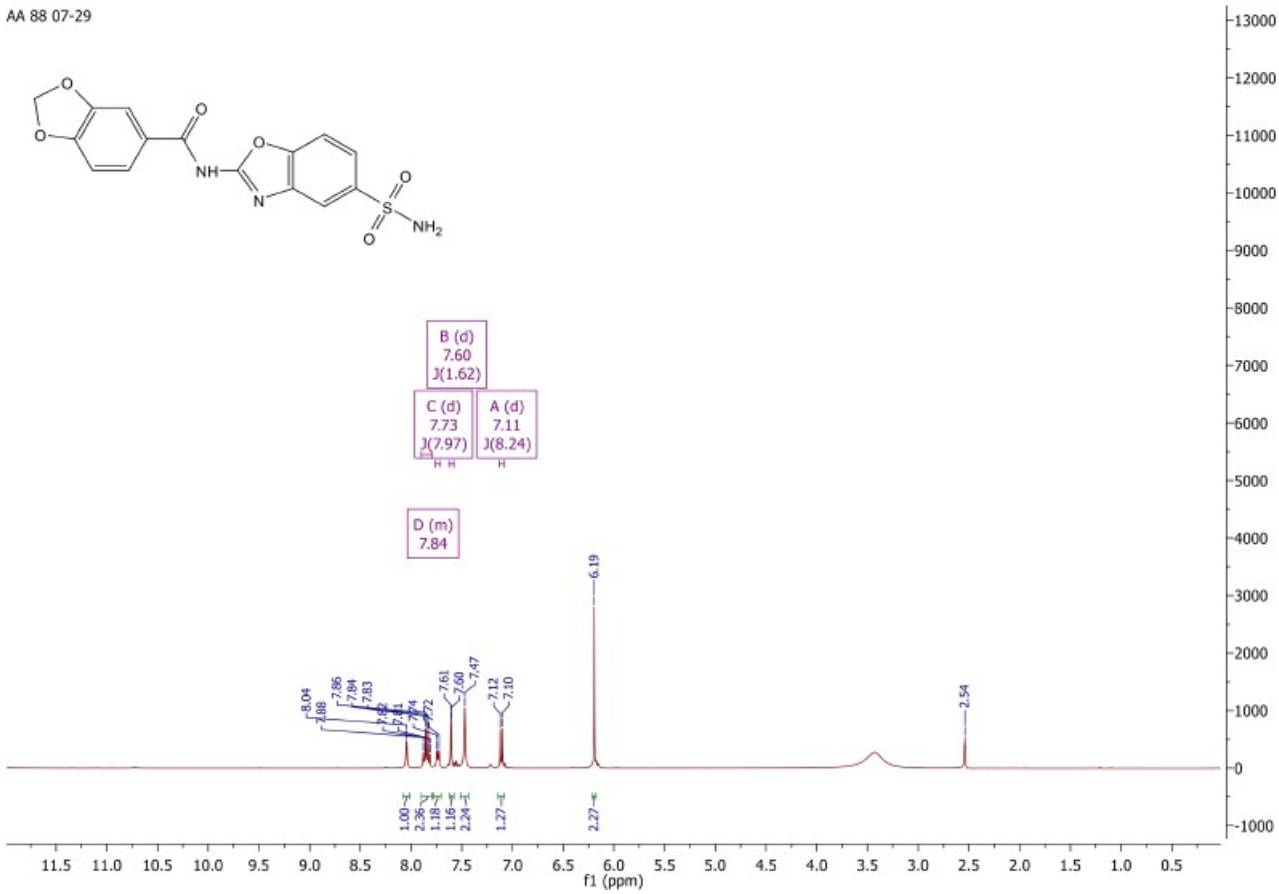
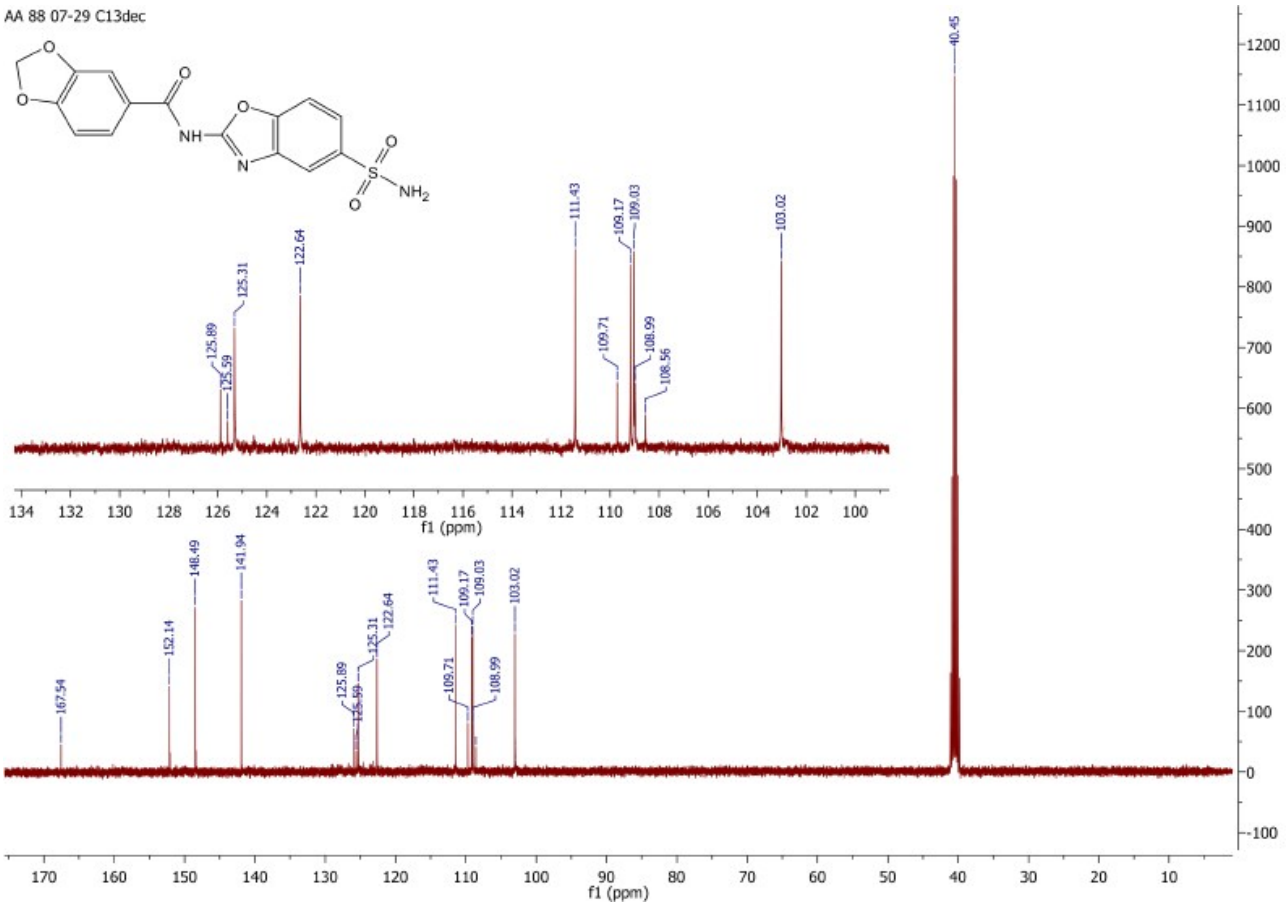


13C-NMR Compound 17



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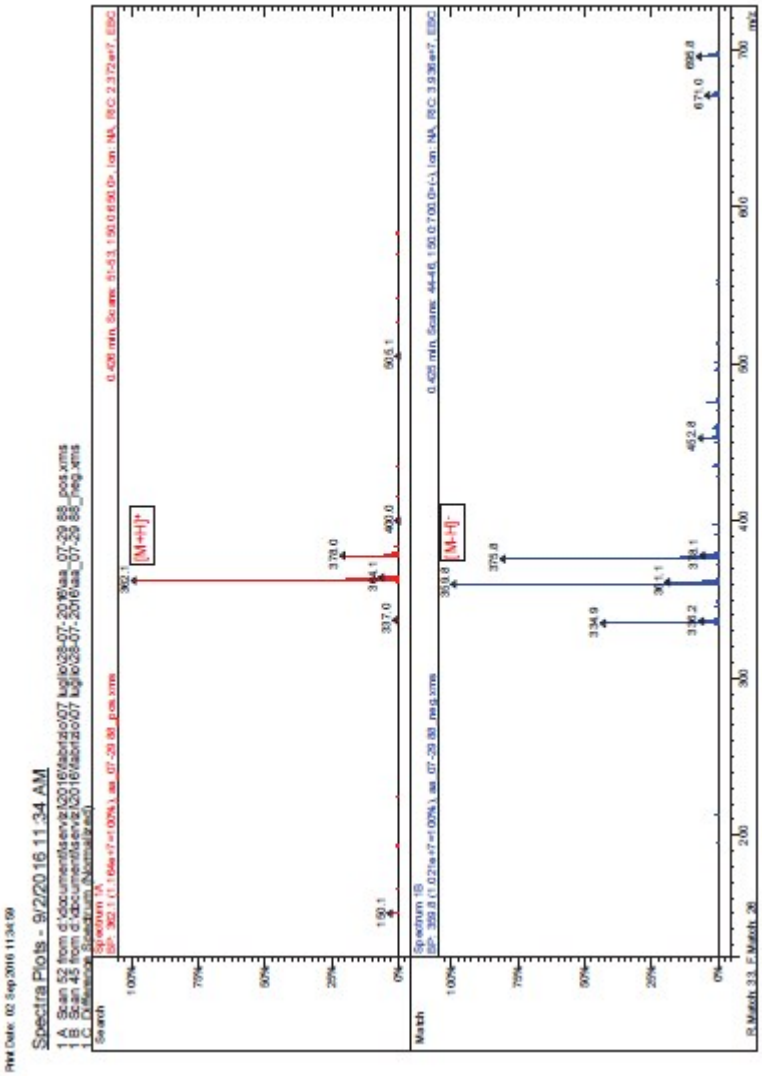
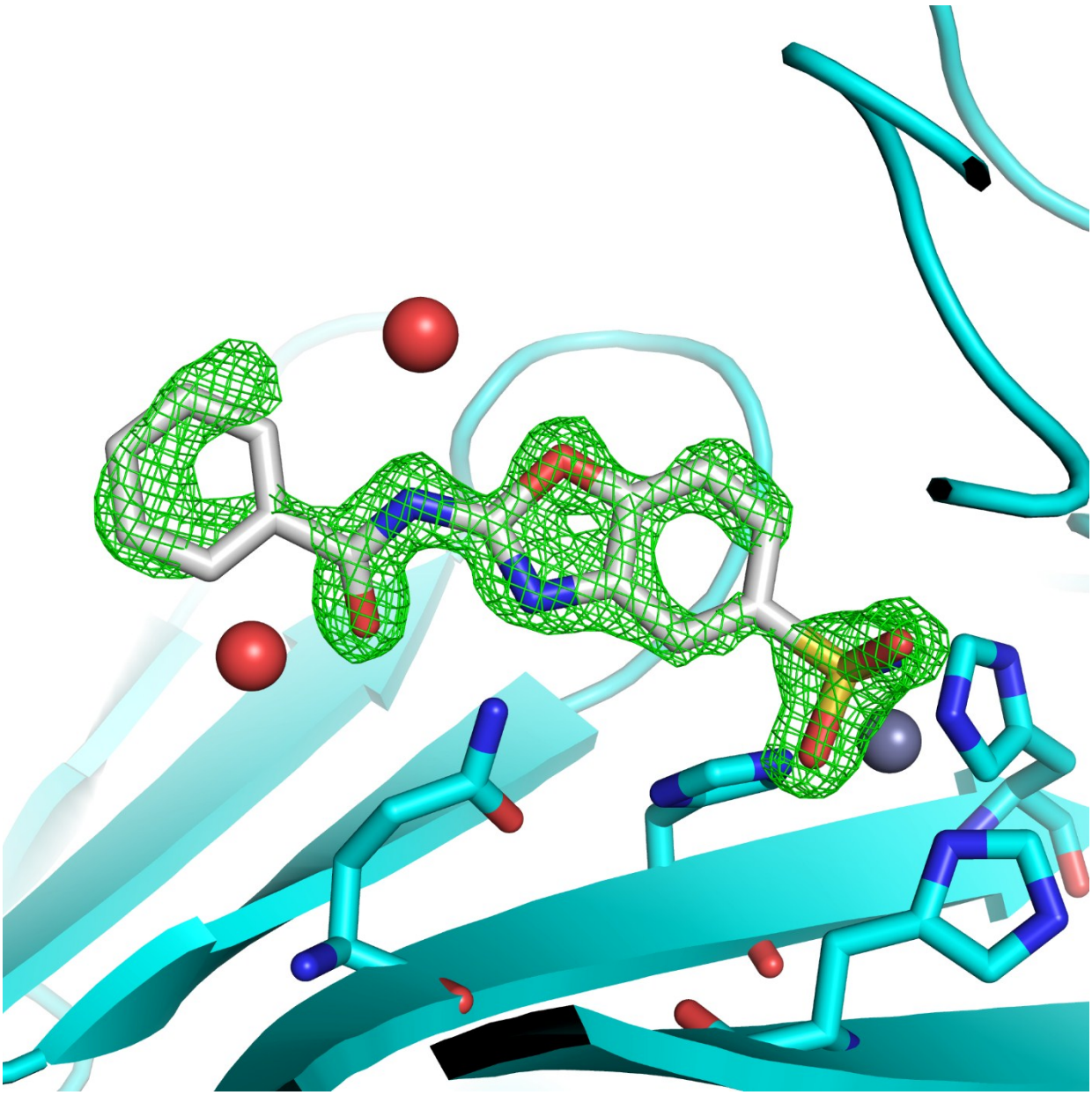


Summary of Data Collection and Atomic Model Refinement Statistics

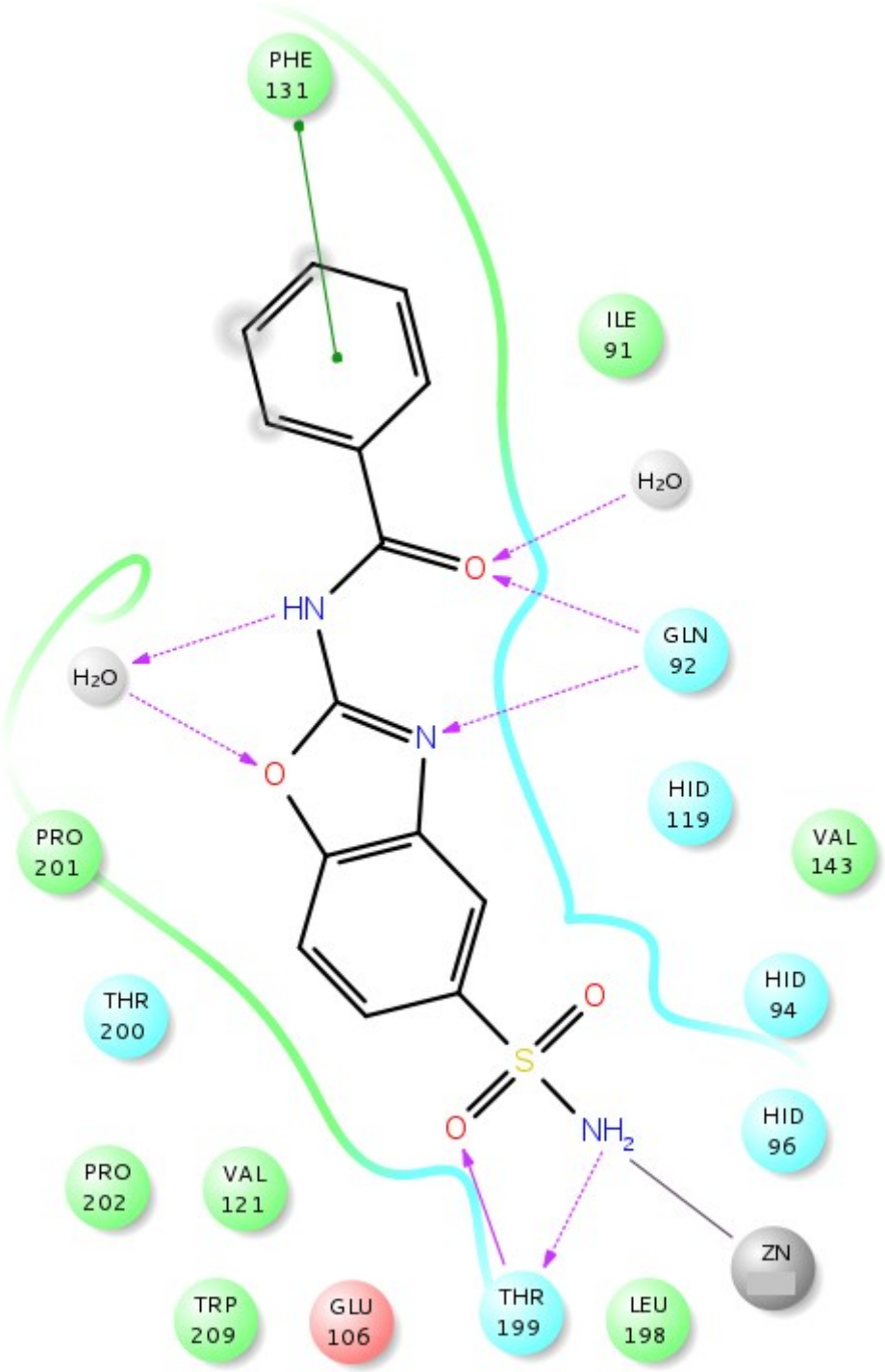
PDB	5TFX
compound	13
Space group	P21
Cell dimensions	
a, b, c	42.6, 41.6, 72.3
alpha, beta, gamma	90, 104.6, 90
Resolution (A)	41.6 - 1.50
Resolution-high (A)	1.52 - 1.50
Rmerge	0.109 (0.783)
Rpim	0.044 (0.365)
CC ½	0.998 (0.668)
I/sigI	13.8 (2.0)
Completeness	96.4 (66.2)
Redundancy	7.1 (5.3)
Refinement	
resolution	41.6-1.50
unique reflections	36248
Rwork/Rfree (%)	18.7 / 24.6
# atoms	2427
Protein	2128
metal (Zn)	1
ligand	22
water	270
B-factors (A2)	13,7
protein	12,8
metal (Zn)	6,3
ligand	13,7
water	24,9
r.m.s. deviations	
Bond length (A)	0,016
Bond angle (o)	1,861

Difference Electron Density Map of Compound 13 within the hCA II Catalytic Site



Legend: A difference (mFo-DFc) electron density map is shown (at 3σ) around the compound; the active site Zn atom is represented as a grey sphere with three His residues in coordination, two bound water molecules are shown as red spheres and Gln92 is highlighted as all are within hydrogen binding distance to the compound. Figure made with Pymol.

2D Interaction Map of Compound 13 within the hCA II Catalytic Site



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