Supporting Information for

Iodine/Oxone® oxidative system for the Synthesis of Selenylindoles Bearing Benzenesulfonamide Moiety as Carbonic Anhydrase I, II, IX, and XII Inhibitors

Martina Palomba,†[a] Andrea Angeli,†[b] Riccardo Galdini,[a] Alexandra Joana Hughineata,[a] Gelson Perin,[c] Eder João Lenardão,[c] Francesca Marini,[a] Claudio Santi,*[a] Claudiu T. Supuran,[b] Luana Bagnoli*[a]

[a]Department of Pharmaceutical Sciences (Group of Catalysis, Synthesis and Organic Green Chemistry), University of Perugia, Via del Liceo 1, 06123 Perugia, Italy.
[b]University of Florence, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy.
[c] Laboratório de Síntese Orgânica Limpa (LASOL), Centro de Ciências Químicas, Farmacêuticas e de Alimentos (CCQFA) Universidade Federal de Pelotas (UFPel), P.O. Box 354, CEP: 96010-900 Pelotas, RS, Brazil.
† These authors contributed equally to this work

e-mail: luana.bagnoli@unipg.it

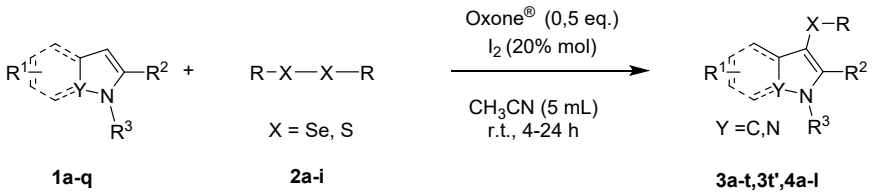
Table of Contents

1. General Information and materials

Reagents and solvents were purchased from Sigma Aldrich, Alfa Aesar and VWR International and employed without further purification, except for the starting products indole carboxamides **1f** -**1g** and diselenides **2b-g,i** that are prepared according to the literature procedures.[1-7] Thin layer chromatography (TLC) was performed in 60 F254 (Merck, KGaA, Darmstadt, Germany) silica gel supported on aluminium sheets. Reaction products were purified by column chromatography on Merck 60 (70-230 mesh) silica gel. Yields correspond to isolated compounds. Purity is estimated to be ≥ 95% based on ^{1}H NMR spectroscopic analysis. NMR experiments were carried out at 25 °C on a Bruker Avance NEO 400 MHz spectrometer equipped with Sample Case operating at 400 MHz for ^{1}H and 100.62 MHz for ^{13}C or a Bruker Avance NEO 600 MHz spectrometer equipped with a Prodigy^{TM}BBO-Cryoprobe operating at 600.13 MHz for ^{1}H, 150.90 MHz for ^{13}C in $CDCl_3$, CD_3OD, DMSO-d_6 and CD_3COCD_3. ^{77}Se NMR spectra are referenced to diphenyl diselenide external standard $(PhSe)_2$ and were recorded at 114 MHz.[8] Chemical shifts (δ) are reported in ppm. The ^{1}H NMR spectra registered in CD_3OD did not show signals for the exchangeable protons of indole N**H**, $SO_2N\mathbf{H_2}$, such protons are visible in DMSO-d_6. High-resolution mass spectrometry (HRMS) measurements were performed using Synapt G2-Si mass spectrometer (Waters) equipped with an APCI/ ESI source and quadrupole-Time-of-Flight mass analyzer. The mass spectrometer operated in the positive ion detection mode. To ensure accurate mass measurements, data were collected in centroid mode and mass was corrected during acquisition using leucine enkephalin solution as an external reference. The results of the measurements were processed using the MassLynx 4.1 software (Waters) incorporated with the instrument. Melting points were determined in Kofler melting apparatus and value are uncorrected.

2. General Procedure

The indole **1a-k, m-q** (1.0 eq., 0.5 mmol) or the pyrazole **1l** (1.0 eq. 0.5 mmol), the diselenide **2a**-**g, 2i** (0.5 eq., 0.25 mmol) or disulfide **2h** (0.5 eq., 0.25 mmol), Oxone® triple salt (0.5 eq., 0.25 mmol) and the iodine (20 mol%) were dissolved in 5 mL of CH_3CN. The reaction mixture was vigorously stirred at room temperature for 4 to 24 hours. Then, the reaction was quenched with a saturated solution of $Na_2S_2O_3$ and extracted with ethyl acetate (3 x 10 mL), dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The crude mixture was purified by column chromatography on silica gel to afford the 3-selenylindoles **3a-q**, 4-selenylpyrazoles **3r-s**, mono- and bis- sulfenylindoles **3t-3t'**, and 3-selenylindoles containing benzensulfonamide moiety **4a-l**.



3. ^{1}H NMR, ^{13}C and ^{77}Se NMR spectra of compounds 3 and 4

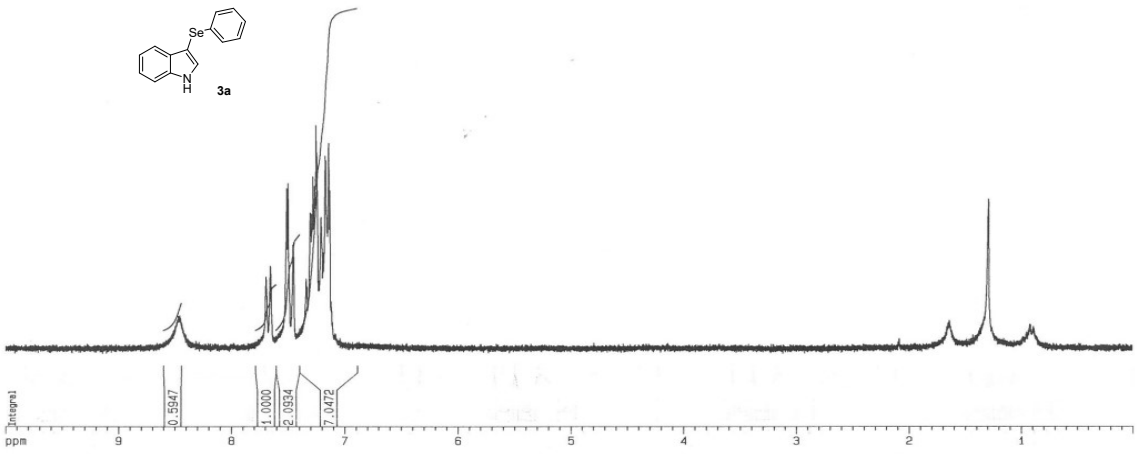


Figure 1. ^{1}H NMR spectrum of compound **3a**[9] in $CDCl_3$

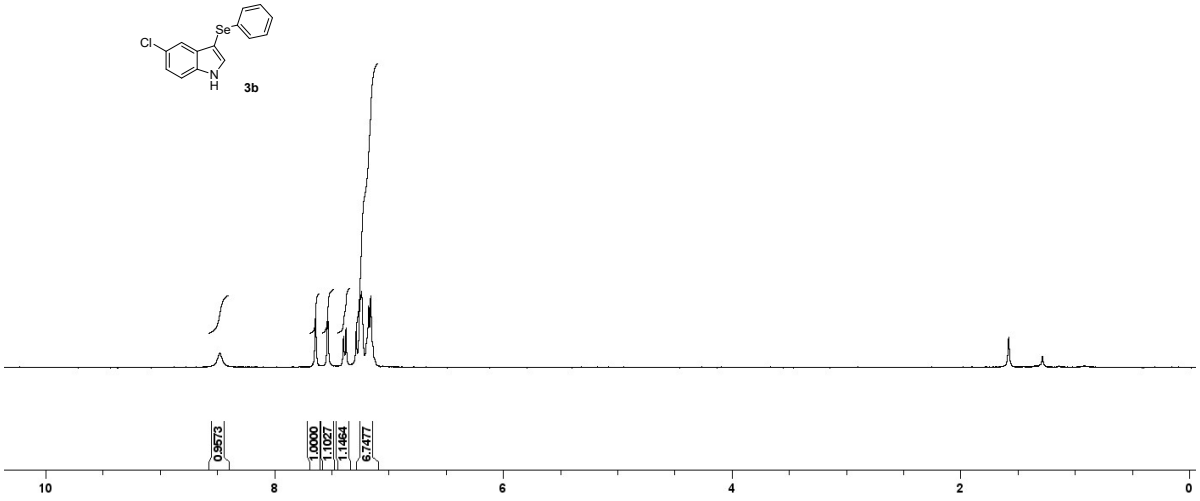


Figure 2. ^{1}H NMR spectrum of compound **3b**[9] in $CDCl_3$

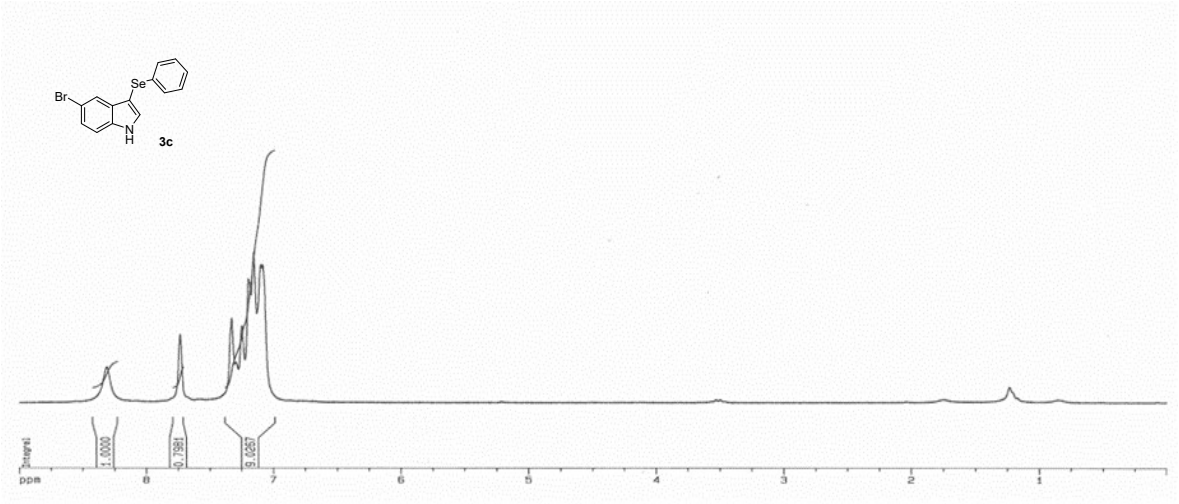


Figure 3. ^{1}H NMR spectrum of compound **3c**[9] in $CDCl_3$

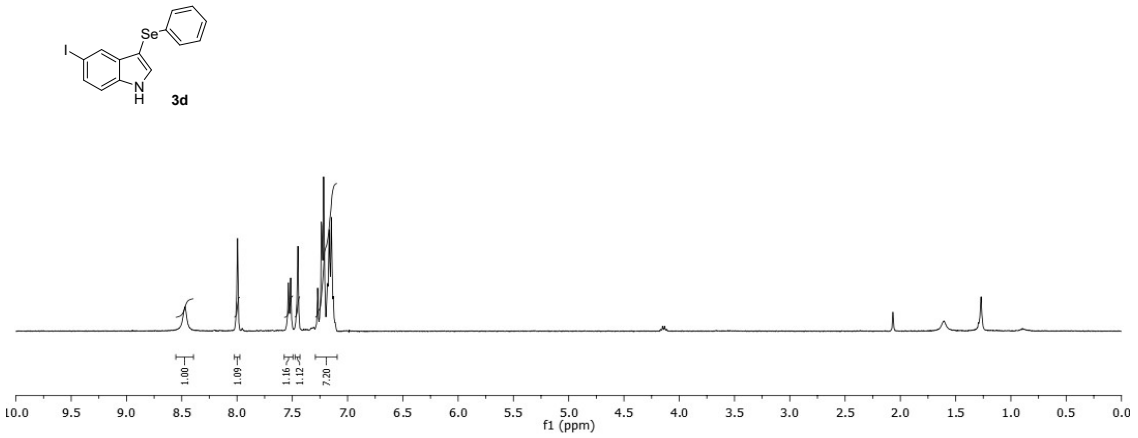


Figure 4. ^{1}H NMR spectrum of compound **3d**[9] in $CDCl_3$

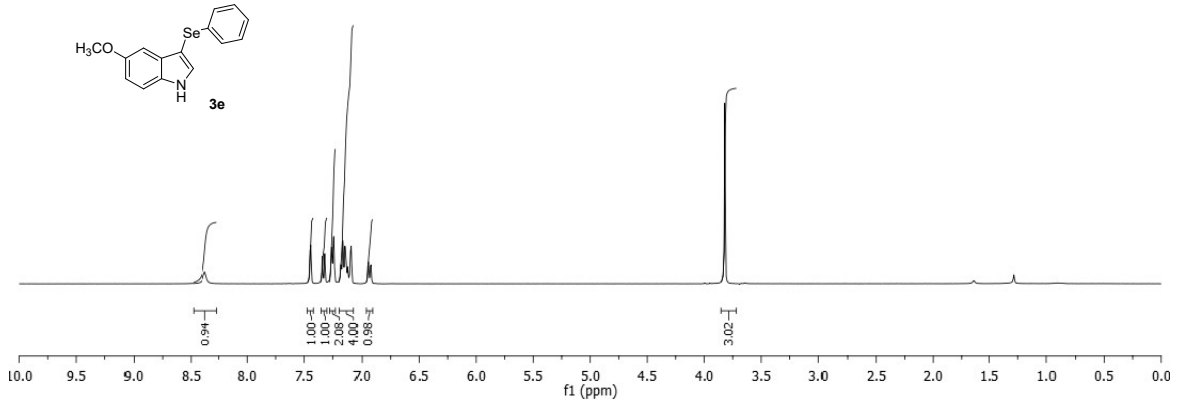


Figure 5. ^{1}H NMR spectrum of compound **3e**[9] in $CDCl_3$

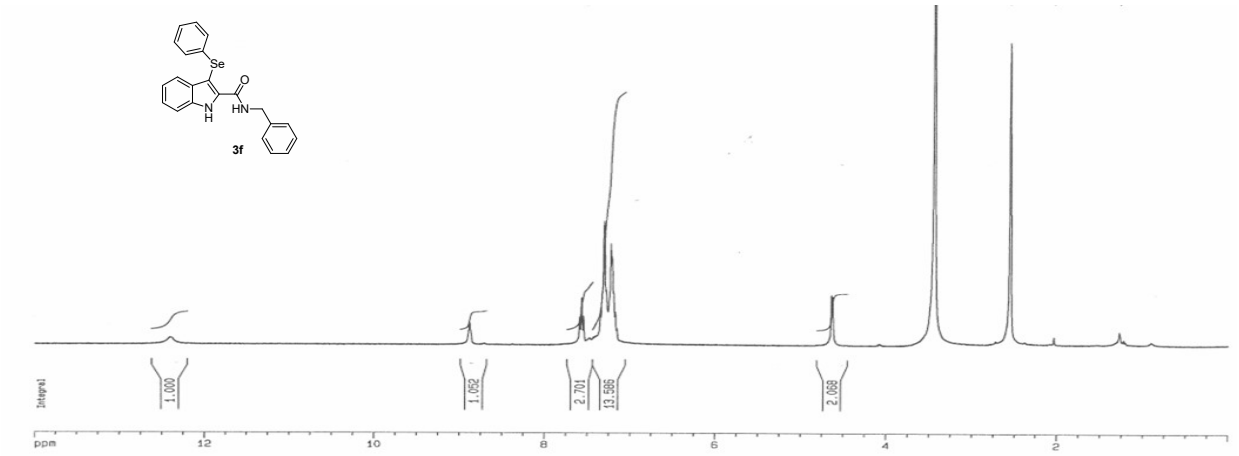


Figure 6. ^{1}H NMR spectrum of compound **3f** in DMSO-d_6

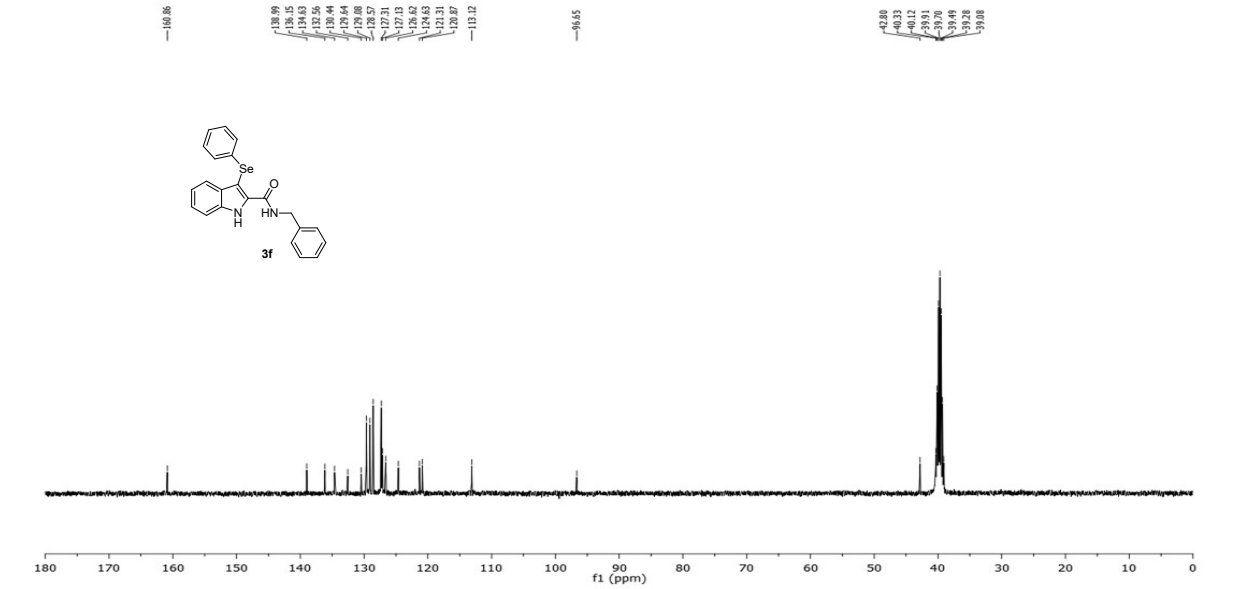


Figure 7. ^{13}C NMR spectrum of compound **3f** in DMSO-d_6

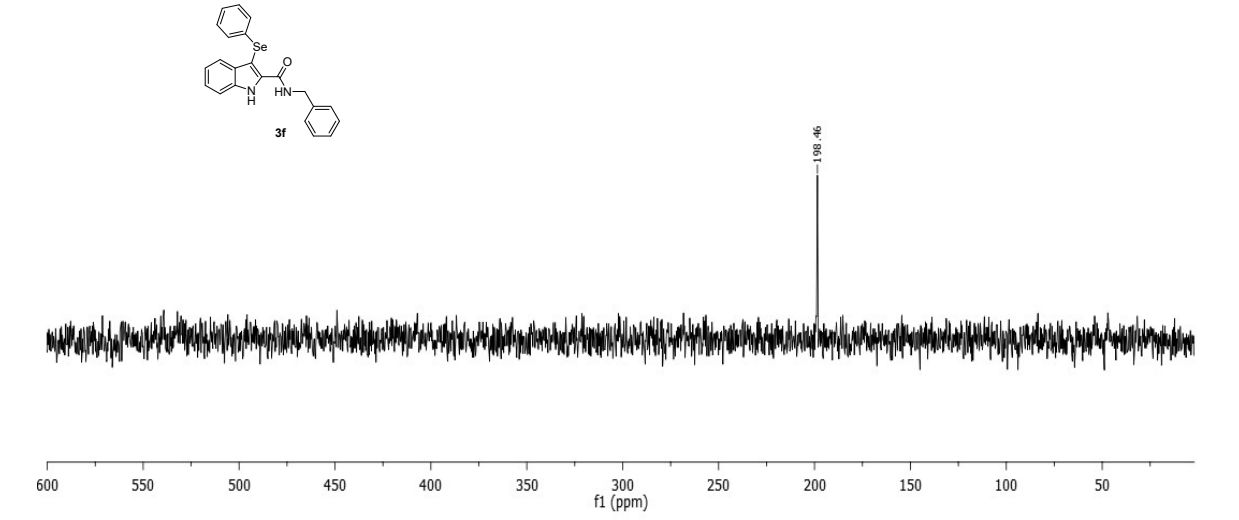


Figure 8. ^{77}Se NMR spectrum of compound **3f** in DMSO-d_6

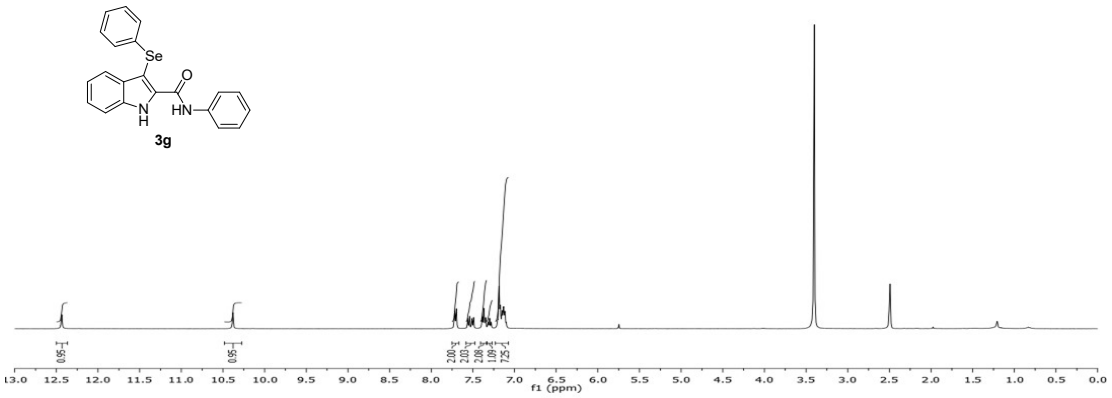


Figure 9. ^{1}H NMR spectrum of compound **3g** in DMSO-d_6

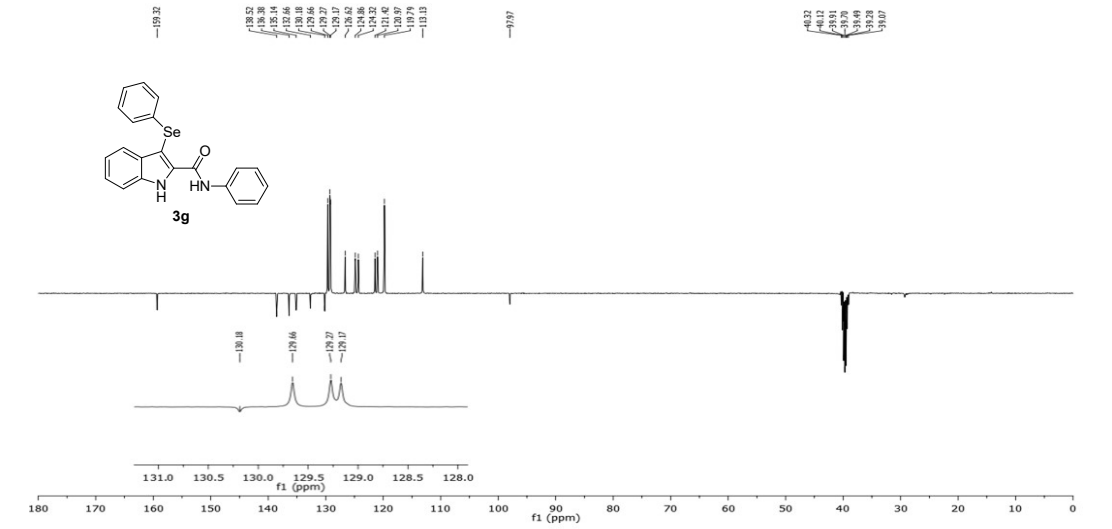


Figure 10. ^{13}C NMR spectrum of compound **3g** in DMSO-d_6

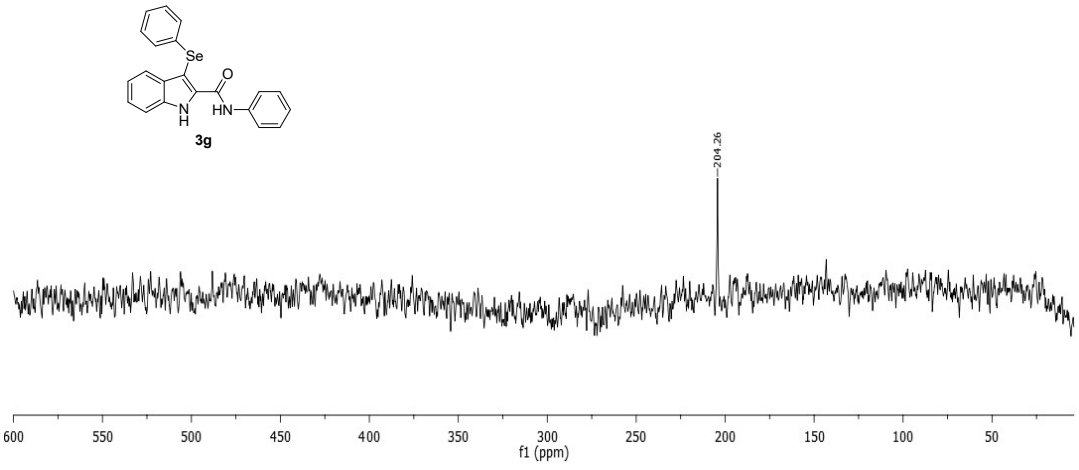


Figure 11. ^{77}Se NMR spectrum of compound **3g** in DMSO-d_6

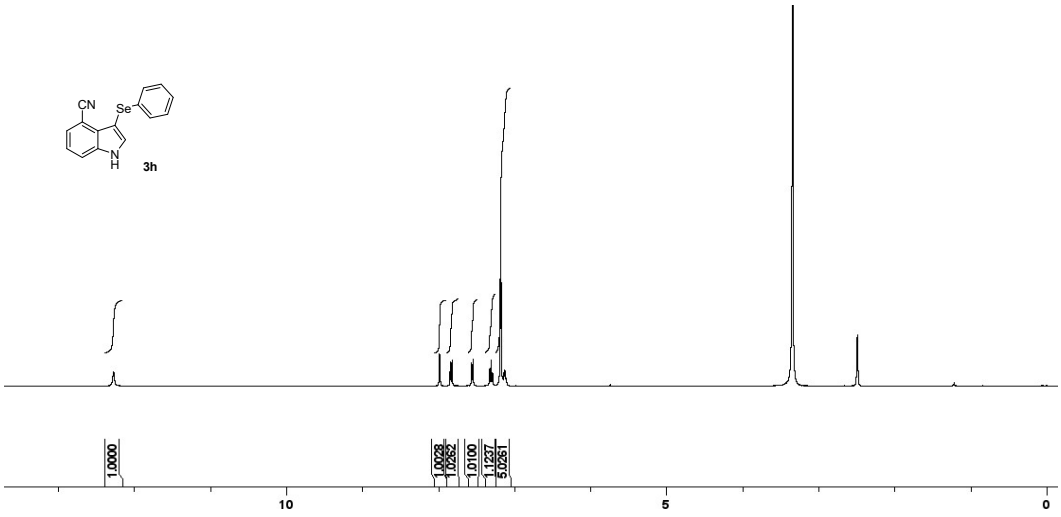


Figure 12. ^{1}H NMR spectrum of compound **3h**[9] in DMSO-d_6

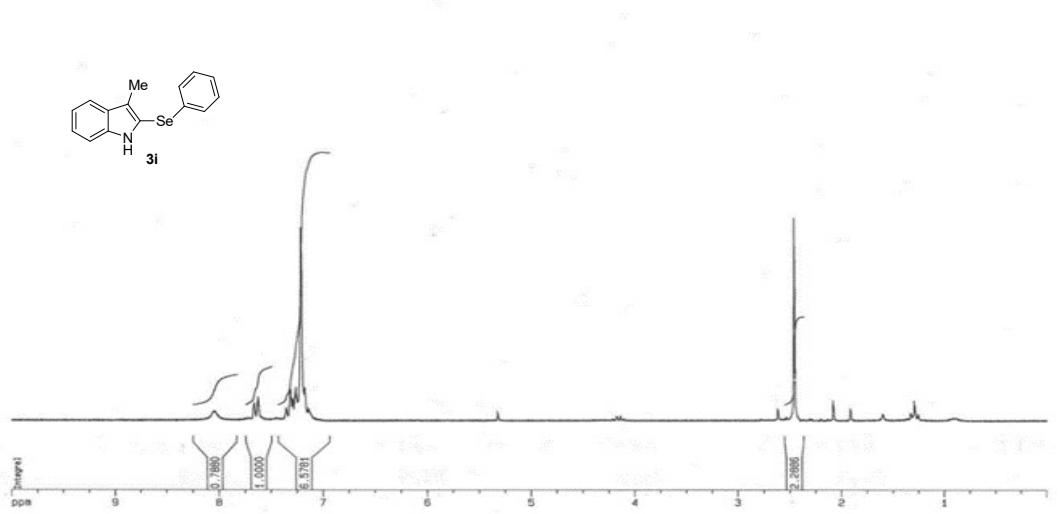


Figure 13. ^{1}H NMR spectrum of compound **3i**[10] in $CDCl_3$

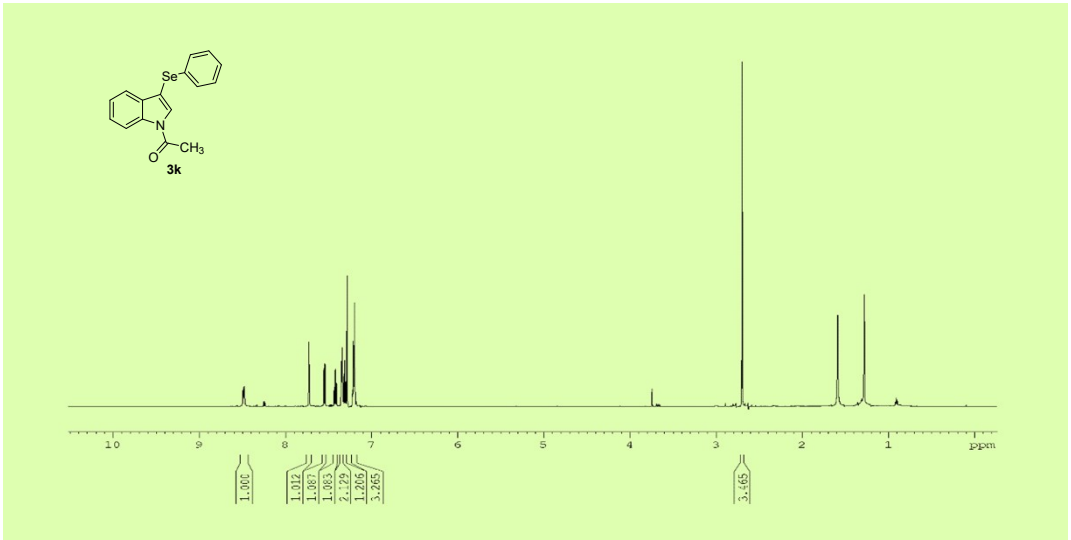


Figure 14. ^{1}H NMR spectrum of compound **3k** in $CDCl_3$

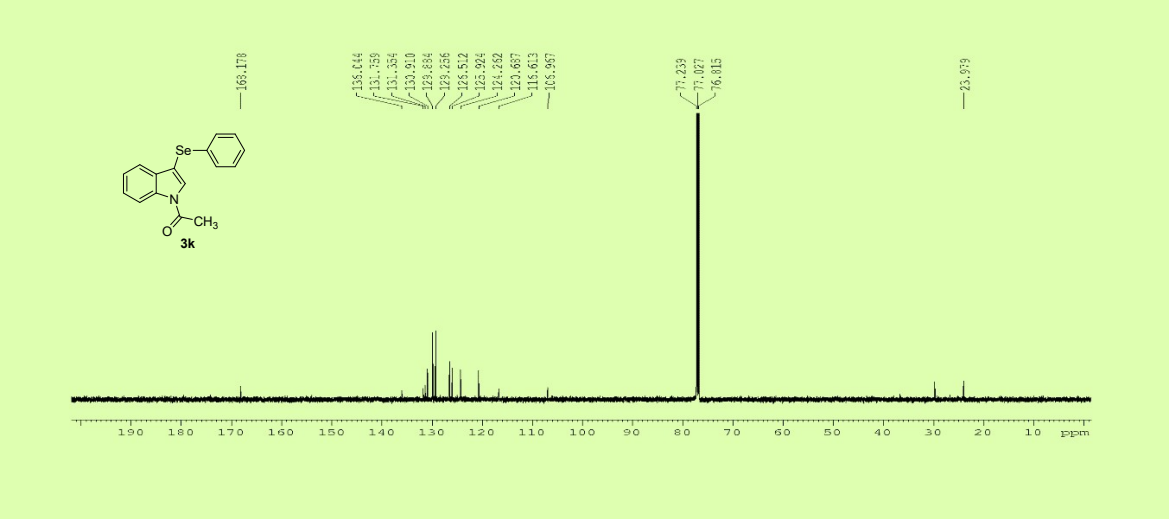


Figure 15. ^{13}C NMR spectrum of compound **3k** in $CDCl_3$

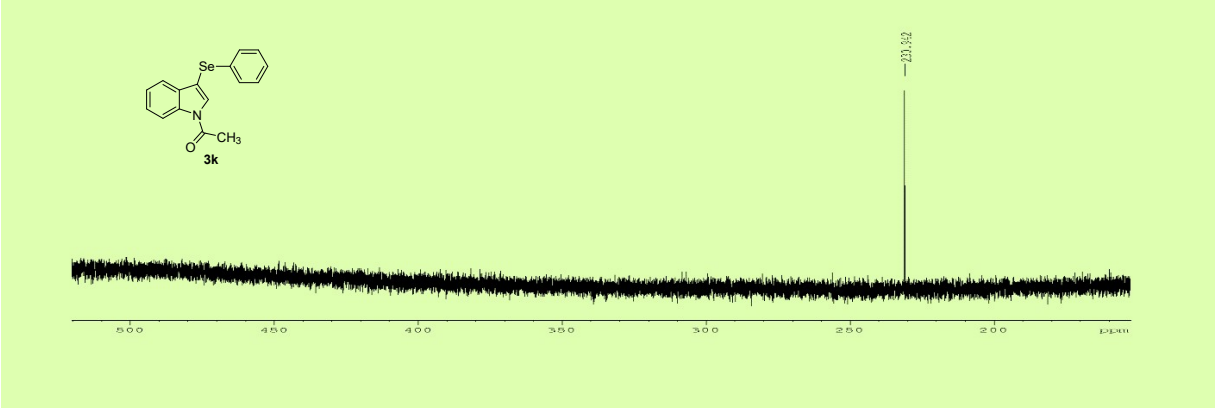


Figure 16. ^{77}Se NMR spectrum of compound **3k** in $CDCl_3$

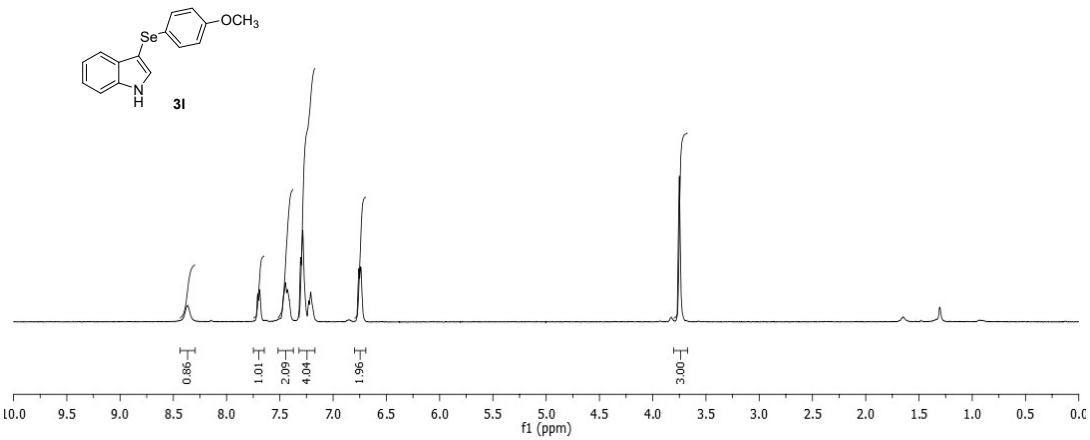


Figure 17. ^{1}H NMR spectrum of compound **3l**[9] in $CDCl_3$

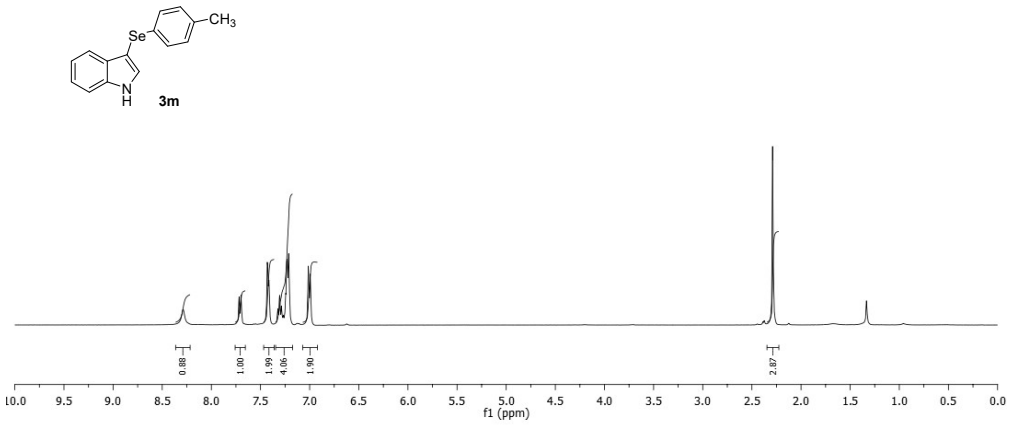


Figure 18. ^{1}H NMR spectrum of compound **3m**[9] in $CDCl_3$

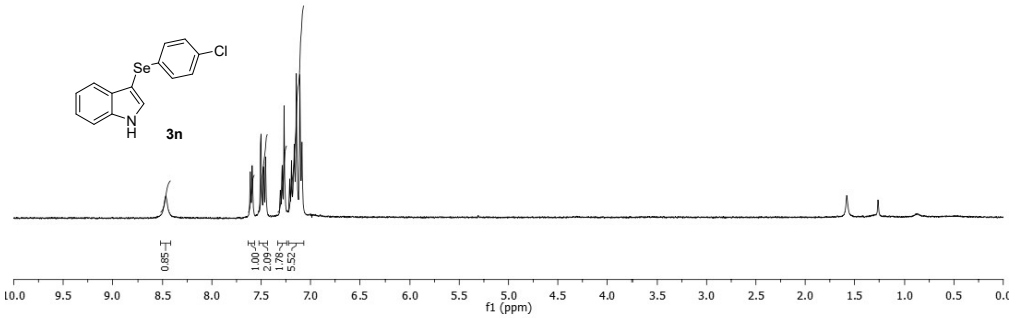


Figure 19. ^{1}H NMR spectrum of compound **3n**[9] in $CDCl_3$

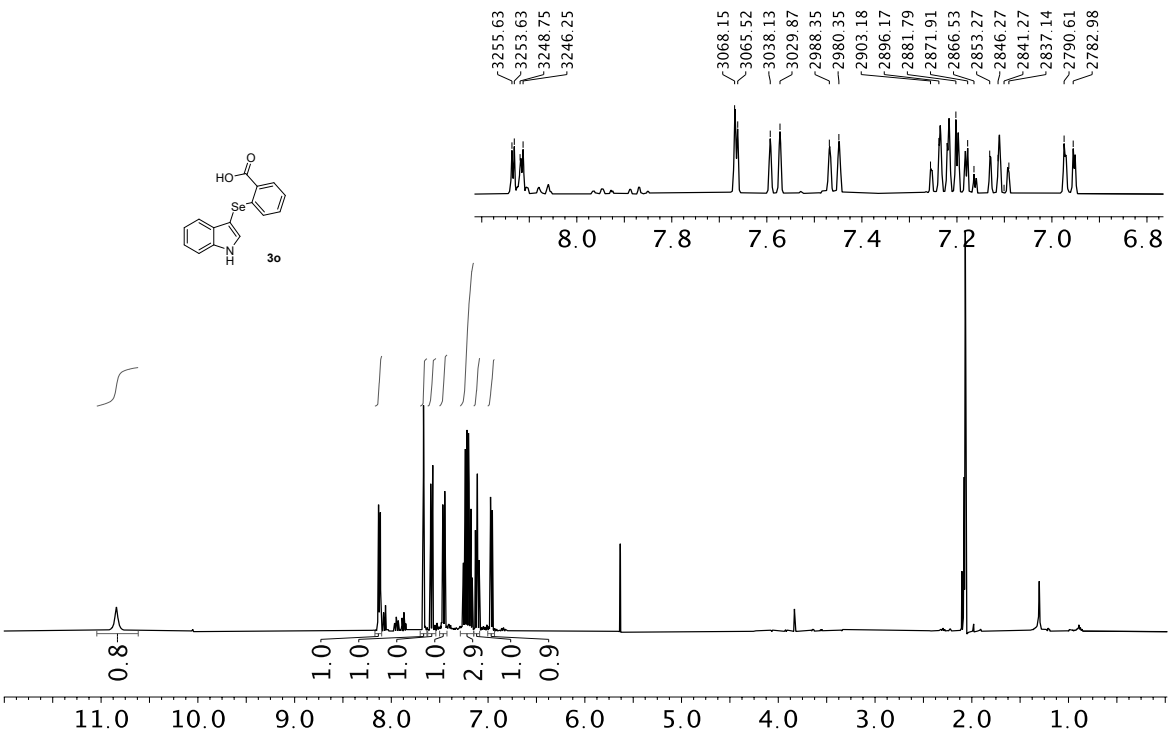


Figure 20. ^{1}H NMR spectrum of compound **3o**[9] in CD_3COCD_3

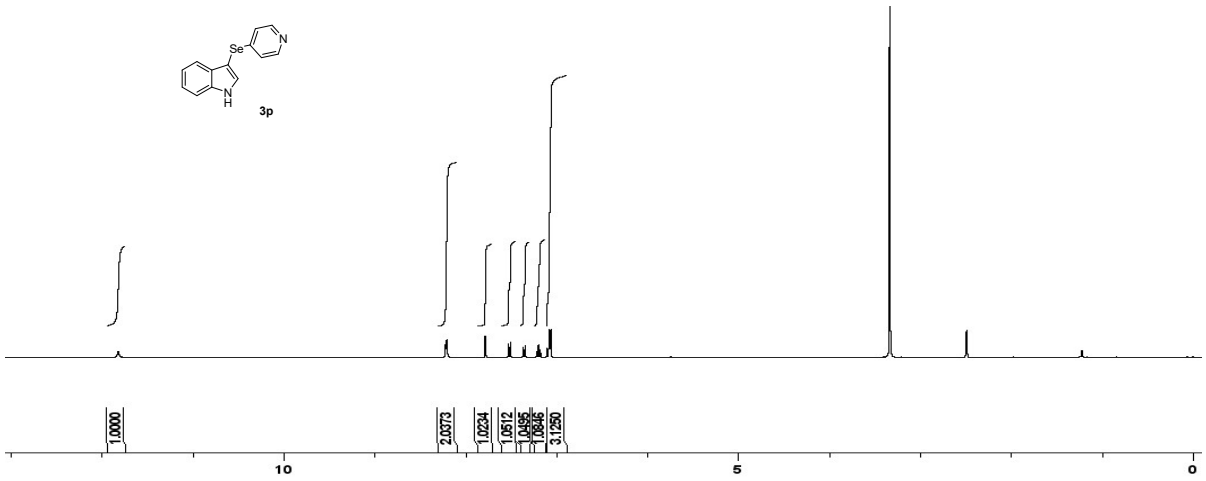


Figure 21. ^{1}H NMR spectrum of compound **3p**[11] in DMSO-d_6

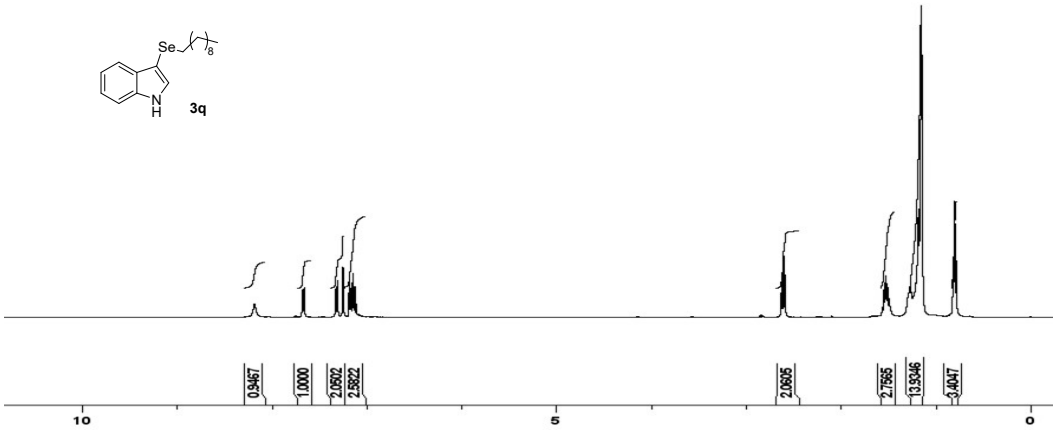


Figure 22. ^{1}H NMR spectrum of compound **3q** in $CDCl_3$

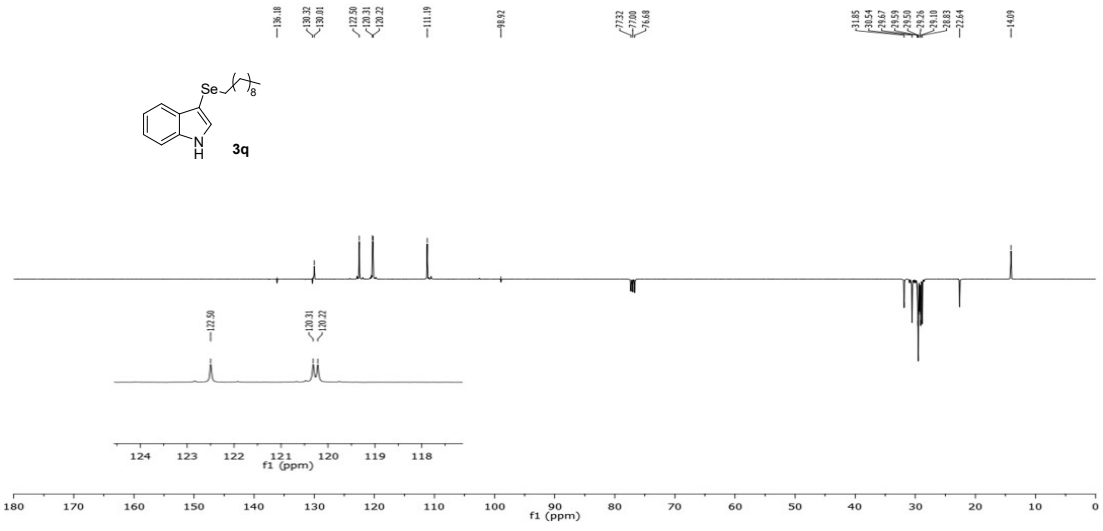


Figure 23. ^{13}C NMR spectrum of compound **3q** in $CDCl_3$

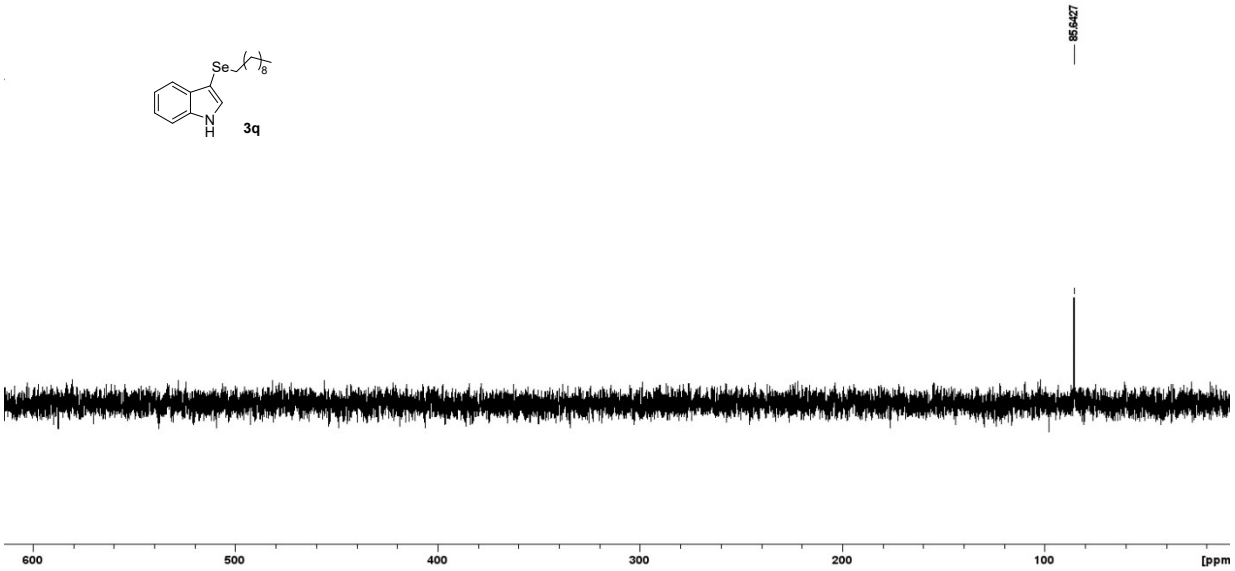


Figure 24. ^{77}Se NMR spectrum of compound **3q** in $CDCl_3$

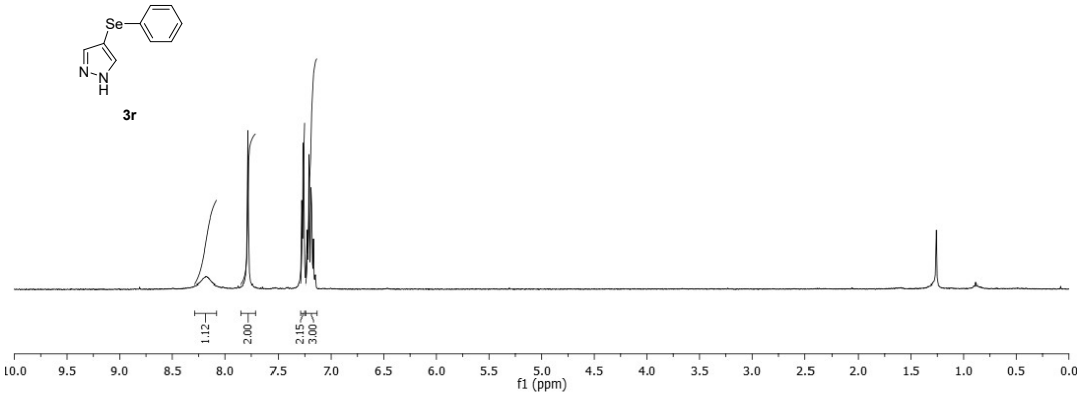


Figure 25. ^{1}H NMR spectrum of compound **3r**[12] in $CDCl_3$

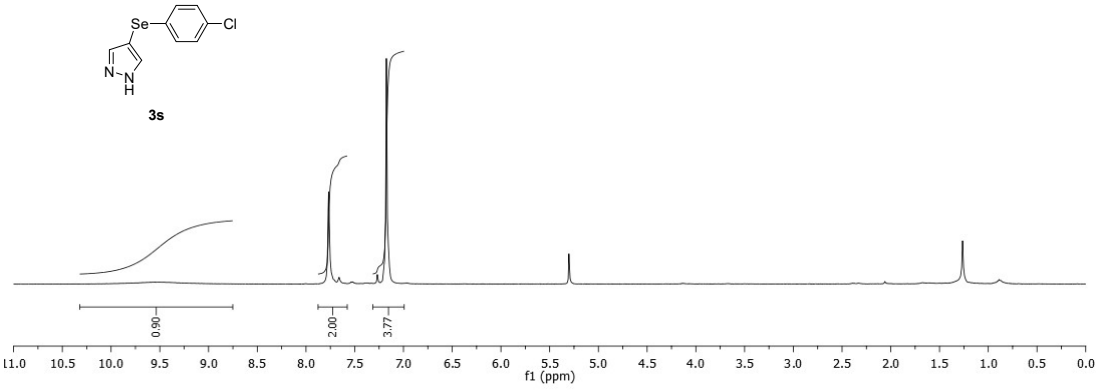


Figure 26. ^{1}H NMR spectrum of compound **3s** in $CDCl_3$

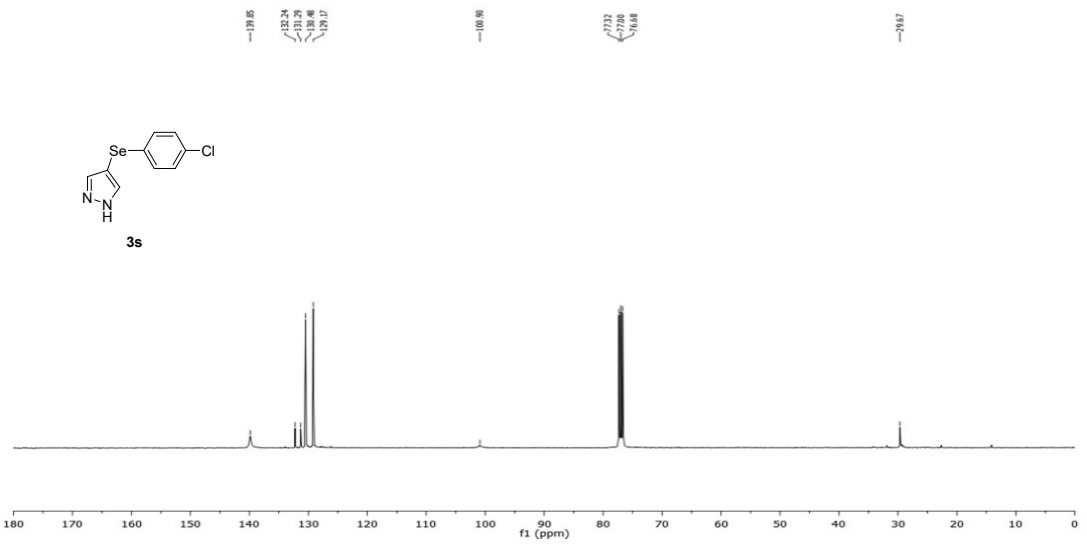


Figure 27. ^{13}C NMR spectrum of compound **3s** in $CDCl_3$

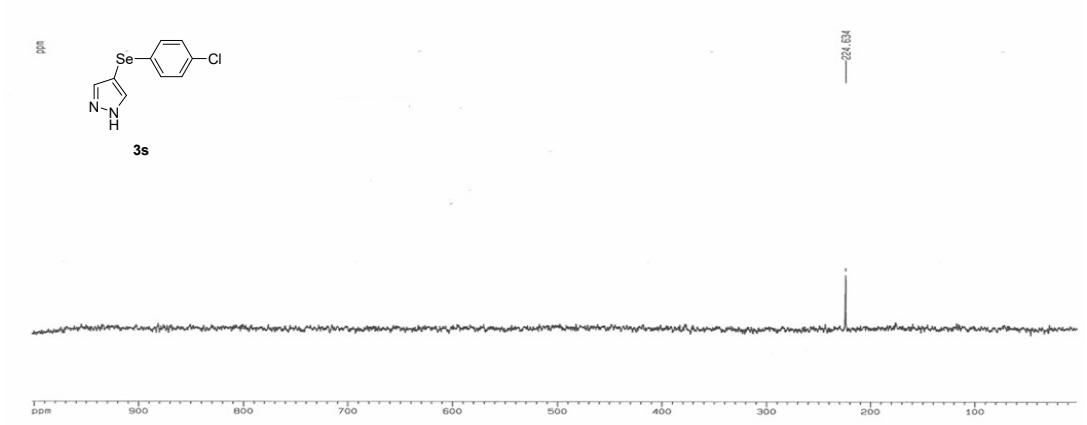


Figure 28. ^{77}Se NMR spectrum of compound **3s** in $CDCl_3$

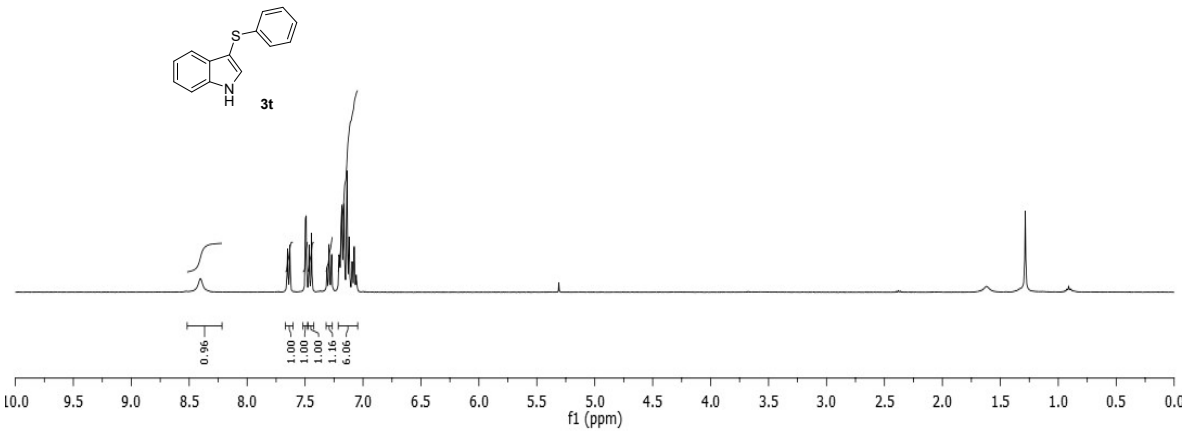


Figure 29. ^{1}H NMR spectrum of compound **3t**[13] in $CDCl_3$

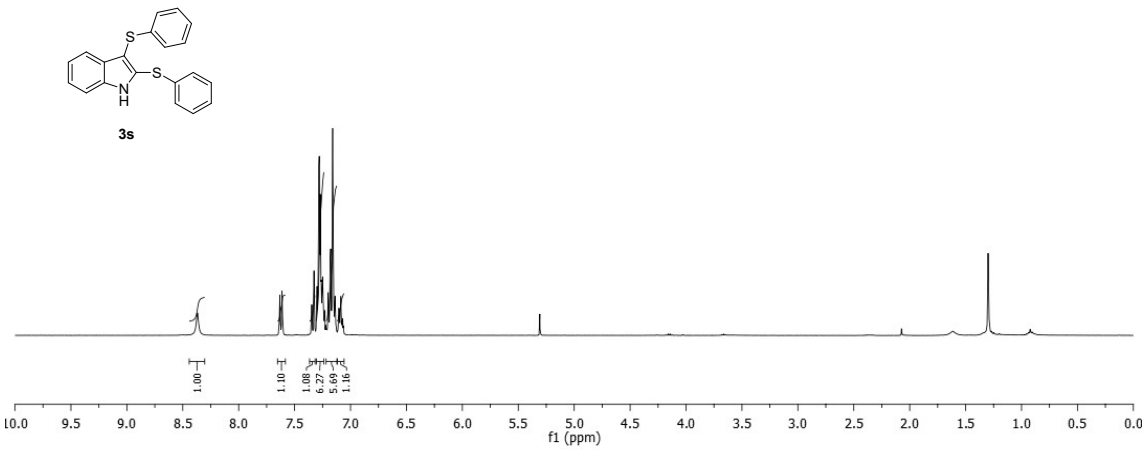


Figure 30. ^{1}H NMR spectrum of compound **3t'**[14] in $CDCl_3$

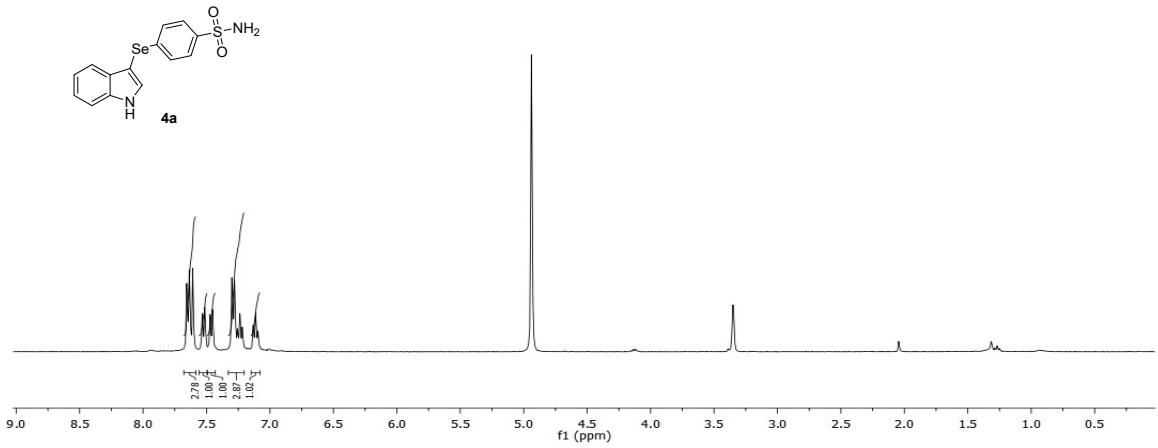


Figure 31. ^{1}H NMR spectrum of compound **4a** in CD_3OD

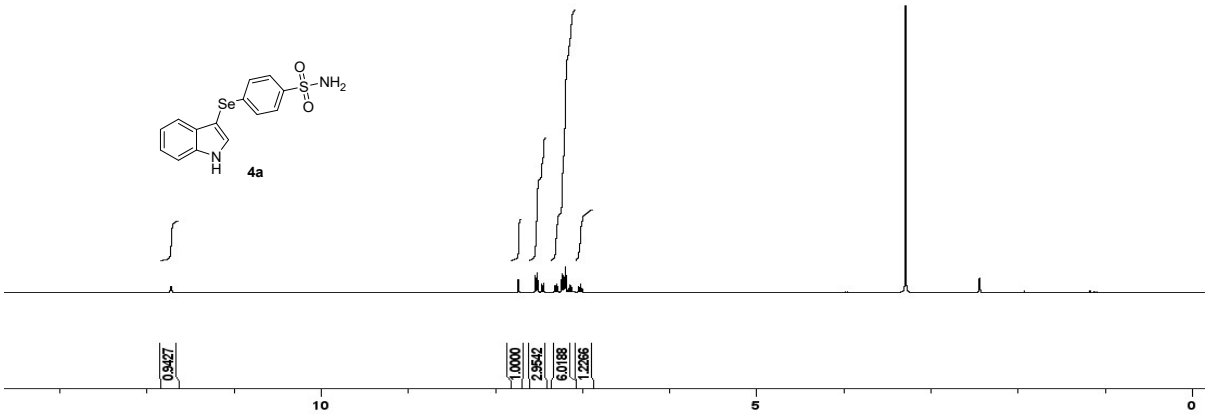


Figure 32. ^{1}H NMR spectrum of compound **4a** in DMSO-d_6

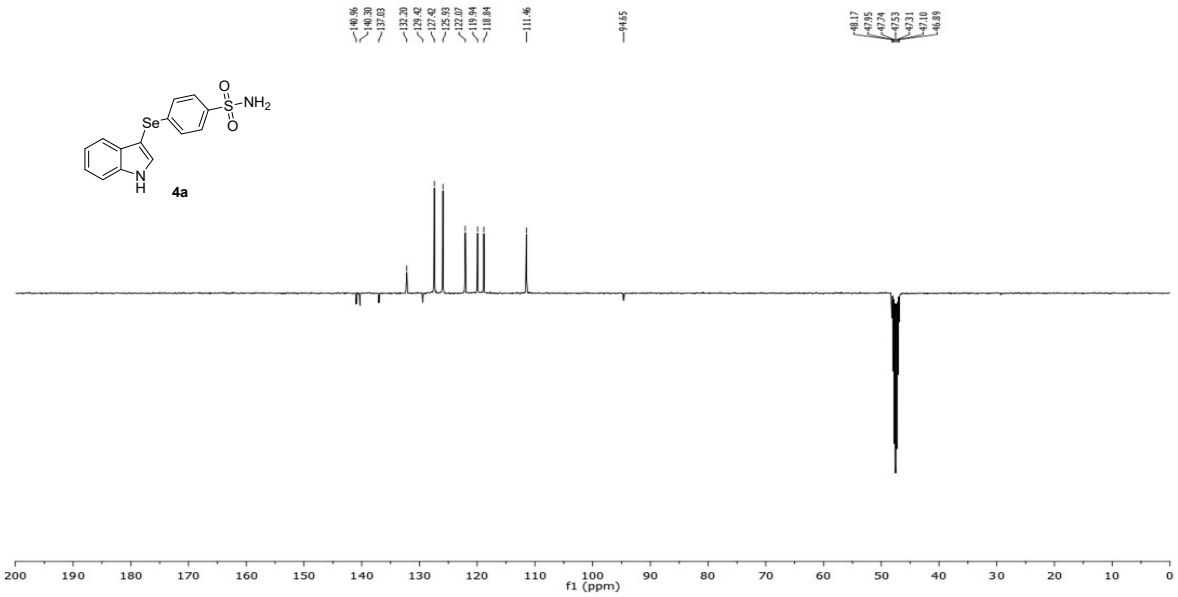


Figure 33. ^{13}C NMR spectrum of compound **4a** in CD_3OD

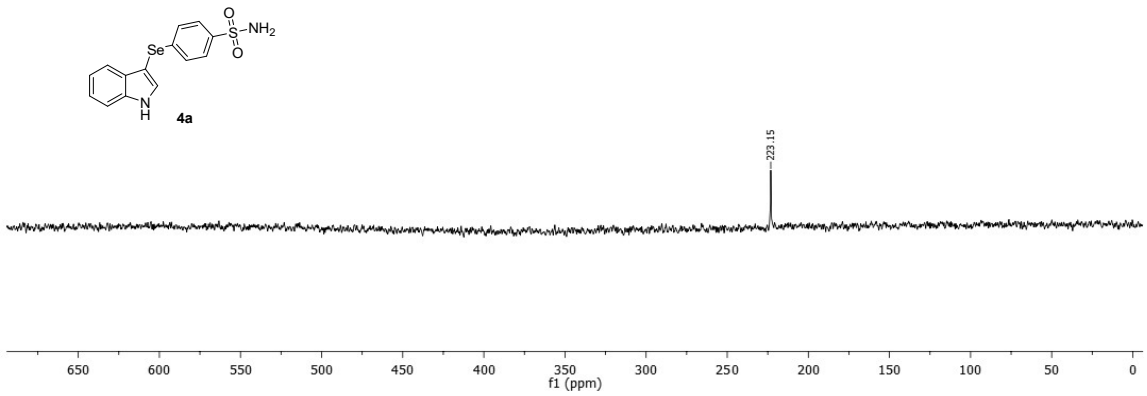


Figure 34. ^{77}Se NMR spectrum of compound **4a** in CD_3OD

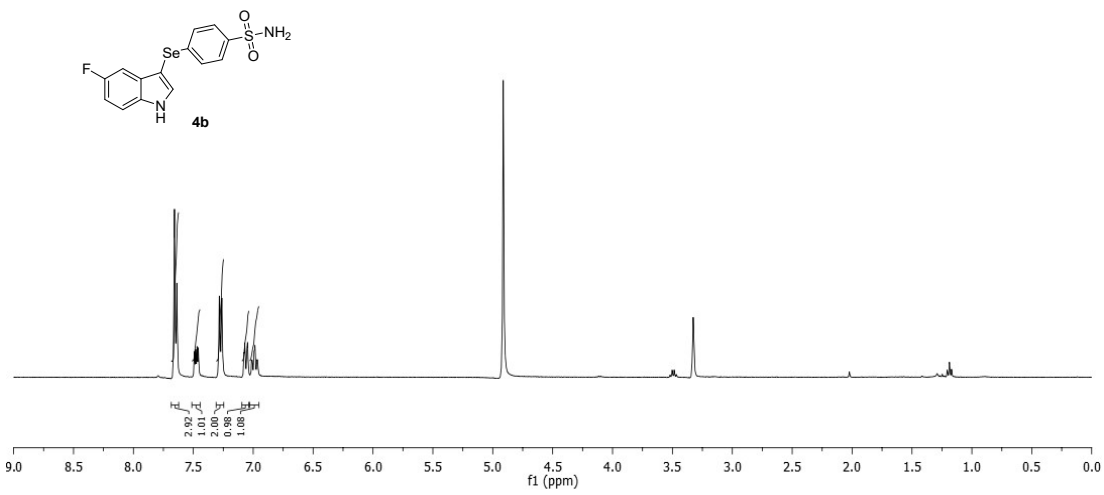


Figure 35. ^{1}H NMR spectrum of compound **4b** in CD_3OD

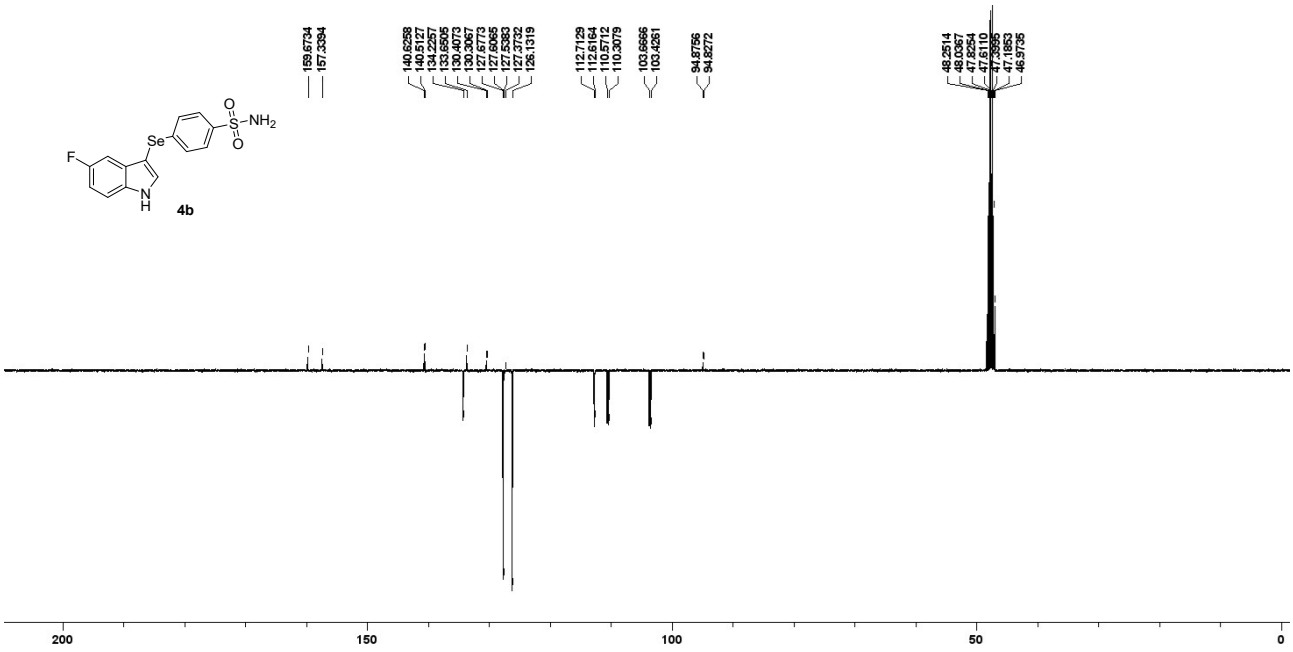


Figure 36. ^{13}C NMR spectrum of compound **4b** in CD_3OD

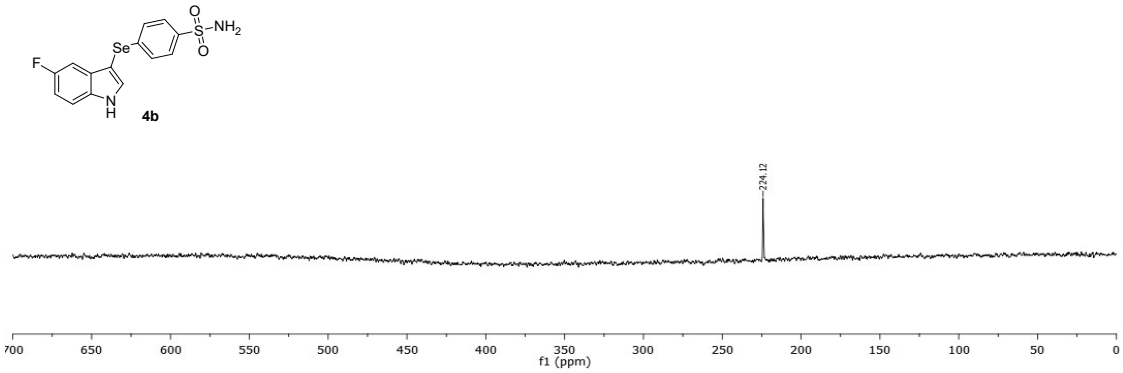


Figure 37. ^{77}Se NMR spectrum of compound **4b** in CD_3OD

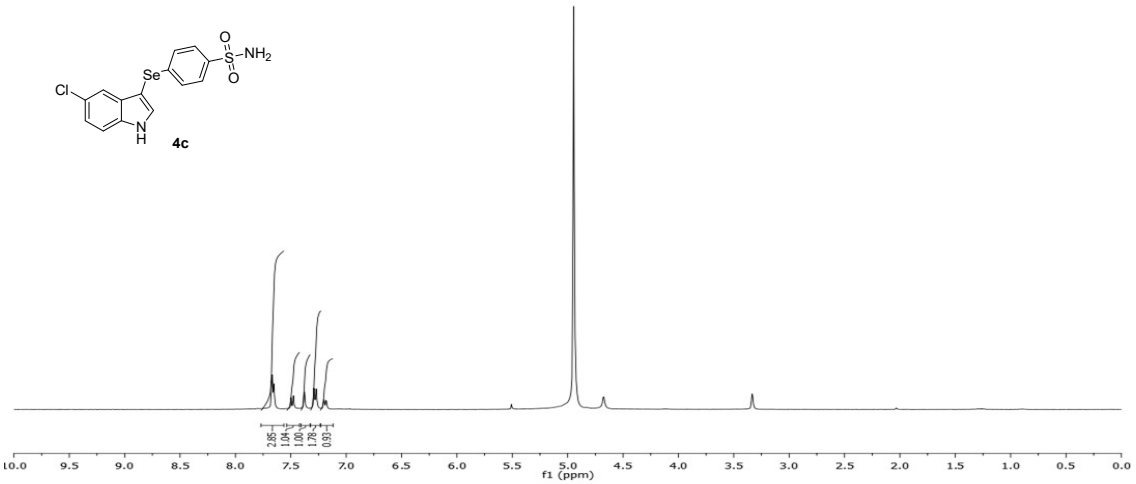


Figure 38. ^{1}H NMR spectrum of compound **4c** in CD_3OD

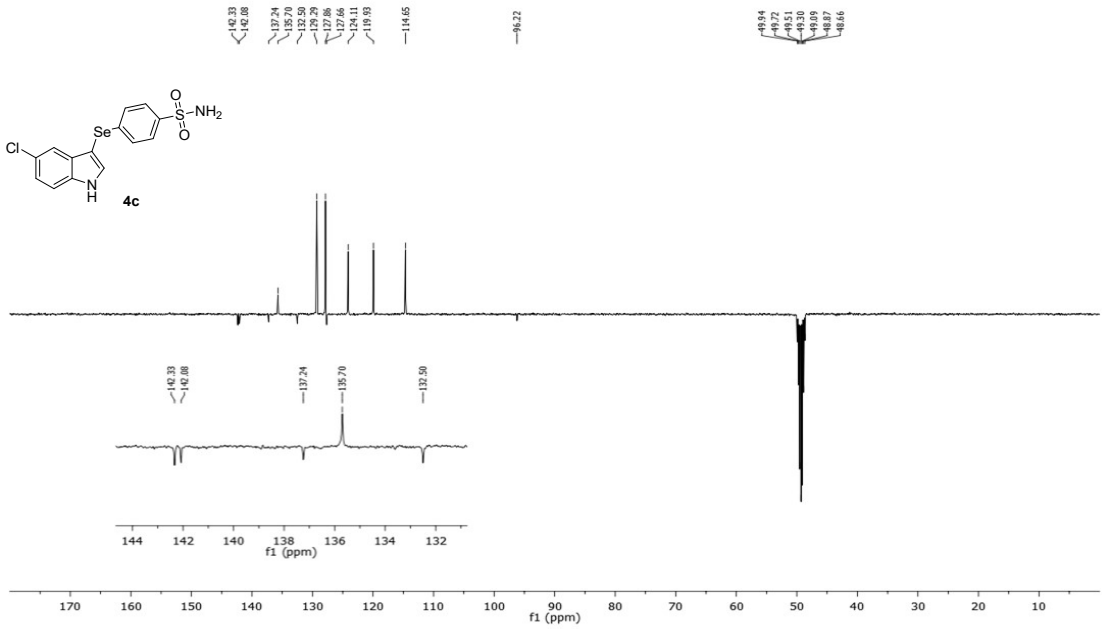


Figure 39. ^{13}C NMR spectrum of compound **4c** in CD_3OD

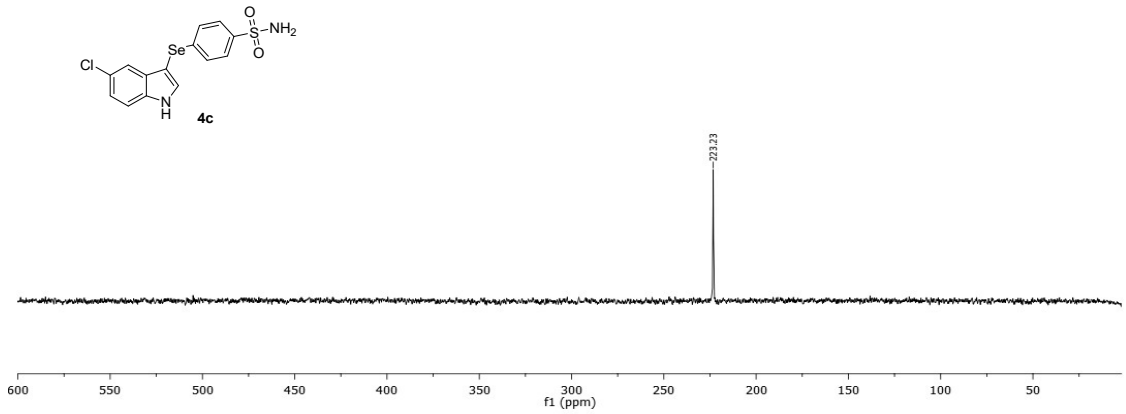


Figure 40. ^{77}Se NMR spectrum of compound **4c** in CD_3OD

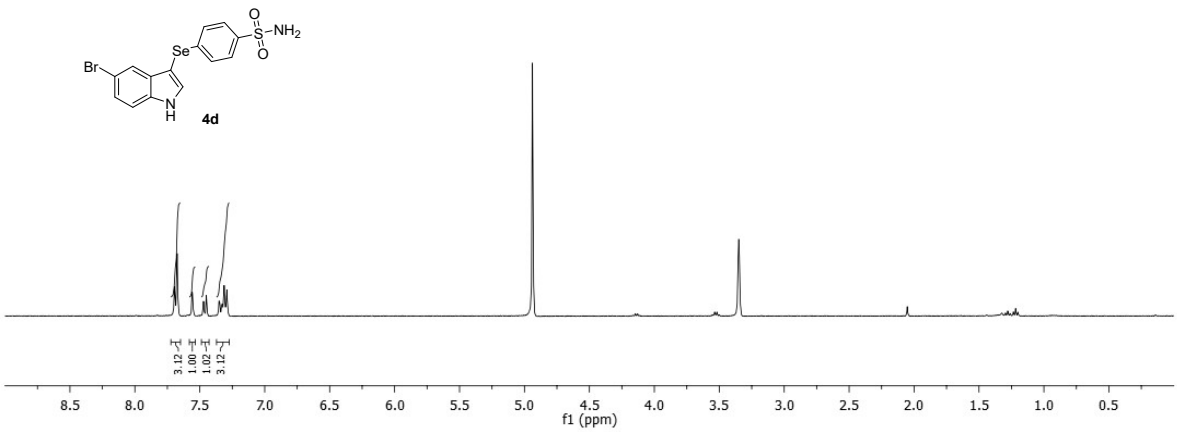


Figure 41. ^{1}H NMR spectrum of compound **4d** in CD_3OD

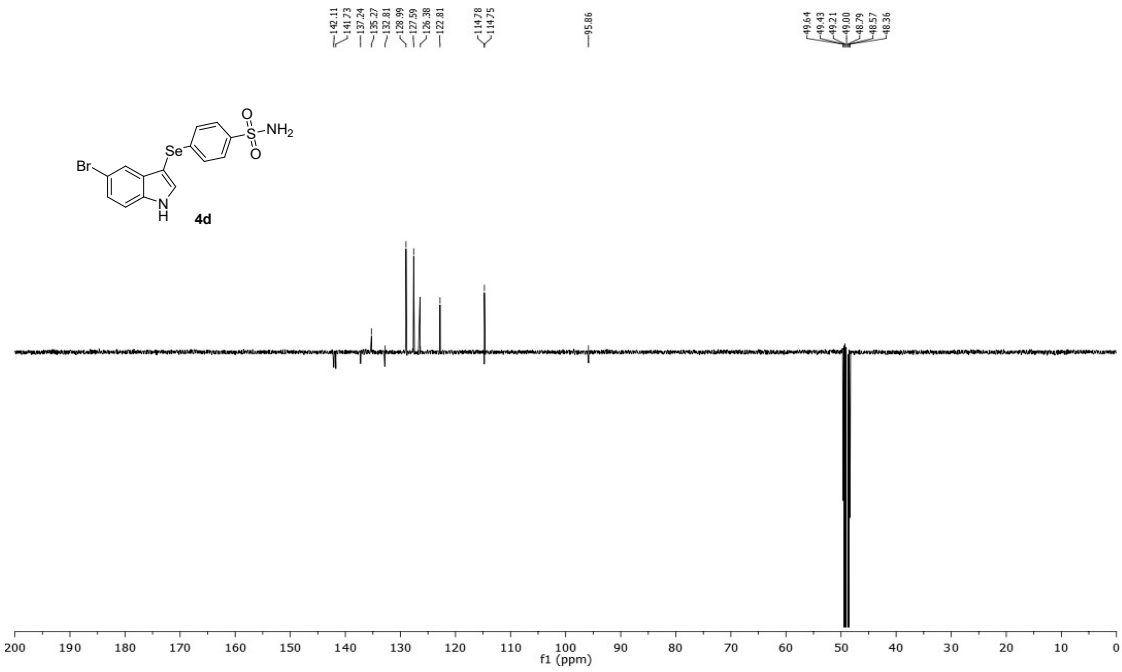


Figure 42. ^{13}C NMR spectrum of compound **4d** in CD_3OD

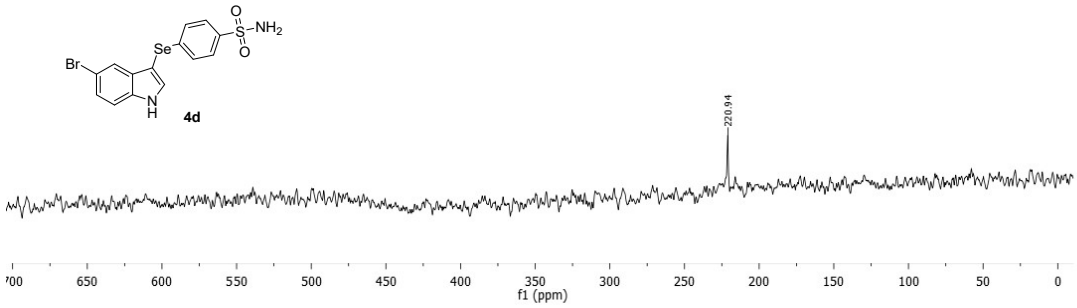


Figure 43. ^{77}Se NMR spectrum of compound **4d** in CD_3OD

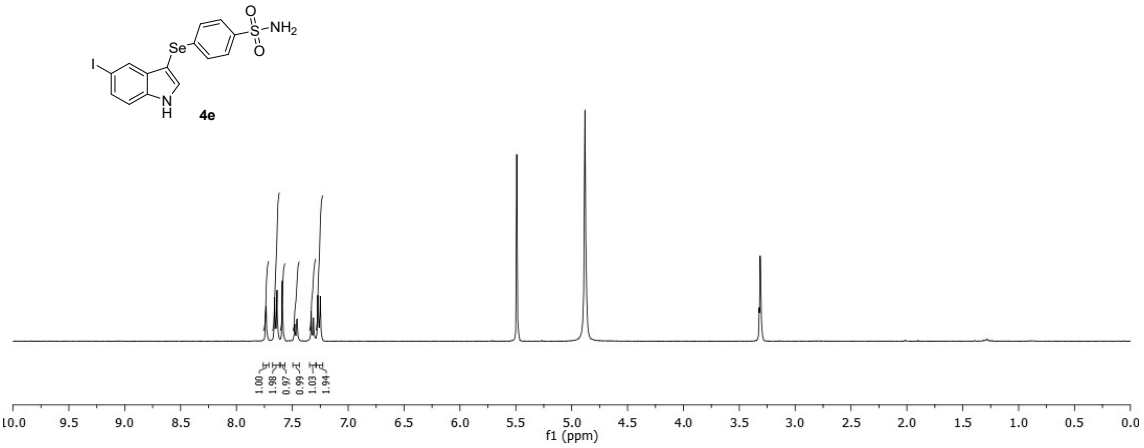


Figure 44. ^{1}H NMR spectrum of compound **4e** in CD_3OD

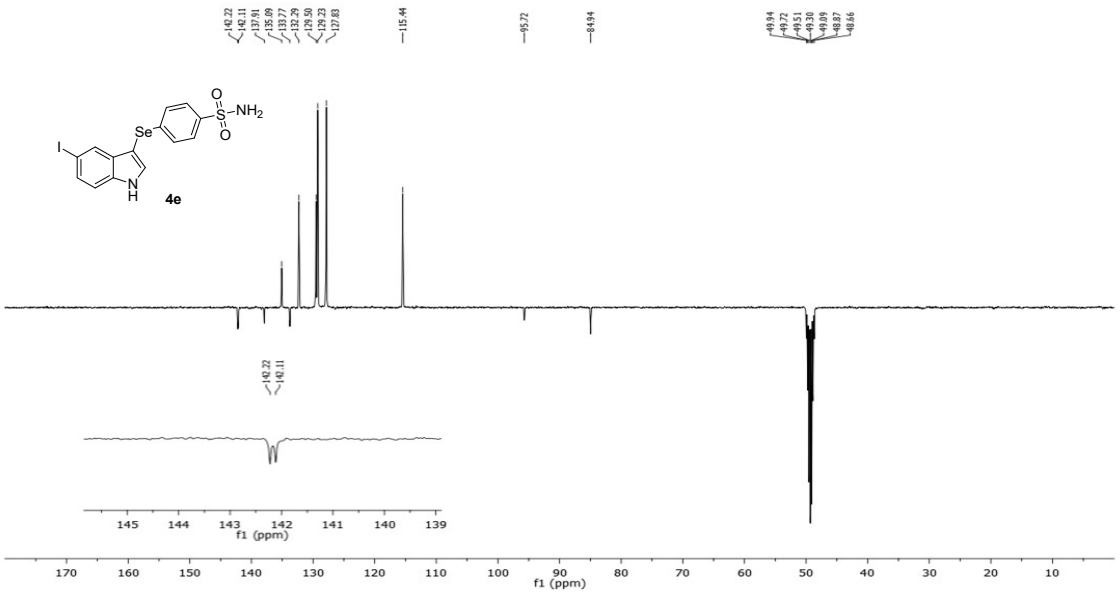


Figure 45. ^{13}C NMR spectrum of compound **4e** in CD_3OD

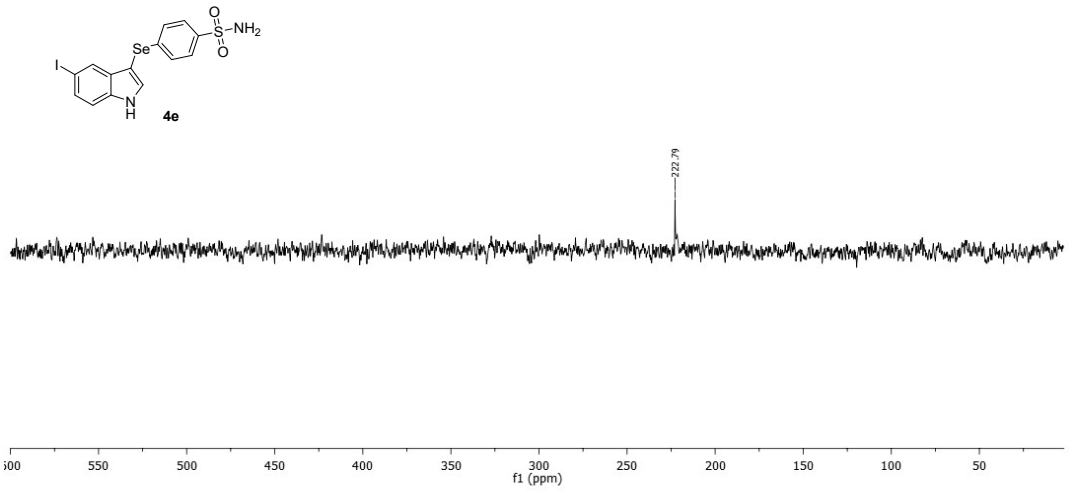


Figure 46. ^{77}Se NMR spectrum of compound **4e** in CD_3OD

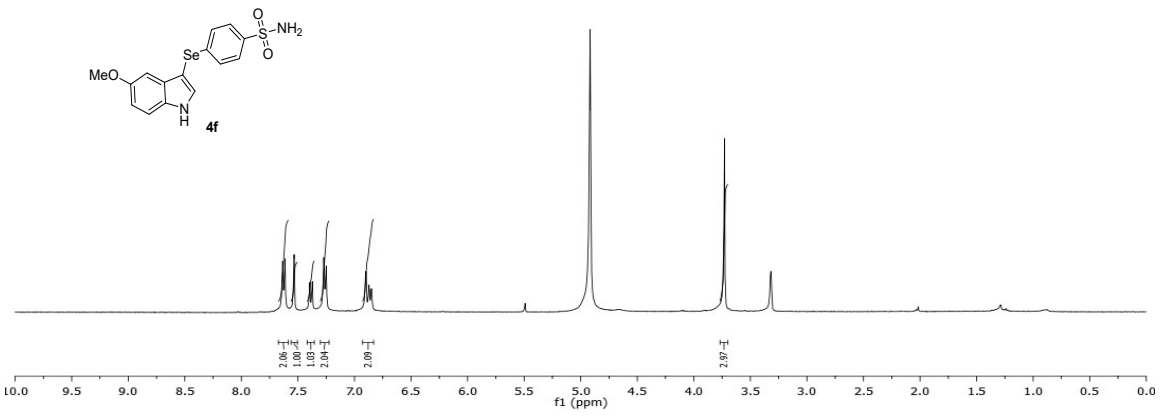


Figure 47. ^{1}H NMR spectrum of compound **4f** in CD_3OD

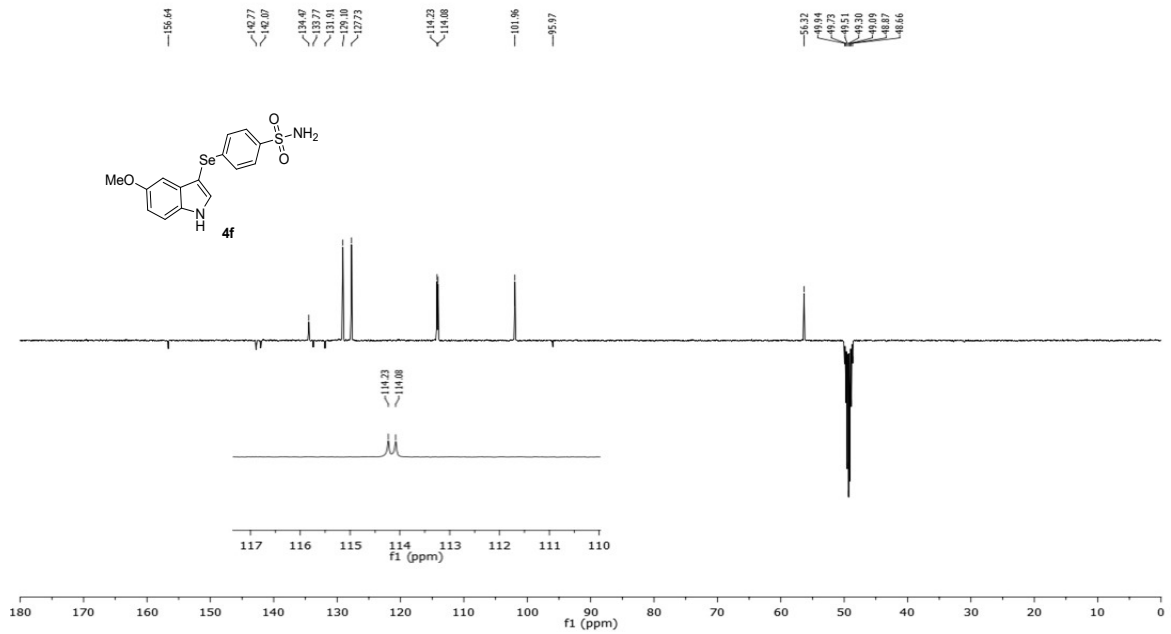


Figure 48. ^{13}C NMR spectrum of compound **4f** in CD_3OD

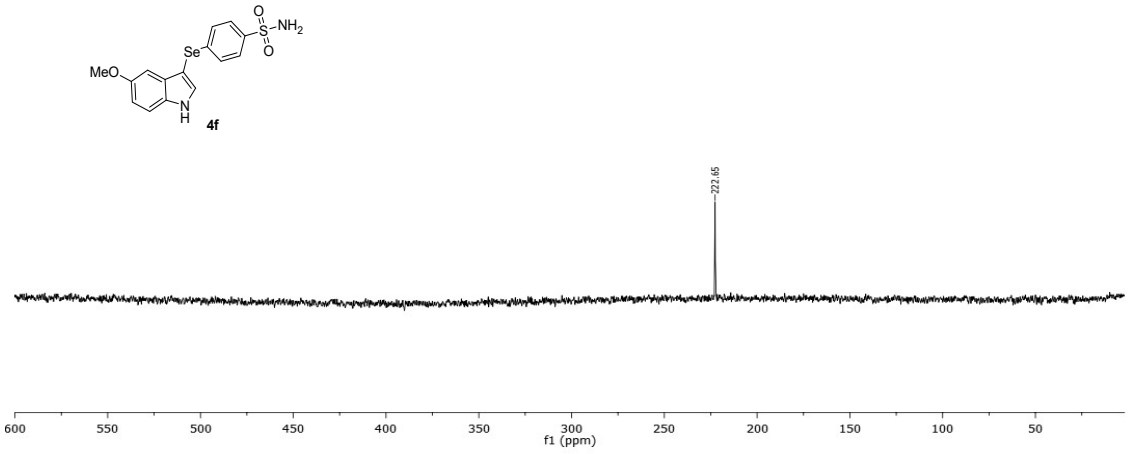


Figure 49. ^{77}Se NMR spectrum of compound **4f** in CD_3OD

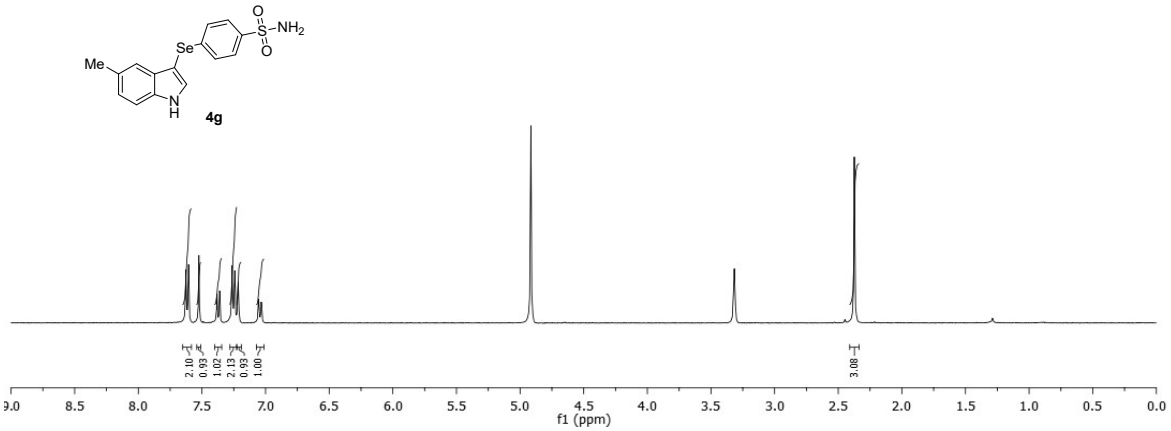


Figure 50. ^{1}H NMR spectrum of compound **4g** in CD_3OD

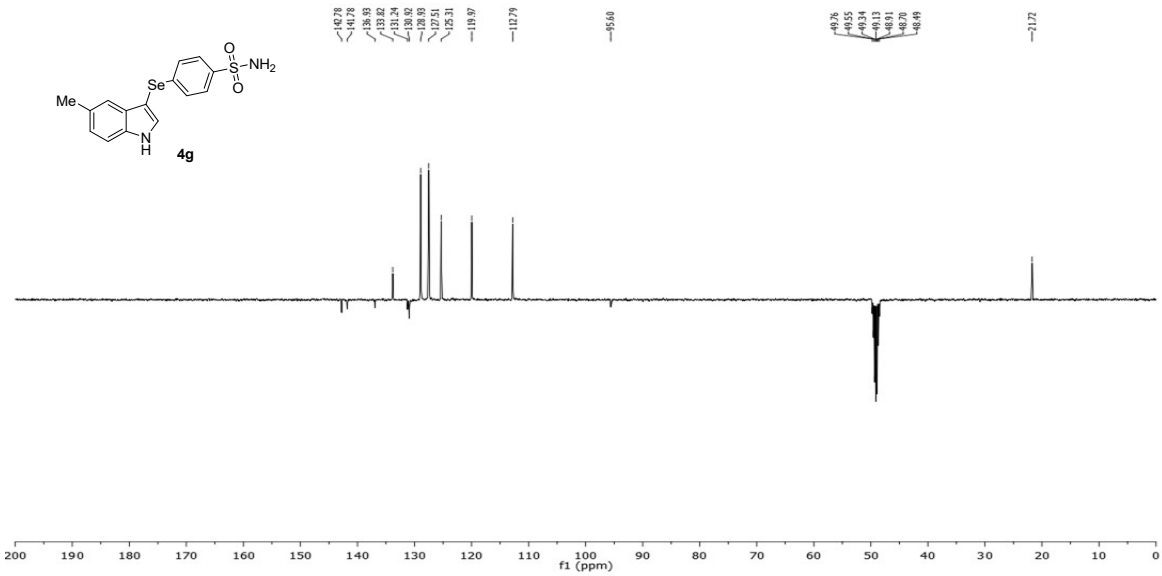


Figure 51. ^{13}C NMR spectrum of compound **4g** in CD_3OD

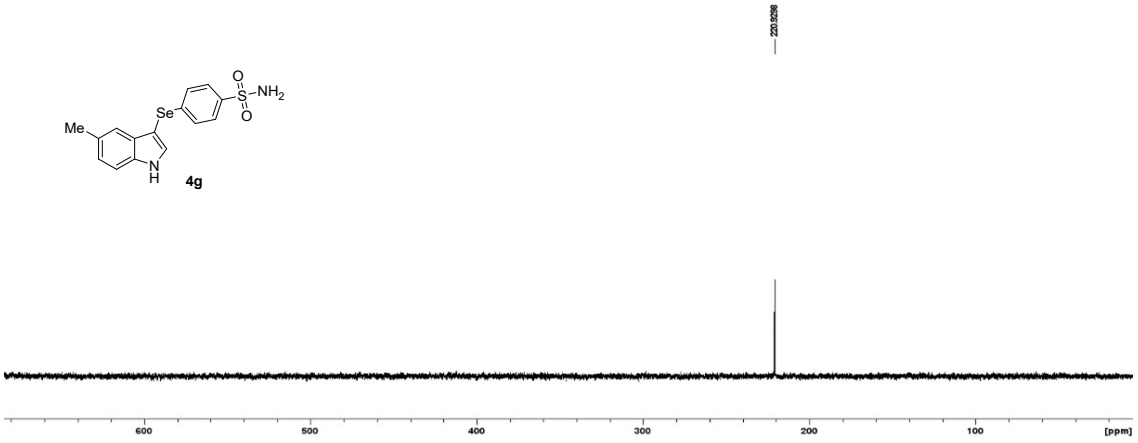


Figure 52. ^{77}Se NMR spectrum of compound **4g** in CD_3OD

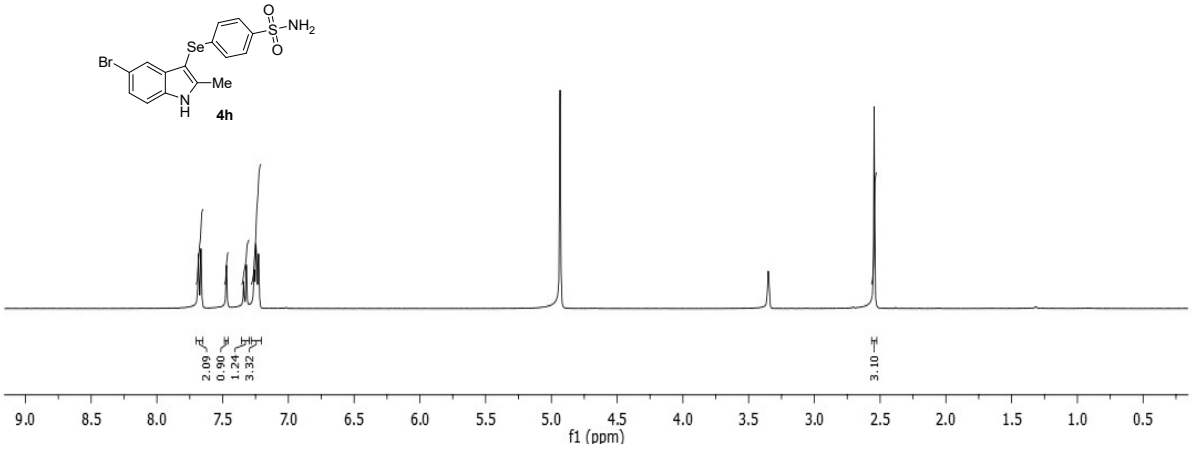


Figure 53. ^{1}H NMR spectrum of compound **4h** in CD_3OD

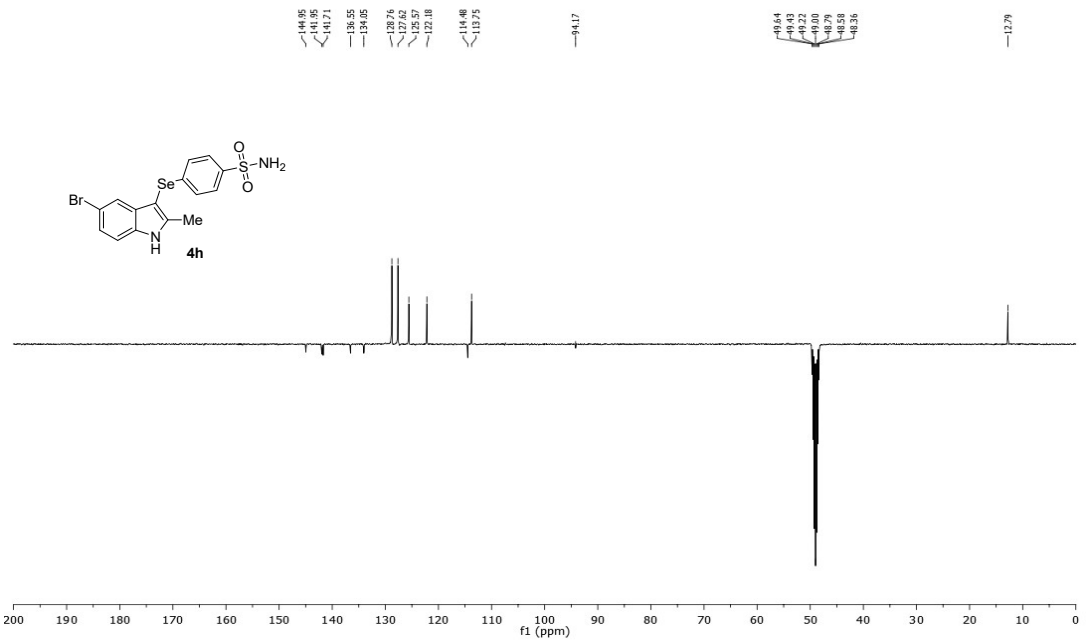


Figure 54. ^{13}C NMR spectrum of compound **4h** in CD_3OD

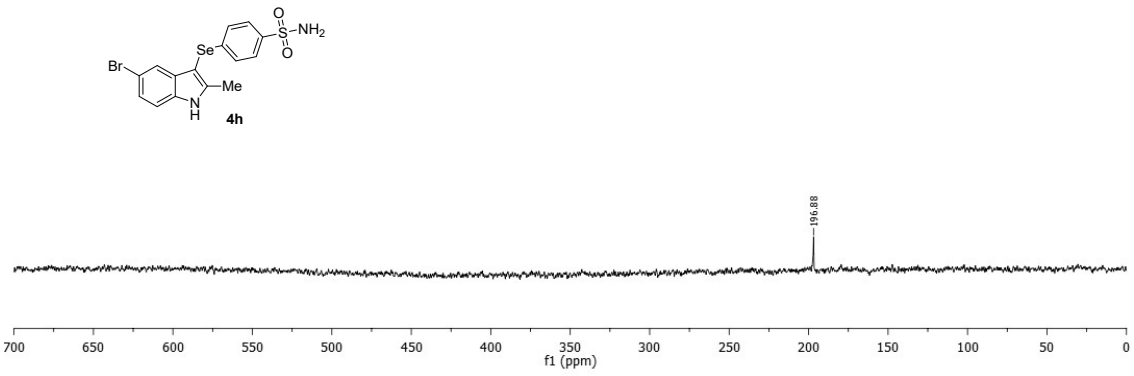


Figure 55. ^{77}Se NMR spectrum of compound **4h** in CD_3OD

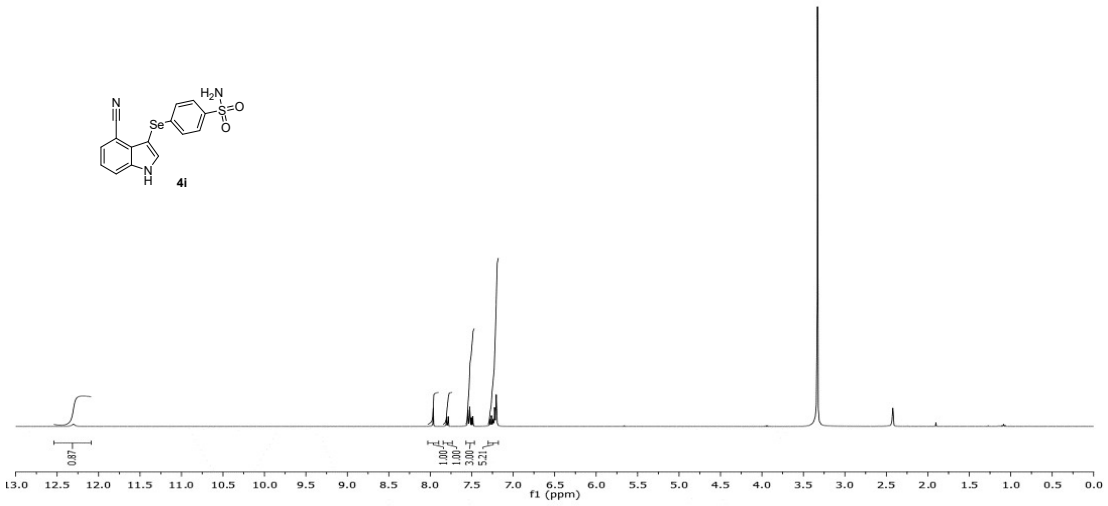


Figure 56. ^{1}H NMR spectrum of compound **4i** in DMSO-d_6

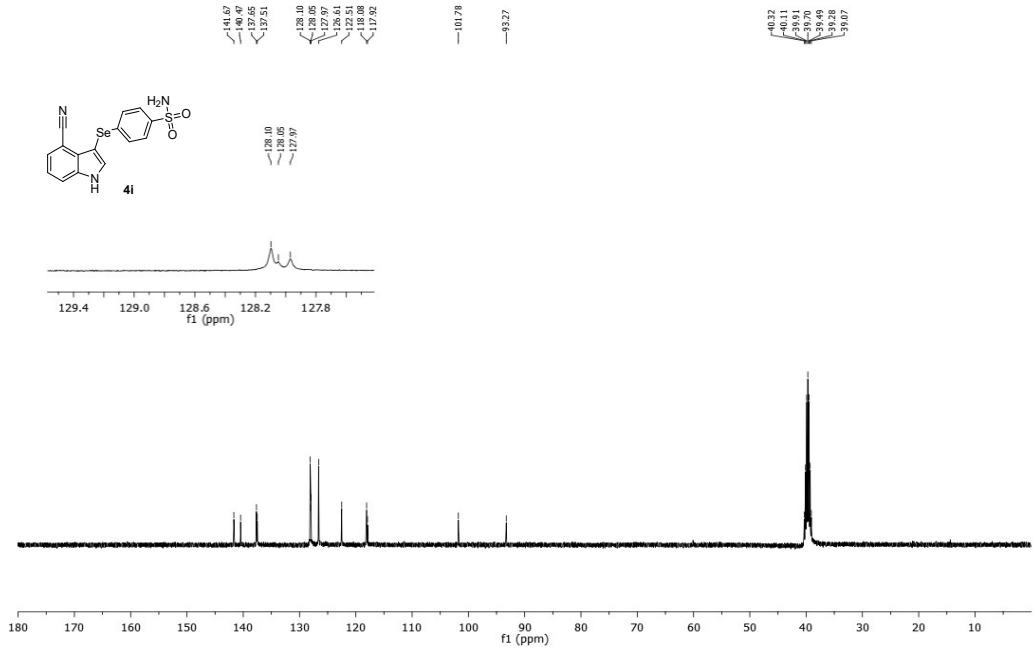


Figure 57. ^{13}C NMR spectrum of compound **4i** in DMSO-d_6

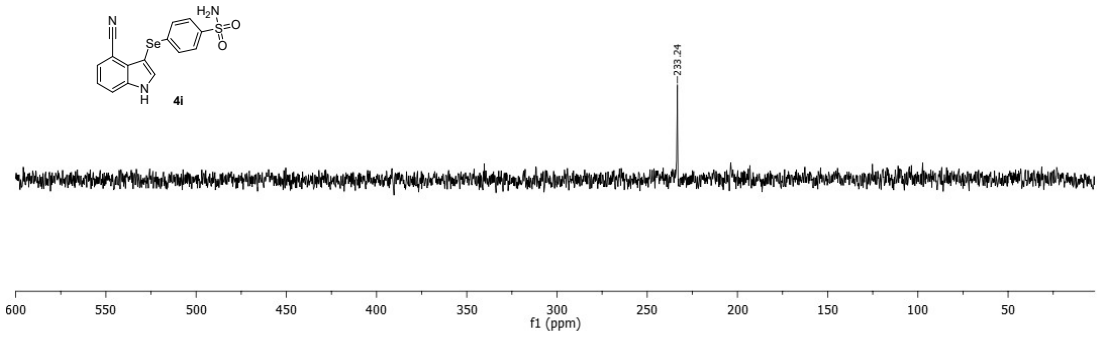


Figure 58. ^{77}Se NMR spectrum of compound **4i** in DMSO-d_6

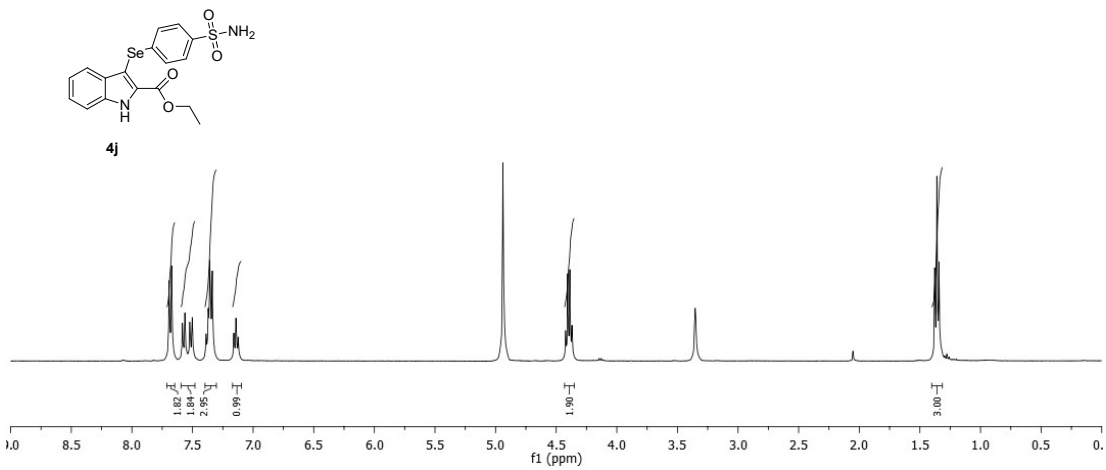


Figure 59. ^{1}H NMR spectrum of compound **4j** in CD_3OD

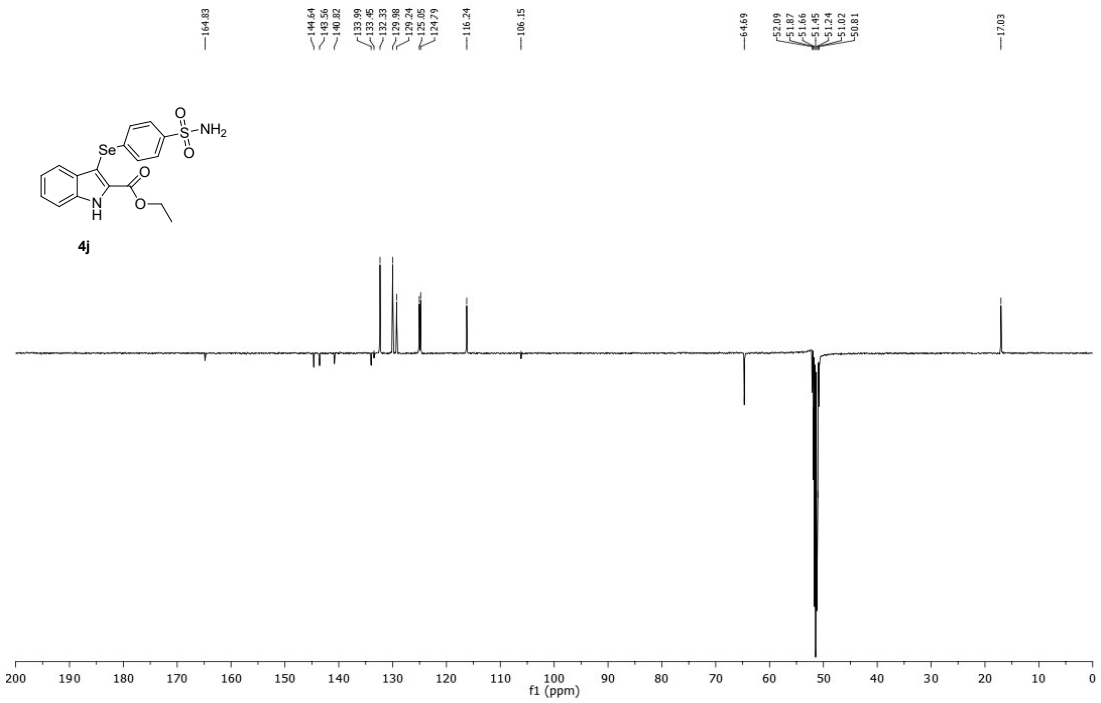


Figure 60. ^{13}C NMR spectrum of compound **4j** in CD_3OD

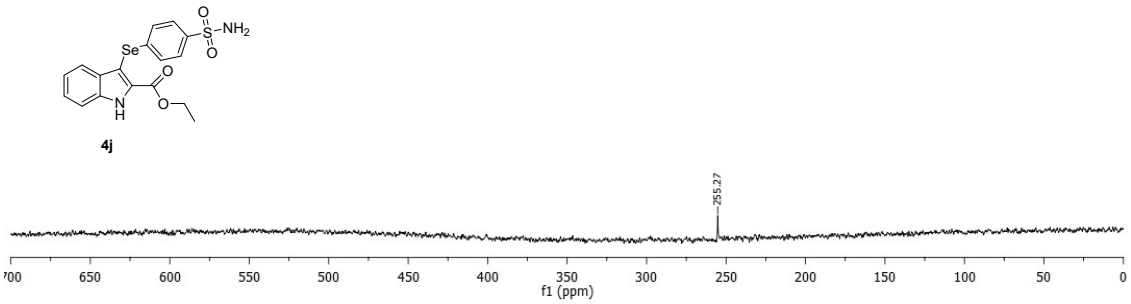


Figure 61. ^{77}Se NMR spectrum of compound **4j** in CD_3OD

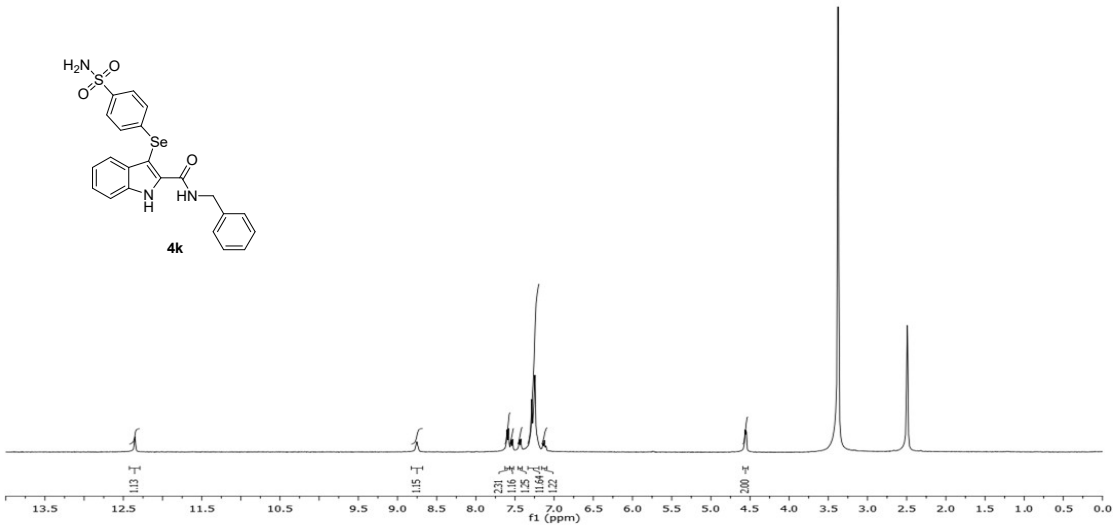


Figure 62. ^{1}H NMR spectrum of compound **4k** in DMSO-d_6

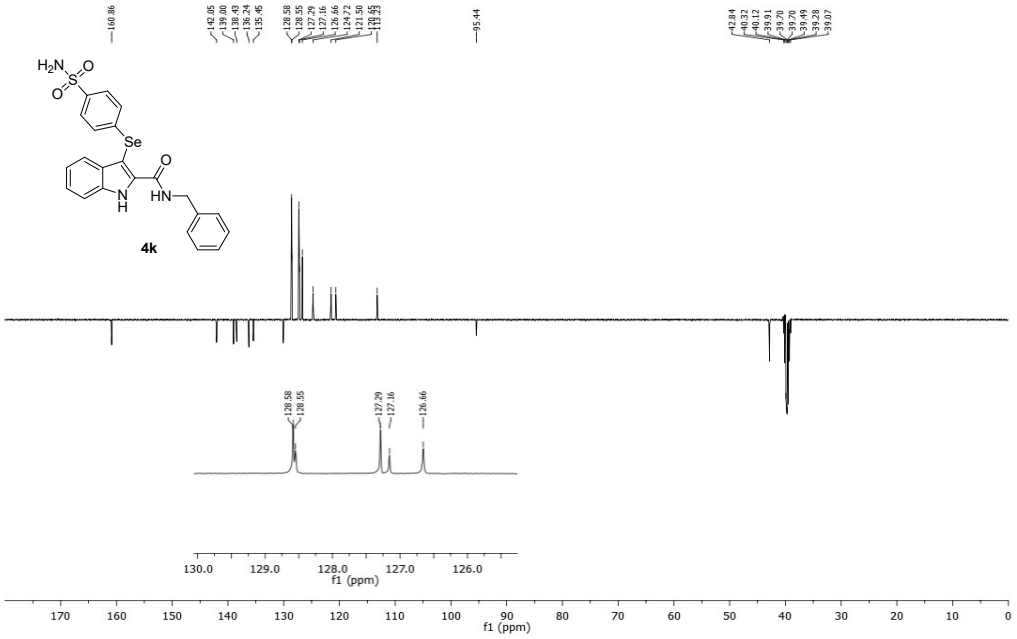


Figure 63. ^{13}C NMR spectrum of compound **4k** in DMSO-d_6

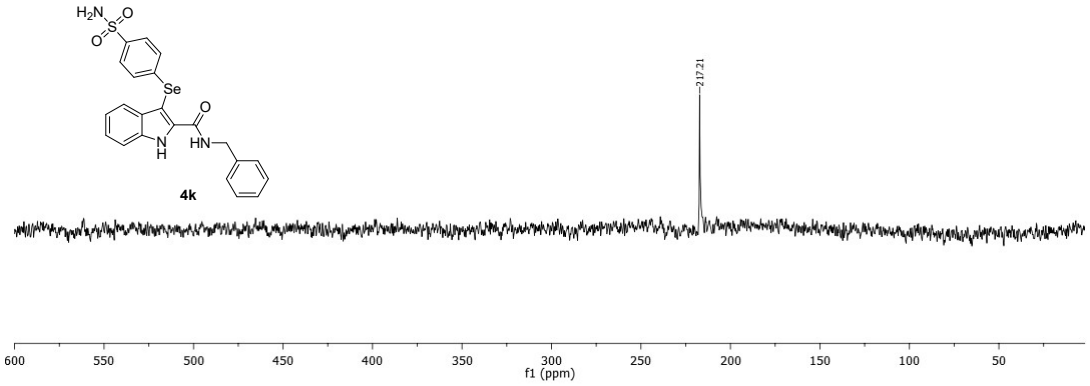


Figure 64. ^{77}Se NMR spectrum of compound **4k** in DMSO-d_6

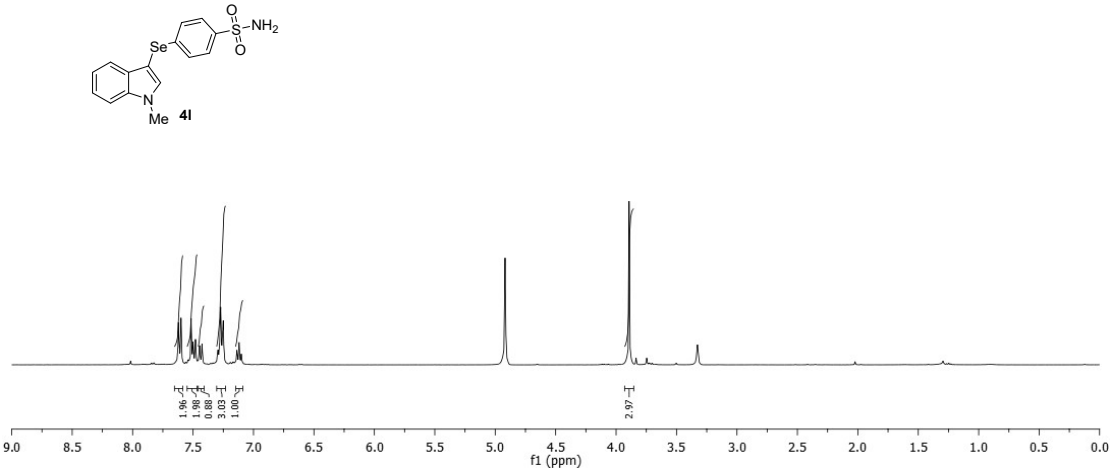


Figure 65. ^{1}H NMR spectrum of compound **4l** in CD_3OD

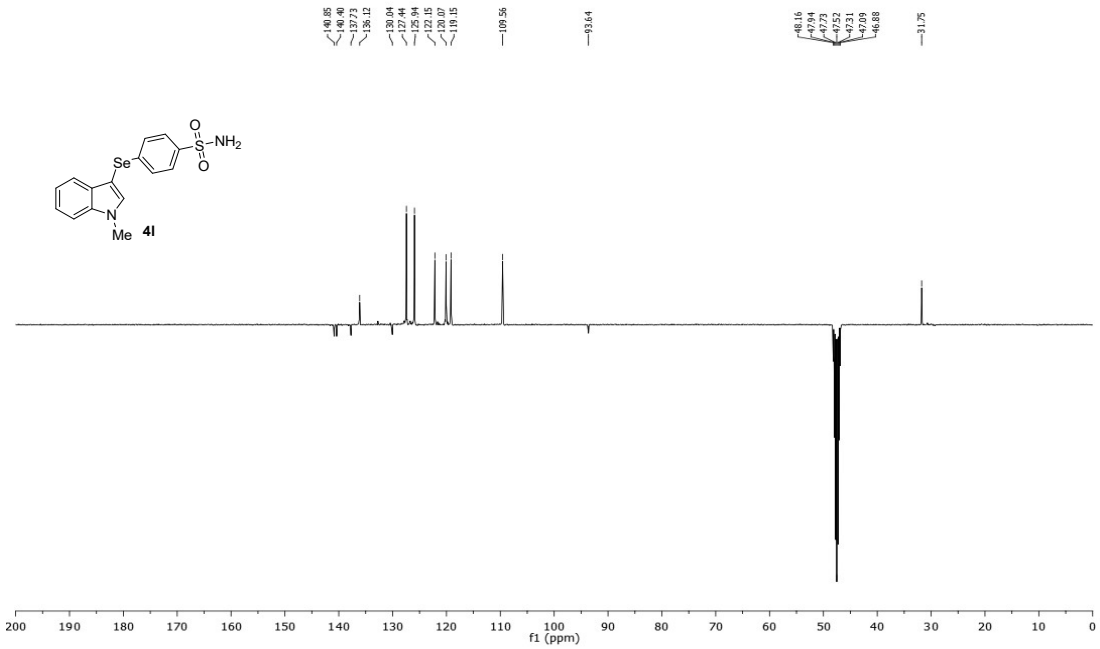


Figure 66. ^{13}C NMR spectrum of compound **4l** in CD_3OD

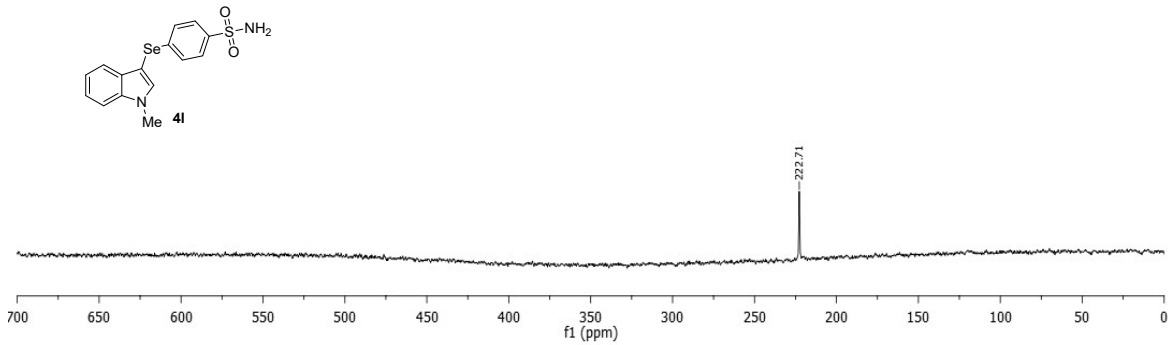
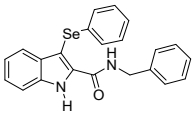


Figure 67. ^{77}Se NMR spectrum of compound **4l** in CD_3OD

4. HMRS Spectra of new compounds

Elemental Composition Report

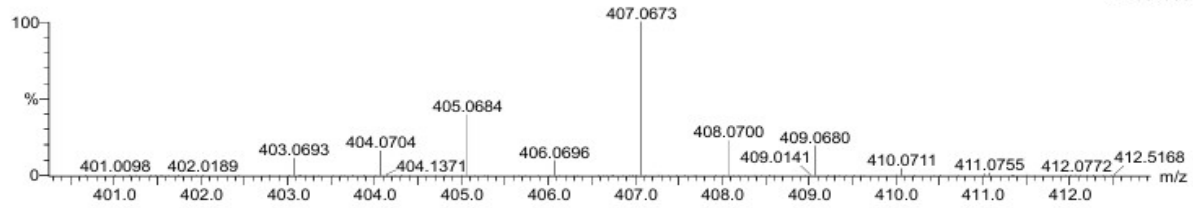
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



3f

Monoisotopic Mass, Even Electron Ions
75 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 O: 0-3 Se: 1-1
210311_AH_40_A 17 (0.197) Cm (14:21-(3:8+53:60))

TOF MS ES+
7.15e+006

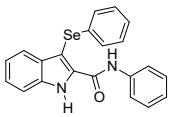


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
407.0673	407.0663	1.0	2.5	15.5	601.8	n/a	n/a	C22 H19 N2 O Se

Figure 68. HRMS *spectrum of compound* ***3f***

Elemental Composition Report

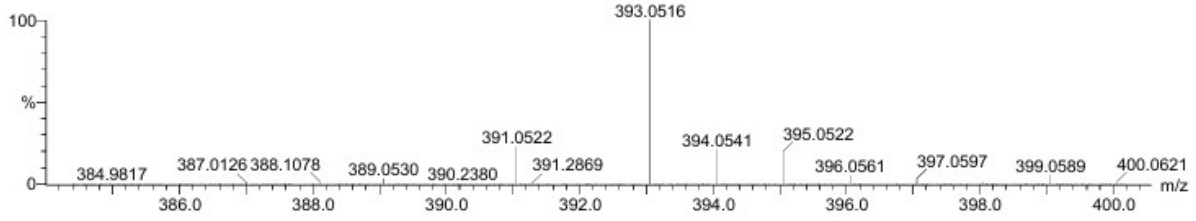
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



3g

Monoisotopic Mass, Even Electron Ions
72 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 O: 0-3 Se: 1-1
210311_AH_25_A 22 (0.240) Cm (22:37-2:9)

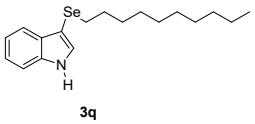
TOF MS ES+
2.10e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
393.0516	393.0506	1.0	2.5	15.5	1521.6	n/a	n/a	C21 H17 N2 O Se

Figure 69. HRMS *spectrum of compound* ***3g***

Elemental Composition Report



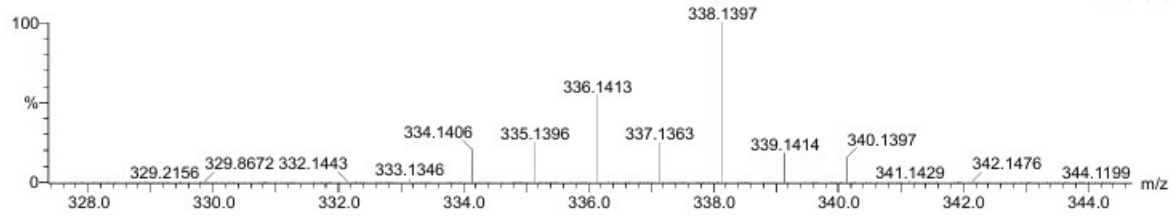
3q

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
16 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 Se: 1-1

210311_AH_22A 16 (0.177) Cm (13:25-(3:8+52:60))

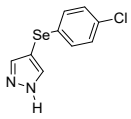
TOF MS ES+
6.39e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
338.1397	338.1387	1.0	3.0	6.5	688.5	n/a	n/a	C18 H28 N Se

Figure 70. HRMS *spectrum of compound* ***3q***

Elemental Composition Report



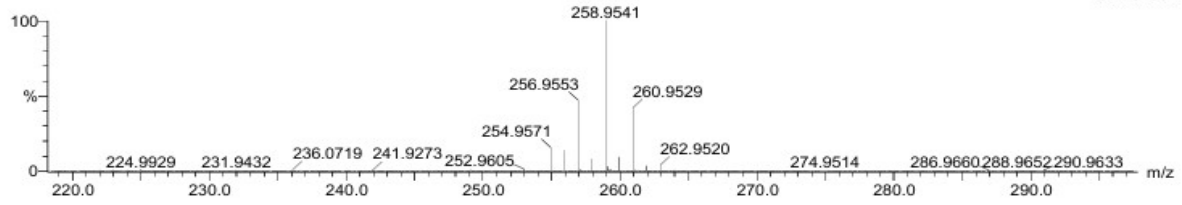
3s

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 Cl: 0-1 Se: 1-1

210311_AH_52 27 (0.294) Cm (27:40-3:8)

1: TOF MS ES+
5.44e+007

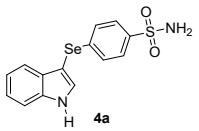


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
258.9541	258.9541	0.0	0.0	7.5	2910.0	n/a	n/a	C9 H8 N2 Cl Se

Figure 71. HRMS *spectrum of compound* ***3s***

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

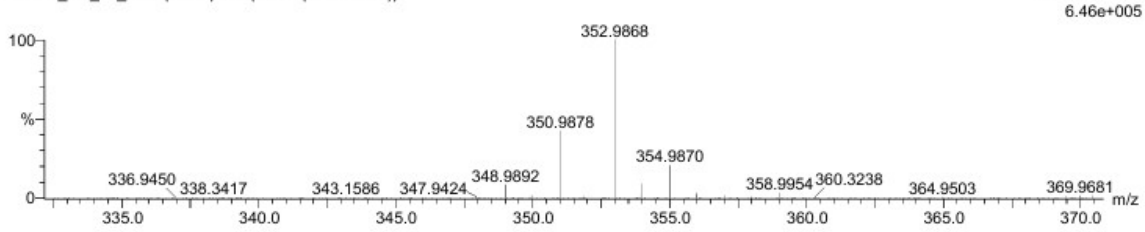


4a

Monoisotopic Mass, Even Electron Ions
155 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-2 S: 0-1 Se: 0-1

210311_AH_02_A 19 (0.214) Cm (19:32-(3:11+49:61))

TOF MS ES+
6.46e+005

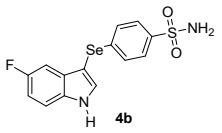


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
352.9868	352.9863	0.5	1.4	10.5	606.5	0.067	93.52	C14 H13 N2 O2 S Se
	352.9869	-0.1	-0.3	19.5	609.2	2.736	6.48	C22 H9 Se

Figure 72. HRMS *spectrum of compound* ***4a***

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

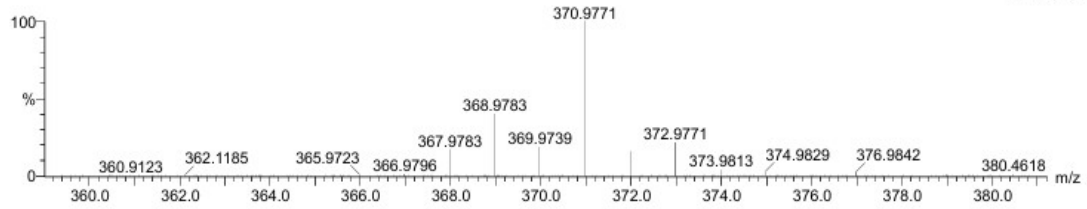


4b

Monoisotopic Mass, Even Electron Ions
141 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-2 S: 0-1 Se: 1-1 F: 0-1

210311_RG_54_A 19 (0.214) Cm (19:26-(4:8+38:45))

TOF MS ES+
6.78e+005

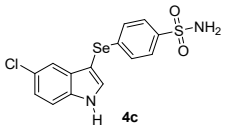


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
370.9771	370.9769	0.2	0.5	10.5	723.2	0.025	97.52	C14 H12 N2 O2 S Se F
	370.9757	1.4	3.8	14.5	726.9	3.753	2.34	C17 H11 N2 O S Se
	370.9775	-0.4	-1.1	19.5	729.8	6.590	0.14	C22 H8 Se F

Figure 73. HRMS *spectrum of compound* ***4b***

Elemental Composition Report

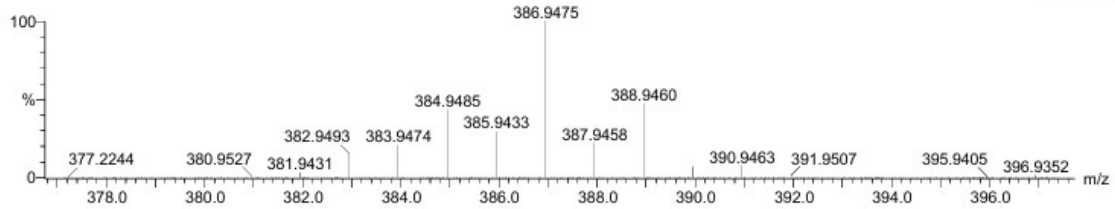
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



4c

Monoisotopic Mass, Even Electron Ions
147 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-2 S: 0-1 Cl: 0-1 Se: 1-1
210311_AH_30_A 17 (0.197) Cm (14:25-(3:8+57:60))

TOF MS ES+
2.05e+006

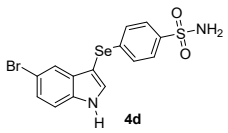


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
386.9475	386.9473	0.2	0.5	10.5	1257.0	3.007	4.94	C14 H12 N2 O2 S Cl Se
	386.9480	-0.5	-1.3	19.5	1254.0	0.051	95.02	C22 H8 Cl Se
	386.9461	1.4	3.6	24.5	1261.8	7.805	0.04	C23 H3 N2 Se

Figure 74. HRMS *spectrum of compound* ***4c***

Elemental Composition Report

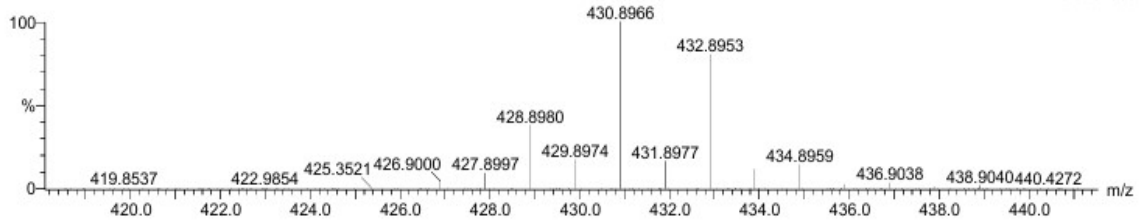
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



4d

Monoisotopic Mass, Even Electron Ions
240 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-2 S: 0-1 Se: 1-1 Br: 0-3
210311_AH_03_A 20 (0.223) Cm (20:25-(3:8+55:60))

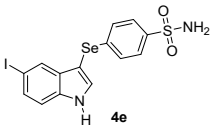
TOF MS ES+
6.92e+005



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
430.8966	430.8968	-0.2	-0.5	10.5	606.3	0.000	99.99	C14 H12 N2 O2 S Se Br
	430.8975	-0.9	-2.1	19.5	615.8	9.484	0.01	C22 H8 Se Br

Figure 75. HRMS *spectrum of compound* ***4d***

Elemental Composition Report

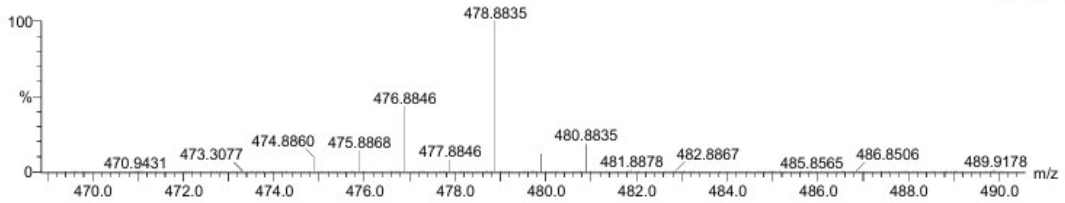


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
162 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-2 S: 0-1 Se: 1-1 I: 0-1

210311_AH_33_A 25 (0.277) Cm (25:37-(1:8+57:60))

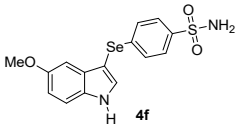
TOF MS ES+
1.38e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
478.8835	478.8829	0.6	1.3	10.5	1061.4	0.026	97.47	C14 H12 N2 O2 S Se I
	478.8836	-0.1	-0.2	19.5	1065.0	3.678	2.53	C22 H8 Se I

Figure 76. HRMS *spectrum of compound* ***4e***

Elemental Composition Report

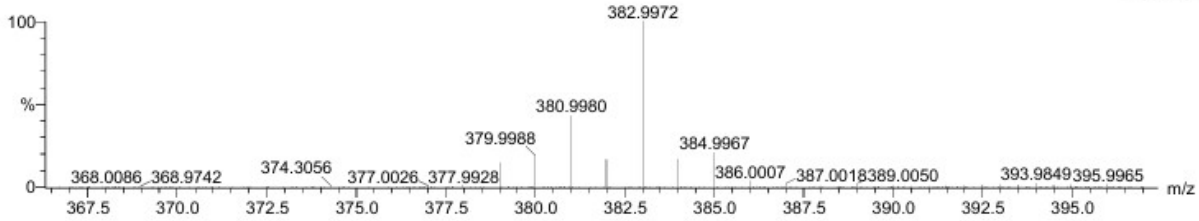


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
124 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-4 S: 0-1 Se: 1-1

210311_AH_12_A 17 (0.197) Cm (14:24-(5:9+42:60))

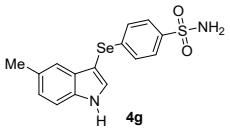
TOF MS ES+
2.26e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
382.9972	382.9969	0.3	0.8	10.5	907.9	0.003	99.67	C15 H15 N2 O3 S Se
	382.9975	-0.3	-0.8	19.5	913.6	5.709	0.33	C23 H11 O Se

Figure 77. HRMS *spectrum of compound* ***4f***

Elemental Composition Report

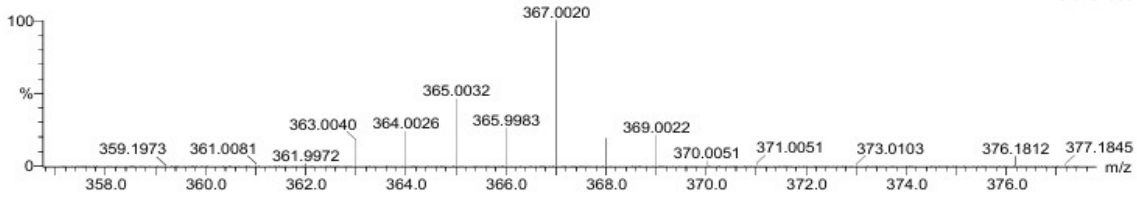


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
119 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-4 S: 0-1 Se: 1-1

210311_RG_71_A 17 (0.197) Cm (16:26-(3:9+52:60))

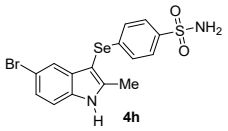
TOF MS ES+
3.91e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
367.0020	367.0019	0.1	0.3	10.5	1089.9	0.030	97.02	C15 H15 N2 O2 S Se
	367.0026	-0.6	-1.6	19.5	1093.4	3.514	2.98	C23 H11 Se

Figure 78. HRMS *spectrum of compound* ***4g***

Elemental Composition Report

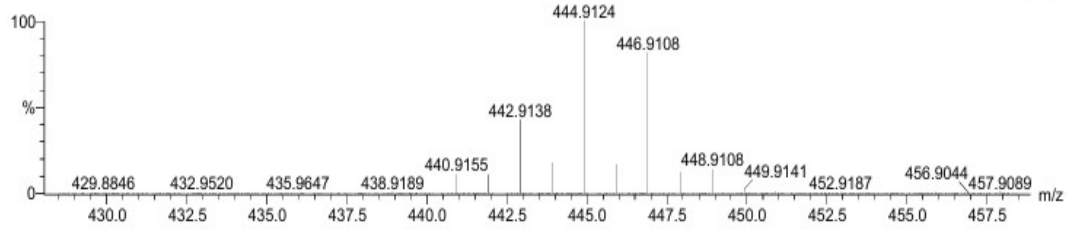


Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
393 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-4 S: 0-1 Se: 1-1 Br: 0-3

210311_RG_60_A 17 (0.197) Cm (15:27-(3:7+53:57))

TOF MS ES+
1.28e+007

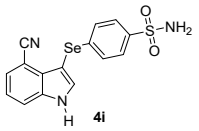


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
444.9124	444.9125	-0.1	-0.2	10.5	1603.1	0.001	99.87	C15 H14 N2 O2 S Se Br
	444.9128	-0.4	-0.9	-0.5	1610.0	6.835	0.11	C10 H23 O4 Se Br2
	444.9131	-0.7	-1.6	19.5	1611.6	8.461	0.02	C23 H10 Se Br

Figure 79. HRMS *spectrum of compound* ***4h***

Elemental Composition Report

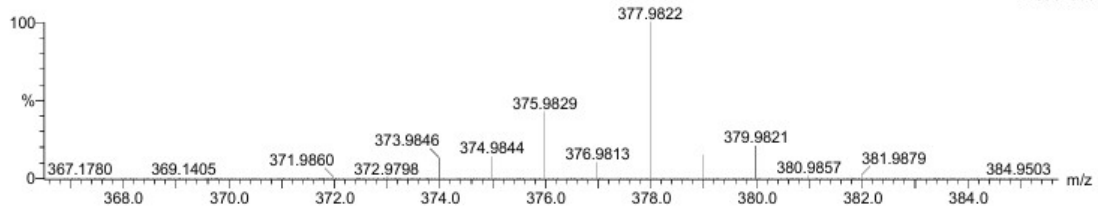
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



4i

Monoisotopic Mass, Even Electron Ions
102 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 O: 0-2 S: 0-1 Se: 1-1
210311_AH_36_A 17 (0.197) Cm (15:26-4:8)

TOF MS ES+
2.89e+006

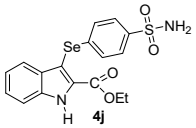


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
377.9822	377.9815	0.7	1.9	12.5	1272.2	0.006	99.40	C15 H12 N3 O2 S Se
	377.9822	0.0	0.0	21.5	1277.3	5.122	0.60	C23 H8 N Se

Figure 80. HRMS *spectrum of compound* ***4i***

Elemental Composition Report

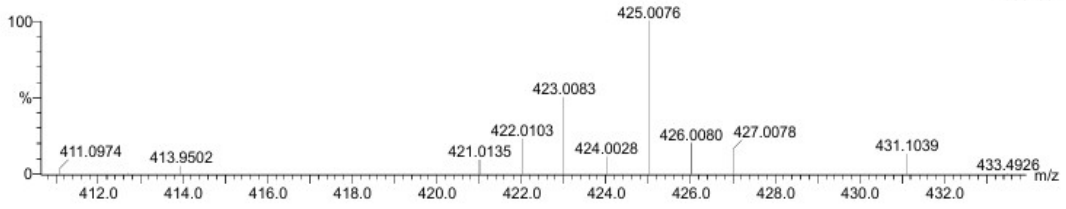
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



4j

Monoisotopic Mass, Even Electron Ions
182 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 O: 0-4 S: 0-1 Se: 1-1
210311_RG_58_AA 12 (0.143)

TOF MS ES+
1.53e+004

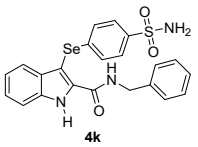


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
425.0076	425.0074	0.2	0.5	11.5	42.1	0.769	46.33	C17 H17 N2 O4 S Se
	425.0081	-0.5	-1.2	20.5	41.9	0.622	53.67	C25 H13 O2 Se

Figure 81. HRMS *spectrum of compound* ***4j***

Elemental Composition Report

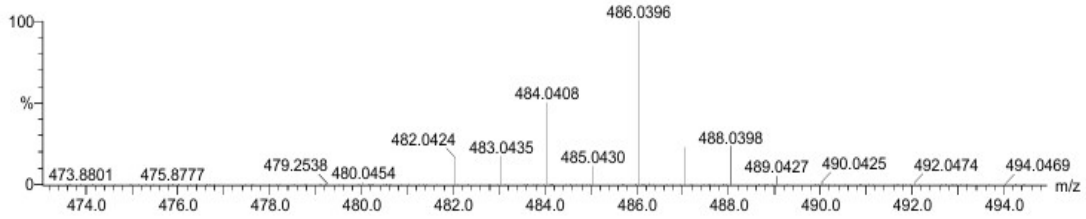
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



4k

Monoisotopic Mass, Even Electron Ions
161 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-3 O: 0-3 S: 0-1 Se: 1-1
210311_AH_46_A 16 (0.177) Cm (15:21-(4:7+43:60))

TOF MS ES+
8.68e+006

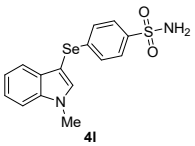


Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
486.0396	486.0391	0.5	1.0	15.5	654.3	0.001	99.90	C22 H20 N3 O3 S Se
	486.0397	-0.1	-0.2	24.5	661.2	6.927	0.10	C30 H16 N O Se

Figure 82. HRMS *spectrum of compound* ***4k***

Elemental Composition Report

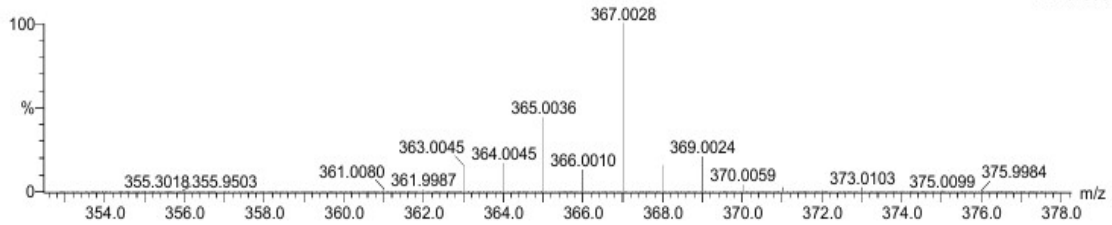
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9



4l

Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-60 H: 0-60 N: 0-2 O: 0-2 S: 0-1 Se: 1-1
210311_AH_35_A 28 (0.303) Cm (28:43-(4:8+56:60))

TOF MS ES+
2.47e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
367.0028	367.0019	0.9	2.5	10.5	1695.9	0.003	99.72	C15 H15 N2 O2 S Se
	367.0026	0.2	0.5	19.5	1701.8	5.866	0.28	C23 H11 Se

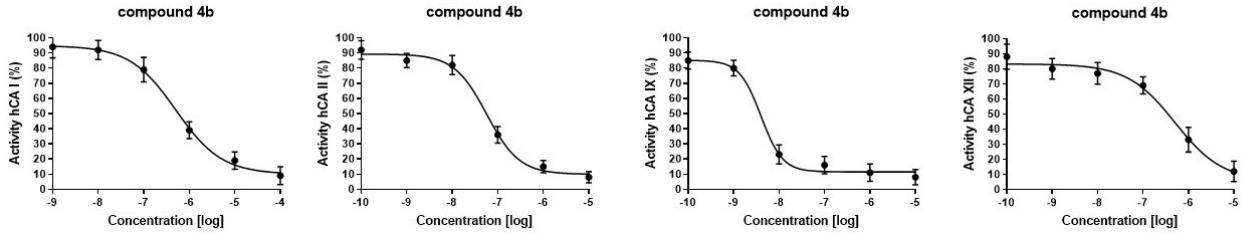
Figure 83. HRMS *spectrum of compound* ***4l***

5. Carbonic anhydrase inhibition

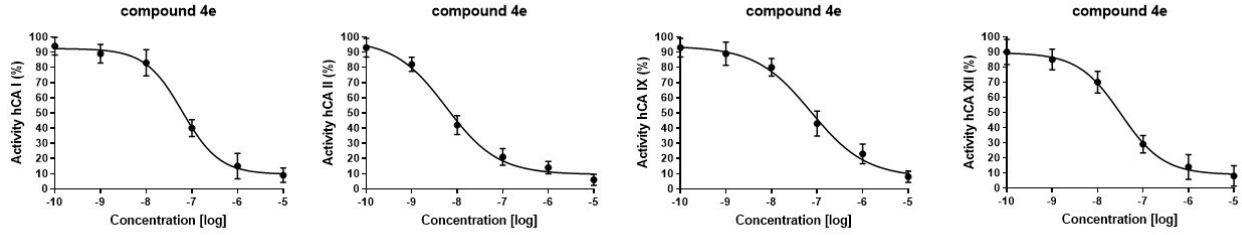
An Applied Photophysics stopped-flow instrument was used to assay the CA catalyzed CO_2 hydration activity.[15] Phenol red (at a concentration of 0.2 mM) was used as an indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) as a buffer, and 20 mM Na_2SO_4 (to maintain constant ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants.[16] Enzyme concentrations ranged between 5-12 nM. For each inhibitor, at least six traces of the initial 5–10% of the reaction were used to determine the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of the inhibitor (0.1 mM) were prepared in distilled–deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to the assay, to allow for the formation of the E–I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earlier and represent the mean from at least three different determinations. All CA isoforms were recombinant proteins obtained in house, as reported earlier.[17-19]

6. Human Carbonic Anhydrase activity

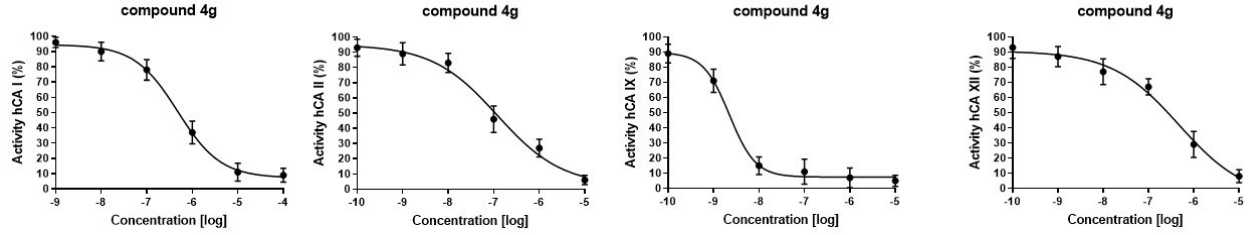
Compound 4b



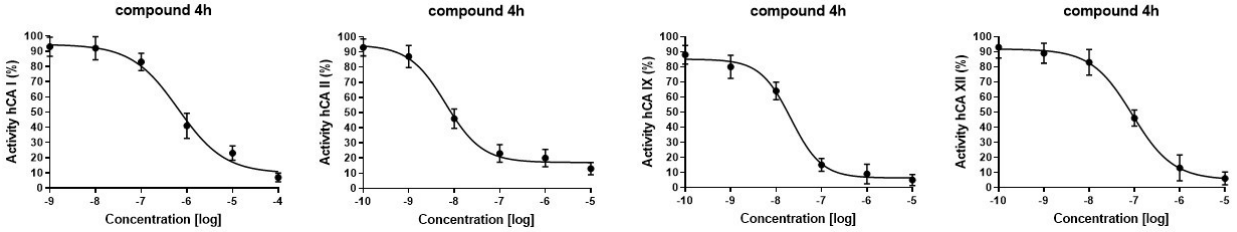
Compound 4e



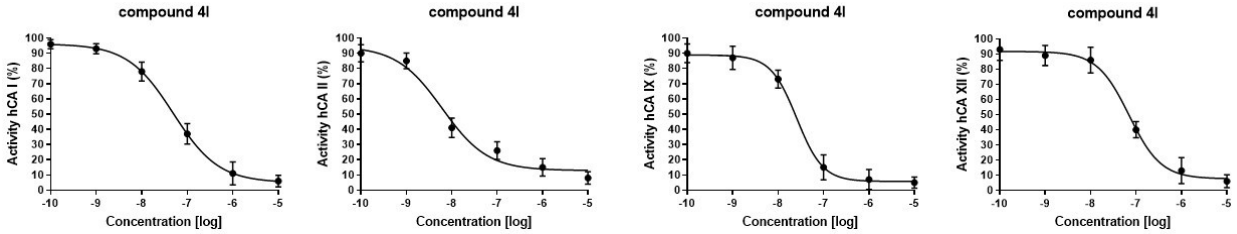
Compound 4g



Compound 4h



Compound 4l



7. References

1. S. K. Sharma, A. K. Mandadapu, B. Kumar and B. Kundu, *J. Org. Chem,* 2011, **76**, 6798.
2. L. Sancineto, A. Mariotti, L. Bagnoli, F. Marini, J. Desantis, N. Iraci, C. Santi, C. Pannecouque and O. Tabarrini, *J. Med. Chem.,* 2015, **58**, 9601.
3. A. Angeli, D. Tanini, A. Capperucci and C. T. Supuran, *ACS Med. Chem. Lett*., 2017, **8**, 1213.
4. A. Krief and M. Derock, *Synlett,* 2005, **6**, 1012.
5. K. B. Sharpless and M. W. Young, *J. Org. Chem.,* 1975, **40**, 947.
6. D. Singh, A. M. Deobald, L. R. S. Camargo, G. Tabarelli, O. E. D. Rodrigues, and A. L. Braga, *Org. Lett.*, 2010, **12**, 3288.
7. Q. B. Zhang, Y. L. Ban, P. F. Yuan, S. J. Peng, J. G. Fang, L. Z. Wu and Q. Liu, *Green Chem.,* 2017, **19**, 5559.
8. R. H. Bartz, P. S. Hellwig, G. Perin, L. E. B. Iarocz, A. Madabeni, L. Orian and M. S. Silva, *New. J. Chem.*, 2024, **48**, 2971.
9. S. Saba, J. Rafique, M. S. Franco, A. R. Schneider, L. Espindola, D. O. Silva and A. L. Braga, *Org. Biomol. Chem.,* 2018, **16**, 880.
10. E. Q. Luz, D. Seckler, J. S. Araujo, L. Angst, D. B. Lima, E. A. M. Rios, R. R. Ribeiro and D. S. Rampon, *Tetrahedron,* 2019, **75**, 1258.
11. Y. Yu, Y. Zhou, Z. Song and G. Liang, *Org. Biomol. Chem*., 2018, **16**, 4958.
12. A. L. Belladonna, R. Cervo, D. Alves, T. Barcellos, R. Cargnelutti and R. F. Schumacher, *Tetrahedron Lett.,* 2020, **61**, 152035.
13. J. B. Azeredo, M. Godoi, G. M. Martins, C. C. Silveira and A. L. Braga, *J. Org. Chem*., 2014, **79**, 4125.
14. H. Zhang, X. Bao, Y. Song, J. Qu, B. Wang, *Tetrahedron*, 2015, **71**, 8885.
15. R. G. Khalifah, *J. Biol. Chem.,* 1971, **246**, 2561.
16. C. T. Supuran, *Nat. Rev. Drug Discov*., 2008, ***7***, 168.
17. A. Angeli, L. Micheli, R. Turnaturi, L. Pasquinucci, C. Parenti, V. Alterio, A. Di Fiore, G. De Simone, S. M. Monti, F. Carta, L. Di Cesare Mannelli, C. Ghelardini and C. T. Supuran, *Eur J Med Chem.*, 2023, **260**, 115783.
18. A. Angeli, E. Trallori, F. Carta, L. Di Cesare Mannelli, C. Ghelardini and C. T. Supuran, *ACS Med Chem Lett*., 2018, **9**, 947.
19. D. Tanini, A. Capperucci, M. Ferraroni, F. Carta, A. Angeli, C. T. Supuran, *Eur J Med Chem*., 2020, **185**, 111811.