

Development of Trisubstituted Thiophene-3-Carboxamide Selenide Derivatives as Novel EGFR Kinase Inhibitors with Cytotoxic Activity

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1. Drug response curve

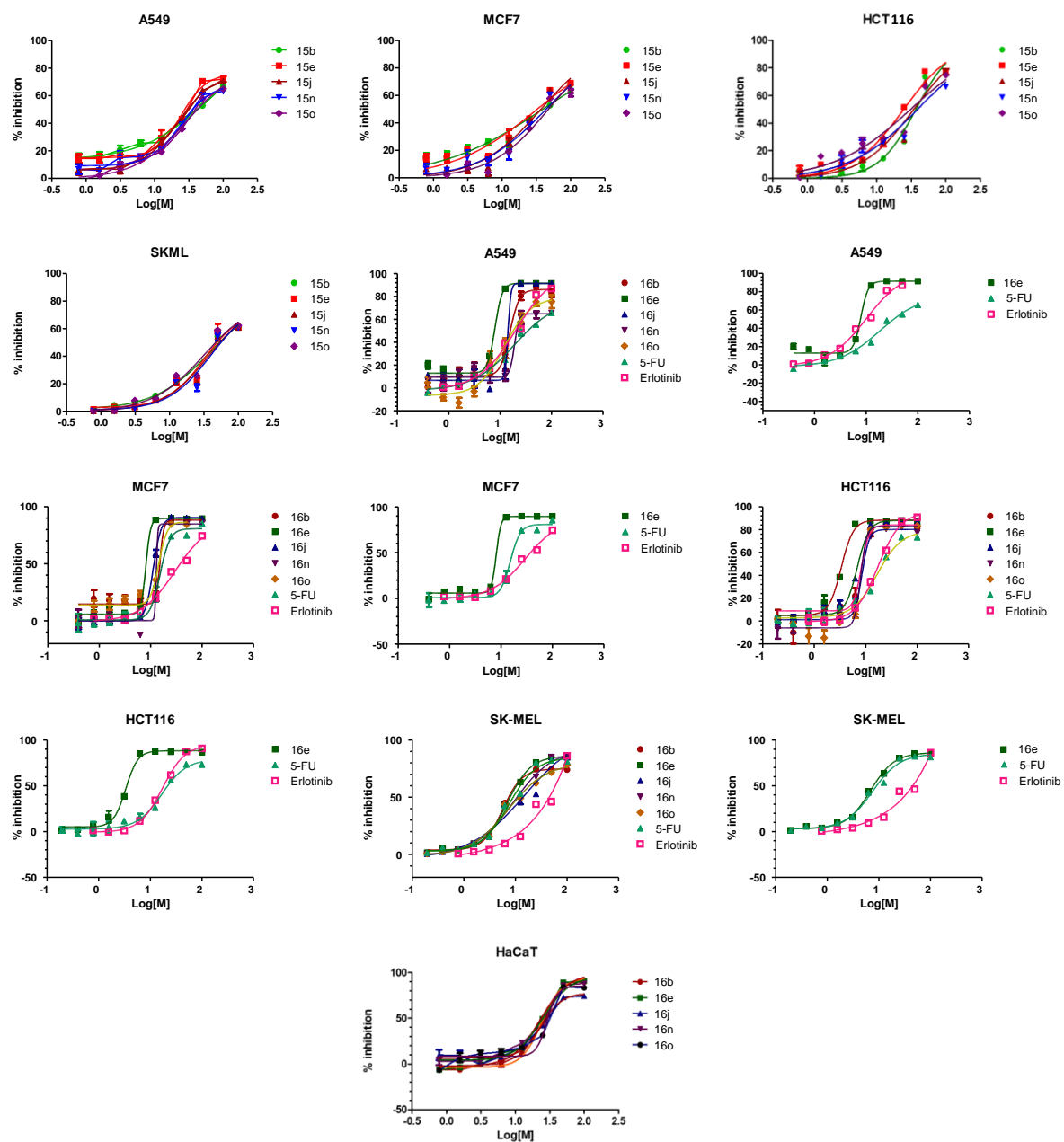
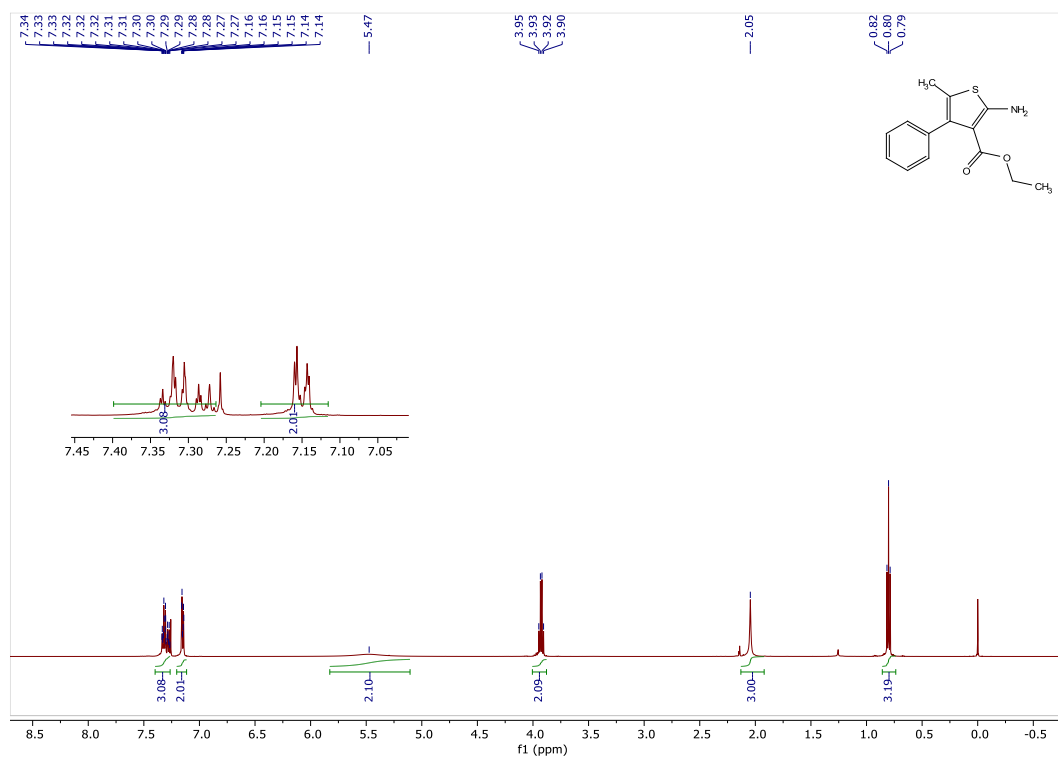


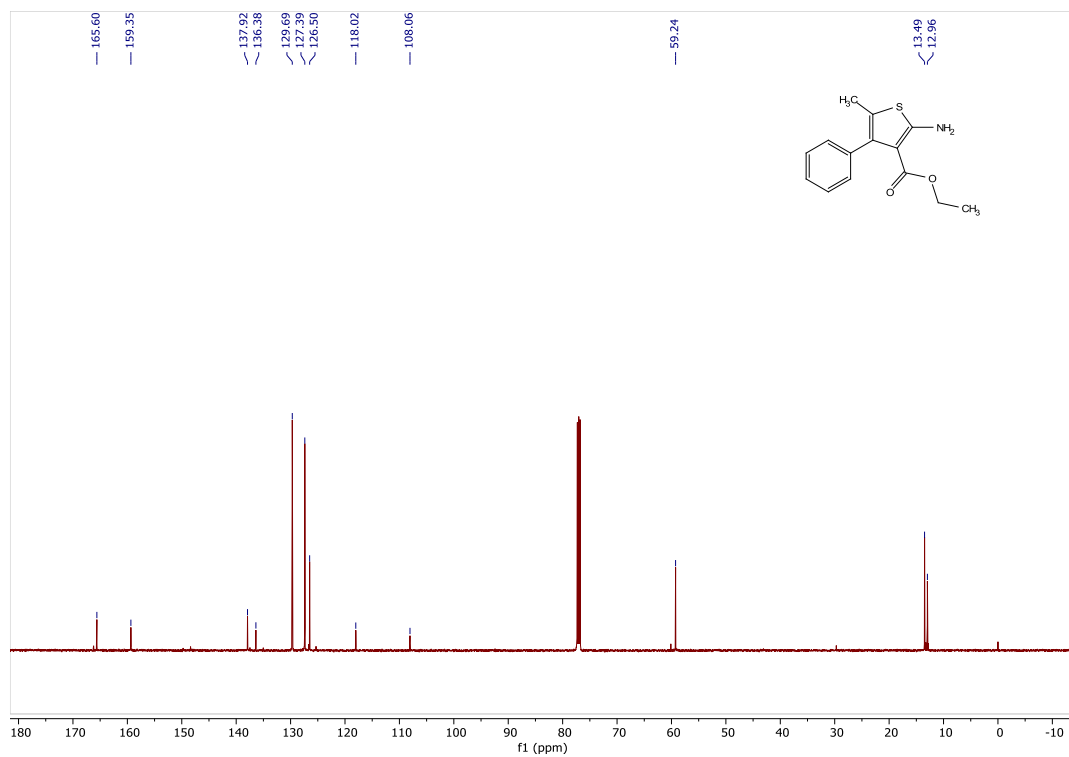
Figure S1: Concentration Response Curves of MTT data of compounds on different cell lines.

2. Spectral Data

19a: Ethyl 2-amino-5-methyl-4-phenylthiophene-3-carboxylate

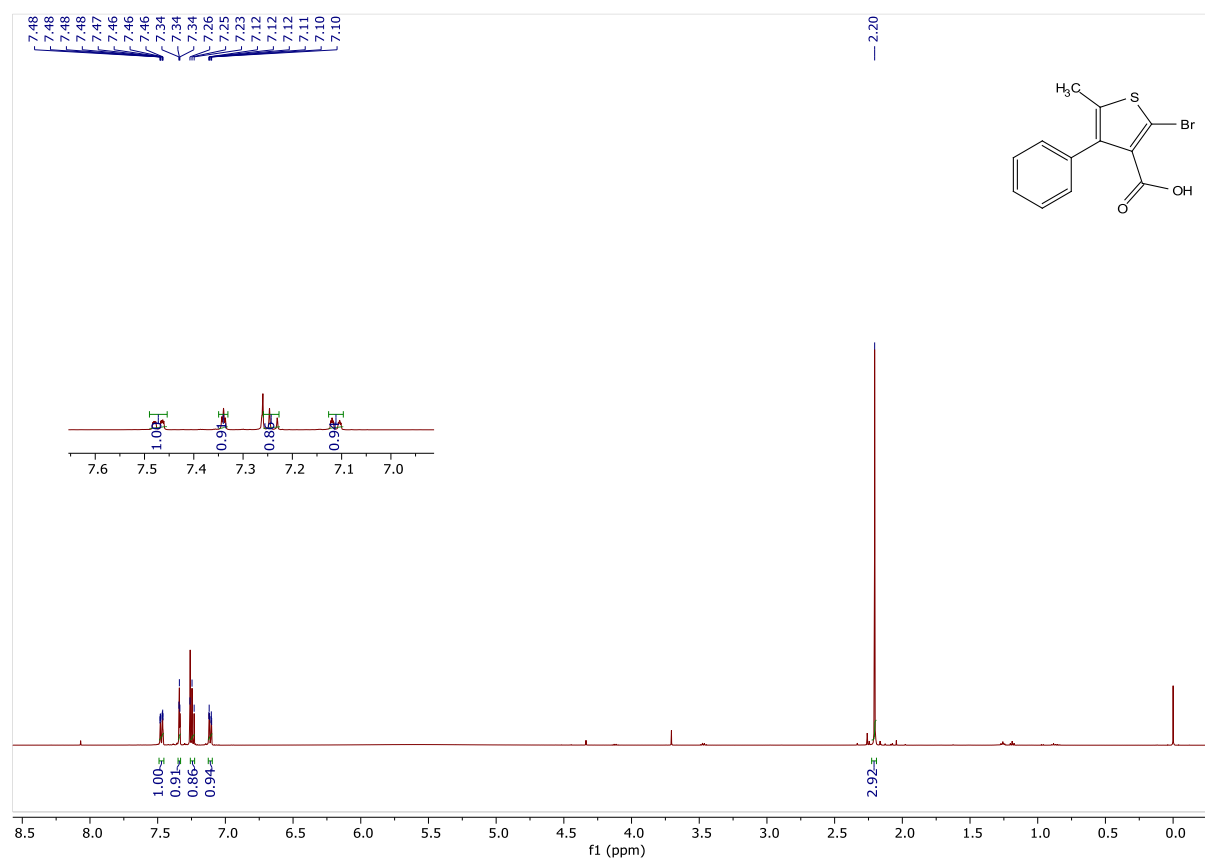


¹H NMR spectrum of compound **19a** (CDCl₃, 500 MHz)



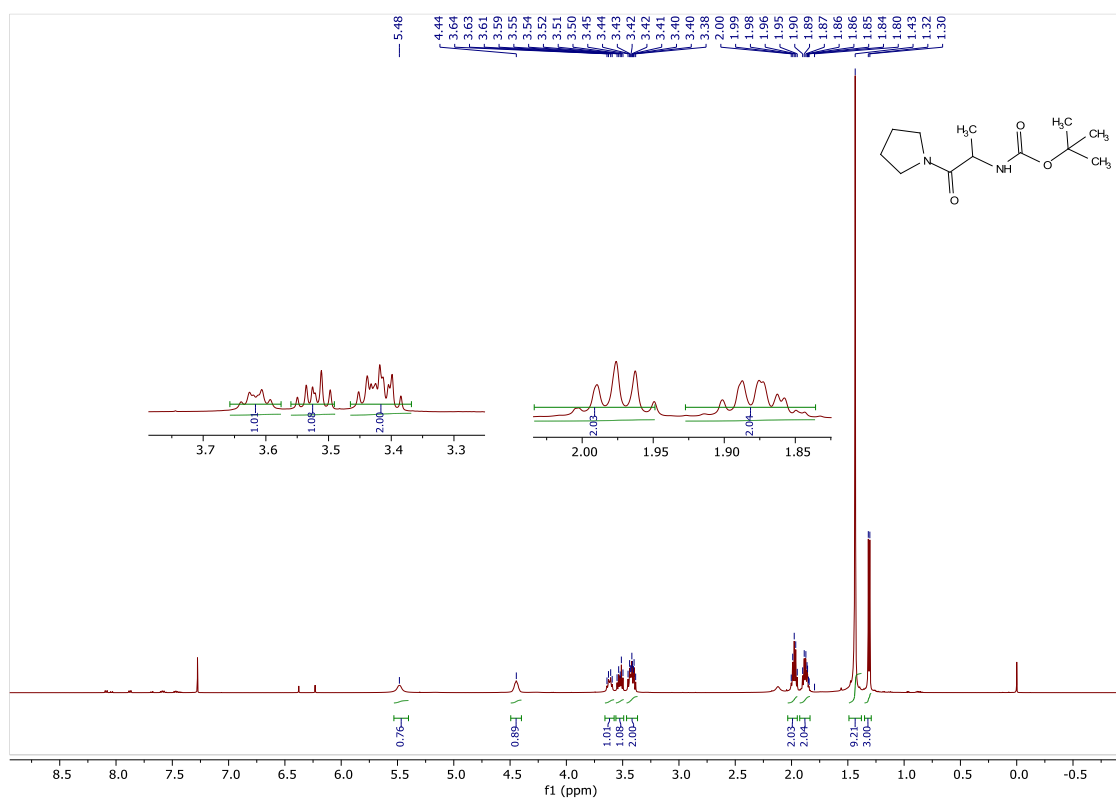
¹³C NMR spectrum of compound **19a** (CDCl₃, 500 MHz)

21a: 2-bromo-5-methyl-4-phenylthiophene-3-carboxylic acid

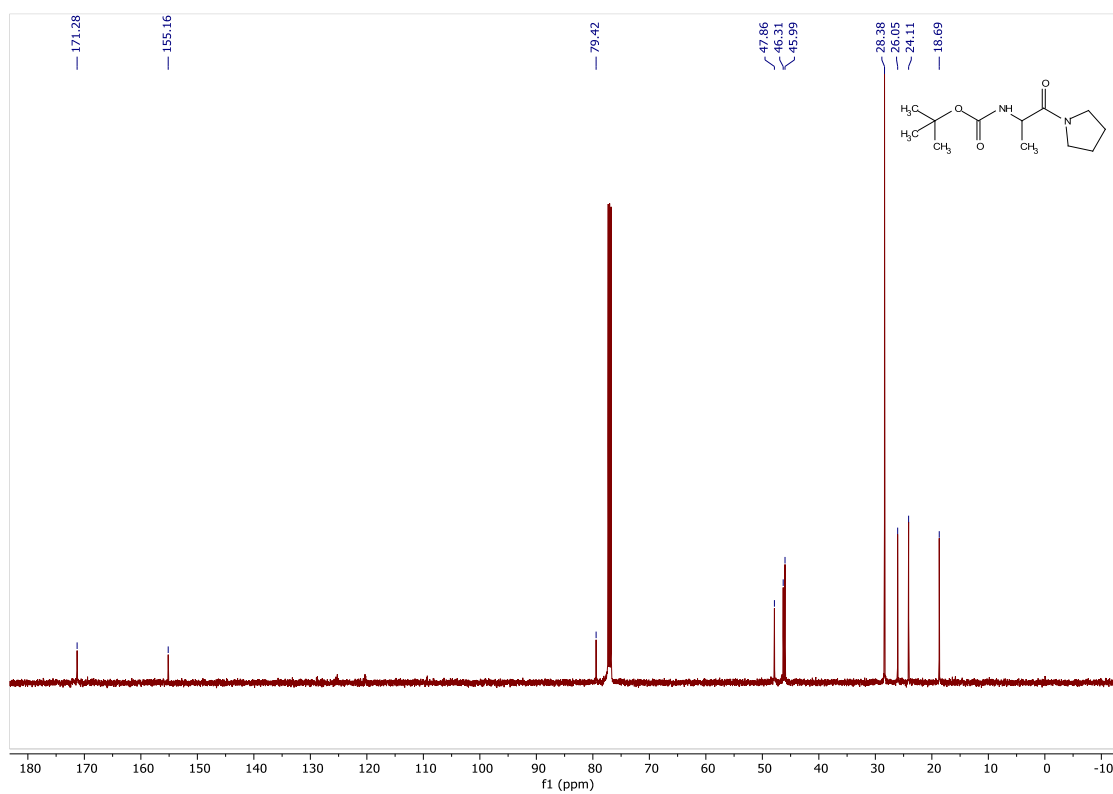


¹H NMR spectrum of compound **21a** (CDCl₃, 500 MHz)

24b: *tert*-butyl(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)carbamate

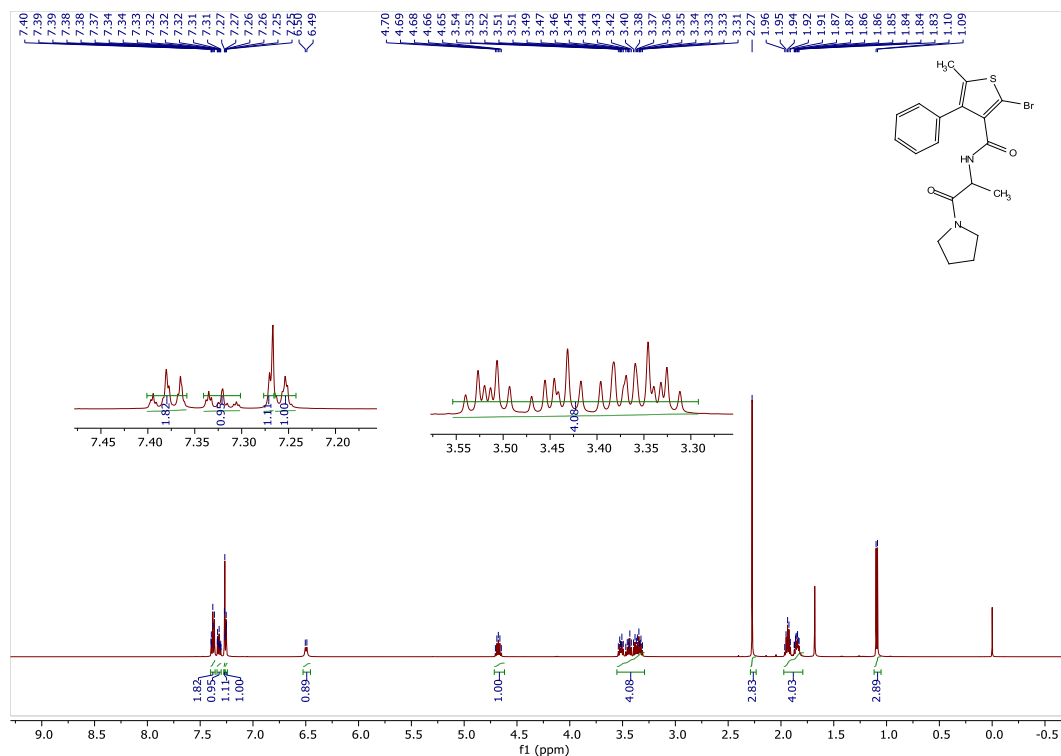


¹H NMR spectrum of compound **24b** (CDCl₃, 500 MHz)

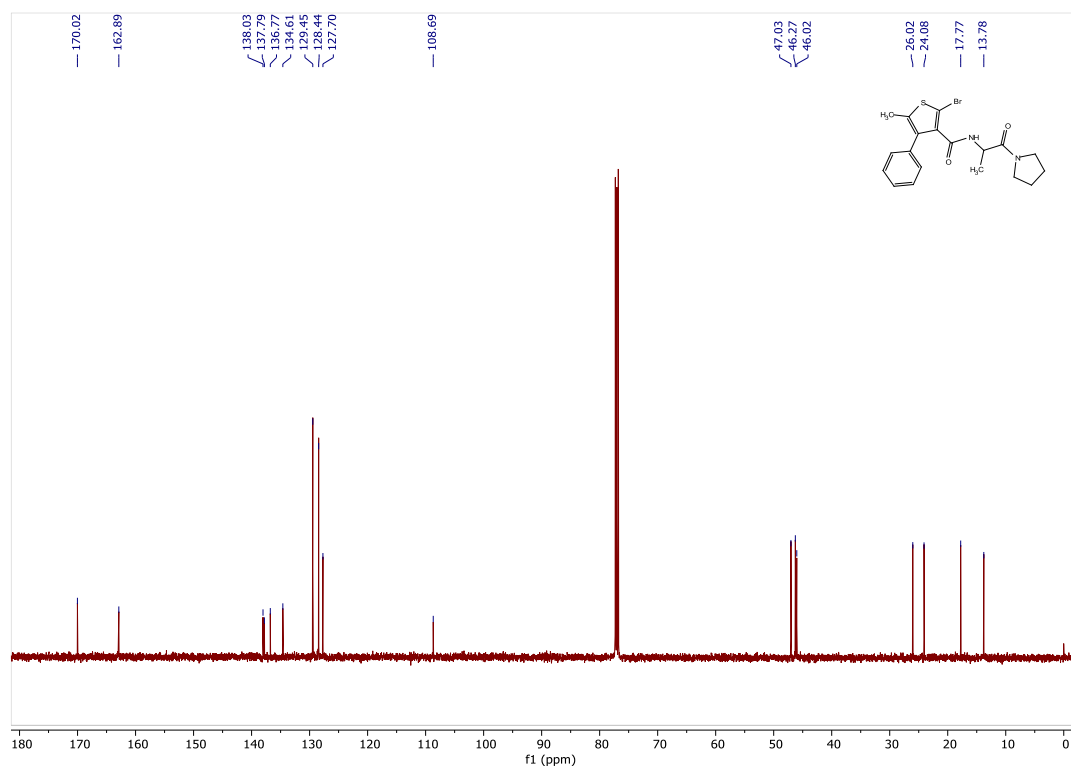


¹³C NMR spectrum of compound **24b** (CDCl₃, 125 MHz)

15b: 2-bromo-5-methyl-N-(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-4-phenylthiophene-3-carboxamide

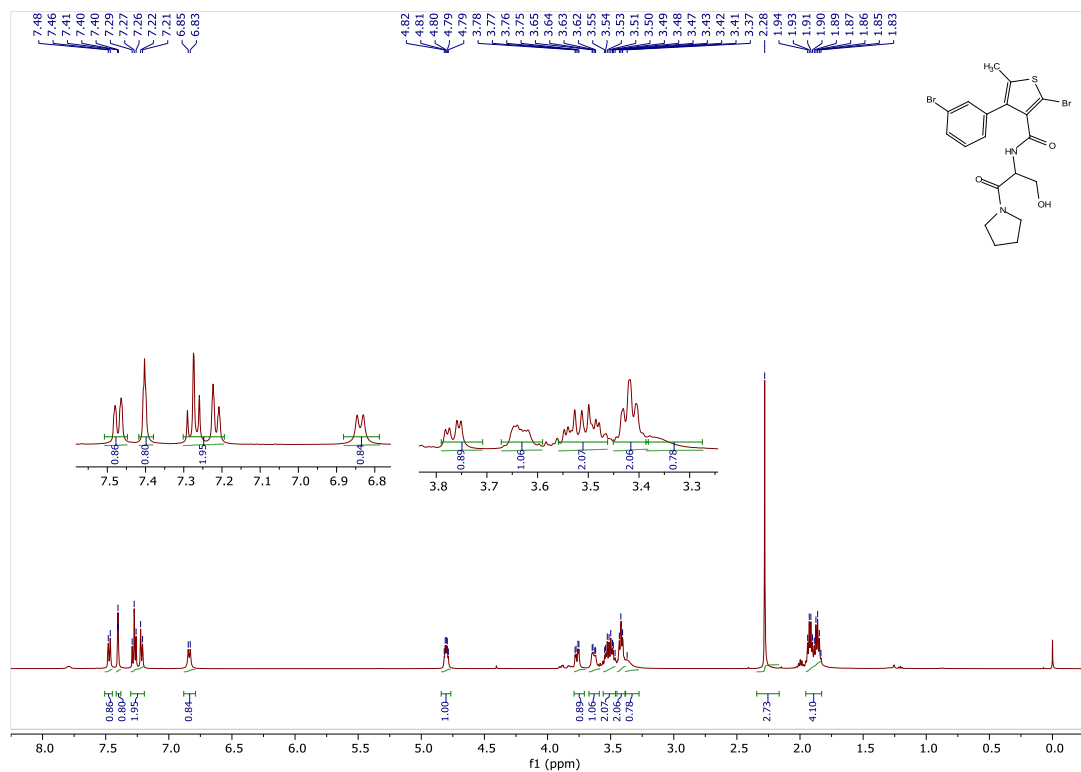


¹H NMR spectrum of compound **15b** (CDCl₃, 500 MHz)

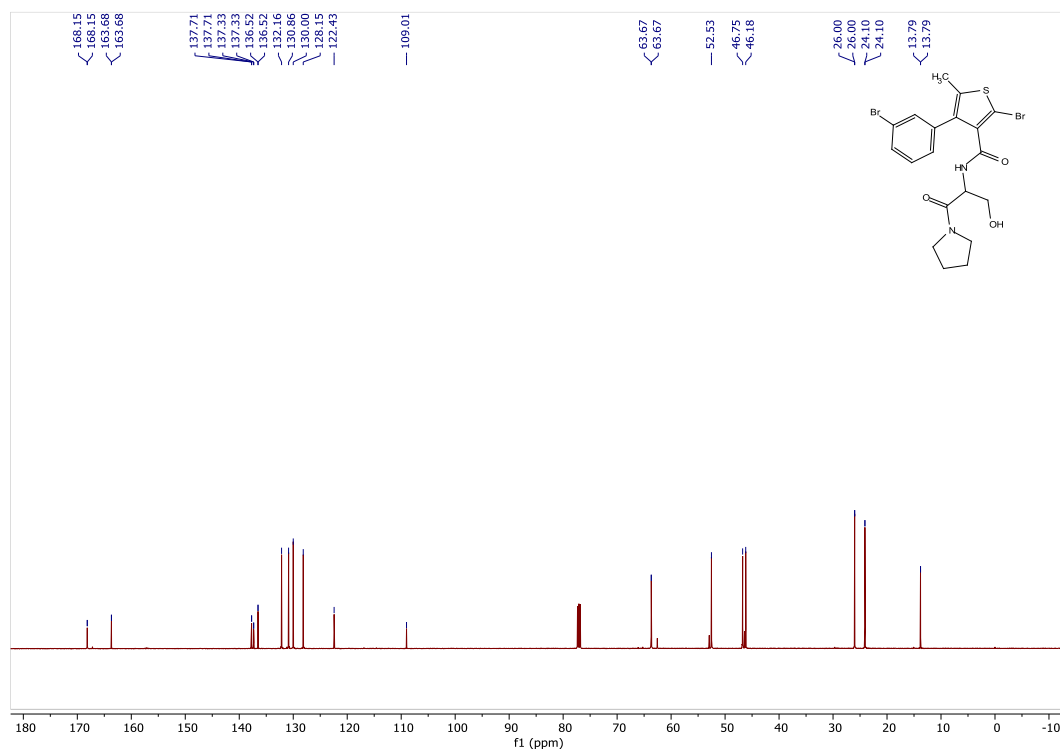


¹³C NMR spectrum of compound **15b** (CDCl₃, 125 MHz)

15e: 2-bromo-4-(3-bromophenyl)-N-(3-hydroxy-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-5-methylthiophene-3-carboxamide

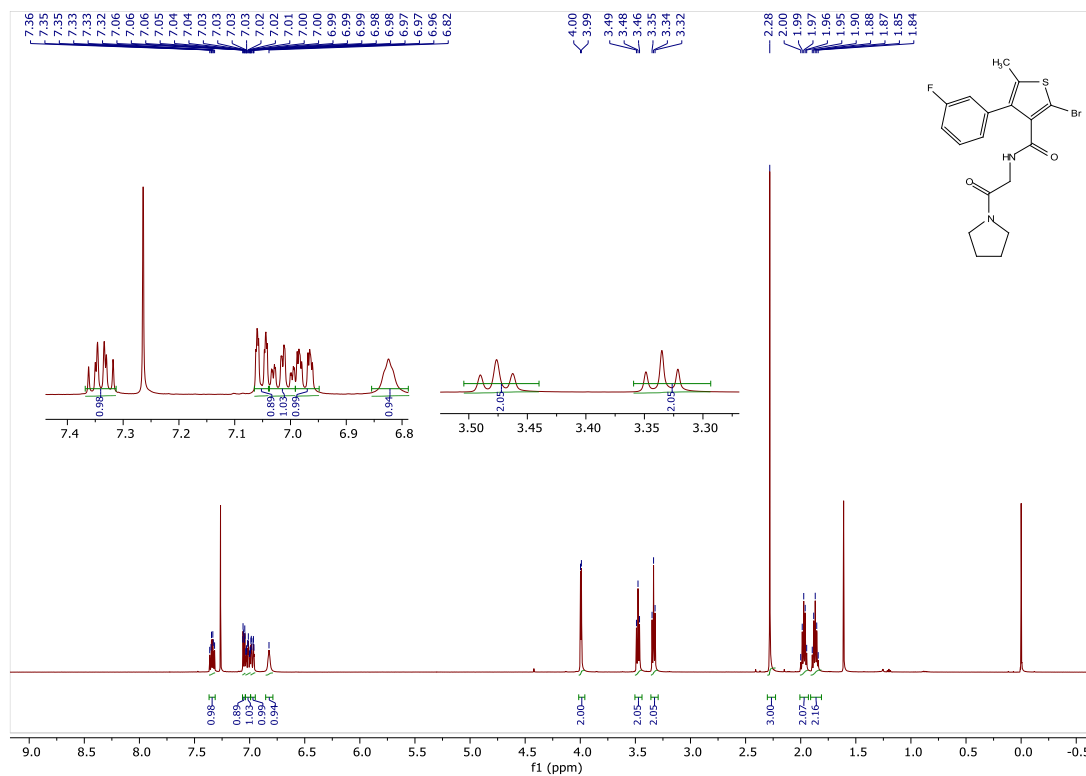


¹H NMR spectrum of compound 15e (CDCl₃, 500 MHz)

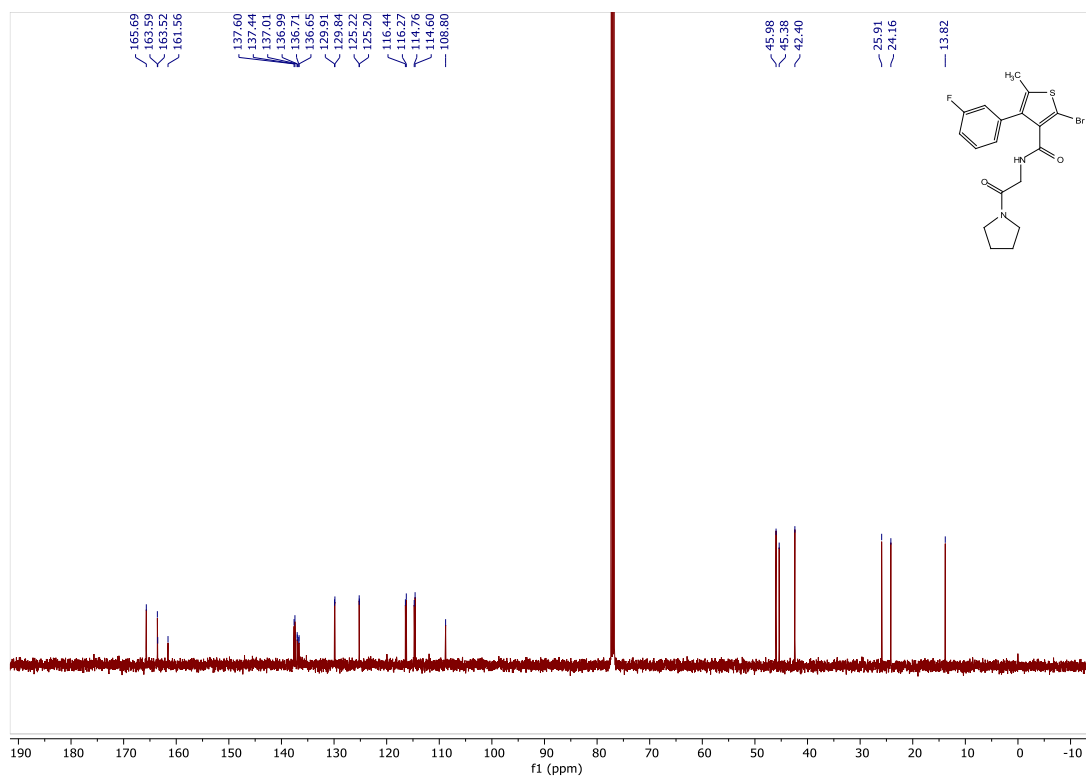


¹³C NMR spectrum of compound 15e (CDCl₃, 125 MHz)

15n: 2-bromo-4-(3-fluorophenyl)-5-methyl-N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)thiophene-3-carboxamide

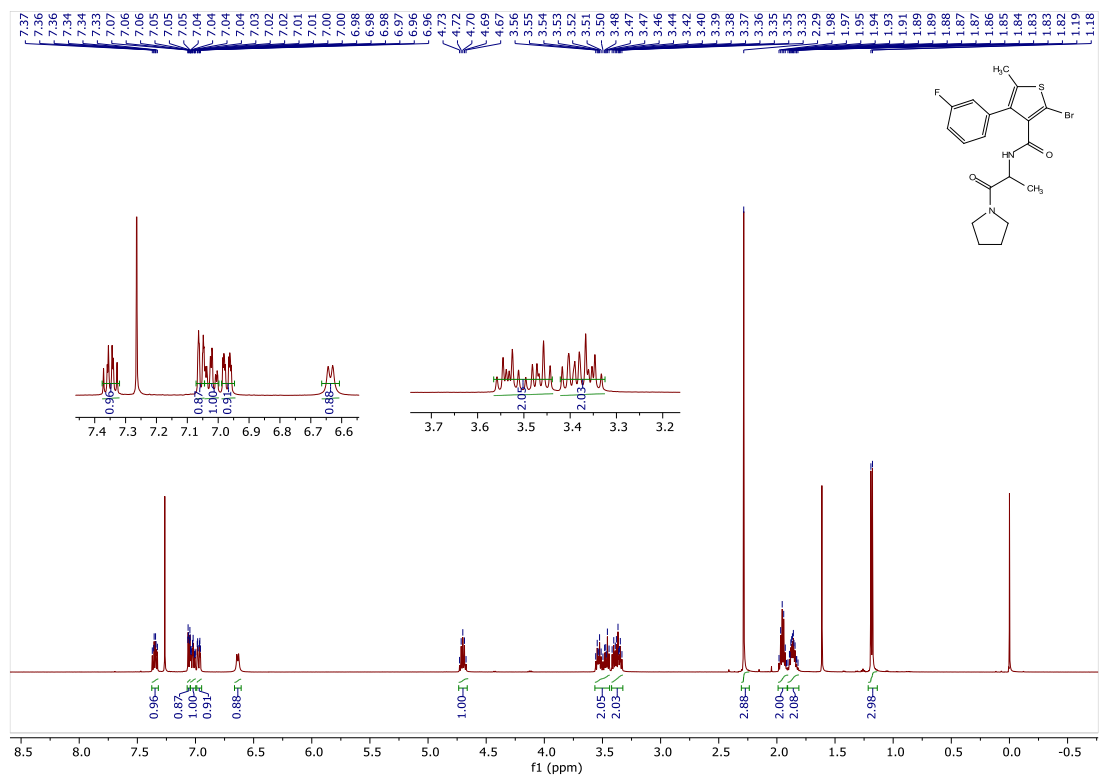


¹H NMR spectrum of compound **15n** (CDCl₃, 500 MHz)

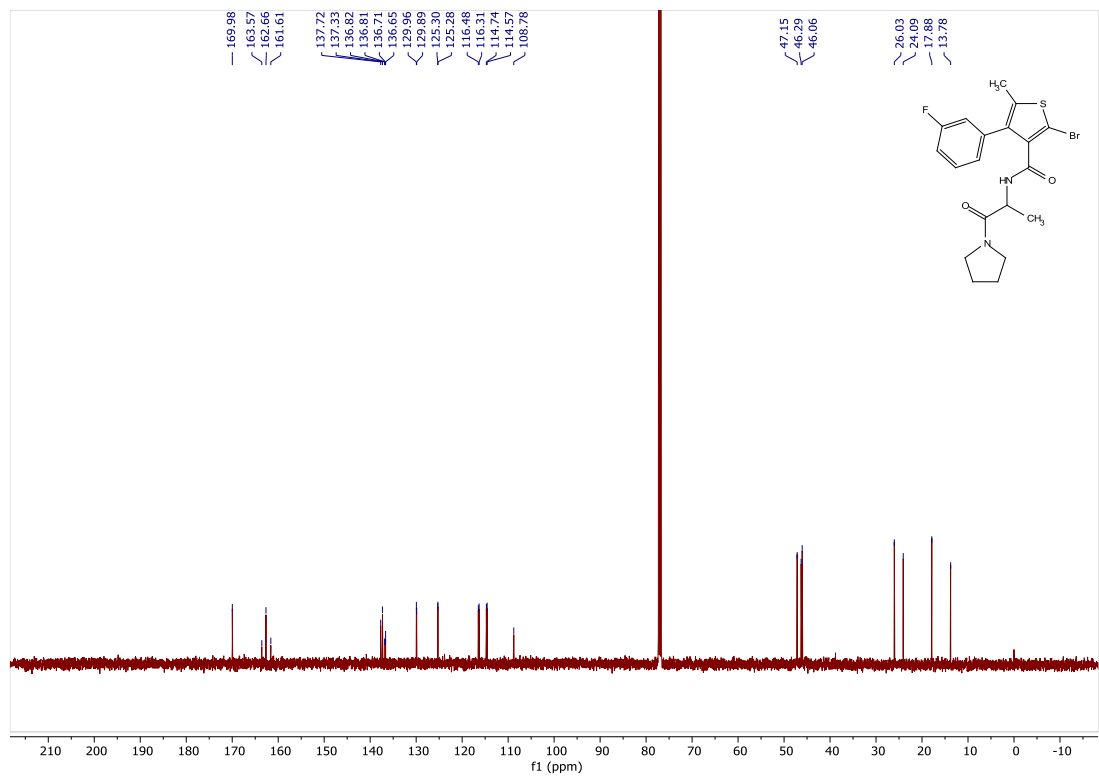


¹³C NMR spectrum of compound **15n** (CDCl₃, 125 MHz)

15o: 2-bromo-4-(3-fluorophenyl)-5-methyl-N-(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide

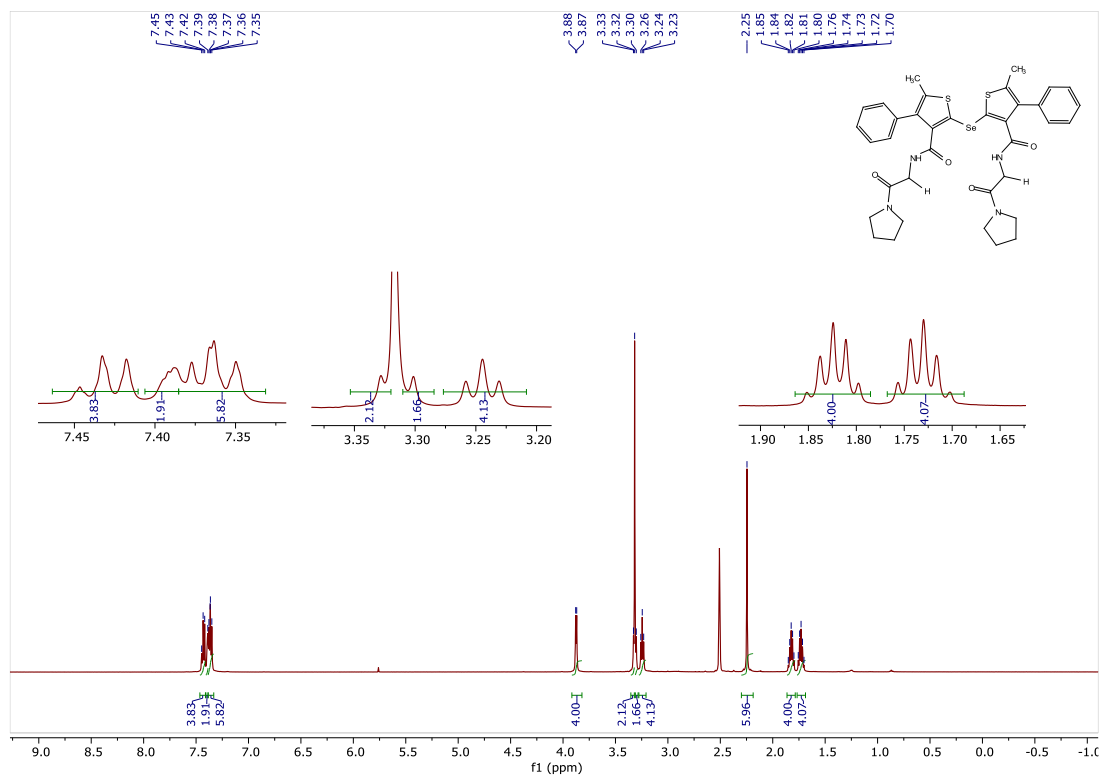


¹H NMR spectrum of compound 15o (CDCl₃, 500 MHz)

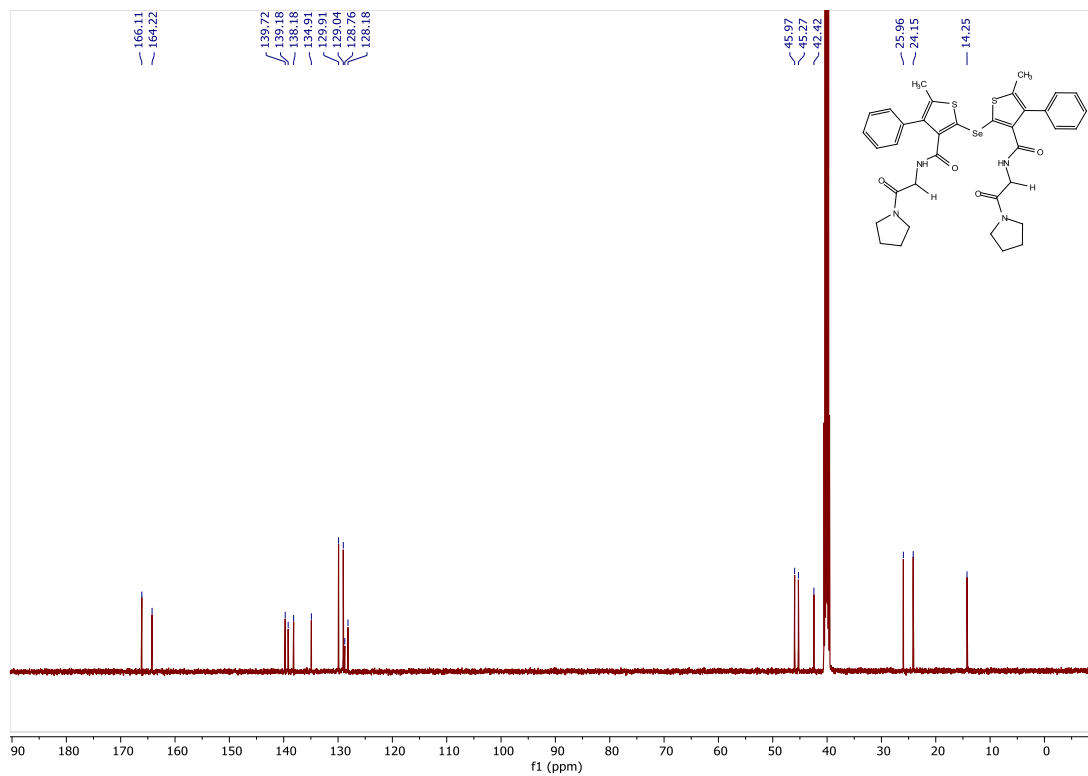


¹³C NMR spectrum of compound 15o (CDCl₃, 125 MHz)

16a: 2,2'-selenobis(5-methyl-N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-4-phenylthiophene-3-carboxamide):

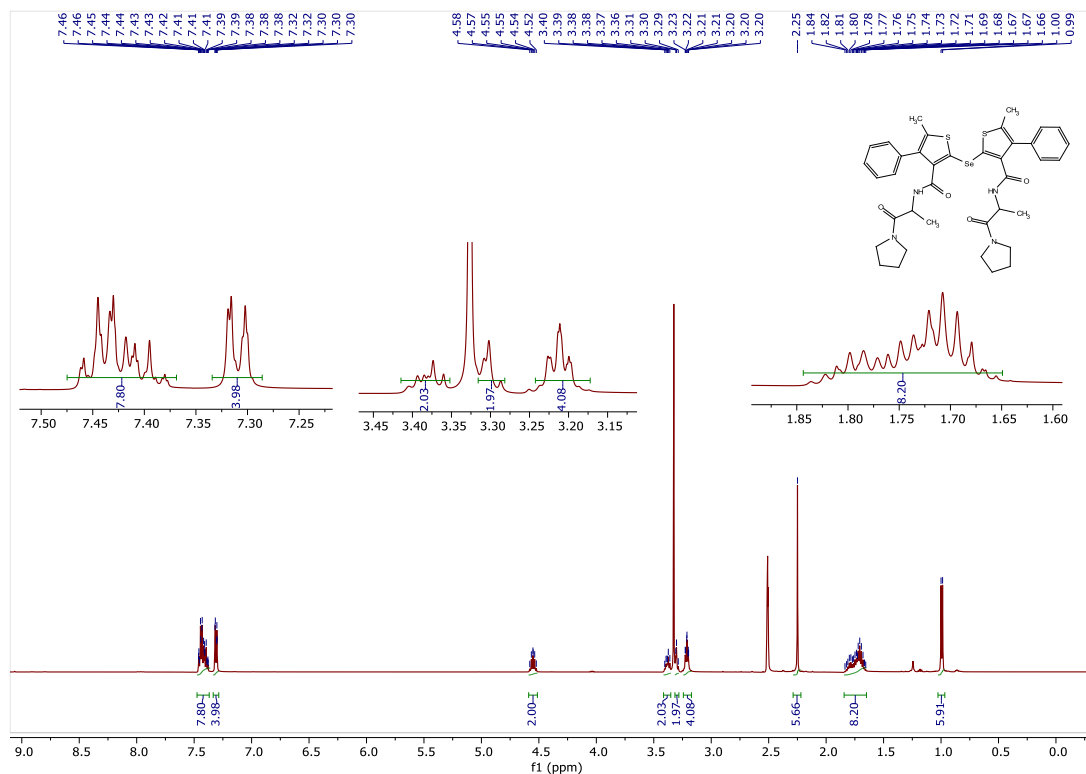


¹H NMR spectrum of compound **16a** (DMSO-*d*₆, 500 MHz)

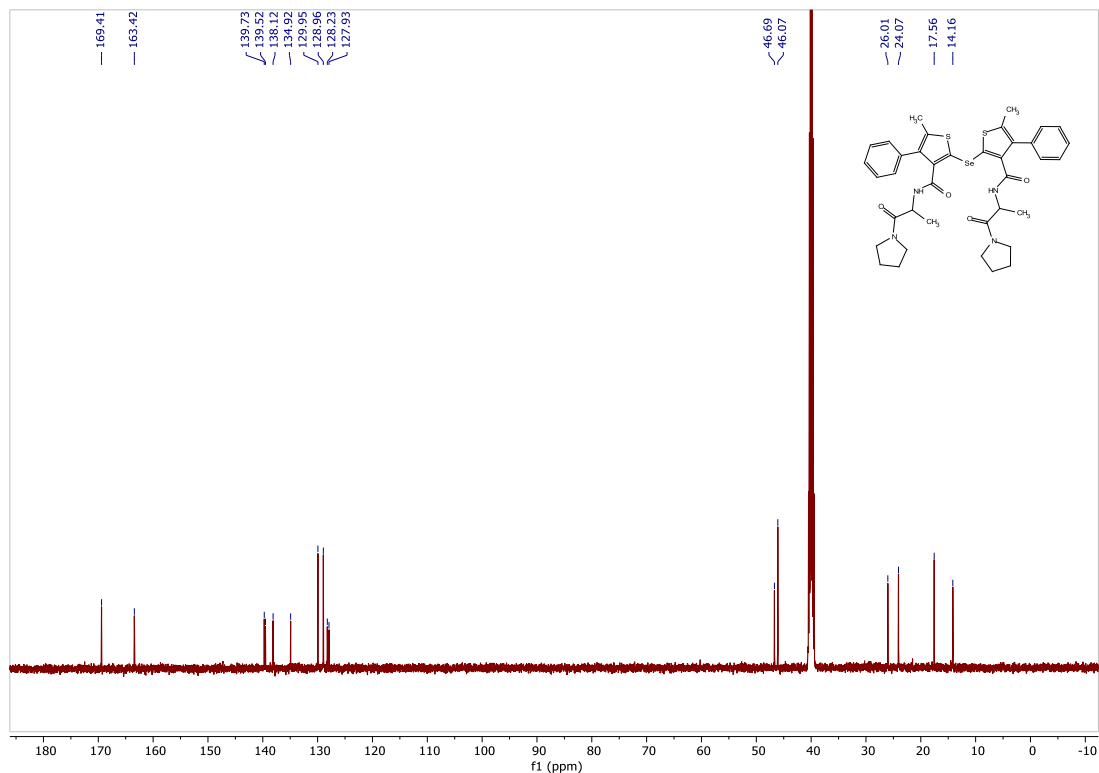


¹³C NMR spectrum of compound **16a** (DMSO-*d*₆, 125 MHz)

16b: 2,2'-selenobis(5-methyl-N-(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-4-phenylthiophene-3-carboxamide)

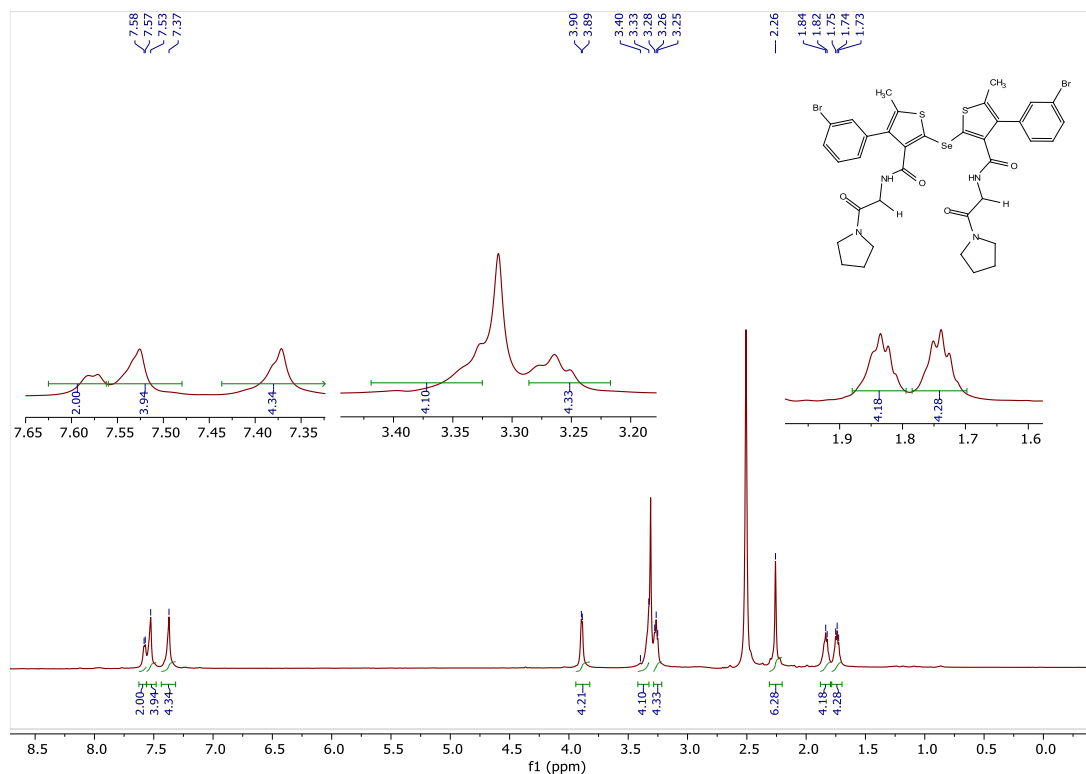


¹H NMR spectrum of compound **16b** (DMSO-*d*₆, 500 MHz)

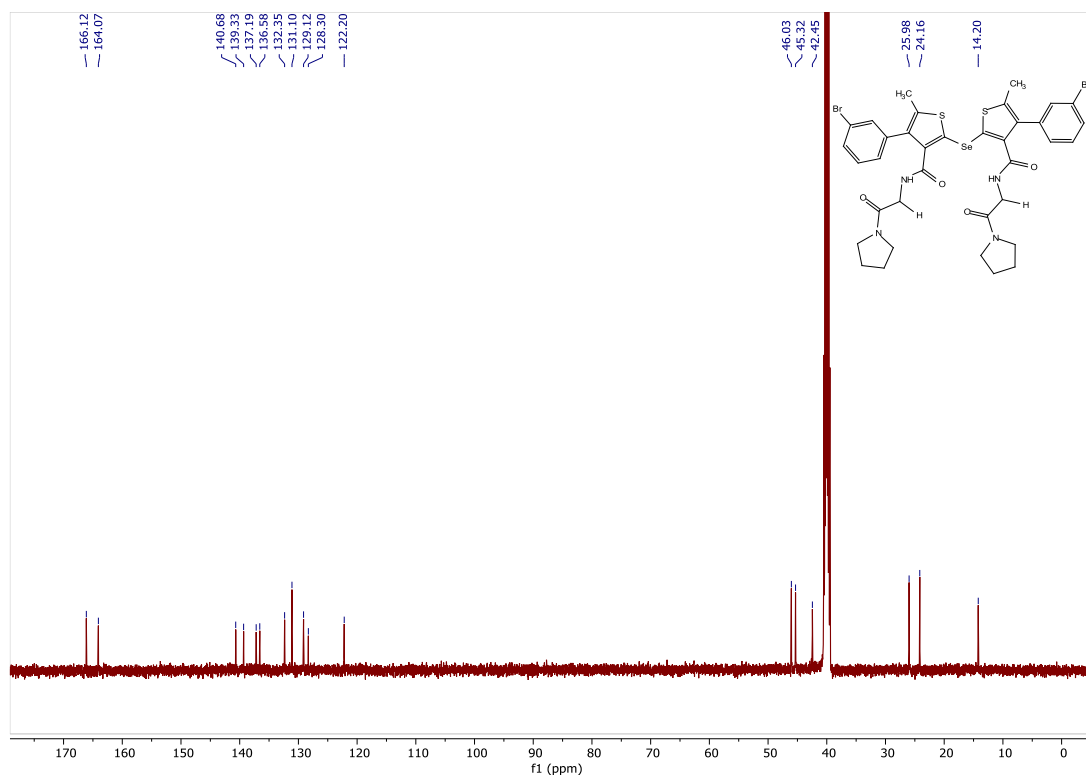


¹³C NMR spectrum of compound **16b** (DMSO-*d*₆, 125 MHz)

16c: 2,2'-selenobis(4-(3-bromophenyl)-5-methyl-N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)thiophene-3-carboxamide)

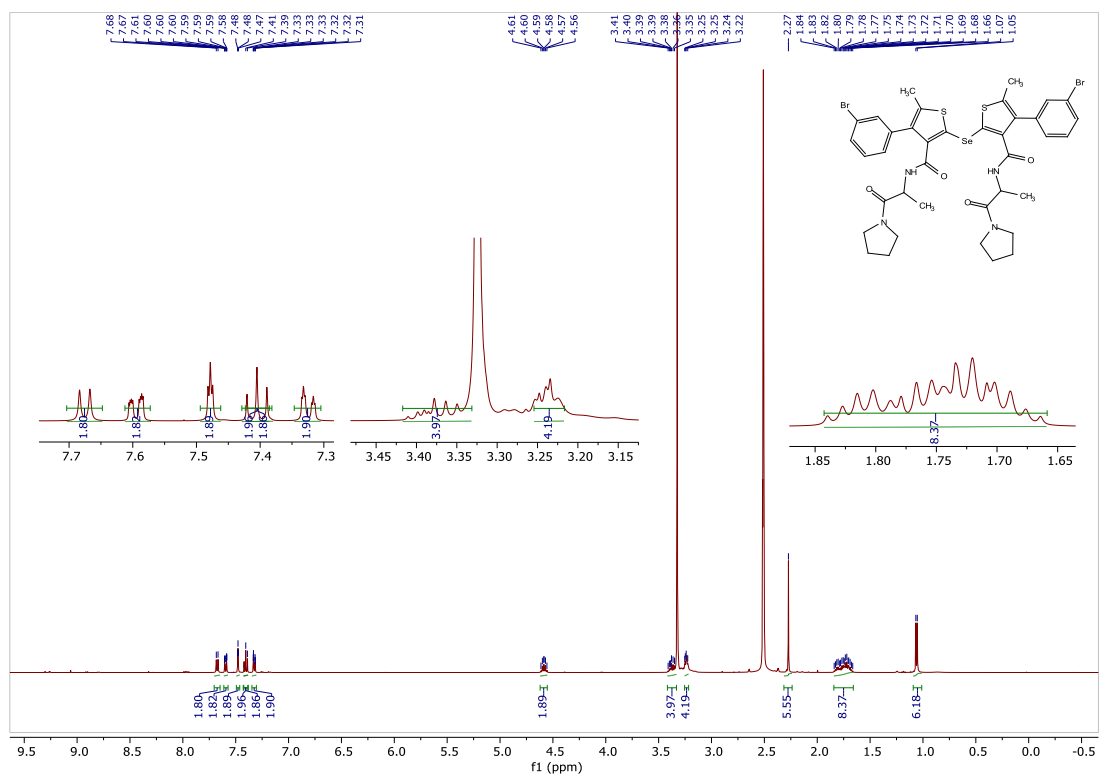


¹H NMR spectrum of compound **16c** (DMSO-*d*₆, 500 MHz)

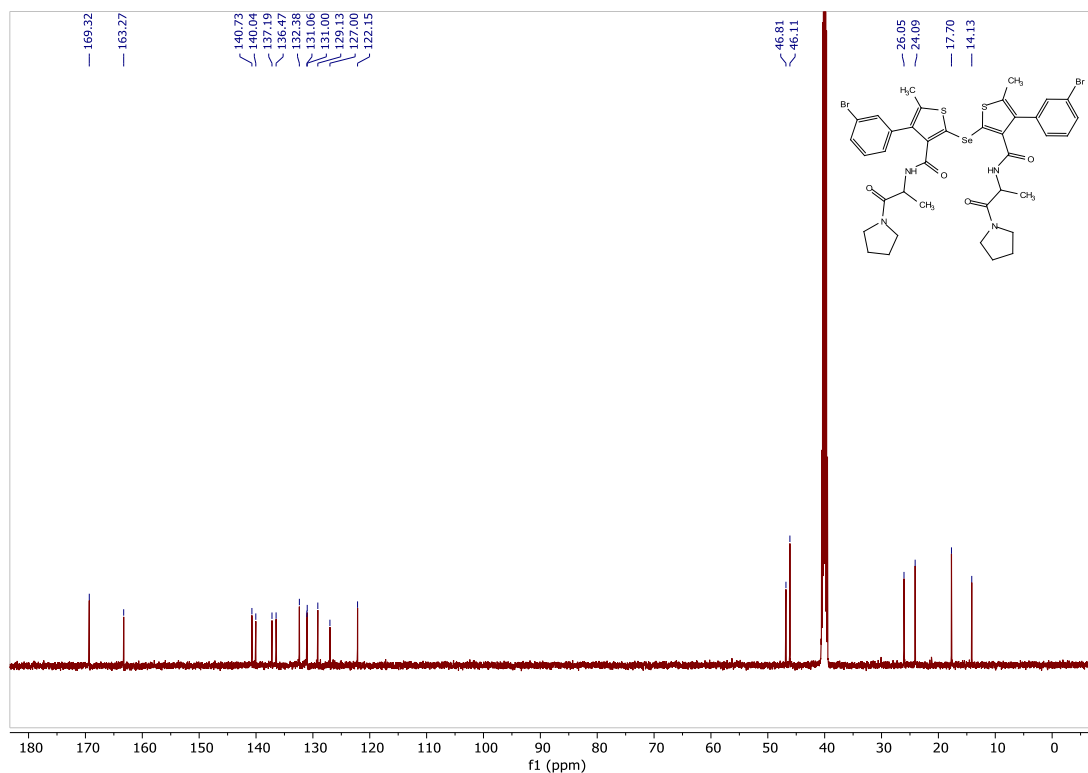


¹³C NMR spectrum of compound **16c** (DMSO-*d*₆, 125 MHz)

16d: 2,2'-selenobis(4-(3-bromophenyl)-5-methyl-N-(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide)

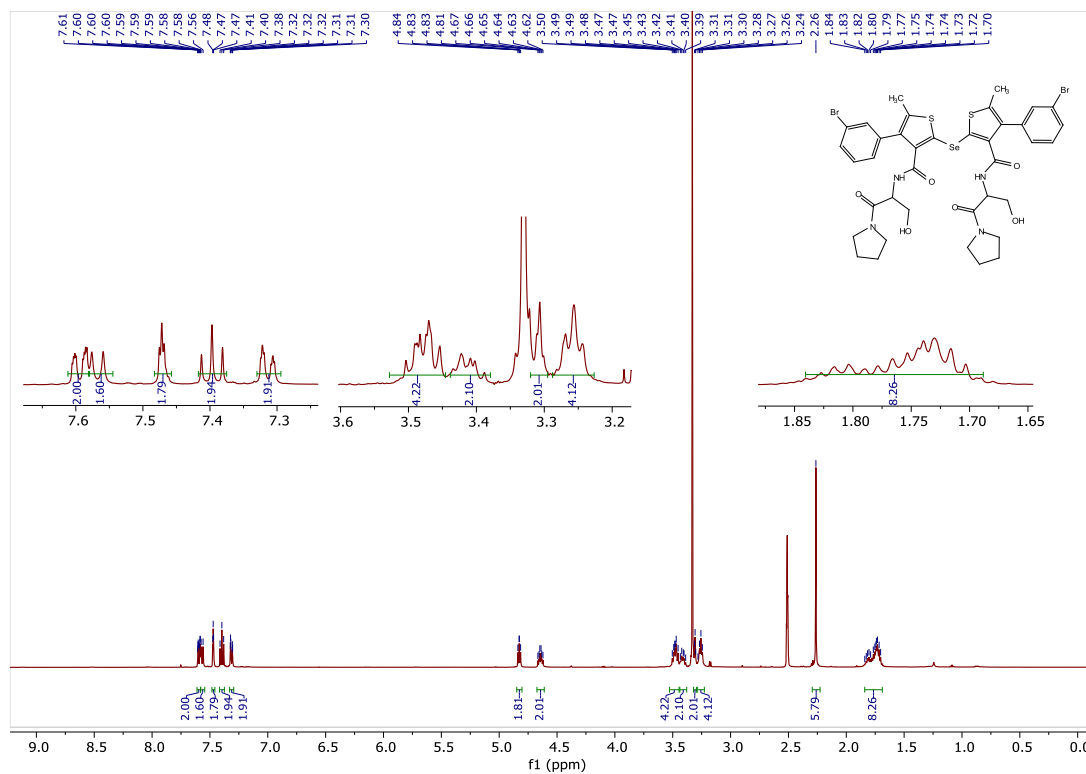


¹H NMR spectrum of compound **16d** (DMSO-*d*₆, 500 MHz)

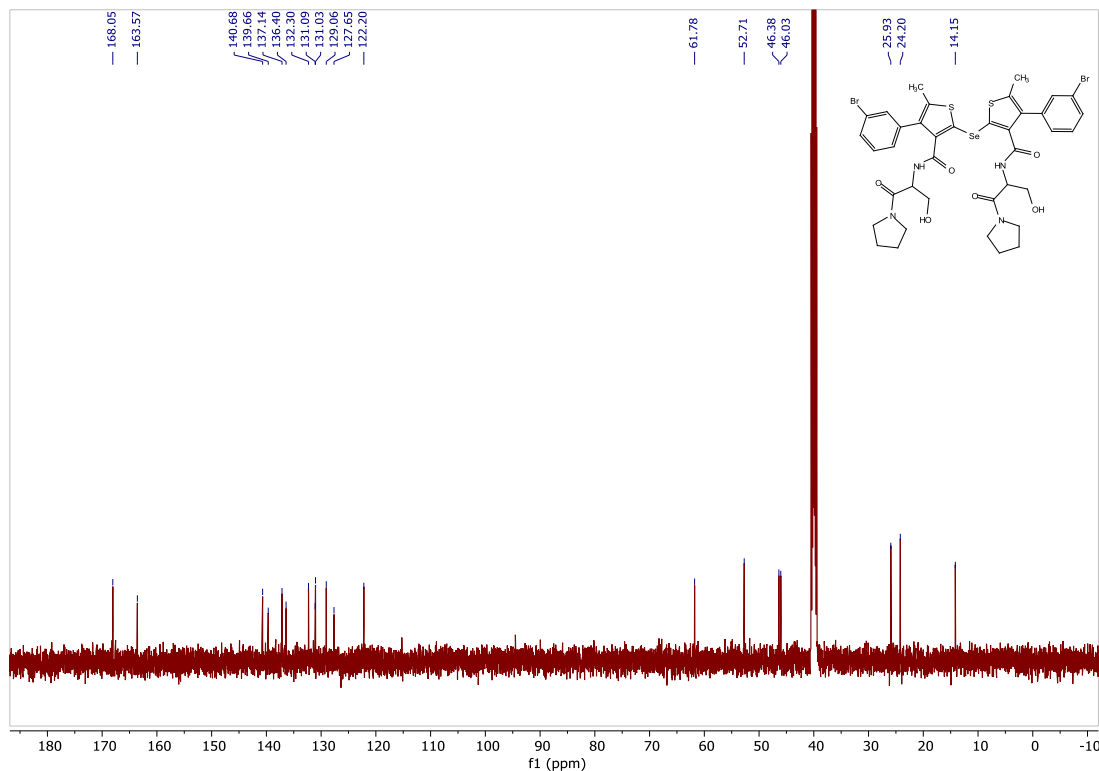


¹³C NMR spectrum of compound **16d** (DMSO-*d*₆, 125 MHz)

16e: 2,2'-selenobis(4-(3-bromophenyl)-N-(3-hydroxy-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-5-methylthiophene-3-carboxamide)

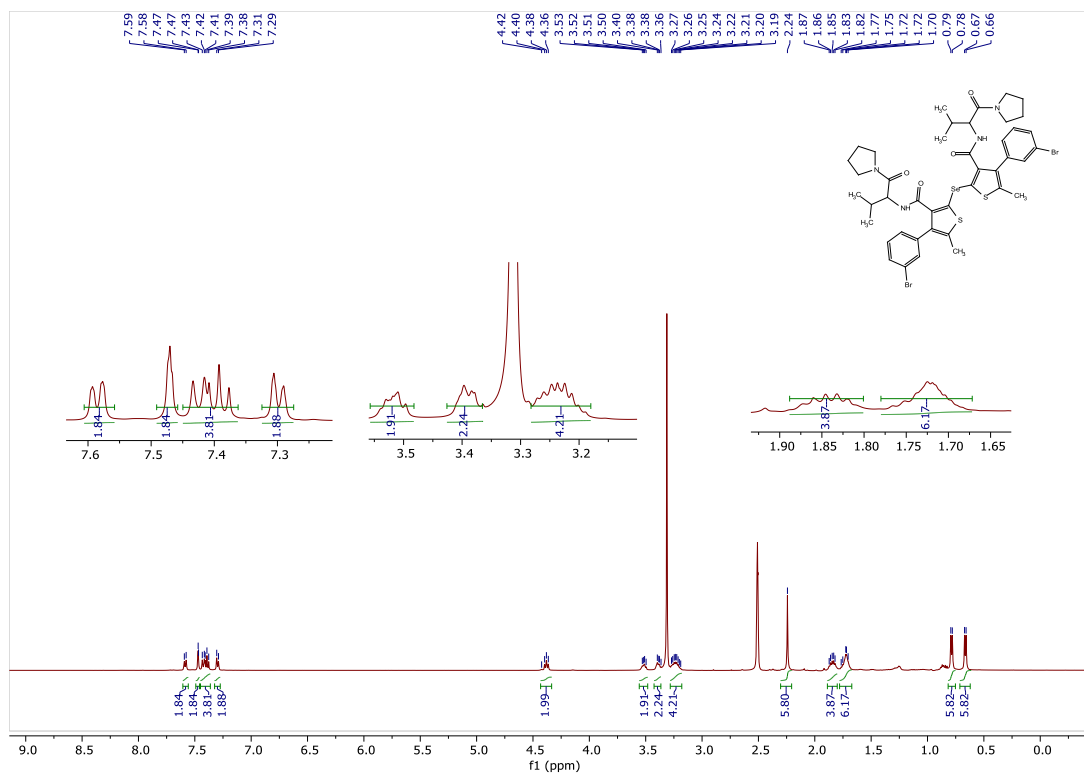


¹H NMR spectrum of compound **16e** (DMSO-*d*₆, 500 MHz)

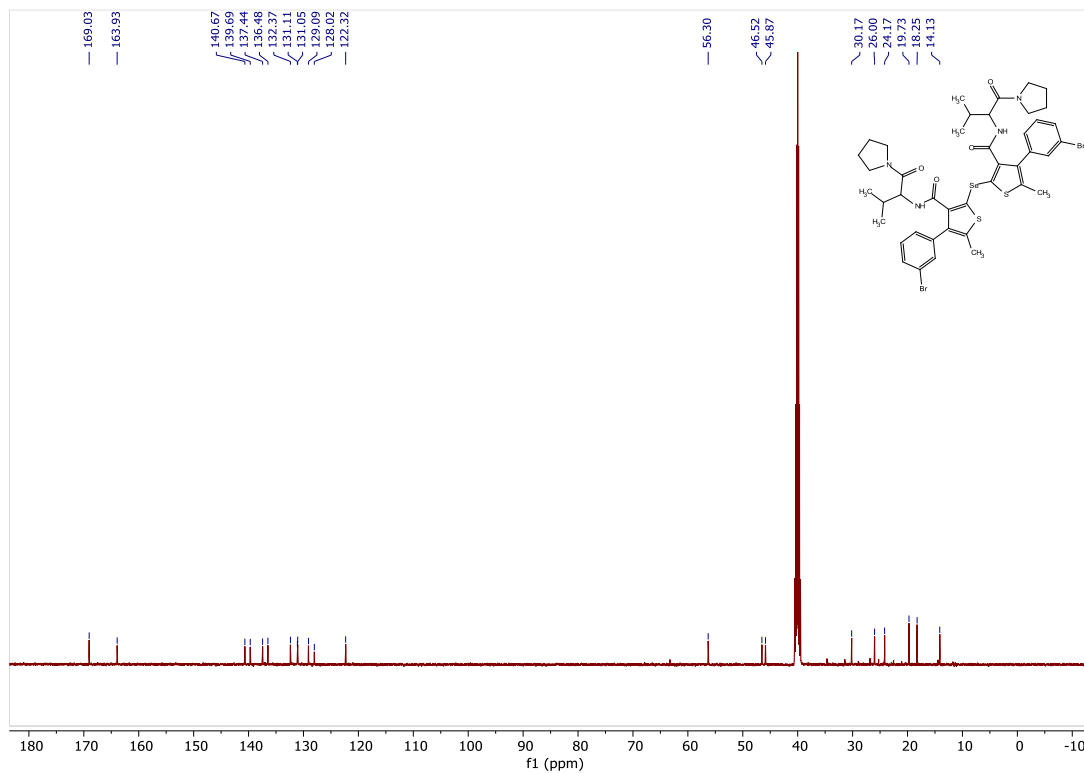


¹³C NMR spectrum of compound **16e** (DMSO-*d*₆, 125 MHz)

16f: 2,2'-selenobis(4-(3-bromophenyl)-5-methyl-N-(3-methyl-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)thiophene-3-carboxamide)

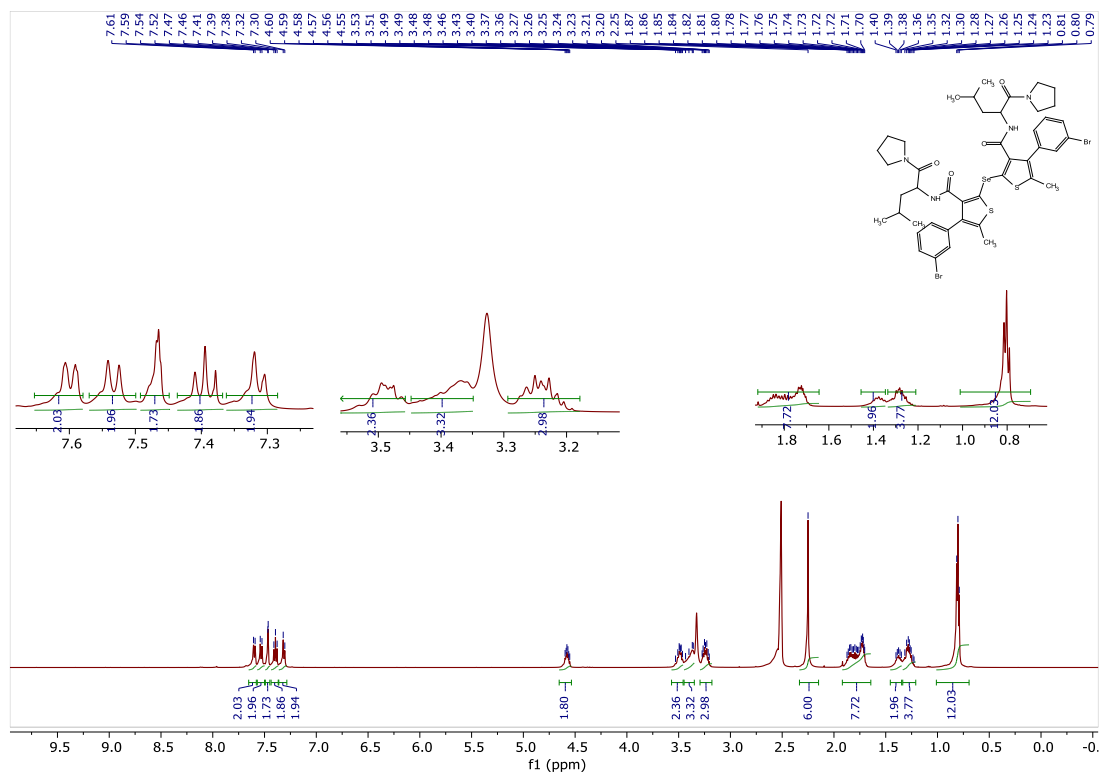


¹H NMR spectrum of compound **16f** (DMSO-*d*₆, 500 MHz)

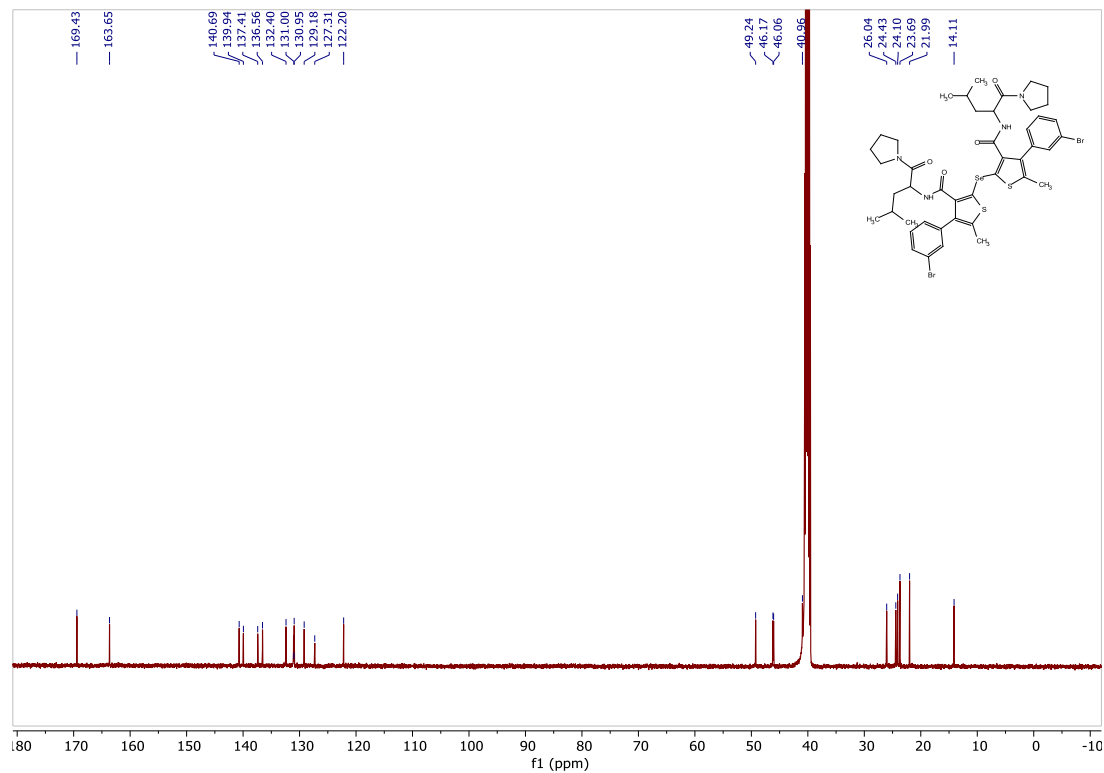


¹³C NMR spectrum of compound **16f** (DMSO-*d*₆, 125 MHz)

16g: 2,2'-selenobis(4-(3-bromophenyl)-5-methyl-N-(3-methyl-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)thiophene-3-carboxamide)

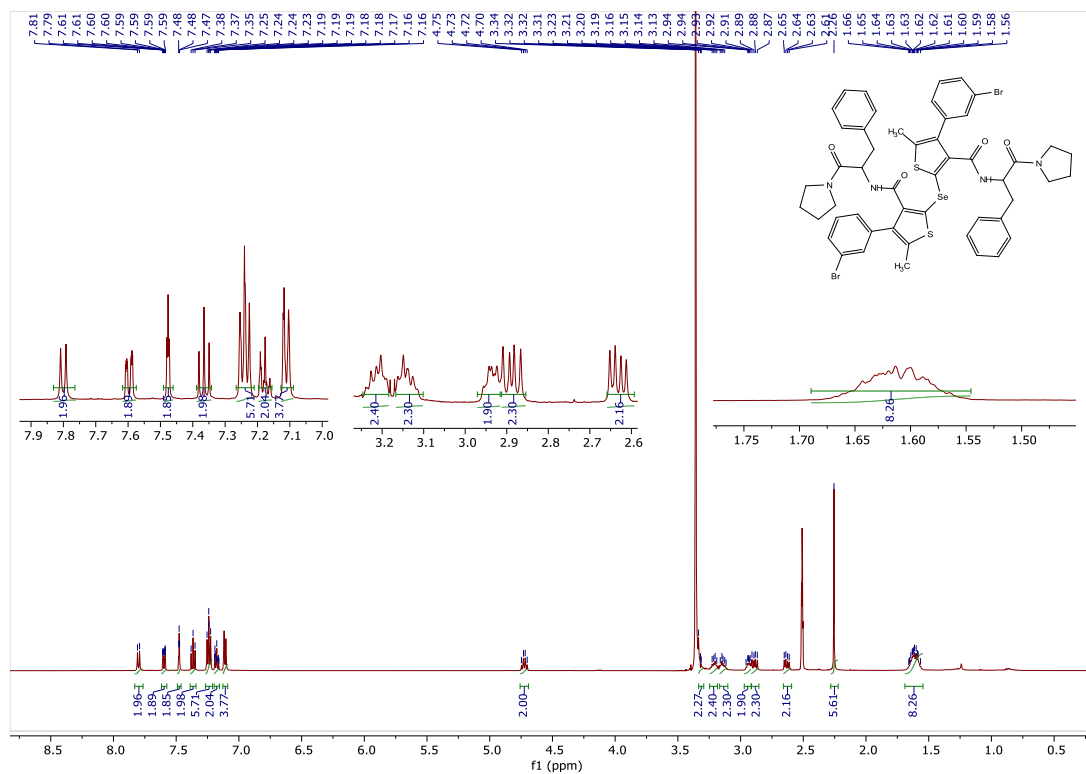


¹H NMR spectrum of compound **16g** (DMSO-*d*₆, 500 MHz)

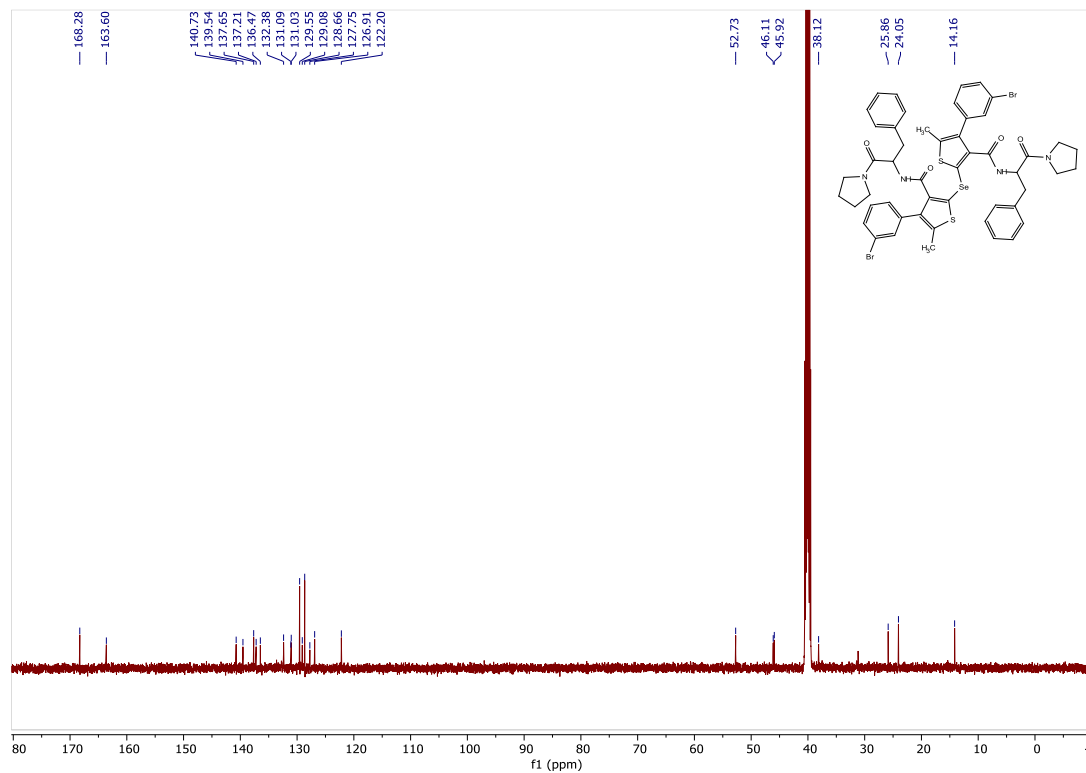


¹³C NMR spectrum of compound **16g** (DMSO-*d*₆, 125 MHz)

16h: 2,2'-selenobis(4-(3-bromophenyl)-5-methyl-N-(1-oxo-3-phenyl-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide)

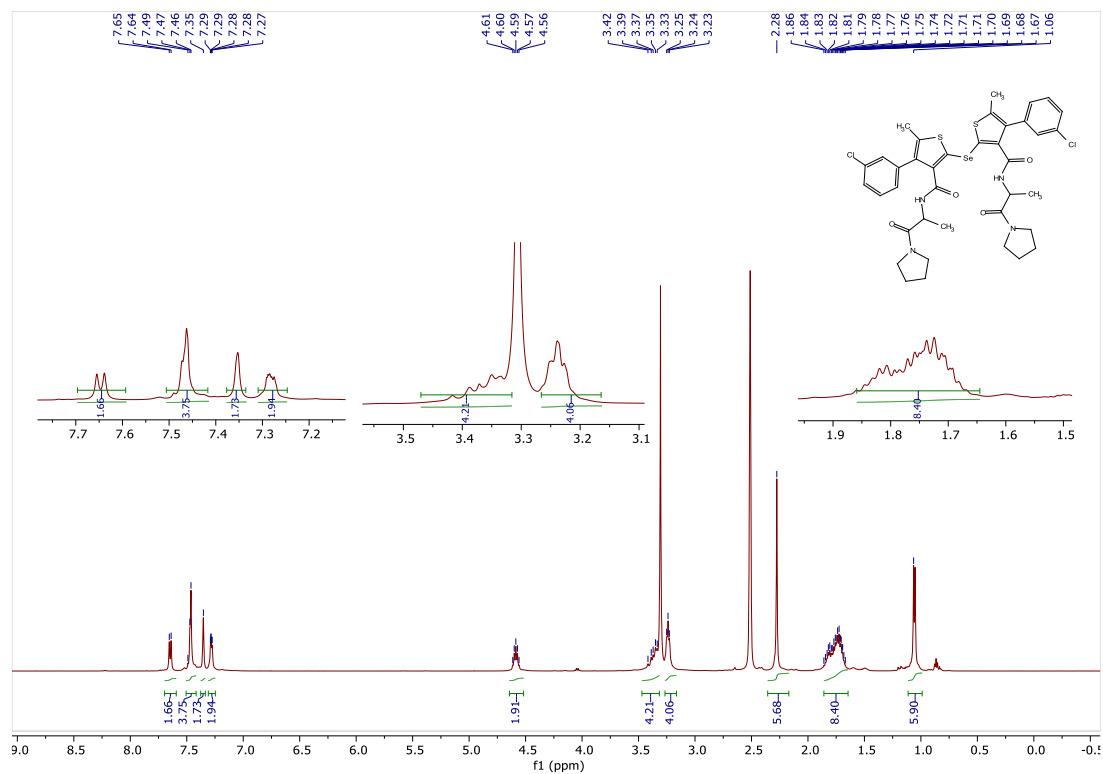


¹H NMR spectrum of compound **16h** (DMSO-*d*₆, 500 MHz)

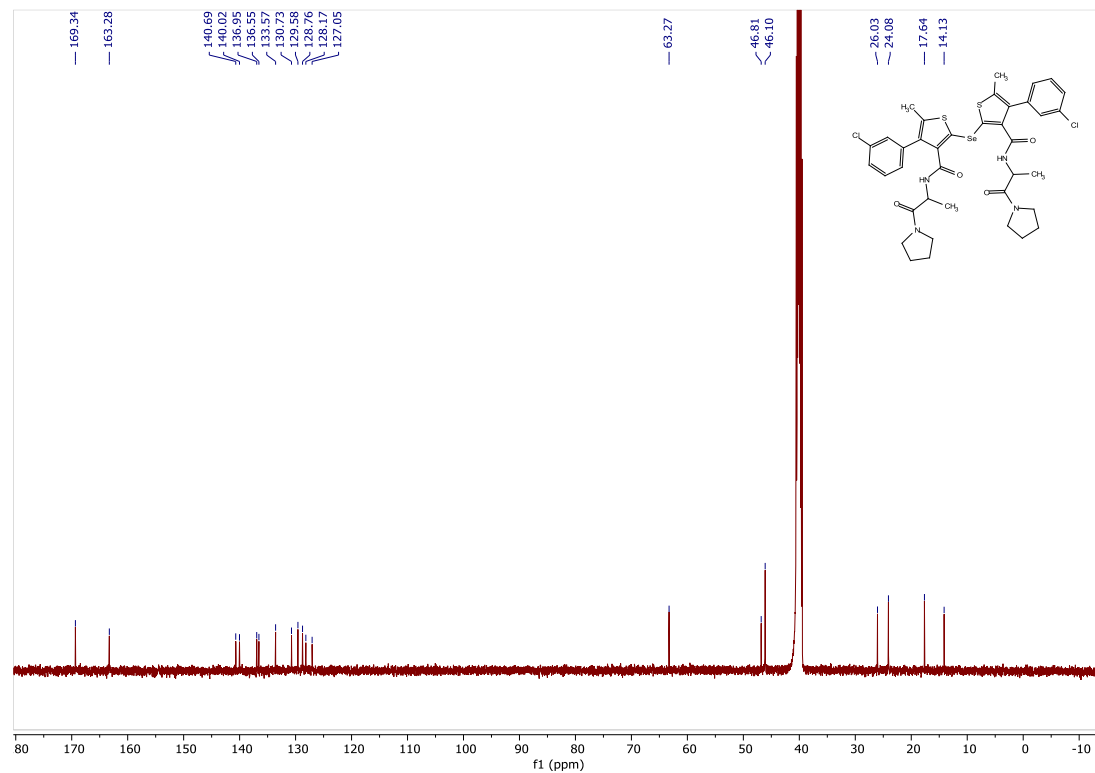


¹³C NMR spectrum of compound **16h** (DMSO-*d*₆, 125 MHz)

16i: 2,2'-selenobis(4-(3-chlorophenyl)-5-methyl-N-(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide)

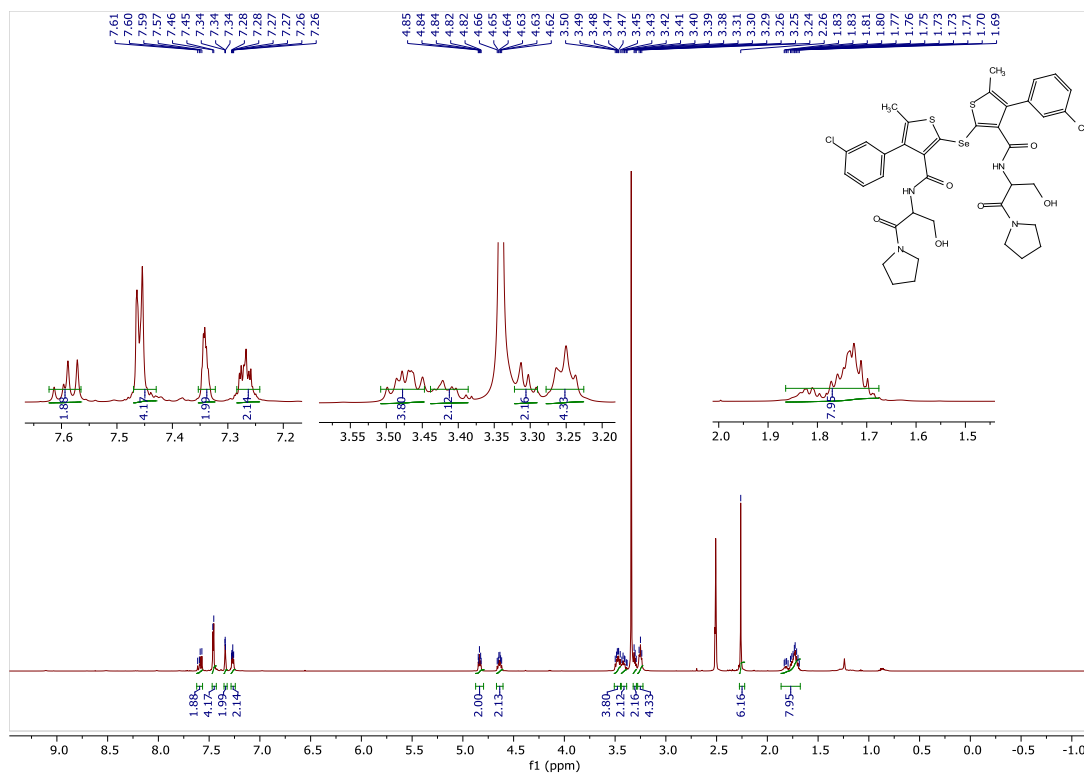


¹H NMR spectrum of compound **16i** (DMSO-*d*₆, 500 MHz)

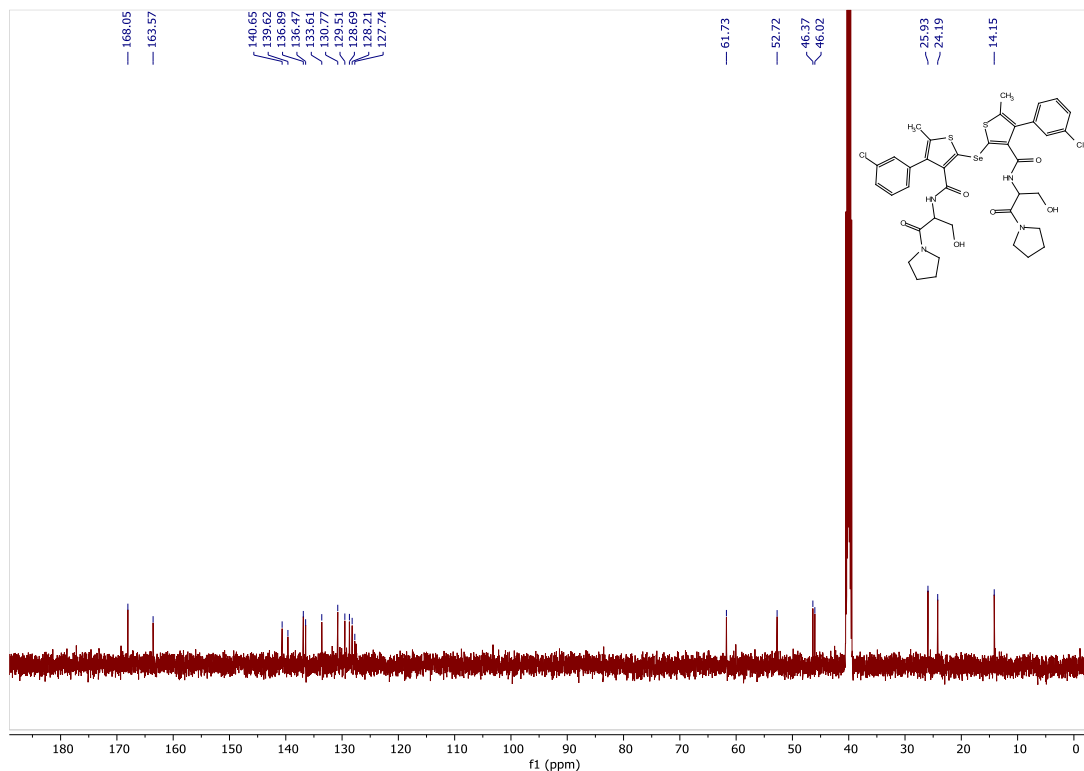


¹³C NMR spectrum of compound **16i** (DMSO-*d*₆, 125 MHz)

16j: 2,2'-selenobis(4-(3-chlorophenyl)-N-(3-hydroxy-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-5-methylthiophene-3-carboxamide)

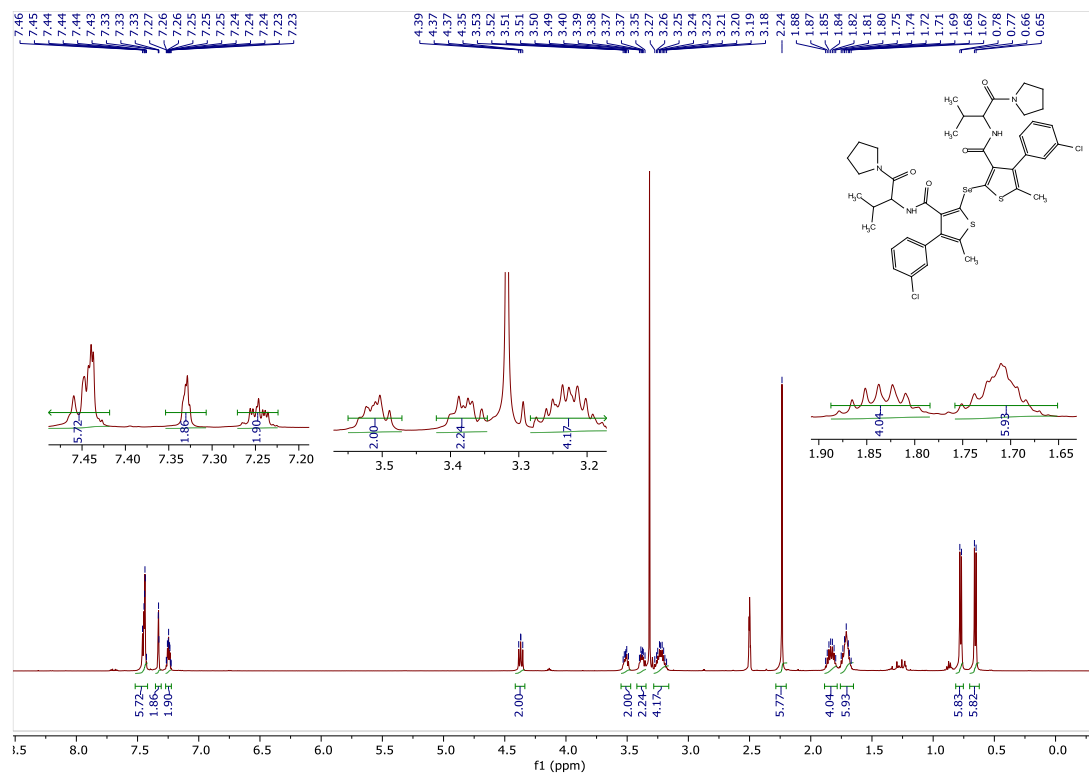


¹H NMR spectrum of compound **16j** (DMSO-*d*₆, 500 MHz)

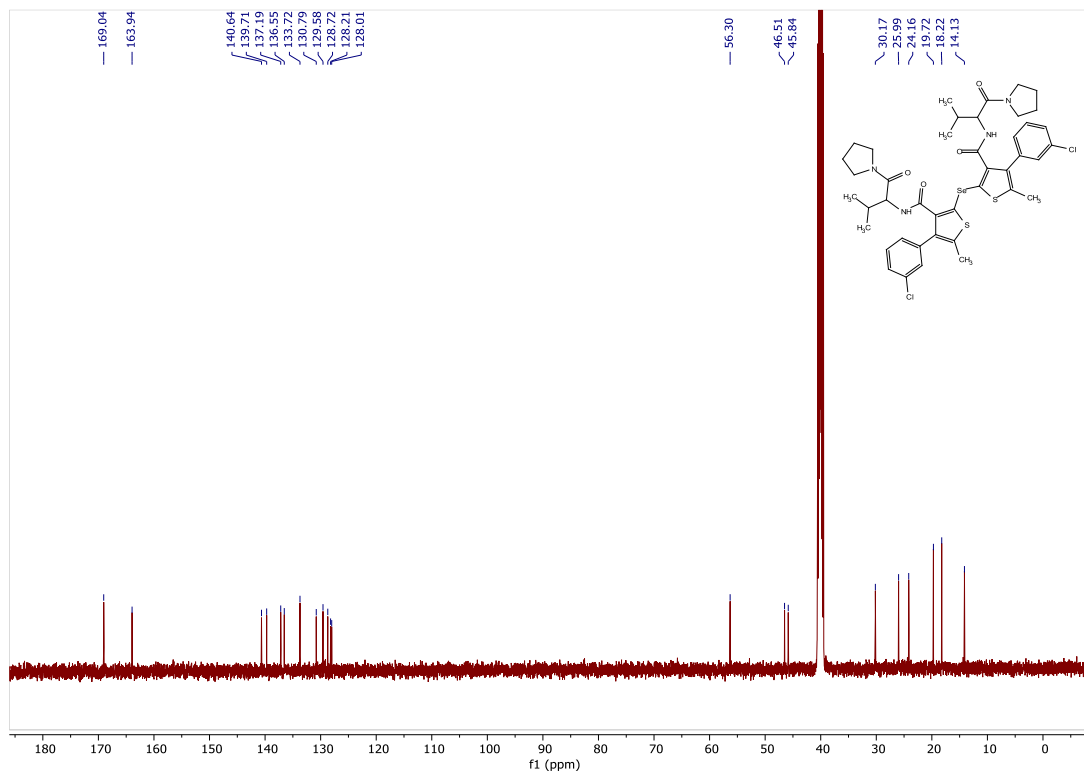


¹³C NMR spectrum of compound **16j** (DMSO-*d*₆, 125 MHz)

16k: 2,2'-selenobis(4-(3-chlorophenyl)-5-methyl-N-(3-methyl-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)thiophene-3-carboxamide)

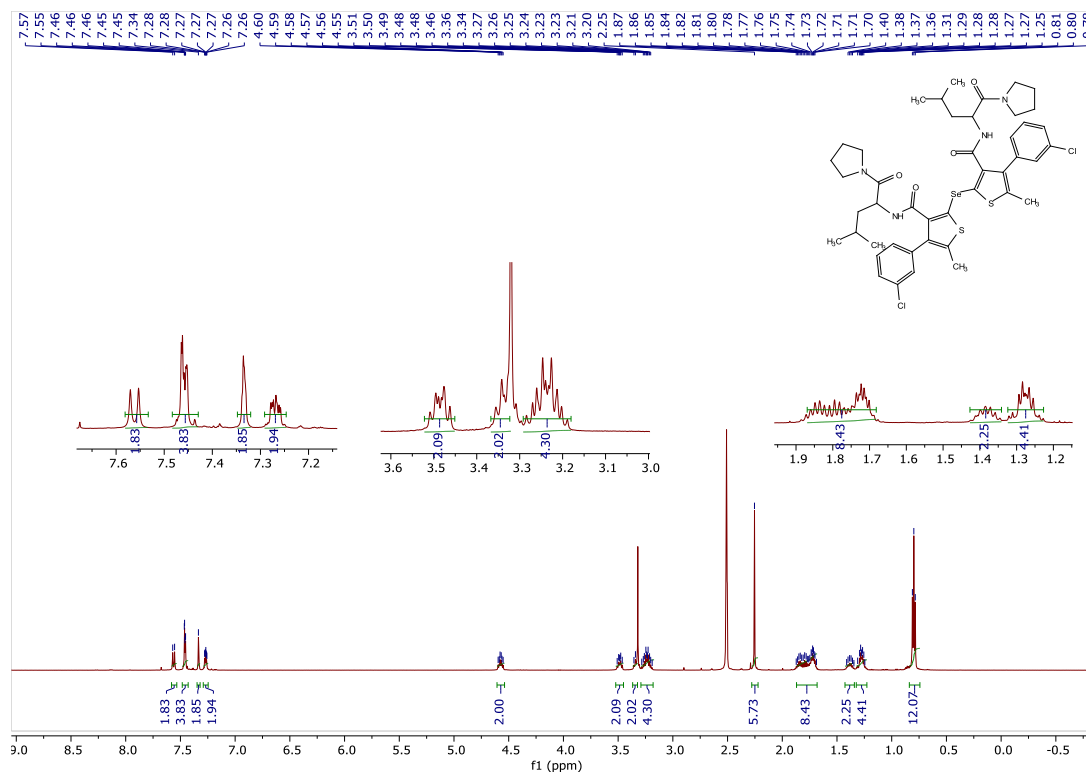


¹H NMR spectrum of compound **16k** (DMSO-*d*₆, 500 MHz)

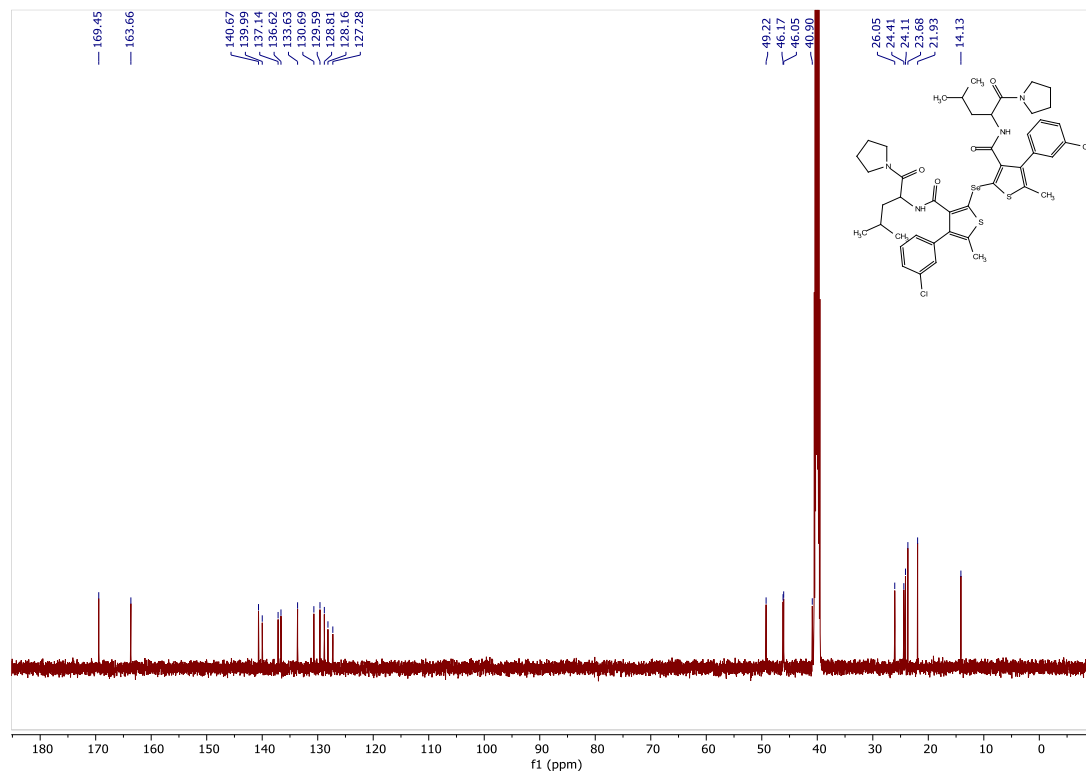


¹³C NMR spectrum of compound **16k** (DMSO-*d*₆, 125 MHz)

16l: 2,2'-selenobis(4-(3-chlorophenyl)-5-methyl-N-(4-methyl-1-oxo-1-(pyrrolidin-1-yl)pentan-2-yl)thiophene-3-carboxamide)

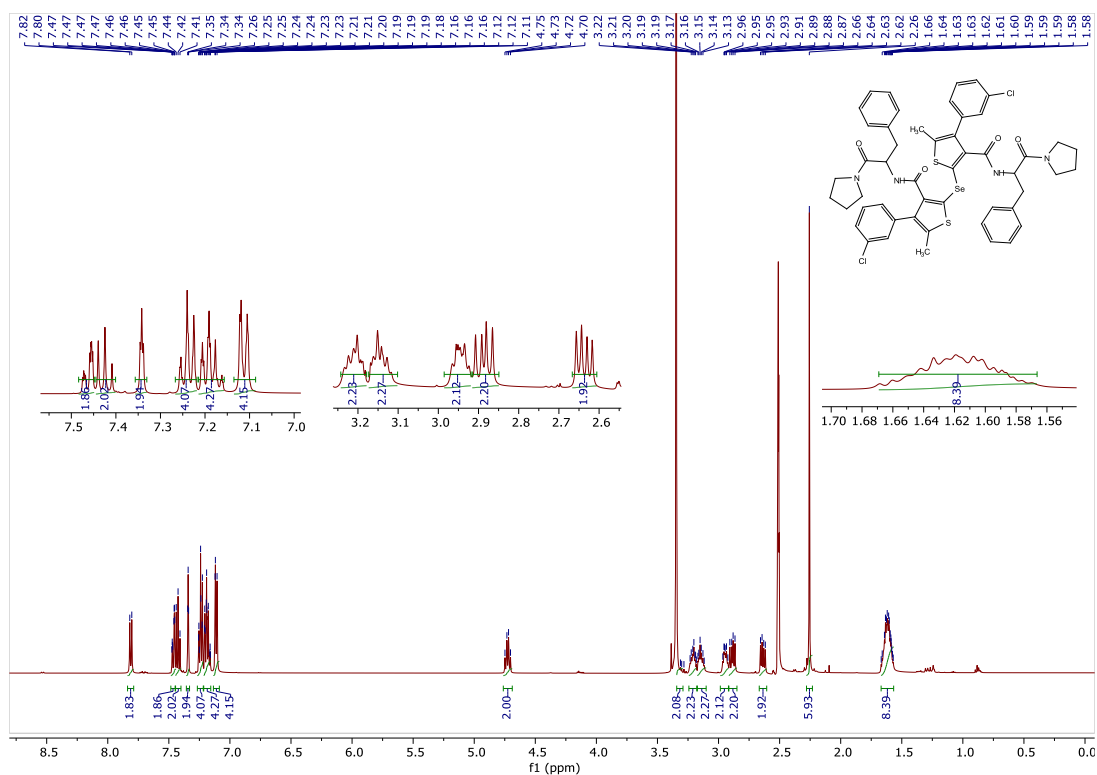


¹H NMR spectrum of compound **16l** (DMSO-*d*₆, 500 MHz)

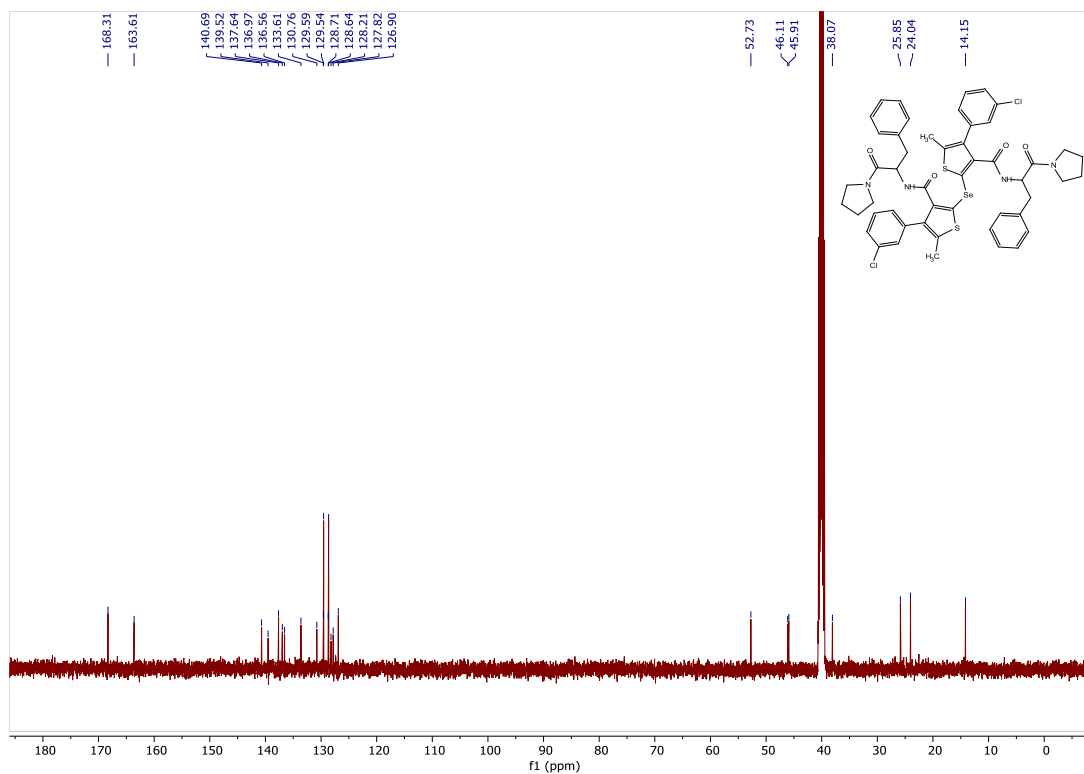


¹³C NMR spectrum of compound **16k** (DMSO-*d*₆, 125 MHz)

16m: 2,2'-selenobis(4-(3-chlorophenyl)-5-methyl-N-(1-oxo-3-phenyl-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide)

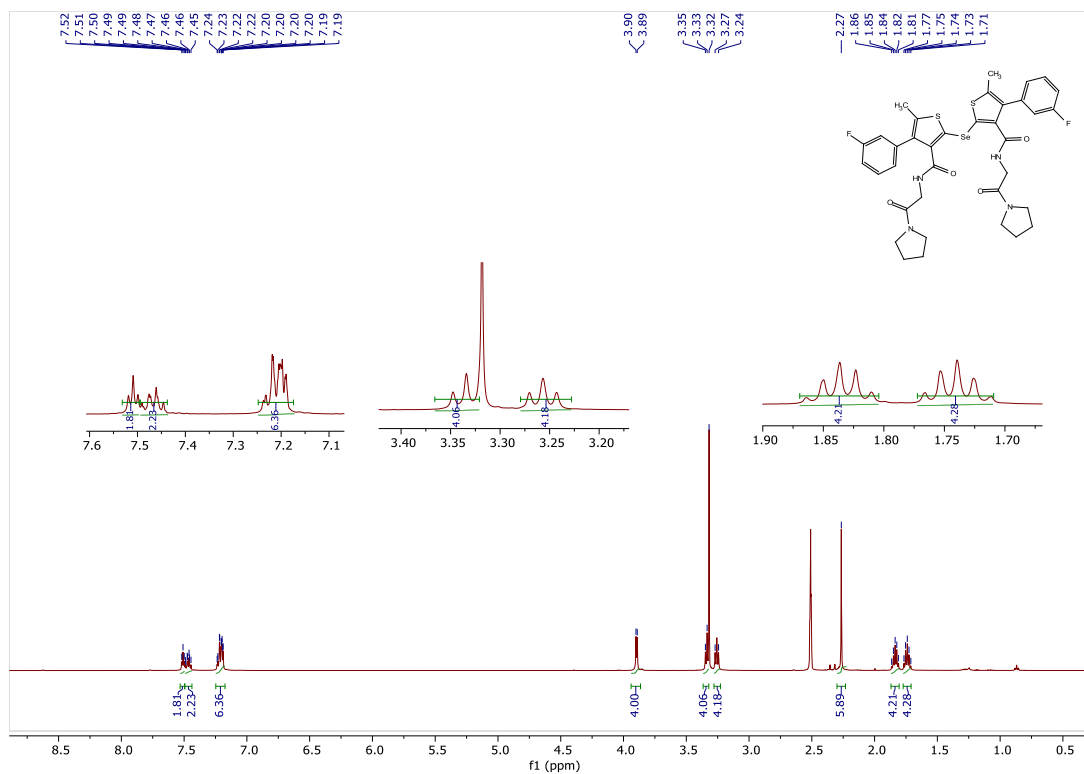


¹H NMR spectrum of compound **16m** (DMSO-*d*₆, 500 MHz)

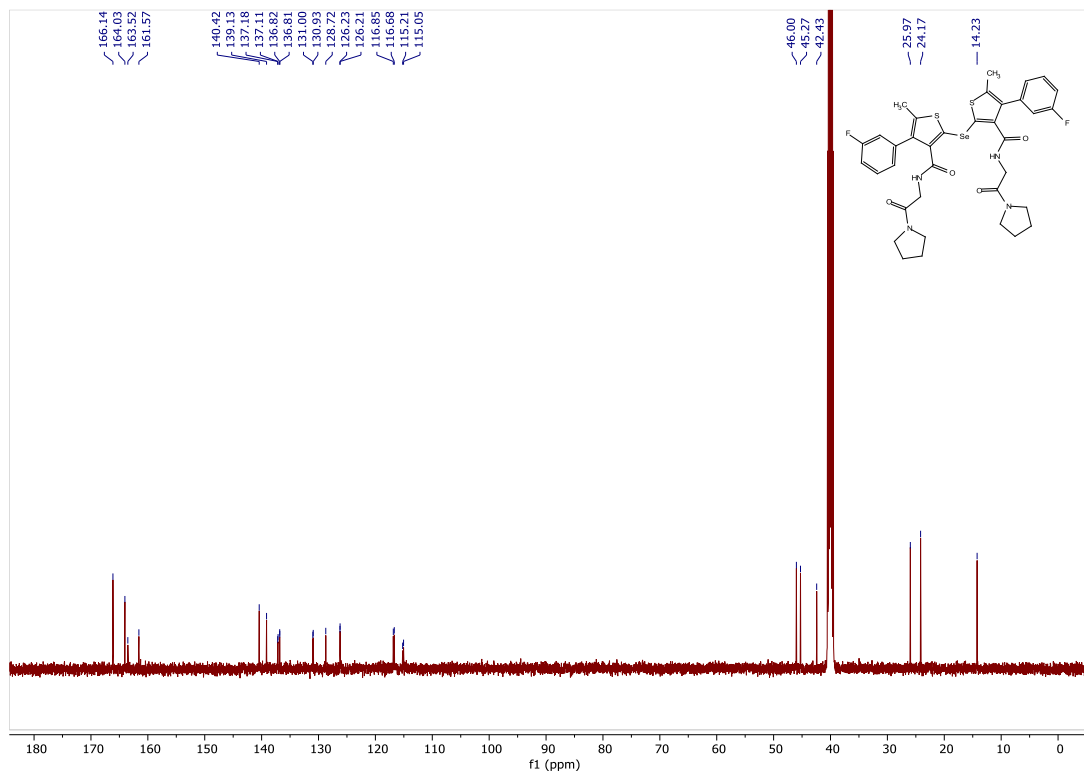


¹³C NMR spectrum of compound **16m** (DMSO-*d*₆, 125 MHz)

16n: 2,2'-selenobis(4-(3-fluorophenyl)-5-methyl-N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)thiophene-3-carboxamide)

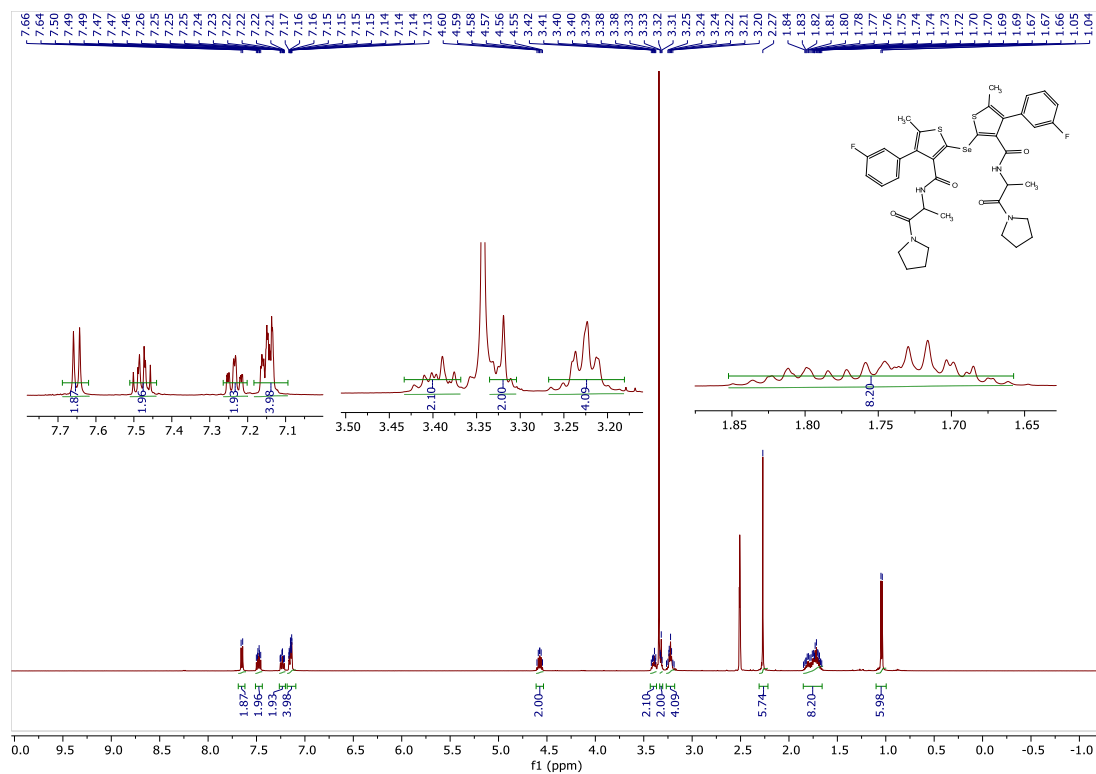


¹H NMR spectrum of compound **16n** (DMSO-*d*₆, 500 MHz)

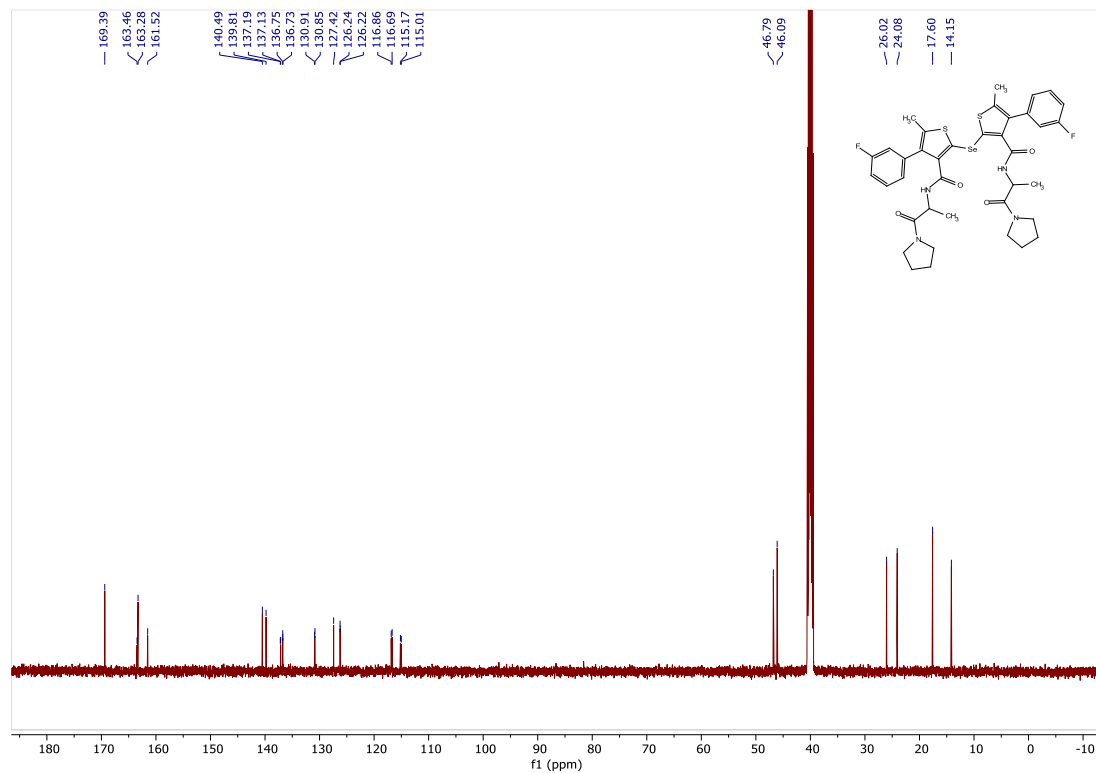


¹³C NMR spectrum of compound **16n** (DMSO-*d*₆, 125 MHz)

16o: 2,2'-selenobis(4-(3-fluorophenyl)-5-methyl-N-(1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide)

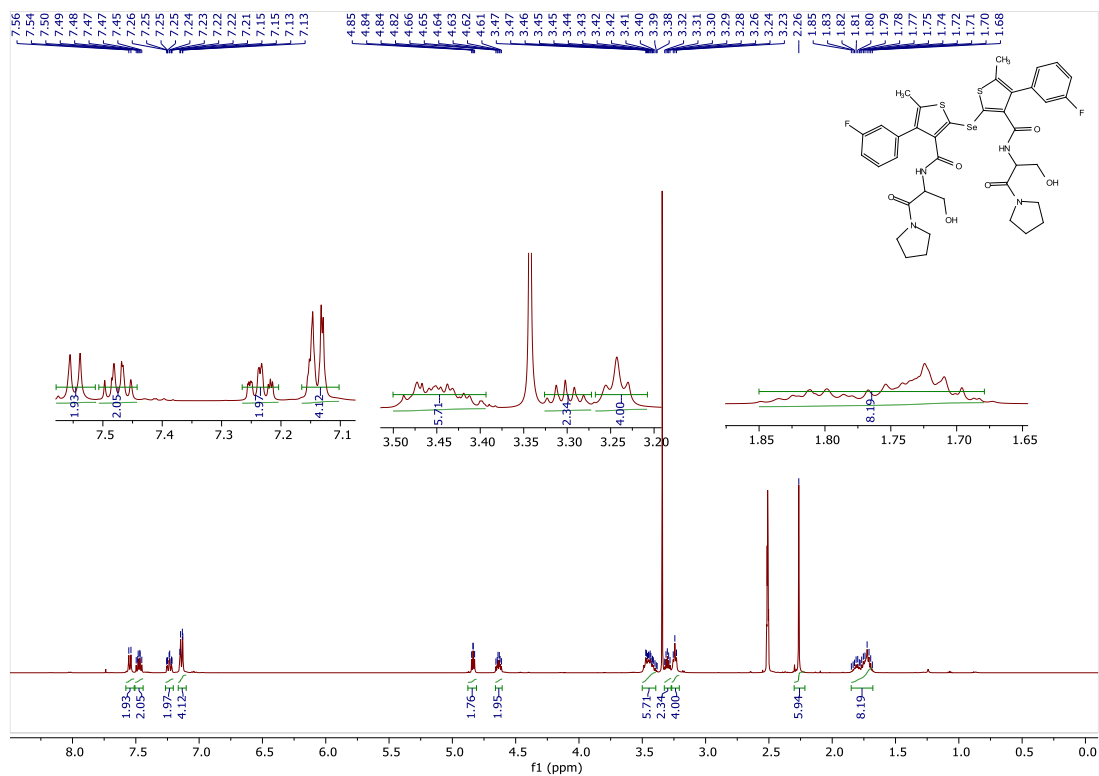


¹H NMR spectrum of compound **16o** (DMSO-*d*₆, 500 MHz)

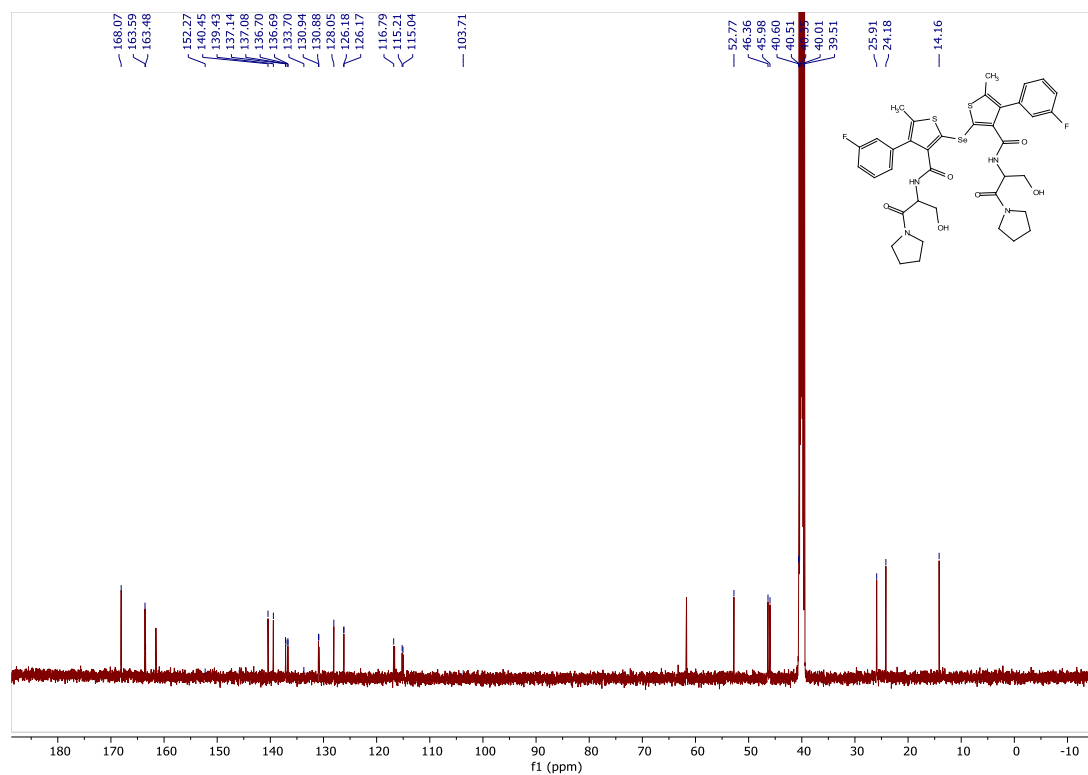


¹³C NMR spectrum of compound **16o** (DMSO-*d*₆, 125 MHz)

16p: 2,2'-selenobis(4-(3-fluorophenyl)-N-(3-hydroxy-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-5-methylthiophene-3-carboxamide)

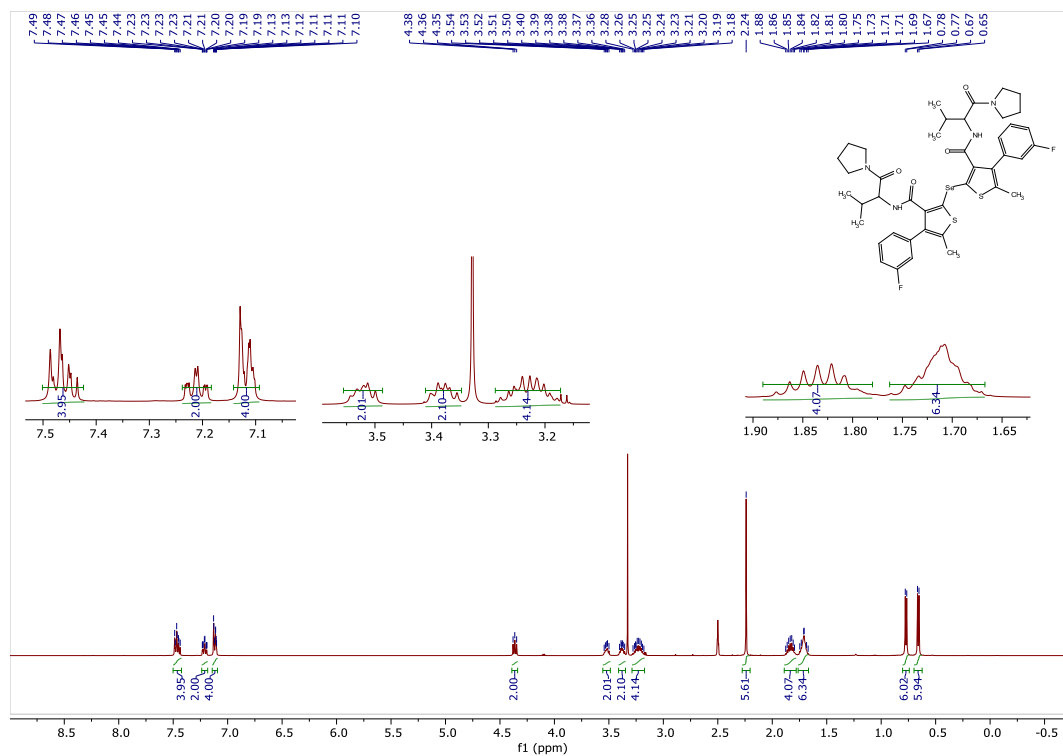


¹H NMR spectrum of compound **16p** (DMSO-*d*₆, 500 MHz)

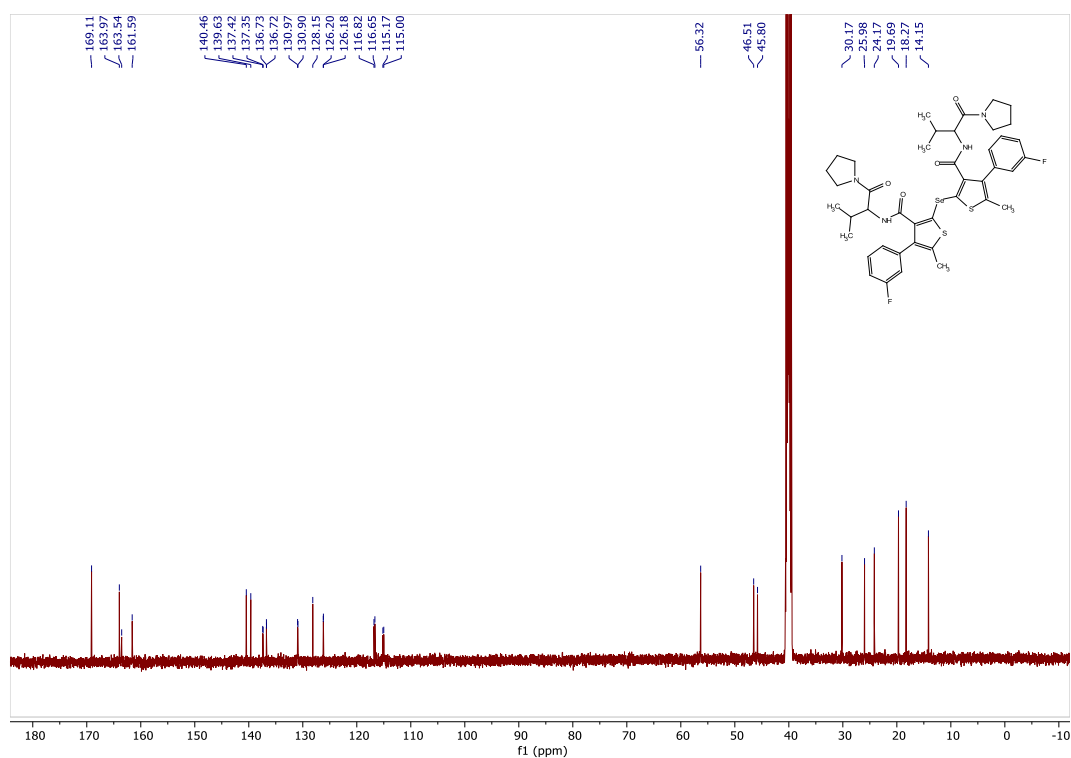


¹³C NMR spectrum of compound **16p** (DMSO-*d*₆, 125 MHz)

16q: 2,2'-selenobis(4-(3-fluorophenyl)-5-methyl-N-(3-methyl-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)thiophene-3-carboxamide)

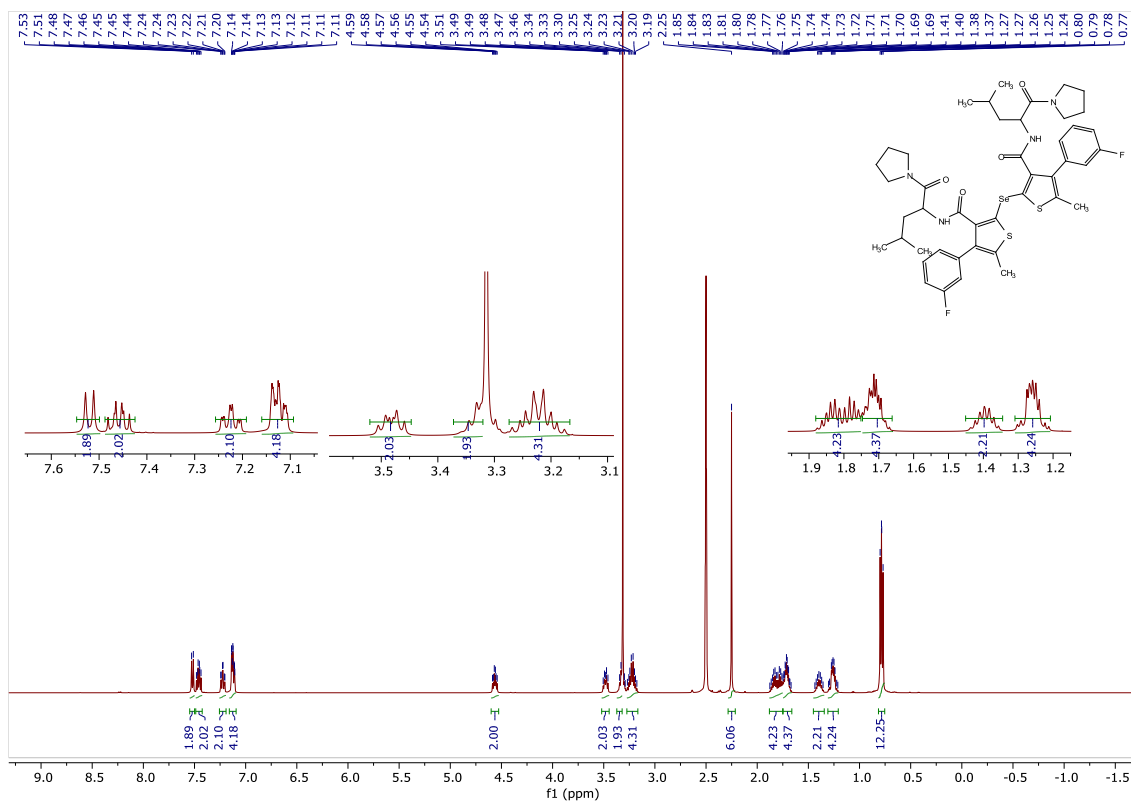


¹H NMR spectrum of compound **16q** (DMSO-*d*₆, 500 MHz)

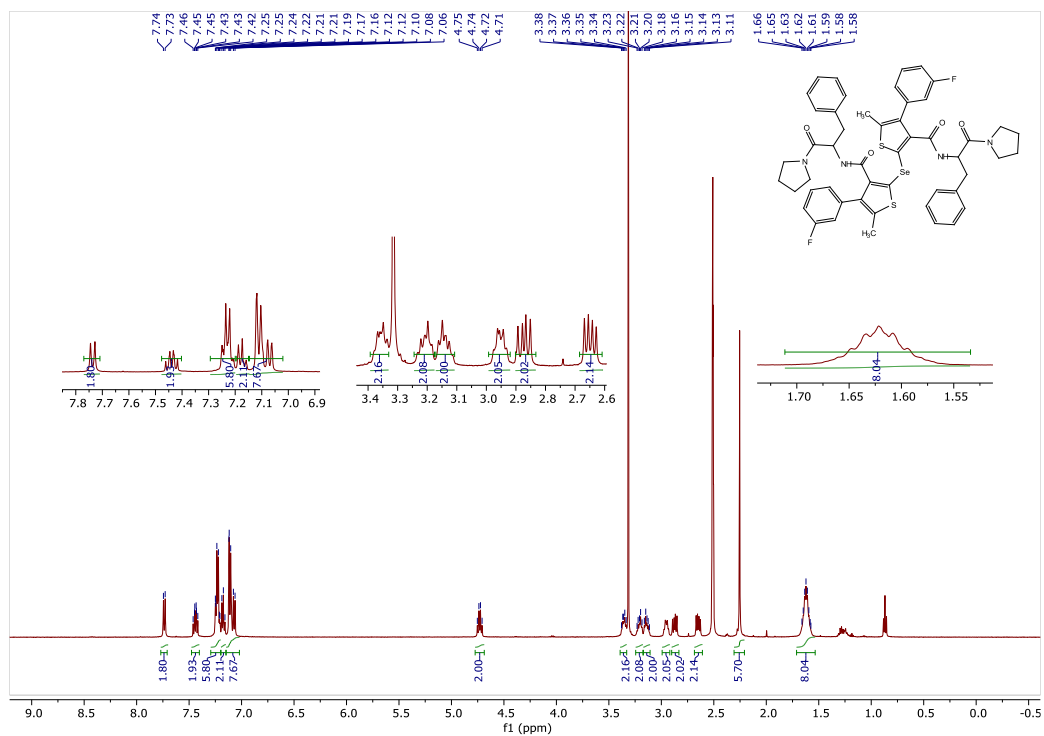


¹³C NMR spectrum of compound **16q** (DMSO-*d*₆, 125 MHz)

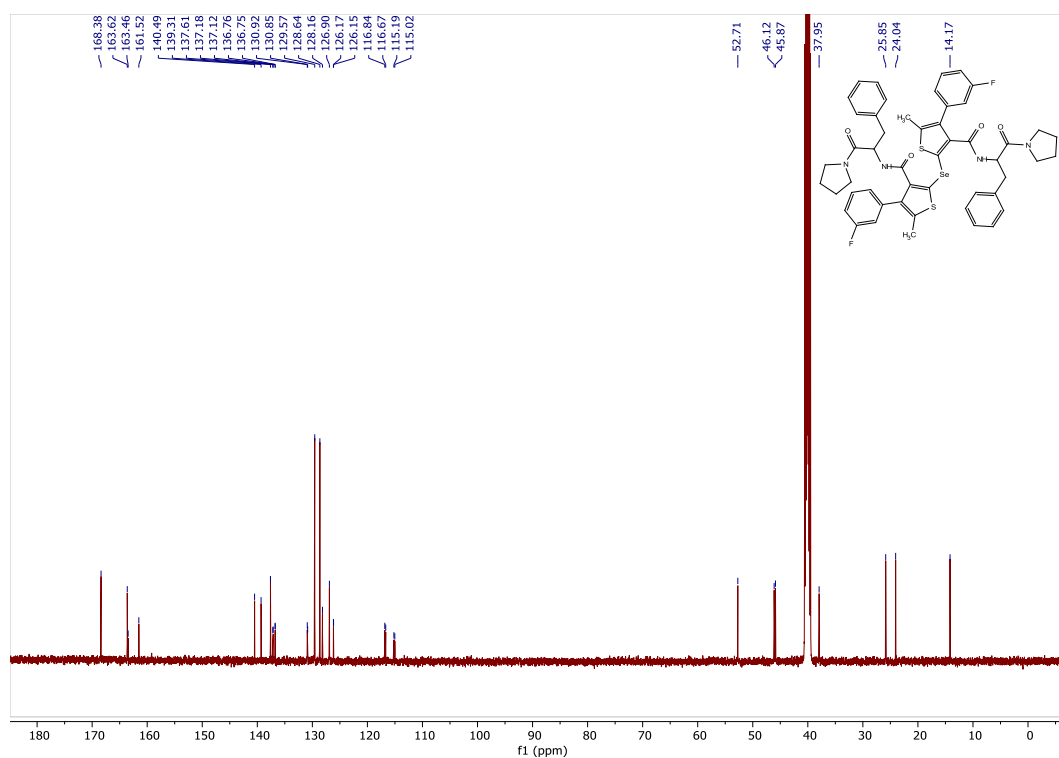
16r: 2,2'-selenobis(4-(3-fluorophenyl)-5-methyl-N-(4-methyl-1-oxo-1-(pyrrolidin-1-yl)pentan-2-yl)thiophene-3-carboxamide)



16s: 2,2'-selenobis(4-(3-fluorophenyl)-5-methyl-N-(1-oxo-3-phenyl-1-(pyrrolidin-1-yl)propan-2-yl)thiophene-3-carboxamide)



¹H NMR spectrum of compound 16s (DMSO-*d*₆, 500 MHz)



¹³C NMR spectrum of compound 16s (DMSO-*d*₆, 125 MHz)

3. HPLC Method conditions

Sample preparation: The compound is dissolved in the mixture of acetonitrile and methanol : H₂O (1:1) with the help of sonication.

Mobile Phase:

- A) 0.1% Formic acid: dissolve 1 ml of formic acid into 1000 ml distilled water.
- B) Acetonitrile 90% and methanol 10 %

Chromatographic condition:

Column: Xbridge C18 , 5 μ 4.6 mm x 250 mm

Column temp: 30 °C

Sample temp: 15 °C

Flow rate: 1 ml/min

Injection volume: 10 μ L

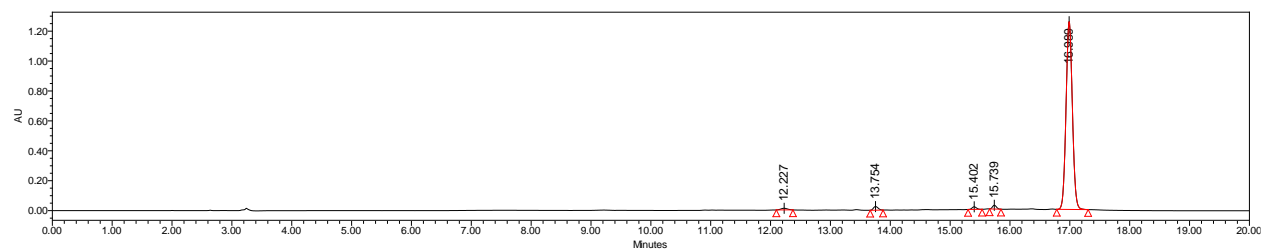
Wavelength: 254 nm

Gradient program:

Time	%B
0.1	10
3	10
8	50
12	90
14	90
16	50
18	10
20	10

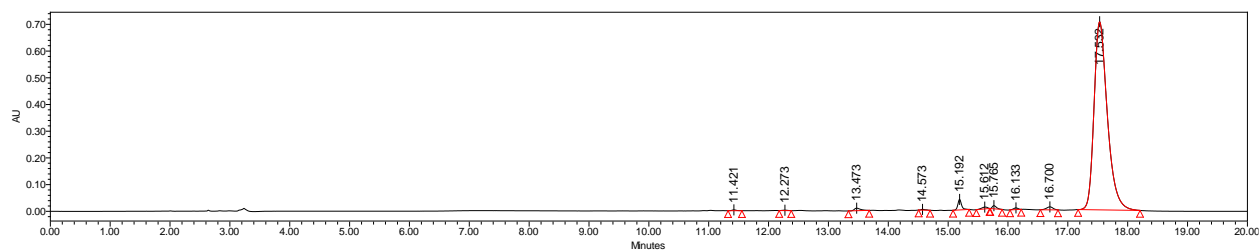
4. HPLC results

Compound 16b



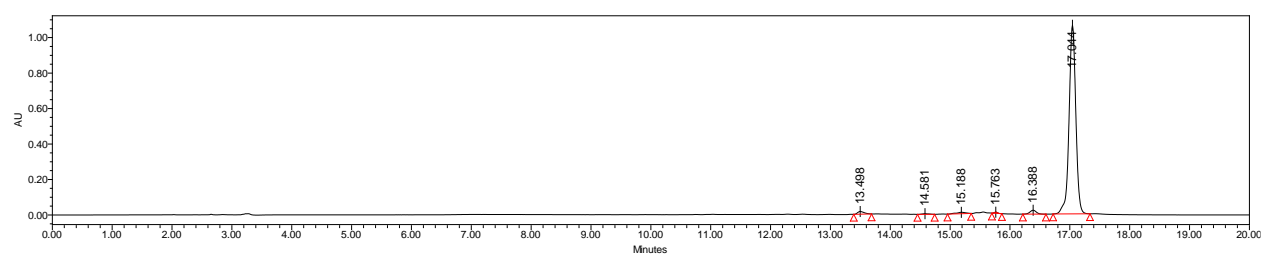
Entry	Name	Retention Time	Area	% Area
1		12.227	86714	0.90
2		13.754	132691	1.38
3		15.402	87153	0.90
4		15.739	115803	1.20
5	16b	16.989	9221073	95.62

Compound 16e



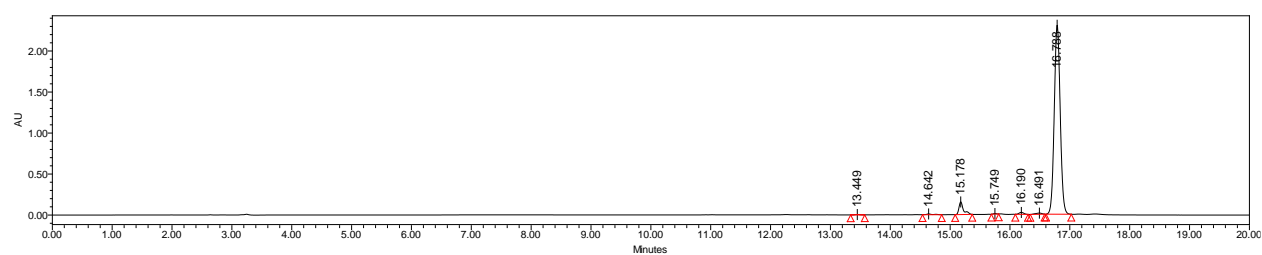
Entry	Name	Retention Time	Area	% Area
1		11.421	15541	0.14
2		12.273	10254	0.09
3		13.473	69326	0.61
4		14.573	16049	0.14
5		15.192	170497	1.50
6		15.612	47487	0.42
7		15.765	57180	0.50
8		16.133	32912	0.29
9		16.700	84647	0.74
10	16e	17.532	10858832	95.57

Compound **16j**



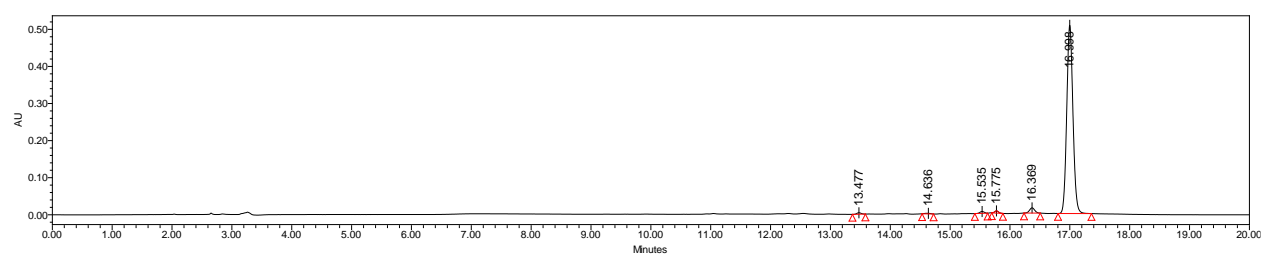
Entry	Name	Retention Time	Area	% Area
1		13.498	117353	1.34
2		14.581	28007	0.32
3		15.188	93077	1.06
4		15.763	25387	0.29
5		16.388	161061	1.84
6	16j	17.044	8335716	95.15

Compound **16n**



Entry	Name	Retention Time	Area	% Area
1		13.449	22370	0.13
2		14.642	66920	0.39
3		15.178	805153	4.72
4		15.749	26384	0.15
5		16.190	130324	0.76
6		16.491	110618	0.65
7	16n	16.788	15901504	93.19

Compound **16o**



Entry	Name	Retention Time	Area	% Area
1		13.477	19551	0.51
2		14.636	11153	0.29
3		15.535	25324	0.66
4		15.775	25572	0.67
5		16.369	84272	2.21
6	16o	16.998	3650214	95.65