Supporting Information

N-Sulfonylphenoxazines as neuronal calcium ion channel blockers

Matthieu Schmit,[a,b] Md. Mahadhi Hasan,[c†] Yashad Dongol,[c] Fernanda C. Cardoso,[c] Michael J. Kuiper,[d] Richard J. Lewis,[c] Peter J. Duggan,[b,e]* Kellie L. Tuck[a]*

[a] *School of Chemistry, Monash University, Victoria 3800, Australia*
[b] *CSIRO Manufacturing, Research Way, Clayton, Victoria 3168, Australia*
[c] *Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia.*
[d] *CSIRO Data 61, Clunies Ross Street, Acton ACT 2601, Australia*
[e] *College of Science and Engineering, Flinders University, Adelaide, South Australia 5042, Australia.*
† current address: Pharmacy Discipline, Life Science School, Khulna University, Khulna, 9208, Bangladesh

E-mail: Kellie.Tuck@monash.au, Peter.Duggan@csiro.au.

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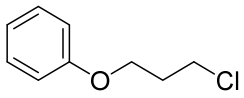
Synthesis of 4-(3-chloropropoxy)benzenesulfonyl chloride (2)

(3-Chloropropoxy)benzene

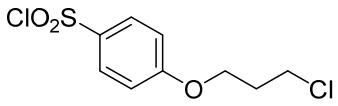


Adapted from Rampa *et al.*[1] Phenol (4.70 g, 50.0 mmol), 1-bromo-3-chloropropane (6.40 mL, 65.0 mmol, 1.3 eq.) and potassium carbonate (13.8 g, 100 mmol, 2 eq.) were dissolved in acetone (100 mL) and the mixture was left stirring at reflux for 3 days. The solvent was then evaporated and the product was redissolved in ethyl acetate (30 mL) and petroleum ether (30 mL) and washed with 1 M $NaOH_{(aq)}$ (3x50 mL). The organic phase was dried on magnesium sulfate, filtered and concentrated. The product was then eluted over a pad of 20-40 nm silica by hexane to afford the title compound as a yellowish oil (7.09 g, 41.4 mmol, 83%). Spectral data was consistent with literature reports.[1]

^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) = 7.32-7.26 (2H, m), 6.96 (1H, tt, *J* = 7.4, 1.0 Hz), 6.93-6.89 (2H, m), 4.12 (2H, t, *J* = 5.7 Hz), 3.75 (2H, t, *J* = 6.4 Hz), 2.24 (2H, quint, *J* = 6.2 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) = 158.7, 129.5, 120.9, 114.5, 64.2, 41.6, 32.3.

4-(3-Chloropropoxy)benzenesulfonyl chloride (2)



Adapted from Ceras *et al*.[2] (3-Chloropropoxy)benzene (7.09 g, 41.4 mmol) was dissolved in DCM (100 mL) and was cooled to approximately -10°C with an ice/salt bath, then a solution of chlorosulfonic acid (5.80 mL, 87.0 mmol, 2.1 eq) in DCM (15 mL) was added dropwise. After 30 min no trace of the starting material was found on TLC (petroleum ether, R_f=0.2). DMF (0.5 mL) was added to the mixture, then a solution of thionyl chloride (3.00 mL, 41.0 mmol, 1 eq.) in DCM (15 mL) was added dropwise. The mixture was left stirring overnight at room temperature, then poured over ice and water (approximately 50 mL). The aqueous phase was extracted with DCM (3x50 mL) and the combined organic layers were dried with magnesium sulfate, filtered and concentrated. A dark oil was obtained, which was purified by eluting with DCM over a pad of 20-40 nm silica to afford the title compound **2** as a yellowish solid (5.12 g, 18.0 mmol, 43%).

MP: 56-58 °C.

^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) = 8.01-7.96 (2H, m), 7.08-7.03 (2H, m), 4.24 (2H, t, *J* = 5.8 Hz), 3.76 (2H, t, 6.0 Hz), 2.30 (2H, quint, *J* = 6.2 Hz).

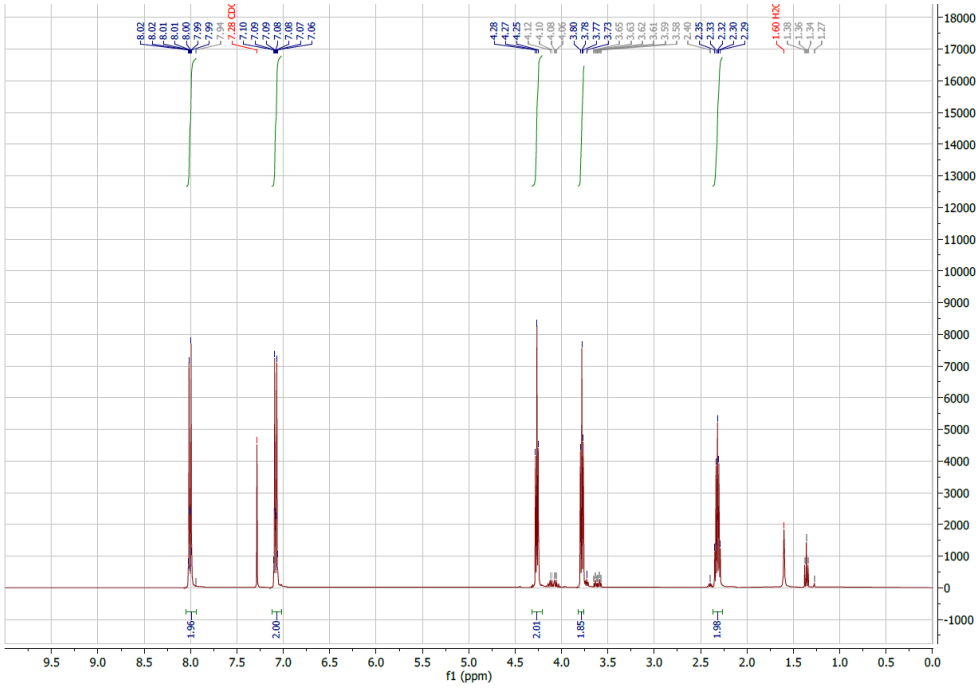
^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) = 164.1, 137.4, 129.6, 115.2, 65.3, 40.9, 31.9.

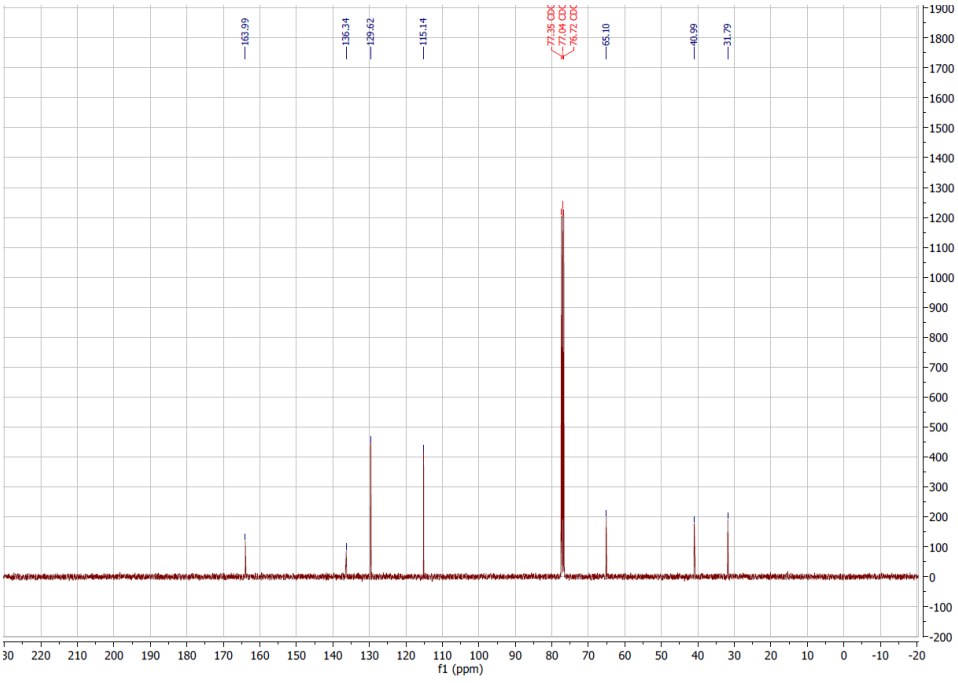
Liquid chromatography methods:

Instrument	Method	Detection
Preparative HPLC		
Agilent 1260 Infinity Phenomenex Luna C8 column (150 x 21.5 mm, 5 µm)	Flowrate: 10 mL / min Eluent A: MQ water, 0.1% TFA Eluent B: ACN, 20% MQ water, 0.1% TFA **Method 1** (Standard): 90% A: 10% B to 10% A: 90% B from 0 to 30 min 10% A: 90% B plateau from 30 to 40 min 10% A: 90% B to 90% A: 10% B from 40 to 41 min **Method 2**: 90% A: 10% B to 10% A: 90% B from 0 to 40 min 10% A: 90% B to 90% A: 10% B from 40 to 41 min **Method 3**: 90% A: 10% B to 10% A: 90% B from 0 to 80 min 10% A: 90% B plateau from 80 to 90 min 10% A: 90% B to 90% A: 10% B from 90 to 91 min	Agilent 1290 Infinity II Diode-Array Detector 250, 254 nm and 210 nm
Analytical HPLC		
Agilent 1220 Infinity Eclipse XDB-C18 column (150mm x 4.6 mm, 5 µm)	Flowrate: 1 mL / min Eluent A: MQ water, 0.1% TFA Eluent B: ACN, 20% MQ water, 0.1% TFA **Method 4** (Standard): 90% A: 10% B from 0 to 2 min 90% A: 10% B to 10% A: 90% B from 2 to 28 min 10% A: 90% B to 90% A: 10% B from 28 to 30 min	Agilent 1290 Infinity II Diode-Array Detector 250, 254 nm and 210 nm
LC/HRMS (QToF)		
Agilent 1200 Series Agilent XDB-C18-bonded silica column (4.6 x 50 mm, 1.8 µm)	Flowrate: 0.4 mL / min Eluent A: MQ water, 0.1% FA Eluent B: ACN, 0.1% FA **Method 5** (Standard): 92% A: 8% B to 20% A: 80% B from 0 to 8 min 20% A: 80% B to 92% A: 8% B from 8 to 12 min	Agilent 6540 Q-TOF MS system

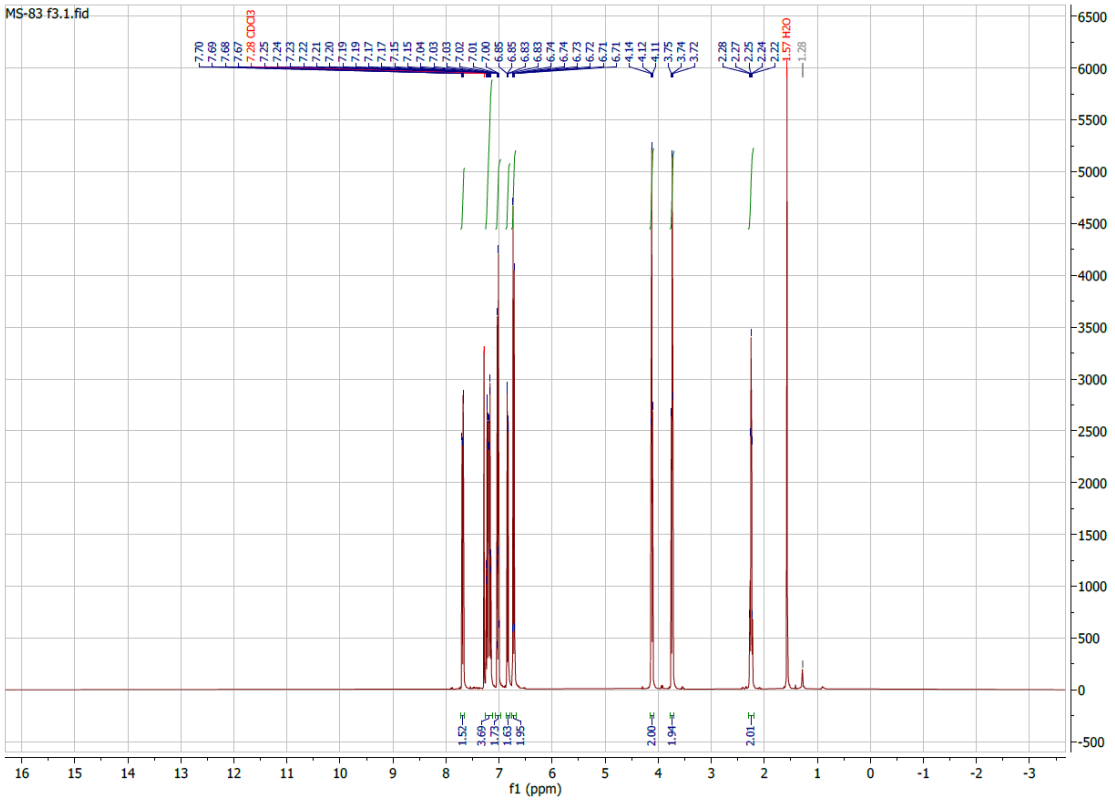
Spectra of synthesised compounds

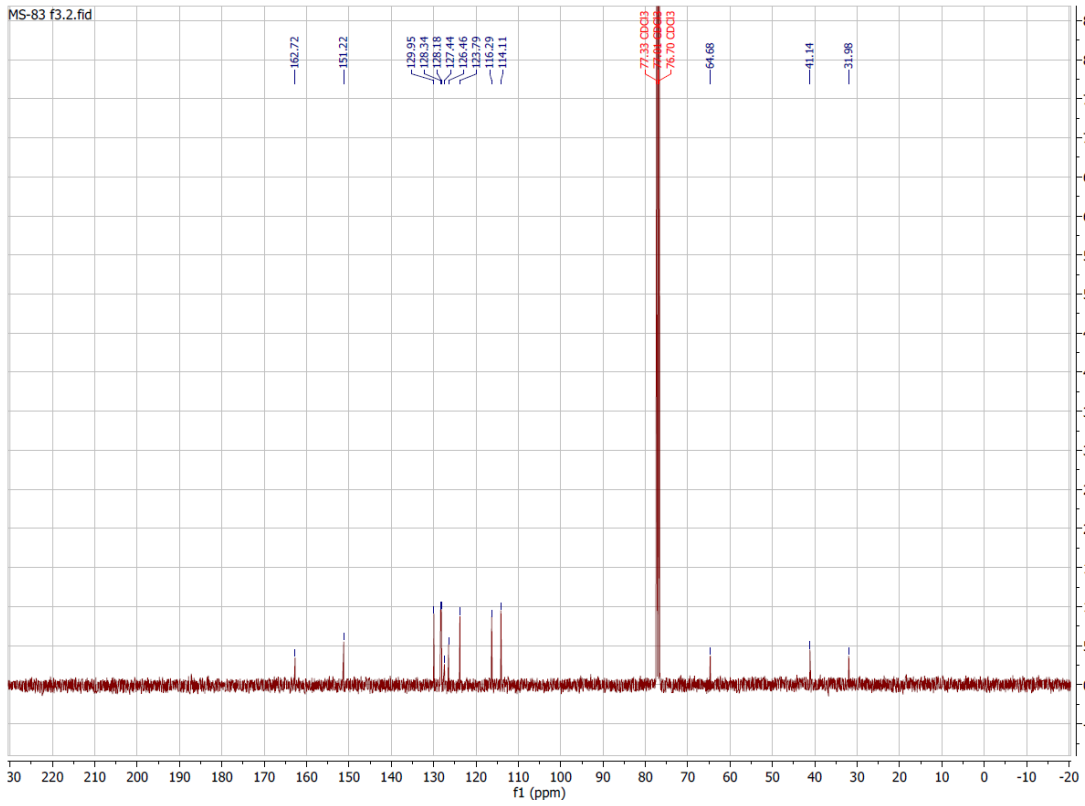
4-(3-Chloropropoxy)benzenesulfonyl chloride (2)



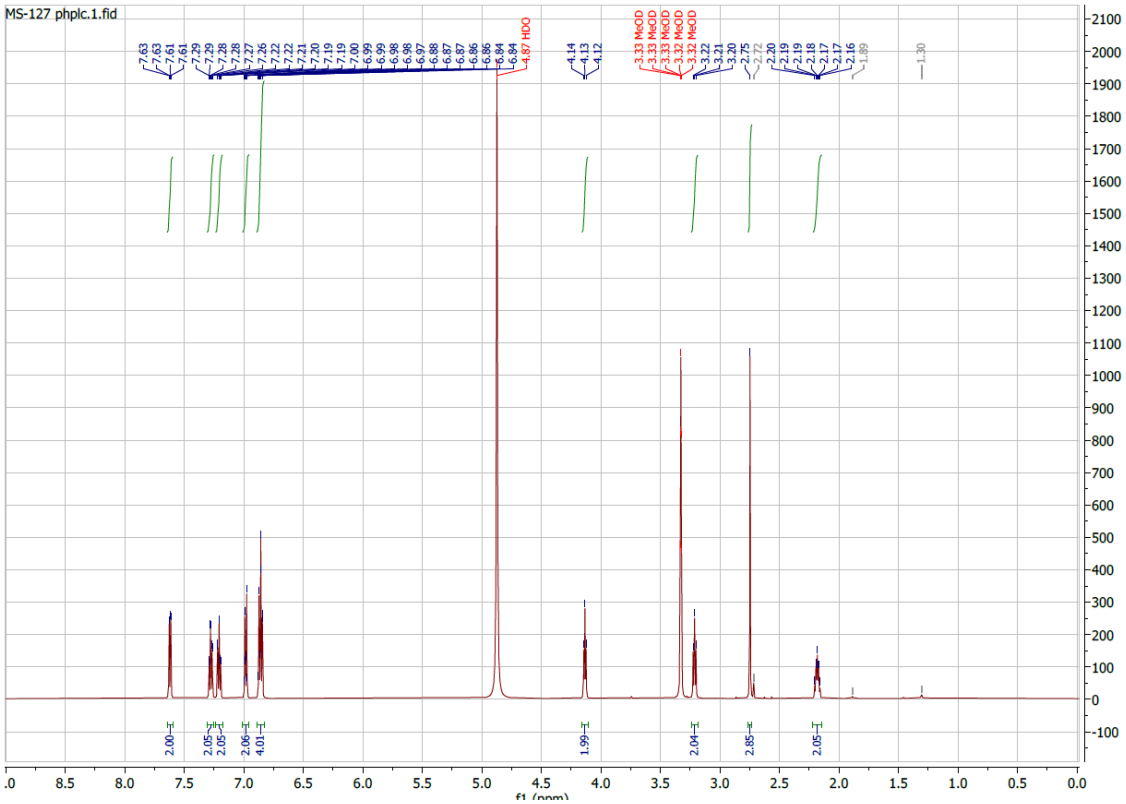


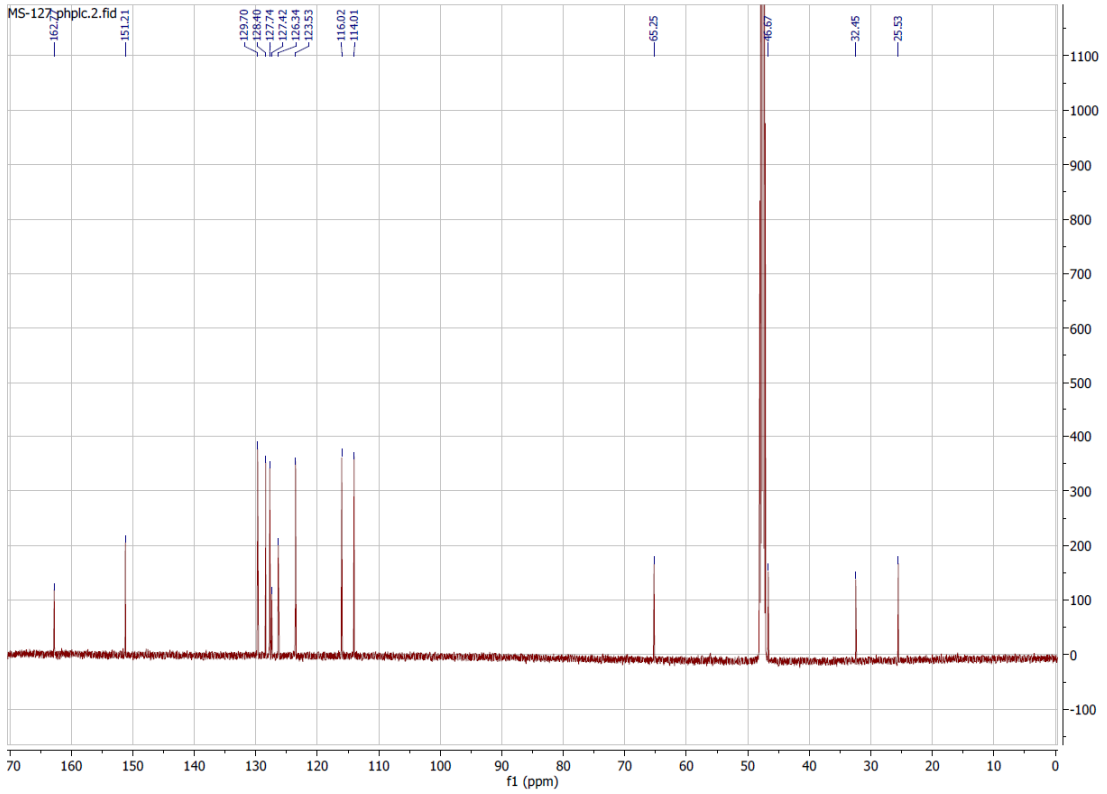
10-((4-(3-Chloropropoxy)phenyl)sulfonyl)-10H-phenoxazine (3)



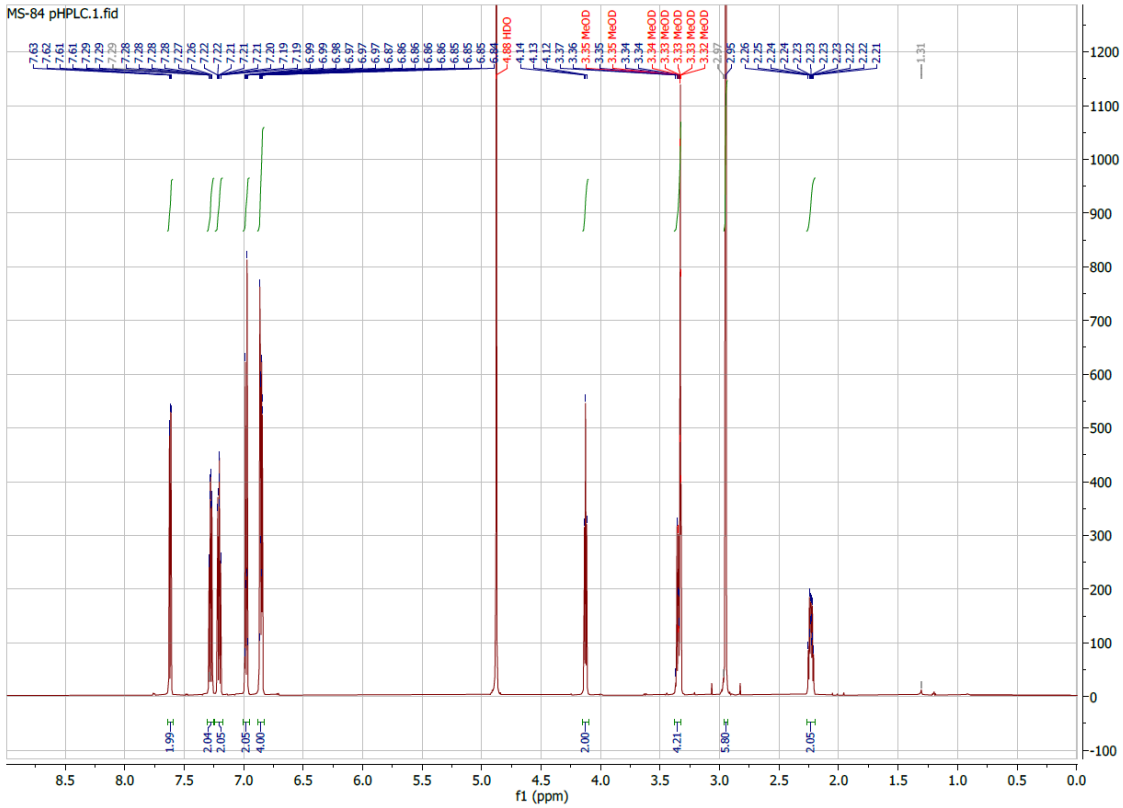


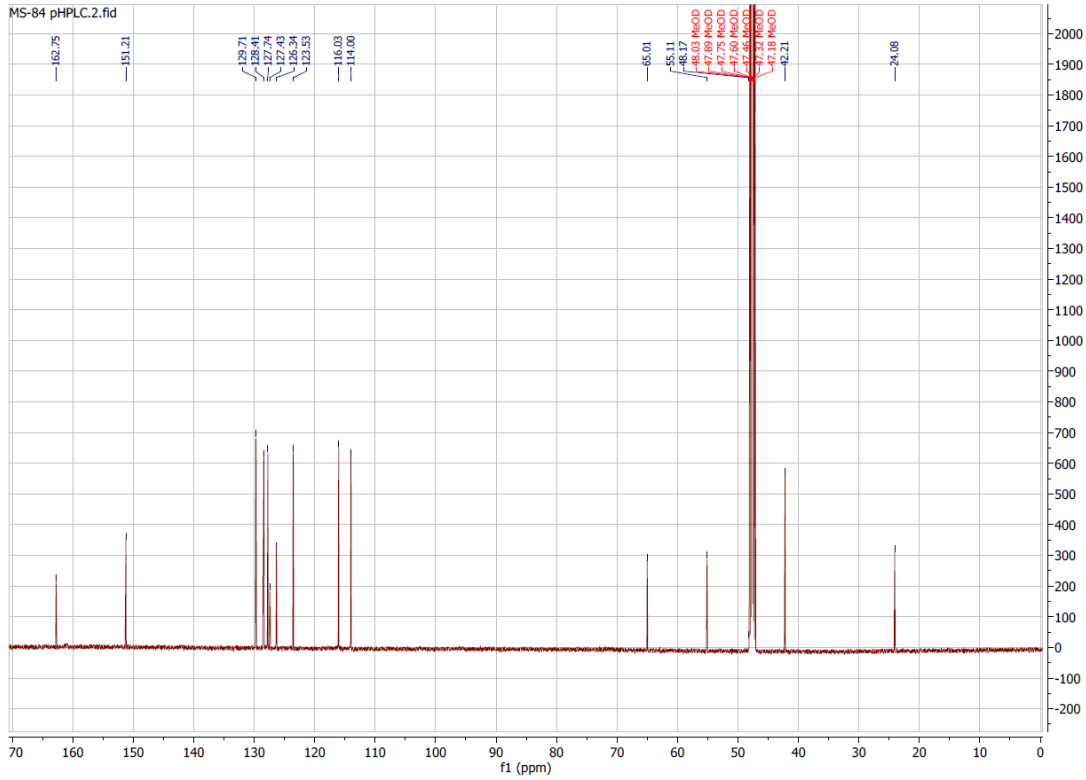
3-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenoxy)-N-methylpropan-1-amine.TFA (4a)



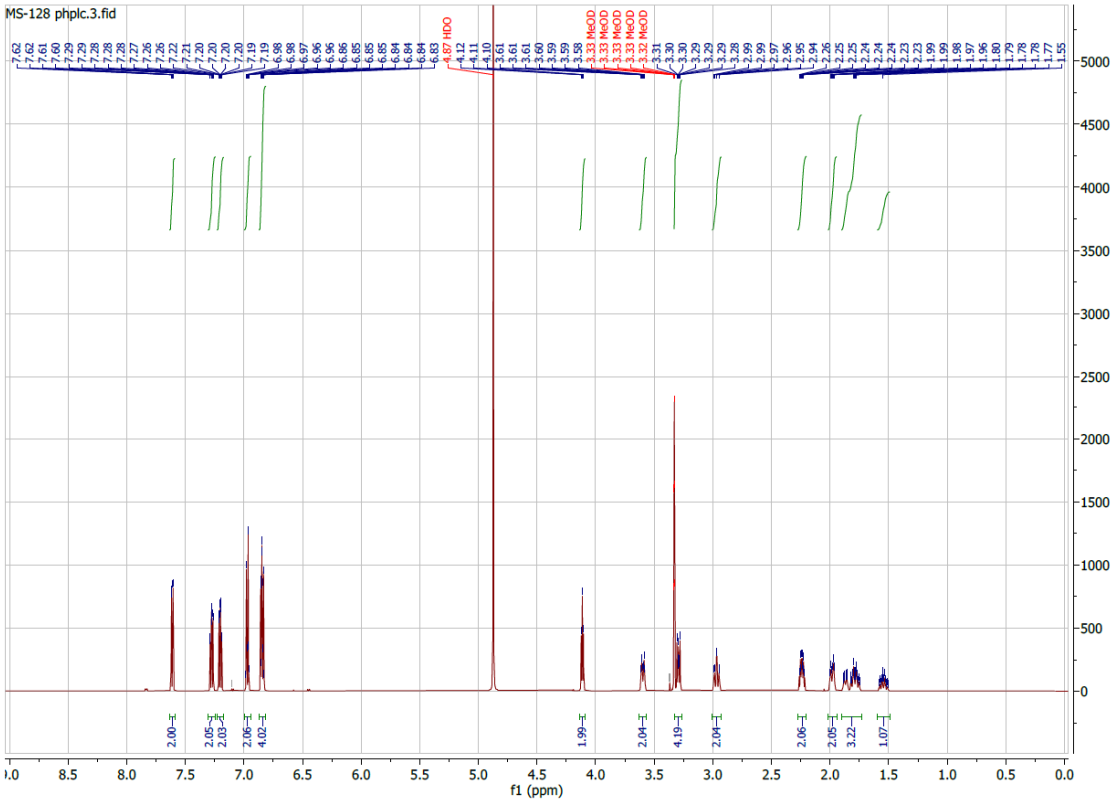


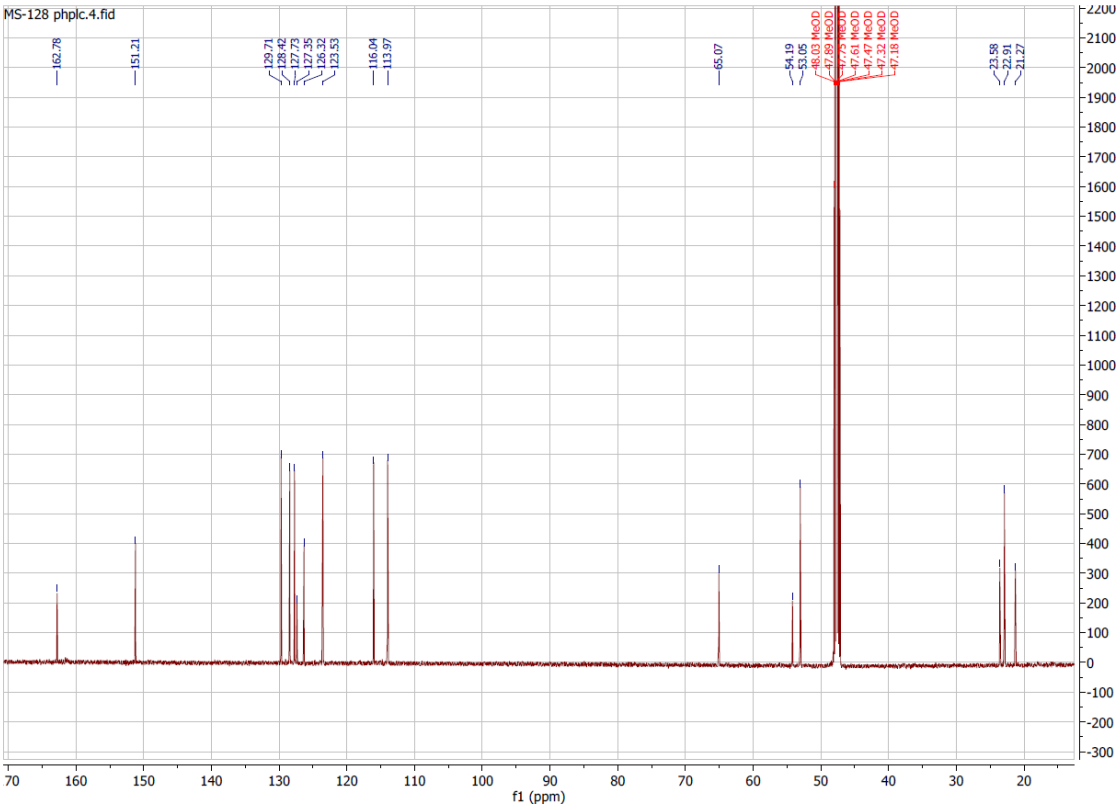
3-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenoxy)-N,N-dimethylpropan-1-amine.TFA (4b)



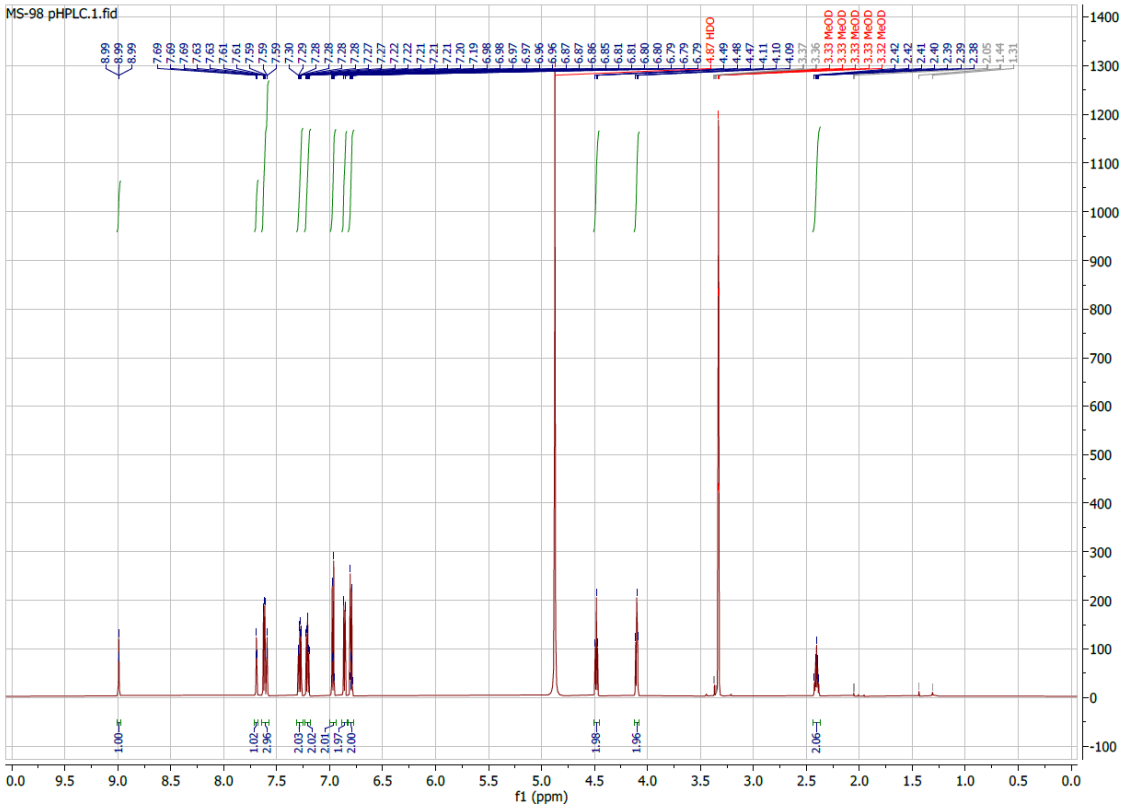


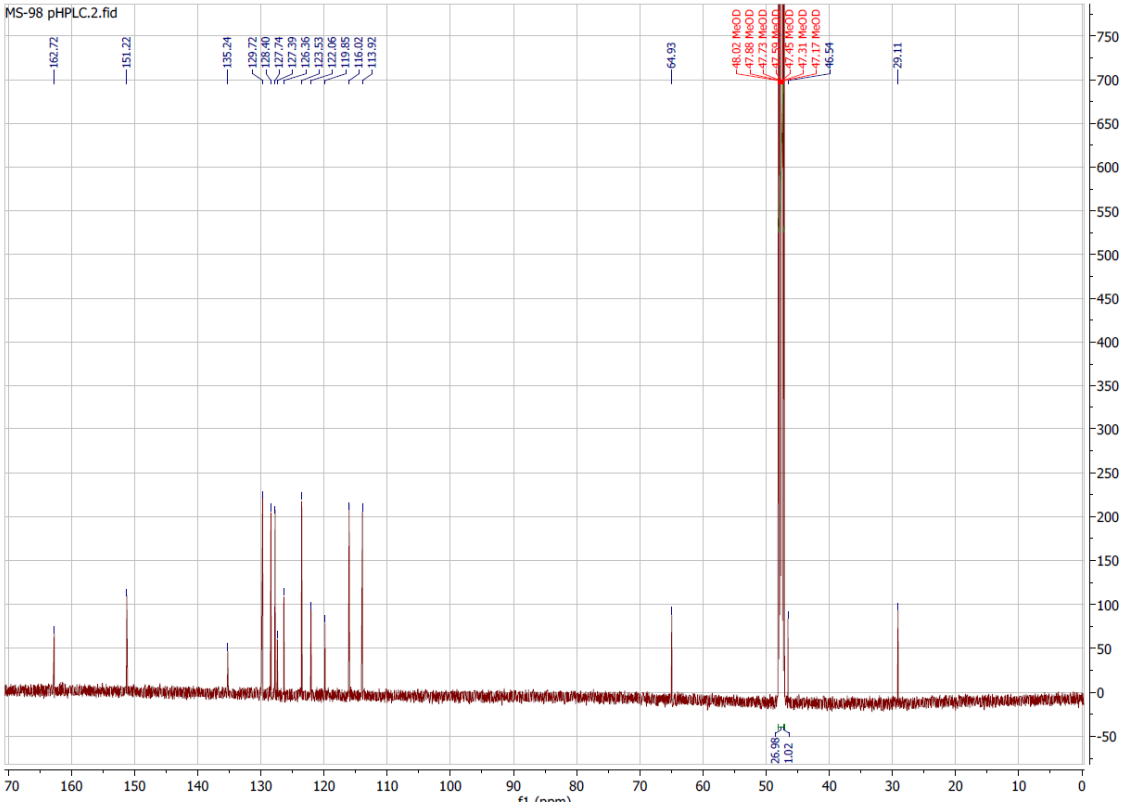
10-((4-(3-(Pyrrolidin-1-yl)propoxy)phenyl)sulfonyl)-10H-phenoxazine.TFA (4c)



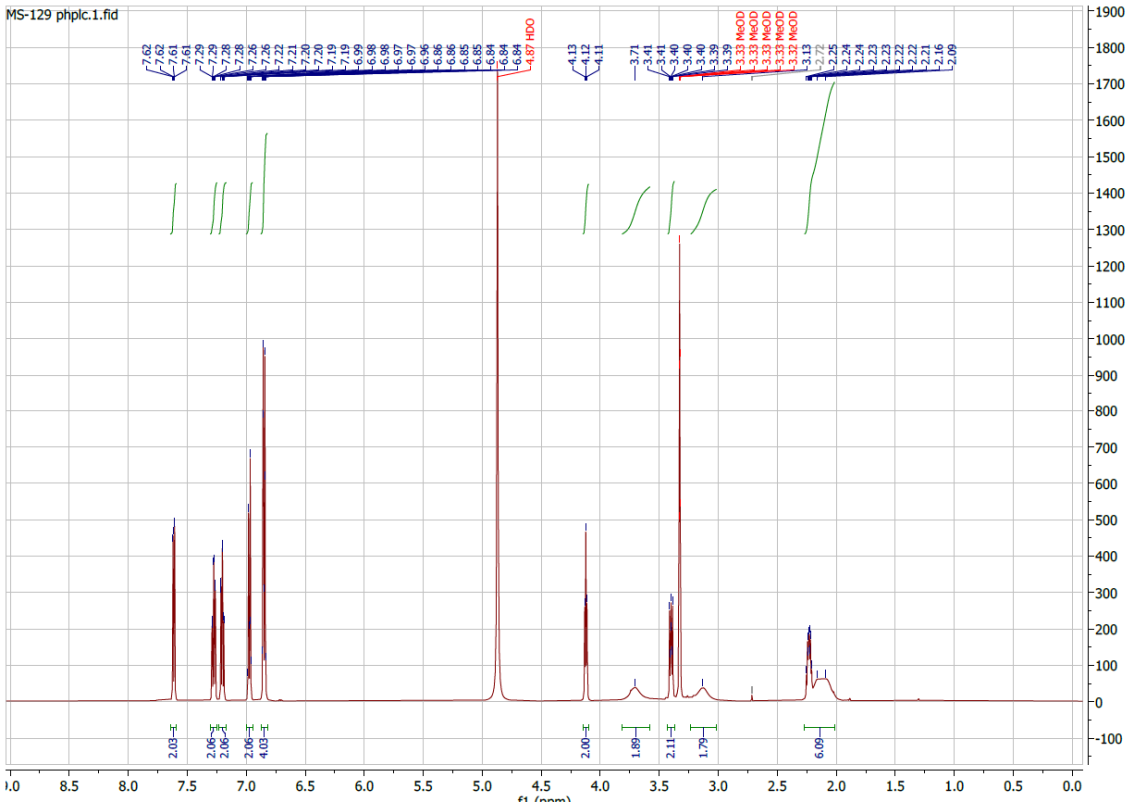


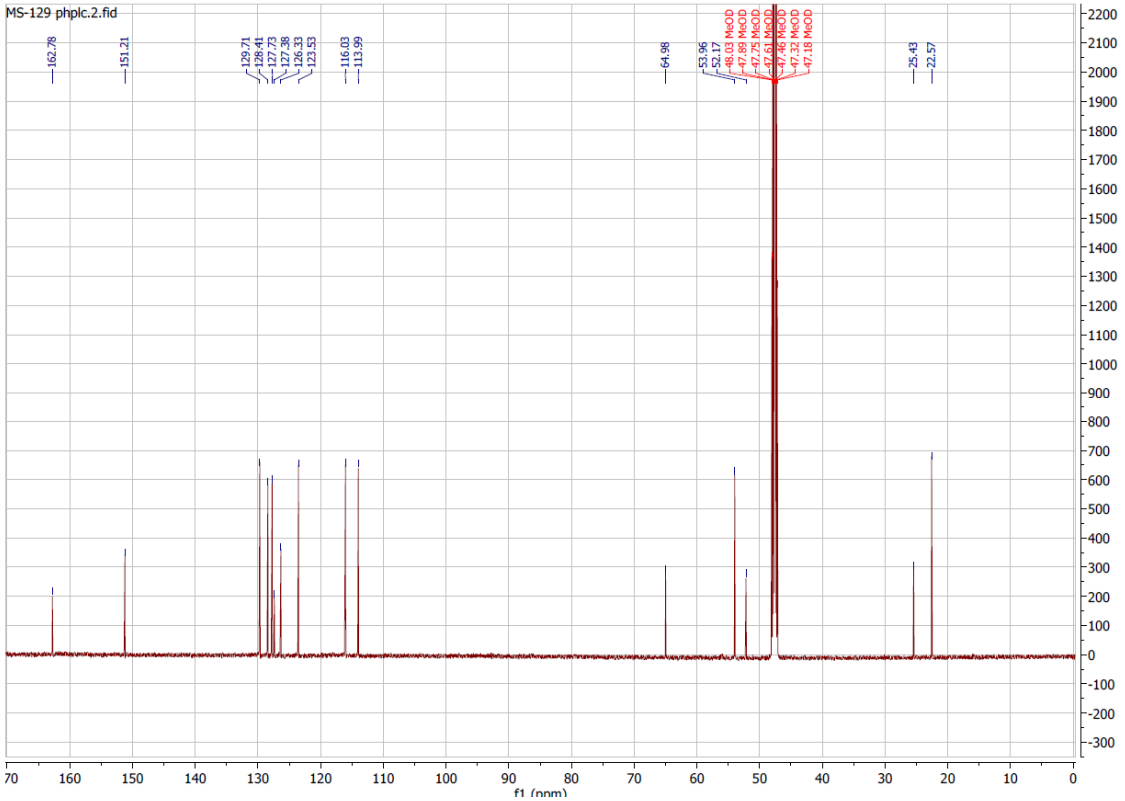
10-((4-(3-(1H-Imidazol-1-yl)propoxy)phenyl)sulfonyl)-10H-phenoxazine.TFA (4d)



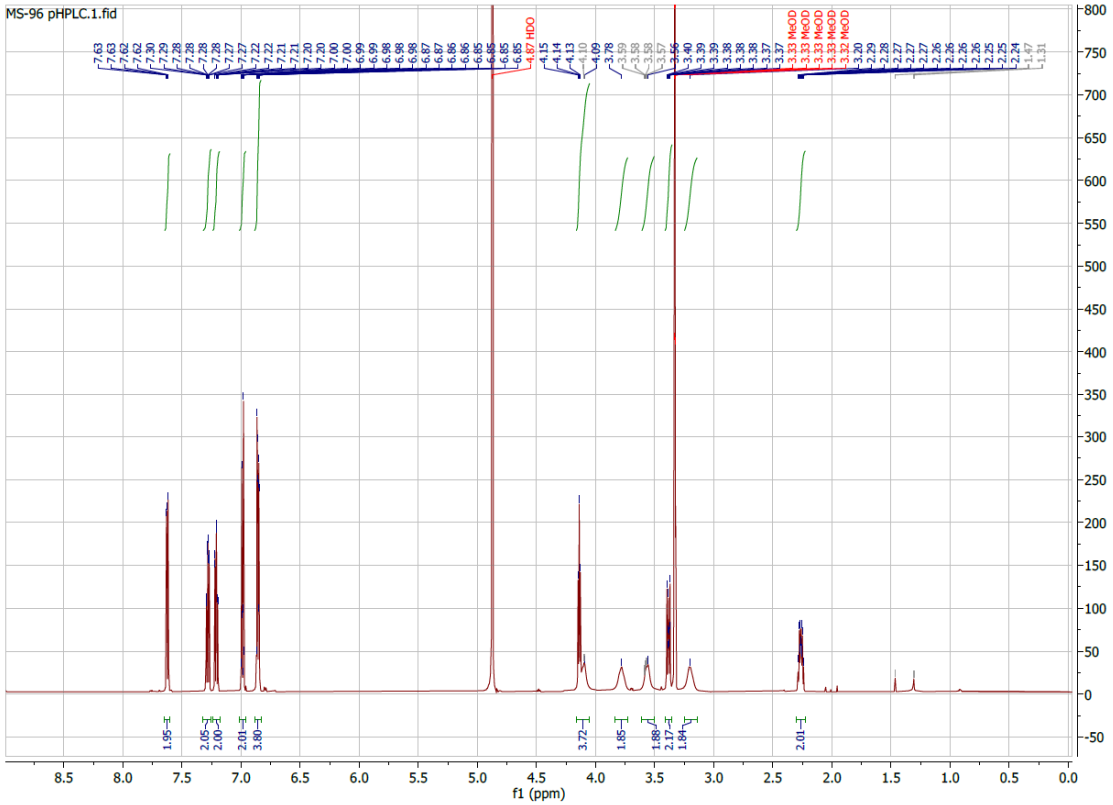


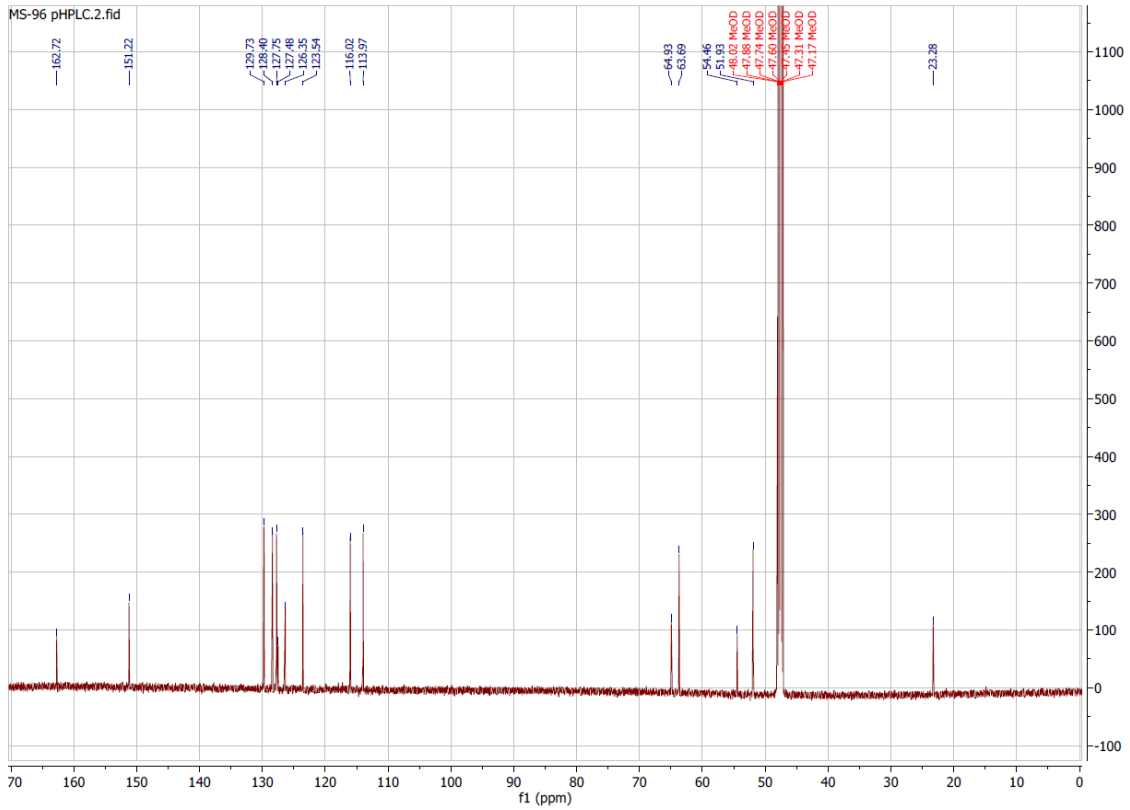
10-((4-(3-(Piperidin-1-yl)propoxy)phenyl)sulfonyl)-10H-phenoxazine.TFA (4e)



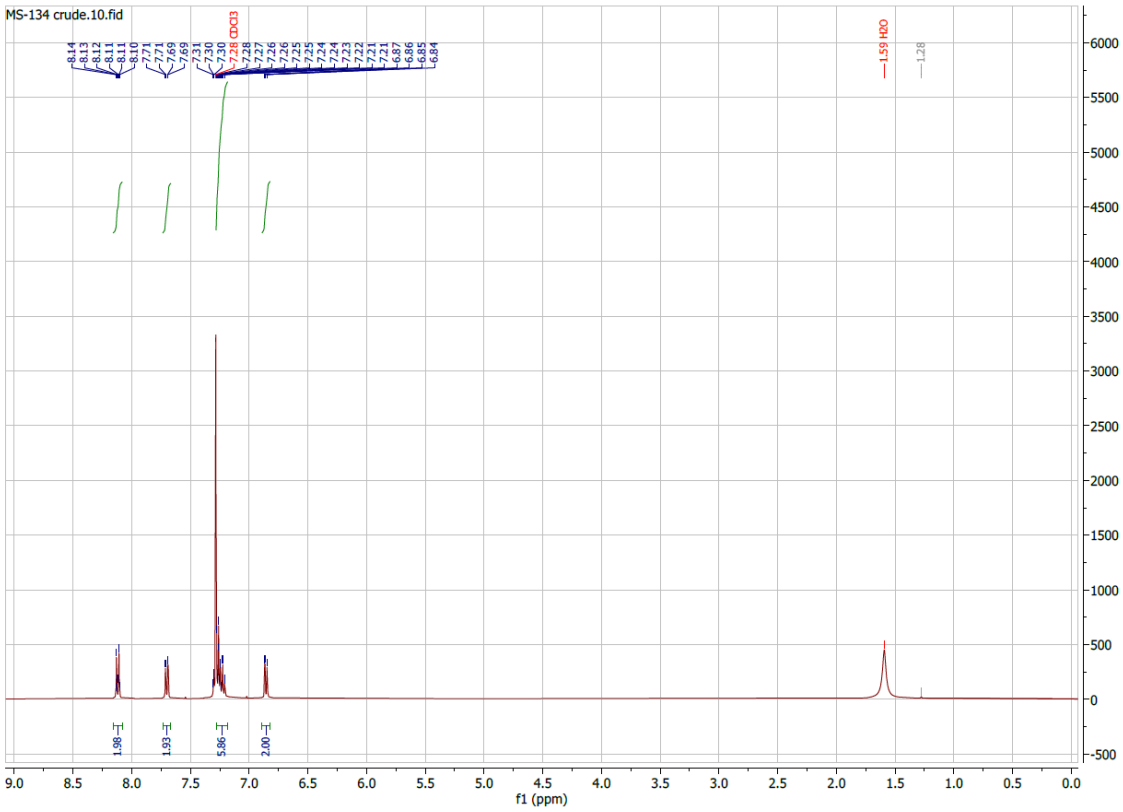


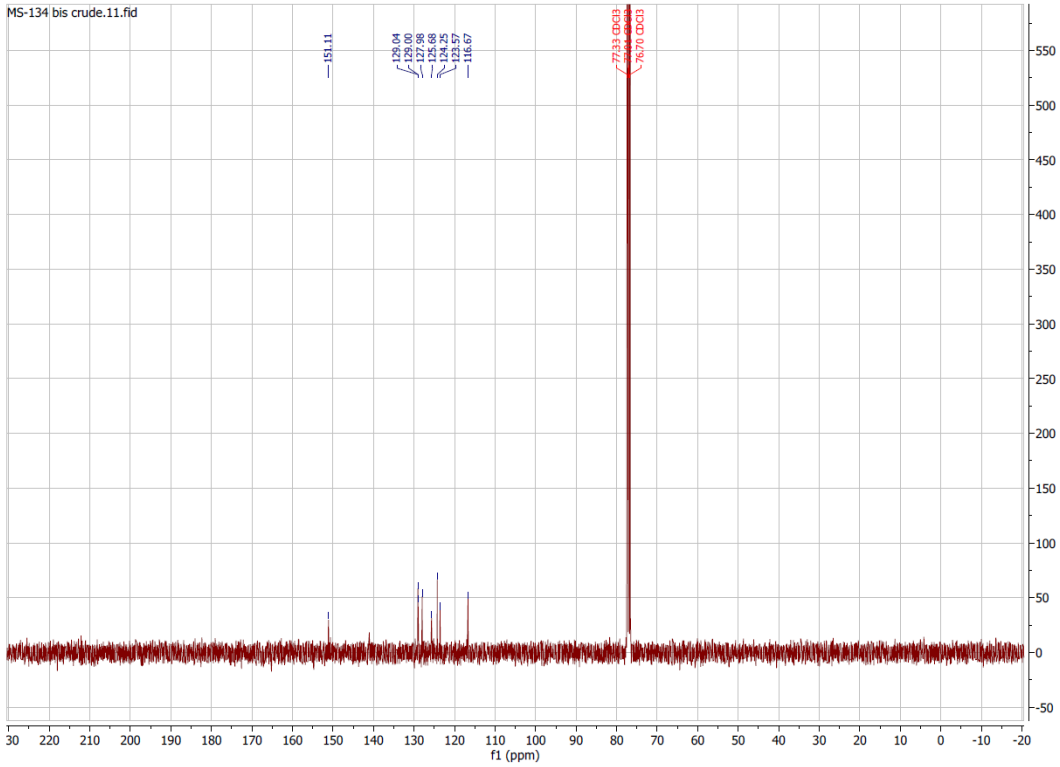
10-((4-(3-Morpholinopropoxy)phenyl)sulfonyl)-10H-phenoxazine.TFA (4f)

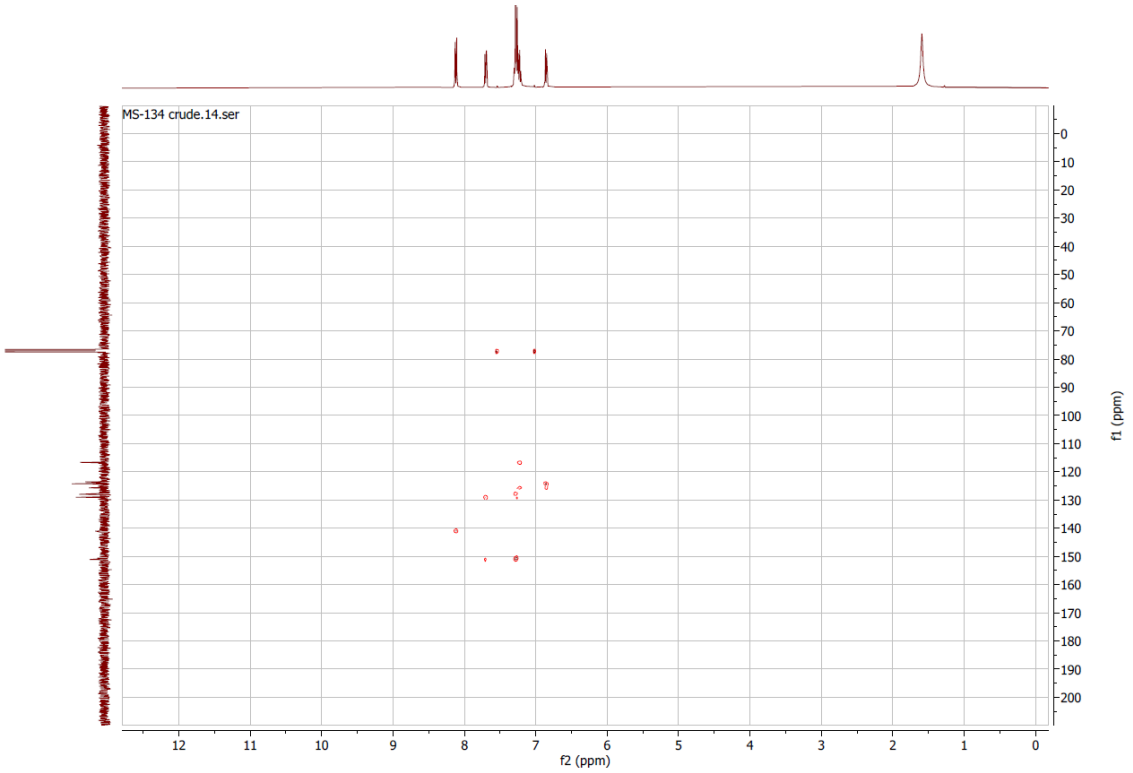




10-((4-Nitrophenyl)sulfonyl)-10H-phenoxazine (6)



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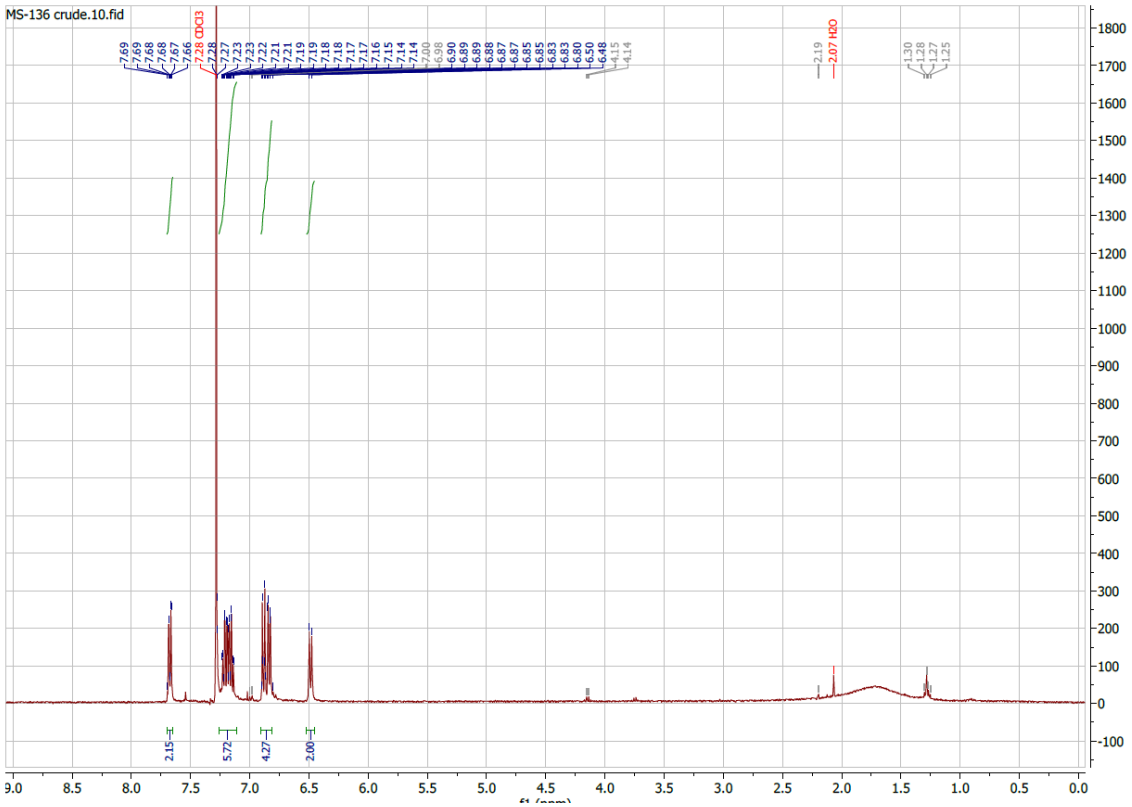
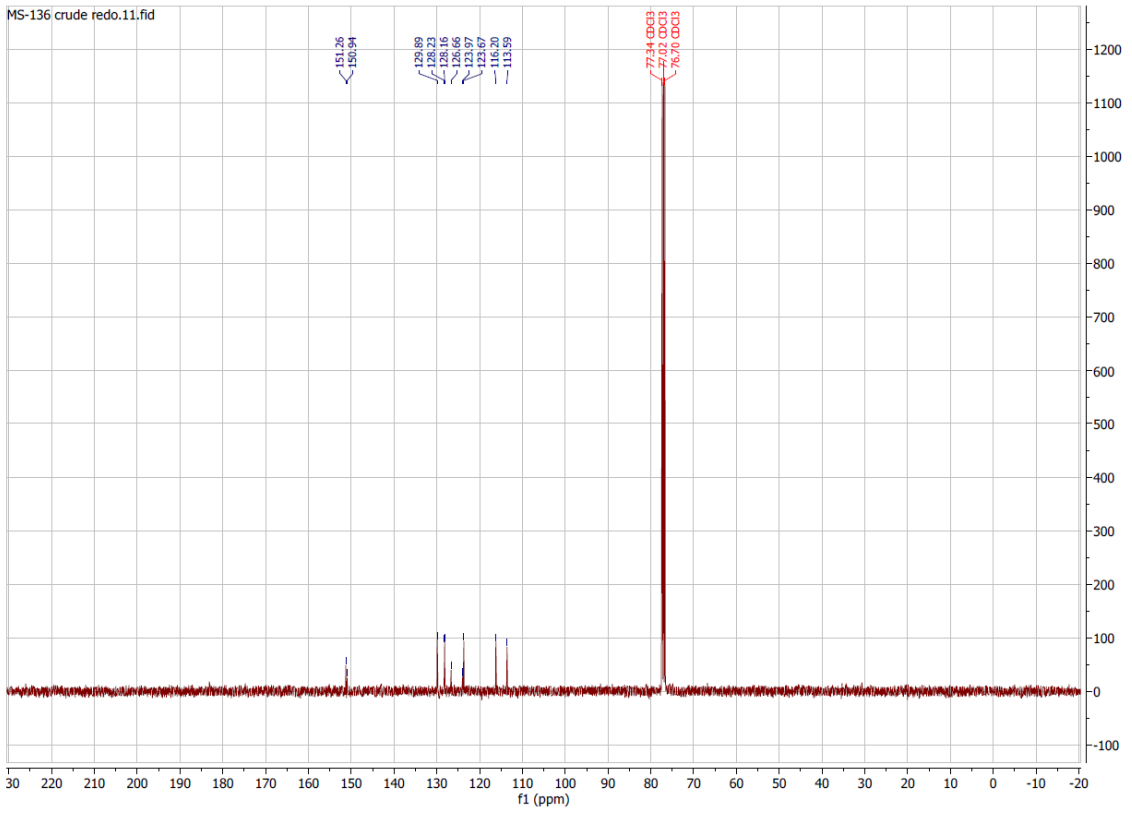
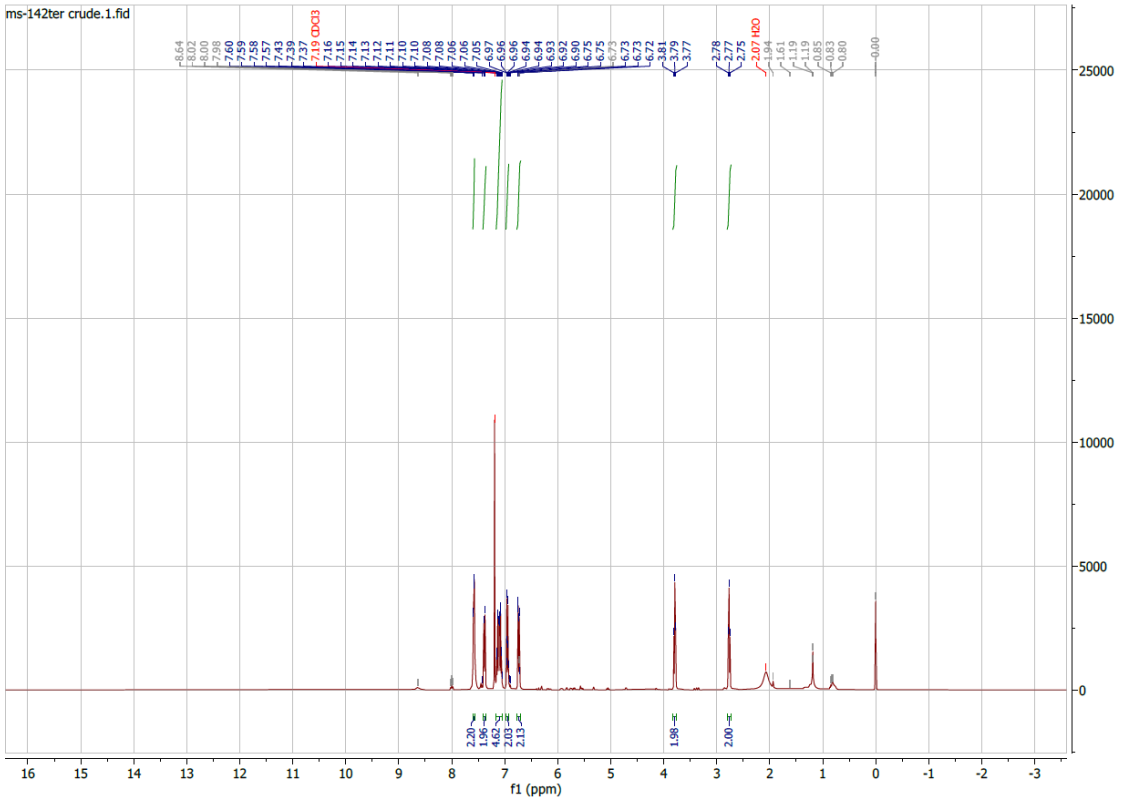
f2 (ppm)
f1 (ppm)

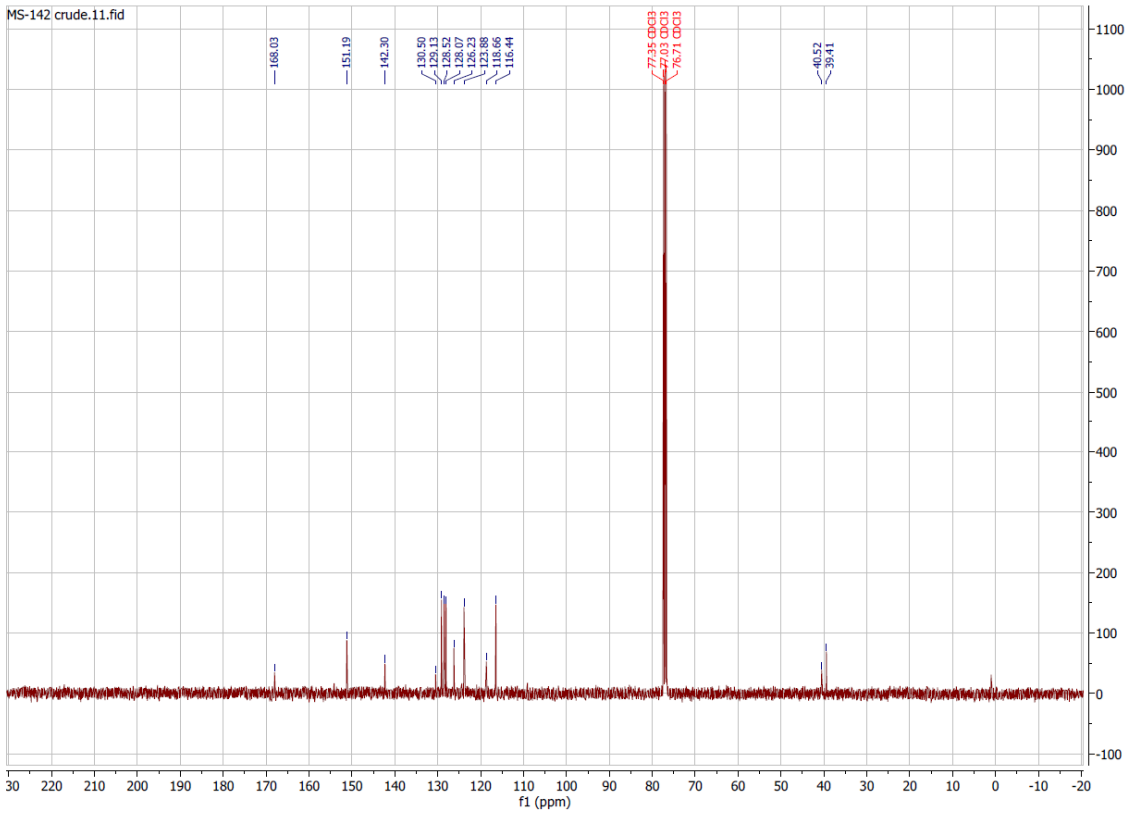
4-((10H-Phenoxazin-10-yl)sulfonyl)aniline (7)



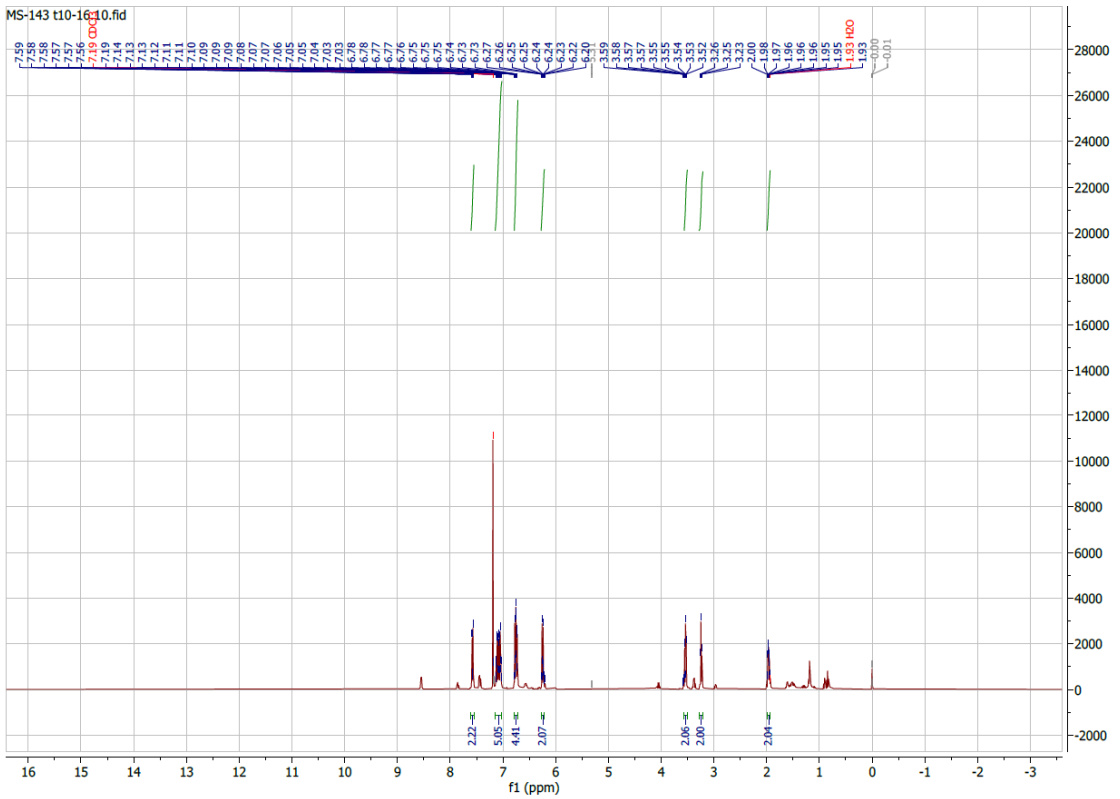


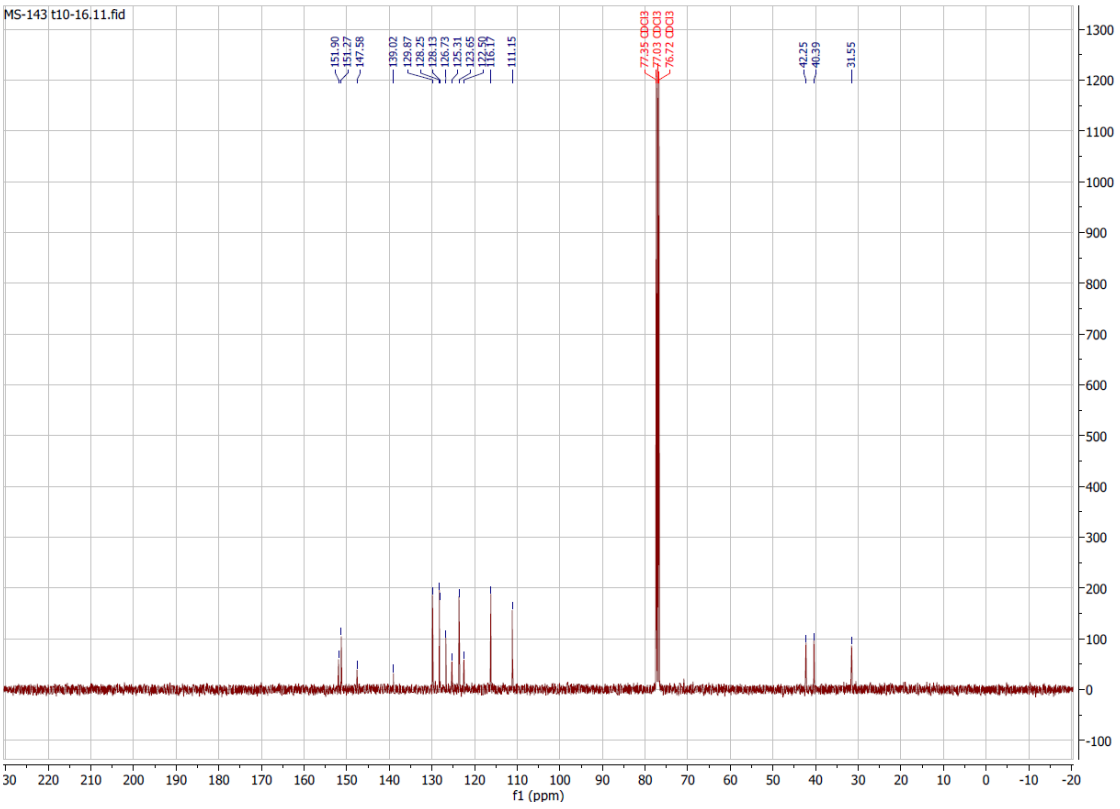
N-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenyl)-3-chloropropanamide (8)



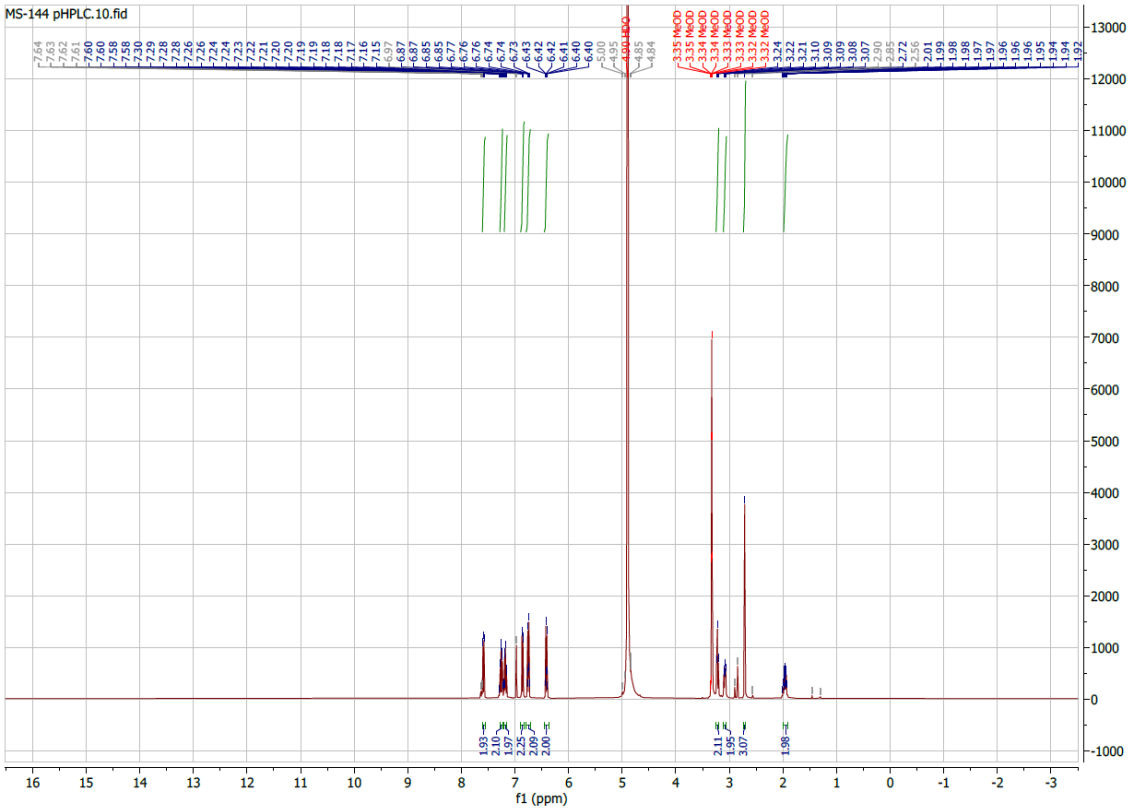


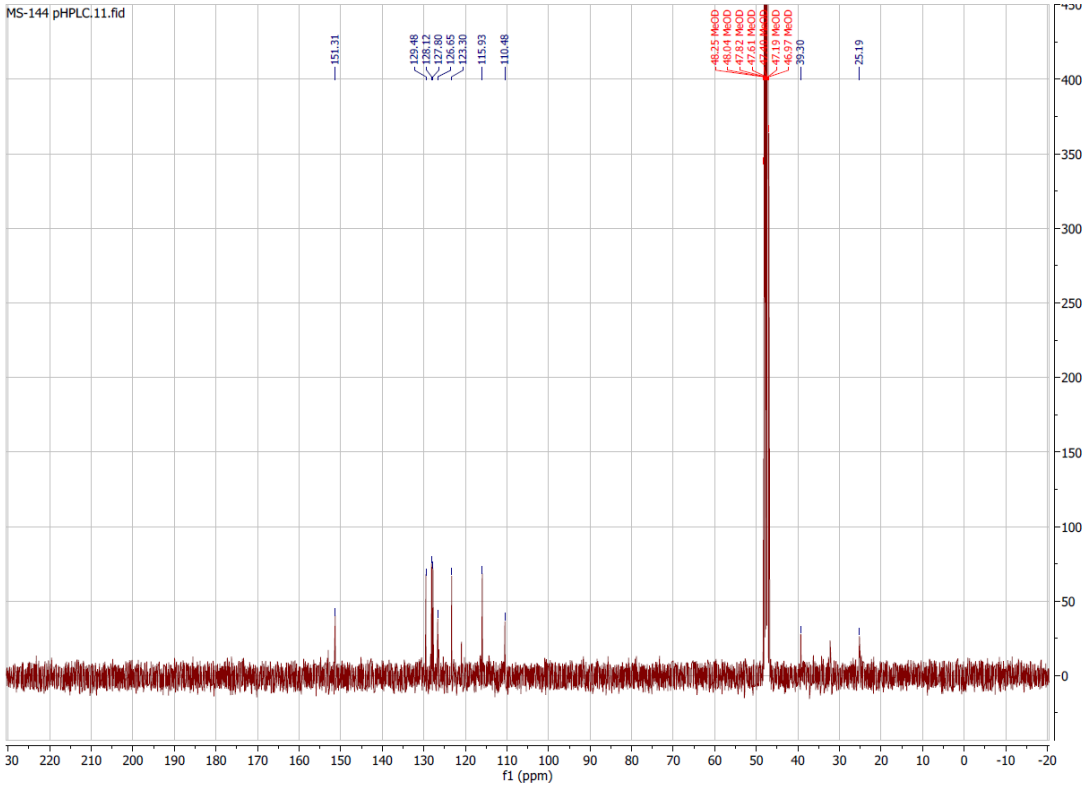
4-((10H-Phenoxazin-10-yl)sulfonyl)-N-(3-chloropropyl)aniline (9)

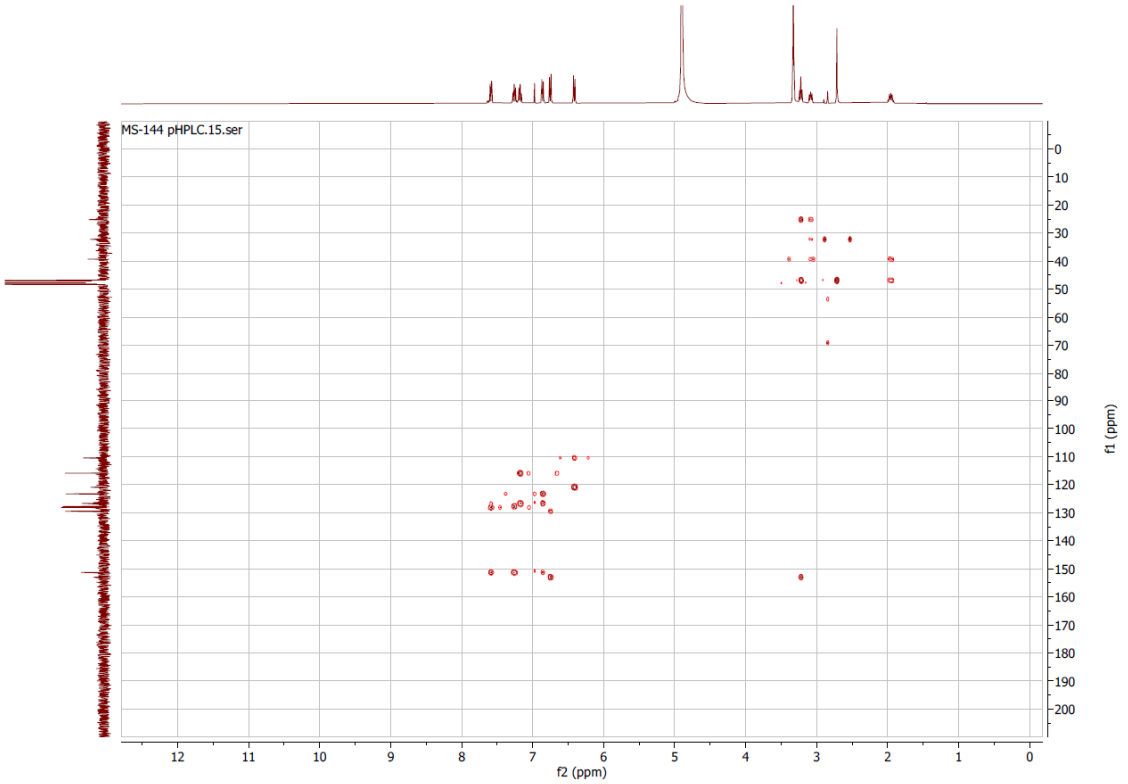




N^1-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenyl)-N^3-methylpropane-1,3-diamine.2TFA (10a)



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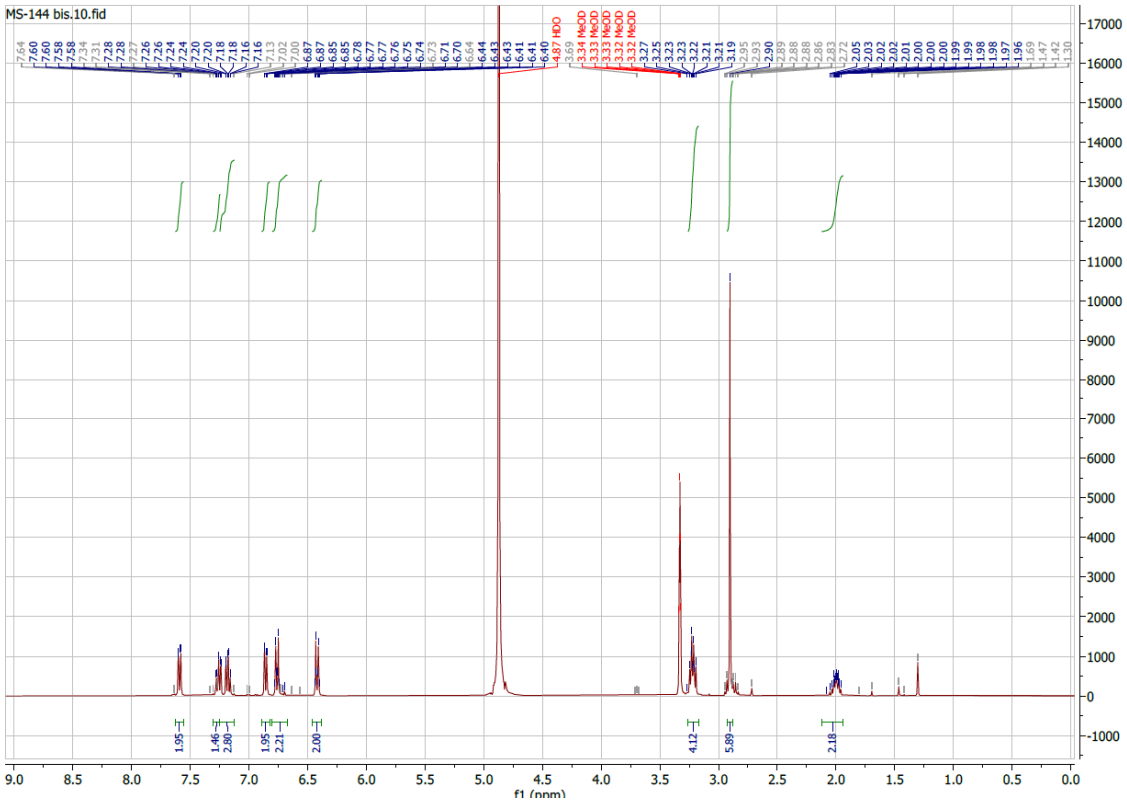
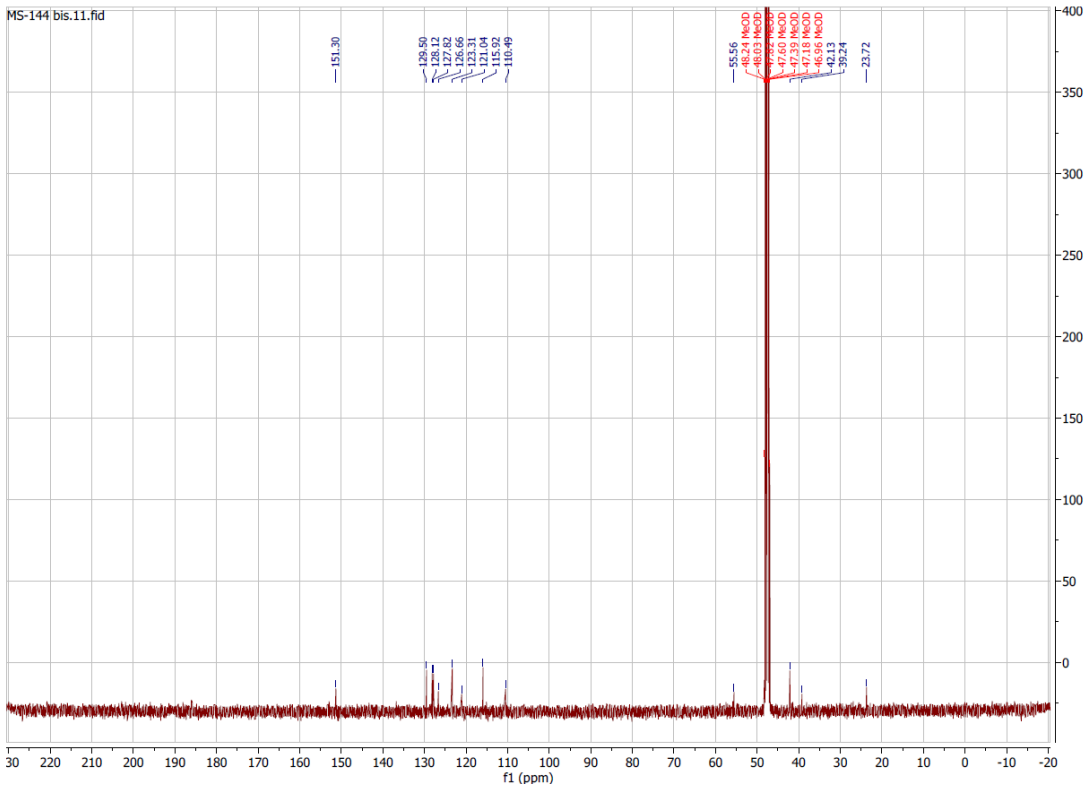
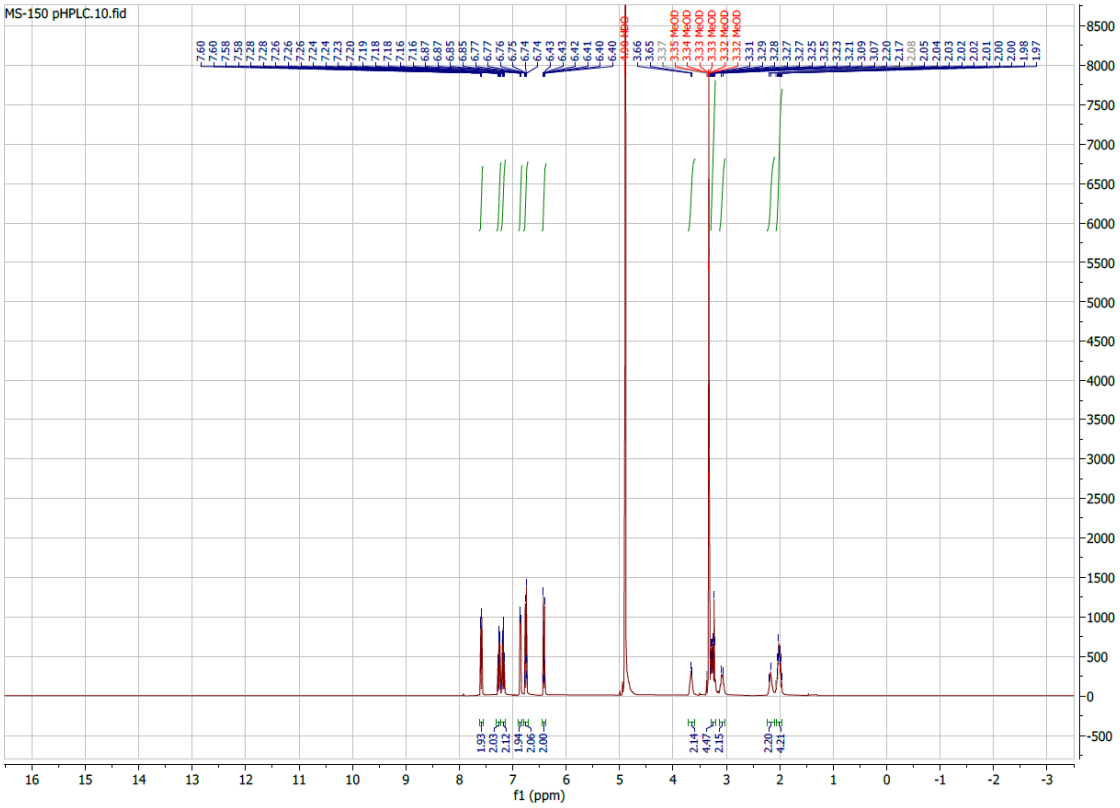
f2 (ppm)
f1 (ppm)

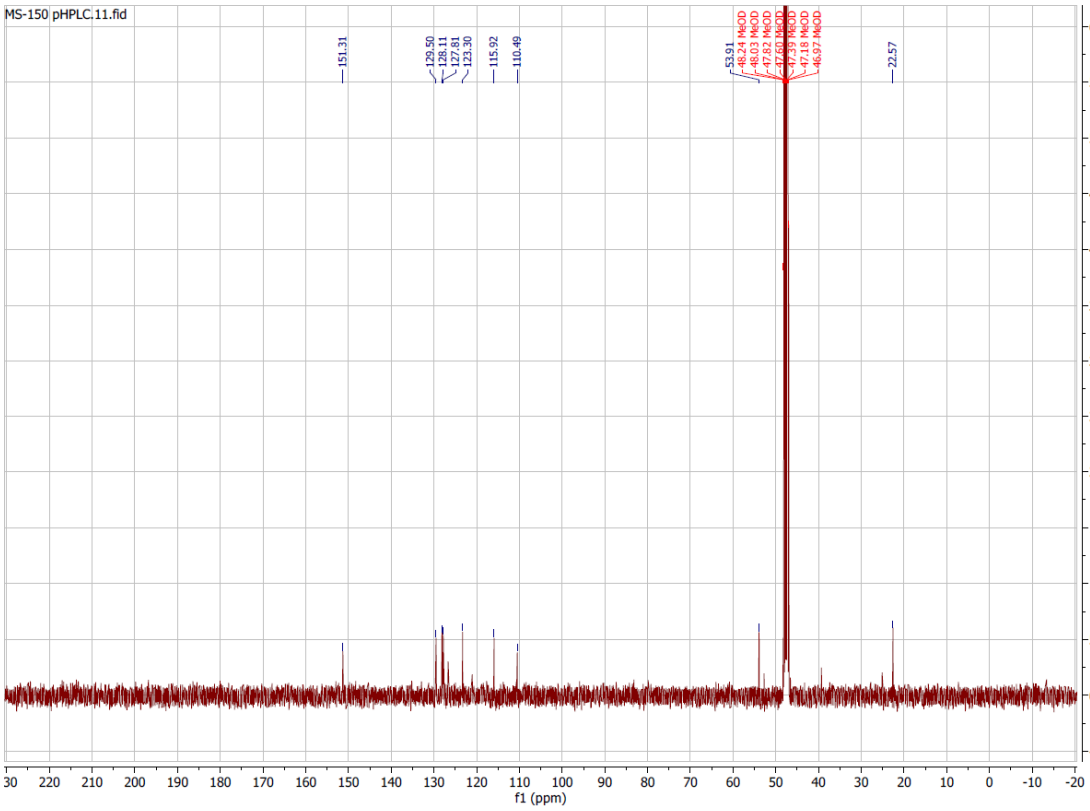
N^1-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenyl)-N^3,N^3-dimethylpropane-1,3-diamine.2TFA (10b)

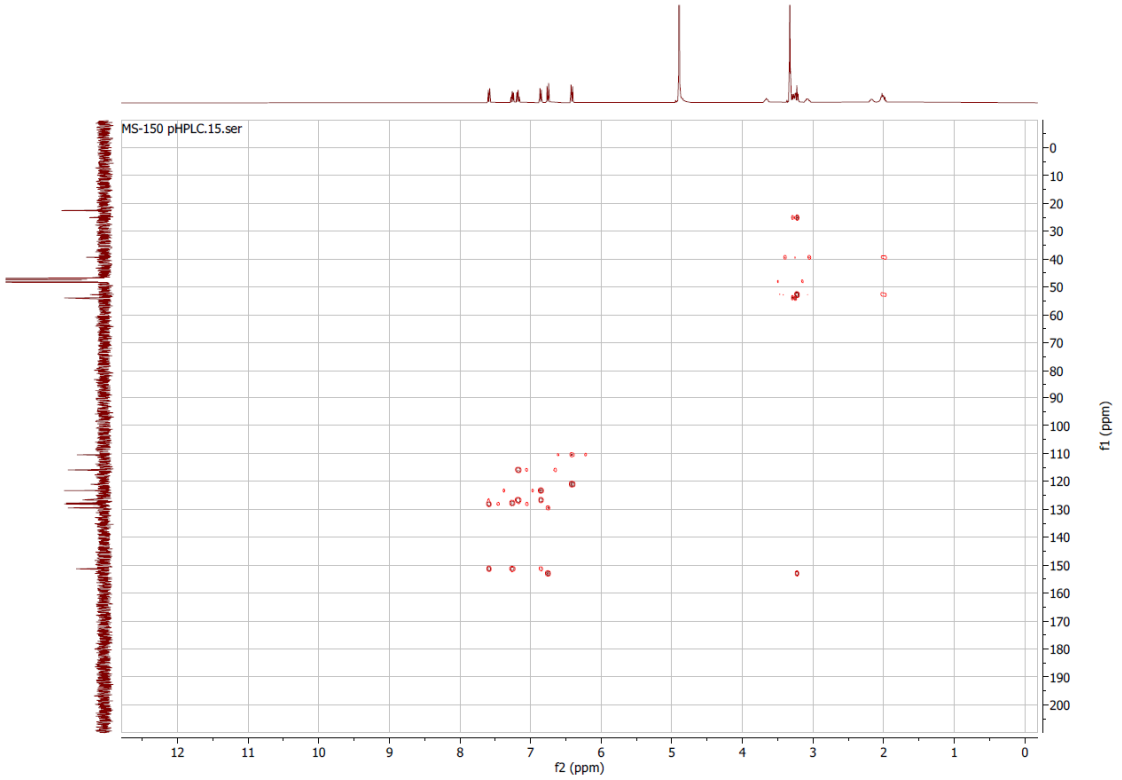




4-((10H-Phenoxazin-10-yl)sulfonyl)-*N*-(3-(pyrrolidin-1-yl)propyl)aniline.2TFA (10c)



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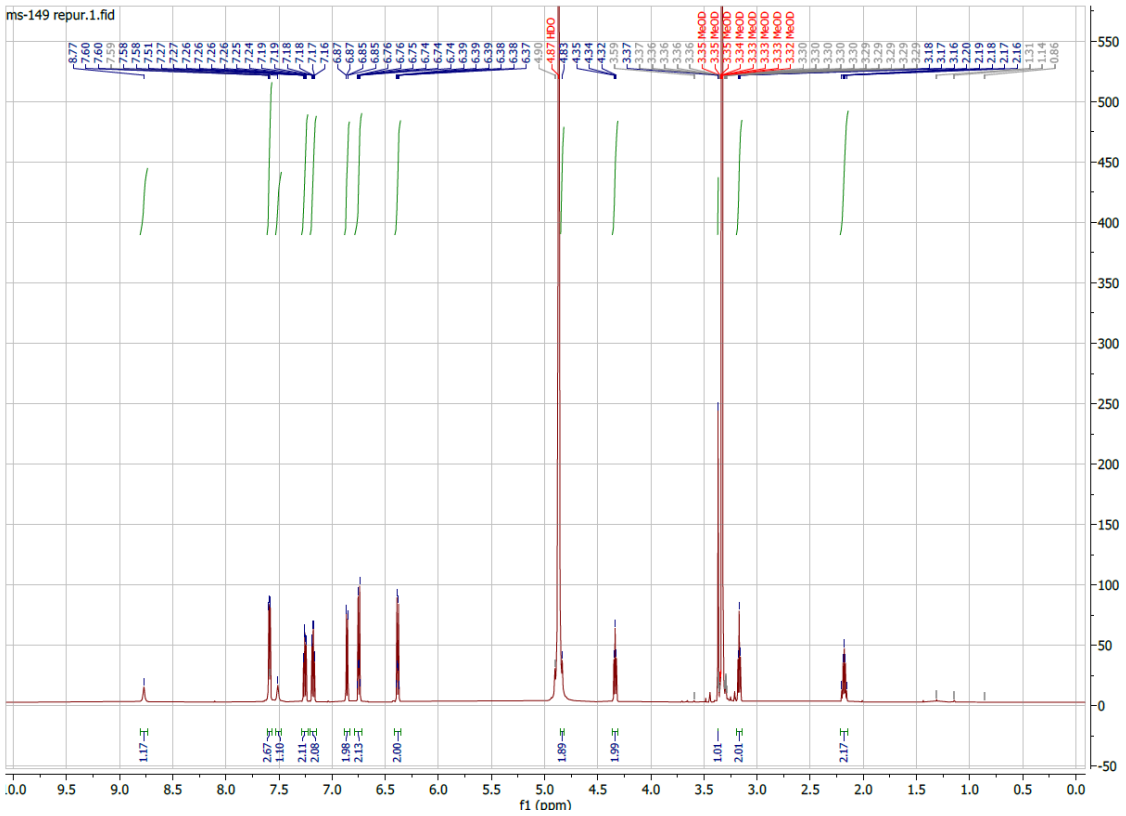
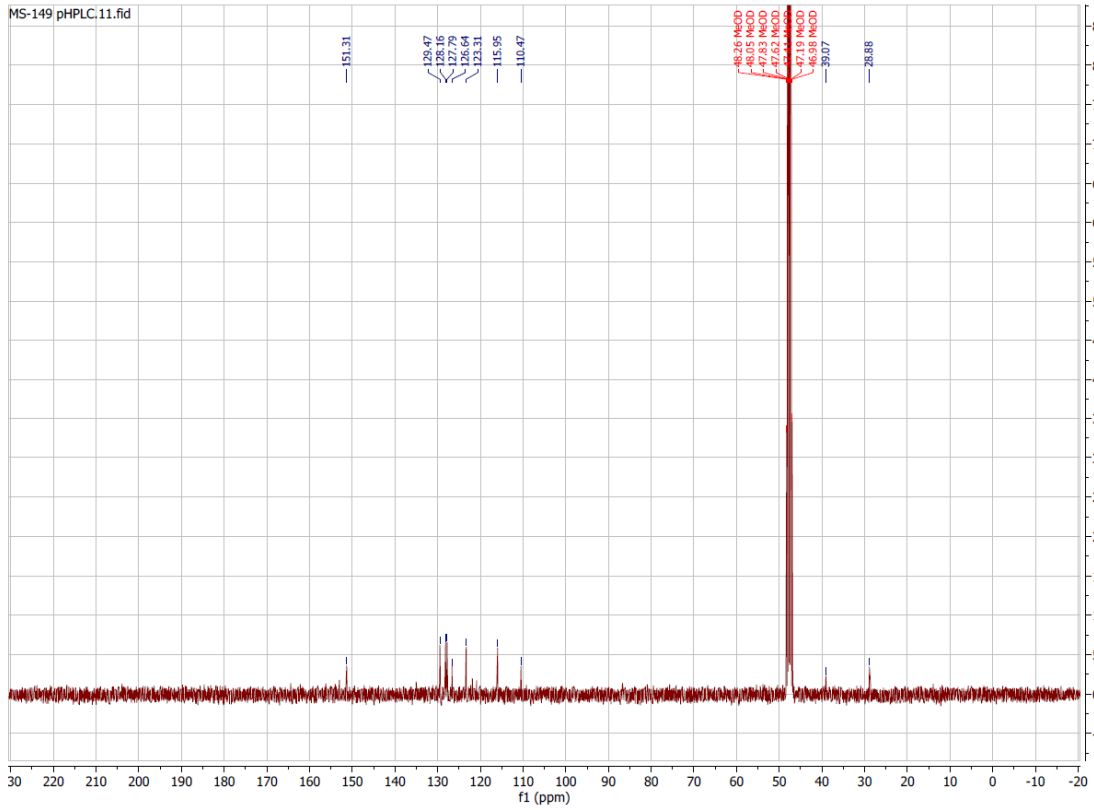
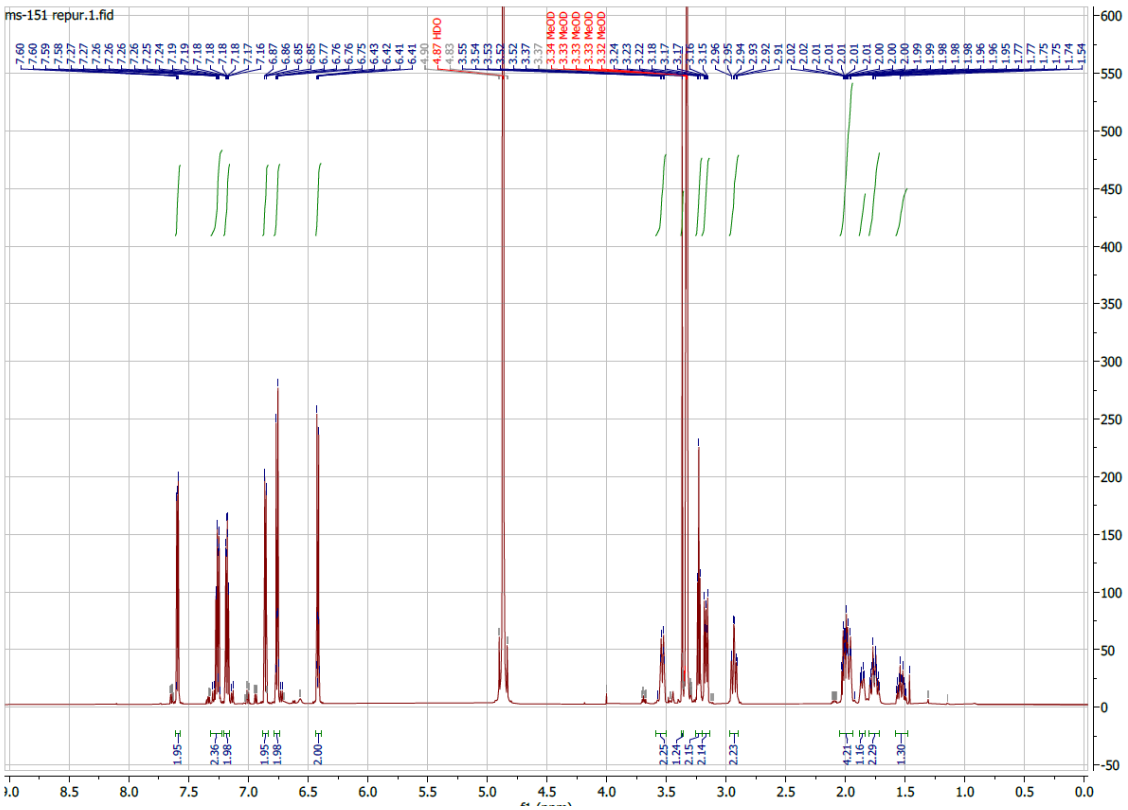
f1 (ppm)

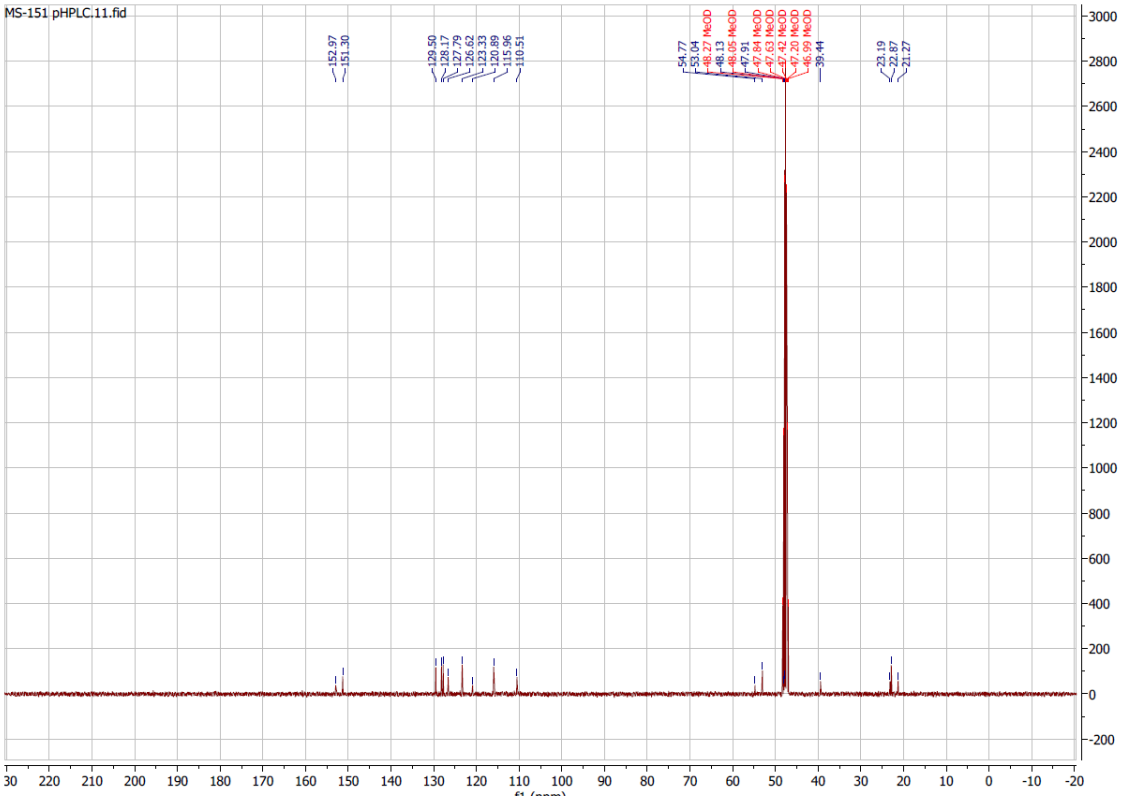
4-((10H-Phenoxazin-10-yl)sulfonyl)-*N*-(3-(1H-imidazol-1-yl)propyl)aniline.2TFA (10d)



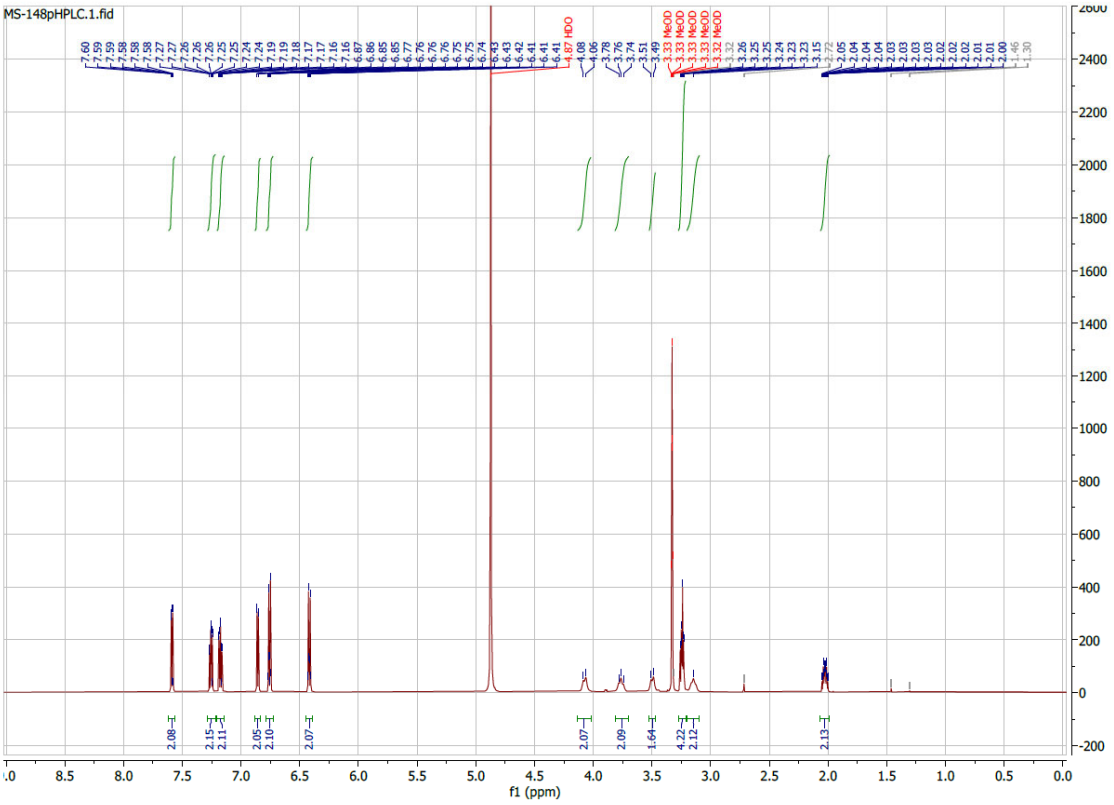


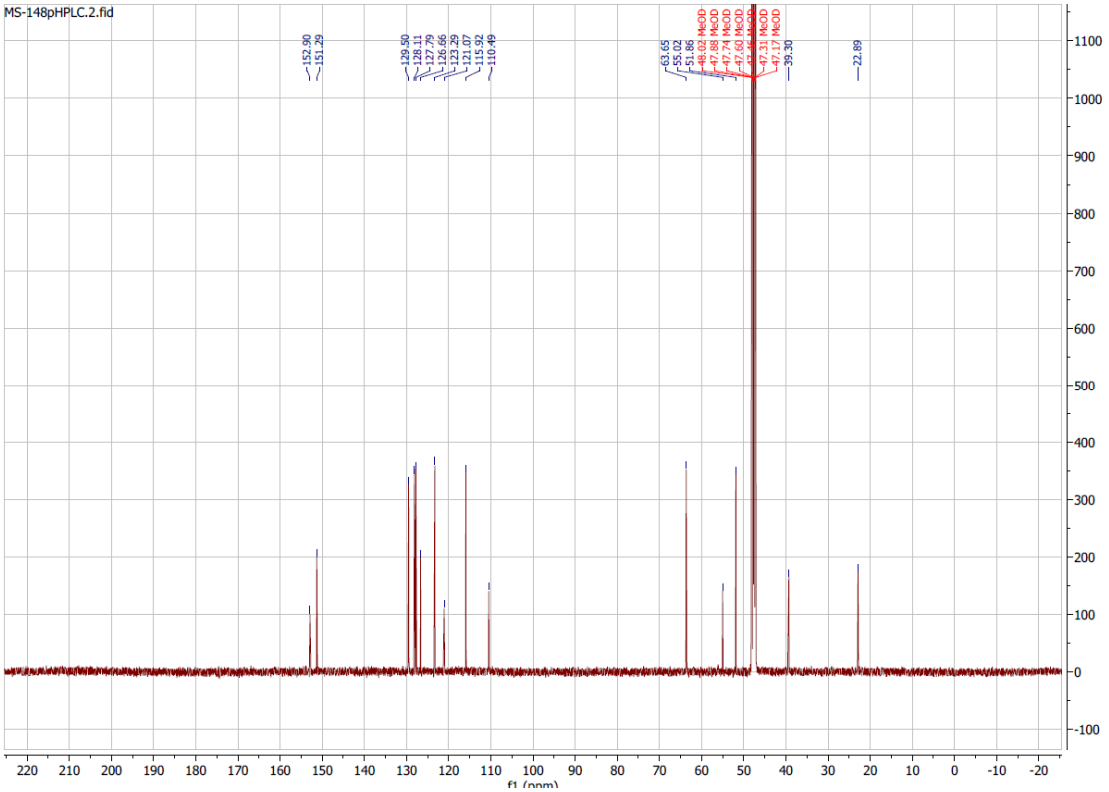
4-((10H-Phenoxazin-10-yl)sulfonyl)-*N*-(3-(piperidin-1-yl)propyl)aniline.2TFA (10e)



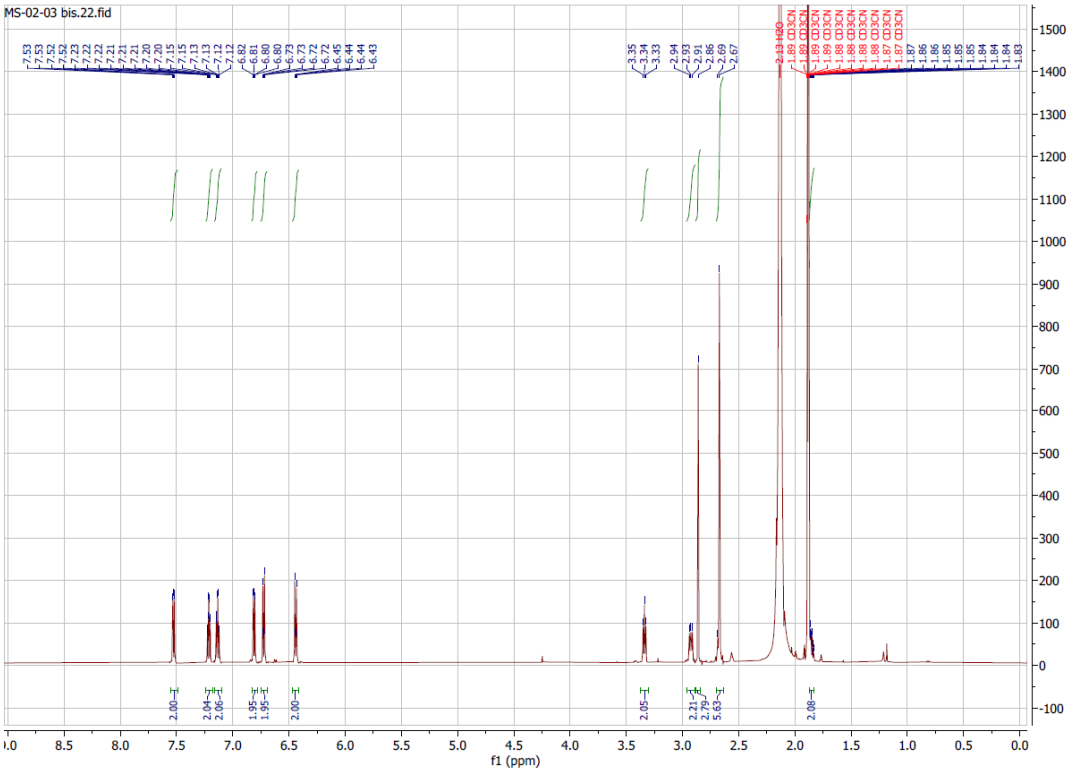


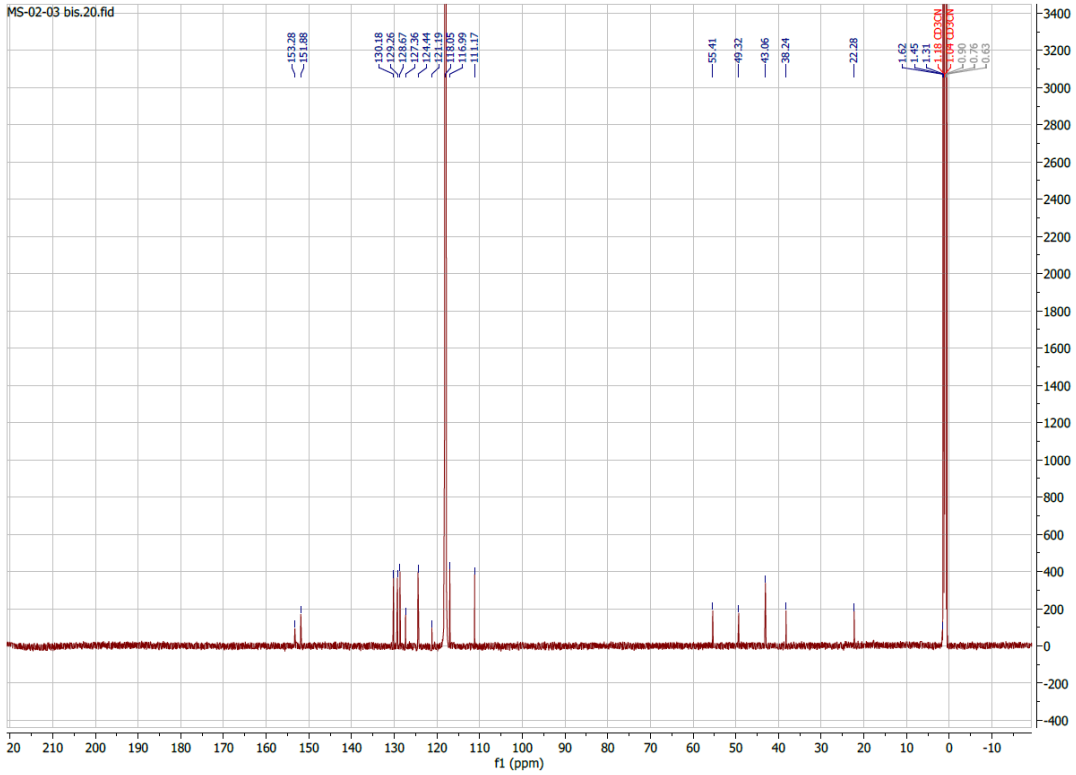
4-((10H-Phenoxazin-10-yl)sulfonyl)-*N*-(3-morpholinopropyl)aniline.2TFA (10f)



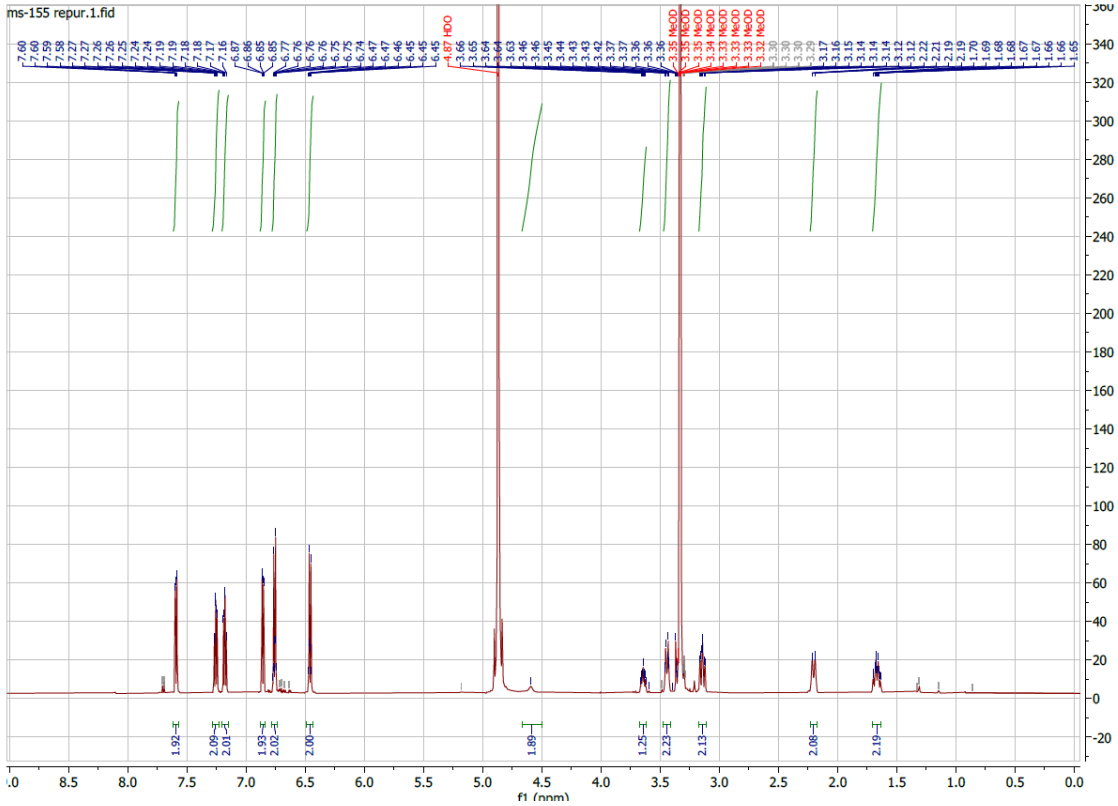


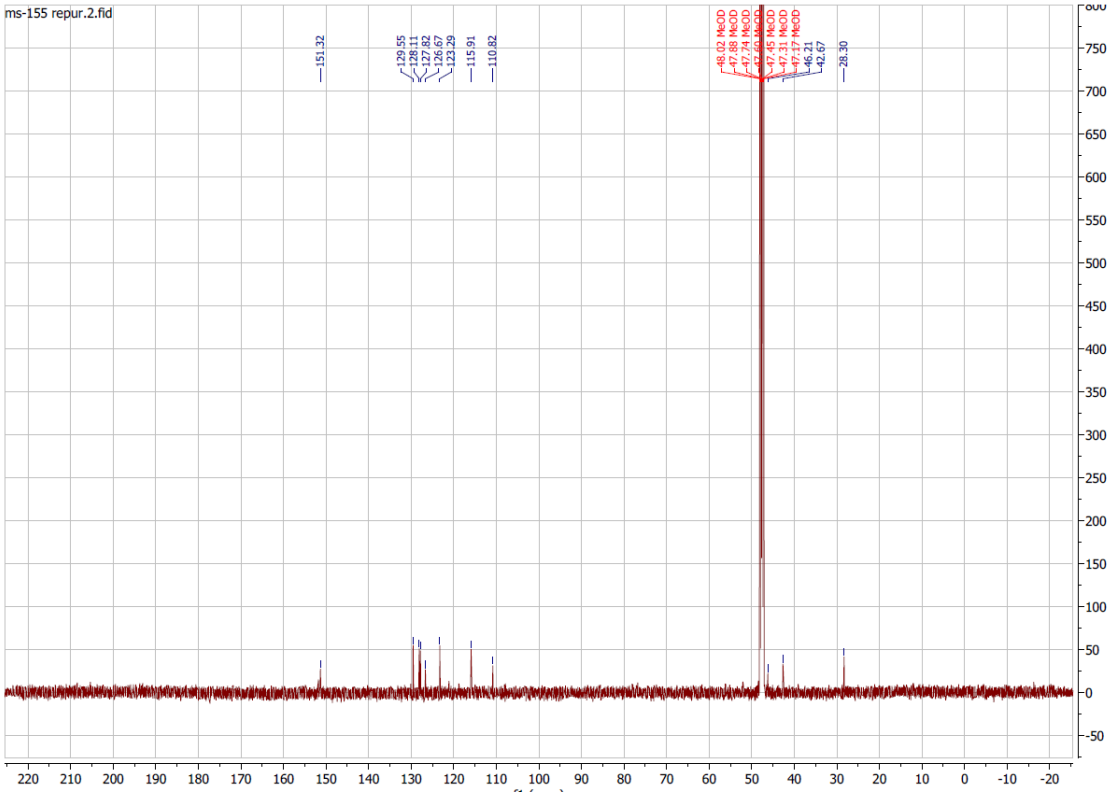
N^1-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenyl)-N^1,N^3,N^3-trimethylpropane-1,3-diamine.2TFA (12)



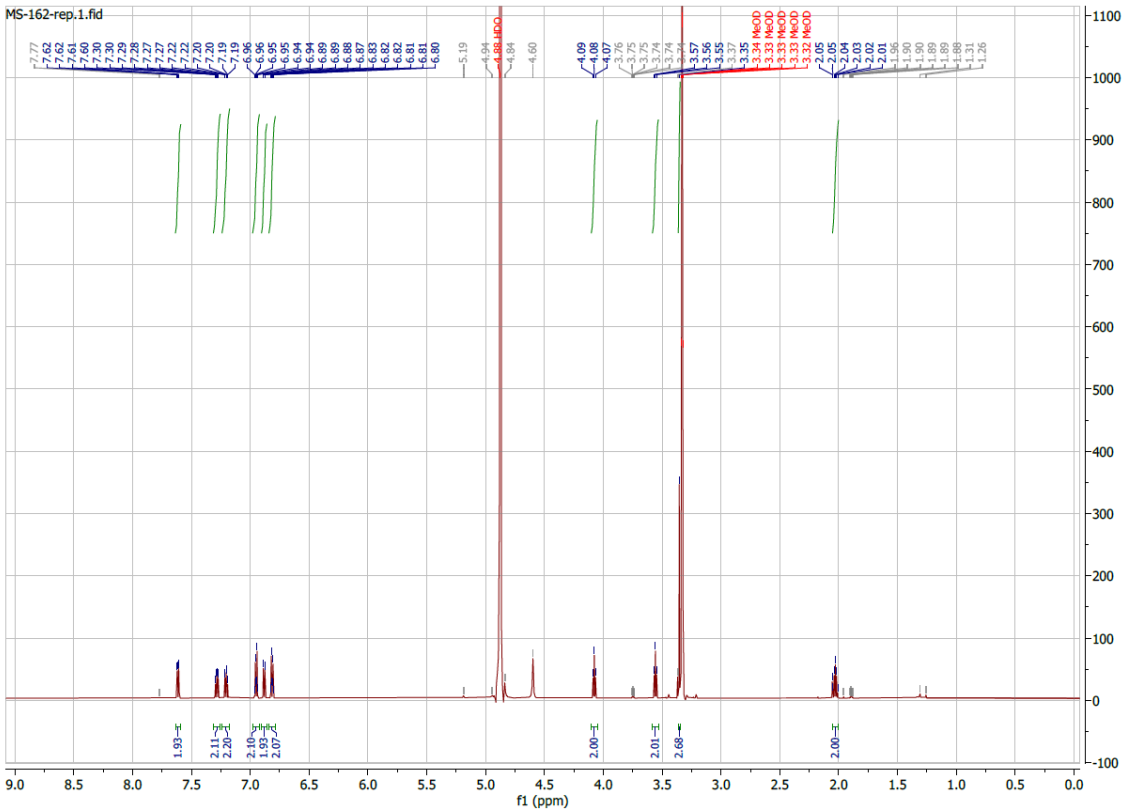


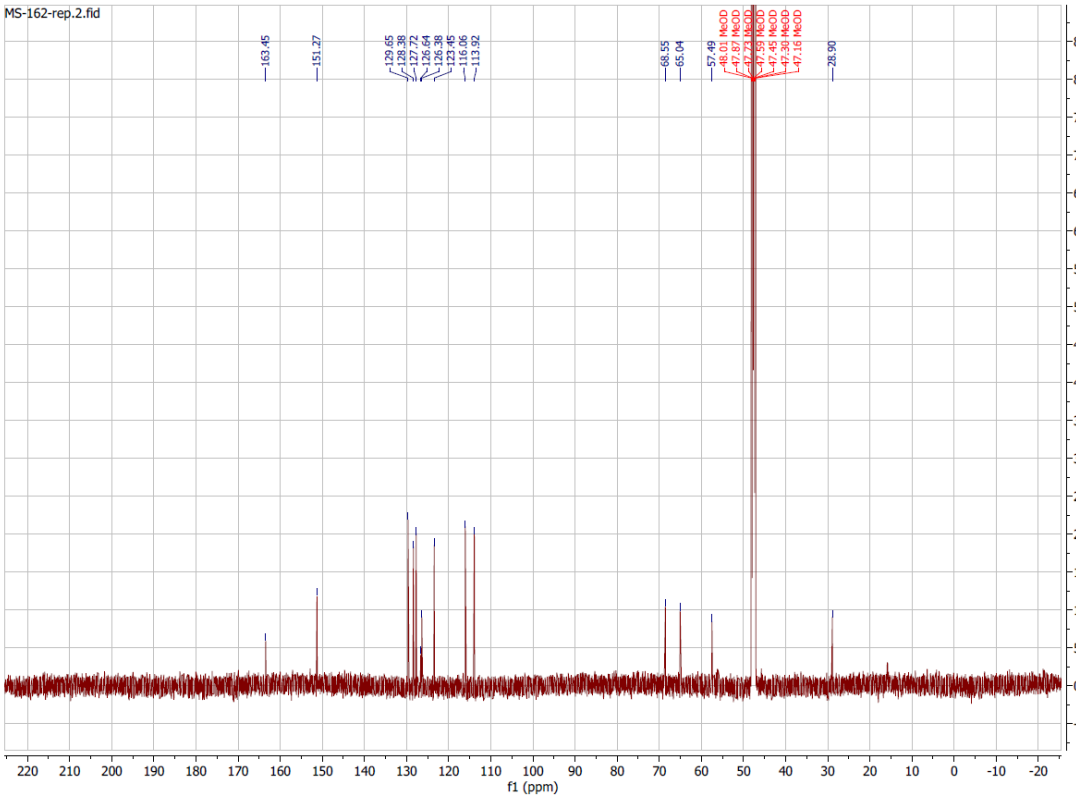
***N*-(4-((10H-Phenoxazin-10-yl)sulfonyl)phenyl)piperidin-4-amine.2TFA (14)**





10-((4-(3-Methoxypropoxy)phenyl)sulfonyl)-10H-phenoxazine (15)





Molecular modelling figure

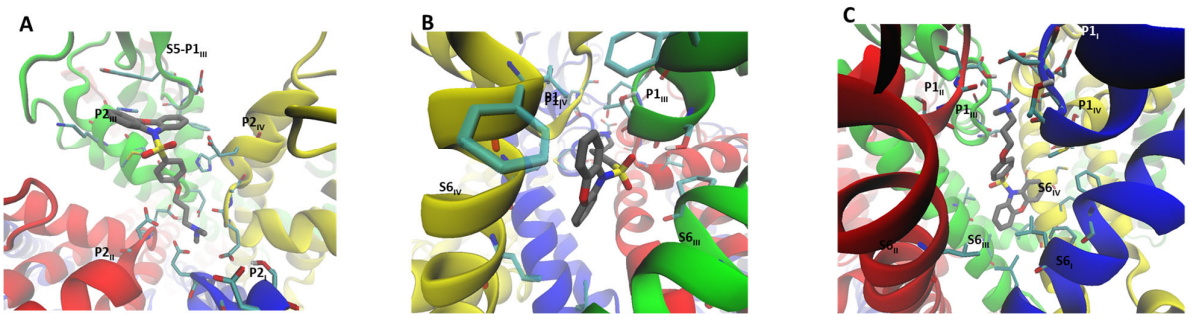


Figure S1. 3D ligand interaction diagrams of protonated **4b** in docking site 1 (**A**), docking site 2 (**B**), and docking site 3 (**C**).

Plasma stability studies

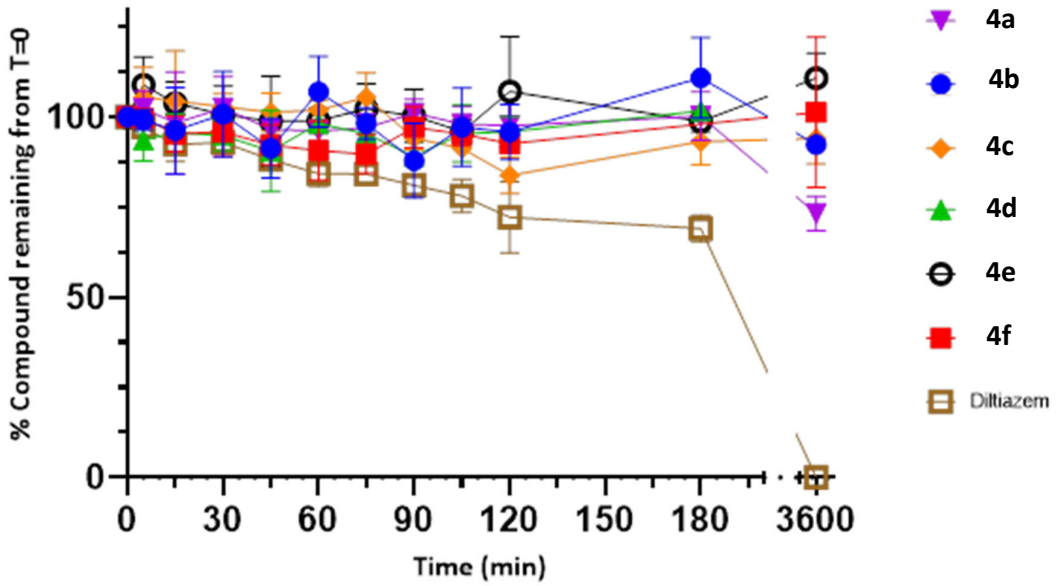


Figure S2. Percentage of compound remaining from t=0 (initial concentration: 150 µM, data averaged over n=3, ±SD) over the course of the rat plasma stability assay for each novel phenoxazine-benzosulfonamide compound, along with diltiazem (positive control).

Liver microsomal stability studies

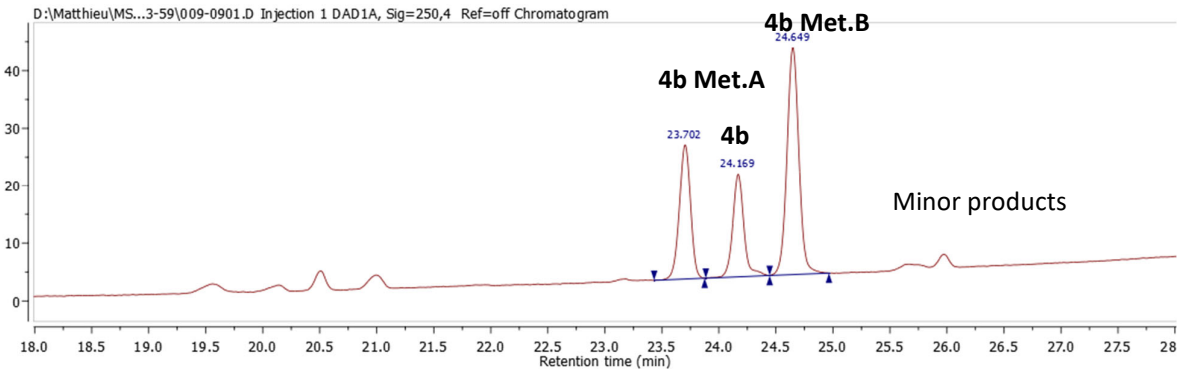


Figure S3. Analytical HPLC chromatogram of the liver microsome stability assay of compound **4b** taken at t = 60 min. Metabolite **4b-B** was consistent with the demethylated product Metabolite **4b-A** was consistent with an hydroxylated metabolite.

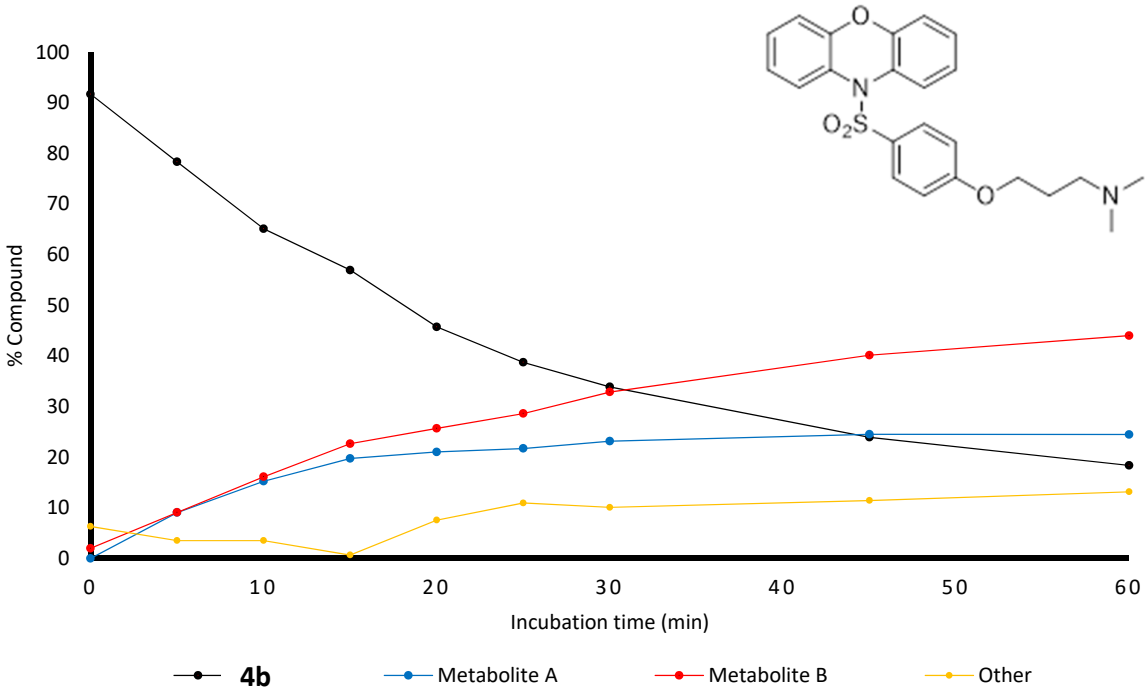


Figure S4. Liver microsome stability assay of **4b**.

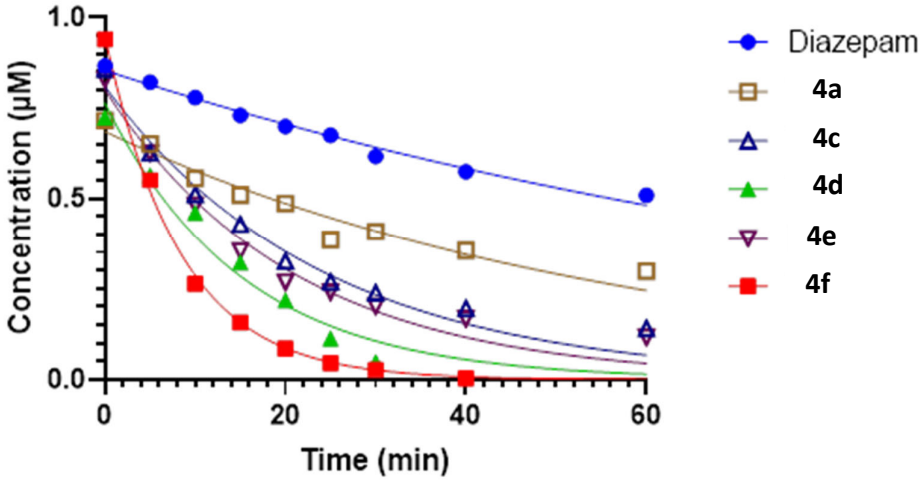


Figure S5. Liver microsome stability of the phenoxazine analogues **4a**, **4c-f**.

References

1. A. Rampa, L. Piazzi, F. Belluti, S. Gobbi, A. Bisi, M. Bartolini, V. Andrisano, V. Cavrini, A. Cavalli, M. Recanatini and P. Valenti, *J. Med. Chem.*, 2001, **44**, 3810.

2. J. Ceras, N. Cirauqui, S. Pérez-Silanes, I. Aldana, A. Monge and S. Galiano, *Eur. J. Med. Chem.*, 2012, **52**, 1.