1-(Arylsulfonyl-isoindol-2-yl)piperazines as 5-HT$_6$R antagonists: mechanochemical synthesis, *in vitro* pharmacological properties and glioprotective activity

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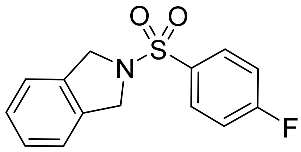
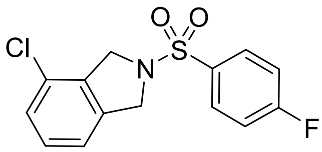
1. Characterization data for intermediate compounds 2a–l

1.1. 2-[(4-Fluorophenyl)sulfonyl]isoindoline **2a**



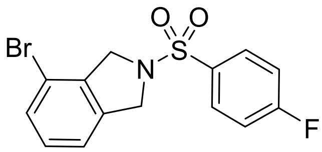
White solid, 194 mg .(isolated yield 98%); UPLC/MS purity 100%, t_R = 7.29; $C_{14}H_{12}FNO_2S$, MW 277.31, Monoisotopic Mass 277.06, $[M+H]^+$ 278.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.62 (s, 4H), 7.15–7.21 (m, 4H), 7.22–7.26 (m, 2H), 7.87–7.93 (m, 2H). ^{13}C NMR (175 MHz, $CDCl_3$) δ ppm 53.8, 116.6 (d, $J_{C\text{-}F}$ = 22.3 Hz), 122.8, 127.9, 130.3 (d, $J_{C\text{-}F}$ = 9.1 Hz), 133.1 (d, $J_{C\text{-}F}$ = 3.0 Hz), 135.9, 165.3(d, $J_{C\text{-}F}$ = 254.7 Hz).

1.2. 4-Chloro-2-[(4-fluorophenyl)sulfonyl]isoindoline **2b**



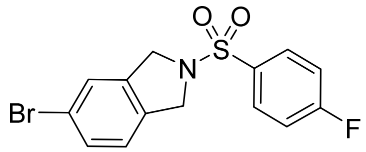
White solid, 168 mg (isolated yield 81%); UPLC/MS purity 100%, t_R = 8.07; $C_{14}H_{11}ClFNO_2S$, MW 311.76, Monoisotopic Mass 311.02, $[M+H]^+$ 312.1. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.60–4.64 (m, 2H), 4.65–4.69 (s, 2H), 7.02–7.09 (m, 1H), 7.16–7.27 (m, 4H), 7.88–7.93 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 53.4, 54.5, 116.7 (d, $J_{C\text{-}F}$ = 2.3 Hz), 121.0, 128.0, 129.2, 129.7, 130.3 (d, $J_{C\text{-}F}$ = 9.7 Hz), 133.0 (d, $J_{C\text{-}F}$ = 3.0 Hz), 134.8, 137.8, 165.4 (d, $J_{C\text{-}F}$ = 255.3 Hz).

1.3. 4-Bromo-2-[(4-fluorophenyl)sulfonyl]isoindoline **2c**



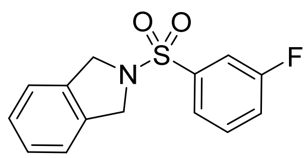
White solid, 177 mg (isolated yield 81%); UPLC/MS purity 100%, t_R = 8.19; $C_{14}H_{11}BrFNO_2S$, MW 356.21, Monoisotopic Mass 354.97, $[M+H]^+$ 356.0/358.0. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.58 (t, J = 2.0 Hz, 2H), 4.70 (s, 2H), 7.08–7.17 (m, 2H), 7.18–7.24 (m, 2H), 7.33–7.39 (m, 1H), 7.88–7.94 (m, 2H). ^{13}C NMR (175 MHz, $CDCl_3$) δ ppm 54.7, 55.1, 116.7 ($J_{C\text{-}F}$ = 22.9 Hz), 117.5, 121.5, 129.8, 130.3 ($J_{C\text{-}F}$ = 9.7 Hz), 131.0, 133.0 ($J_{C\text{-}F}$ = 3.6 Hz), 136.9, 137.6, 165.4 ($J_{C\text{-}F}$ = 255.3 Hz).

1.4. 5-Bromo-2-[(4-fluorophenyl)sulfonyl]isoindoline **2d**



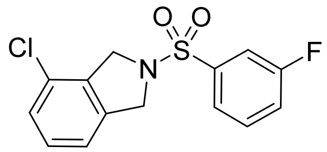
White solid, 189 mg (isolated yield 86%); UPLC/MS purity 98%, t_R = 8.05; $C_{14}H_{11}BrFNO_2S$, MW 356.21, Monoisotopic Mass 354.97, $[M+H]^+$ 356.0/358.1. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.55 (s, 2H), 4.59 (s, 2H), 7.04 (d, J = 8.3 Hz, 1H), 7.16–7.25 (m, 2H), 7.31 (s, 1H), 7.33–7.38 (m, 1H), 7.85–7.91 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 53.3, 53.4, 116.7 ($J_{C\text{-}F}$ = 22.9 Hz), 121.7, 124.3, 126.0, 130.2 ($J_{C\text{-}F}$ = 9.1 Hz), 131.1, 132.9 ($J_{C\text{-}F}$ = 3.0 Hz), 134.9, 138.2, 165.4 ($J_{C\text{-}F}$ = 255.3 Hz).

1.5. 2-[(3-Fluorophenyl)sulfonyl]isoindoline **2e**



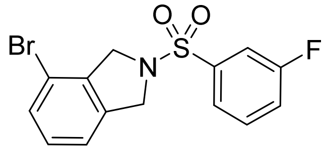
White solid, 190 mg (isolated yield 96%); UPLC/MS purity 100%, t_R = 7.39; $C_{14}H_{12}FNO_2S$, MW 277.31, Monoisotopic Mass 277.06, $[M+H]^+$ 278.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.64 (s, 4H), 7.16–7.20 (m, 2H), 7.22–7.26 (m, 2H), 7.26–7.29 (m, 1H), 7.51 (td, J = 8.1, 5.3 Hz, 1H), 7.59 (dq, J = 8.3, 1.5 Hz, 1H), 7.67 (dq, J = 7.7, 0.9 Hz, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ ppm 53.9, 114.9 ($J_{C\text{-}F}$ = 24.1 Hz), 120.2 ($J_{C\text{-}F}$ = 21.1 Hz), 122.8, 123.3 ($J_{C\text{-}F}$ = 3.0 Hz), 128.0, 131.1 ($J_{C\text{-}F}$ = 7.8 Hz), 135.8, 139.0 ($J_{C\text{-}F}$ = 6.6 Hz), 162.7 ($J_{C\text{-}F}$ = 252.3 Hz).

1.6. 4-Chloro-2-[(3-fluorophenyl)sulfonyl]isoindoline **2f**



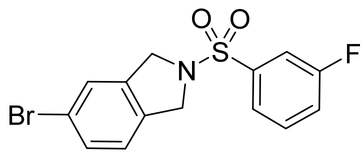
White solid, 156 mg (isolated yield 75%); UPLC/MS purity 100%, t_R = 8.13; $C_{14}H_{11}ClFNO_2S$, MW 311.76, Monoisotopic Mass 311.02, $[M+H]^+$ 312.1. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 4.61–4.66 (m, 2H), 4.67–4.71 (m, 2H), 7.03–7.10 (m, 1H), 7.17–7.22 (m, 2H), 7.26–7.32 (m, 1H), 7.49–7.55 (m, 1H), 7.57–7.61 (m, 1H), 7.66–7.70 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 53.4, 54.5, 114.9 (d, $J_{C\text{-}F}$ = 24.7 Hz), 120.3 (d, $J_{C\text{-}F}$ = 21.1 Hz), 121.0, 123.3 (d, $J_{C\text{-}F}$ = 3.0 Hz), 128.0, 129.2, 129.7, 131.2 (d, $J_{C\text{-}F}$ = 7.2 Hz), 134.7, 137.8, 138.9 (d, $J_{C\text{-}F}$ = 6.6 Hz), 162.7 (d, $J_{C\text{-}F}$ = 251.7 Hz).

1.7. 4-Bromo-2-[(3-fluorophenyl)sulfonyl]isoindoline **2g**



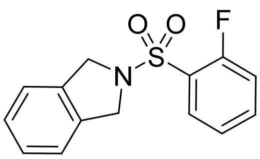
White solid, 173 mg (isolated yield 79%); UPLC/MS purity 100%, t_R = 8.12; $C_{14}H_{11}BrFNO_2S$, MW 356.21, Monoisotopic Mass 354.97, $[M+H]^+$ 356.0/358.0. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 4.60 (t, J = 2.0 Hz, 2H), 4.72 (t, J = 2.0 Hz, 2H), 7.09–7.14 (m, 2H), 7.29 (tdd, J = 8.3, 2.6, 0.9 Hz, 1H), 7.35–7.38 (m, 1H), 7.53 (td, J = 8.0, 5.4 Hz, 1H), 7.60 (ddd, J = 8.0, 2.6, 1.7 Hz, 1H), 7.68 (ddd, J = 7.7, 1.7, 0.9 Hz, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ ppm 54.7, 55.1, 114.9 (J_{C-F} = 24.1 Hz), 117.5, 120.3 (J_{C-F} = 21.1 Hz), 121.6, 123.3 (J_{C-F} = 3.6 Hz), 129.8, 131.0, 131.3 (J_{C-F} = 7.2 Hz), 136.8, 137.6, 138.9 (J_{C-F} = 6.6 Hz), 162.7 (J_{C-F} = 251.7 Hz).

1.8. 5-Bromo-2-[(3-fluorophenyl)sulfonyl]isoindoline **2h**



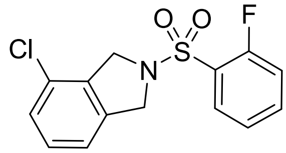
White solid, 186 mg (isolated yield 85%); UPLC/MS purity 100%, t_R = 8.12; $C_{14}H_{11}BrFNO_2S$, MW 356.21, Monoisotopic Mass 354.97, $[M+H]^+$ 356.0/358.0. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 4.58 (d, J = 1.4 Hz, 2H), 4.61 (s, 2H), 7.05 (d, J = 8.3 Hz, 1H), 7.28 (tdd, J = 8.6, 8.6, 2.6, 0.9 Hz, 1H), 7.31–7.33 (m, 1H), 7.34–7.38 (m, 1H), 7.48–7.55 (m, 1H), 7.57 (dt, J = 8.0, 1.7 Hz, 1H), 7.66 (dt, J = 7.8, 1.3 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 53.4, 53.5, 114.9 (J_{C-F} = 24.1 Hz), 120.3 (J_{C-F} = 21.7 Hz), 121.8, 123.3 (J_{C-F} = 3.0 Hz), 124.3, 126.0, 131.2, 131.3, 134.9, 138.1, 138.8 (J_{C-F} = 6.6 Hz), 162.7 (J_{C-F} = 252.3 Hz).

1.9. 2-[(2-Fluorophenyl)sulfonyl]isoindoline **2i**



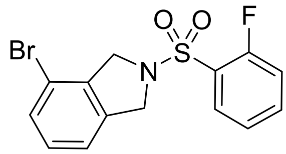
White solid, 184 mg (isolated yield 93%); UPLC/MS purity 100%, t_R = 7.26; $C_{14}H_{12}FNO_2S$, MW 277.31, Monoisotopic Mass 277.06, $[M+H]^+$ 278.2. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 4.75 (s, 4H), 7.14–7.17 (m, 1H), 7.18–7.22 (m, 2H), 7.23–7.29 (m, 3H), 7.52–7.57 (m, 1H), 7.93–7.97 (m, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ ppm 53.5, 117.5 (J_{C-F} = 21.7 Hz), 122.7, 124.48 (J_{C-F} = 3.62 Hz), 126.19 (J_{C-F} = 15.1 Hz), 127.9, 131.4, 135.1 (J_{C-F} = 8.45 Hz), 136.0, 159.0 (J_{C-F} = 245.0 Hz).

1.10. 4-Chloro-2-[(2-fluorophenyl)sulfonyl]isoindoline **2j**



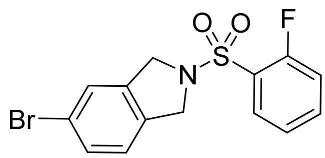
White solid, 164 mg (isolated yield 79%); UPLC/MS purity 100%, t_R = 8.02; $C_{14}H_{11}ClFNO_2S$, MW 311.76, Monoisotopic Mass 311.02, $[M+H]^+$ 312.1. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 4.72–4.75 (m, 2H), 4.80–4.83 (m, 2H), 7.05–7.11 (m, 1H), 7.16–7.23 (m, 3H), 7.29 (td, J = 7.7, 1.1 Hz, 1H), 7.53–7.59 (m, 1H), 7.96 (ddd, J = 7.8, 6.9, 1.9 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 53.1, 54.2, 117.5 (d, $J_{C\text{-}F}$ = 21.7 Hz), 121.0, 124.6 (d, $J_{C\text{-}F}$ = 3.6 Hz), 126.1 (d, $J_{C\text{-}F}$ = 15.1 Hz), 127.9, 129.2, 129.6, 131.4, 134.9, 135.2 (d, $J_{C\text{-}F}$ = 8.5 Hz), 138.0, 159.0 (d, $J_{C\text{-}F}$ = 255.3 Hz).

1.11. 4-Bromo-2-[(2-fluorophenyl)sulfonyl]isoindoline **2k**



White solid, 180 mg (isolated yield 82%); UPLC/MS purity 100%, t_R = 8.15; $C_{14}H_{11}BrFNO_2S$, MW 356.21, Monoisotopic Mass 354.97, $[M+H]^+$ 356.0/358.0. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 4.70 (s, 2H), 4.85 (s, 2H), 7.12–7.16 (m, 2H), 7.16–7.21 (m, 1H), 7.29 (td, J = 8.0, 1.1 Hz, 1H), 7.35–7.41 (m, 1H), 7.53–7.59 (m, 1H), 7.96 (ddd, J = 7.9, 7.0, 1.7 Hz, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ ppm 54.5, 54.8, 117.5 ($J_{C\text{-}F}$ = 23.5 Hz), 117.8, 121.5, 124.6 ($J_{C\text{-}F}$ = 3.6 Hz), 126.1 ($J_{C\text{-}F}$ = 15.1 Hz), 129.7, 130.9, 131.4, 135.2 ($J_{C\text{-}F}$ = 8.5 Hz), 136.9, 137.8, 159.0 ($J_{C\text{-}F}$ = 255.3 Hz).

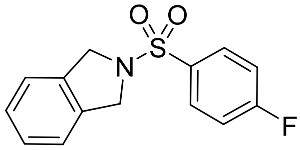
1.12. 5-Bromo-2-[(2-fluorophenyl)sulfonyl]isoindoline **2l**



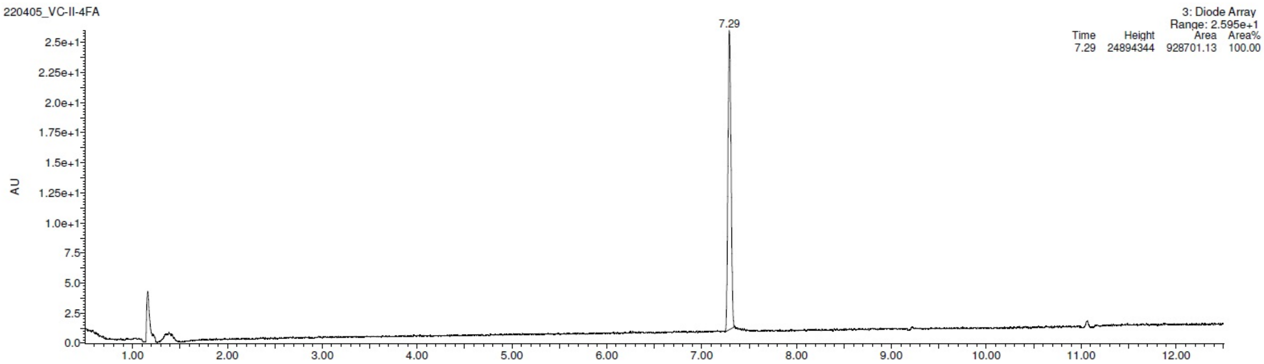
White solid, 189 mg (isolated yield 86%); UPLC/MS purity 100%, t_R = 8.04; $C_{14}H_{11}BrFNO_2S$, MW 356.21, Monoisotopic Mass 354.97, $[M+H]^+$ 356.0/358.1. ^1H NMR (500 MHz, $CDCl_3$) δ ppm 4.68 (s, 2H), 4.72 (s, 2H), 7.06 (d, J = 8.3 Hz, 1H), 7.14–7.21 (m, 1H), 7.28 (td, J = 7.7, 0.9 Hz, 1H), 7.33 (s, 1H), 7.35–7.39 (m, 1H), 7.52–7.58 (m, 1H), 7.92–7.96 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 53.1 (dd, $J_{C\text{-}F}$ = 6.0, 3.0 Hz), 117.5 ($J_{C\text{-}F}$ = 21.7 Hz), 121.7, 124.3, 124.6 ($J_{C\text{-}F}$ = 3.6 Hz), 126.0 ($J_{C\text{-}F}$ = 15.1 Hz), 126.1, 131.1, 131.4, 135.1, 135.2 ($J_{C\text{-}F}$ = 8.5 Hz), 138.3, 159.0 ($J_{C\text{-}F}$ = 255.3 Hz).

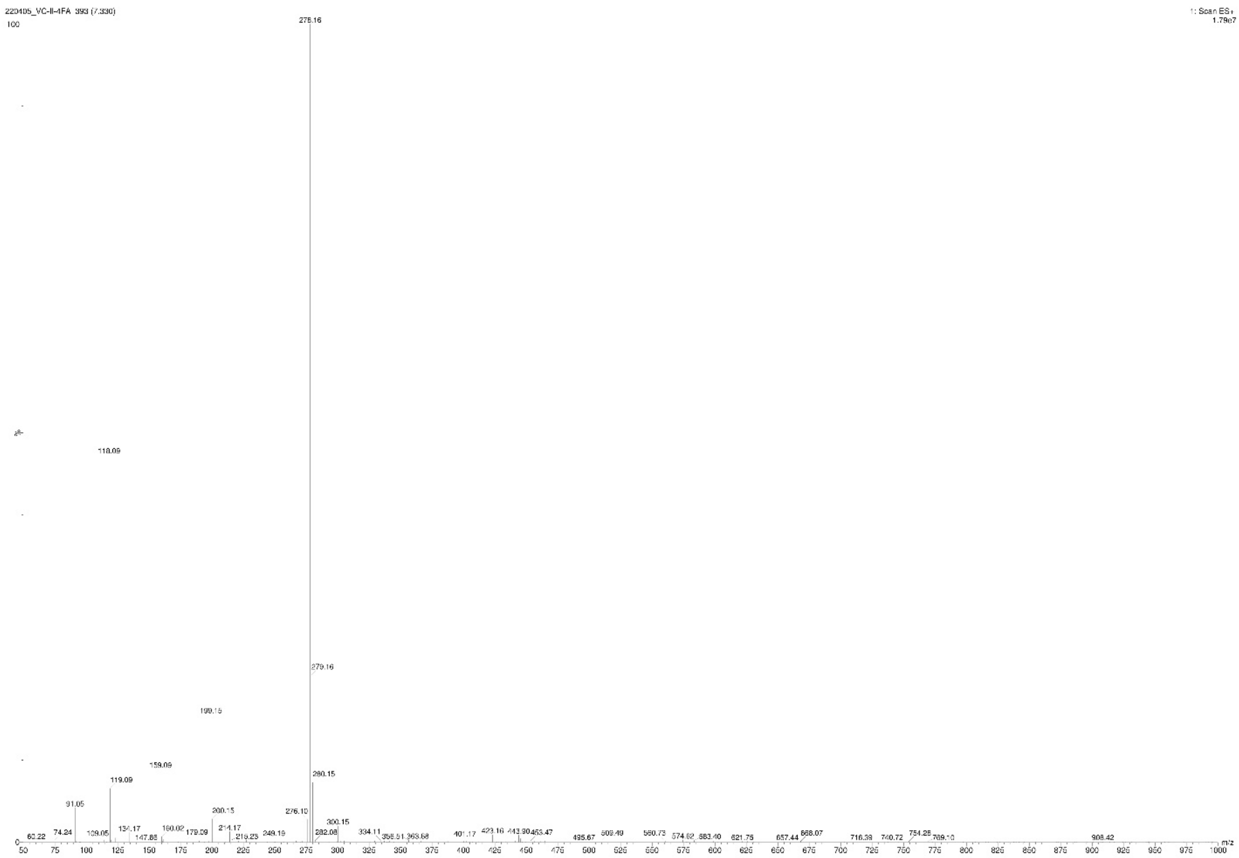
2. UPLC/MS, ^{1}H NMR and ^{13}C NMR spectra of all intermediates and final compounds

2.1. 2-[(4-Fluorophenyl)sulfonyl]isoindoline **2a**

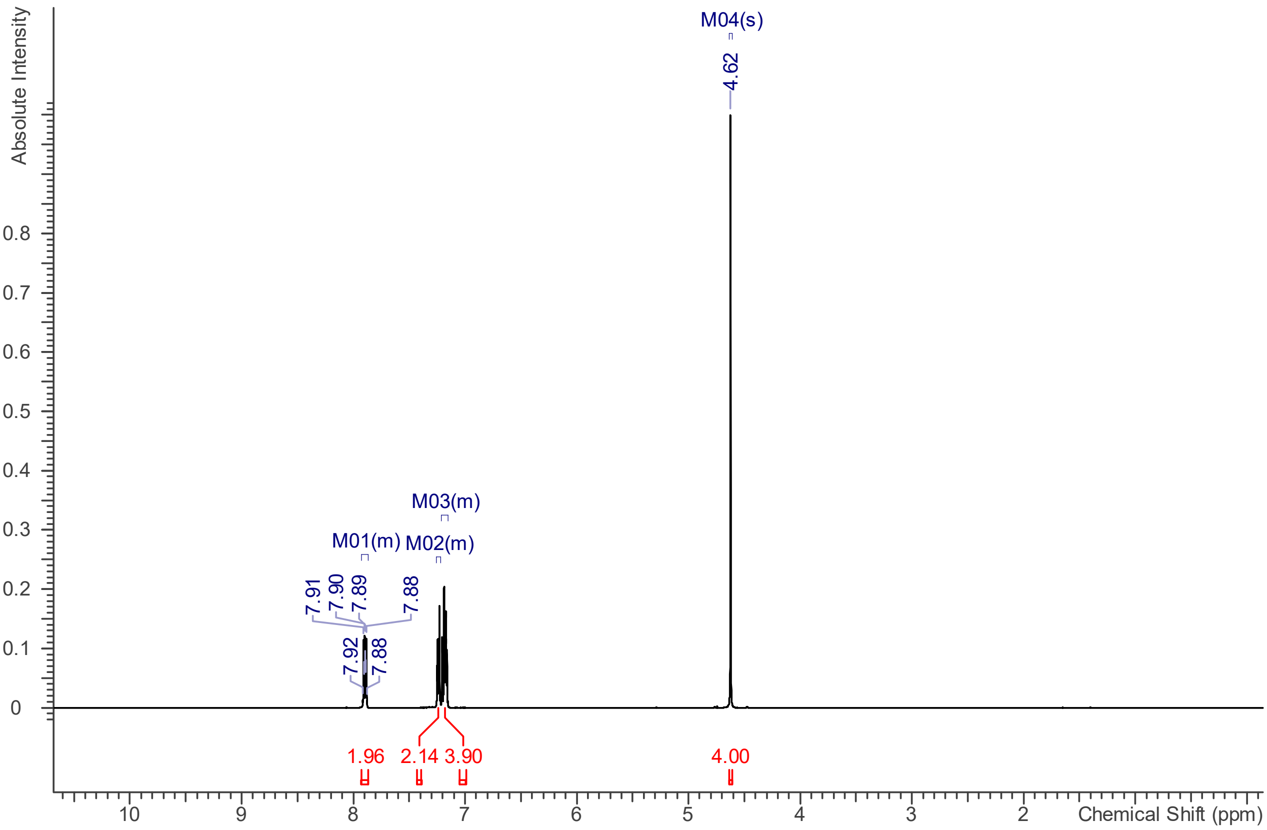


UPLC/MS

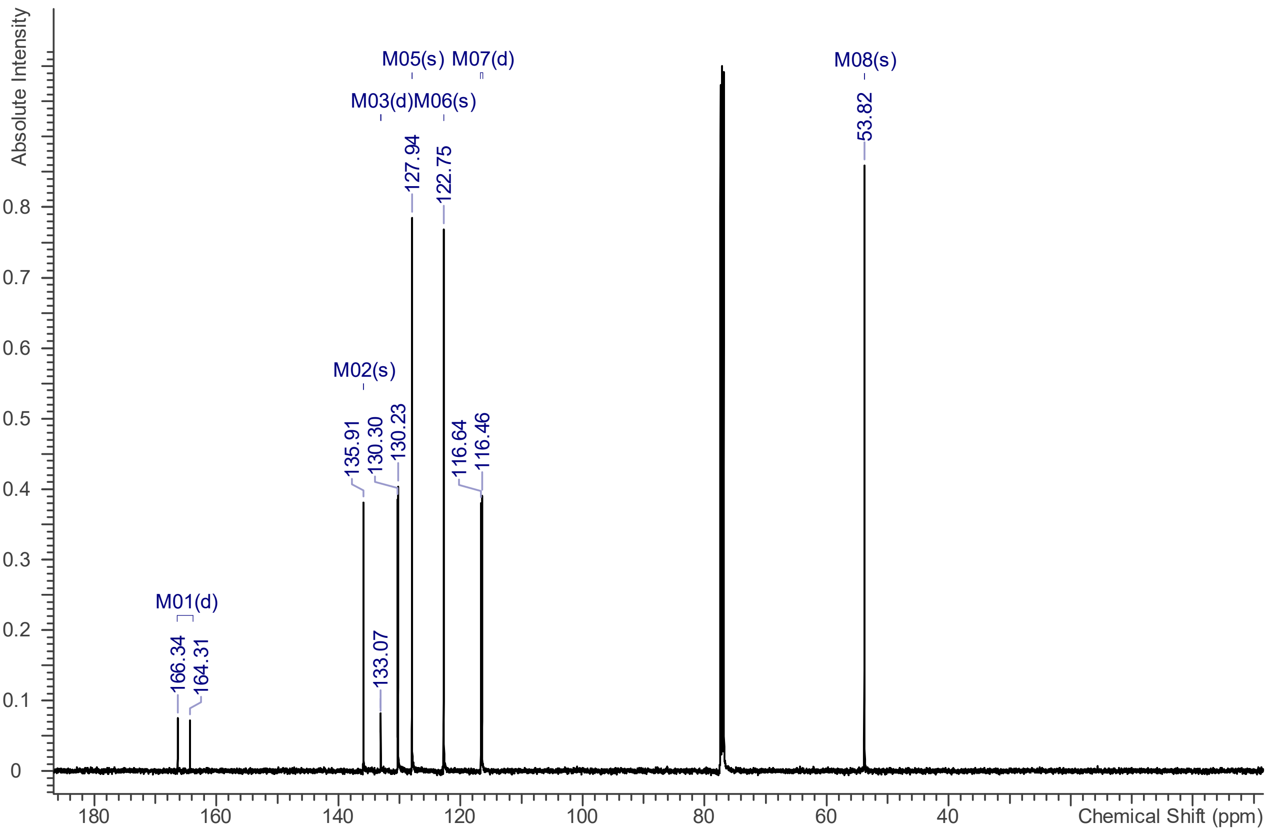




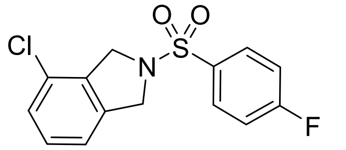
^{1}H-NMR (500 MHz, $CDCl_3$)



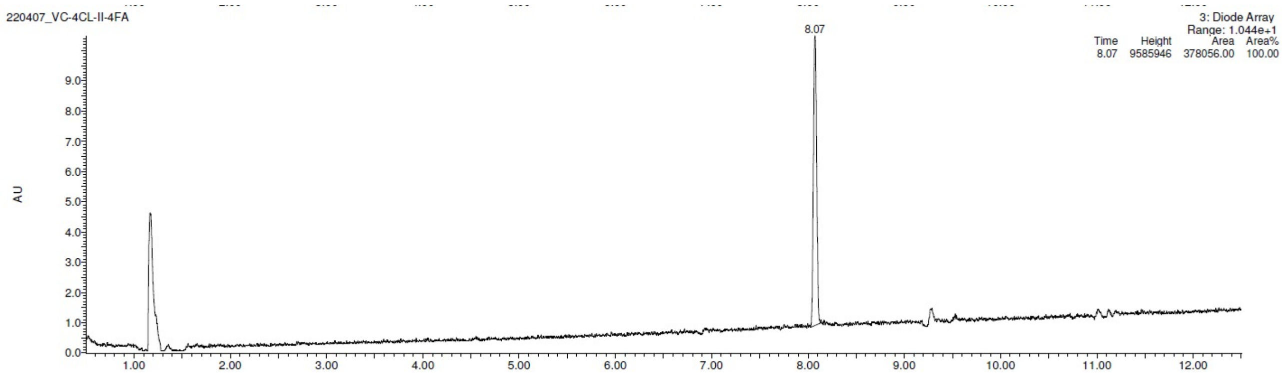
^{13}C-NMR (126 MHz, $CDCl_3$)

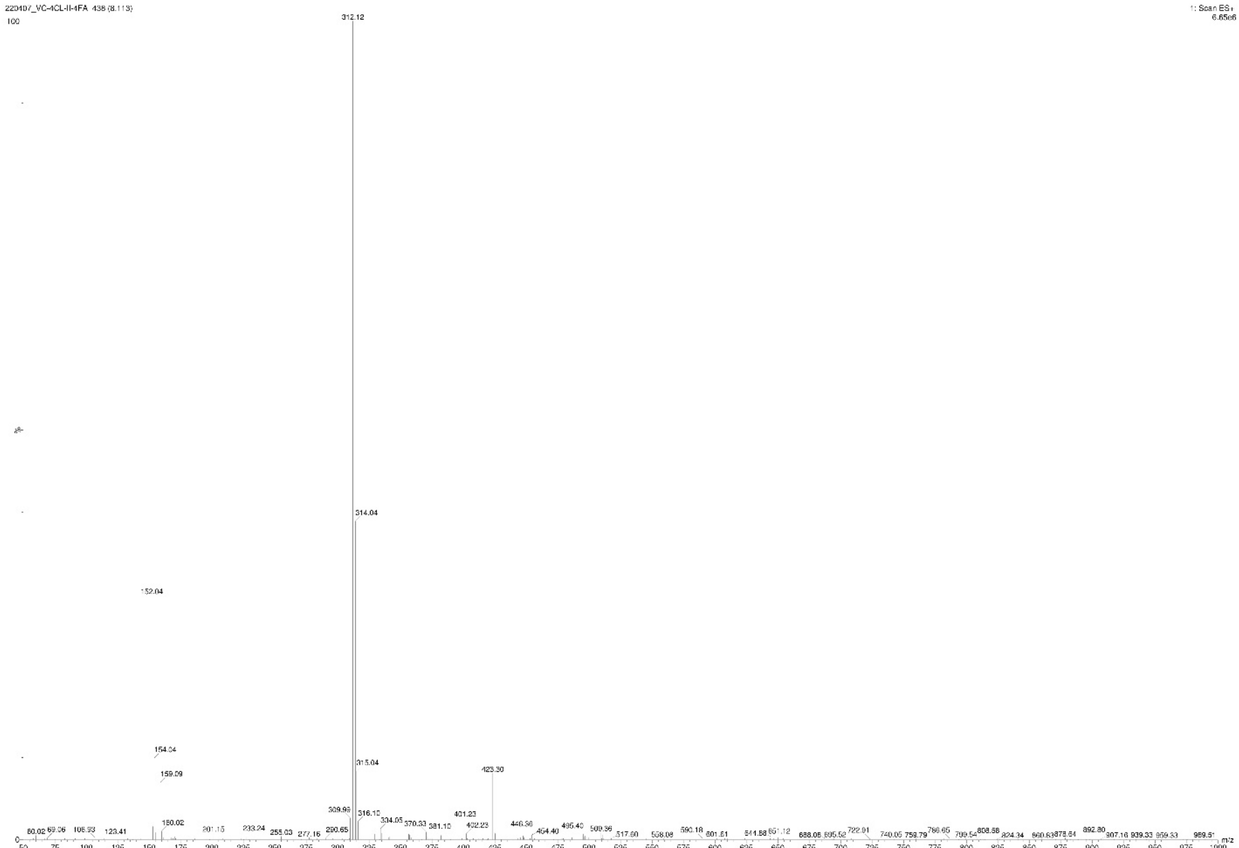


2.2. 4-Chloro-2-[(4-fluorophenyl)sulfonyl]isoindoline **2b**

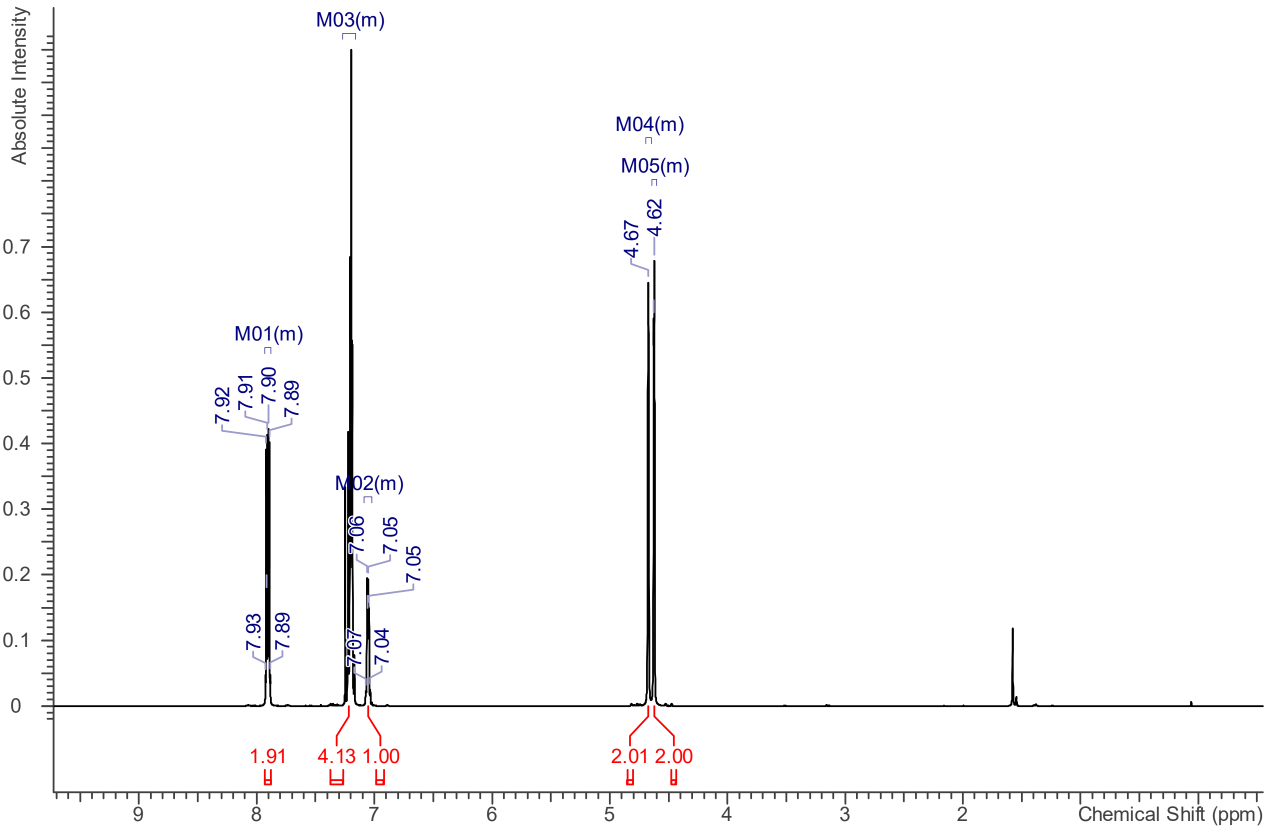


UPLC/MS

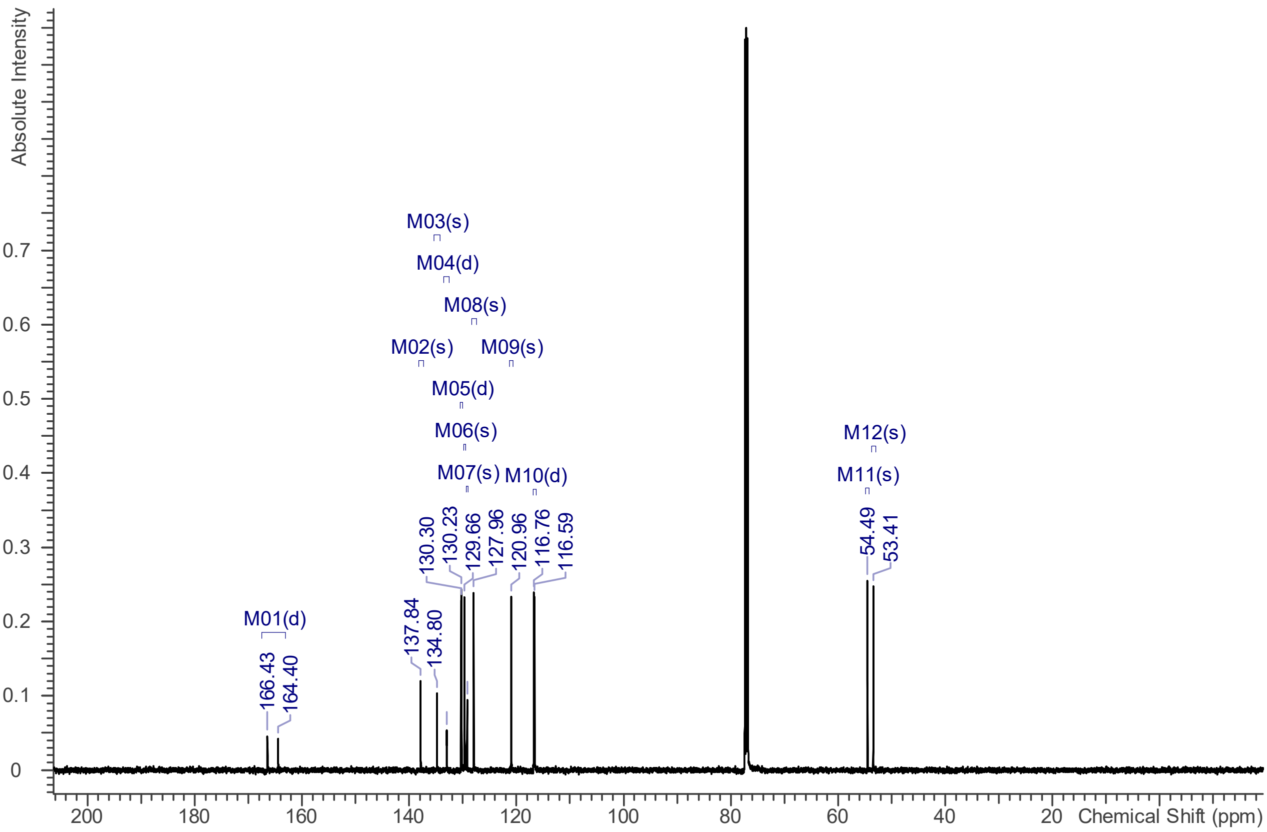




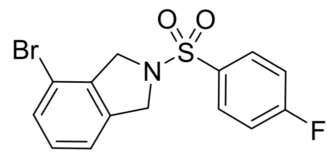
[1]H-NMR (500 MHz, $CDCl_3$)



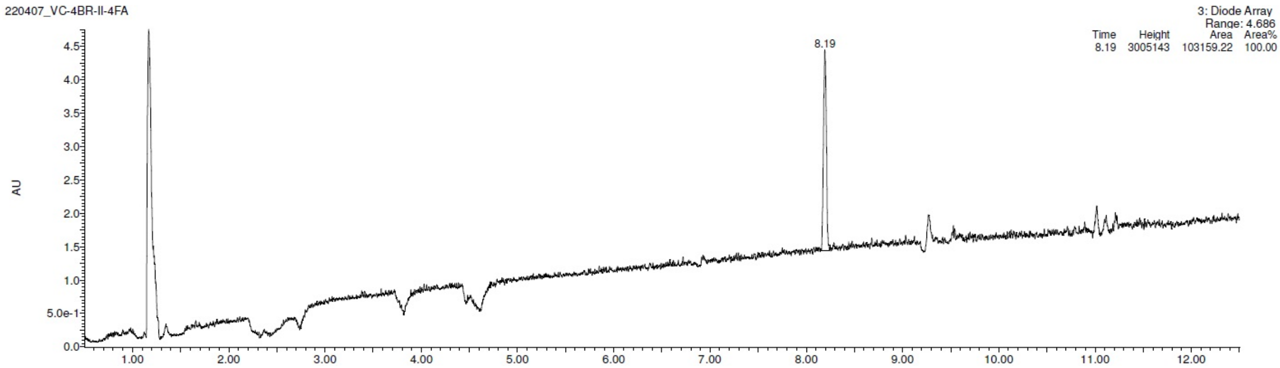
[13]C-NMR (126 MHz, $CDCl_3$)

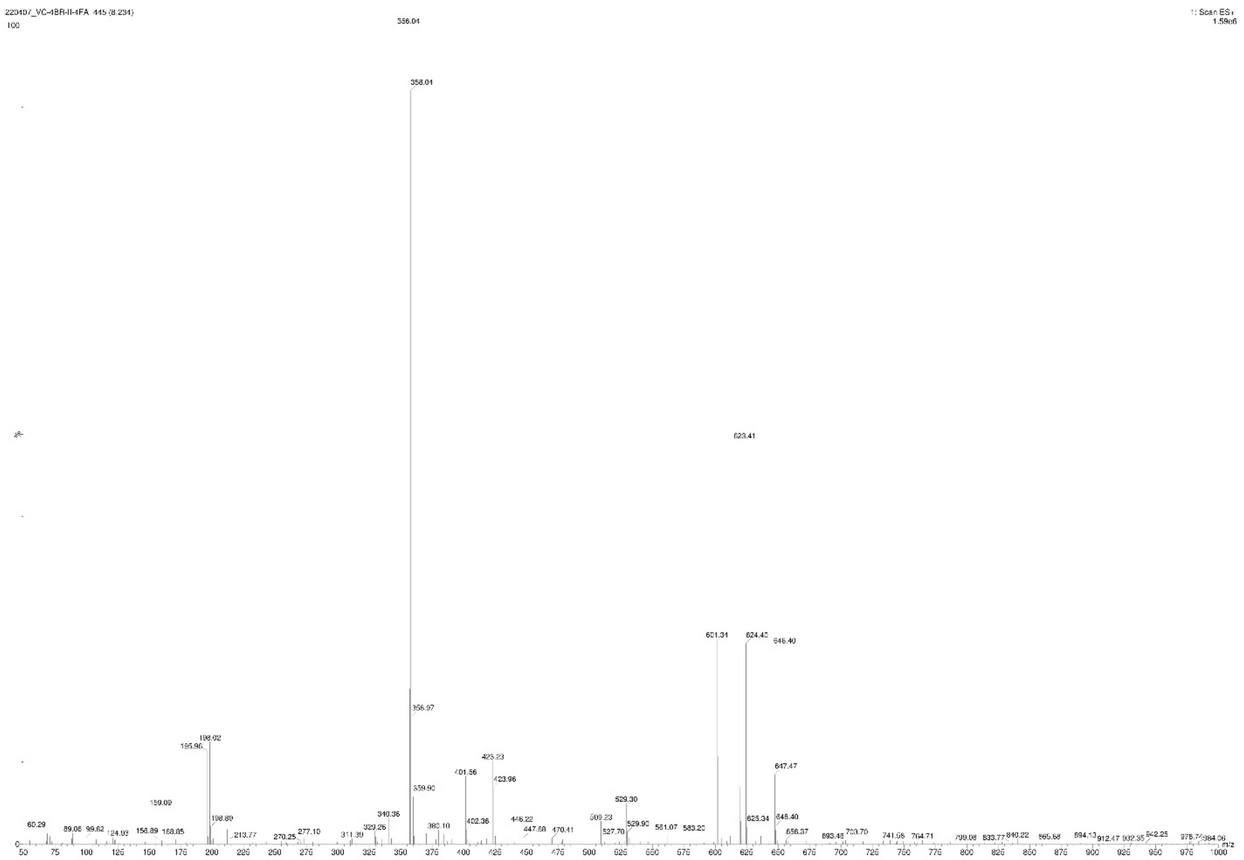


2.3. 4-Bromo-2-[(4-fluorophenyl)sulfonyl]isoindoline **2c**

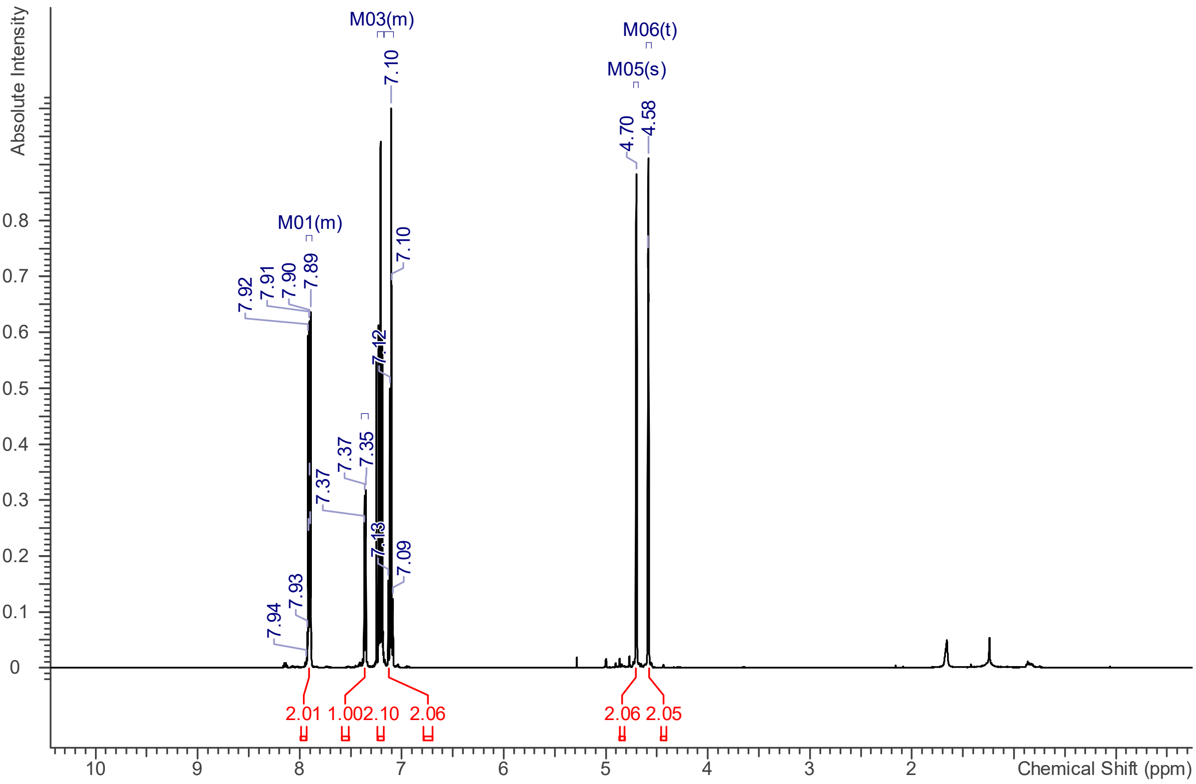


UPLC/MS

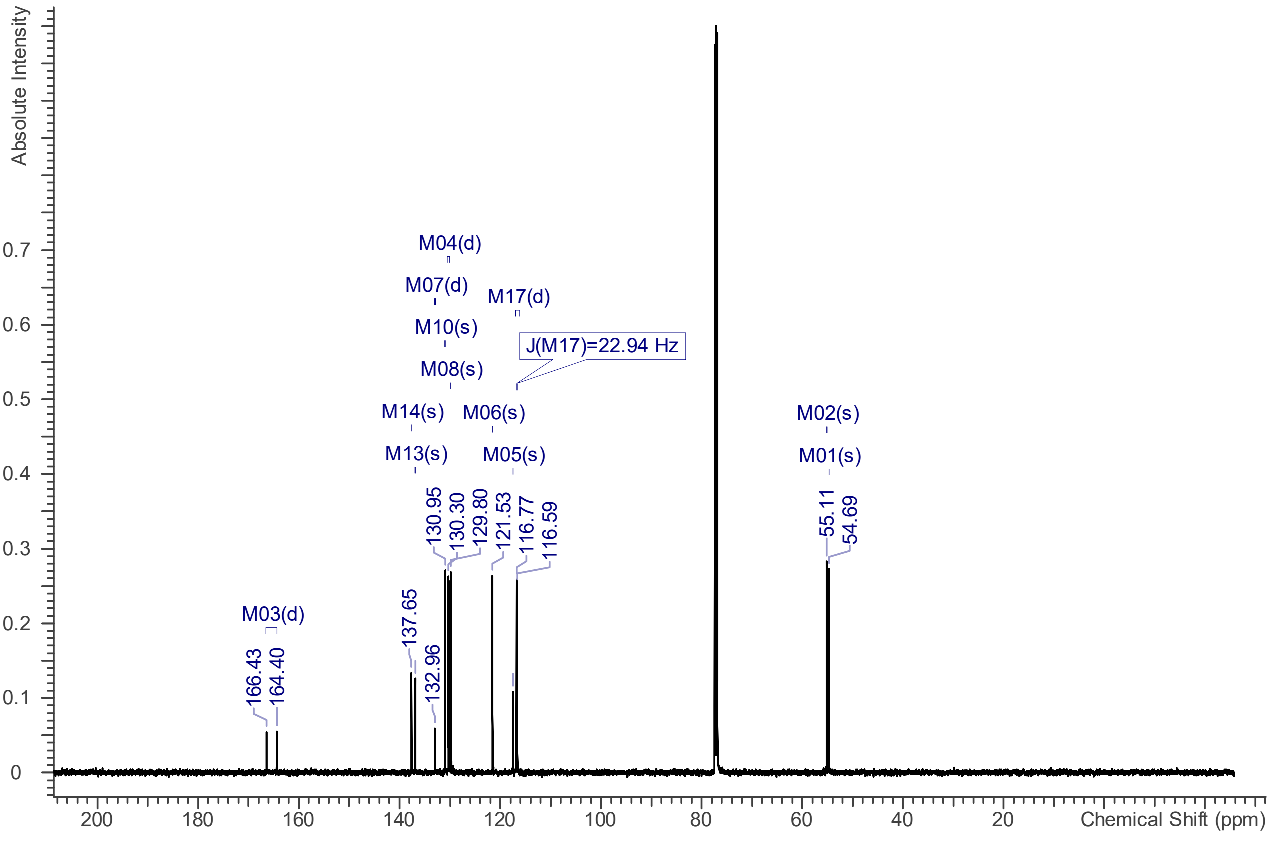




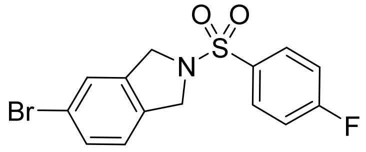
^{1}H-NMR (500 MHz, $CDCl_3$)



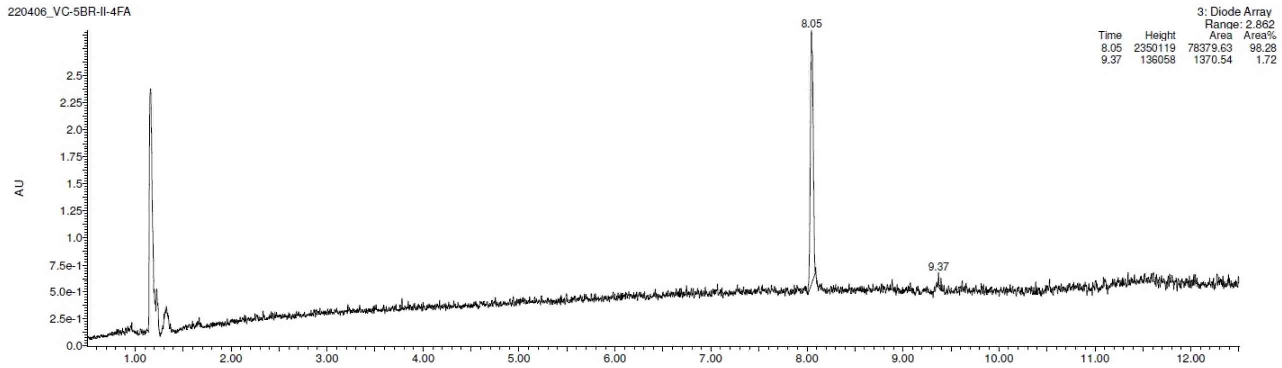
^{13}C-NMR (126 MHz, $CDCl_3$)

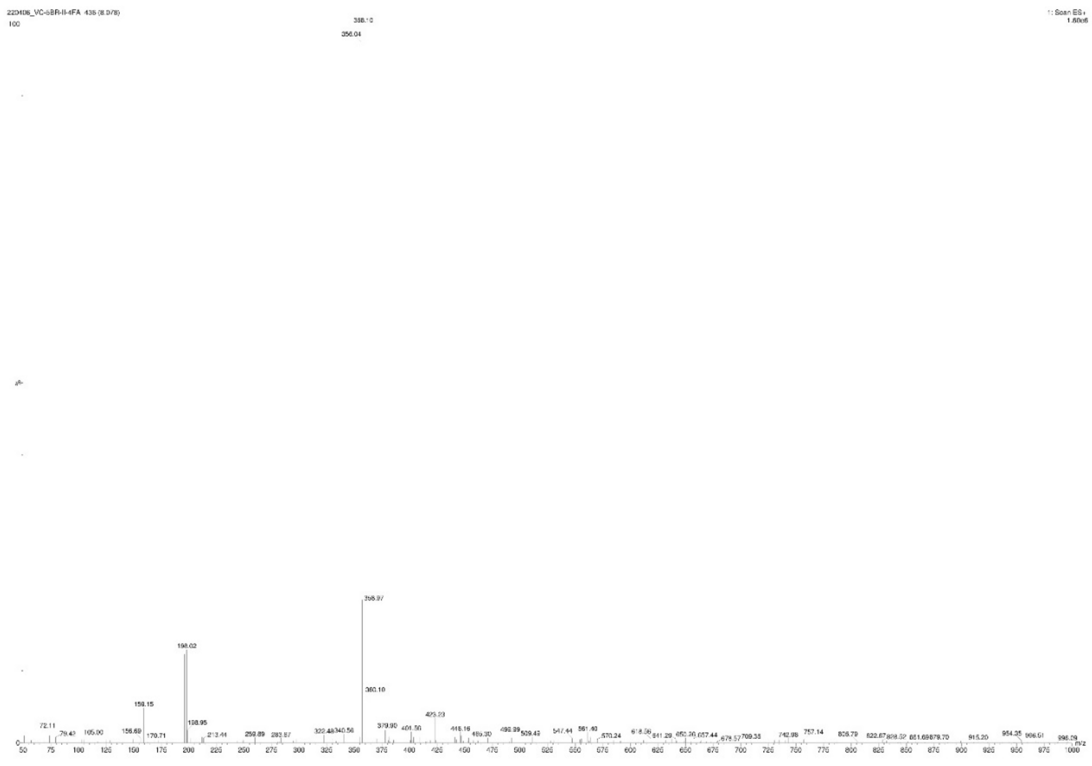


2.4. 5-Bromo-2-[(4-fluorophenyl)sulfonyl]isoindoline **2d**

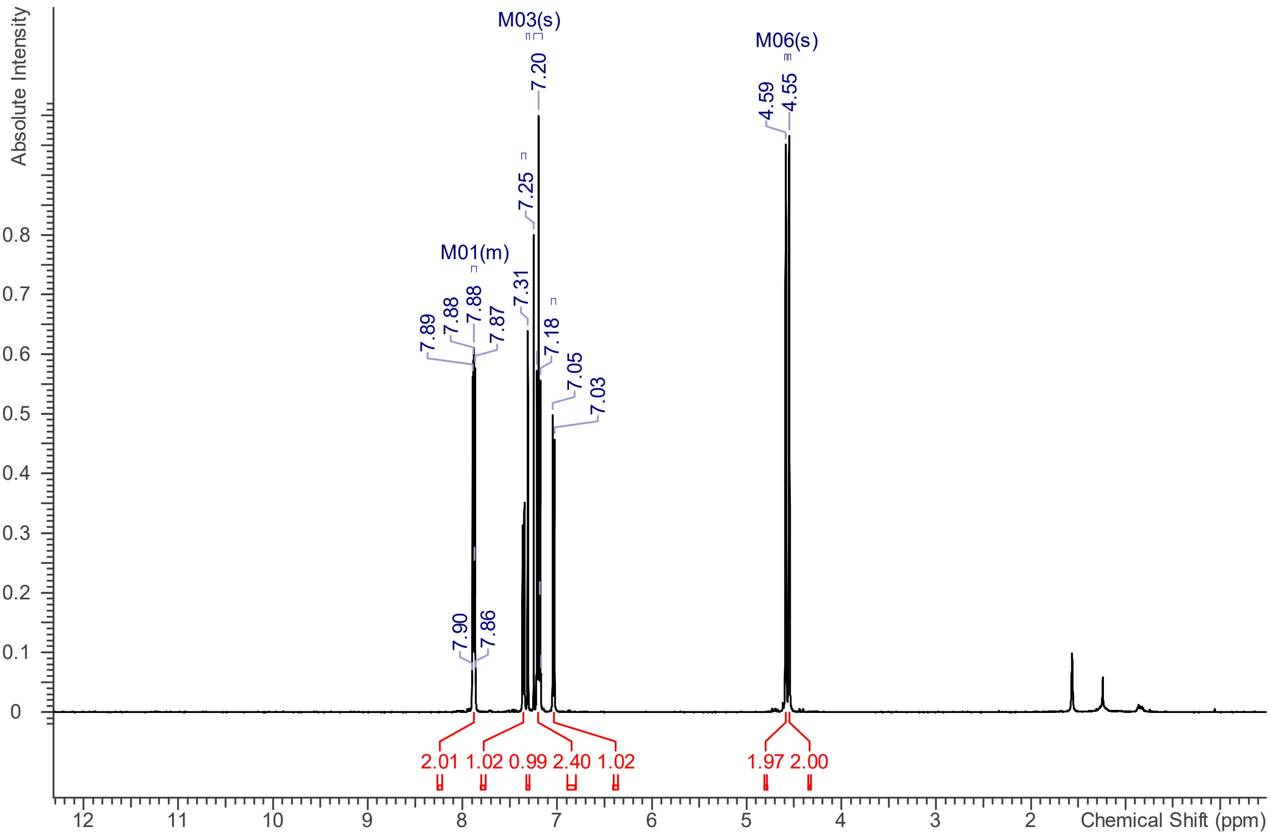


UPLC/MS

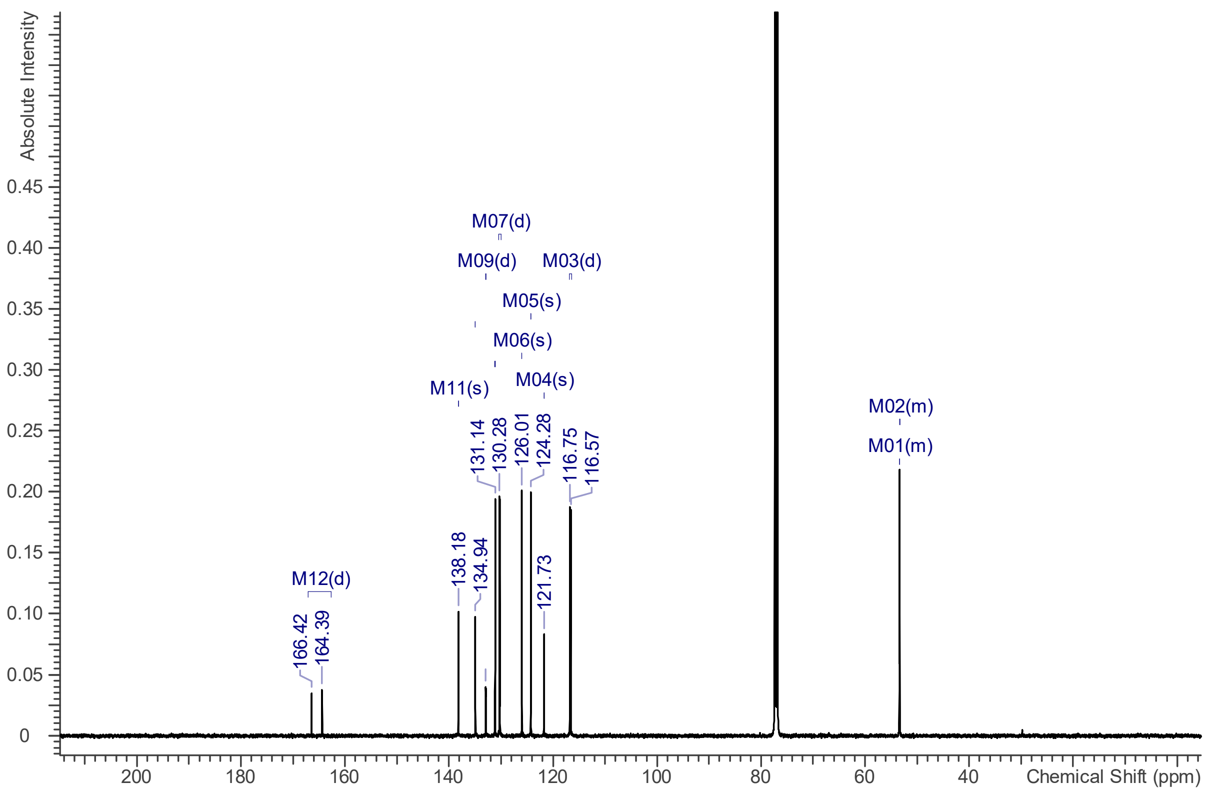




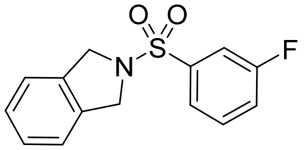
[1]H-NMR (500 MHz, $CDCl_3$)



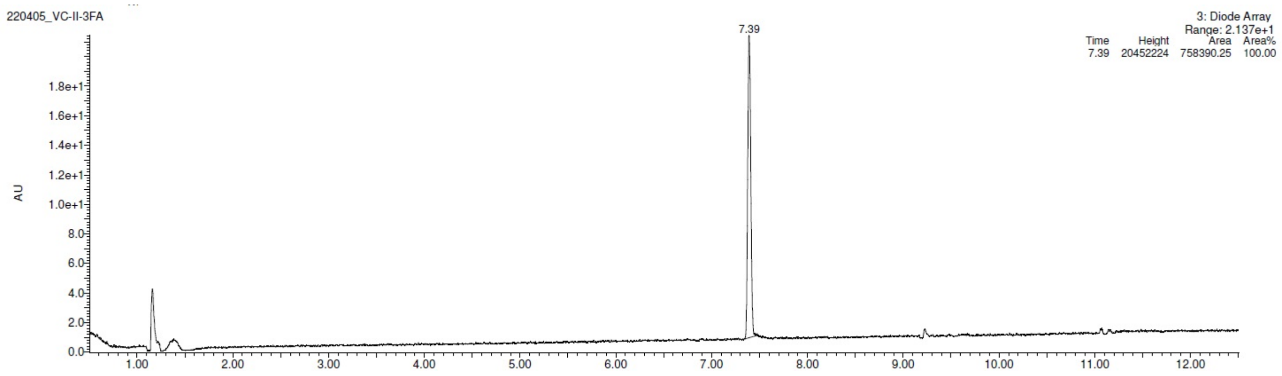
[13]C-NMR (126 MHz, $CDCl_3$)

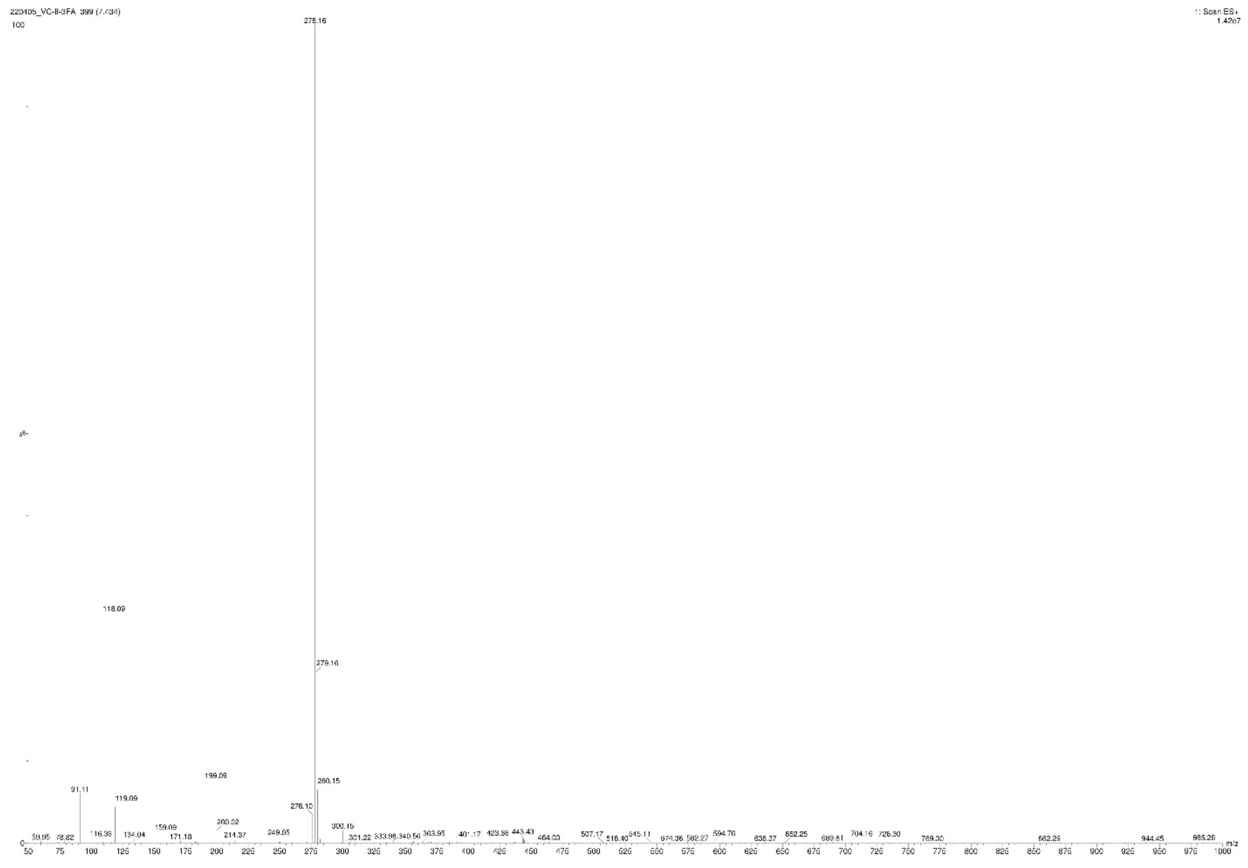


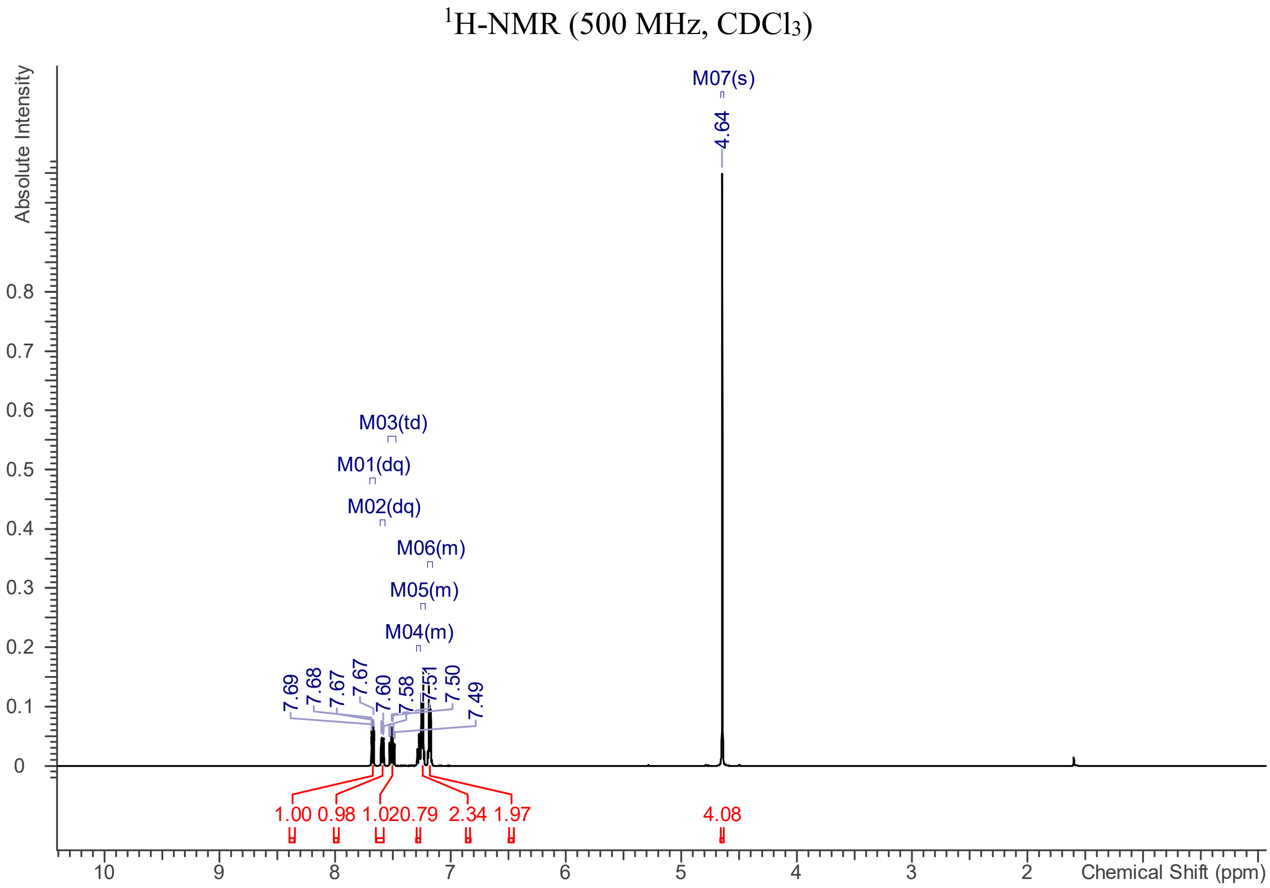
2.5. 2-[(3-Fluorophenyl)sulfonyl]isoindoline **2e**



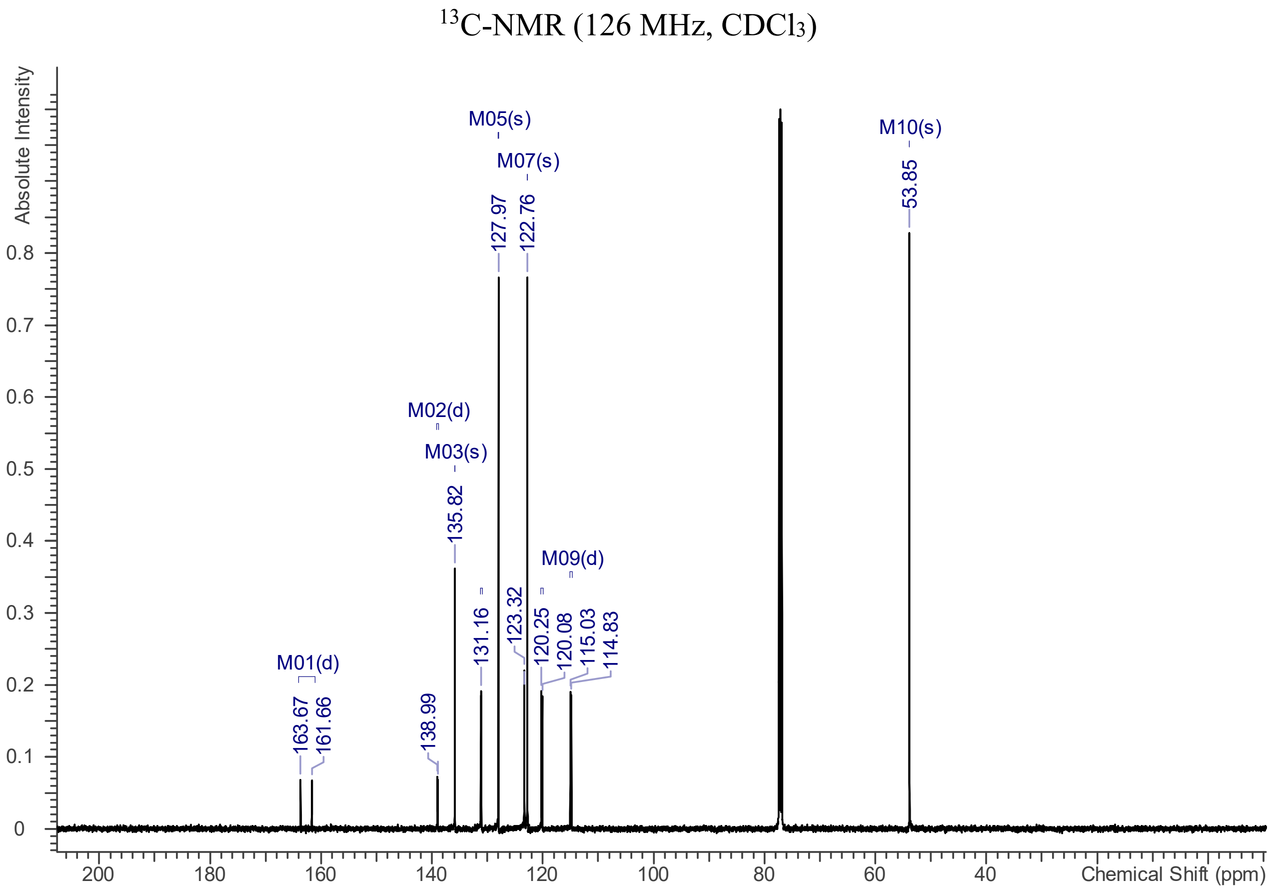
UPLC/MS





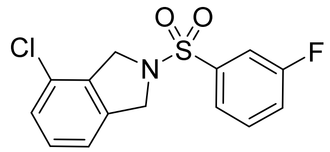


^{1}H-NMR (500 MHz, $CDCl_3$)
M07(s)
4.64
M03(td)
M01(dq)
M02(dq)
M06(m)
M05(m)
M04(m)
7.69
7.68
7.67
7.67
7.60
7.58
7.51
7.50
7.49
1.00 0.98 1.02 0.79 2.34 1.97
4.08
Absolute Intensity
Chemical Shift (ppm)

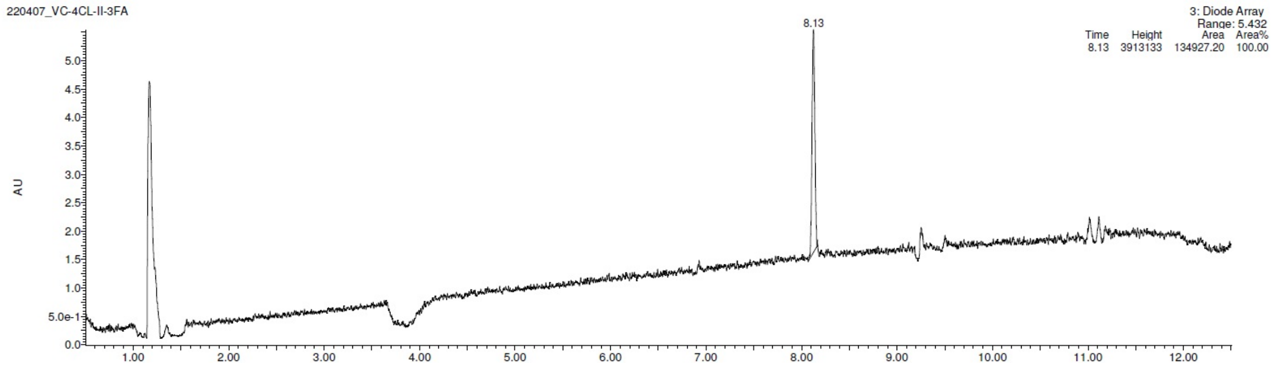


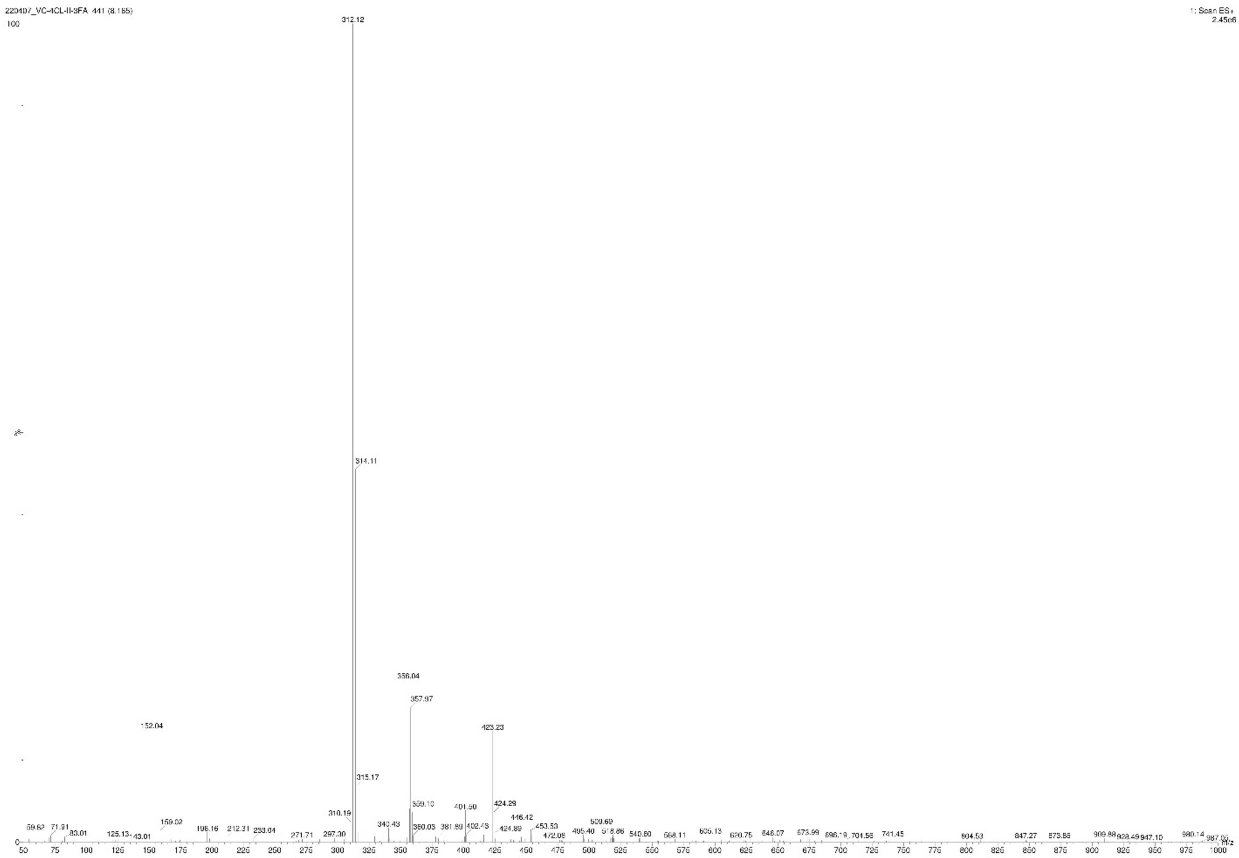
^{13}C-NMR (126 MHz, $CDCl_3$)
M05(s)
M07(s)
M10(s)
127.97
122.76
53.85
M02(d)
M03(s)
135.82
M09(d)
131.16
123.32
120.25
120.08
115.03
114.83
M01(d)
163.67
161.66
138.99
Absolute Intensity
Chemical Shift (ppm)

2.6. 4-Chloro-2-[(3-fluorophenyl)sulfonyl]isoindoline **2f**

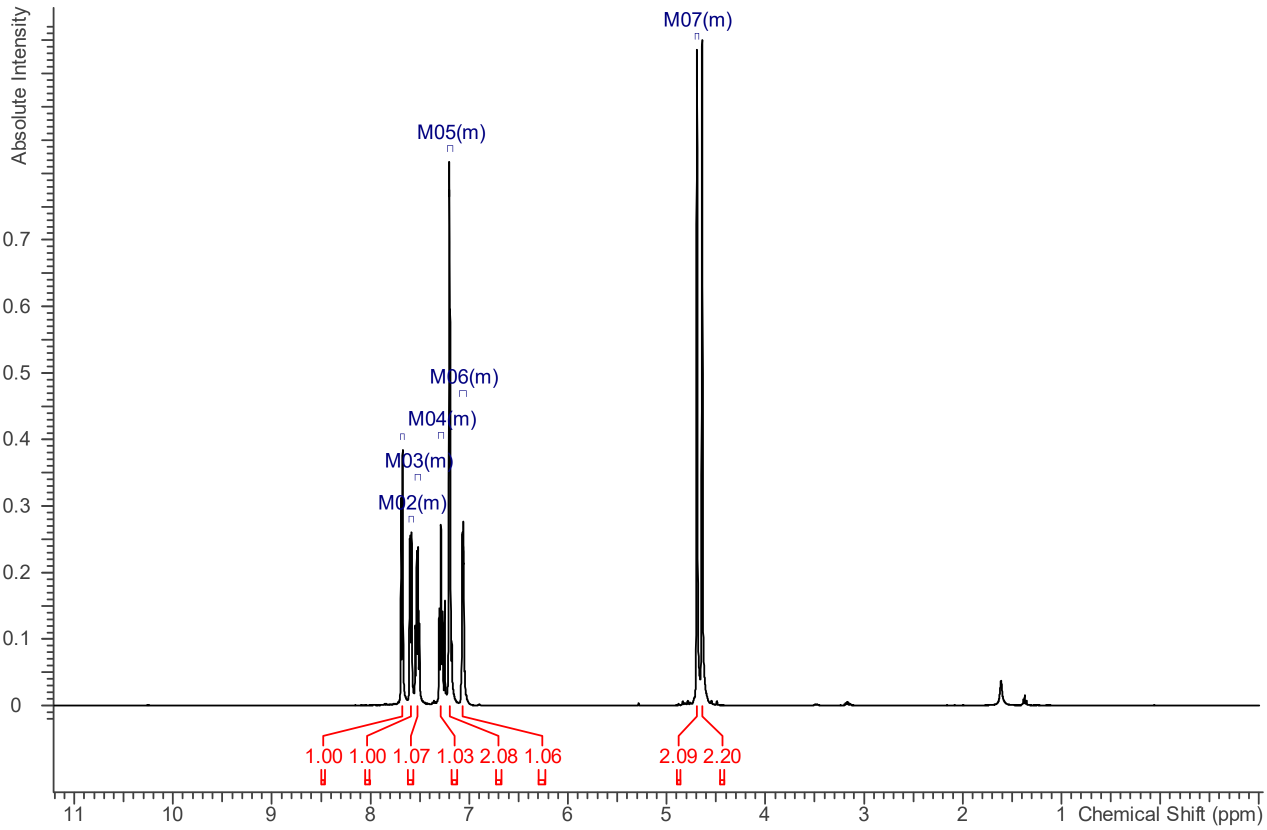


UPLC/MS

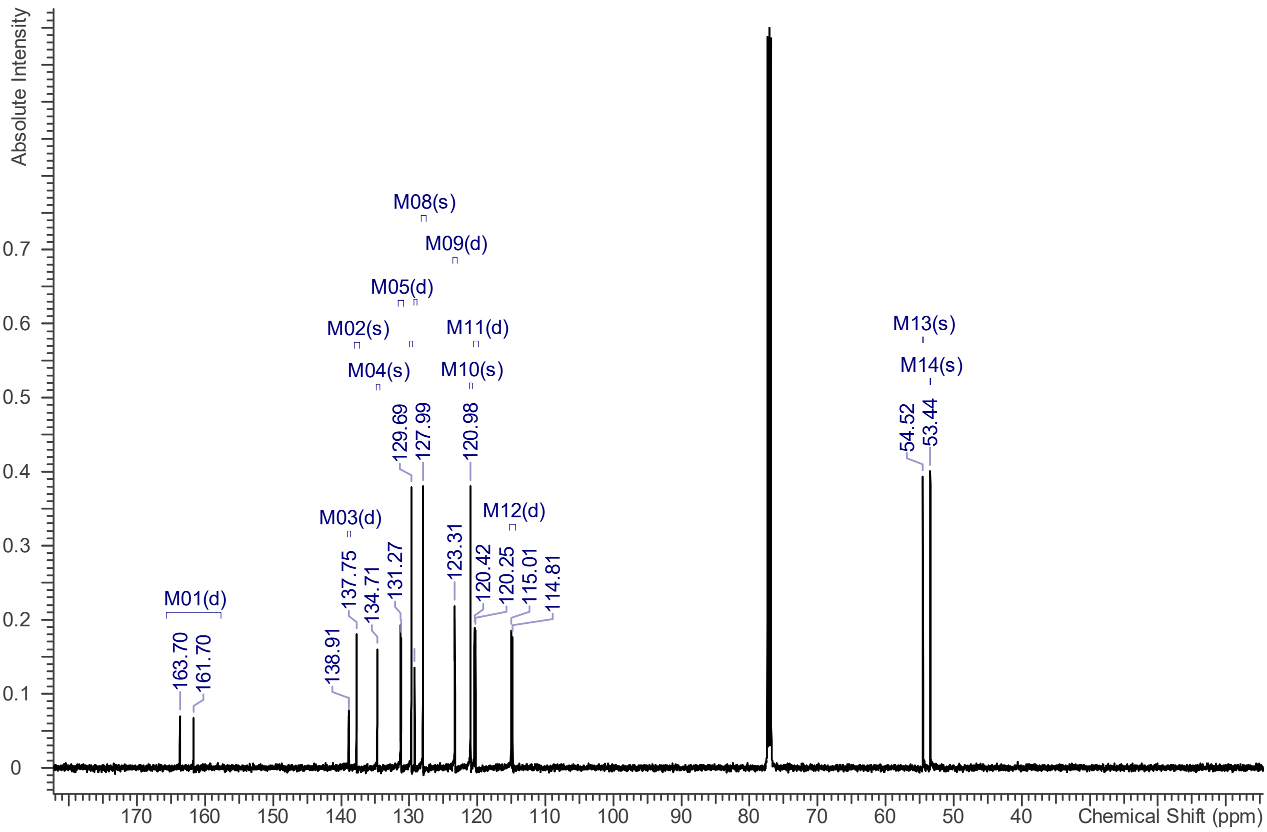




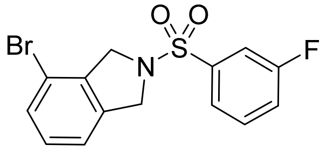
^{1}H-NMR (500 MHz, $CDCl_3$)



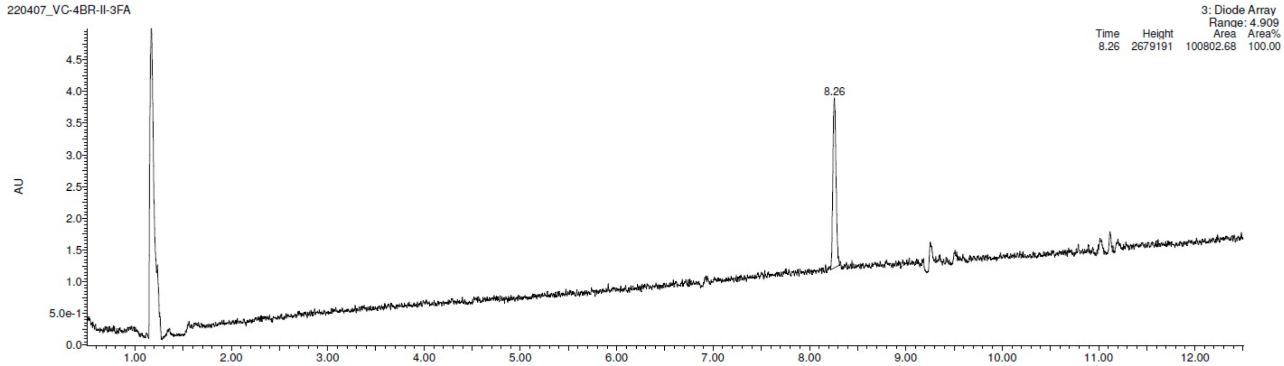
^{13}C-NMR (126 MHz, $CDCl_3$)

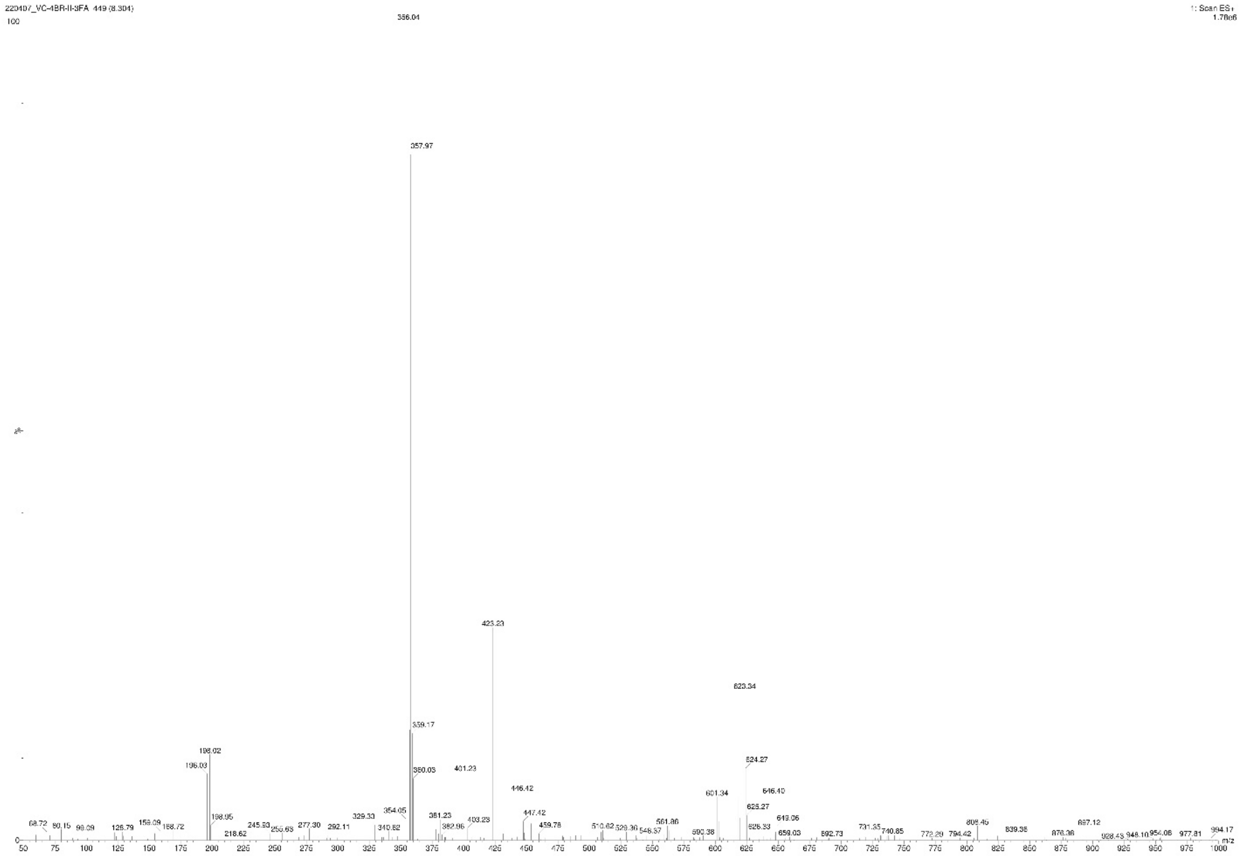


2.7. 4-Bromo-2-[(3-fluorophenyl)sulfonyl]isoindoline **2g**

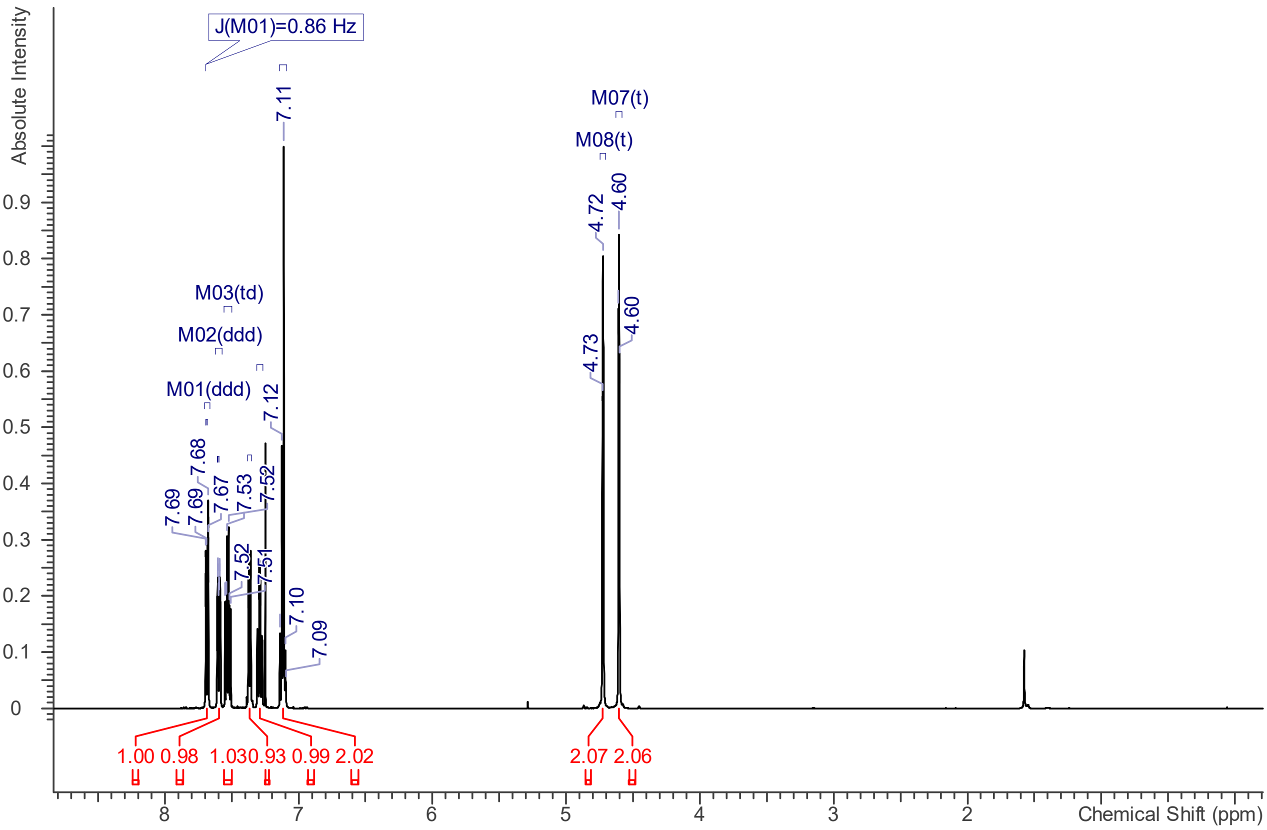


UPLC/MS

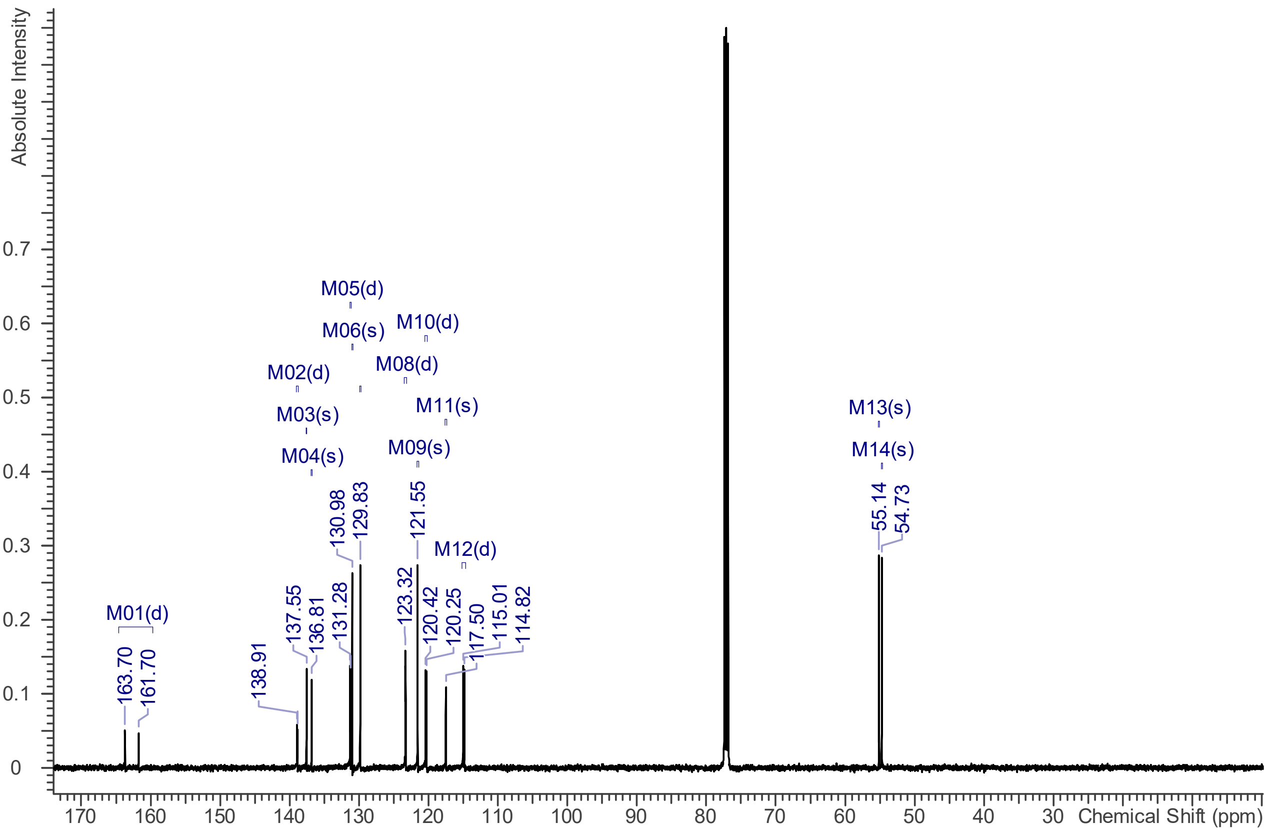




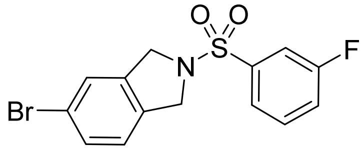
[1]H-NMR (500 MHz, $CDCl_3$)



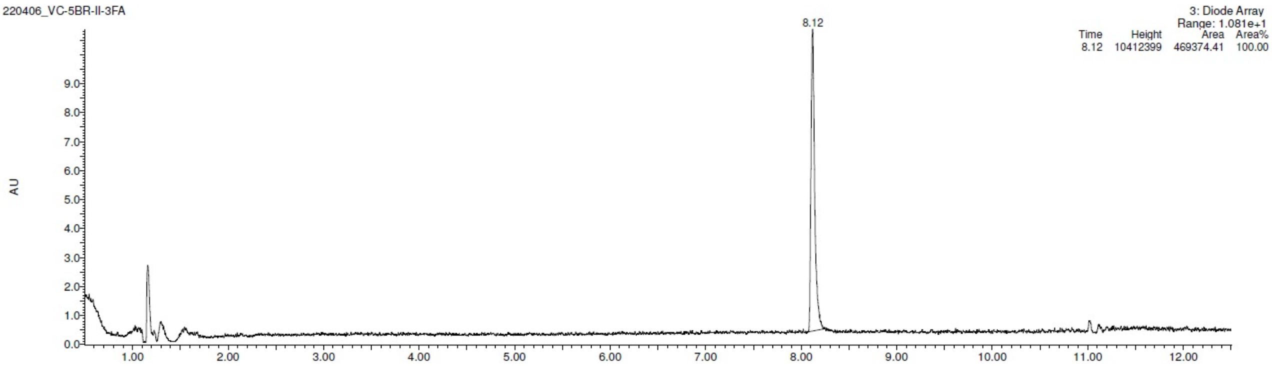
[13]C-NMR (126 MHz, $CDCl_3$)

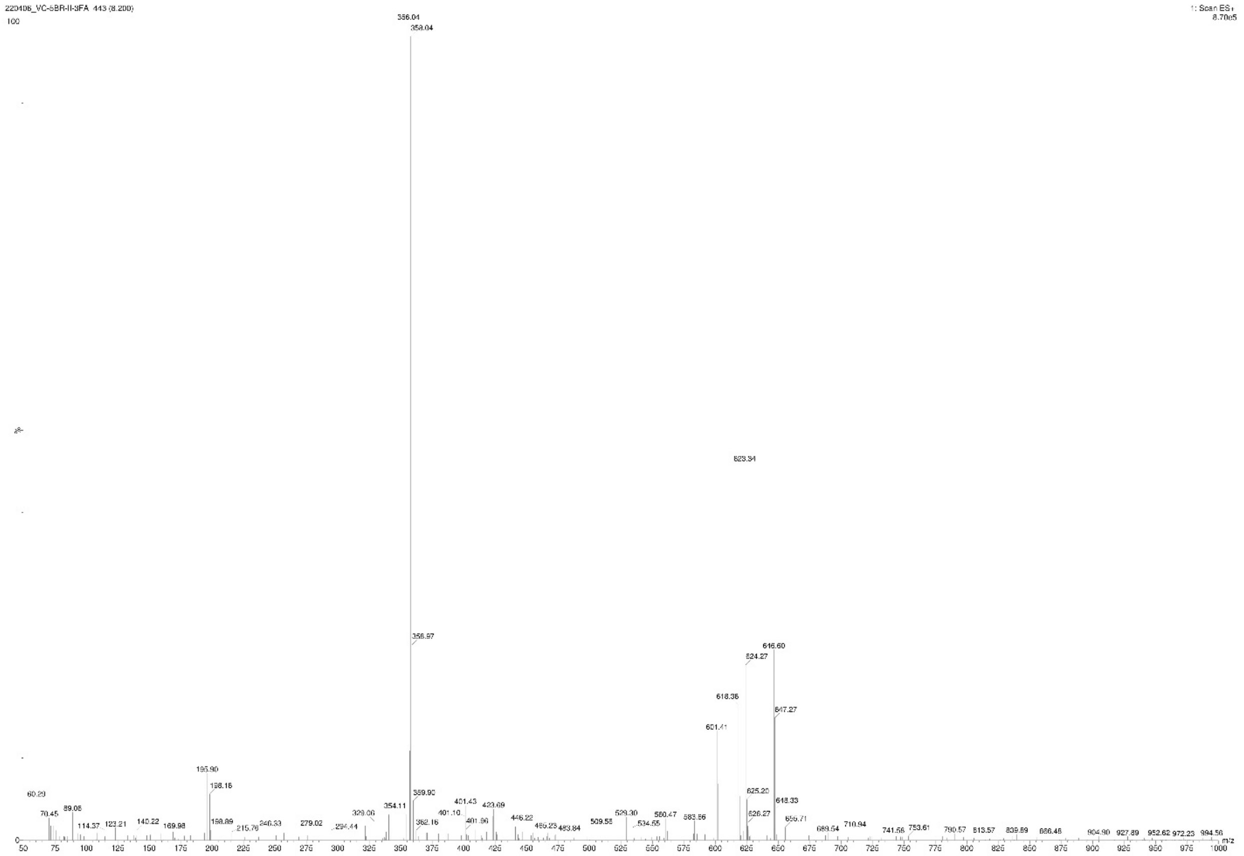


2.8. 5-Bromo-2-[(3-fluorophenyl)sulfonyl]isoindoline **2h**

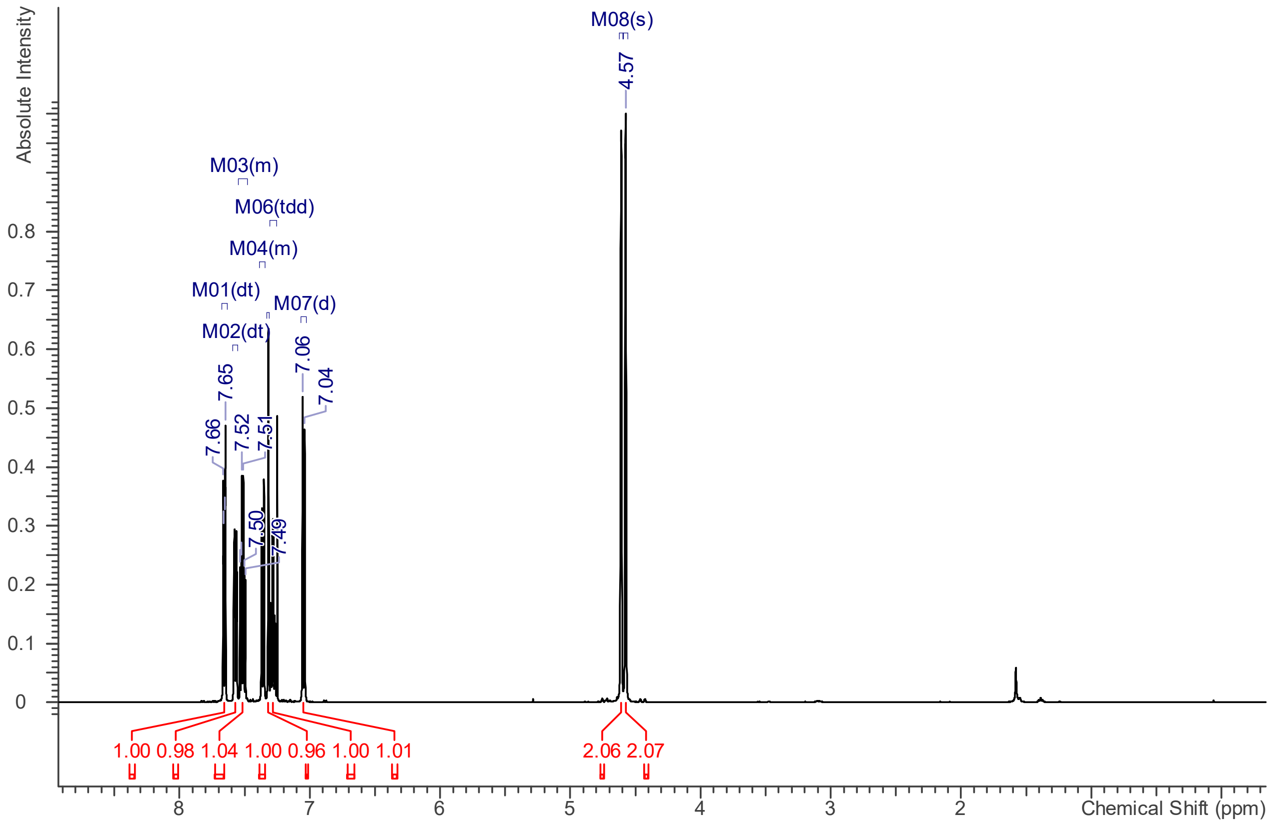


UPLC/MS

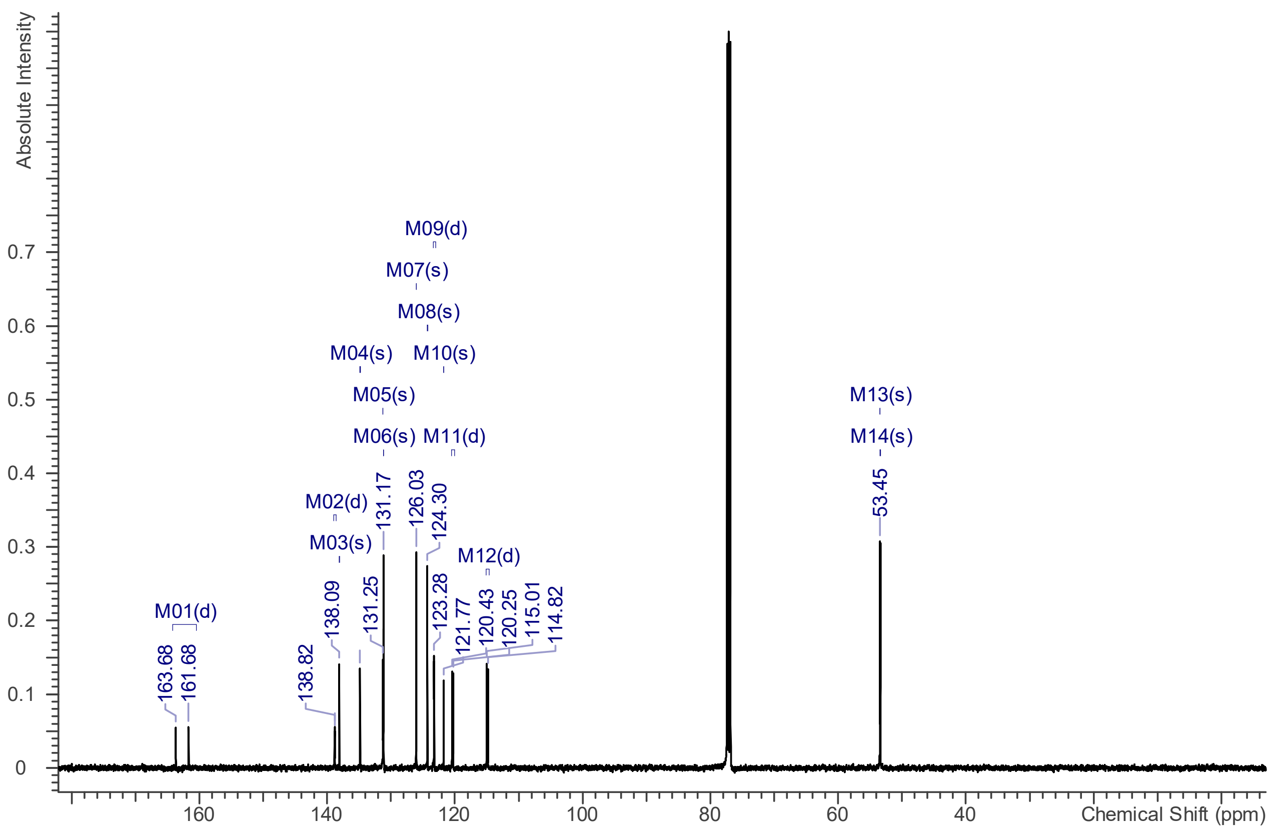




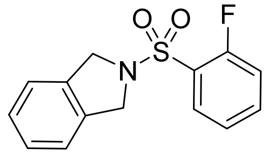
^{1}H-NMR (500 MHz, $CDCl_3$)



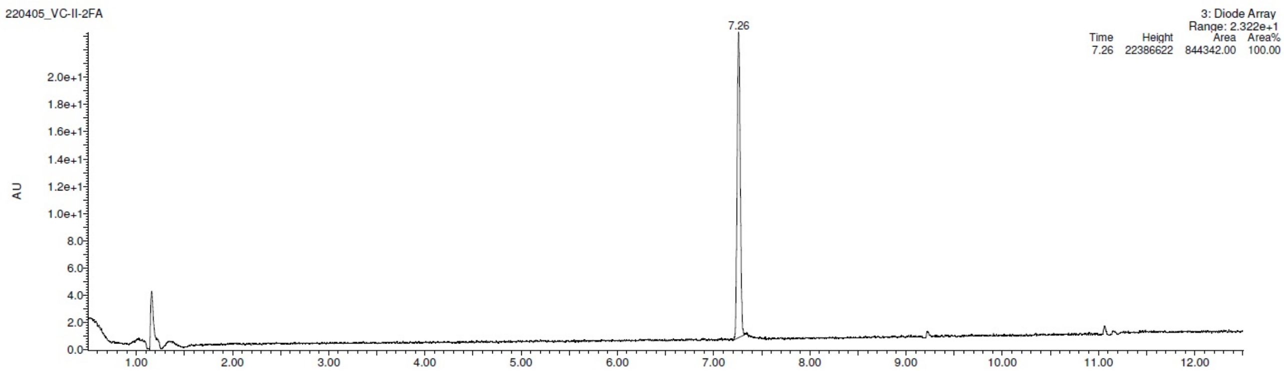
^{13}C-NMR (126 MHz, $CDCl_3$)

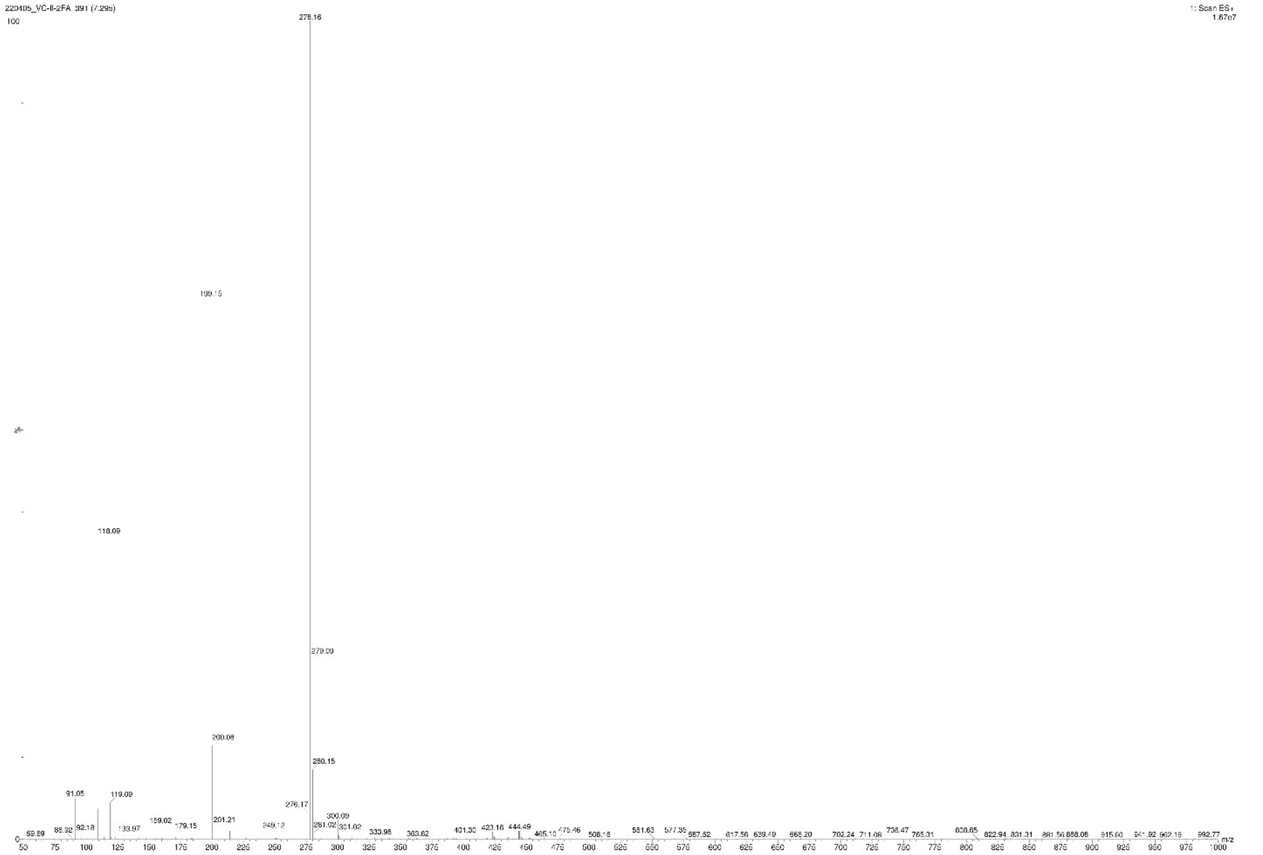


2.9. 2-[(2-Fluorophenyl)sulfonyl]isoindoline **2i**

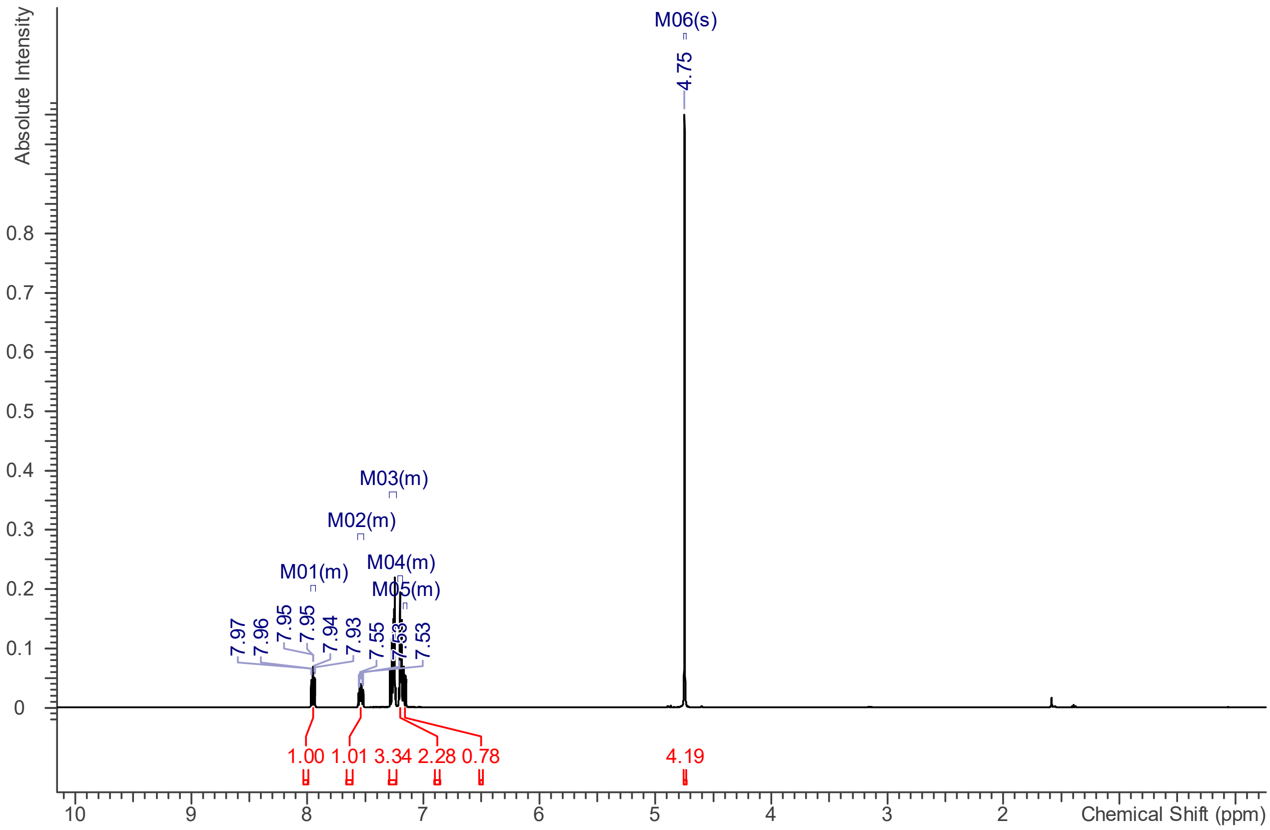


UPLC/MS

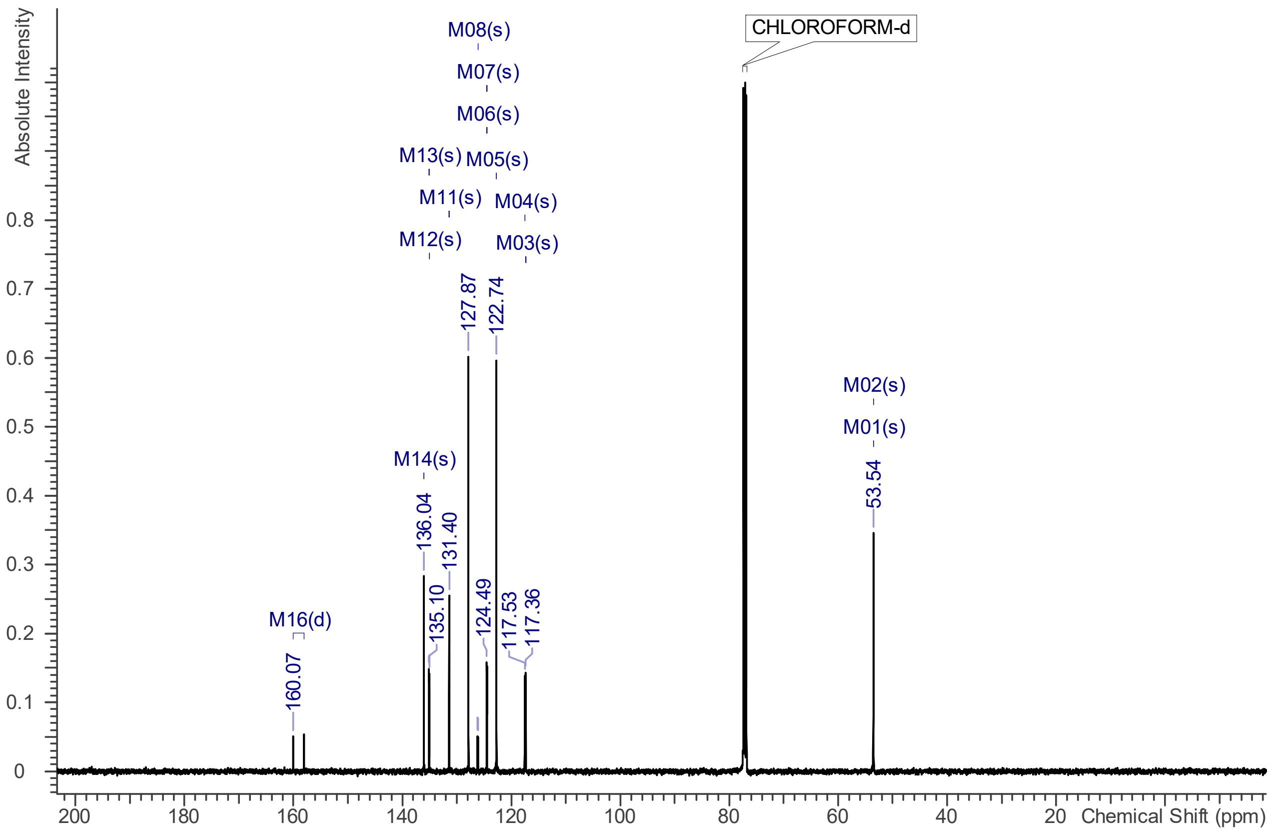




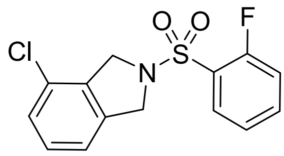
^{1}H-NMR (500 MHz, $CDCl_3$)



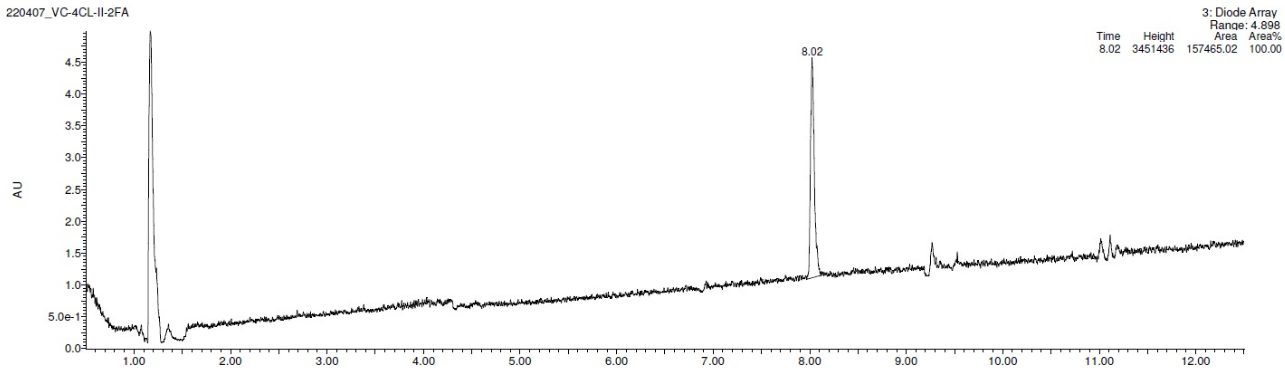
^{13}C-NMR (126 MHz, $CDCl_3$)

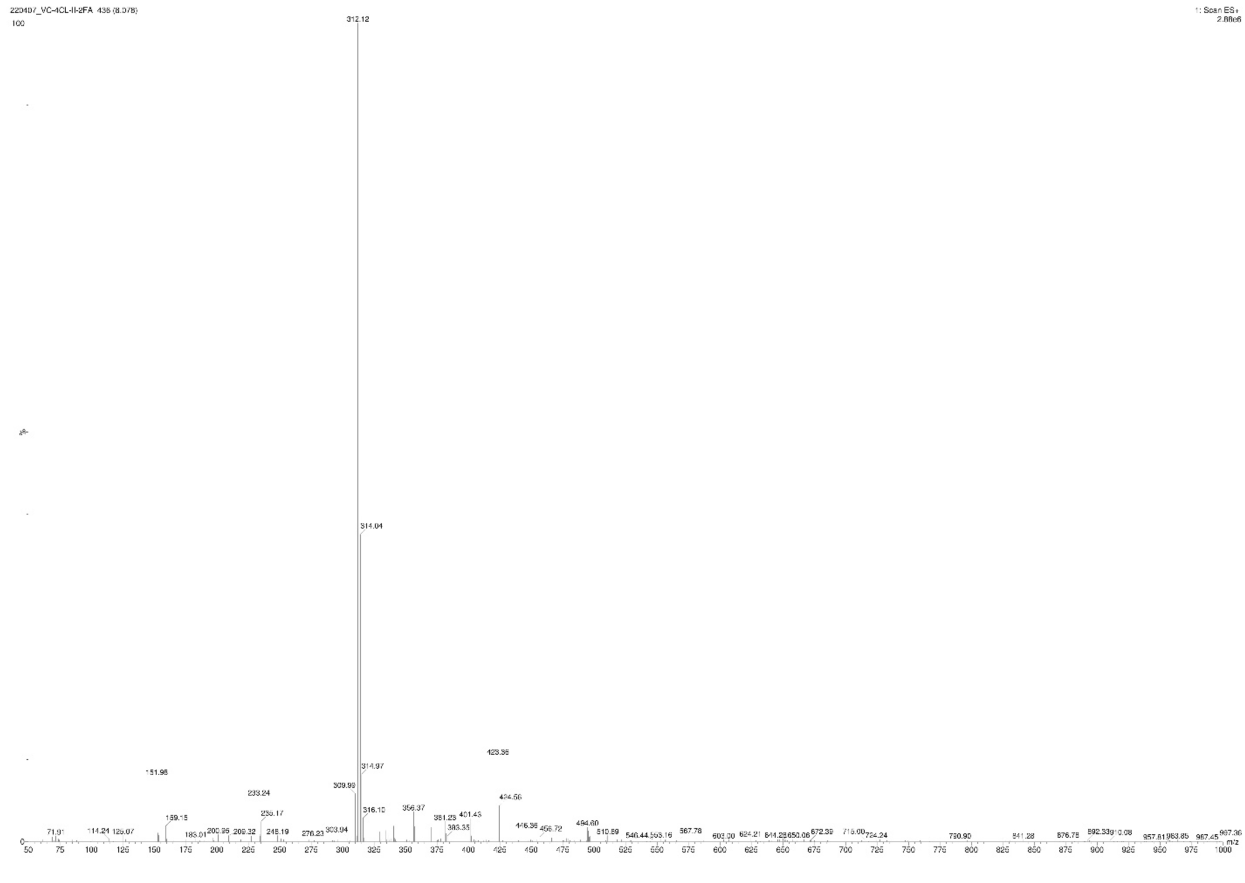


2.10. 4-Chloro-2-[(2-fluorophenyl)sulfonyl]isoindoline **2j**

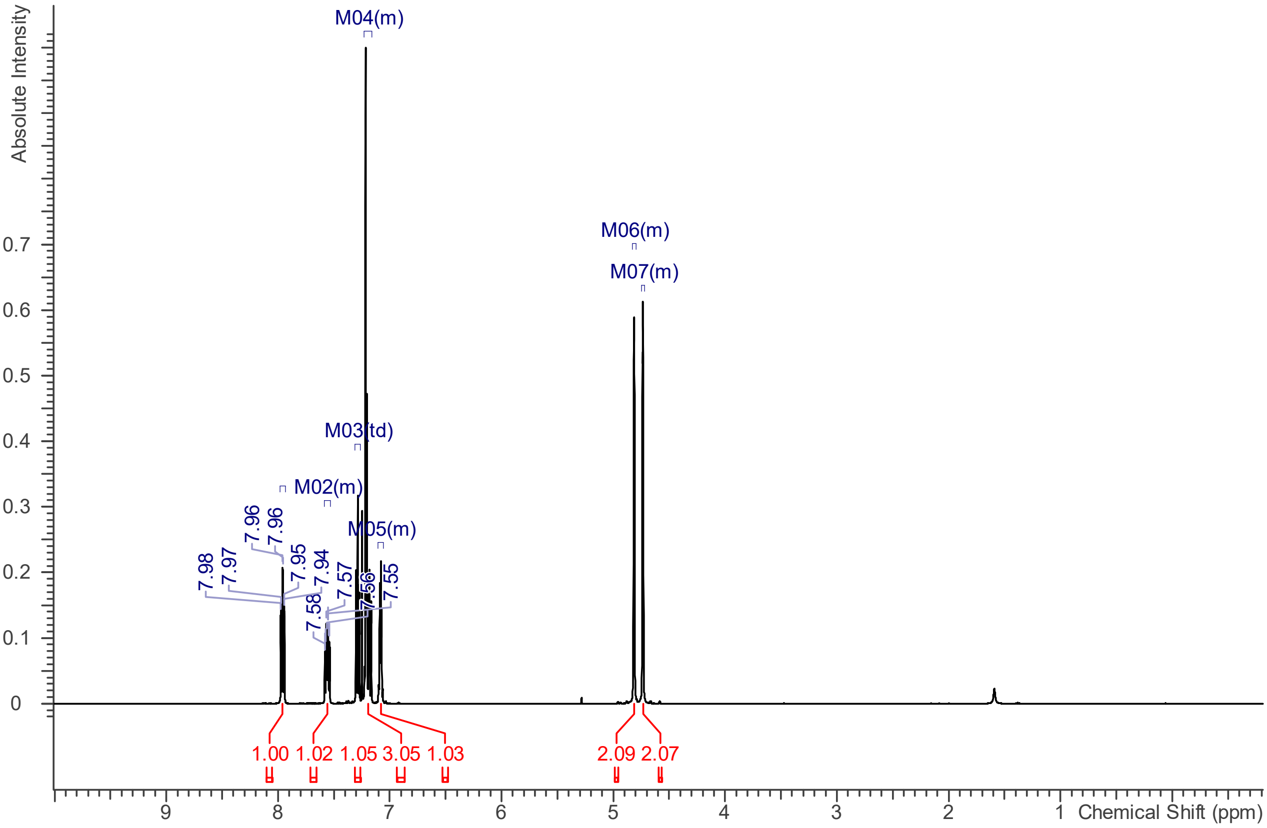


UPLC/MS

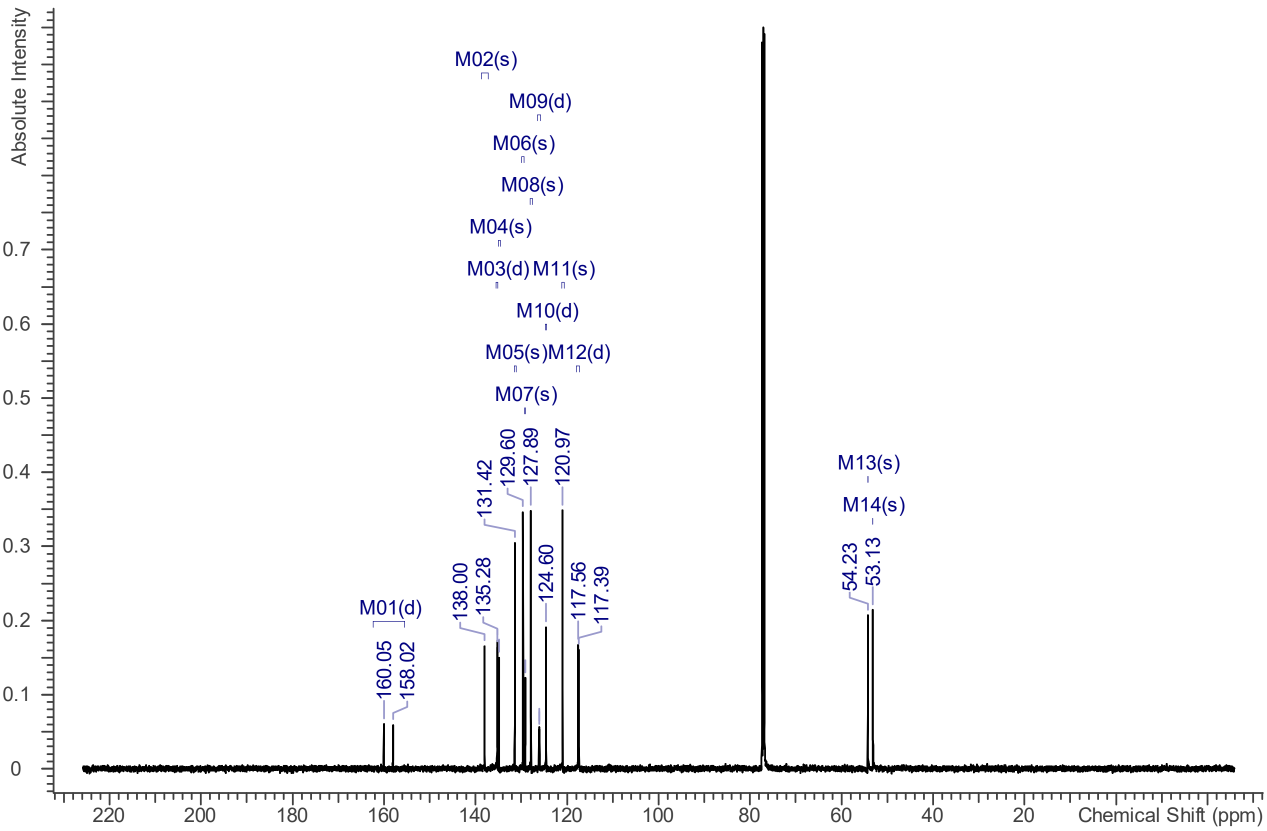




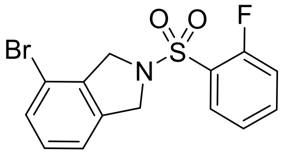
^{1}H-NMR (500 MHz, $CDCl_3$)



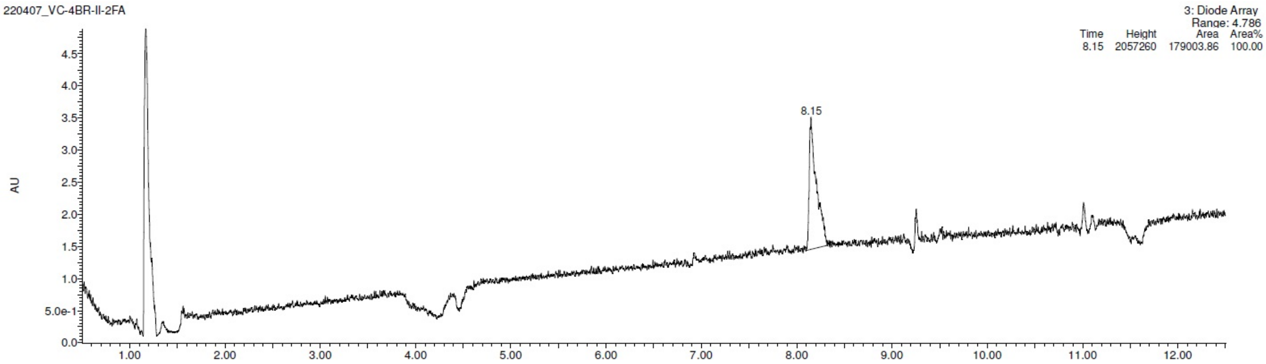
^{13}C-NMR (126 MHz, $CDCl_3$)

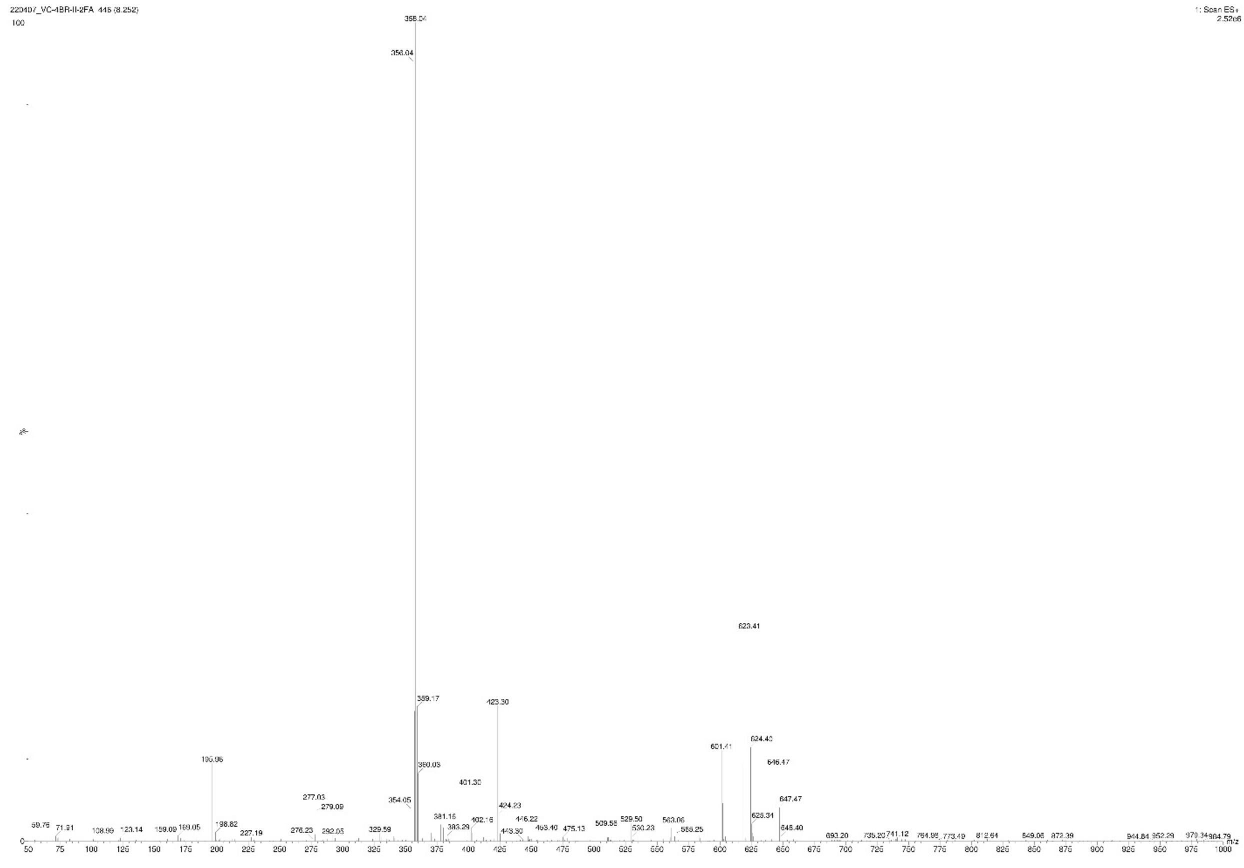


2.11. 4-Bromo-2-[(2-fluorophenyl)sulfonyl]isoindoline **2k**

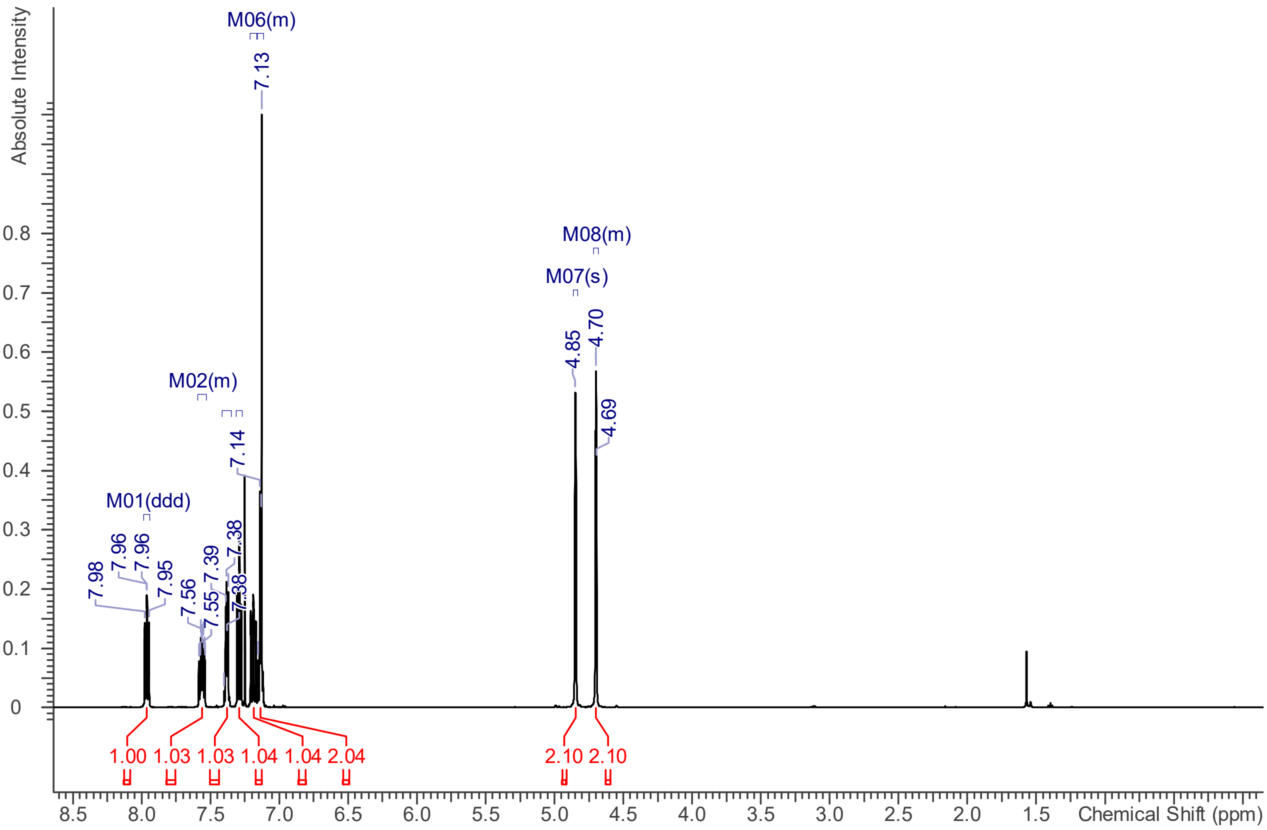


UPLC/MS

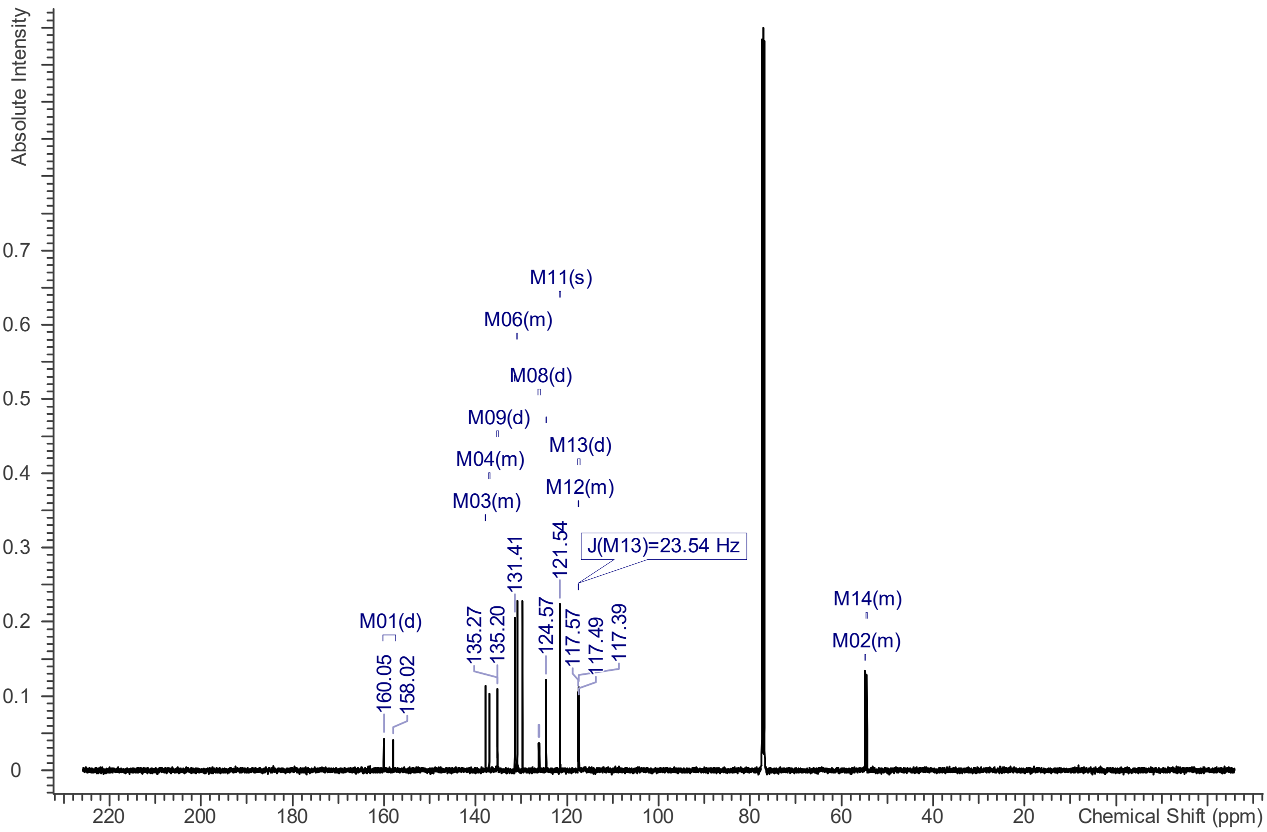




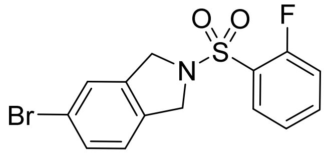
^{1}H-NMR (500 MHz, $CDCl_3$)



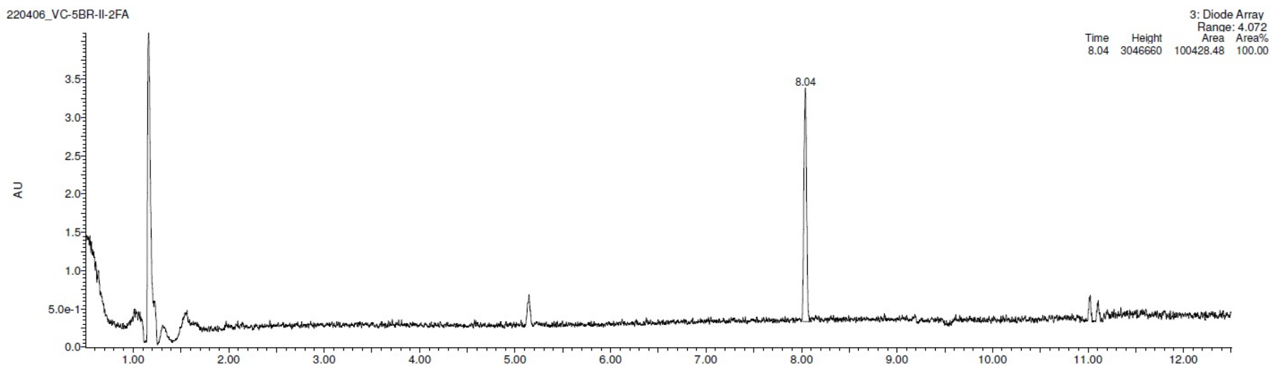
^{13}C-NMR (126 MHz, $CDCl_3$)

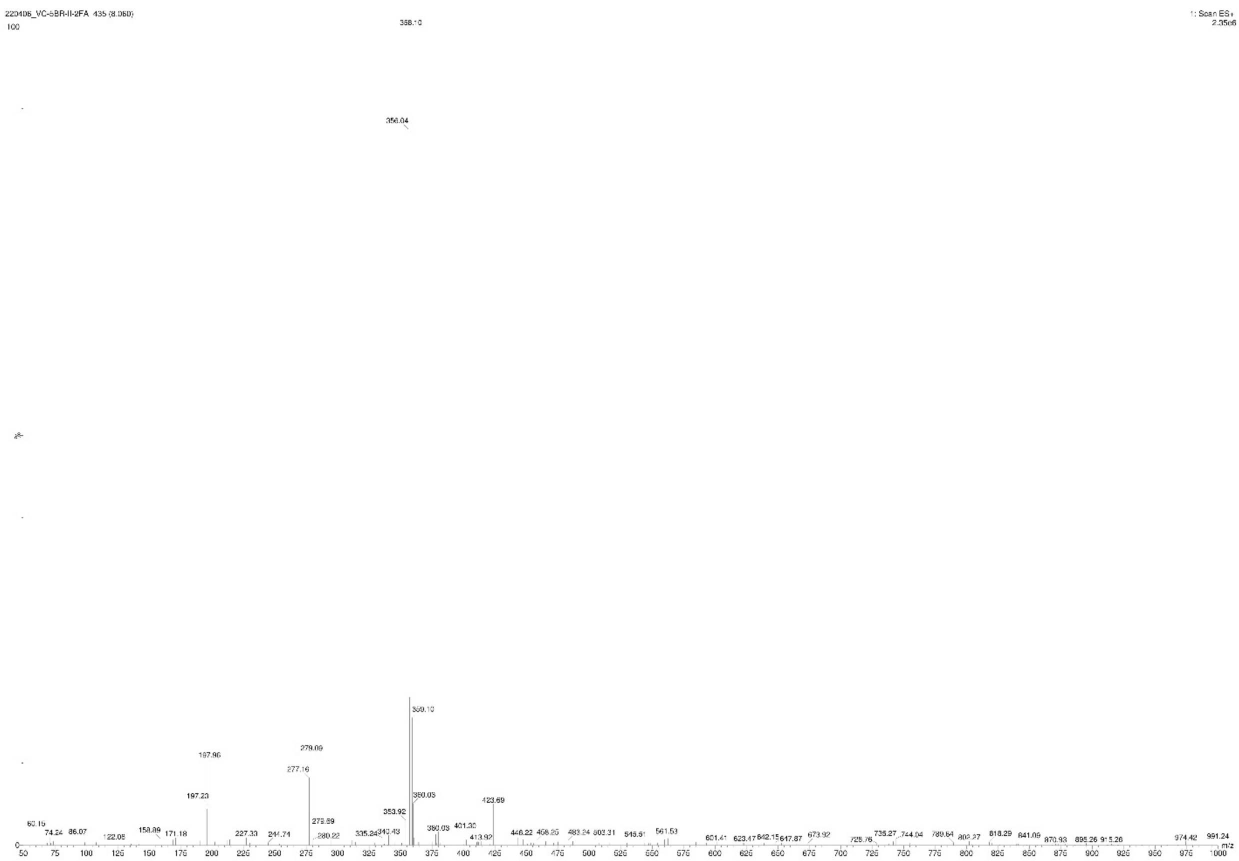


2.12. 5-Bromo-2-[(2-fluorophenyl)sulfonyl]isoindoline **2l**

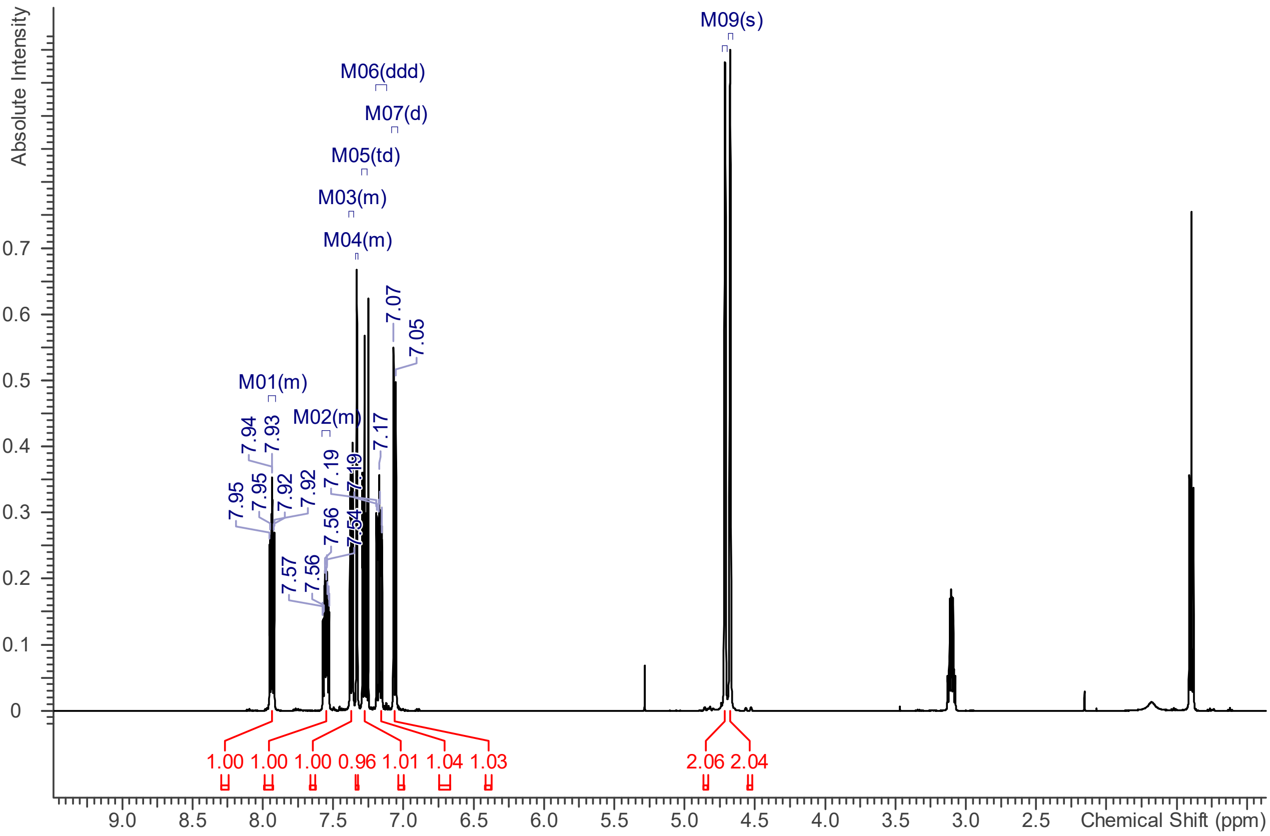


UPLC/MS

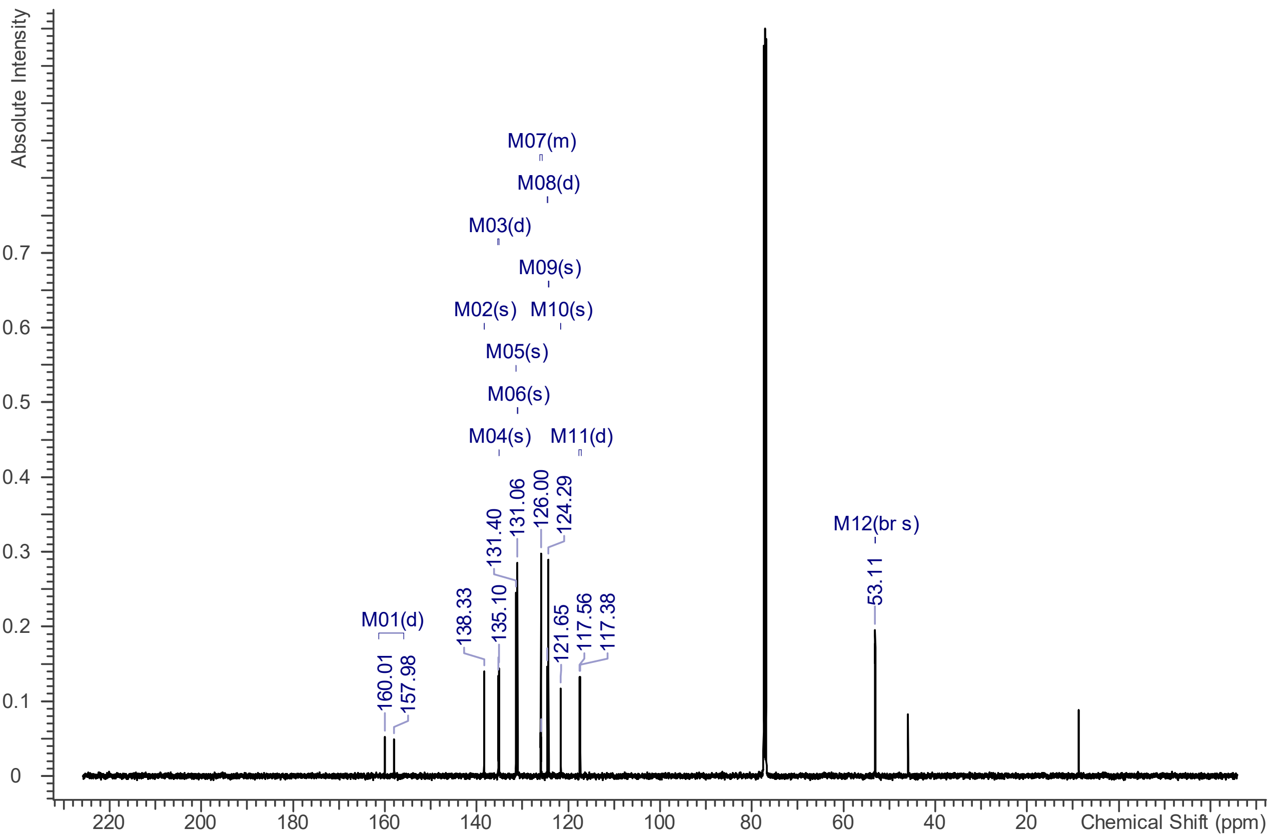




^{1}H-NMR (500 MHz, $CDCl_3$)

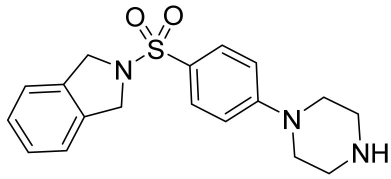


^{13}C-NMR (126 MHz, $CDCl_3$)

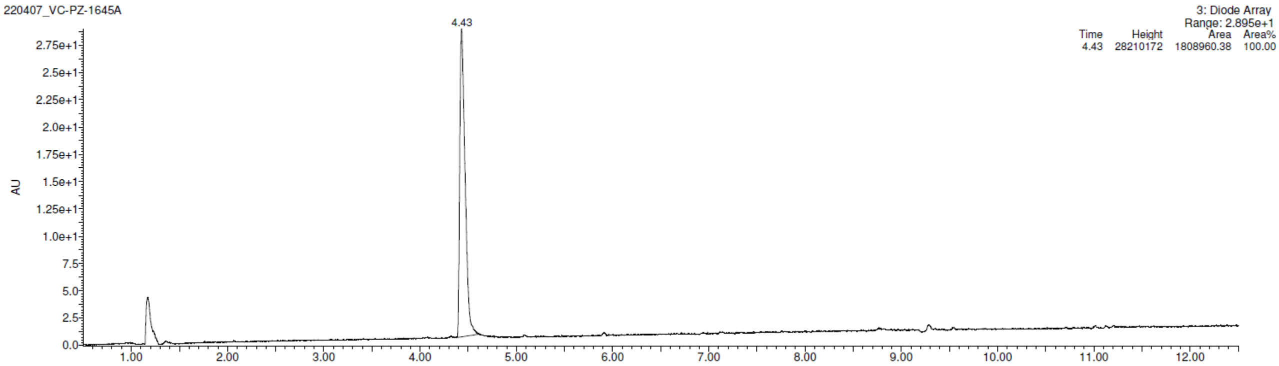


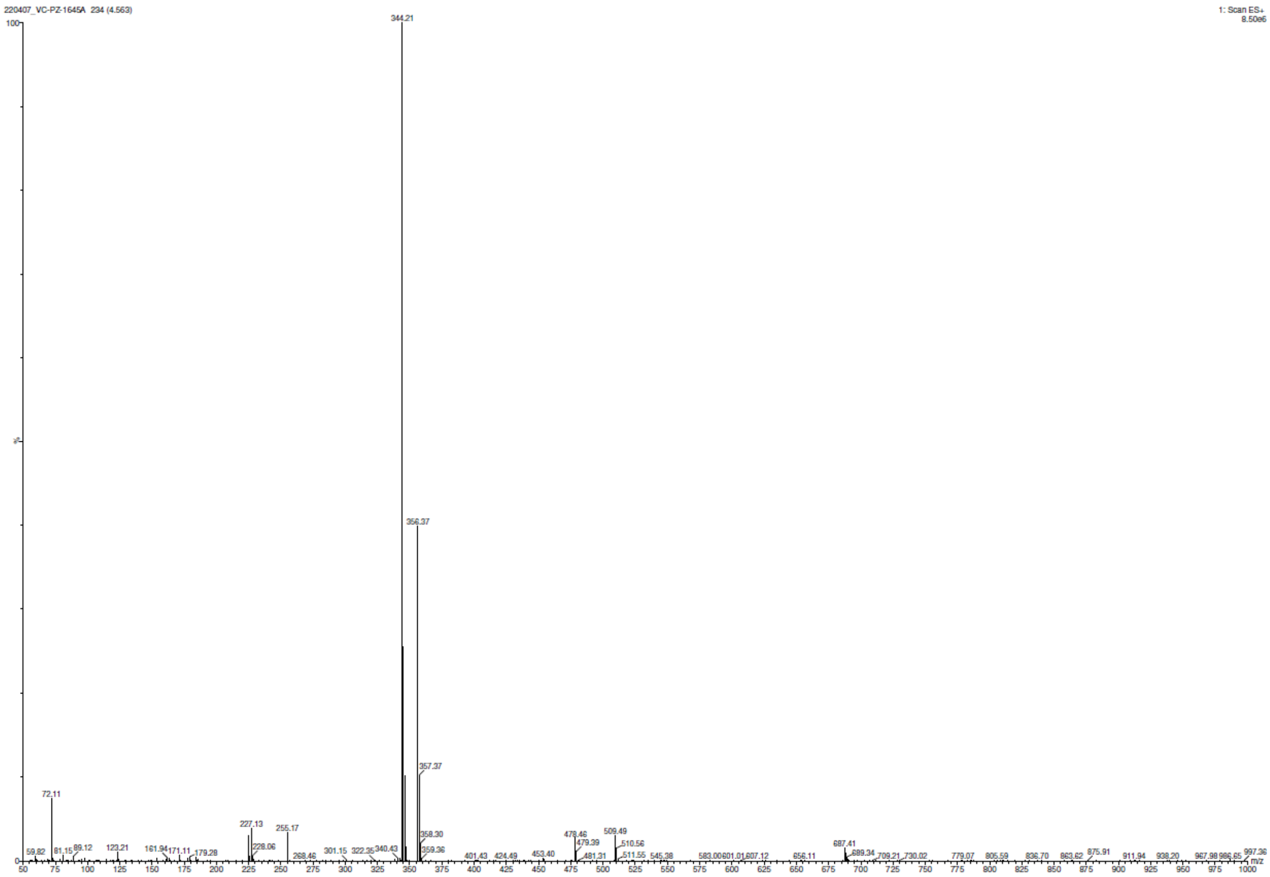
3. UPLC/MS, ^{1}H NMR and ^{13}C NMR spectra of final compounds 3a–l

3.1. 2-{[4-(Piperazin-1-yl)phenyl]sulfonyl}isoindoline **3a**

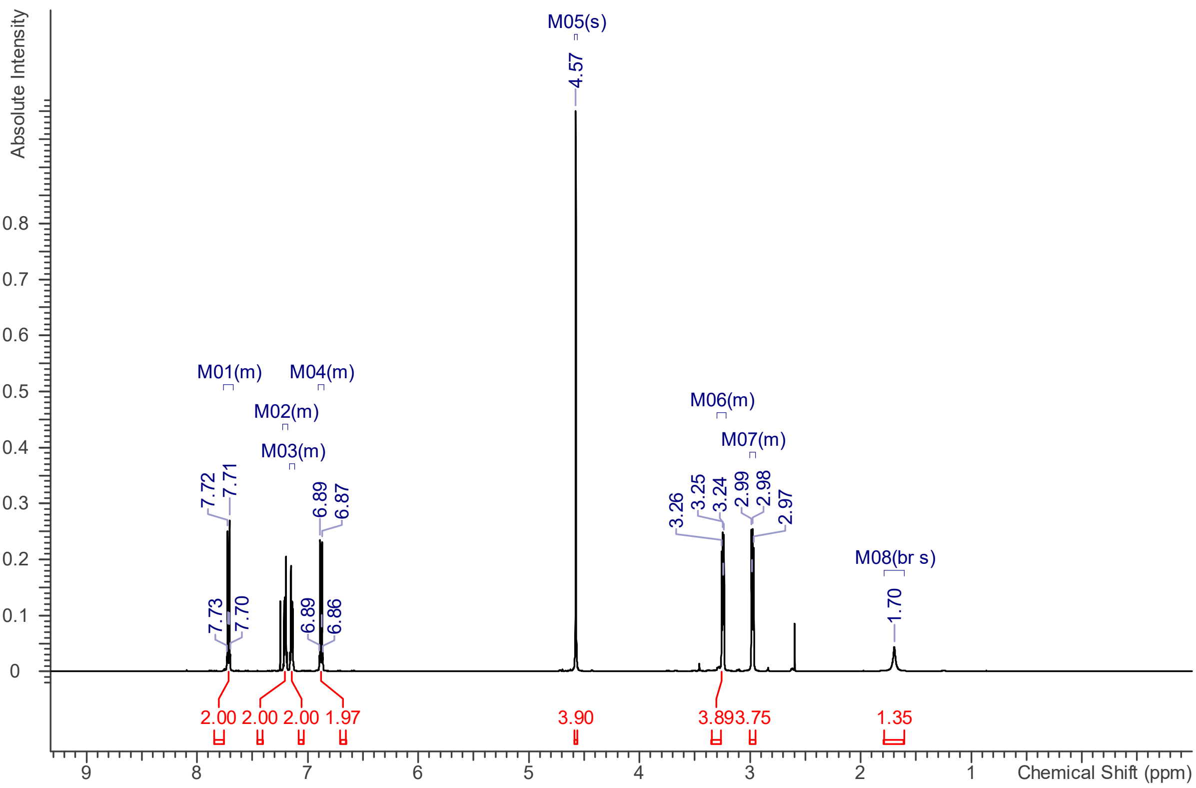


UPLC/MS

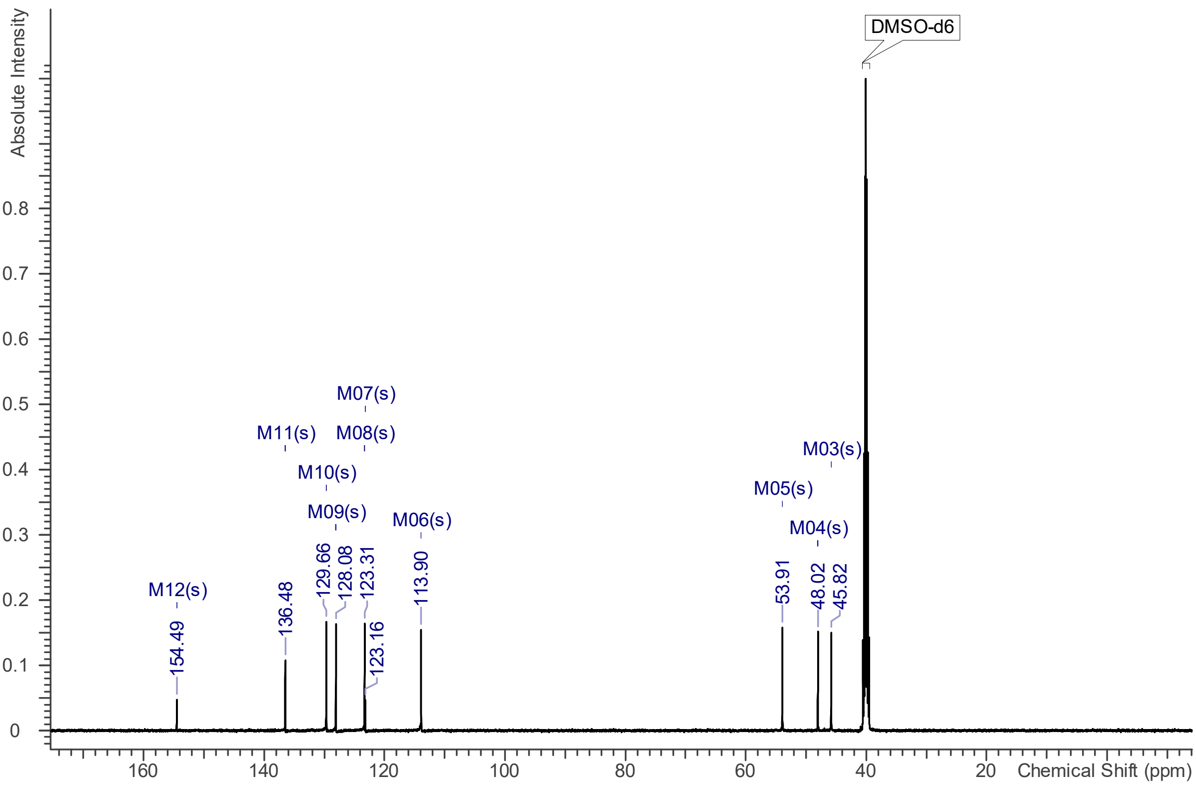




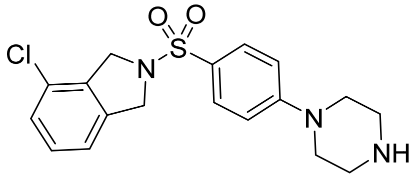
^{1}H-NMR (500 MHz, $CDCl_3$)



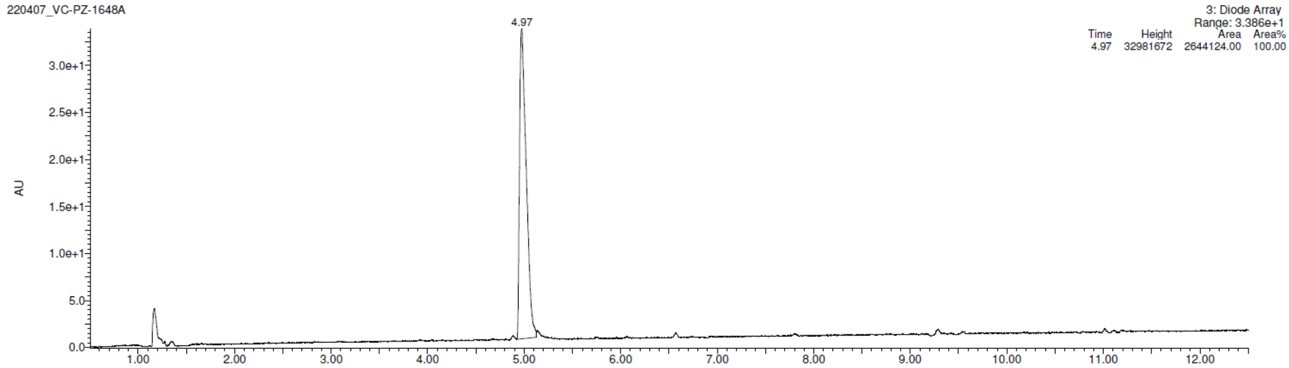
^{13}C-NMR (126 MHz, DMSO-d_6)

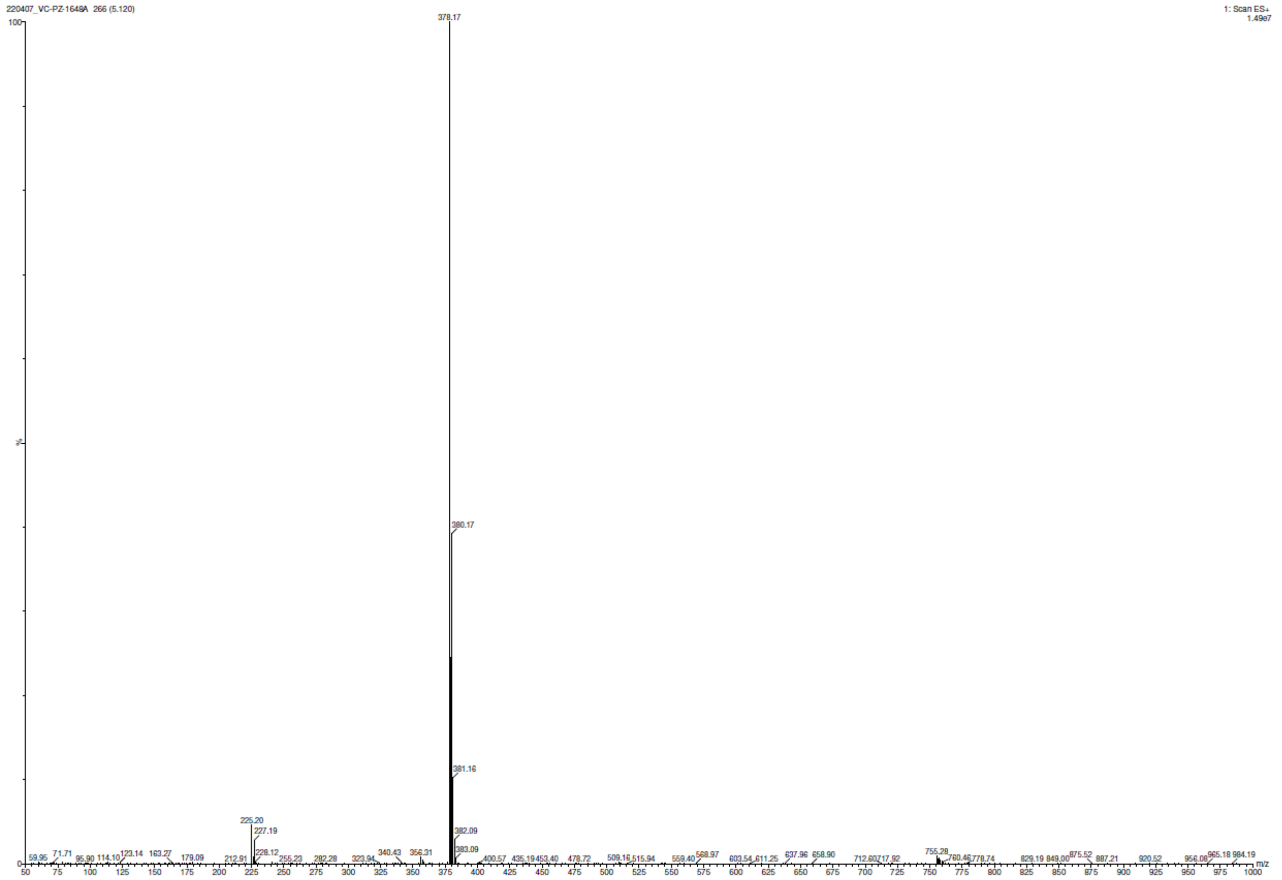


3.2. 4-Chloro-2-{[4-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3b**

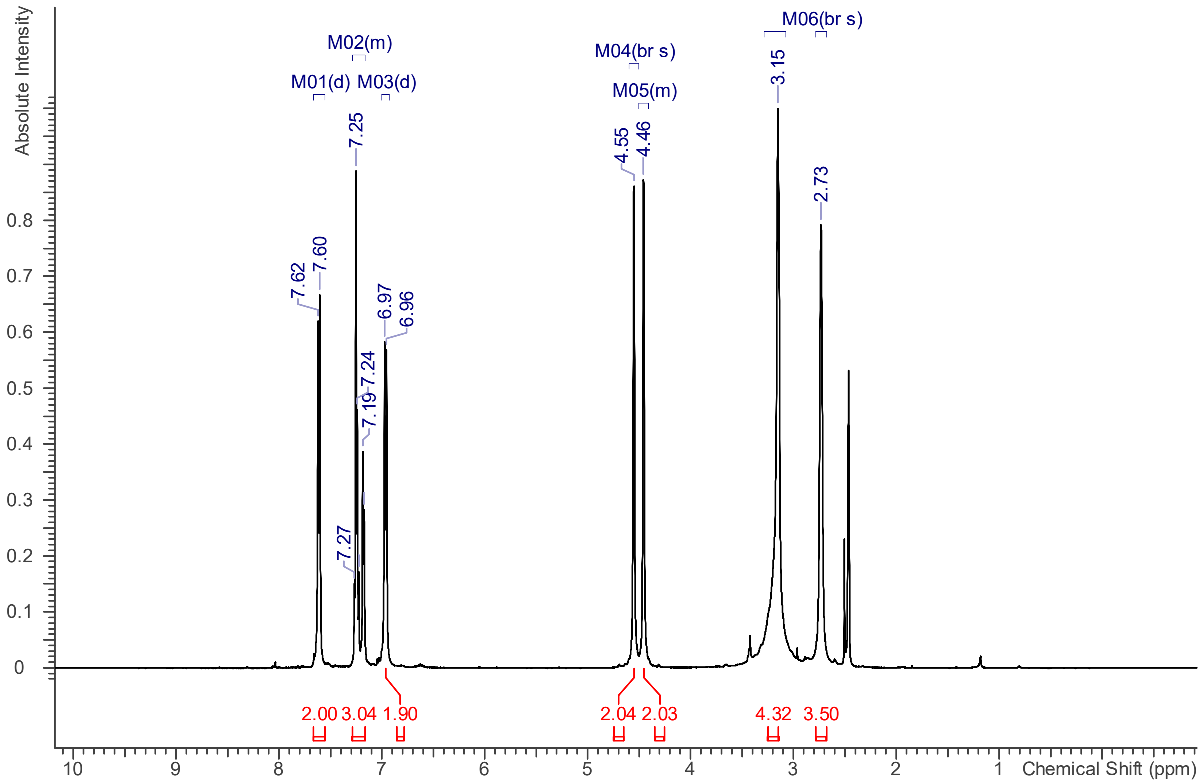


UPLC/MS

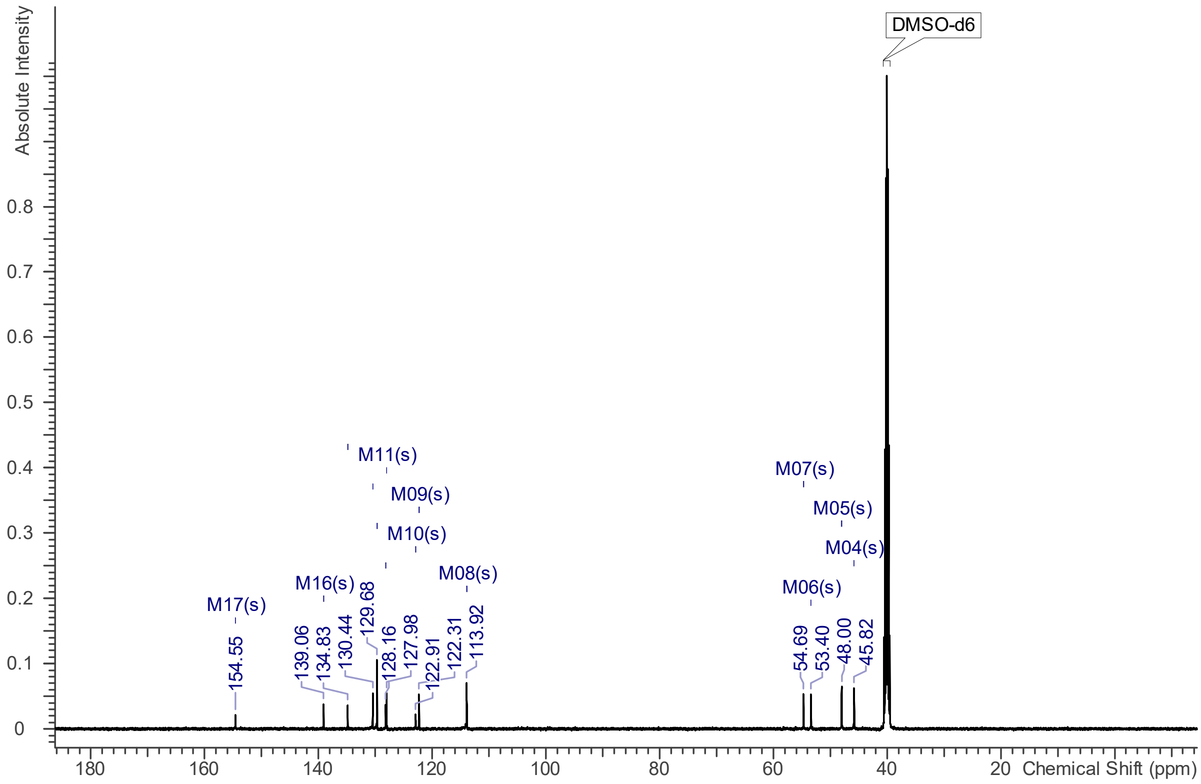




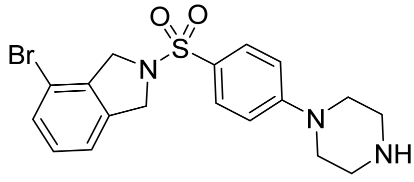
^{1}H-NMR (500 MHz, DMSO-d_6)



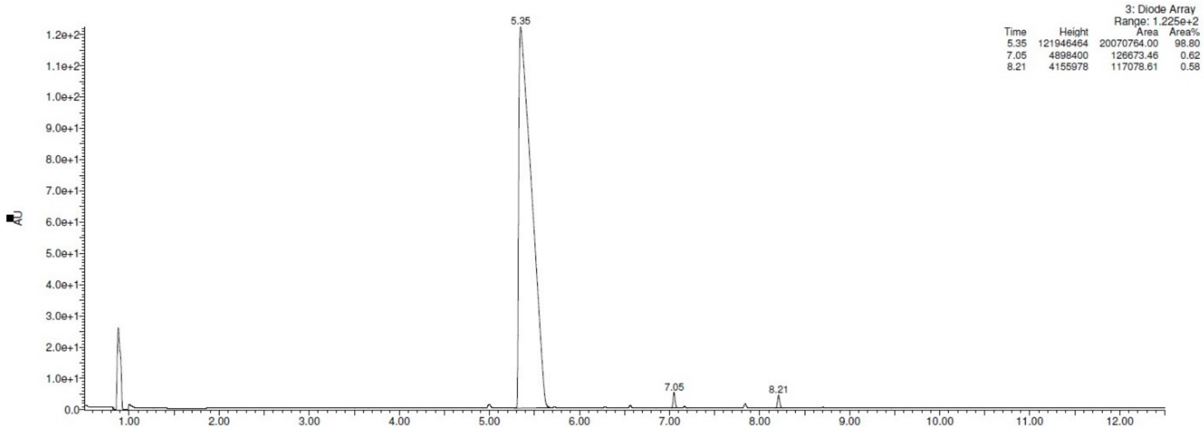
^{13}C-NMR (126 MHz, DMSO-d_6)

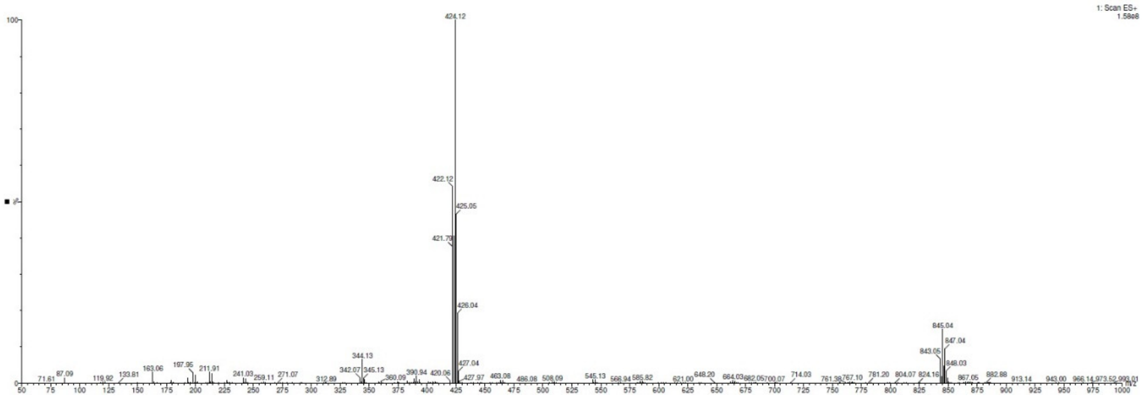


3.3. 4-Bromo-2-{[4-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3c**

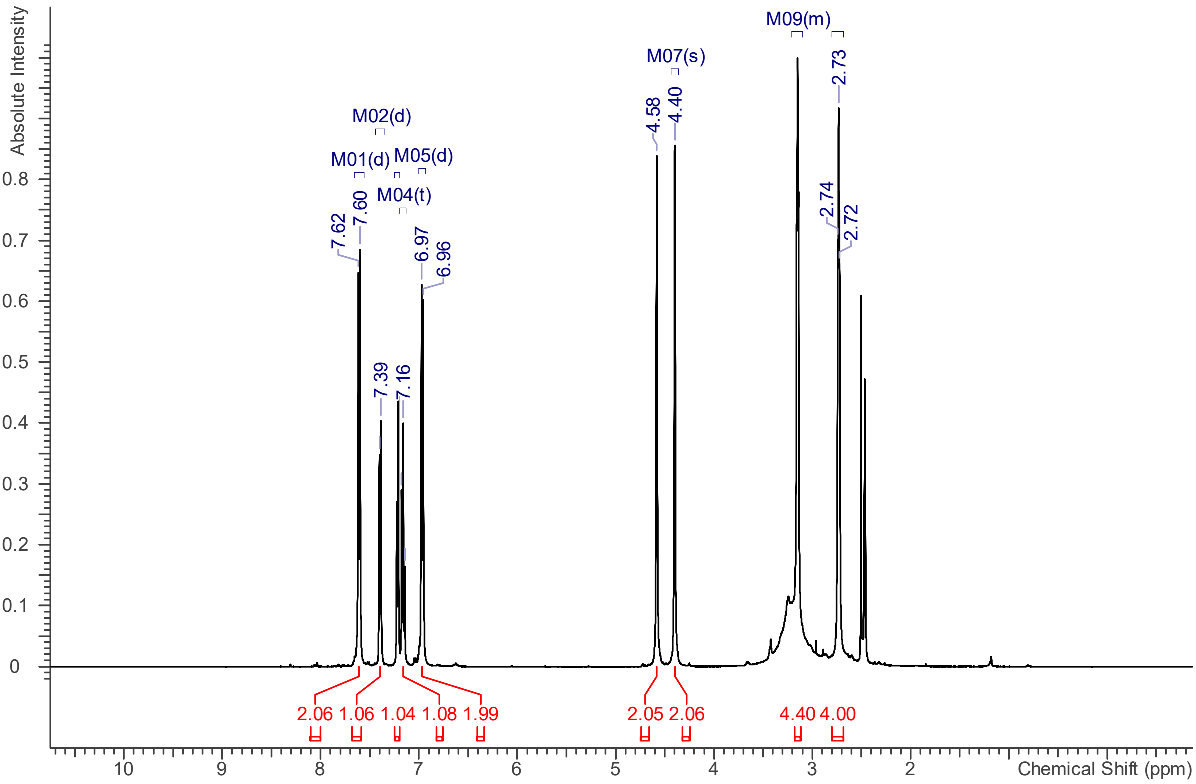


UPLC/MS

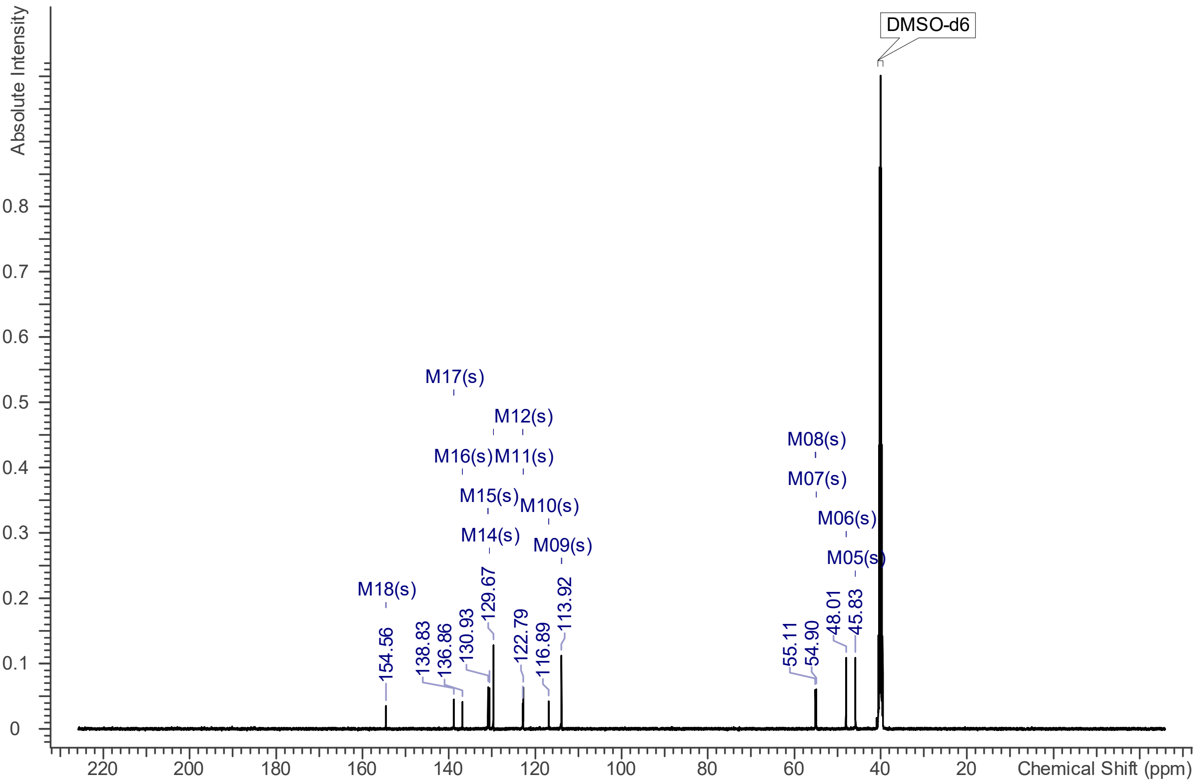




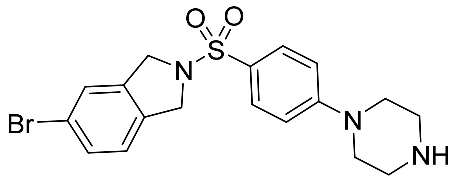
^{1}H-NMR (500 MHz, DMSO-d_6)



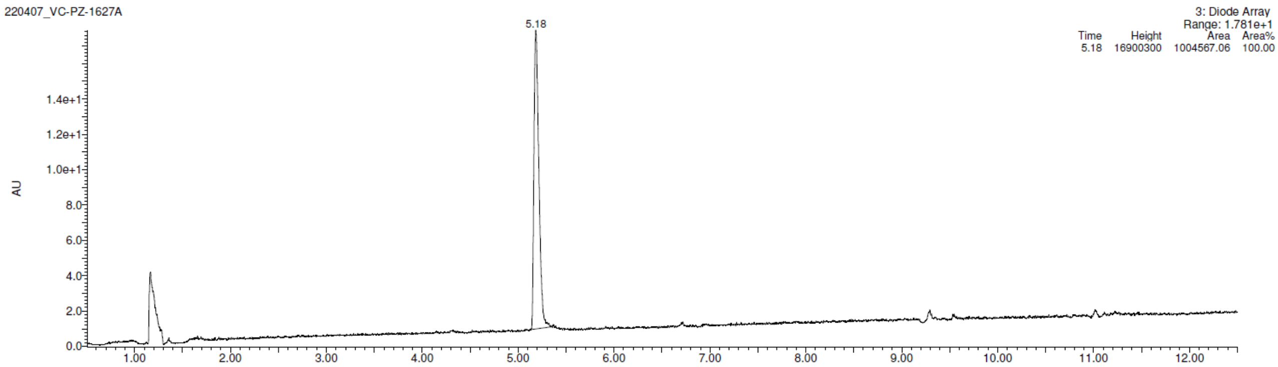
^{13}C-NMR (126 MHz, DMSO-d_6)

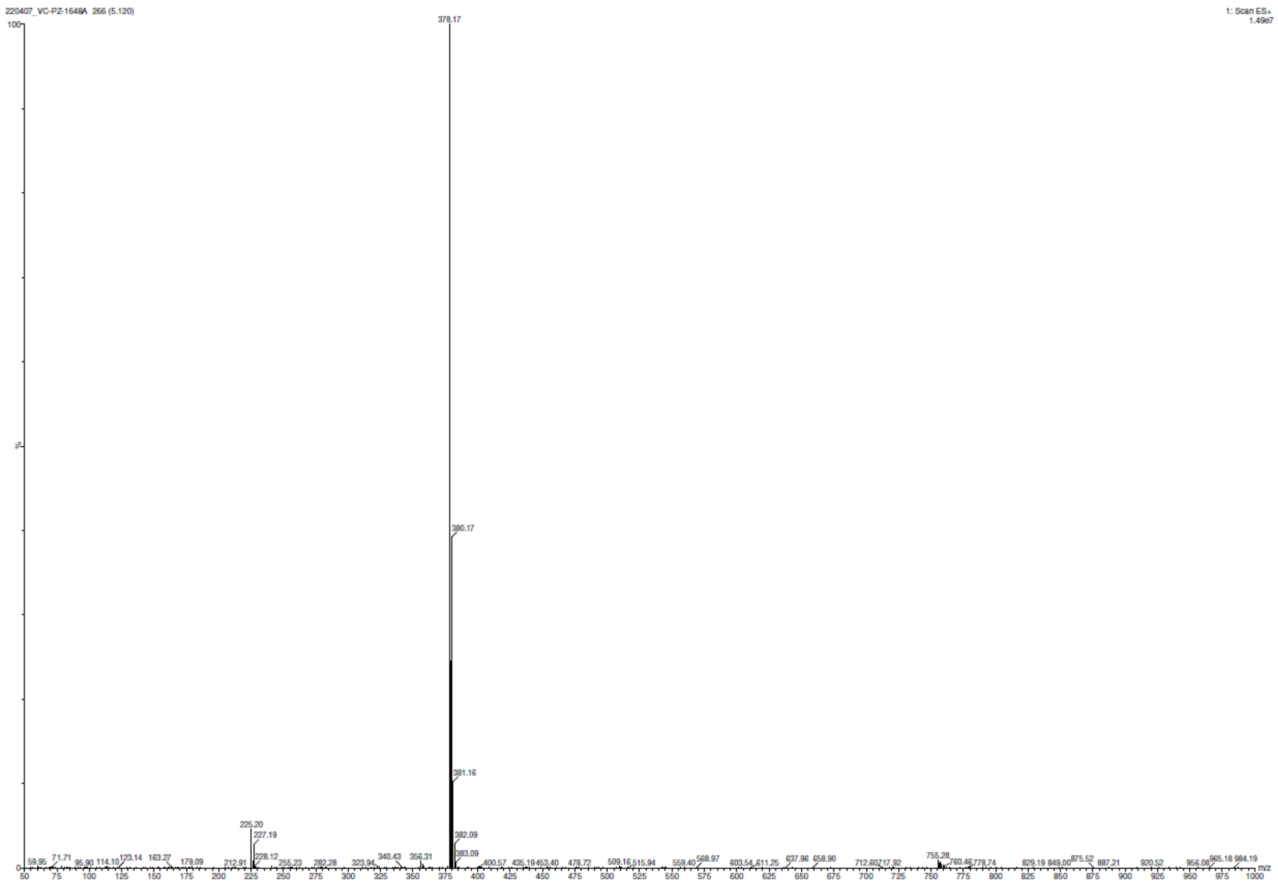


3.4. 5-Bromo-2-{[4-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3d**

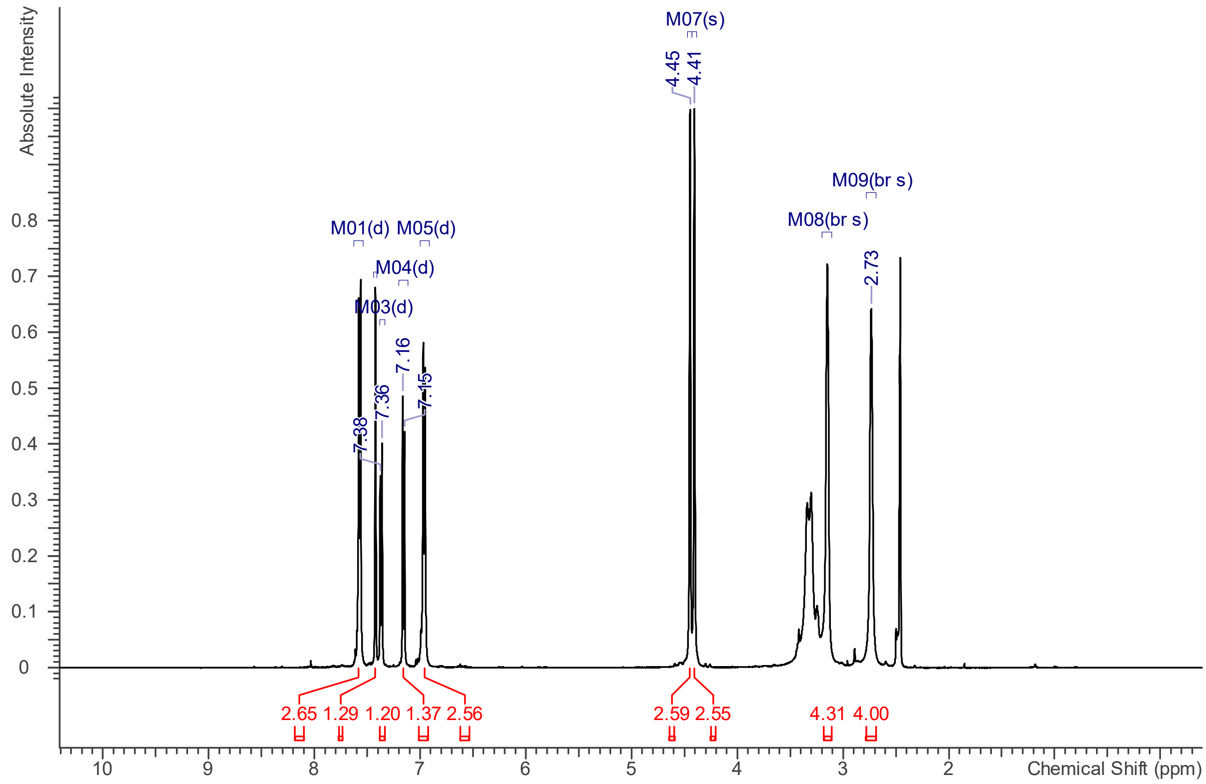


UPLC/MS

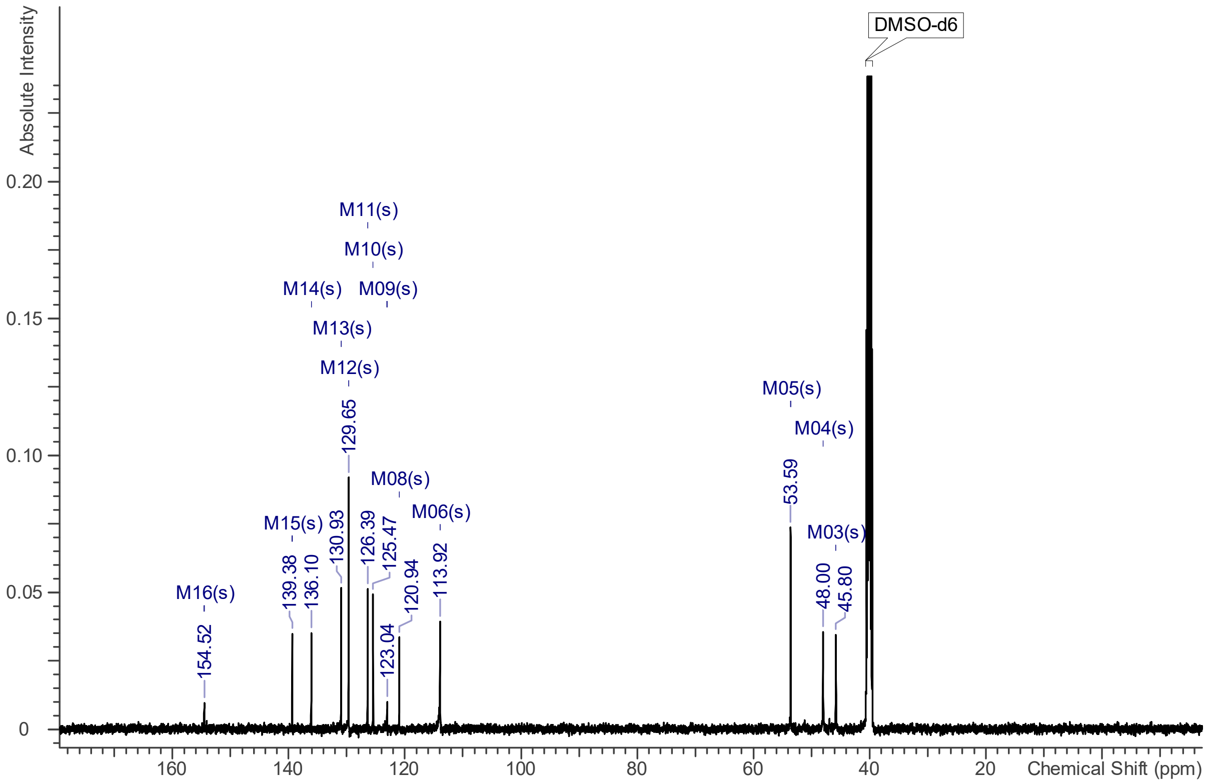




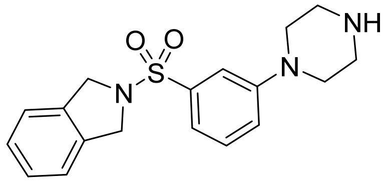
^{1}H-NMR (500 MHz, DMSO-d_6)



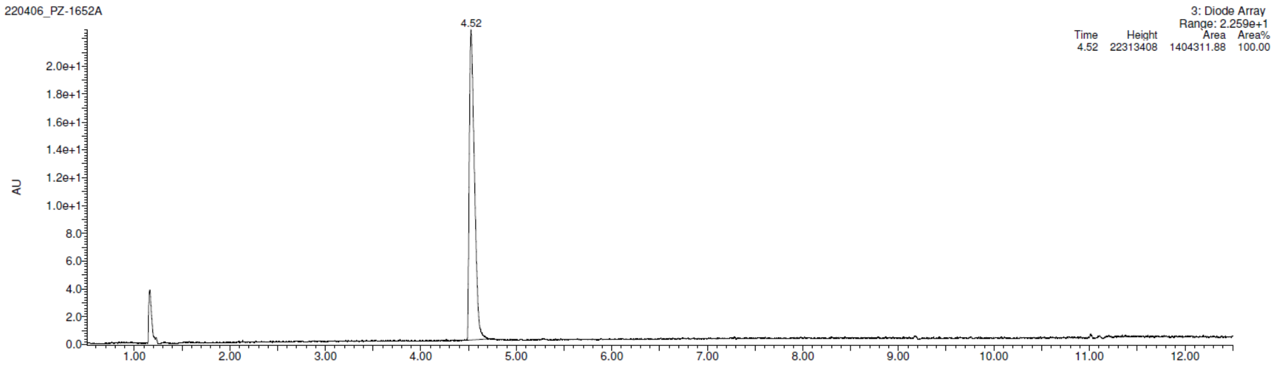
^{13}C-NMR (126 MHz, DMSO-d_6)

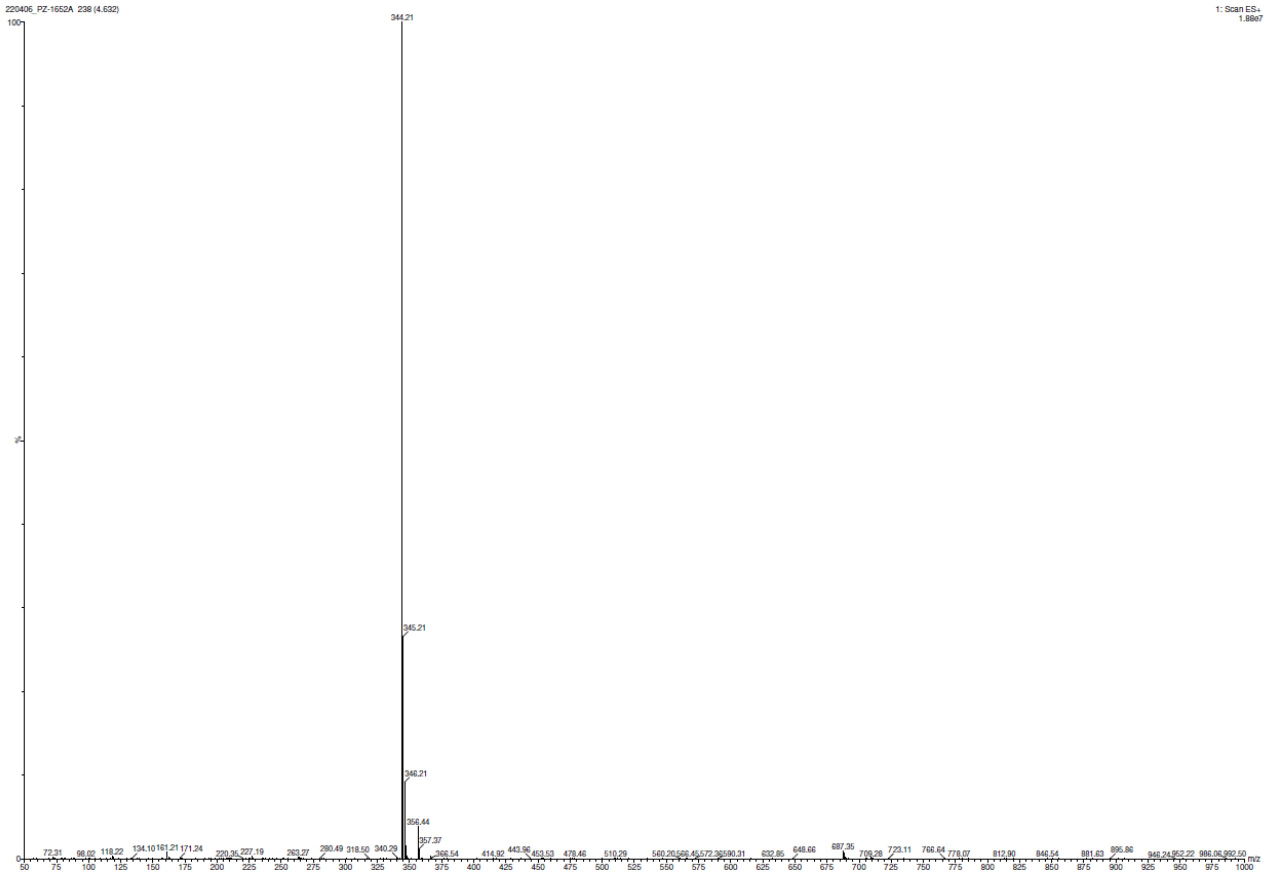


3.5. 2-{[3-(Piperazin-1-yl)phenyl]sulfonyl}isoindoline **3e**

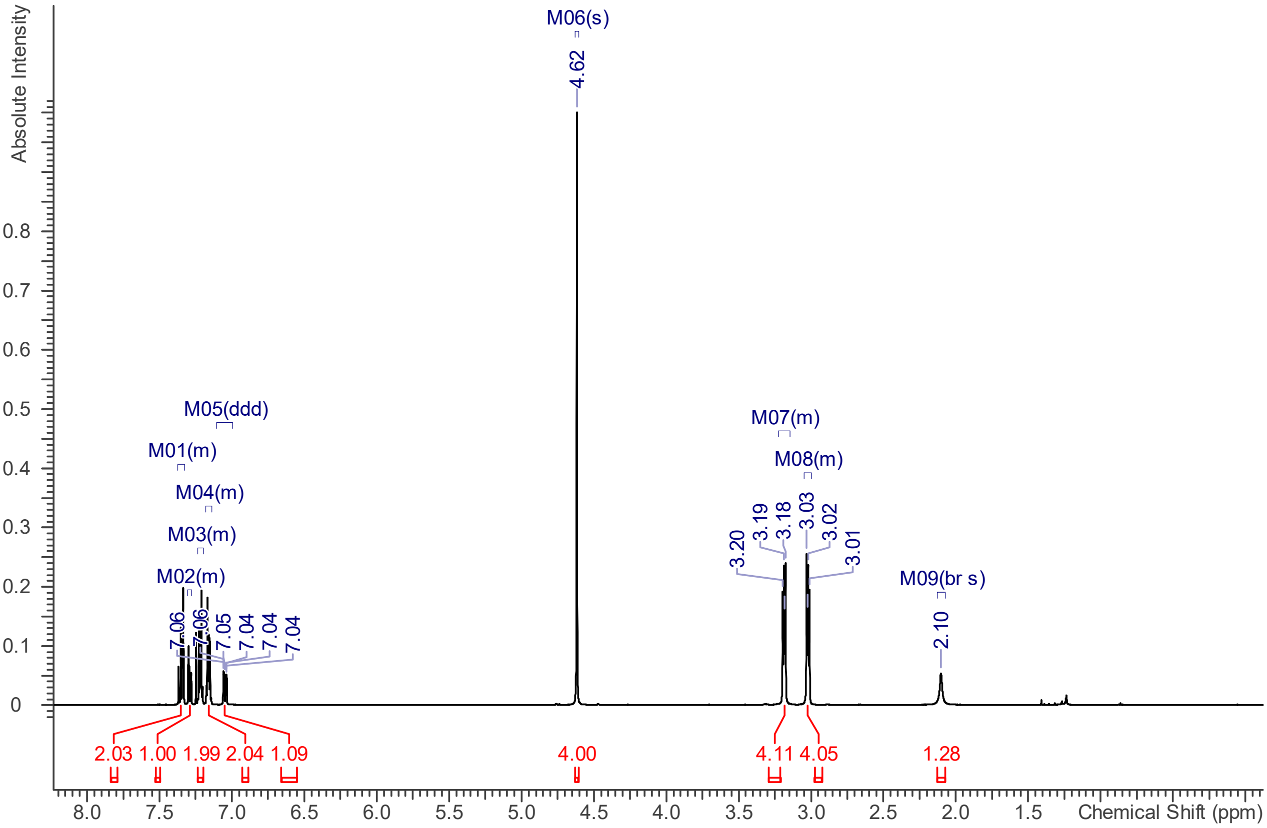


UPLC/MS





[1]H-NMR (500 MHz, $CDCl_3$)



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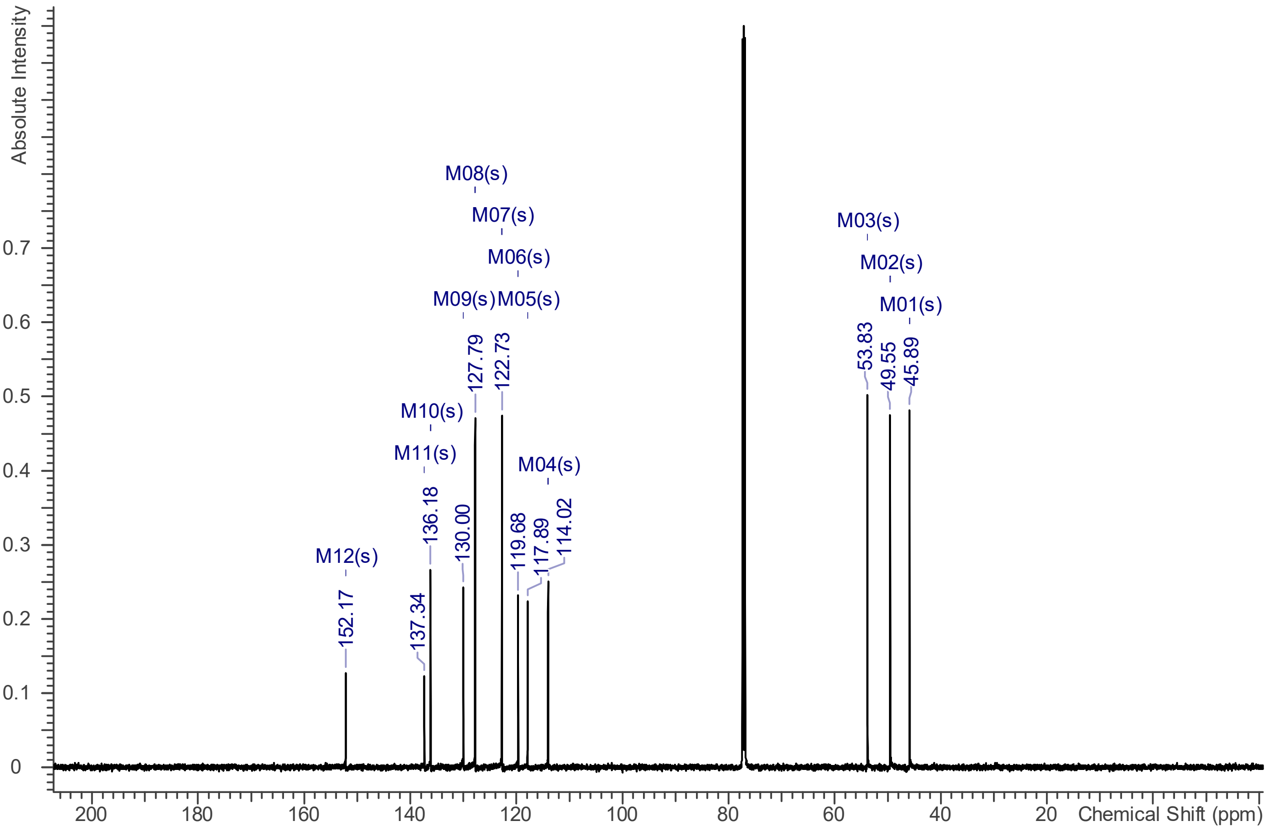
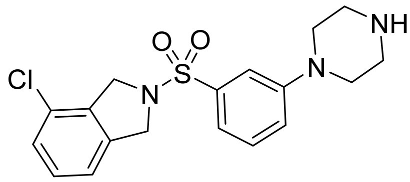
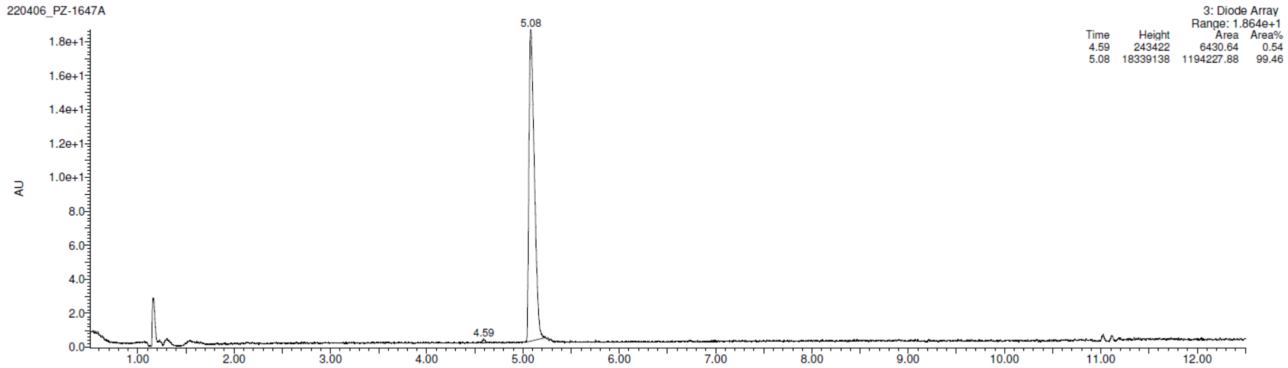
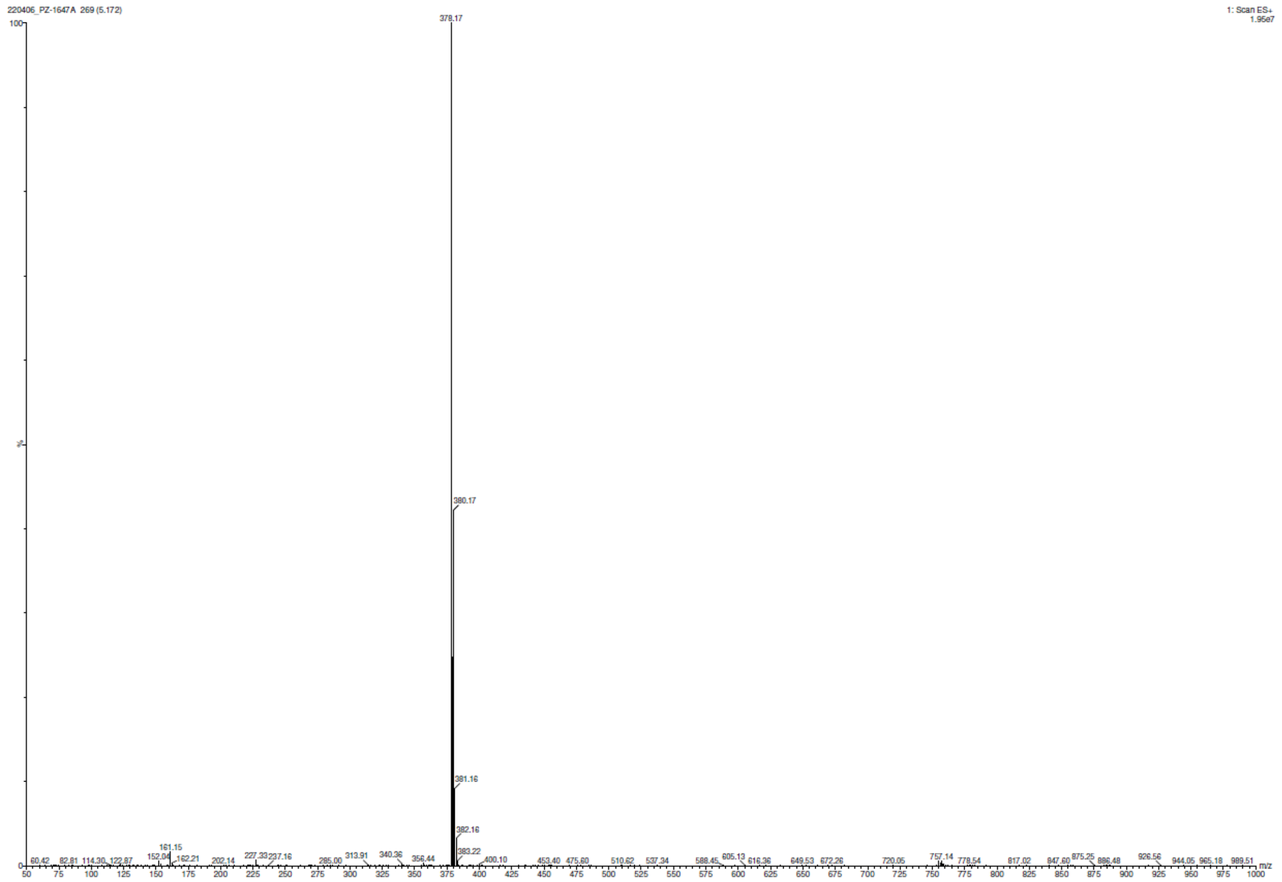


3.6. 4-Chloro-2-{[3-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3f**



UPLC/MS





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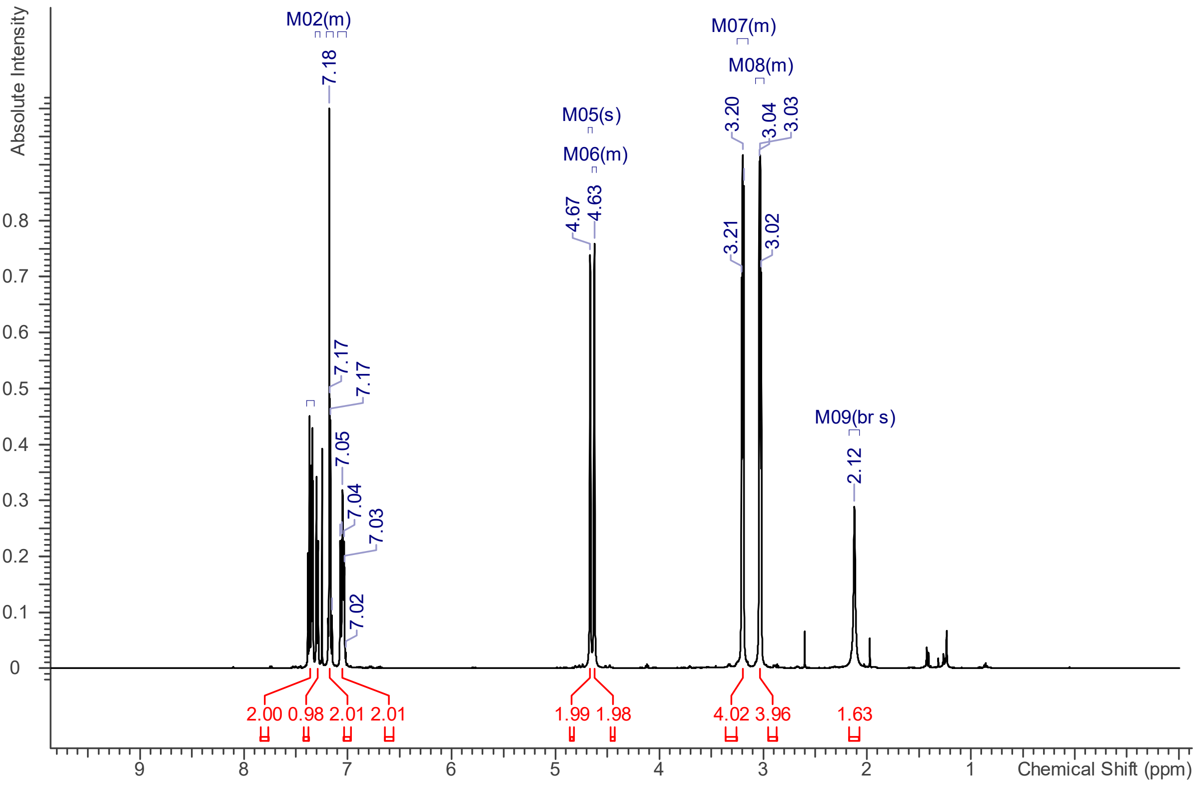

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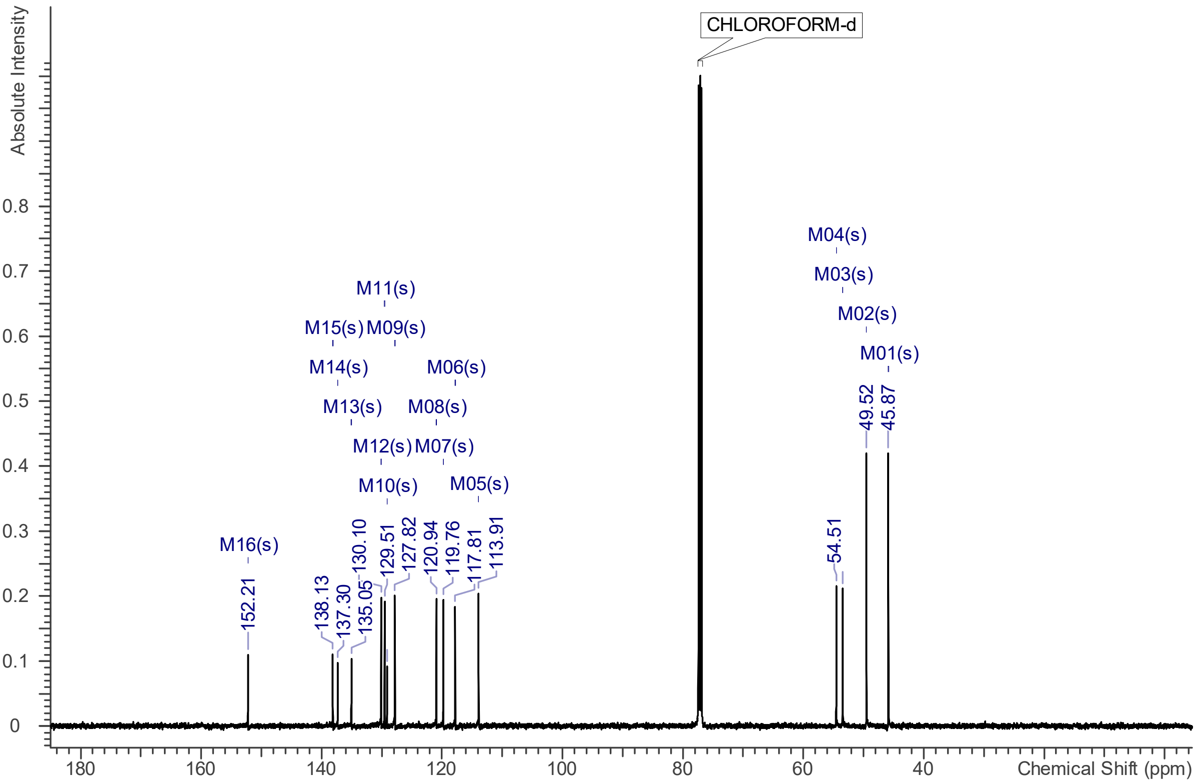
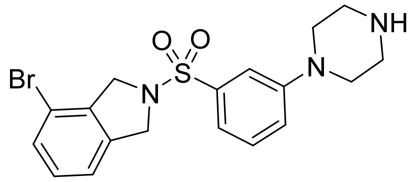
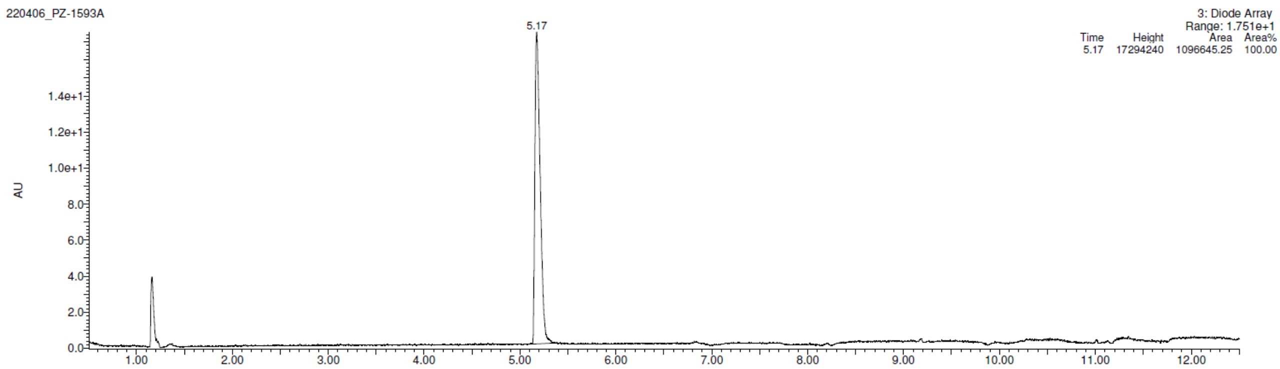
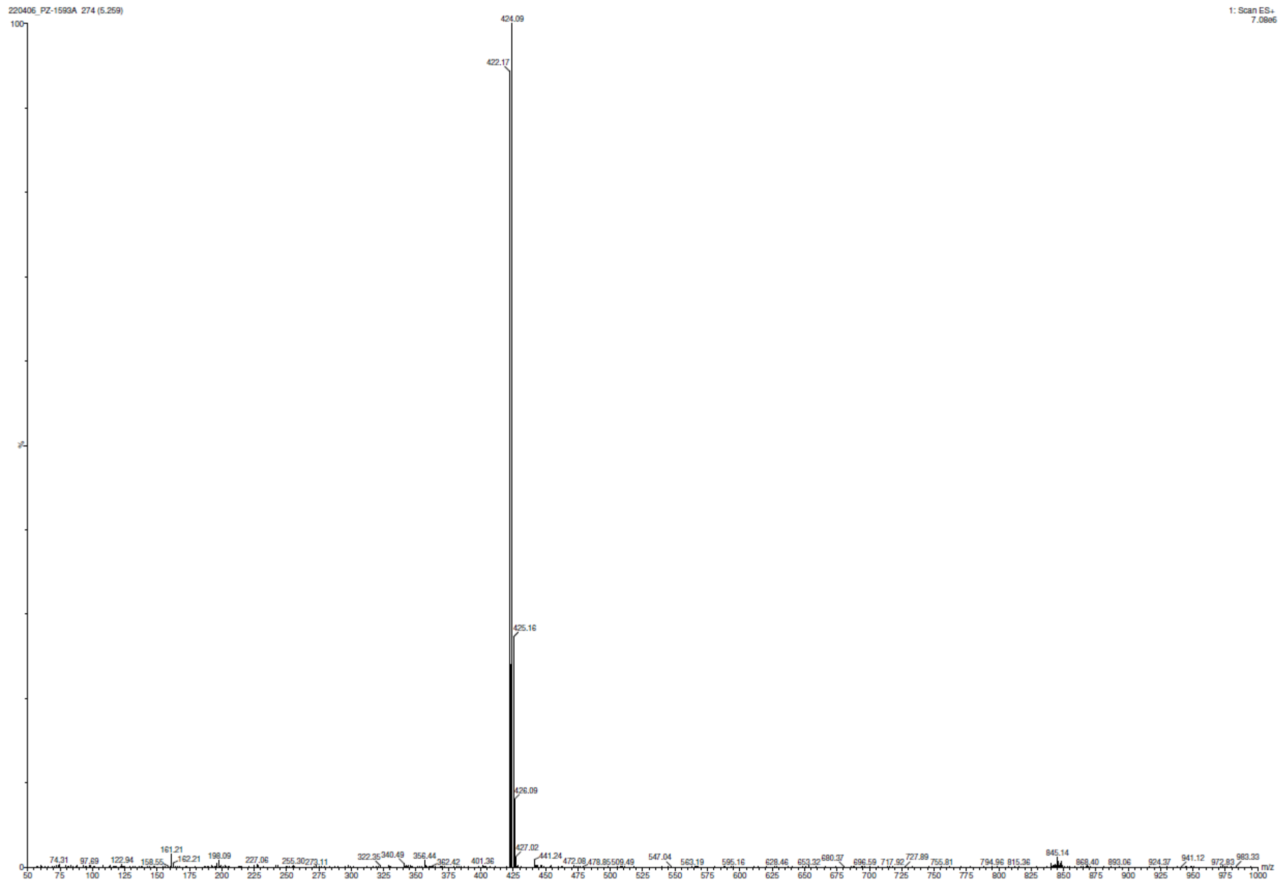


3.7. 4-Bromo-2-{[3-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3g**

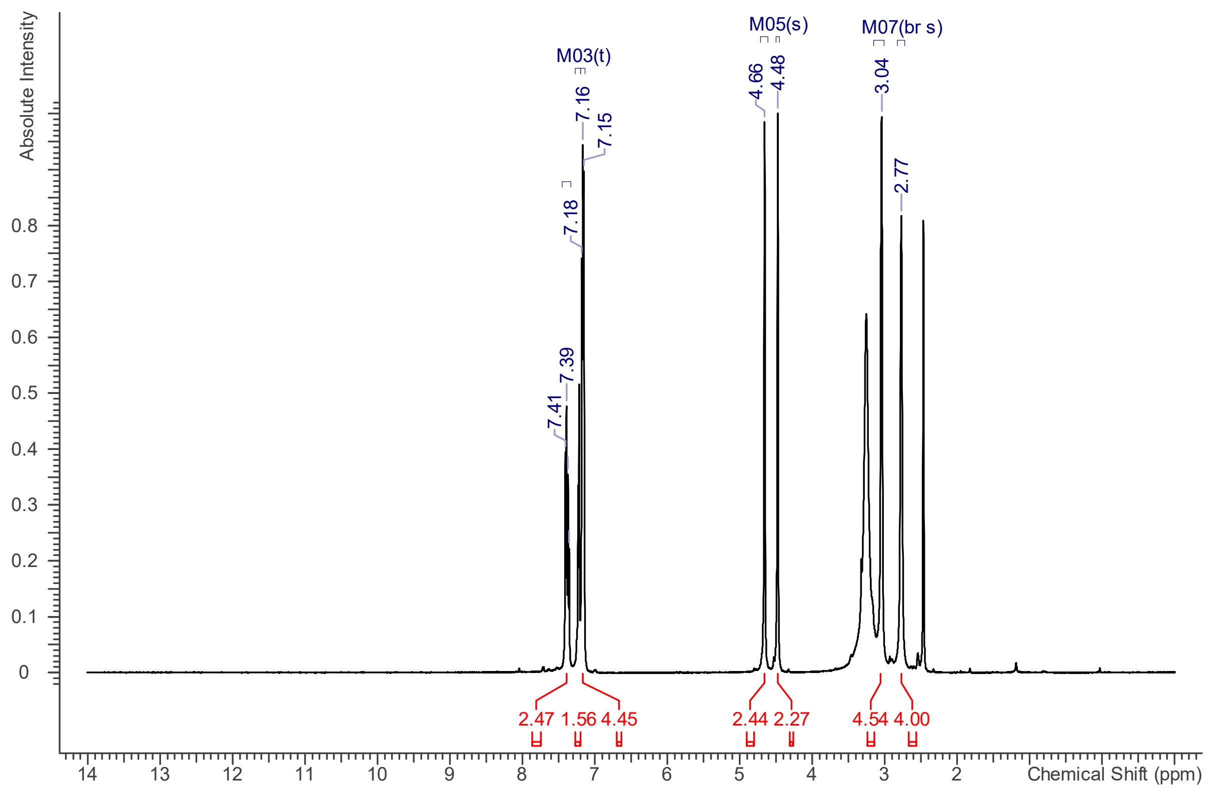


UPLC/MS





[1]H-NMR (500 MHz, DMSO-d_6)



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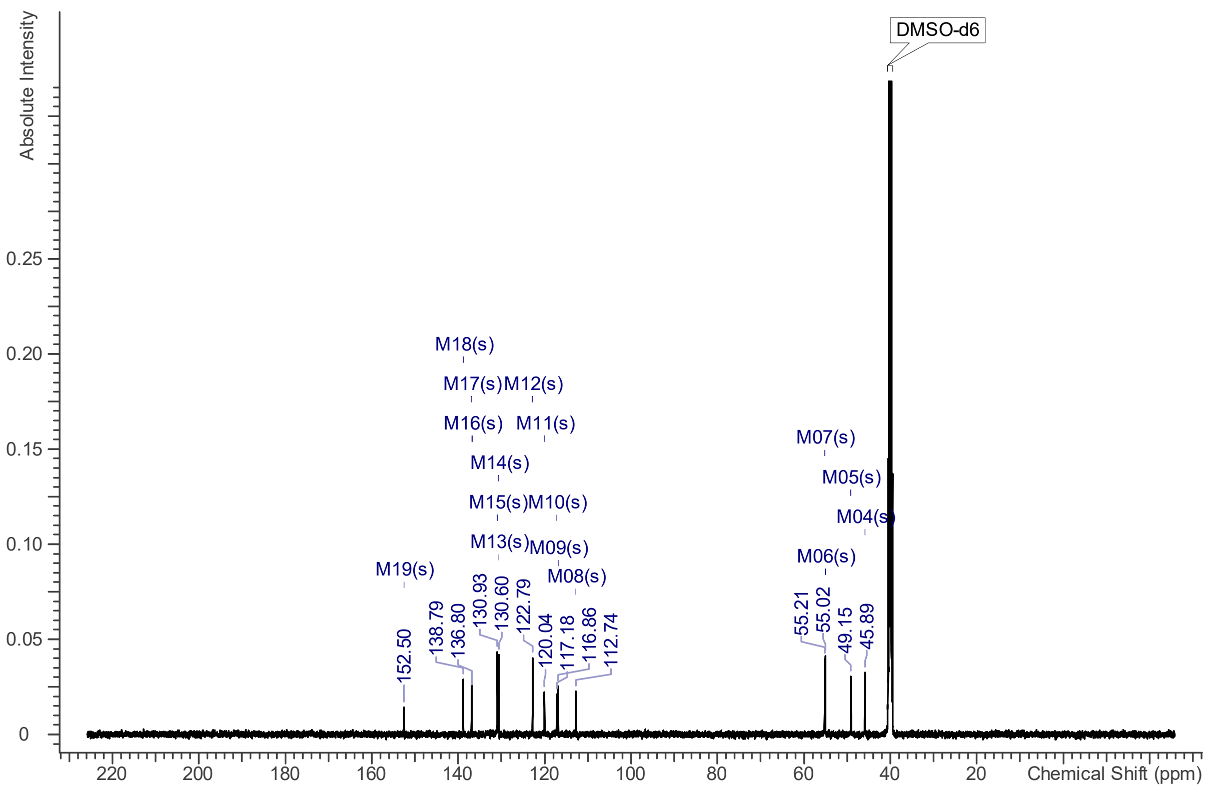
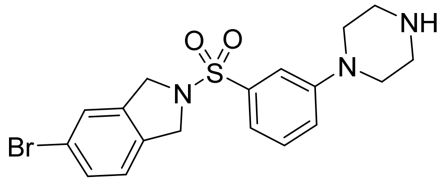


3.8. 5-Bromo-2-{[3-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3h**



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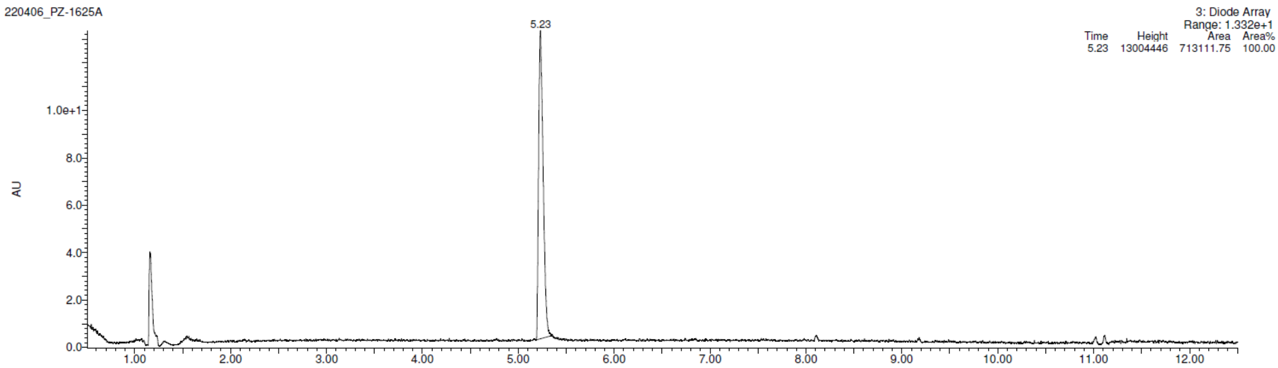

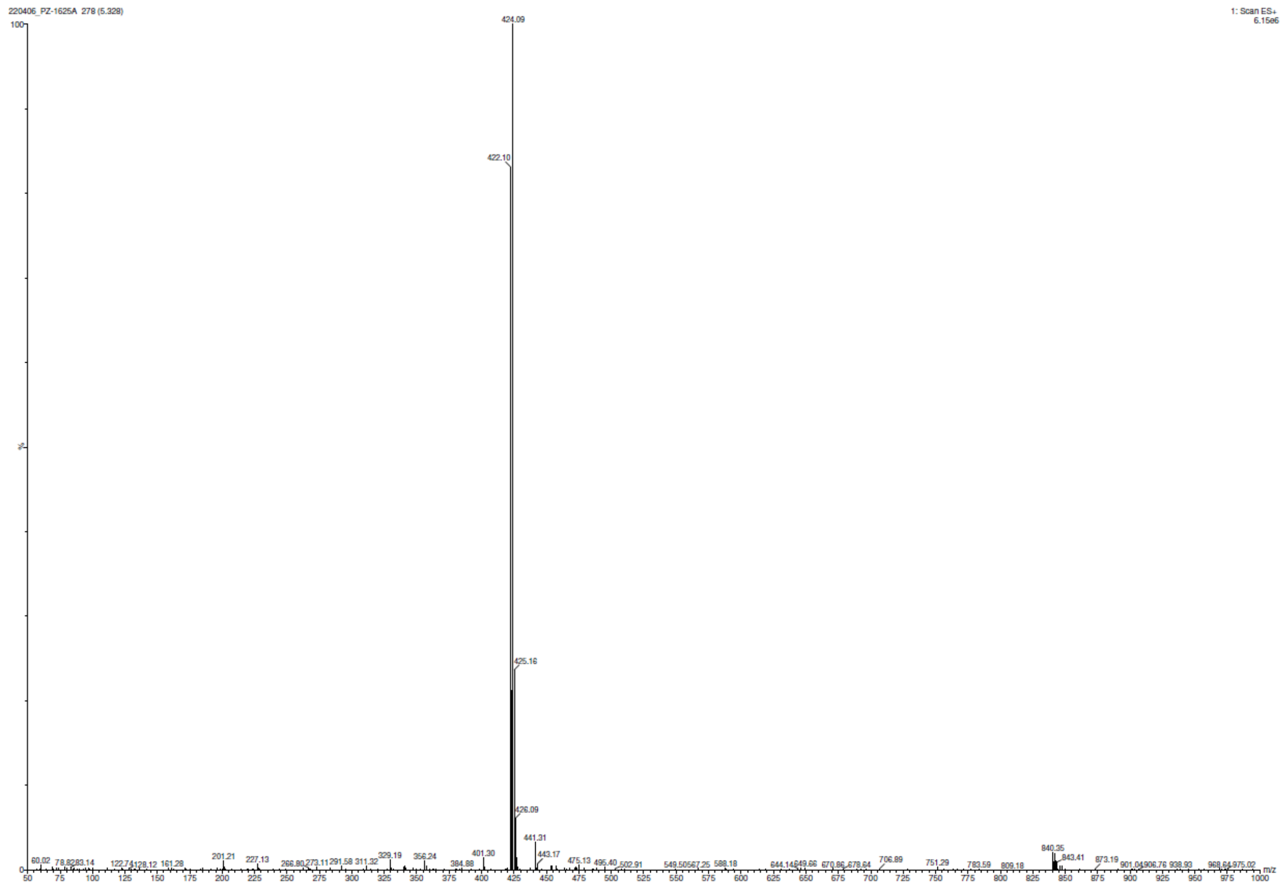

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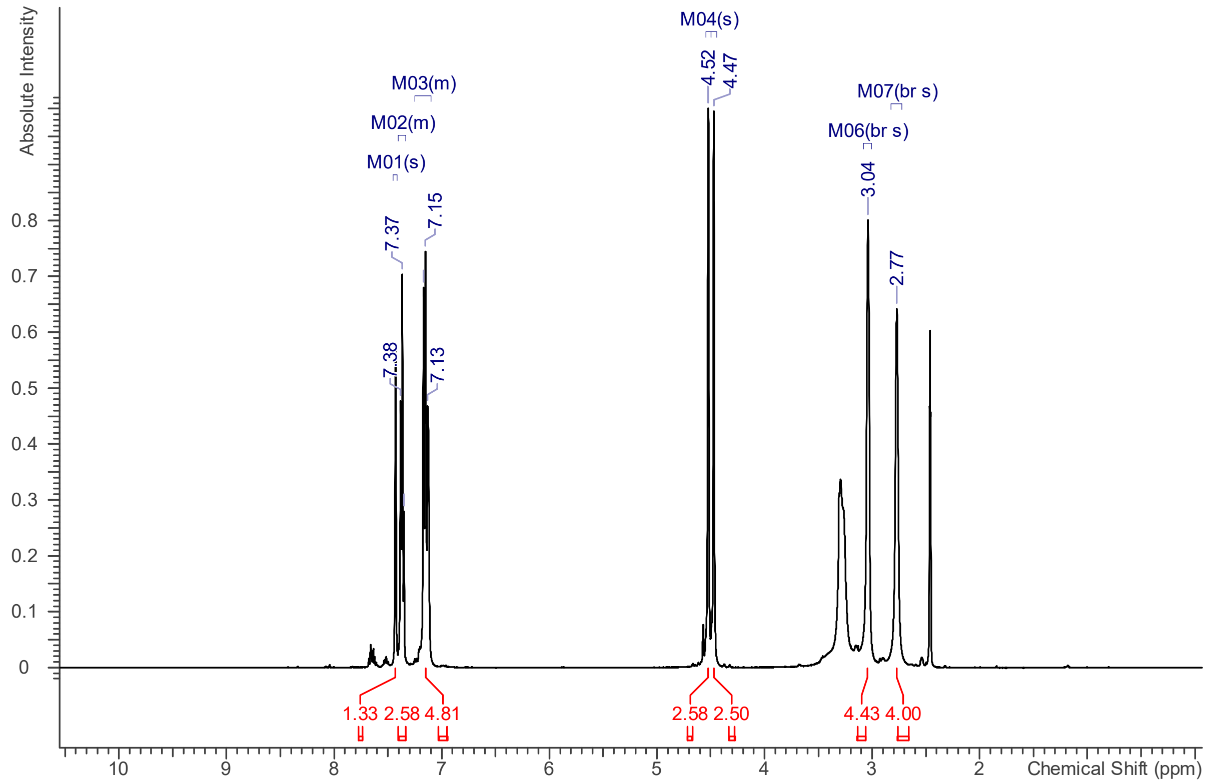

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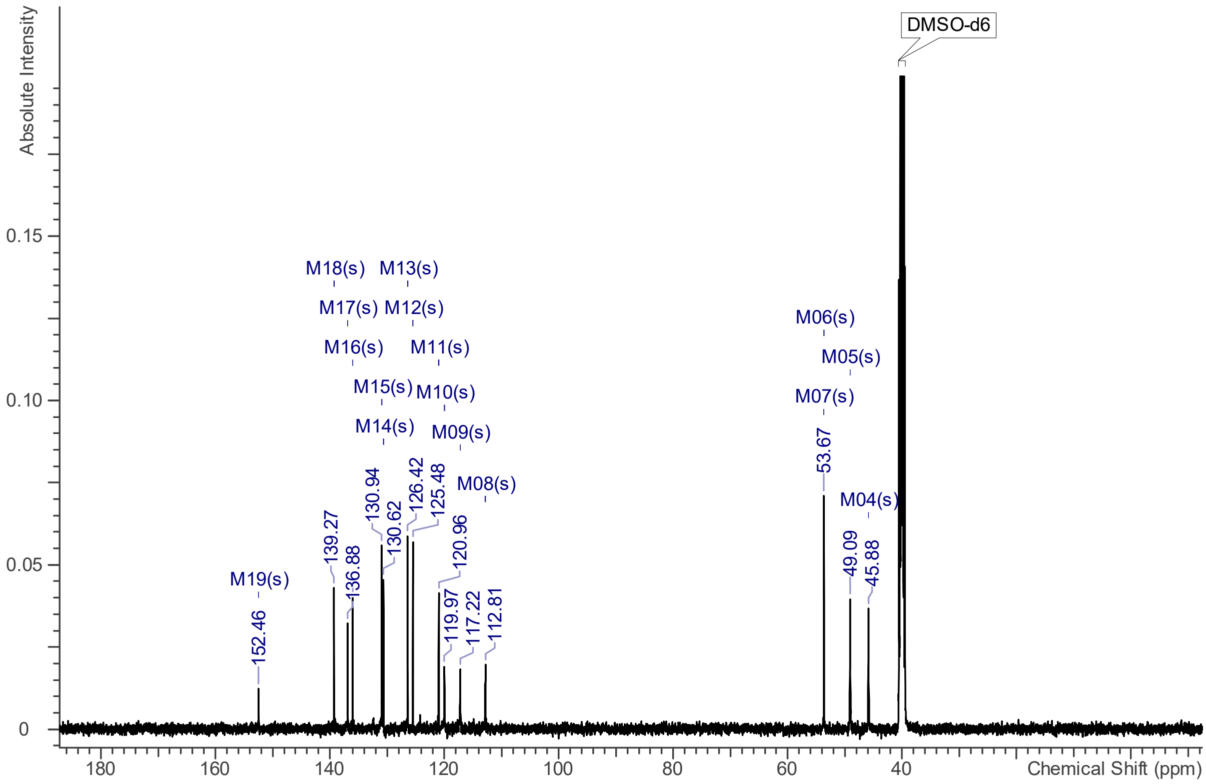
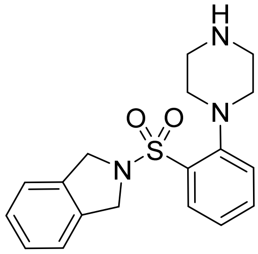


3.9. 2-{[2-(Piperazin-1-yl)phenyl]sulfonyl}isoindoline **3i**



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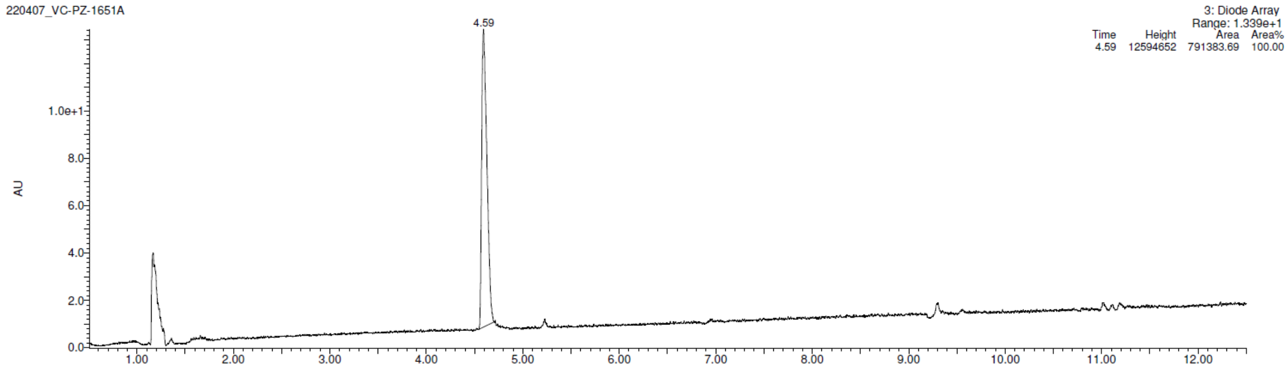

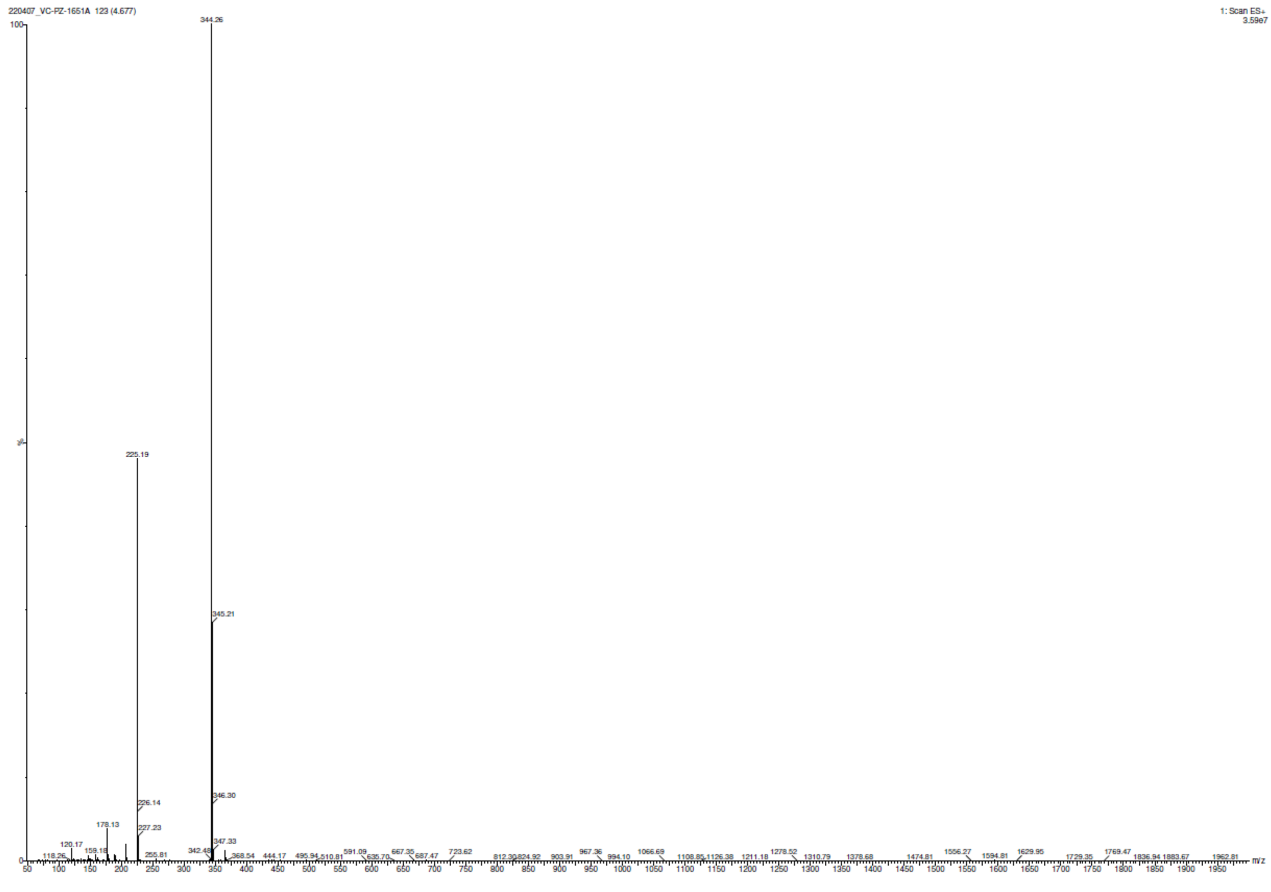

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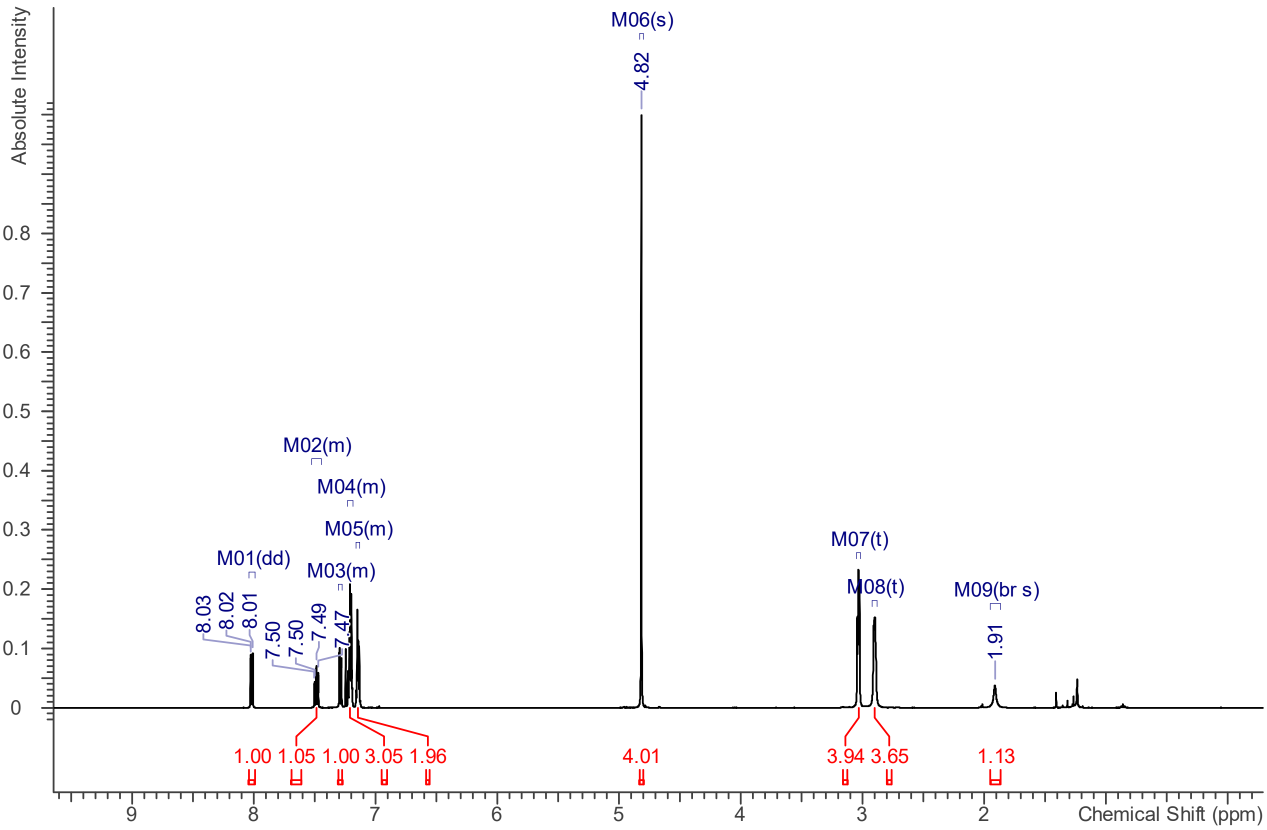

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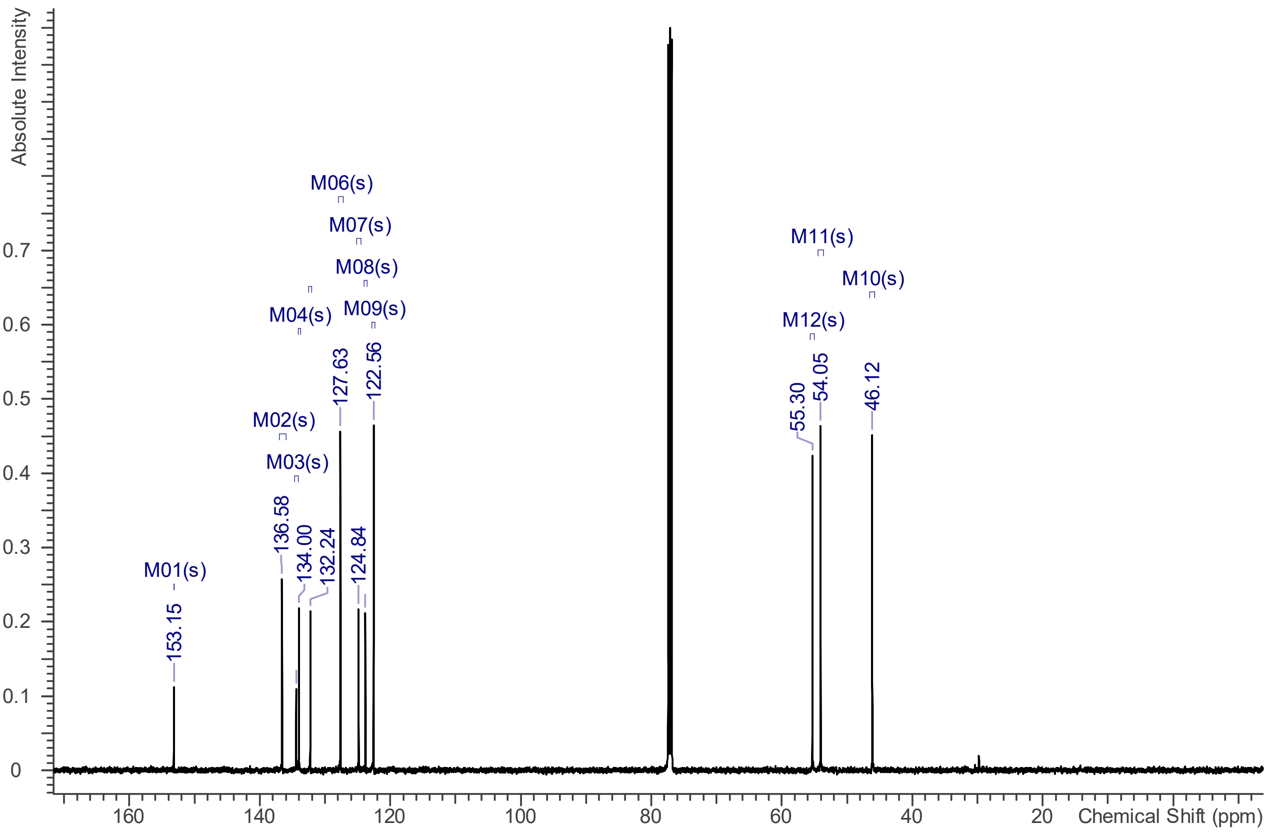
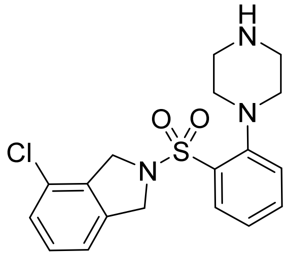


3.10. 4-Chloro-2-{[2-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3j**



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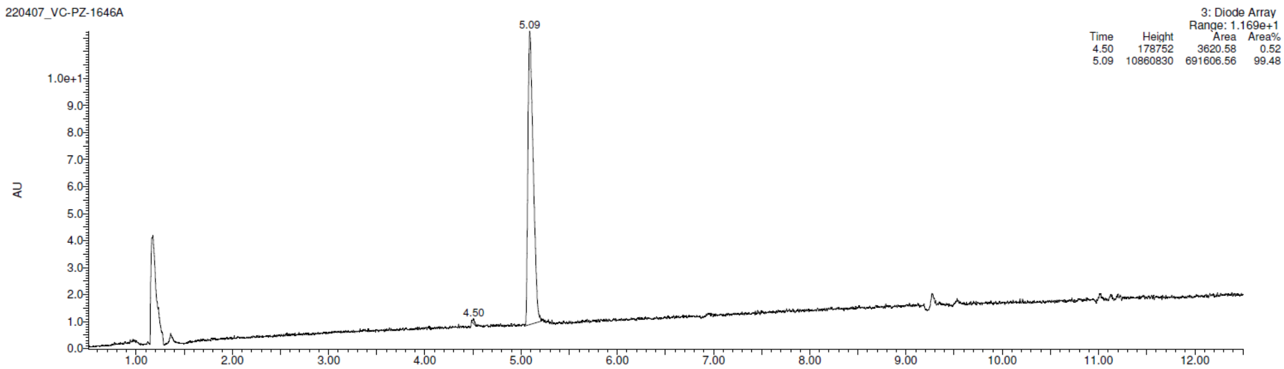

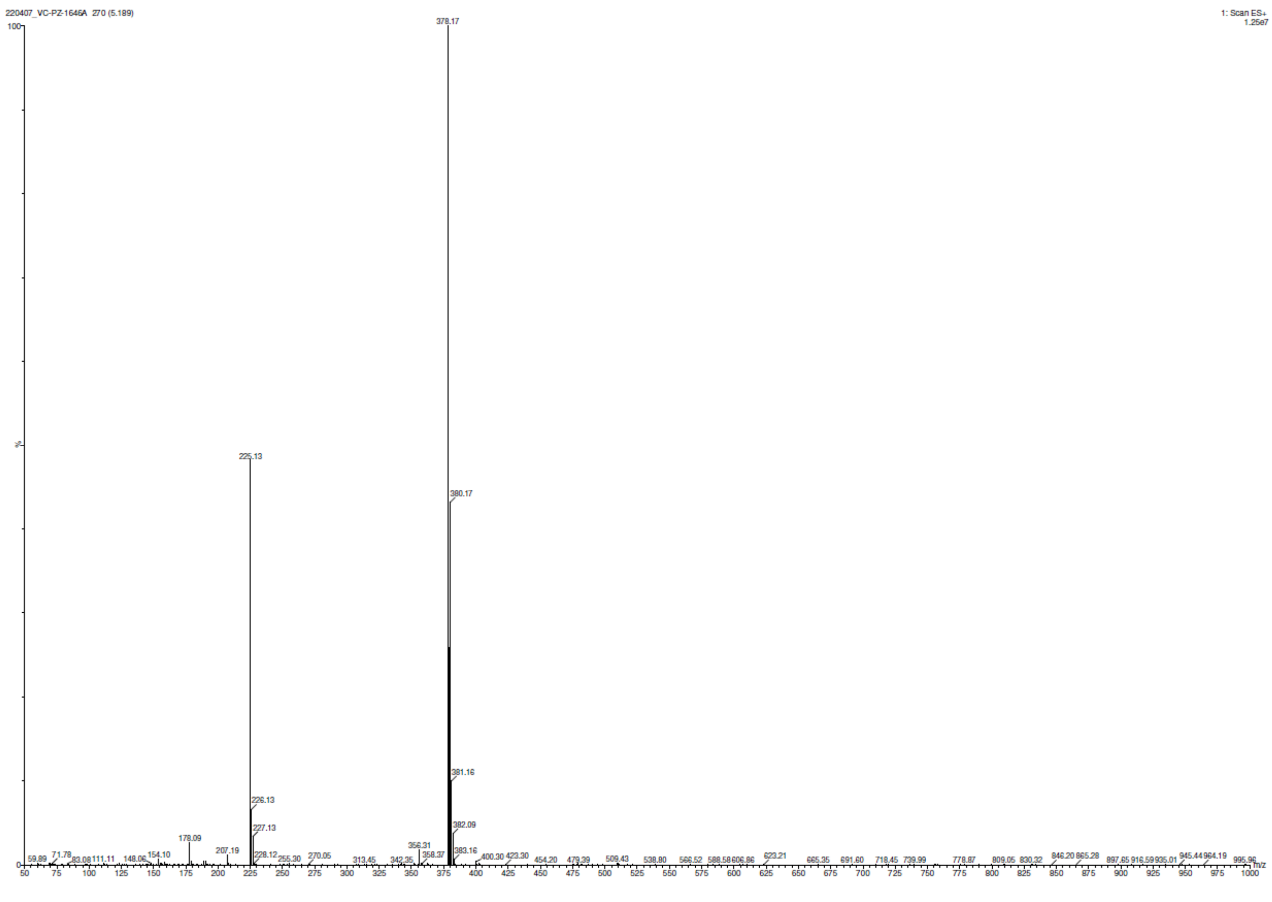

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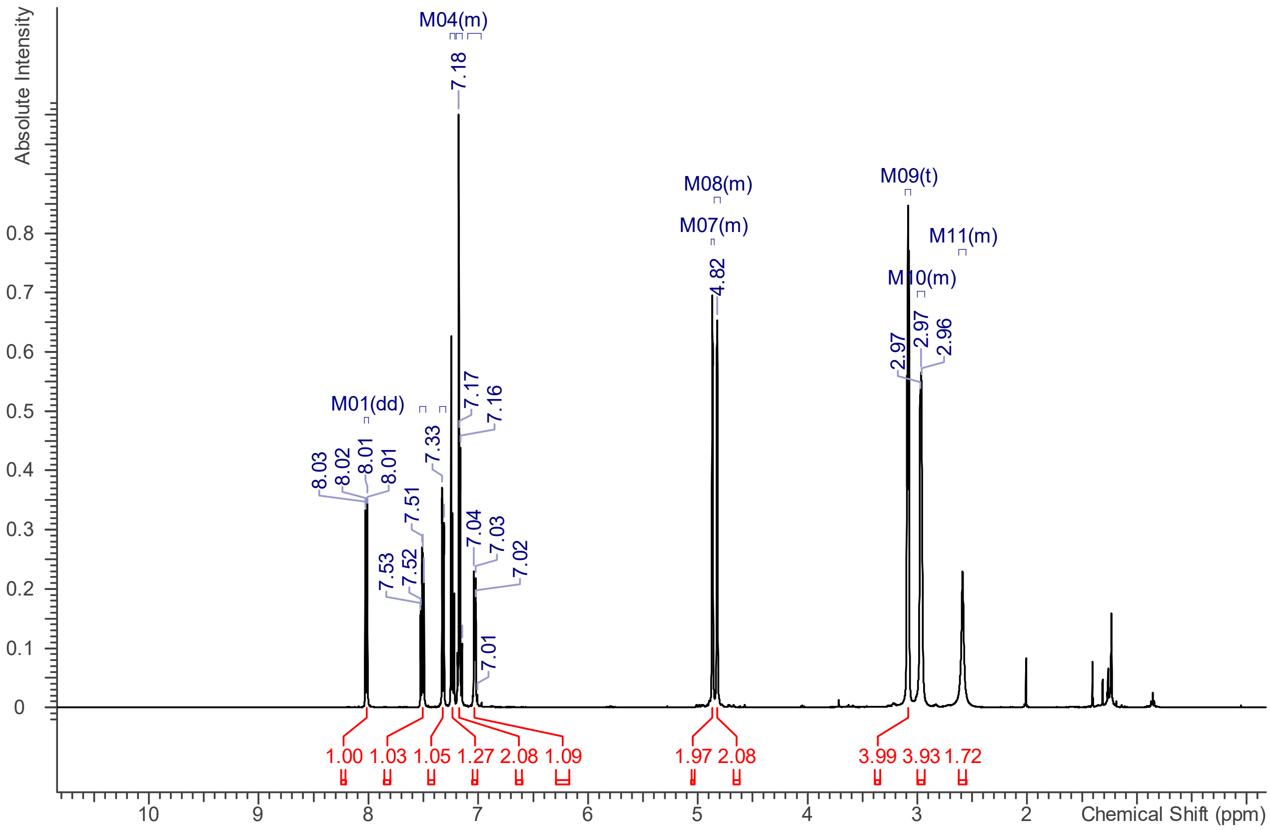

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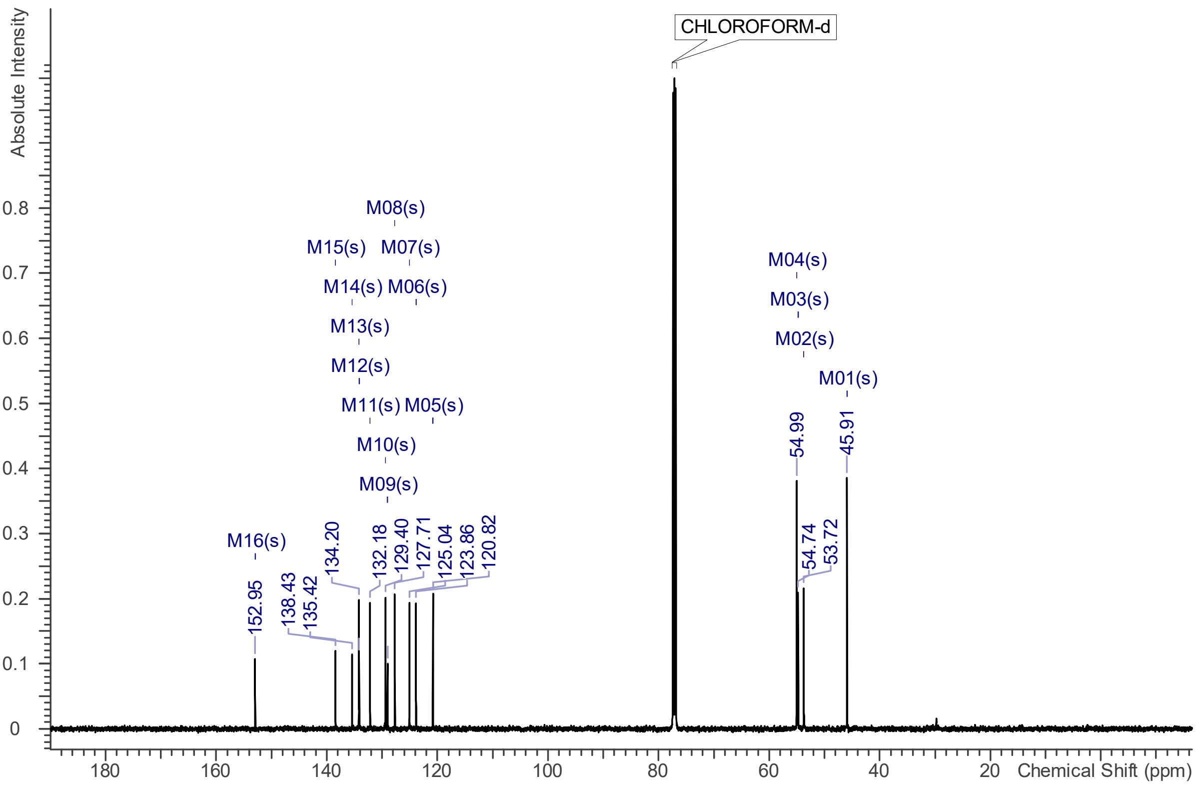
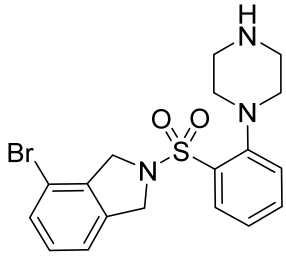


3.11. 4-Bromo-2-{[2-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3k**



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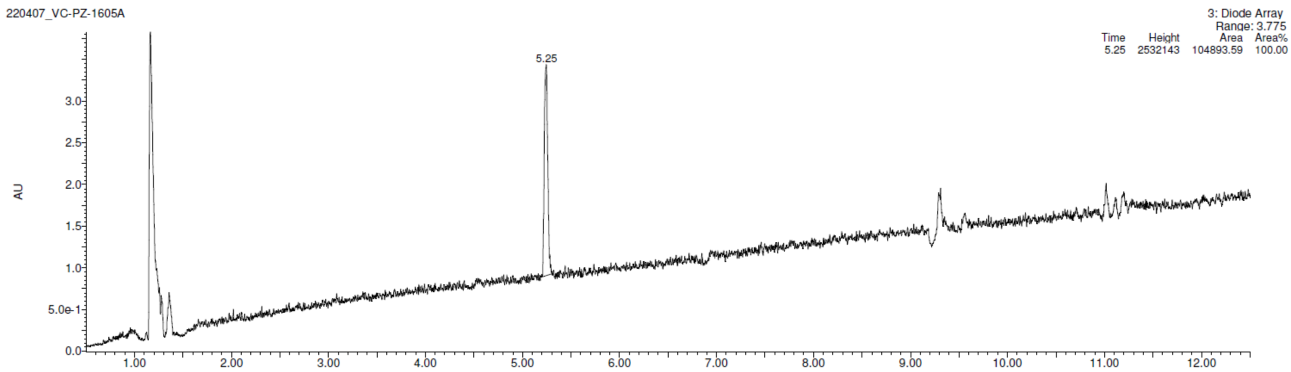

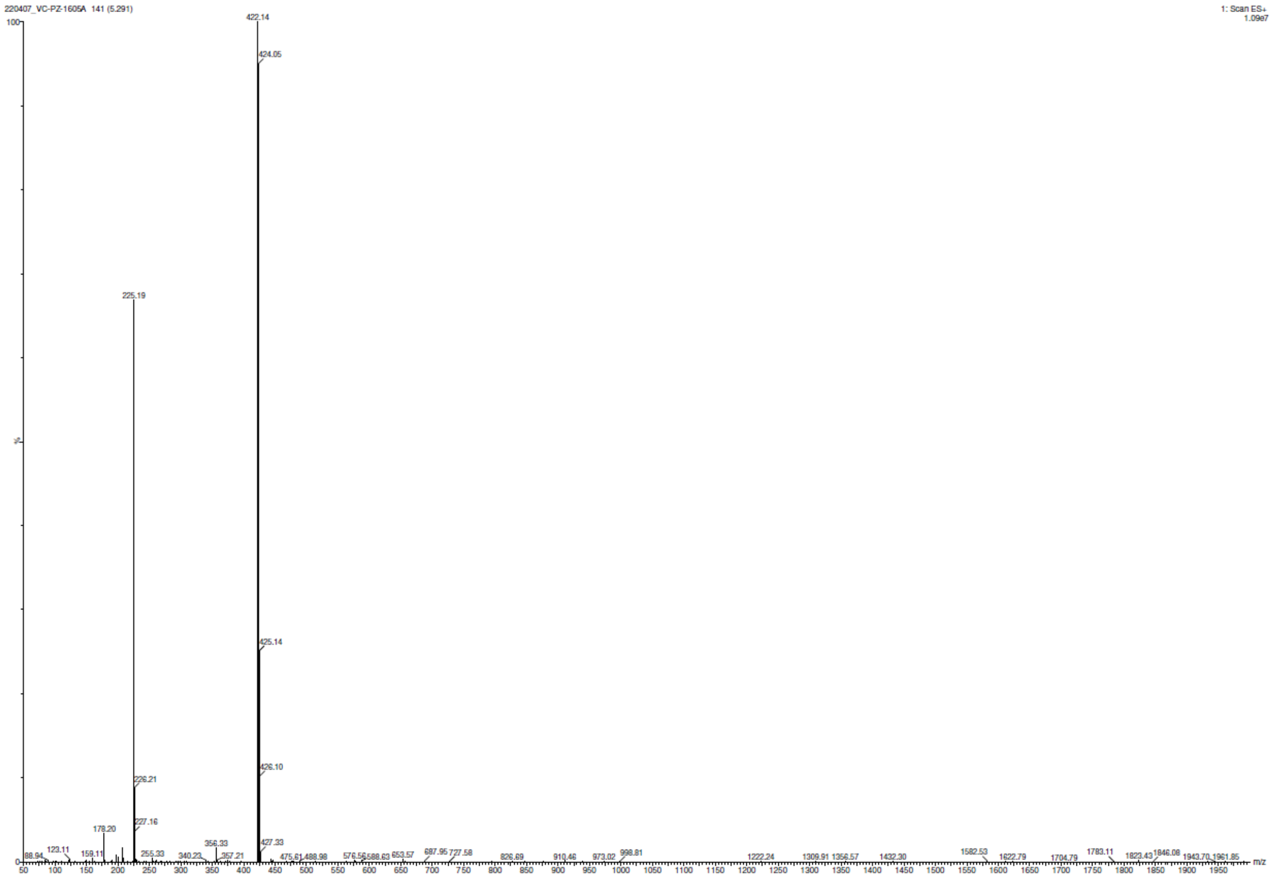

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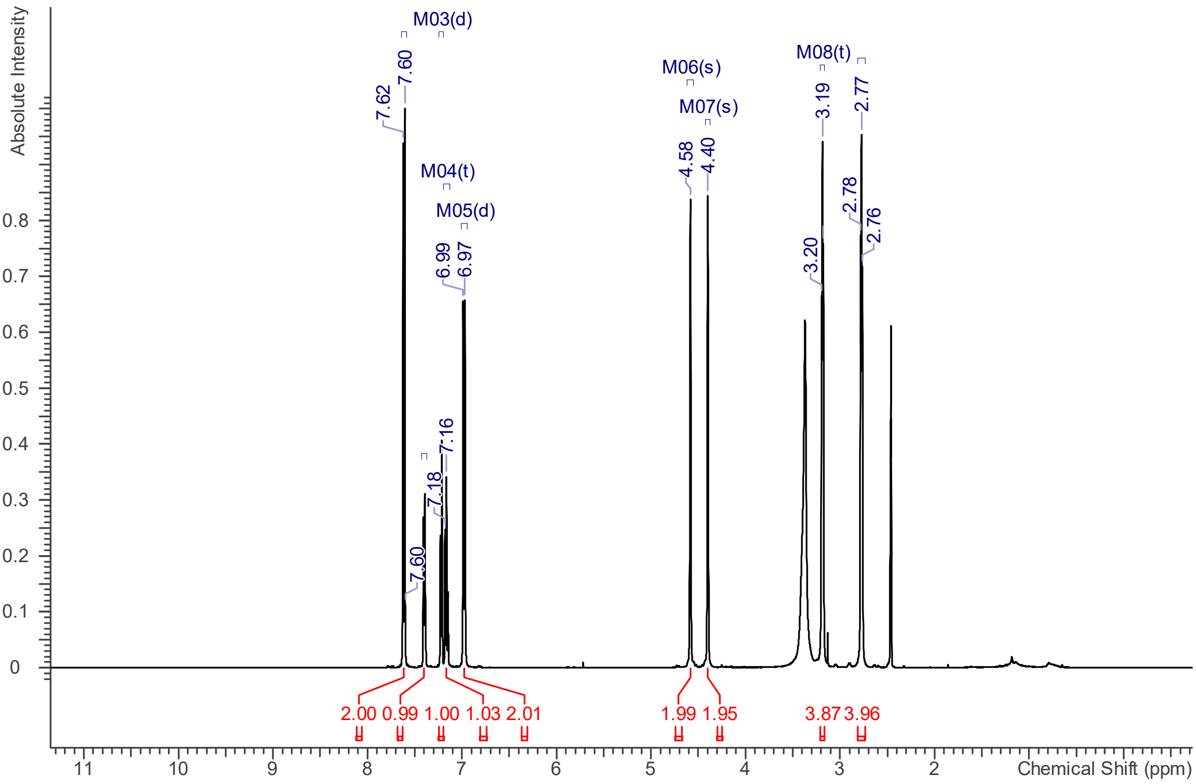
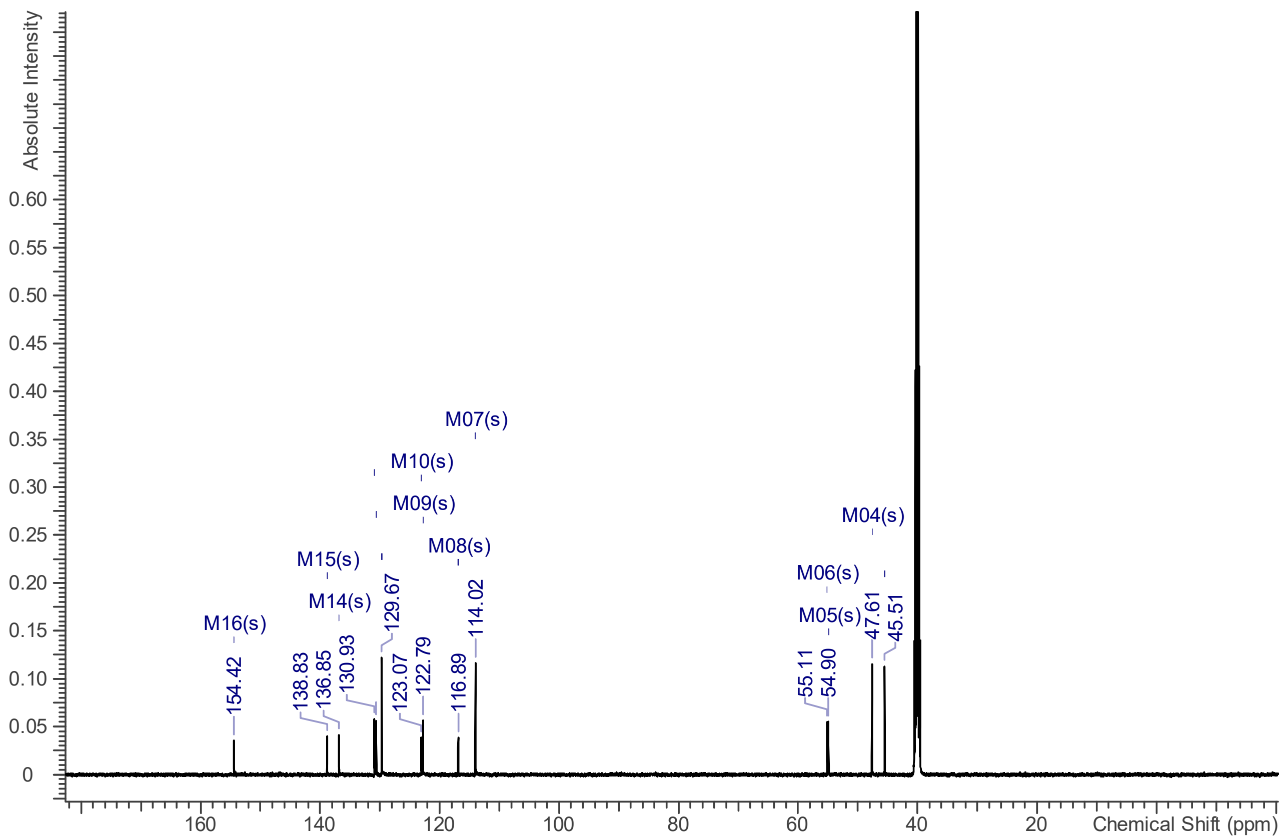
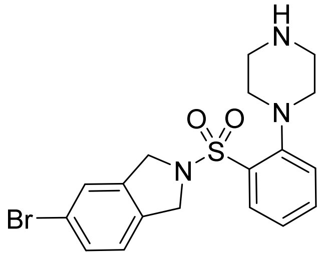


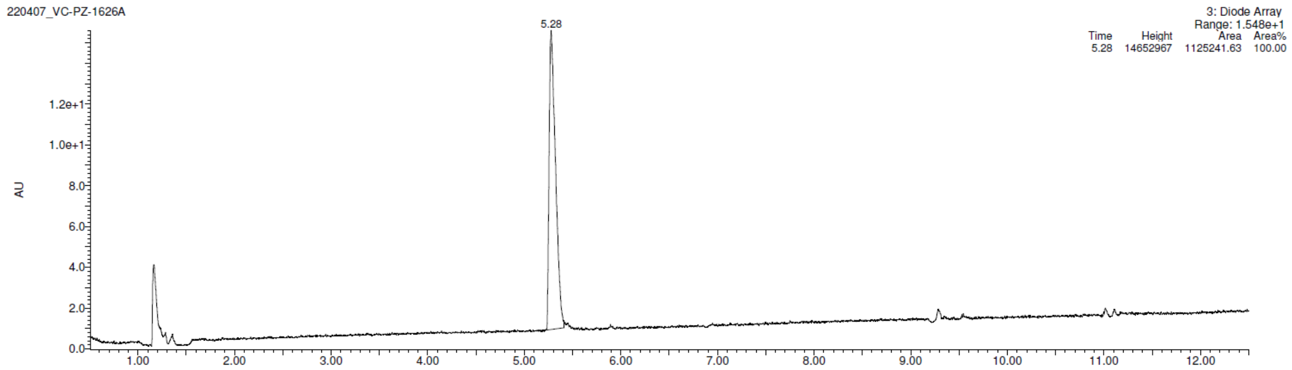
^{13}C-NMR (126 MHz, DMSO-d_6)

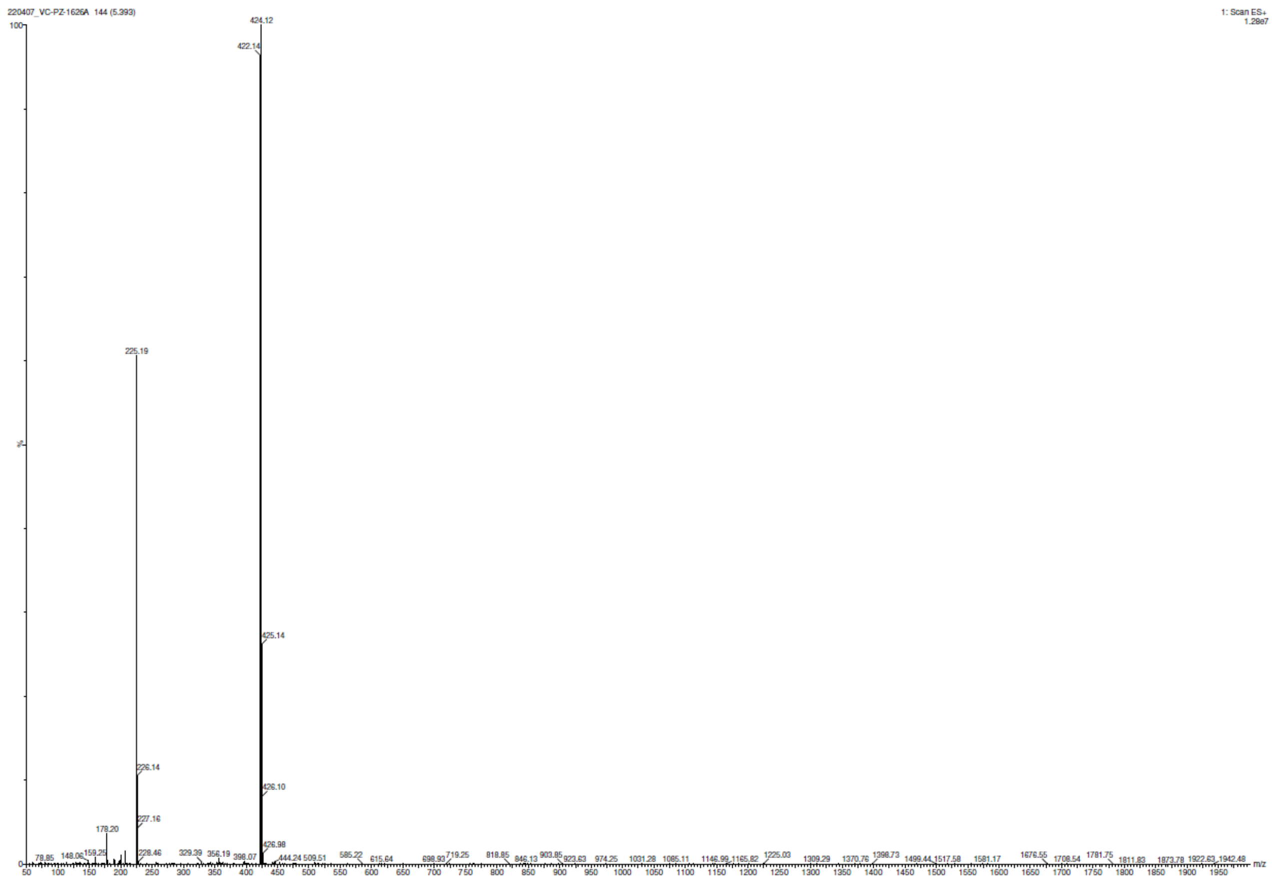


3.12. 5-Bromo-2-{[2-(piperazin-1-yl)phenyl]sulfonyl}isoindoline **3l**



UPLC/MS





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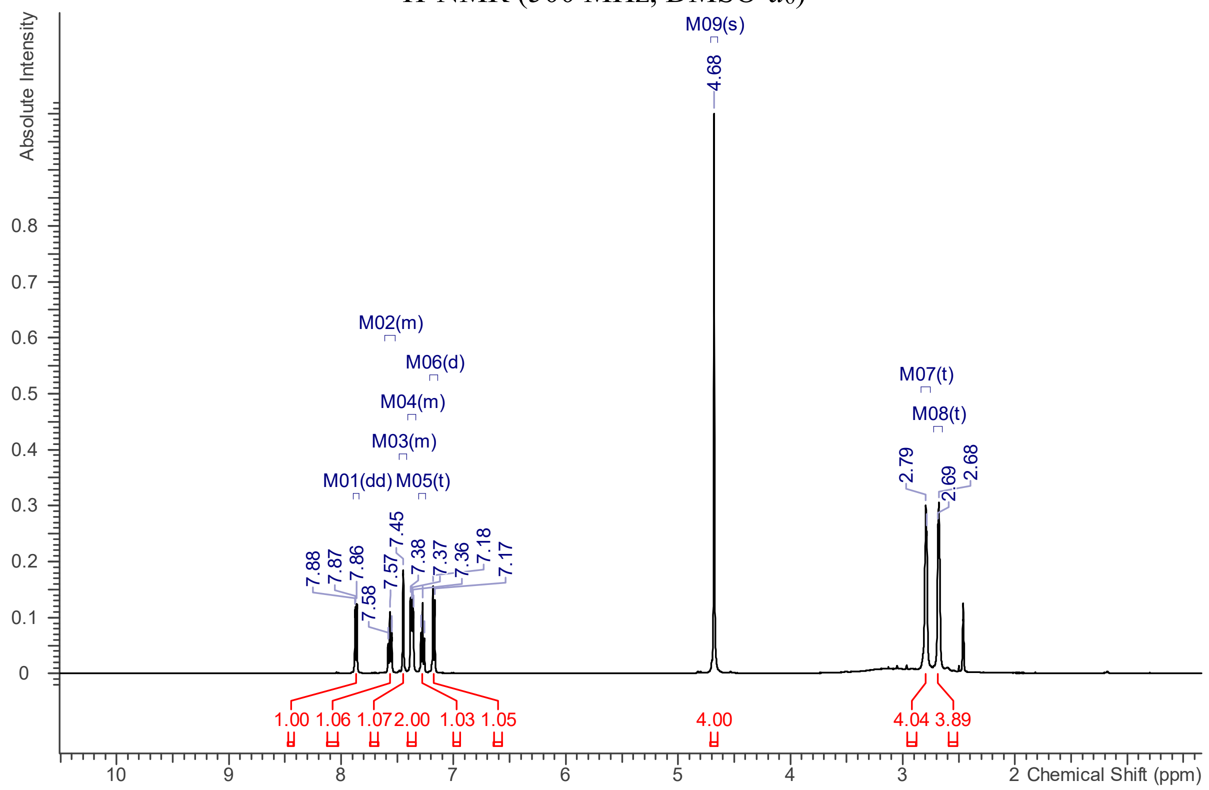

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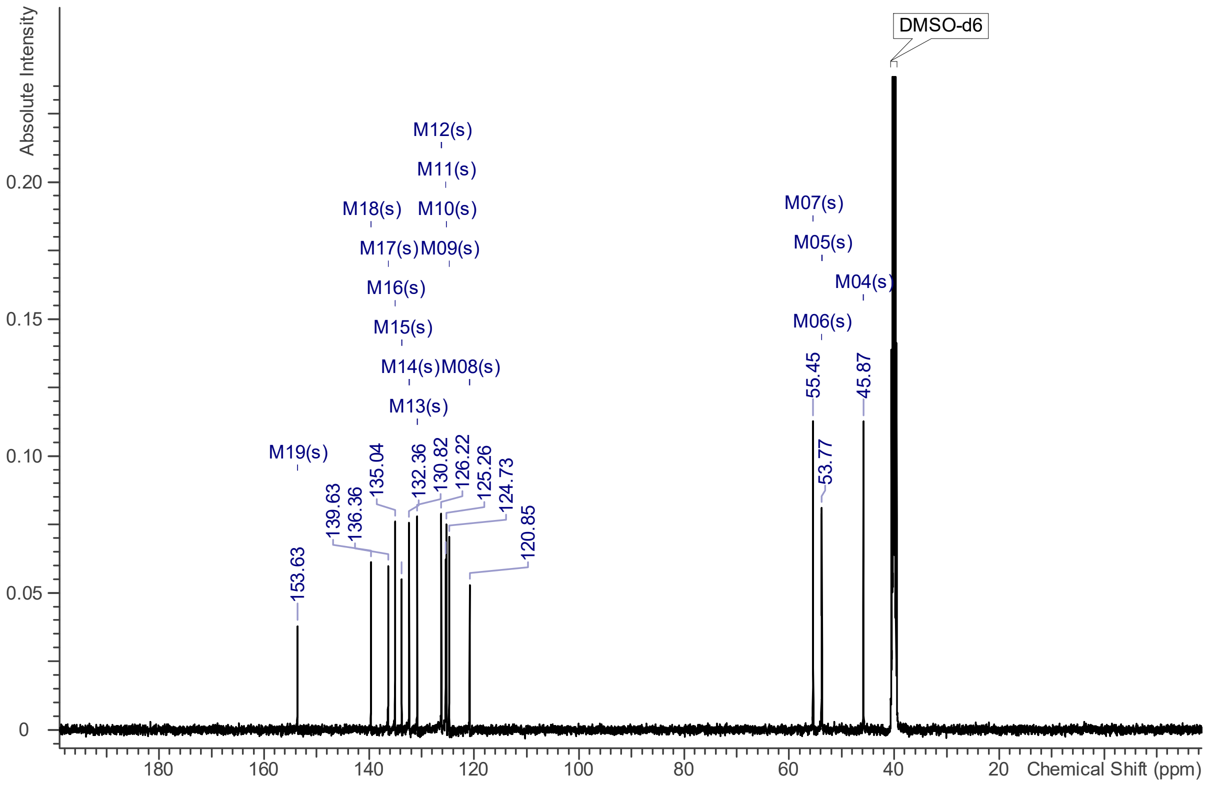
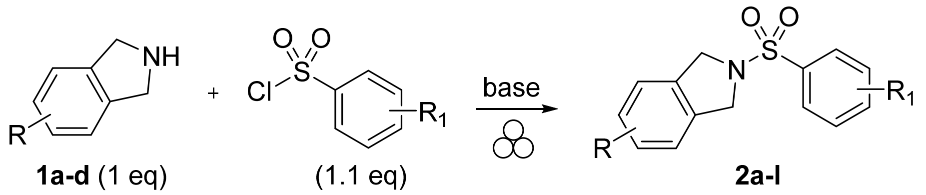


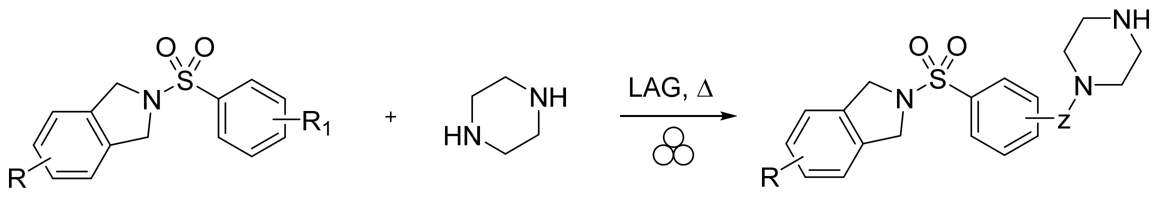
Table S1–SI. Optimization of milling conditions for sulfonylation of isoindolines **1a**–**d**



entry	R	R_1 [eq]	Base [eq]	time [min]	% conversion[a]	% yield[b]	product
1[c]	H	4-F	K_2CO_3 (2)	5	97	95	**2a**
2[c]	H	4-F	$NaHCO_3$ (2)	5	69	ND	**2a**
3[c]	H	4-F	KOH (2)	5	77	ND	**2a**
4[c]	H	4-F	NaOH (2)	5	94	92	**2a**
5[c]	H	4-F	NaOH (3)	5	100	98	**2a**
6[d]	H	4-F	NaOH (3)	5	100	98	**2a**
7[d]	4-Cl	4-F	NaOH (3)	7	85	81	**2b**
8[d]	4-Br	4-F	NaOH (3)	7	83	81	**2c**
9[d]	5-Br	4-F	NaOH (3)	7	89	86	**2d**
10[d]	H	3-F	NaOH (3)	5	98	96	**2e**
11[d]	4-Cl	3-F	NaOH (3)	7	79	75	**2f**
12[d]	4-Br	3-F	NaOH (3)	7	82	79	**2g**
13[d]	5-Br	3-F	NaOH (3)	7	87	85	**2h**
14[d]	H	2-F	NaOH (3)	5	95	93	**2i**
15[d]	4-Cl	2-F	NaOH (3)	7	82	79	**2j**
16[d]	4-Br	2-F	NaOH (3)	7	86	82	**2k**
17[d]	5-Br	2-F	NaOH (3)	7	91	88	**2l**

Reaction conditions: vbm 30 Hz, ϕ_{ball} = 1.5 cm, [a]Conversions were determined by UPLC, [b]Yields were calculated after filtration and washing with H_2O, [c]10 mL SS jar, total mass of reagents = 85 mg, milling load = 10 mg/mL, [d]35 mL SS jar, total mass of reagents = 350 mg, milling load = 10 mg/mL; ND = not determined.

Table S2–SI. Optimization of milling conditions for aromatic substitution of intermediates **2a–l**



entry	R	R_1	LAG [η][a]	T [°C]	time [min]	% conversion[b]	% yield[c]	product
1[d]	H	4-F	-	25	90	0	ND	**3a**
2[d]	H	4-F	-	50	30	24	ND	**3a**
3[d]	H	4-F	-	50	90	55	ND	**3a**
4[e]	H	4-F	MeCN	50	90	72	ND	**3a**
5[f]	H	4-F	MeCN	50	90	90	88	**3a**
6[f]	4-Cl	4-F	MeCN	50	90	90	83	**3f**
7[f]	4-Br	4-F	MeCN	50	90	89	88	**3i**
8[f]	5-Br	4-F	MeCN	50	90	90	86	**3l**
9[f]	H	3-F	MeCN	50	90	59	ND	**3c**
10[f]	H	3-F	DMSO	80	90	78	75	**3c**
11[f]	4-Cl	3-F	DMSO	80	90	80	77	**3e**
12[f]	4-Br	3-F	DMSO	80	90	79	76	**3h**
13[f]	5-Br	3-F	DMSO	80	90	80	78	**3k**
14[f]	H	2-F	MeCN	50	90	88	84	**3a**
15[f]	4-Cl	2-F	MeCN	50	90	86	81	**3d**
16[f]	4-Br	2-F	MeCN	50	90	85	80	**3g**
17[f]	5-Br	2-F	MeCN	50	90	86	82	**3j**

Reaction conditions: vbm 30 Hz, ϕ_{ball} = 1.5 cm, 10 mL SS jar, total mass of reagents = 85 mg, milling load 10 mg/mL, [a]Liquid Assisted Grinding, η = 0.4 µL/mg; [b]Conversions were determined by UPLC, [c]Yield for isolated compound; [d]piperazine anhydrous (1 eq); [e]piperazine anhydrous (2 eq); [f]piperazine anhydrous (3 eq); ND = not determined.

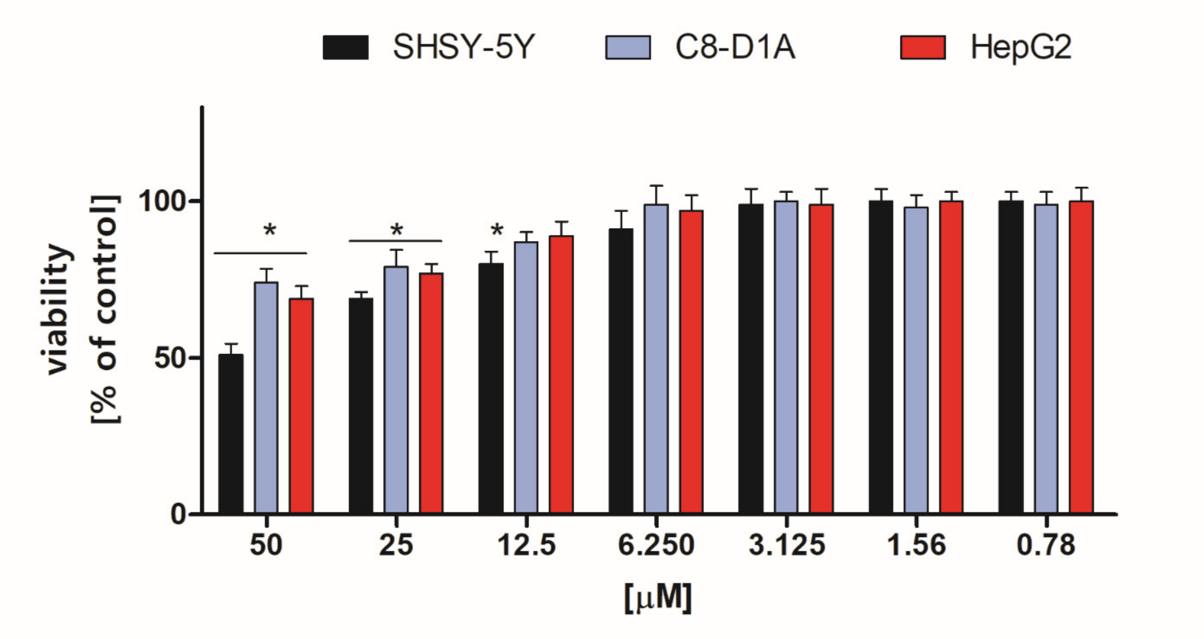


Figure S1–SI. The effect of compound **3g** on cellular viability in (**A**) SH-SY5Y (human neuroblastoma), (**B**) C8-1DA (mouse astrocytes), and (**C**) HepG2 (human hepatocellular carcinoma) cell lines. Cells were incubated in the presence of **3g** in concentration range 0.78–50 μM for 48 hours, then MTT assay was performed to assesses cellular viability. Graph represent cells viability calculated as percent of control (mean ± SD) of three independent experiments. Statistical analysis by one-way ANOVA showed significant differences between the groups ($p < 0.05$) and was followed by the Dunnett's multiple comparisons test, * $p \leq 0.05$.

1. Procedure for radioligand binding assay

All experiments were carried out according to the previously published procedures [1–3]. HEK293 cells stably expressing human 5-HT_{1A}, 5-HT_6, 5-HT_{7b} and D_{2L} receptors (prepared with the use of Lipofectamine 2000) or CHO-K1 cells with plasmid containing the sequence coding for the human serotonin 5-HT_{2A} receptor (Perkin Elmer) were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and grown in Dulbecco's Modified Eagle's Medium containing 10% dialyzed fetal bovine serum and 500 μg/ml G418 sulfate. For membrane preparation, cells were cultured in 150 cm^2 flasks, grown to 90% confluence, washed twice with pre-warmed to 37 °C phosphate buffered saline (PBS) and centrifuged (200 × g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, pellets were stored at –80 °C. Cell pellets were thawed and homogenized in 10 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35,000 × g for 15 min at 4 °C, with incubation for 15 min at 37 °C between the centrifugations. The composition of the assay buffers was experimentally selected to achieve the maximum signal window.

5-HT_{1A}R: 50 mM Tris HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; **5-HT_{2A}R**: 50 mM Tris HCl, 0.1 mM EDTA, 4 mM $MgCl_2$ and 0.1% ascorbate; **5-HT_6R**: 50 mM Tris HCl, 0.5 mM EDTA and 4 mM $MgCl_2$; **5-HT_{7b}R**: 50 mM Tris HCl, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; **D_{2L}R**: 50 mM Tris HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate).

All assays were carried out in a total volume of 200 μL in 96-well plates for 1 h at 37 °C, except 5-HT_{1A}R and 5-HT_{2A}R which were incubated at room temperature and 27 °C, respectively. The process of equilibration was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader (PerkinElmer, USA). For displacement studies the assay samples contained as radioligands (PerkinElmer, USA): 2.5 nM [^{3}H]-8-OH-DPAT (135.2 Ci/ mmol) for 5-HT_{1A}R; 1 nM [^{3}H]-ketanserin (53.4 Ci/mmol) for 5-HT_{2A}R; 2 nM [^{3}H]-LSD (83.6 Ci/mmol) for 5-HT_6R; 0.8 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_7R or 2.5 nM [^{3}H]-raclopride (76.0 Ci/mmol) for D_{2L}R. Non-specific binding was defined in the presence of 10 μM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, 20 μM of mianserin for 5-HT_{2A}R, 10 μM of methiothepine for 5-HT_6R or 10 μM of haloperidol for D_{2L}R bidning assays. Each compound was tested in triplicate at 7 concentrations (10^{-10}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation [4].

2. Procedure for determination of functional activity at Gs signaling

*2.1. Impact of compound **3e**, **3f** and **3g** on cAMP production in 1321N1 cells*

The properties of evaluated compounds to inhibit cAMP production induced by 5-CT (1000 nM), a 5-HT$_6$R agonist, was assessed according to previously published procedures [5,6]. The level of cAMP was measured using frozen recombinant 1321N1 cells expressing the human serotonin 5-HT$_6$R (PerkinElmer). Total cAMP was measured using the LANCE cAMP detection kit (PerkinElmer), according to the manufacturer's recommendations. For quantification of cAMP levels, 2000 cells/well (5 µL) were incubated with mixture of compound **3e**, **3f** and **3g** (5 µL) for 30 min at room temperature in 384-well white opaque microtiter plate. After incubation, the reaction was stopped and cells were lysed by the addition of 10 µL of working solution (5 µL Eu-cAMP and 5 µL ULight-anti-cAMP) for 1 h at room temperature. Time-resolved fluorescence resonance energy transfer (TR-FRET) was detected by an Infinite M1000 Pro (Tecan) using instrument settings from LANCE cAMP detection kit manual. Compound was tested in triplicate at 8 concentrations (10^{-11}–10^{-4} M). K_b values were calculated from Cheng–Prusoff equation specific for the analysis of functional inhibition curves: $K_b = IC_{50}/(1+A/EC_{50})$ where A represents agonist concentration, IC_{50} the concentration of antagonist producing a 50% reduction in the response to agonist, and EC_{50} the agonist concentration which causes a half of the maximal response [4].

1.1.1. Impact of evaluated compounds on cAMP production elicited by constitutively active 5-HT$_6$R in NG108-15 cells

NG108-15 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% dialyzed fetal calf serum, 2% hypoxanthine/aminopterin/thymidine (Life technologies), and antibiotics. cAMP measurement was performed in cells transiently expressing 5-HT$_6$R using the bioluminescence resonance energy transfer (BRET) sensor for cAMP, CAMYEL (cAMP sensor using YFP-Epac-RLuc) [7]. NG108-15 cells were cotransfected in suspension with 5-HT$_6$R (0.5 µg DNA/million cells) and CAMYEL constructs (1 µg DNA/million cells), using Lipofectamine 2000, according to the manufacturer's protocol, and plated in white 96-well plates (Greiner), at a density of 50,000 cells per well in DMEM containing 2% dialyzed fetal calf serum. After 24 h of transfection, cells were washed with PBS containing calcium and magnesium. To test the functional properties of compound **3e**, **3f** and **3g** as well as the reference SB-258585, cells were treated with vehicle or with the tested compound at a concentration ranging from 0.1 nM to 10 µM. Coelanterazine H (Molecular Probes) was added at a final concentration of 5 µM, and incubated at room temperature for 5 min. BRET was measured using a Mithras LB 940 plate reader (Berthold Technologies). Expression of 5-

HT_6R in NG108-15 cells induced a strong decrease in CAMYEL BRET signal, compared with cells transfected with an empty vector instead of the plasmid encoding the 5-HT_6R. This decrease in CAMYEL BRET signal was thus used as an index of 5-HT_6R constitutive activity at Gs signaling.

3. Procedure for evaluation of the effect of tested compounds on Cdk5-dependent neurite growth

In line with previously reported methods [5,8], NG108-15 cells were transfected with plasmids encoding either cytosolic GFP or a GFP-tagged 5-HT$_6$R in suspension using Lipofectamine 2000 (Life technologies) and plated on glass coverslips. Six hours after transfection, cells were treated with either DMSO (control), **3e**, **3f** and **3g** or SB-258585 for 48 h. Cells were fixed in 4% paraformaldehyde (PFA) supplemented with 4% sucrose for 10 min. PFA fluorescence was quenched by incubating the cells in PBS containing 0.1 M glycine, prior to mounting in Prolong Gold antifade reagent (Thermo Fisher Scientific). Cells were imaged using an AxioImagerZ1 microscope equipped with epifluorescence (Zeiss), using a 20 × objective for cultured cells, and neurite length was assessed using the Neuron J plugin of the ImageJ software (NIH).

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