

Rational design, synthesis and pharmacological evaluation of chalcones as dual-acting compounds – histamine H₃ receptor ligands and MAO B inhibitors

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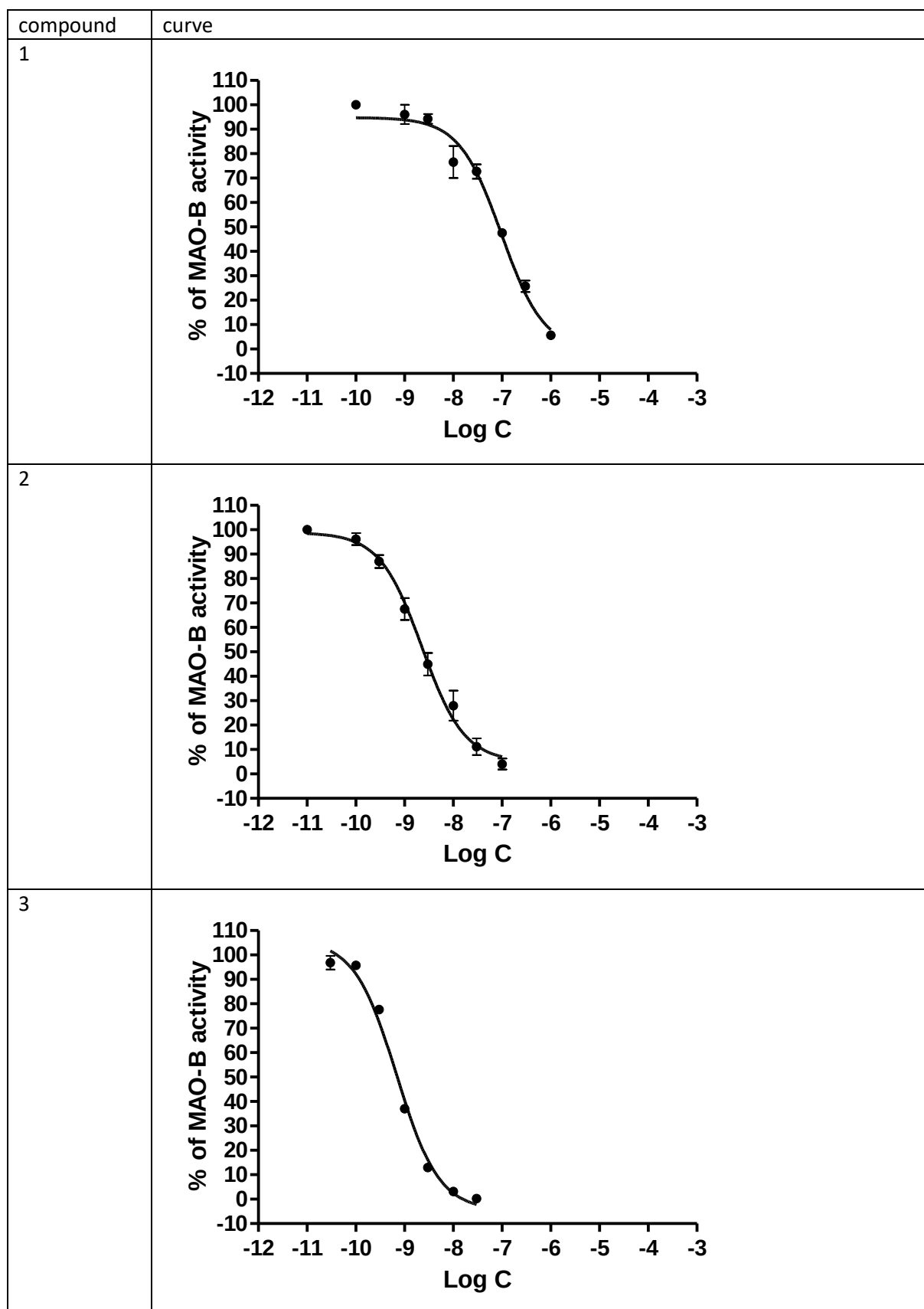
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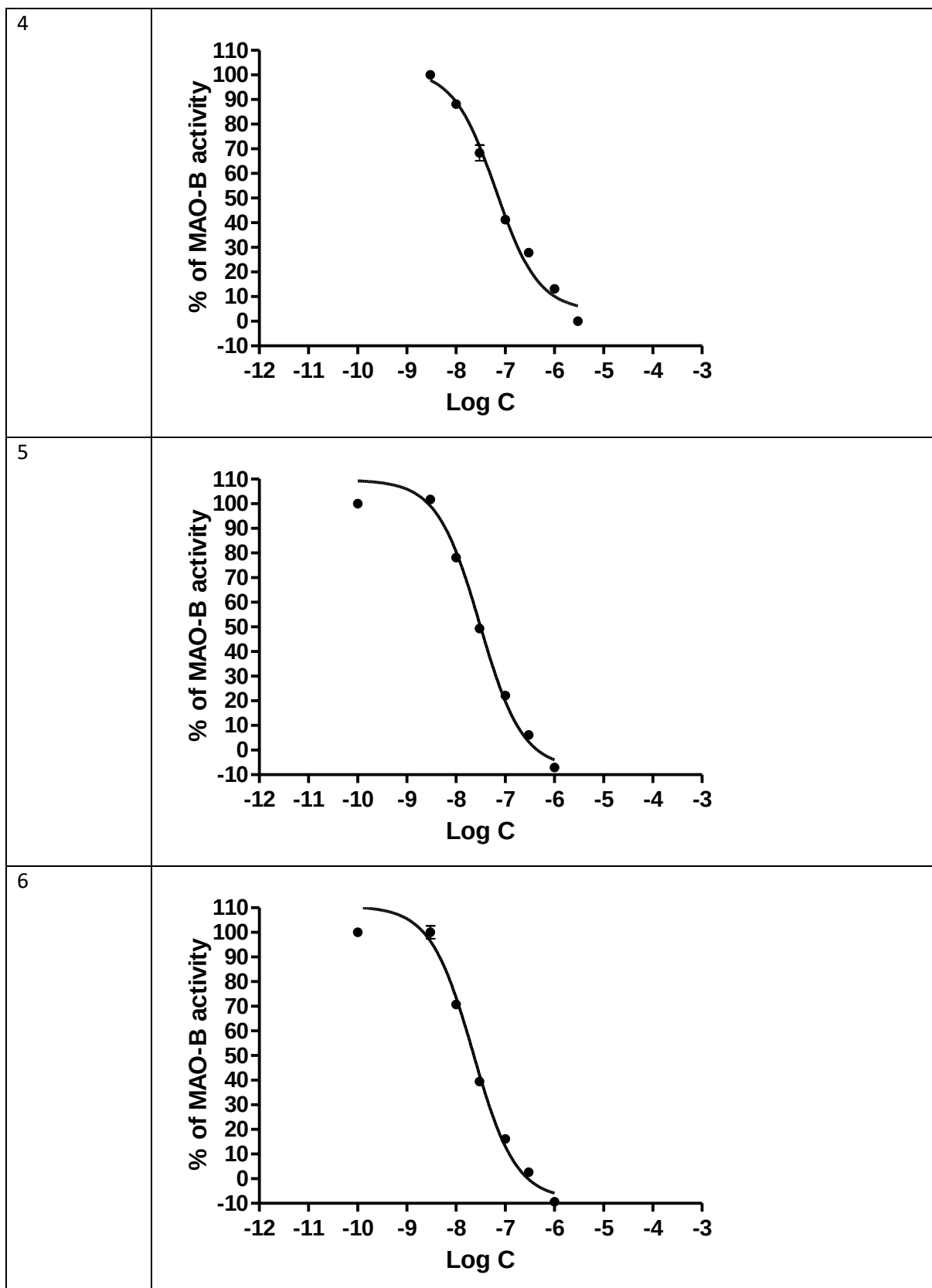
Correspondence: dorota.lazewska@uj.edu.pl (D.Ł.); anna.stasiak@umed.lodz.pl (A.St.)

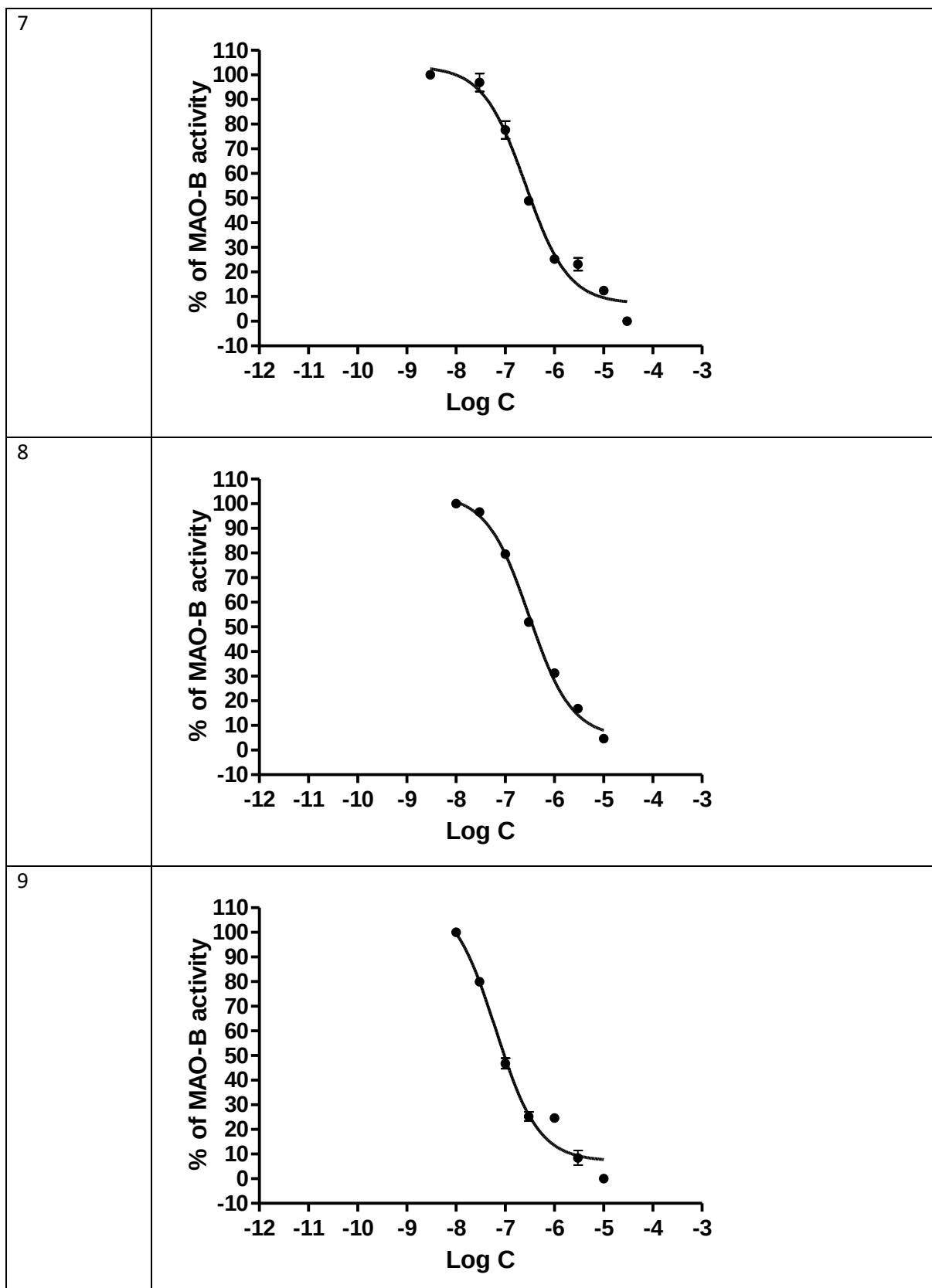
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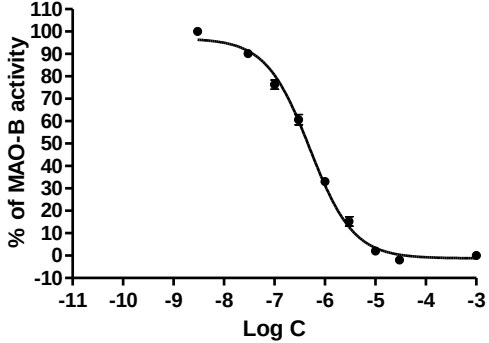
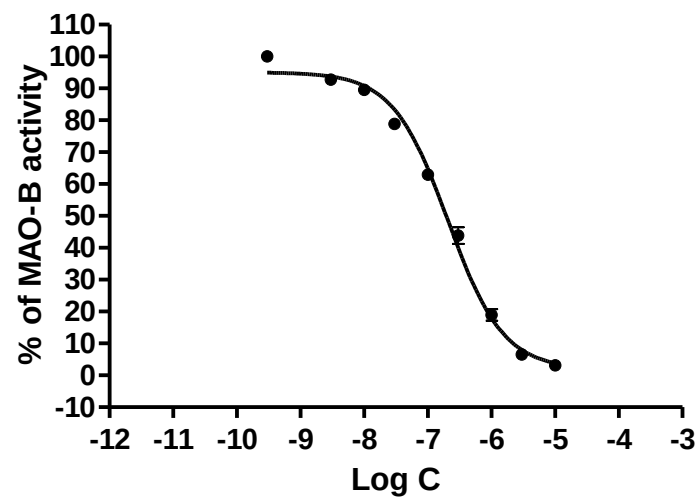
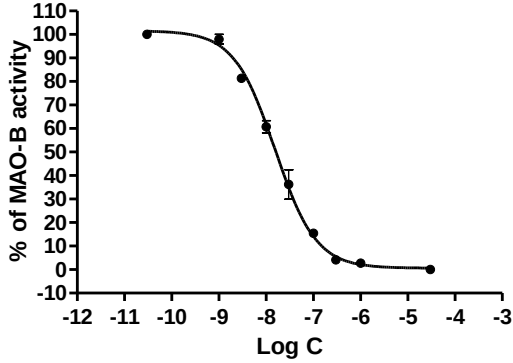
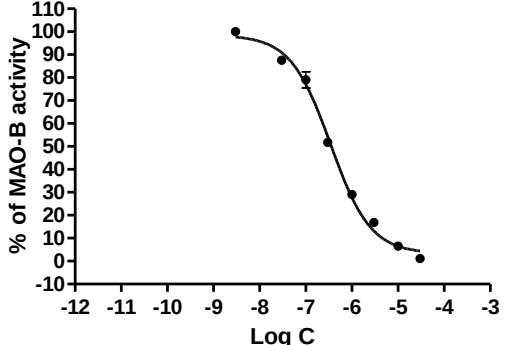
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Graphs showing the data that were used to calculate the IC₅₀ values in the case of MAO B.







12	 Graph 12 shows the percentage of MAO-B activity versus Log C. The activity starts at approximately 100% at Log C = -8.5 and decreases sigmoidally to near 0% at Log C = -3.5. <table border="1"> <thead> <tr> <th>Log C</th> <th>% of MAO-B activity</th> </tr> </thead> <tbody> <tr><td>-8.5</td><td>100</td></tr> <tr><td>-8.0</td><td>90</td></tr> <tr><td>-7.5</td><td>75</td></tr> <tr><td>-7.0</td><td>60</td></tr> <tr><td>-6.5</td><td>35</td></tr> <tr><td>-6.0</td><td>15</td></tr> <tr><td>-5.5</td><td>5</td></tr> <tr><td>-5.0</td><td>2</td></tr> <tr><td>-4.5</td><td>1</td></tr> <tr><td>-3.5</td><td>0</td></tr> </tbody> </table>	Log C	% of MAO-B activity	-8.5	100	-8.0	90	-7.5	75	-7.0	60	-6.5	35	-6.0	15	-5.5	5	-5.0	2	-4.5	1	-3.5	0
Log C	% of MAO-B activity																						
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Data from functional characterisation in cAMP accumulation assay of compounds 12 and 15¹

cAMP H ₃ R													
Agonist mode*					Antagonist mode**								
Compound	E max %	EC ₅₀	pEC ₅₀	SD	Compound	E max %	IC ₅₀	IC ₅₀	pIC ₅₀	K _b	K _b	SD	R ²
		M		pEC ₅₀			M	nM		M	nM	K _b [nM]	K _b
α-Methylhistamine	100	5.93E-09	8.23	0.873	Thioperamide	100	1.11E-07	110	6.96	4.14E-08	41.4	2.81	0.931
12	0	n.c.	n.c.	n.c.	AR-15	109	1.05E-08	10.5	7.98	3.94E-09	3.94	0.85	0.974
15	3	n.c.	n.c.	n.c.	AR-35	105	6.30E-08	63	7.20	3.42E-08	34.2	2.78	0.953

¹Compounds were tested in duplicate in two separate experiments *Results were normalized as percentage of maximal agonist response (α-Methylhistamine 10⁻⁵ M);

**Results were normalised as a percentage of reference antagonist (Thioperamide 10⁻⁵ M); E_{max} is the maximum possible effect; n.c. - not calculable;

Metabolic stability evaluation.

1. *In silico* studies

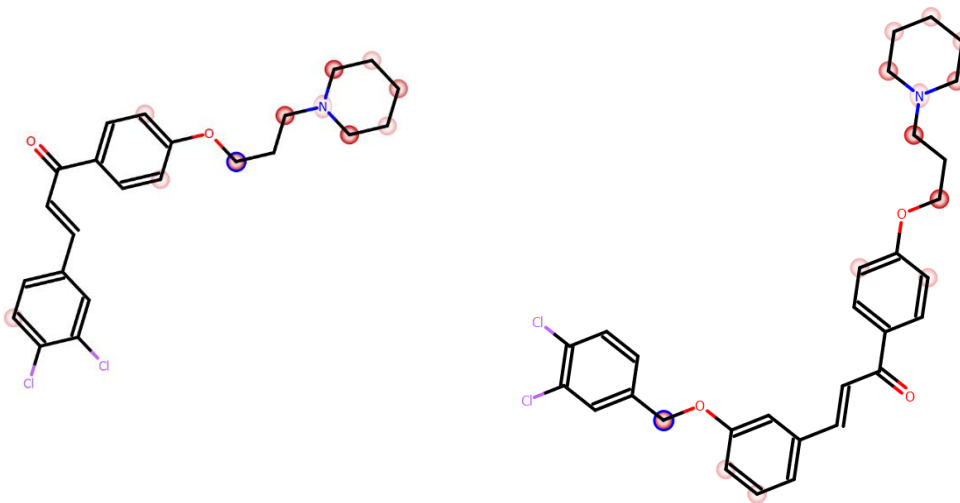


Figure S1. *In silico* prediction of the sites of metabolism by MetaSite 6.01 for compounds: **12** (left) and **15** (right). The darker red color - the higher probability to be involved in the metabolism pathway. The blue circle marked the site of compound with the highest probability of metabolic bioconversion.

2. *In vitro* – determination of metabolic pathways in rat liver microsomes (RLMs)

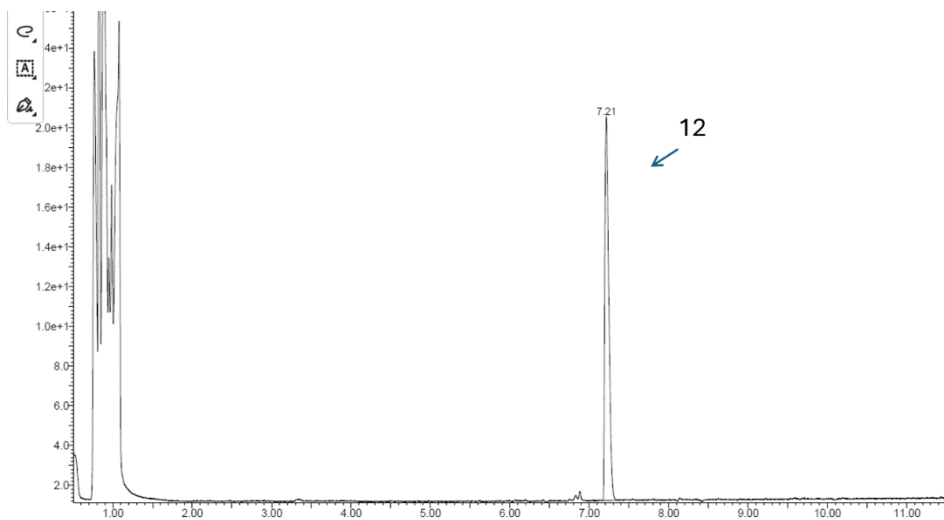


Figure S2. UPLC of control reaction of compound **12** (incubation for 120 min without microsomes)

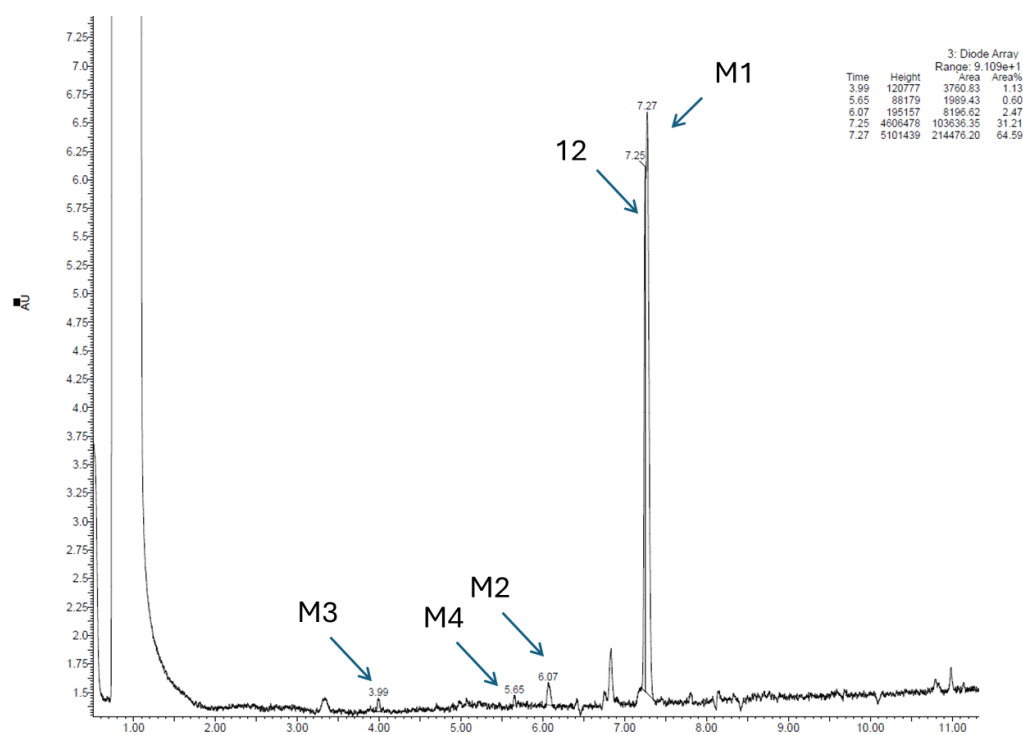
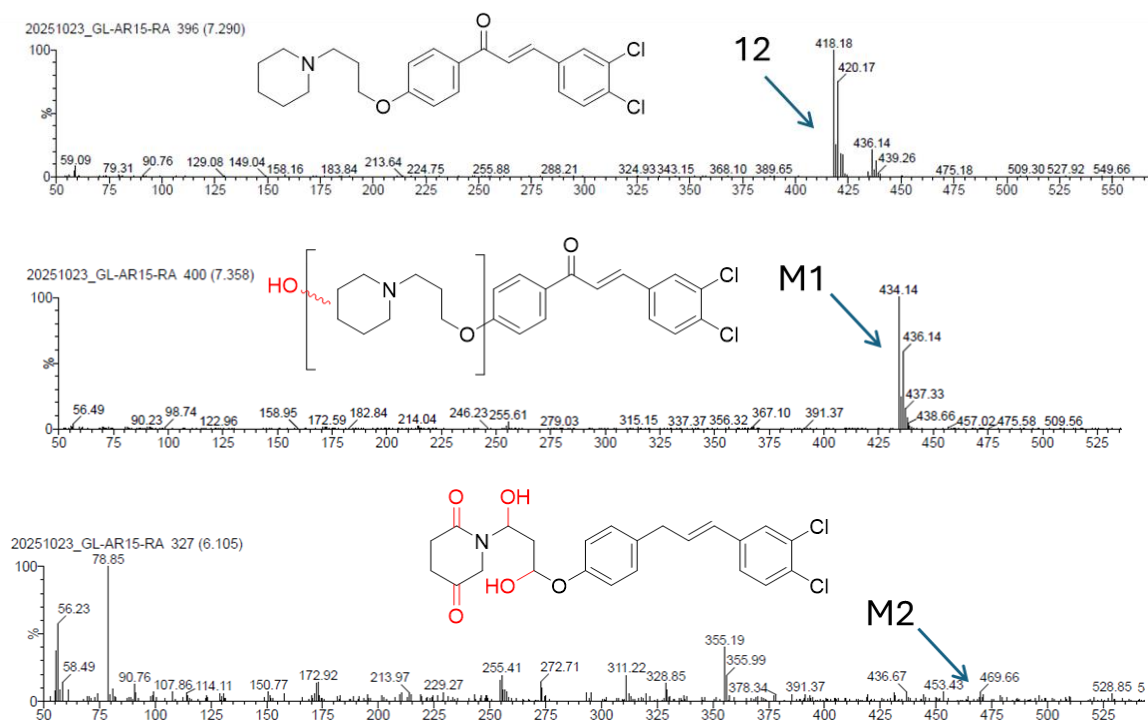


Figure S3. UPLC after incubation of **12** for 120 min with rat liver microsomes.



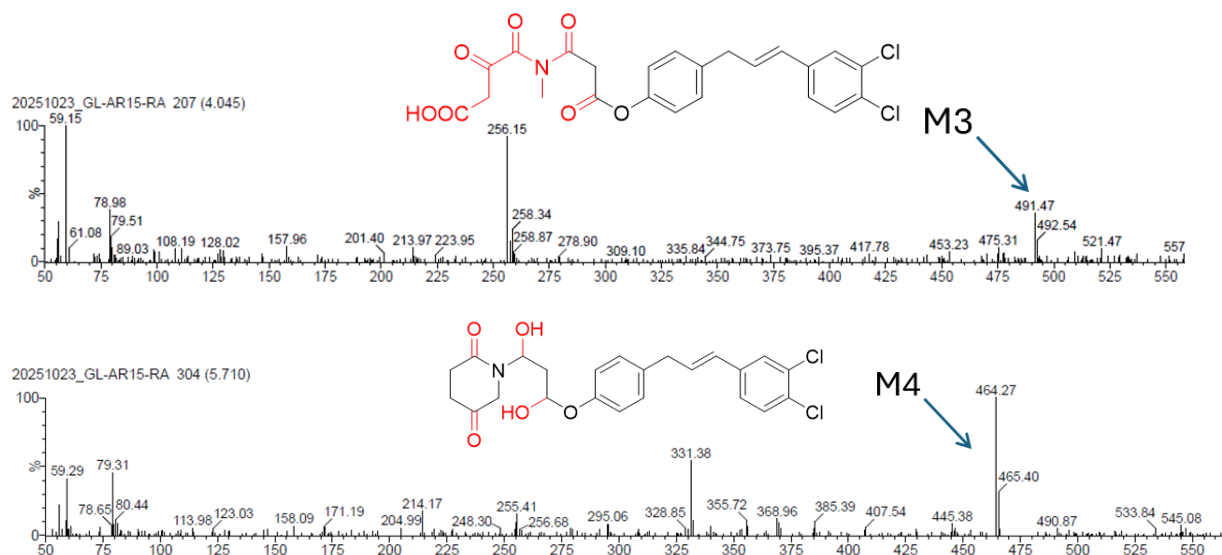


Figure S4. MS spectra of **12** and metabolites after incubation for 120 min with rat liver microsomes.

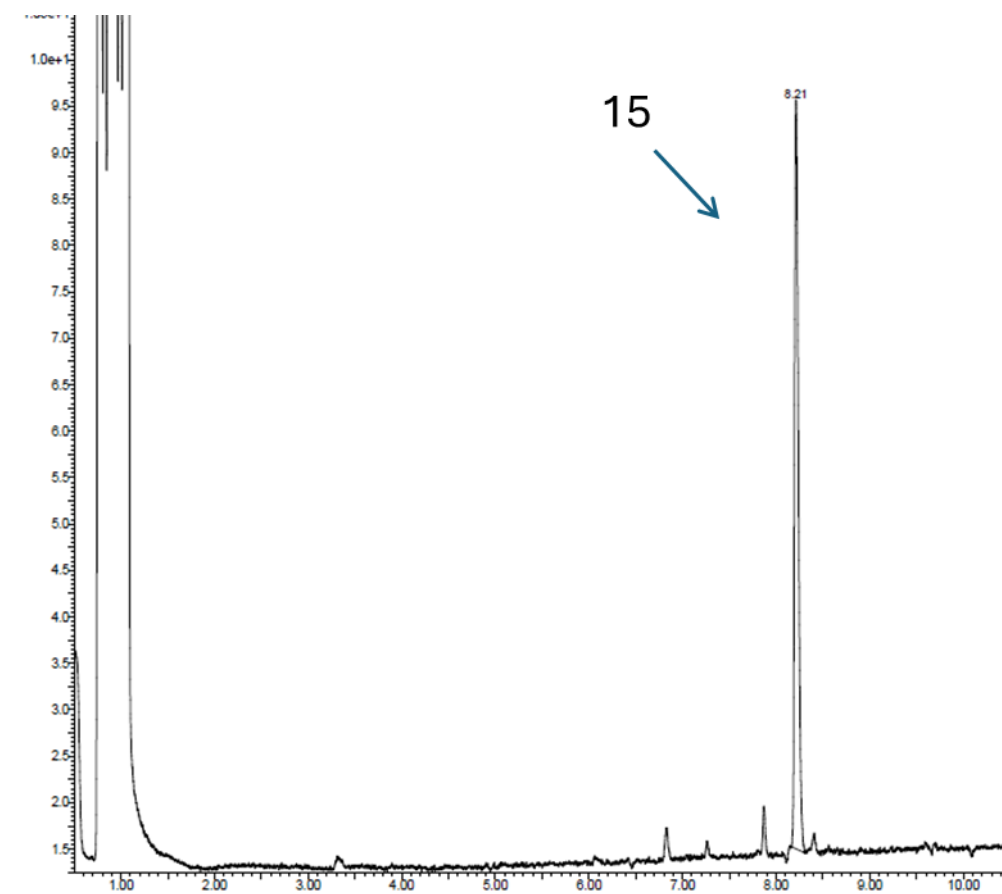


Figure S5. UPLC of control reaction of compound **15** (incubation for 120 min without microsomes)

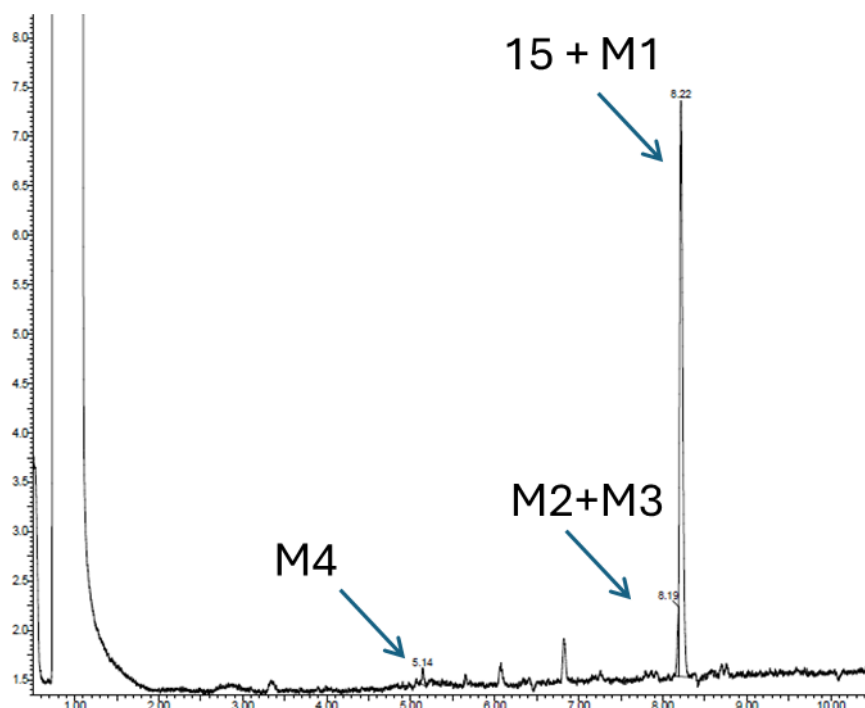
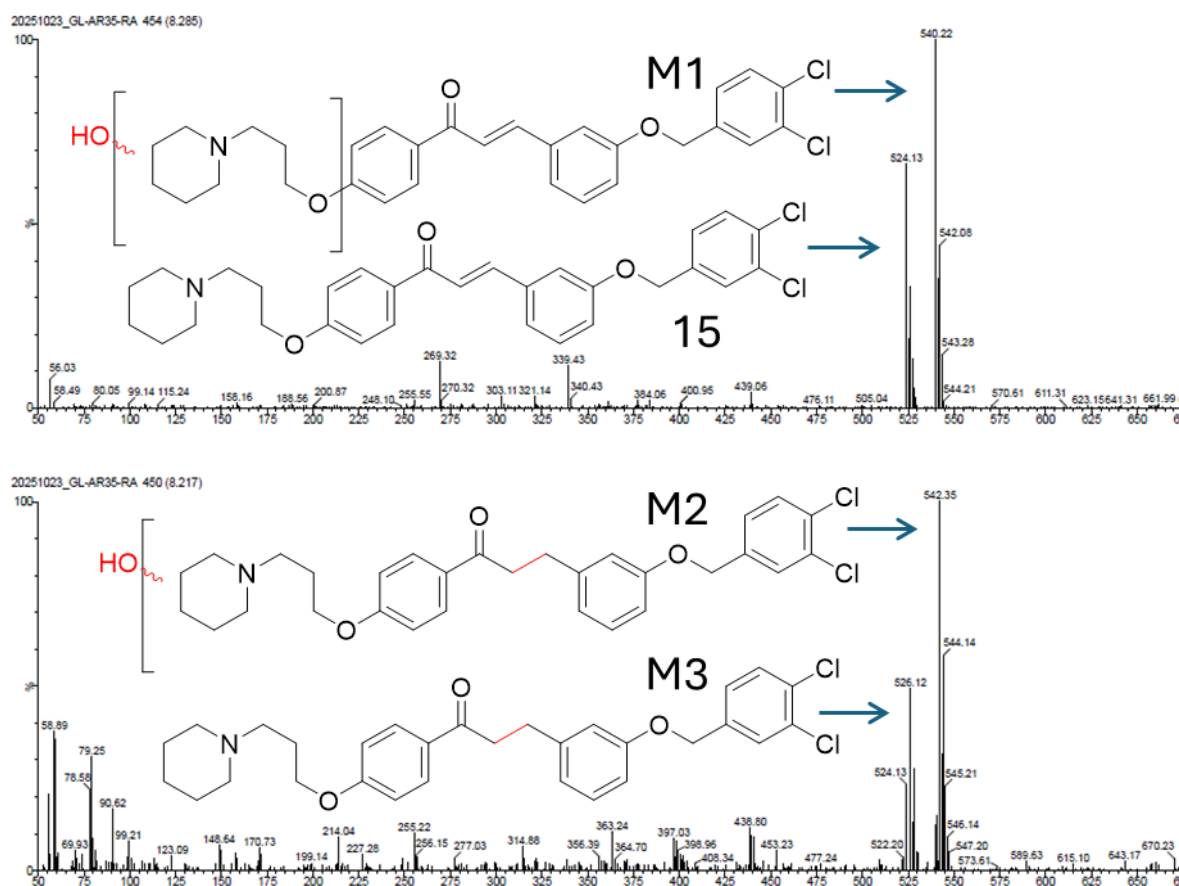


Figure S6. UPLC after incubation of **15** for 120 min with rat liver microsomes.



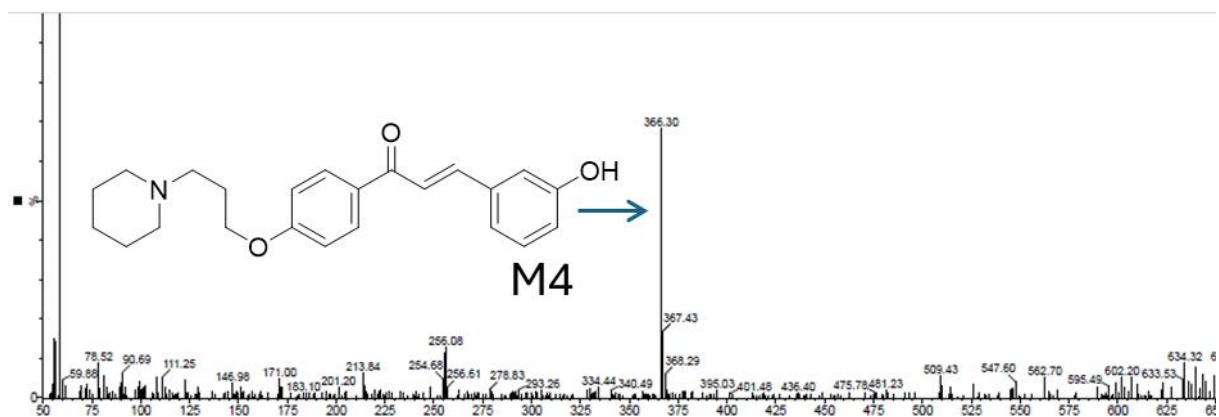
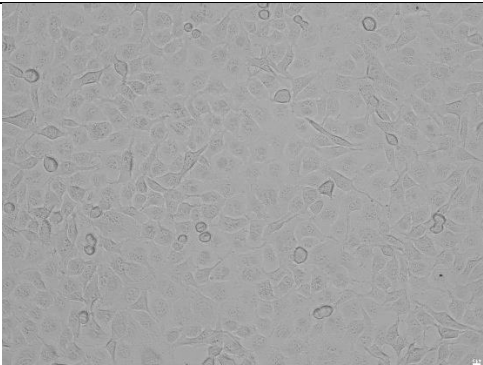
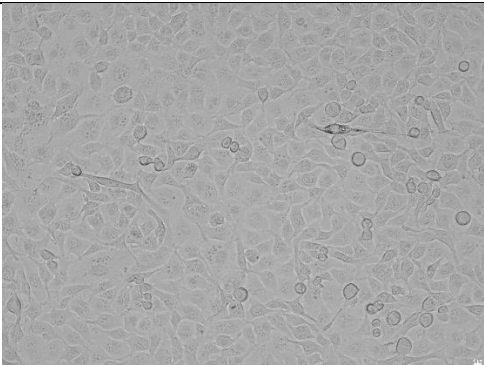
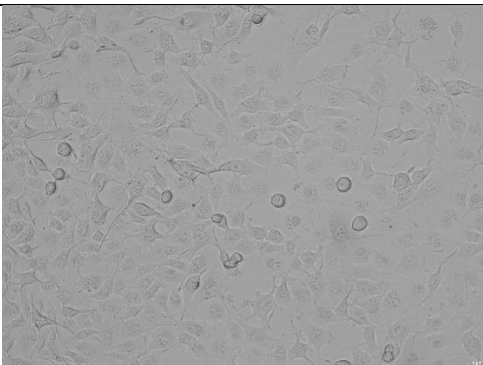
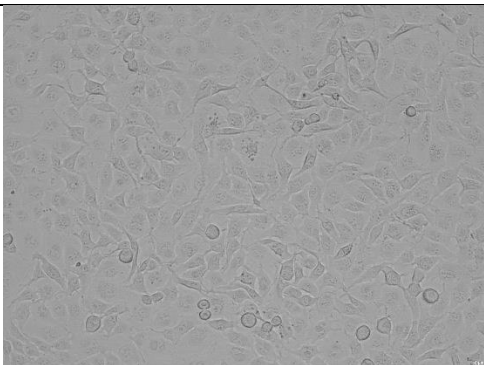
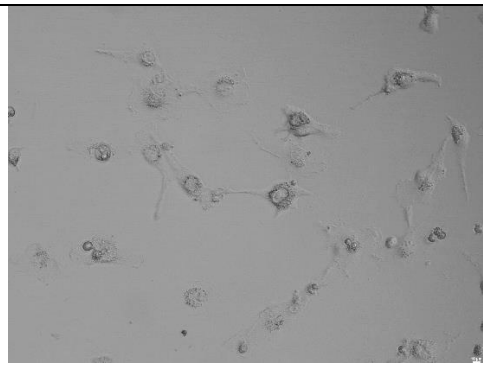
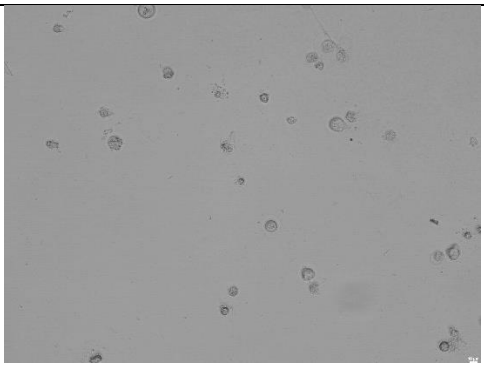
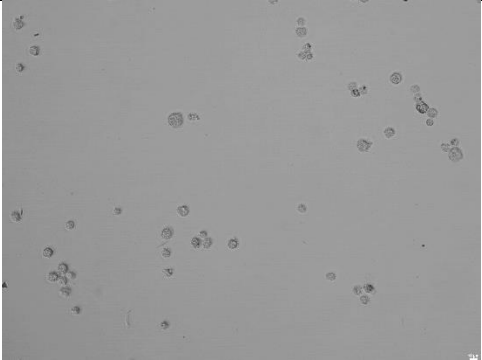
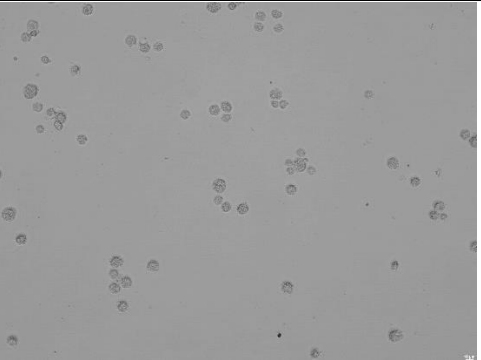
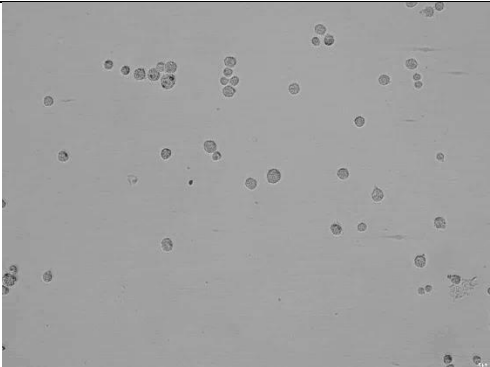
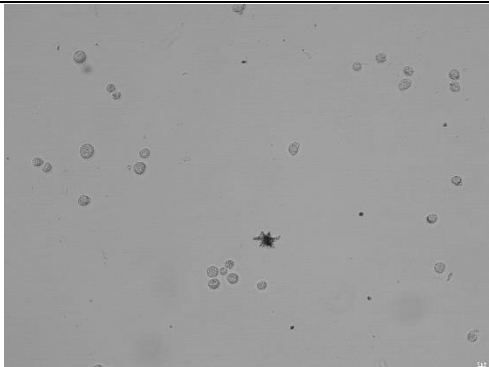
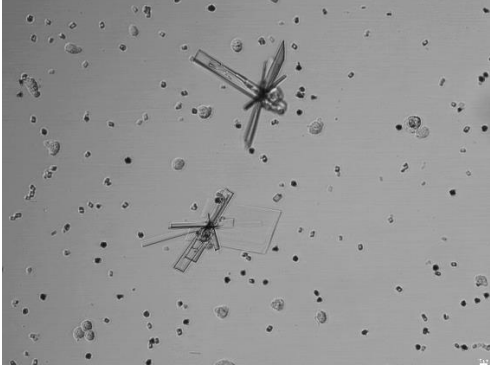
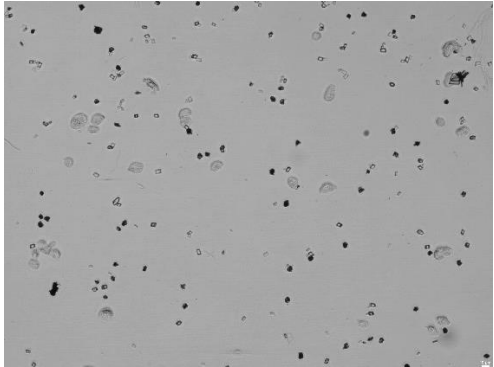
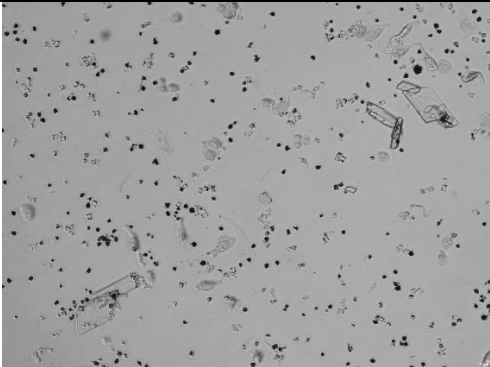
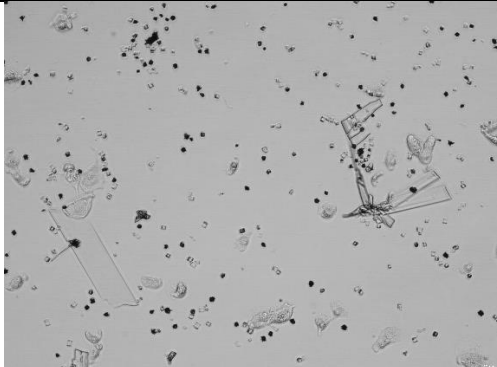


Figure S7. MS spectra of **15** and metabolites after incubation for 120 min with rat liver microsomes.

Views of microscope images taken for compounds 12 and 15 at various concentrations on the tested cell lines. The photomicrographs were done using Leica DMi8 inverted microscope (objective 10x) of the HepG2 and SH-SY5Y cells after 72h of incubation in the presence of compounds 12 and 15 (0.1-100 μ M).

Table .S1 The microscope images of SH-SY5Y cells after incubation with compound 12 at various concentrations. Compound precipitated at 25 -100 μ M.

Compound 12		
0.1 μ M		
1 μ M		
5 μ M		

10 μM		
25 μM		
50 μM		
100 μM		

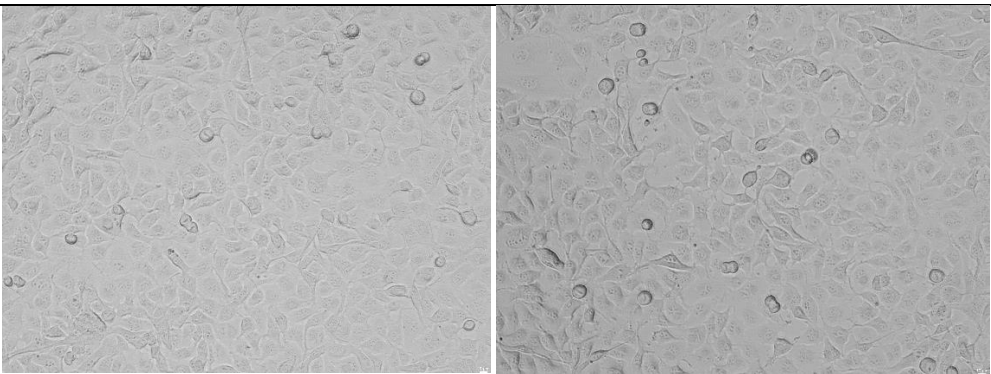
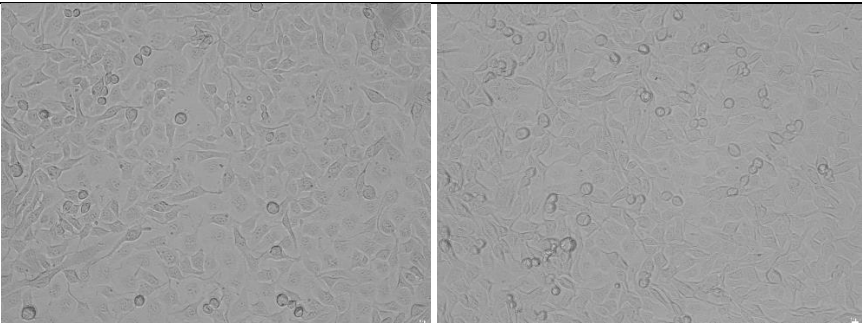
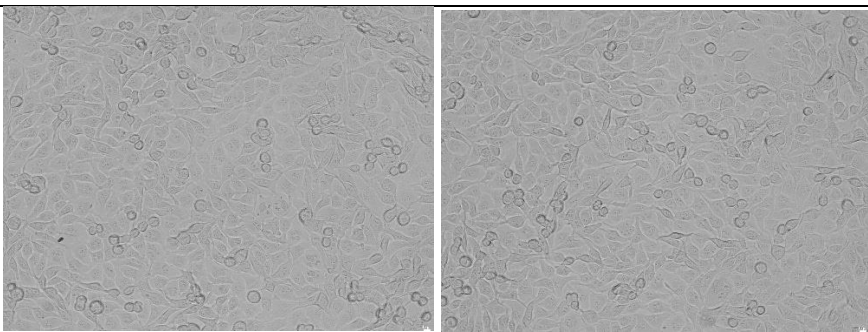
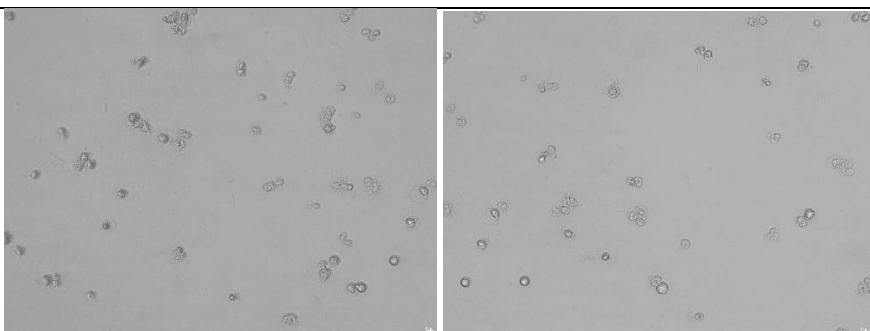
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Table S2. The microscope images of SH-SY5Y cells after incubation with compound **15** at various concentrations. Compound precipitated at 50 and 100 μM .

Compound 15	
0,1 μM	
1 μM	
10 μM	

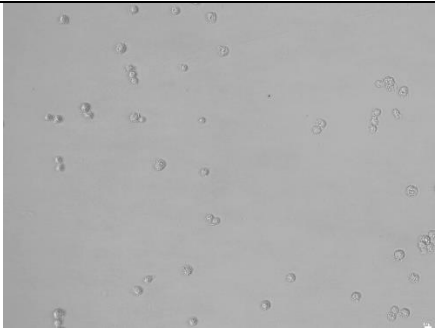
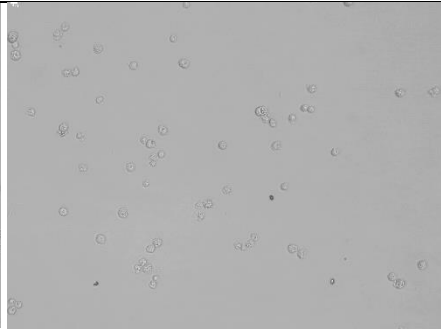
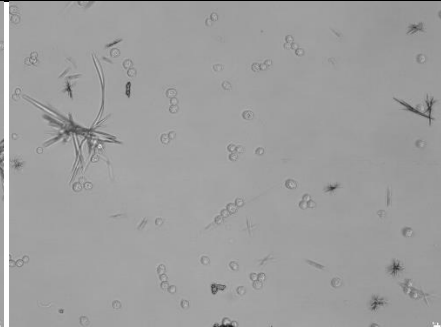
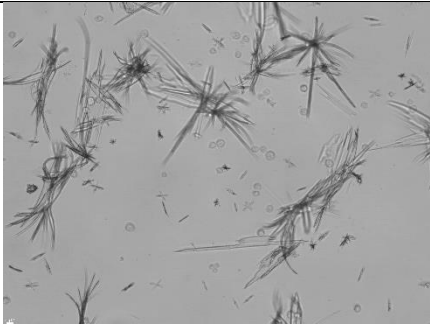
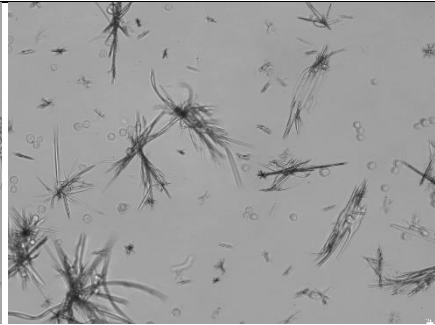
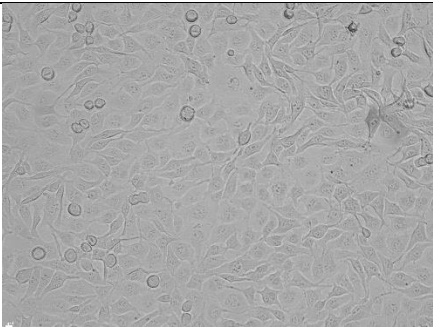
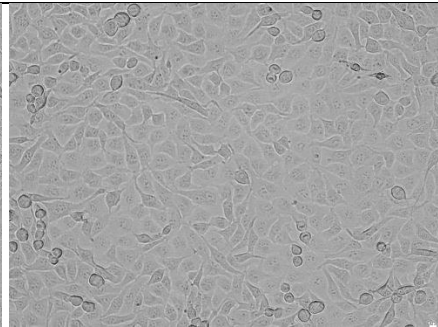
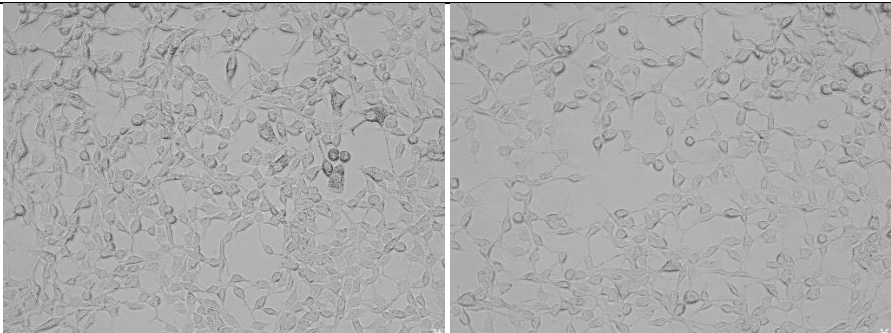
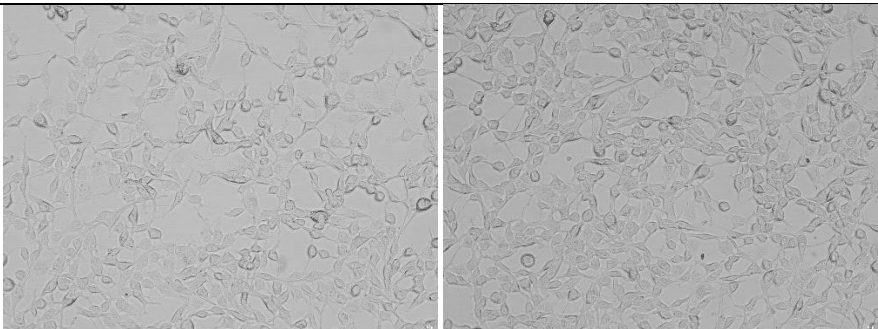
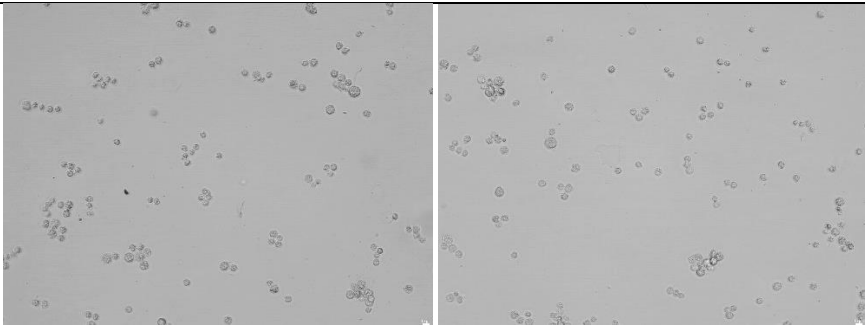
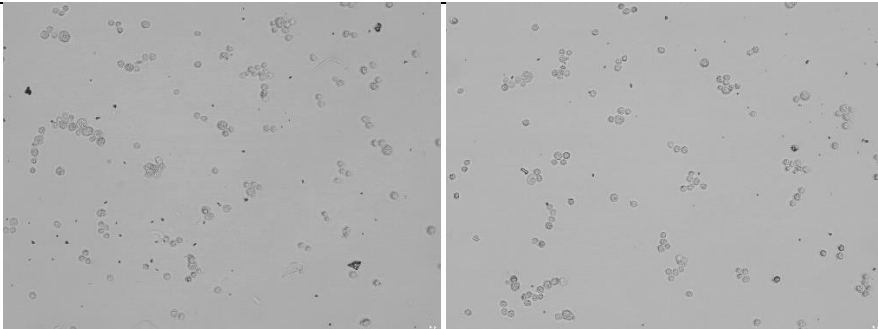
25 Mm		
50 μ M		
100 μ M		
kontrola		

Table S3. The microscope images of HepG2 cells after incubation with compound **12** at various concentrations. Compound precipitated at 25 -100 μ M.

Compound 12	
0,1 μ M	
1 μ M	
10 μ M	
25 μ M	

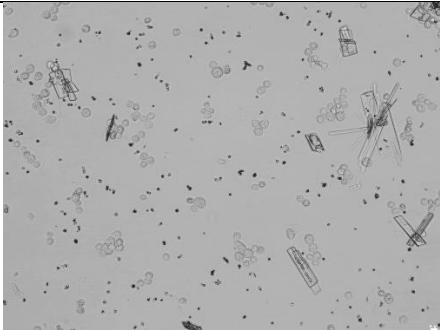
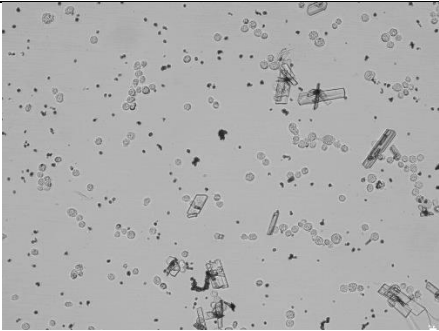
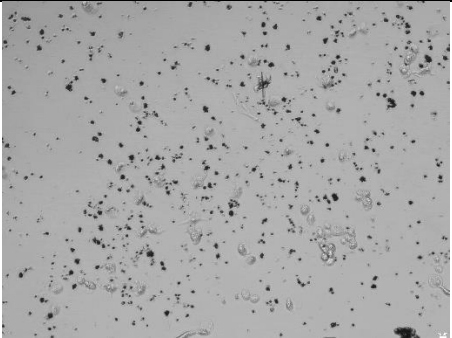
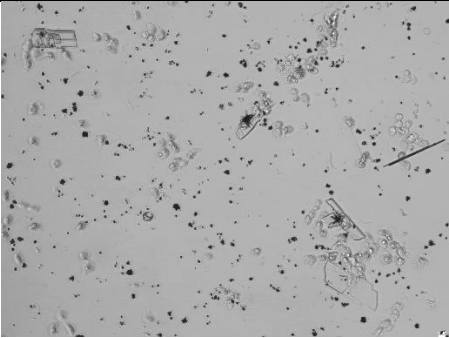
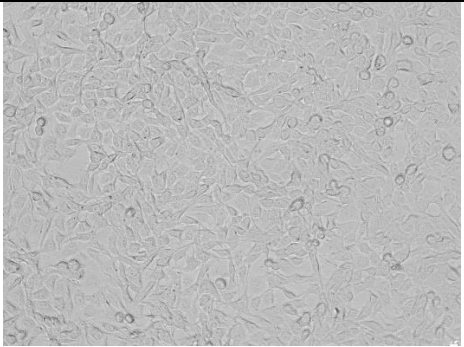
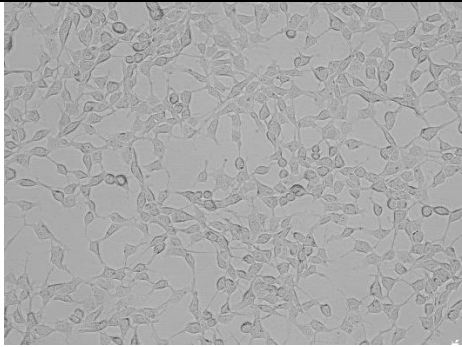
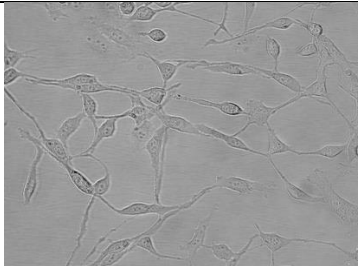
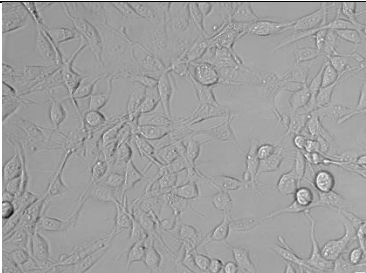
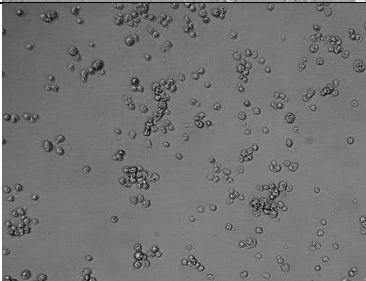
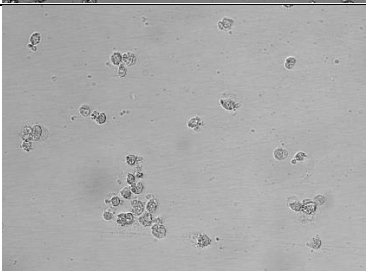
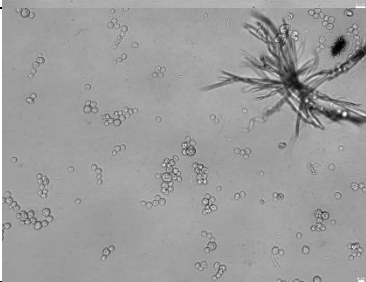
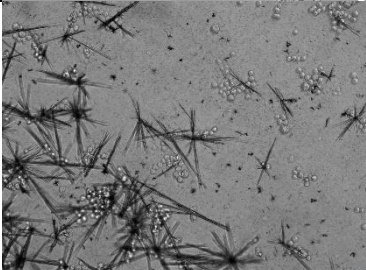
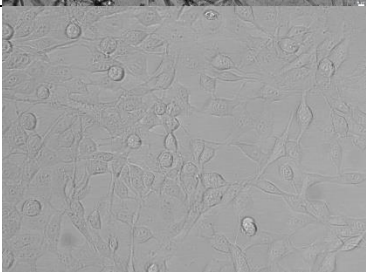
50 μM		
100 μM		
kontrola		

Table S4. The microscope images of HepG2 cells after incubation with compound **15** at various concentrations. Compound precipitated at 50 and 100 μM .

Compound 15	
0,1 μM	

1 μM	
10 μM	
25 μM	
50 μM	
100 μM	
Control	

^1H NMR spectra of compounds 1-9.

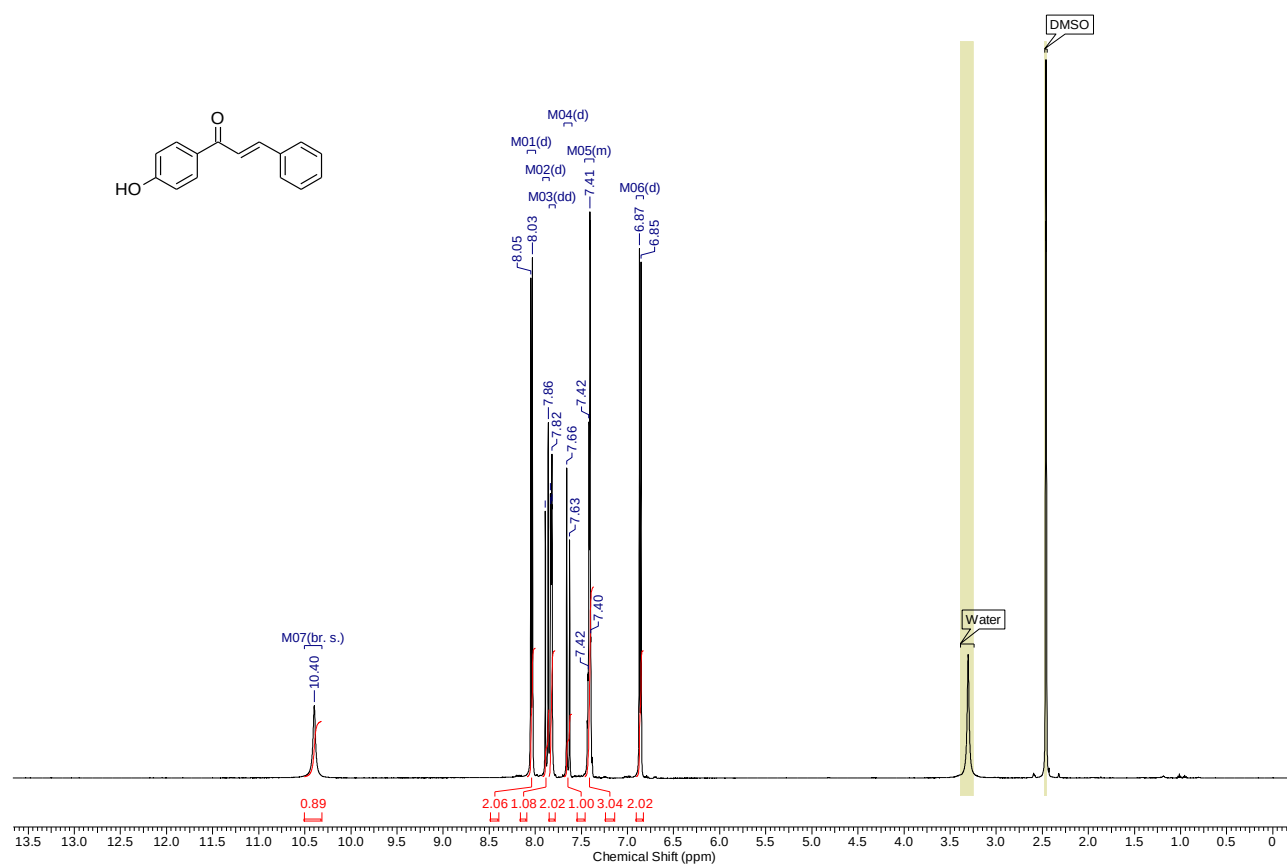


Figure S8. ^1H NMR spectrum of compound 1.

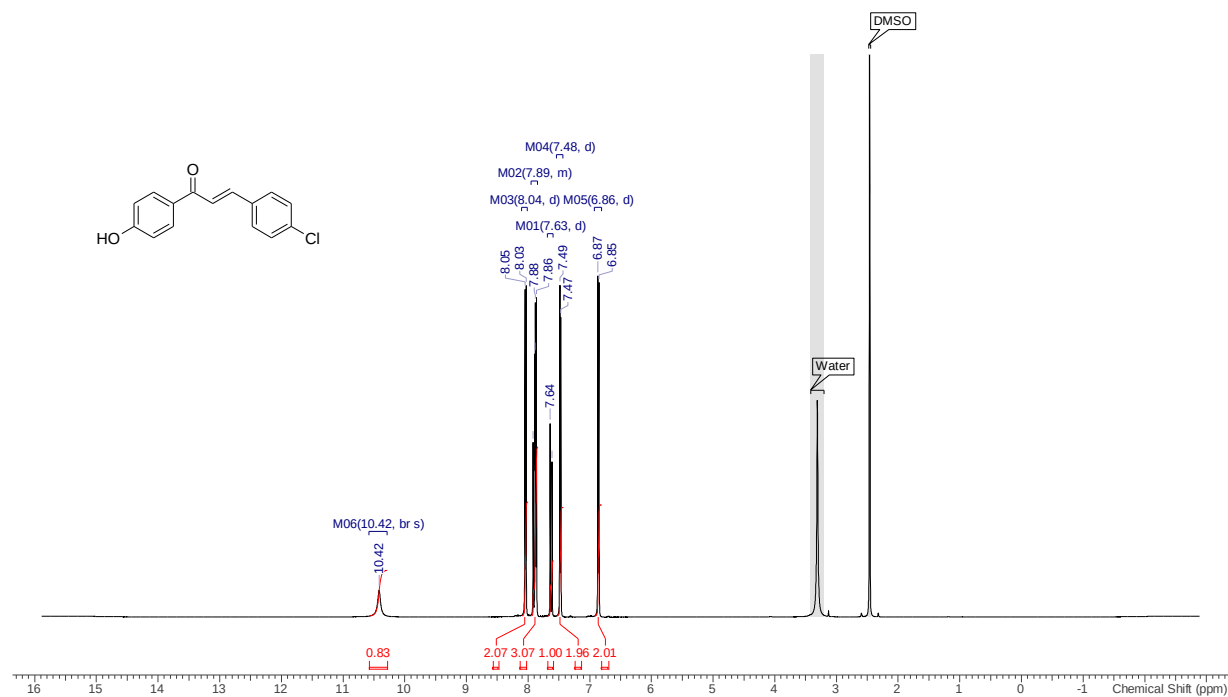


Figure S9. ^1H NMR spectrum of compound 2.

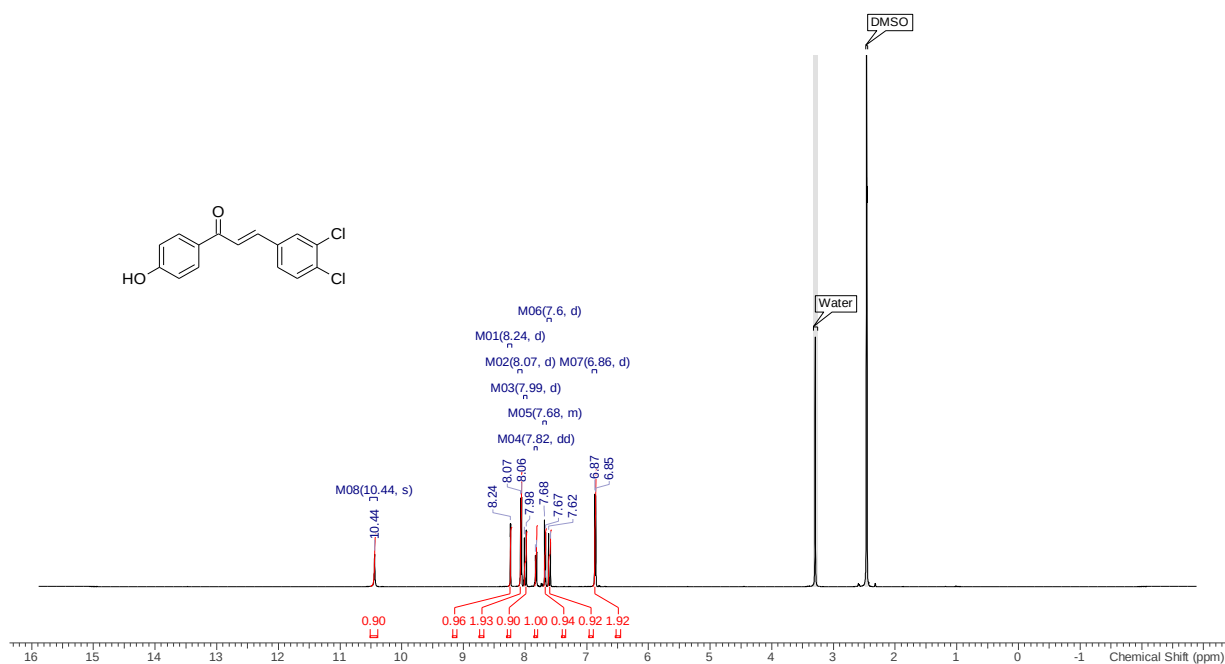


Figure S10. ¹H NMR spectrum of compound 3.

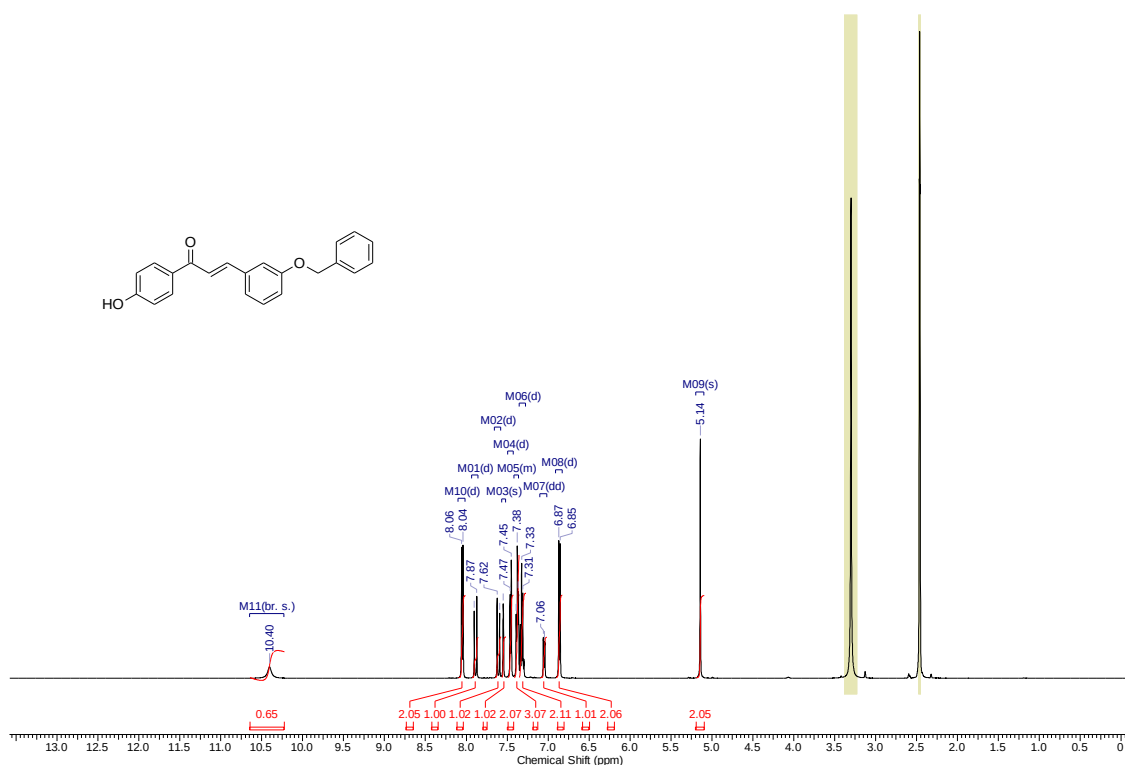


Figure S11. ¹H NMR spectrum of compound 4.

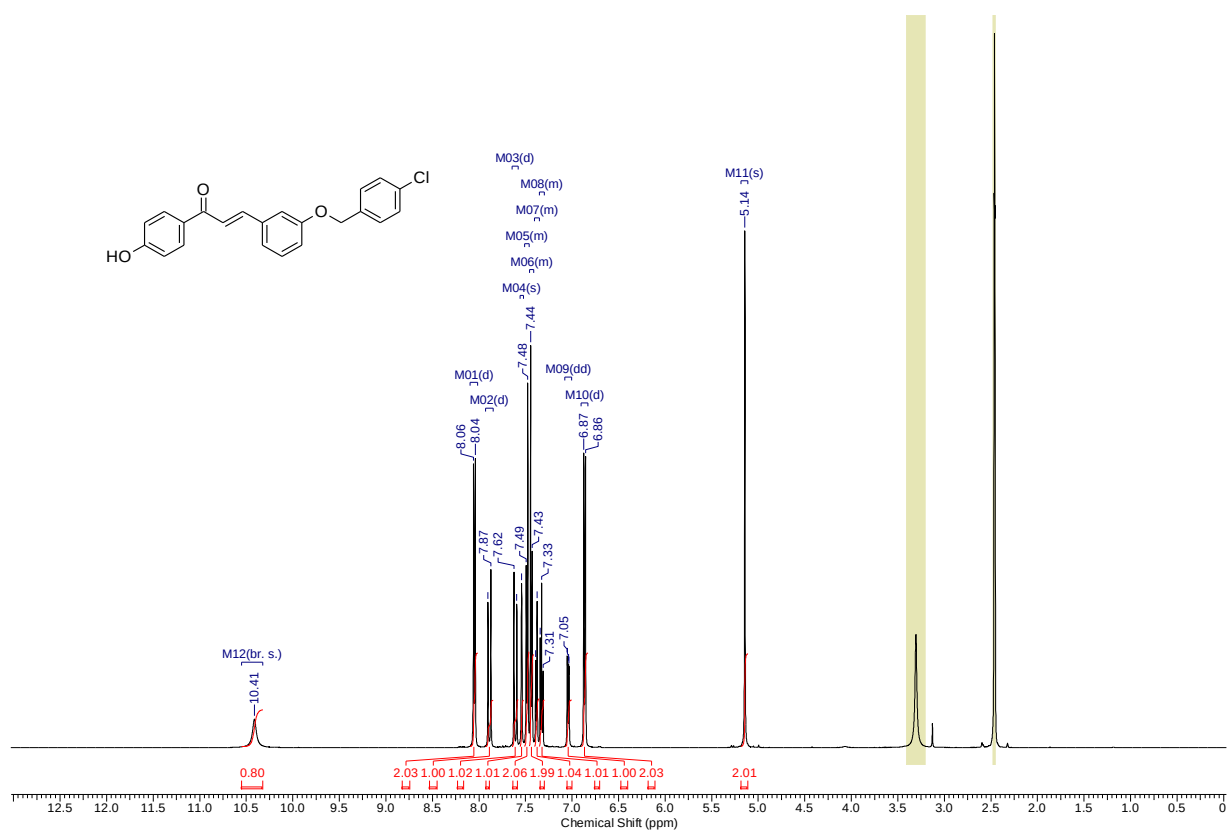


Figure S12. ¹H NMR spectrum of compound 5.

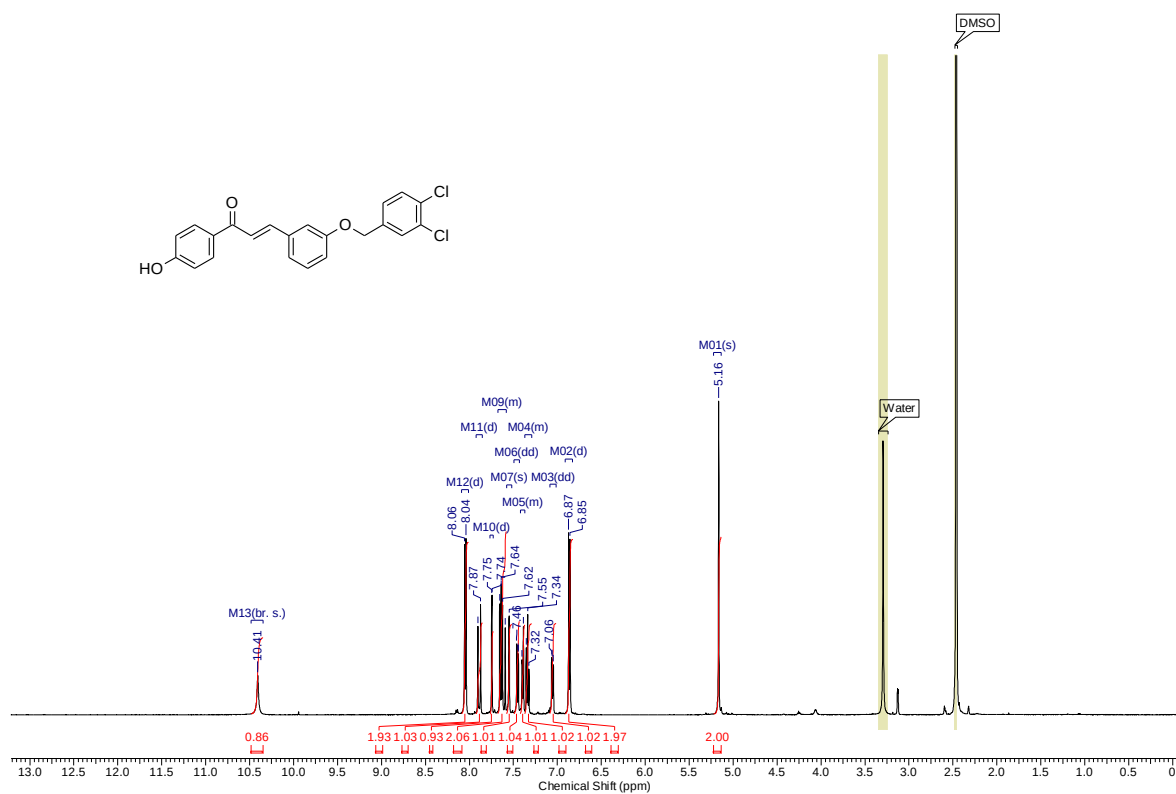


Figure S13. ¹H NMR spectrum of compound 6.

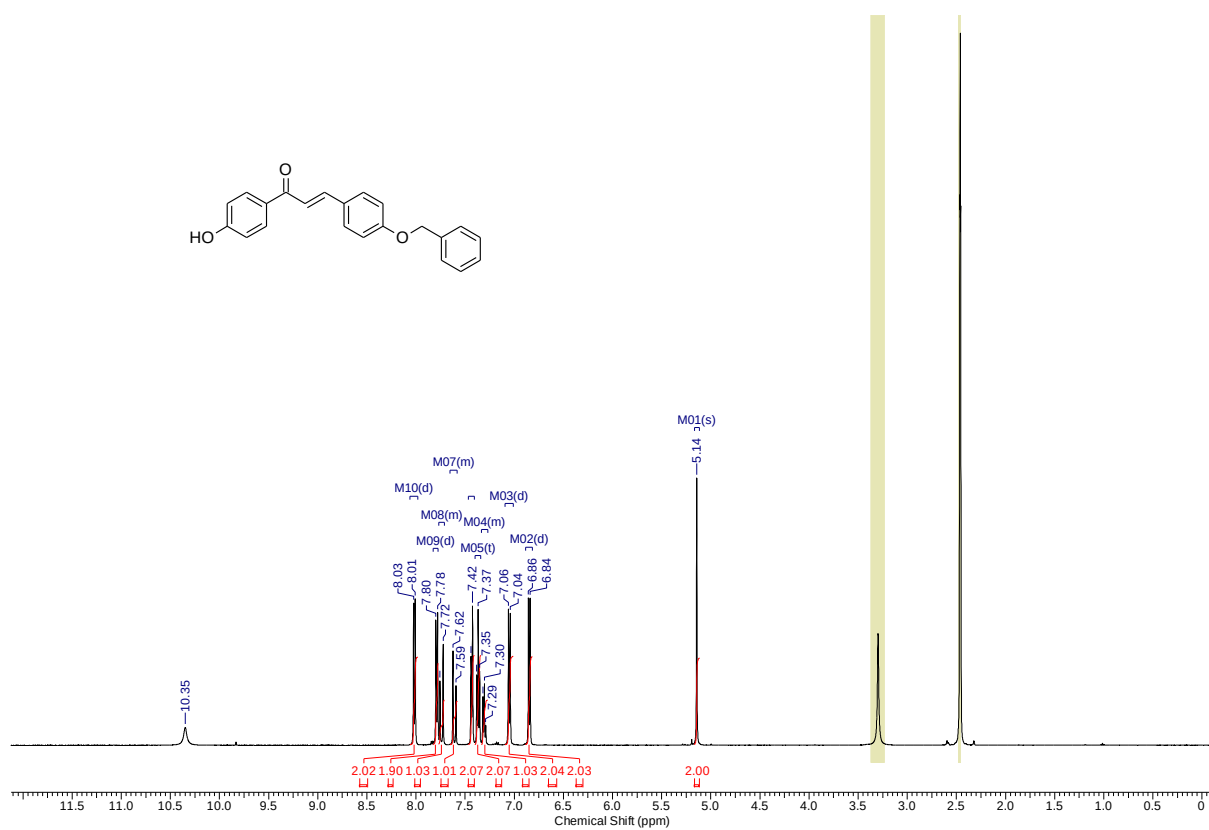


Figure S14. ¹H NMR spectrum of compound 7.

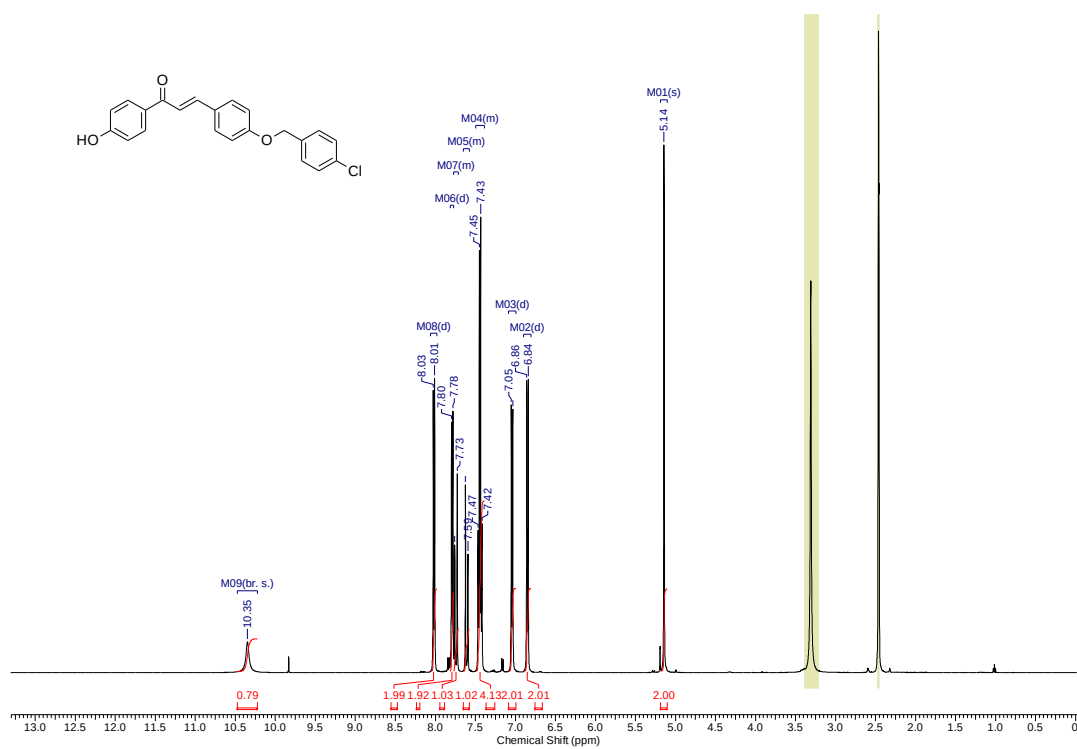


Figure S15. ¹H NMR spectrum of compound 8.

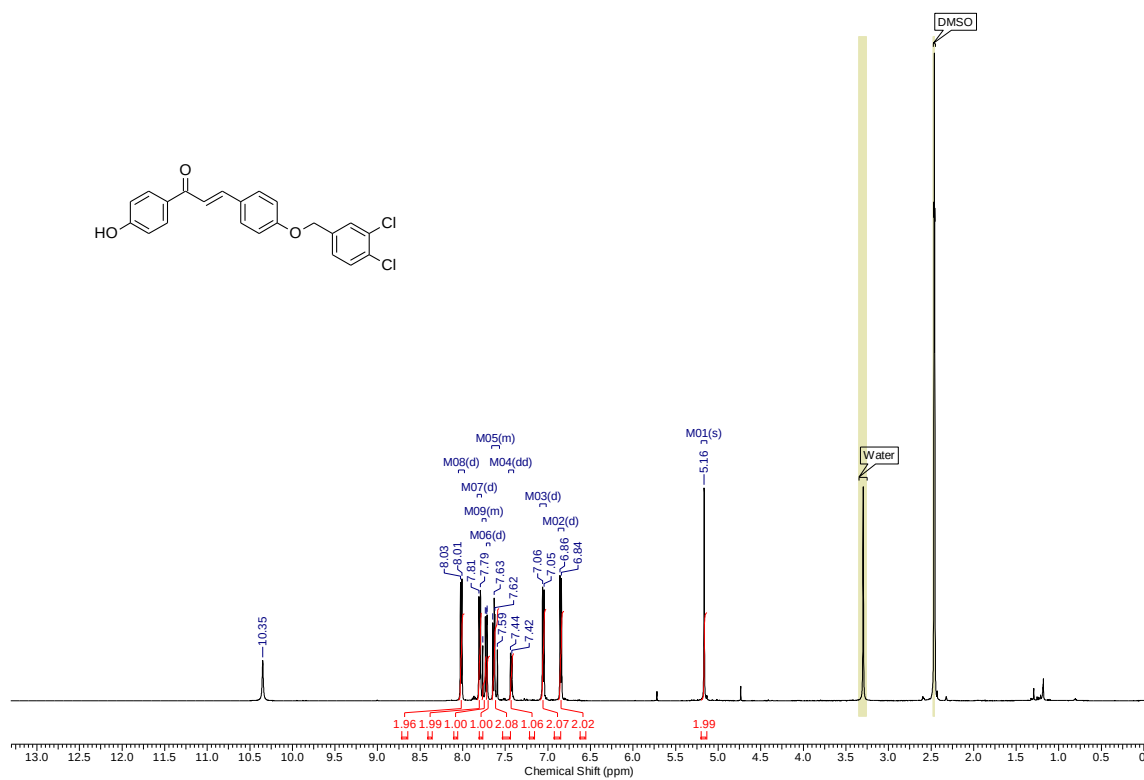


Figure S16. ^1H NMR spectrum of compound **9**.

¹H and ¹³C NMR spectra of compounds 10-18

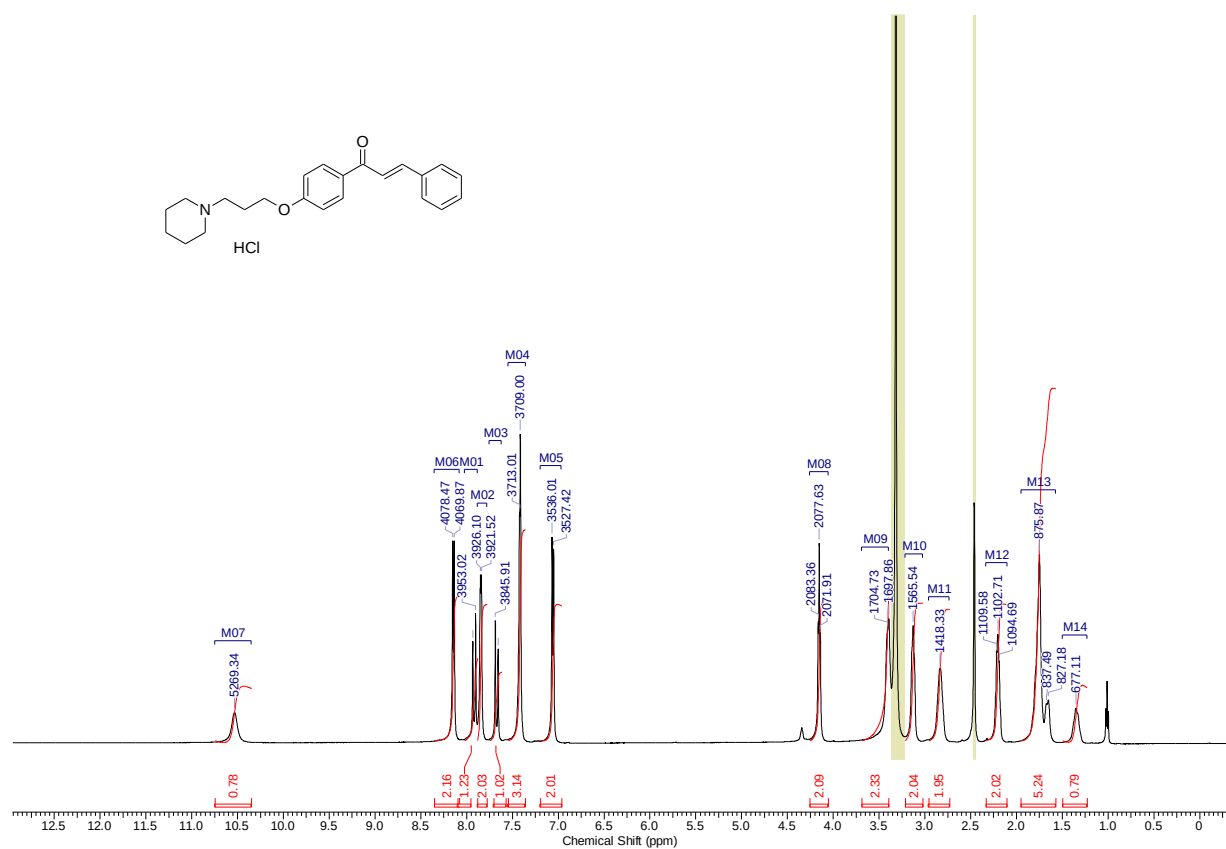


Figure S17. ¹H NMR spectrum of compound 10.

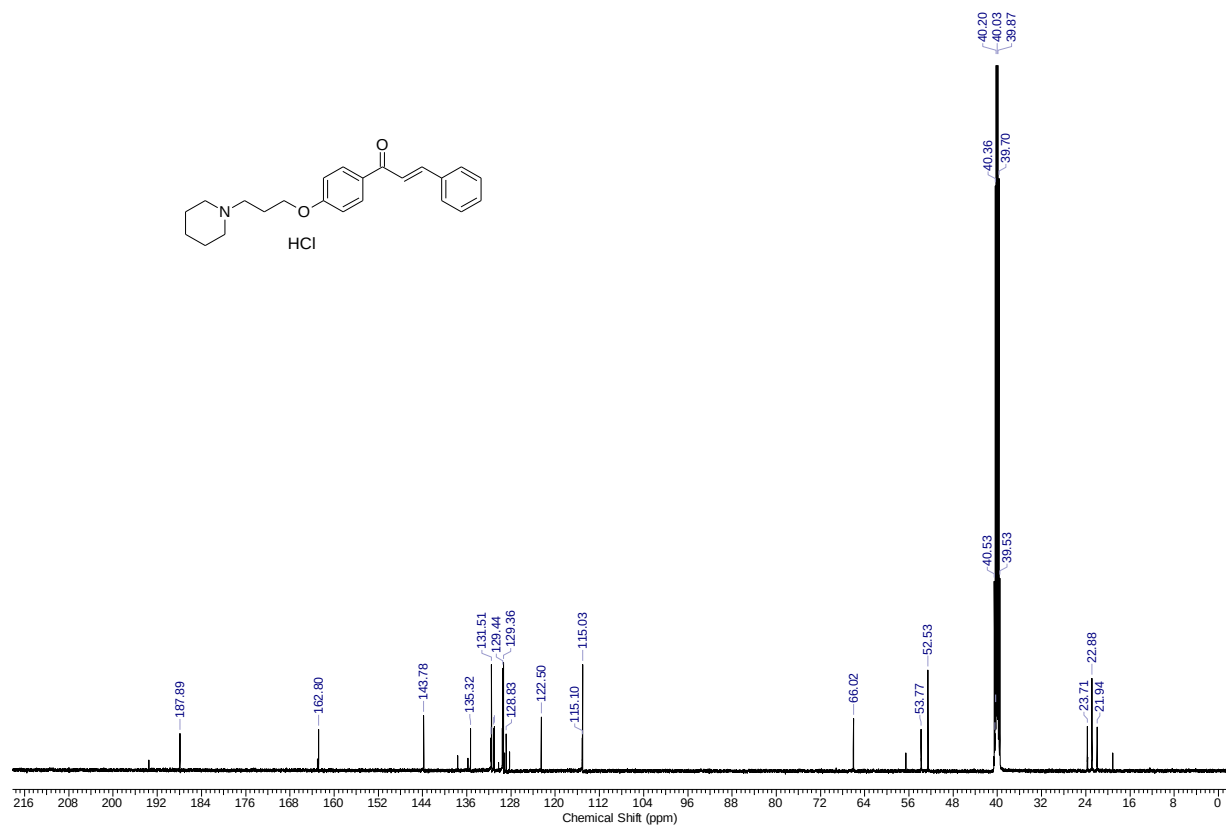


Figure S18. ¹³C NMR spectrum of compound 10.

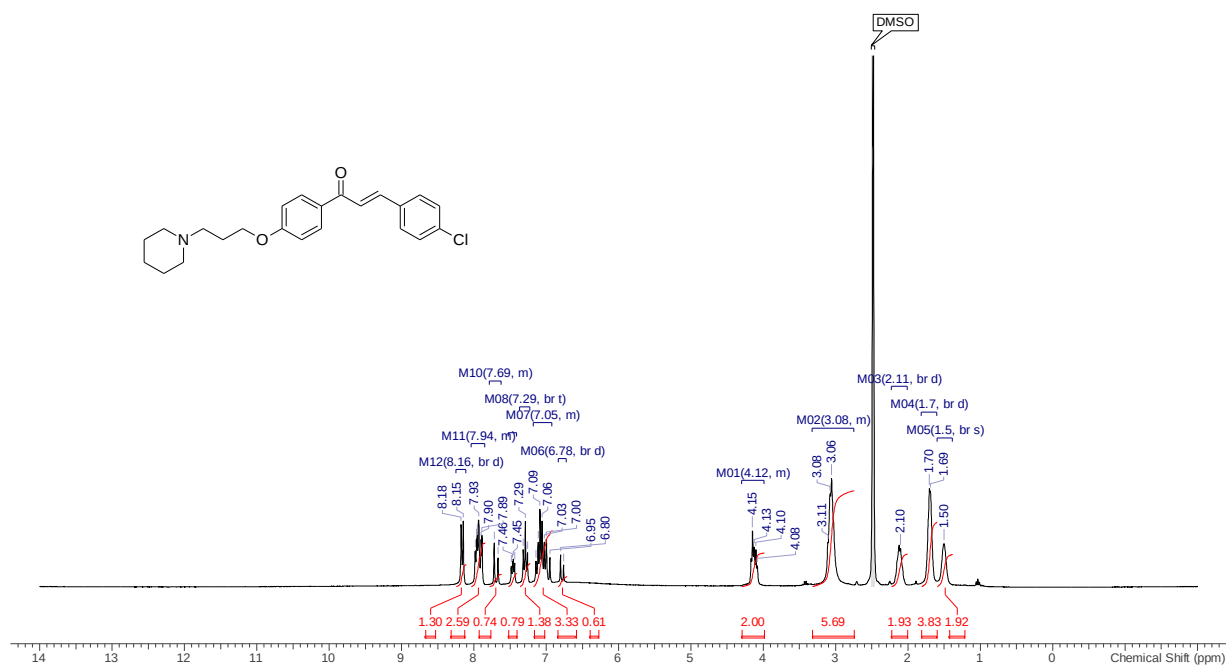


Figure S19. ¹H NMR spectrum of compound 11.

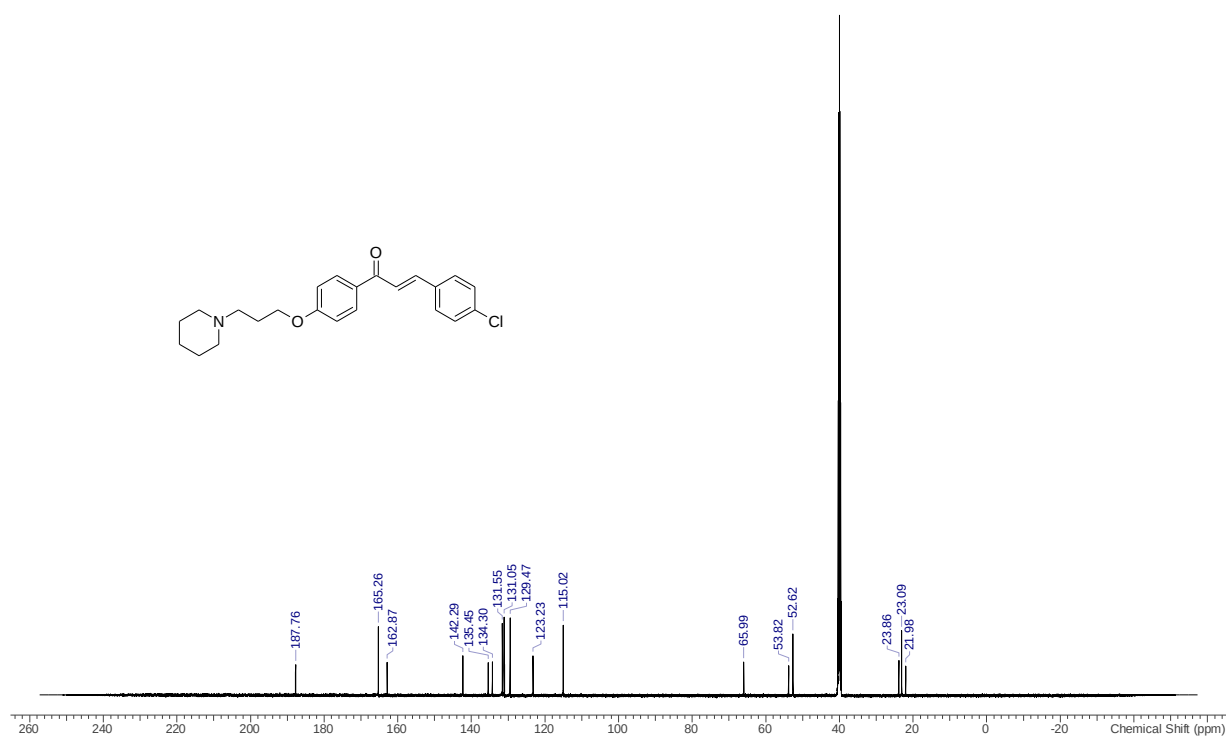


Figure S20. ¹³C NMR spectrum of compound 11.

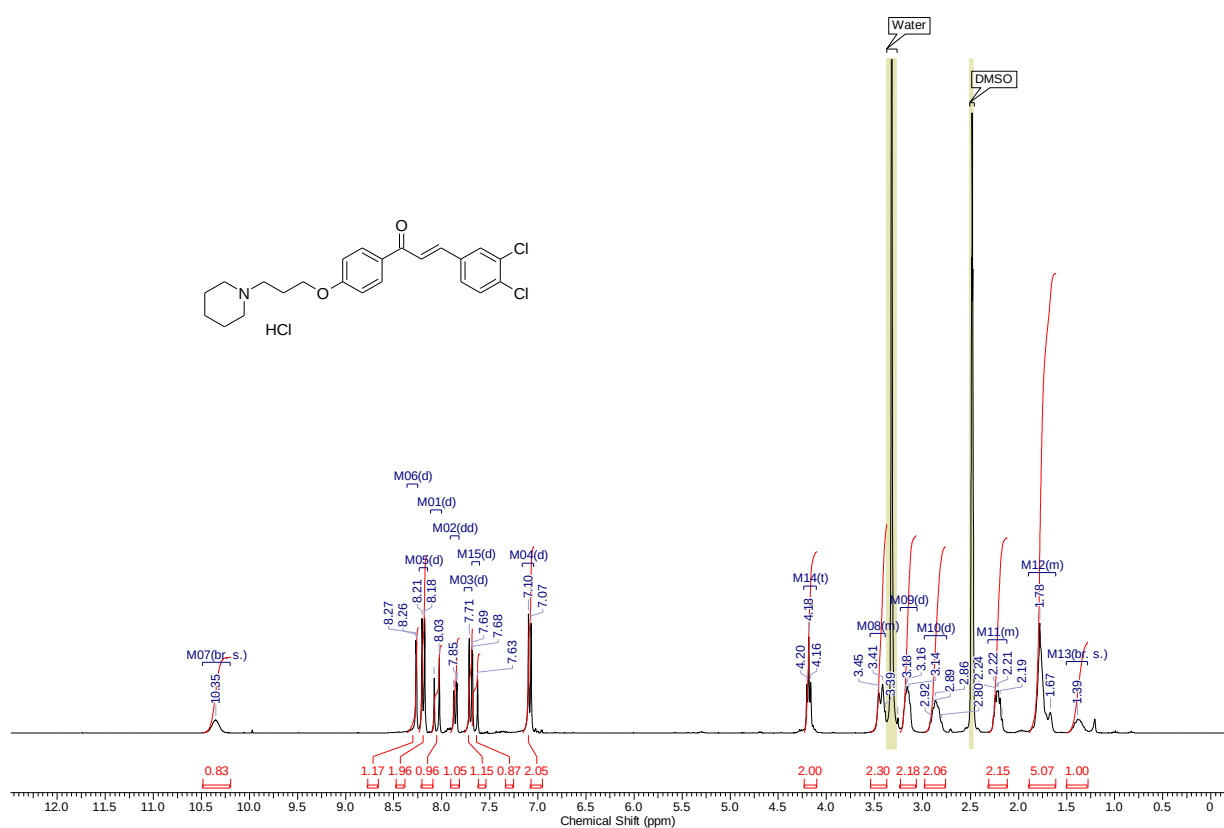


Figure S21. ¹H NMR spectrum of compound 12.

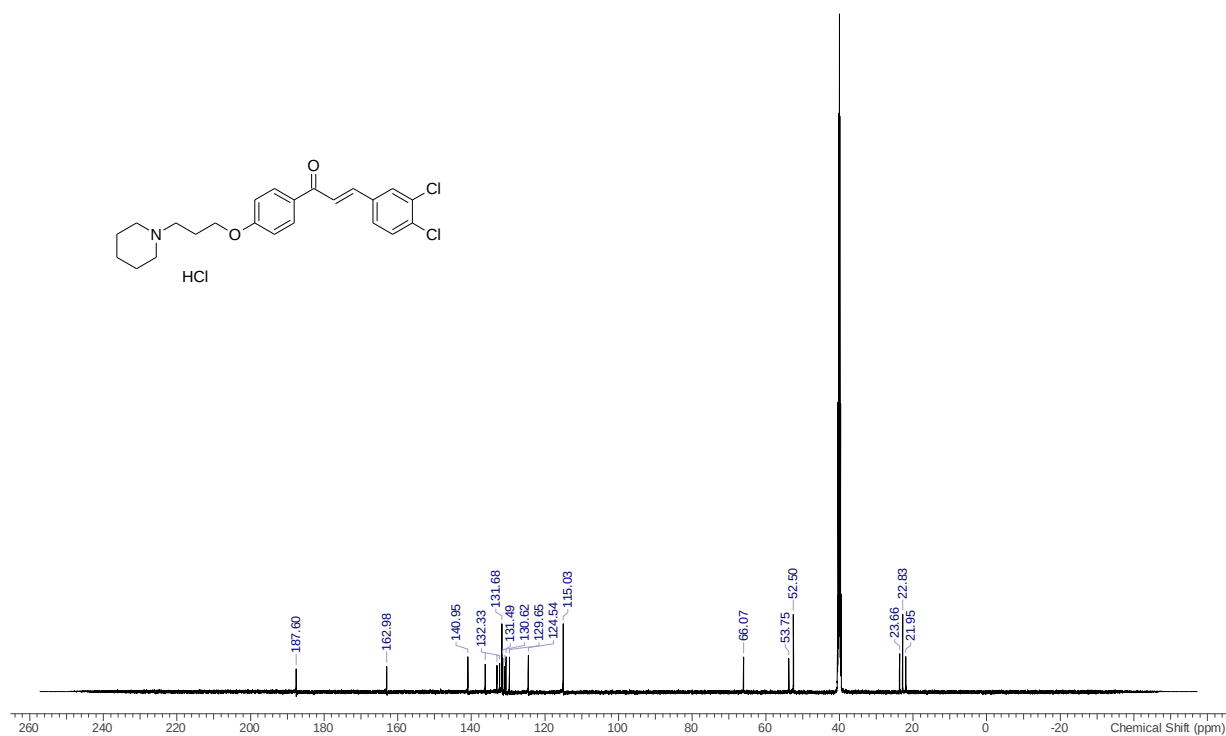


Figure S22. ¹³C NMR spectrum of compound 12.

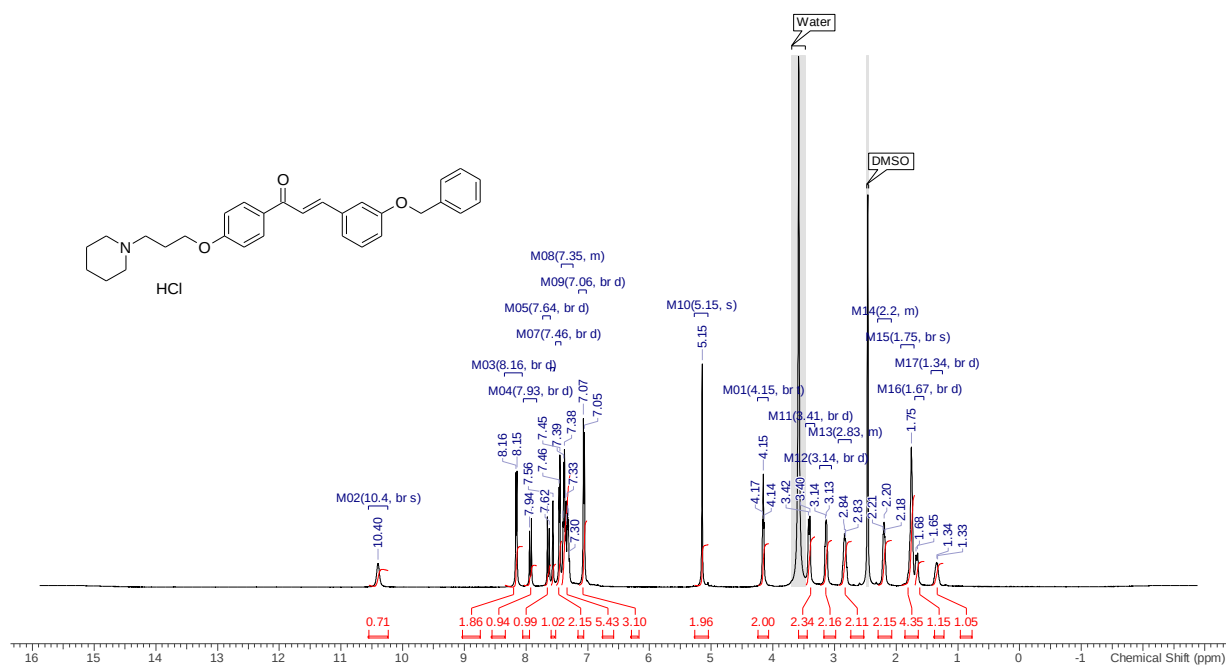


Figure S23. ¹H NMR spectrum of compound 13.

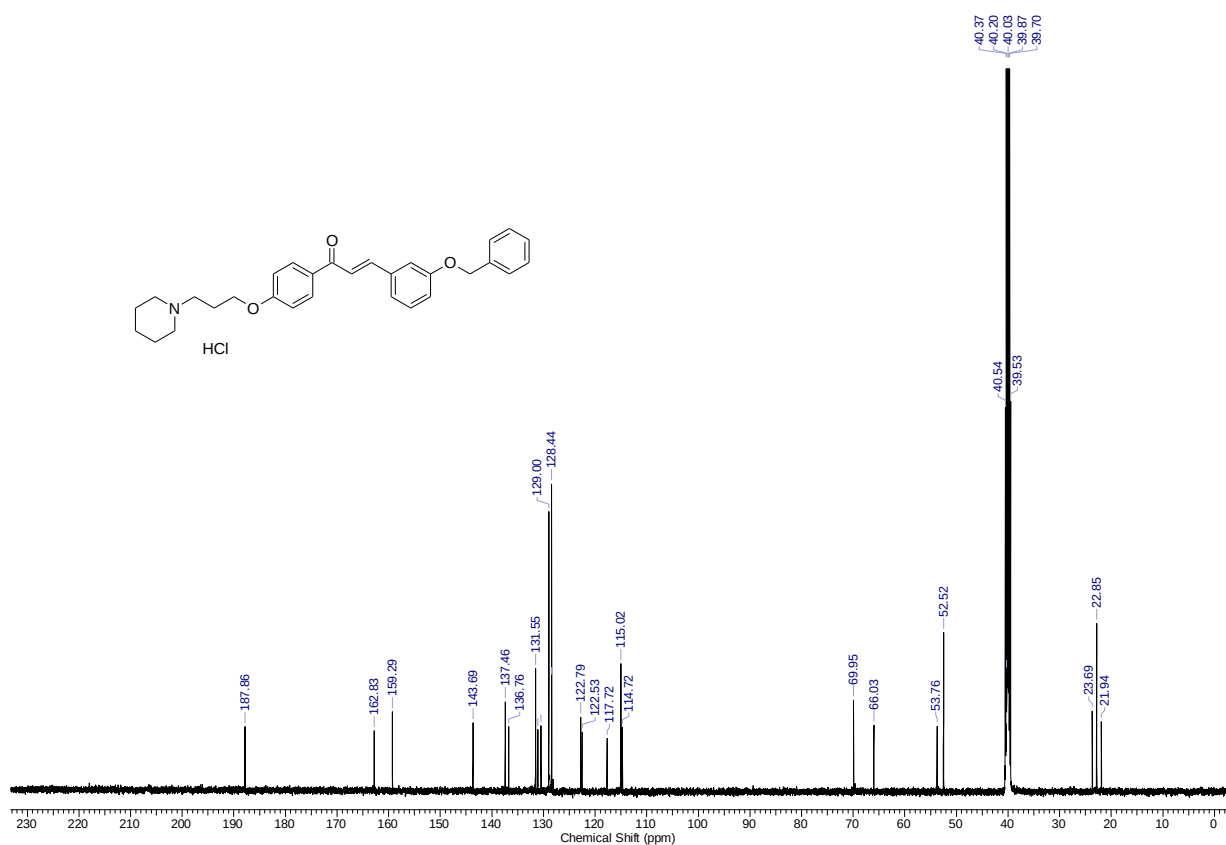


Figure S24. ¹³C NMR spectrum of compound 13.

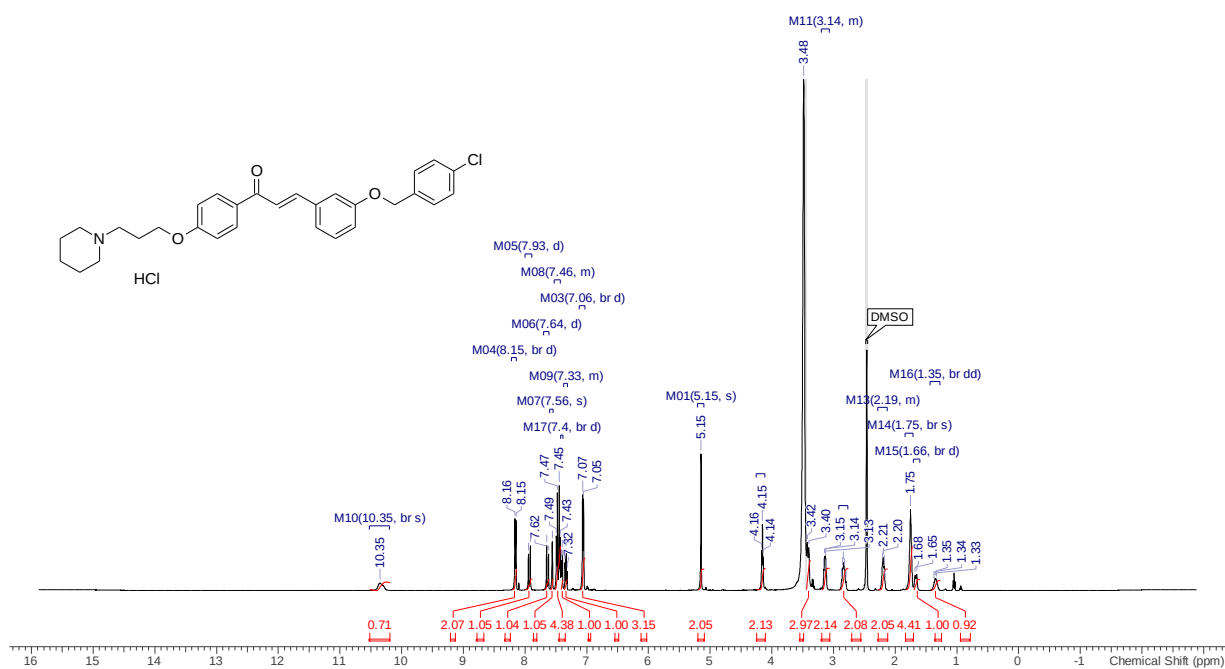


Figure S25. ¹H NMR spectrum of compound 14.

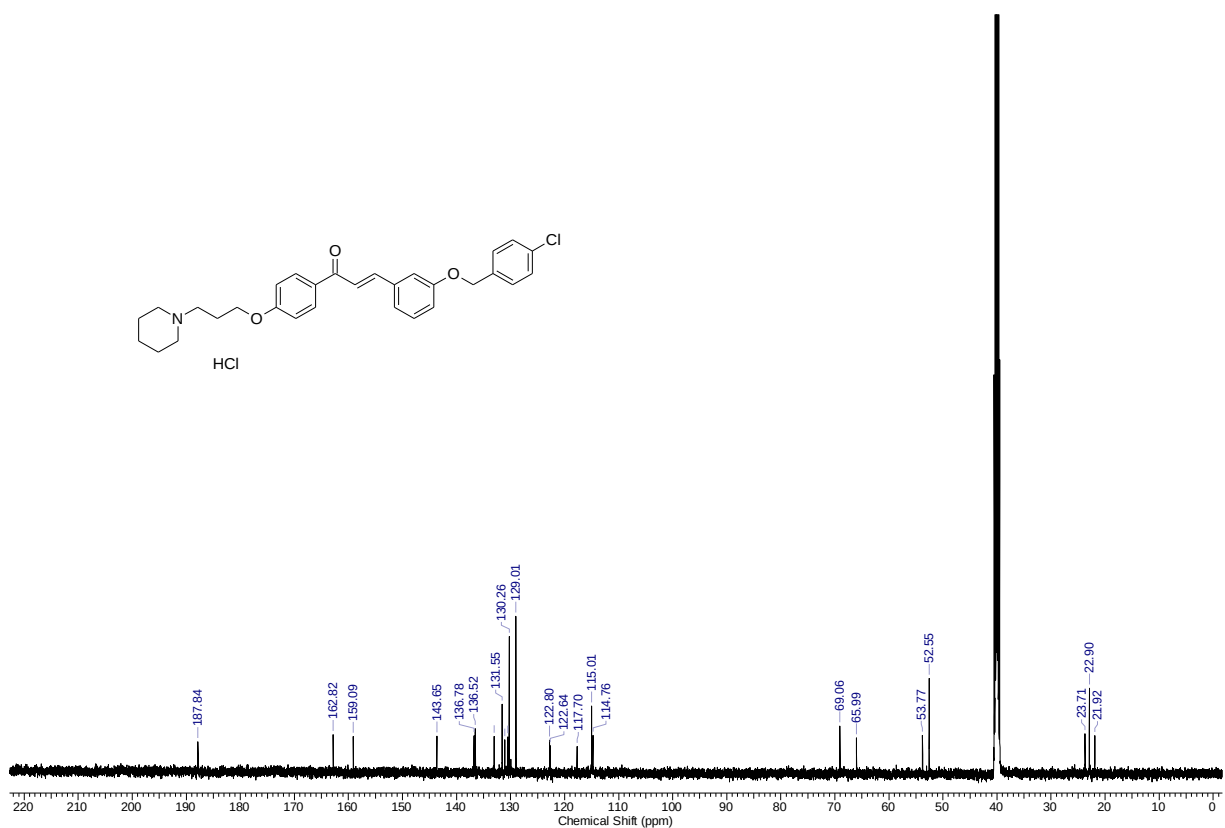


Figure S26. ¹³C NMR spectrum of compound 14.

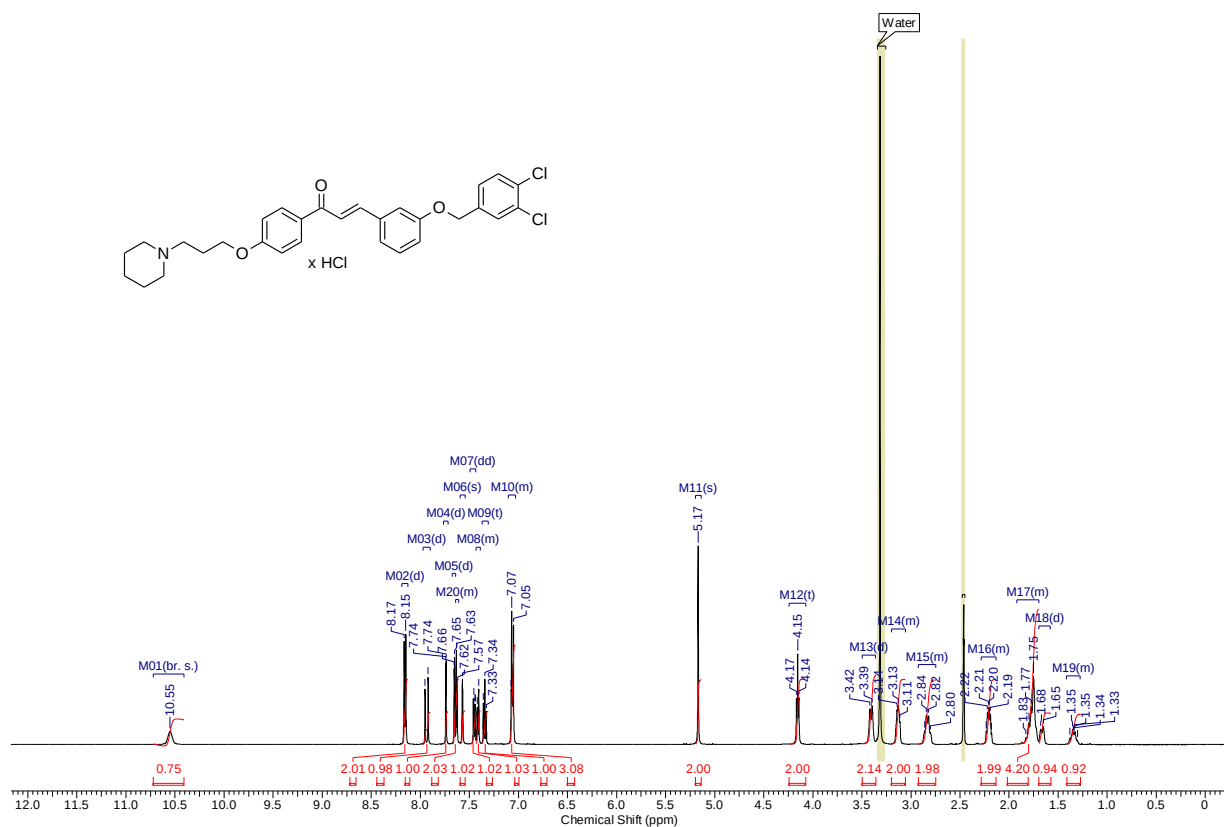


Figure S27. ¹H NMR spectrum of compound 15.

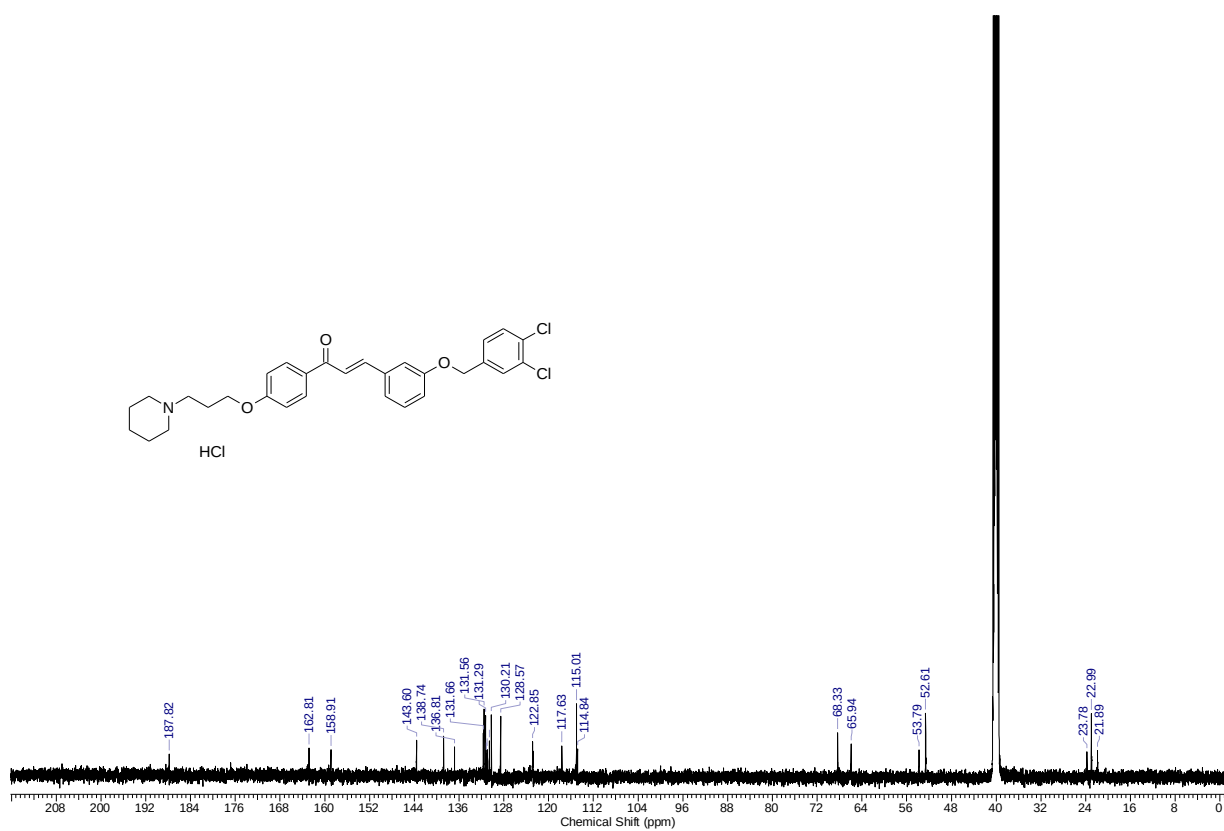


Figure S28. ¹³C NMR spectrum of compound 15.

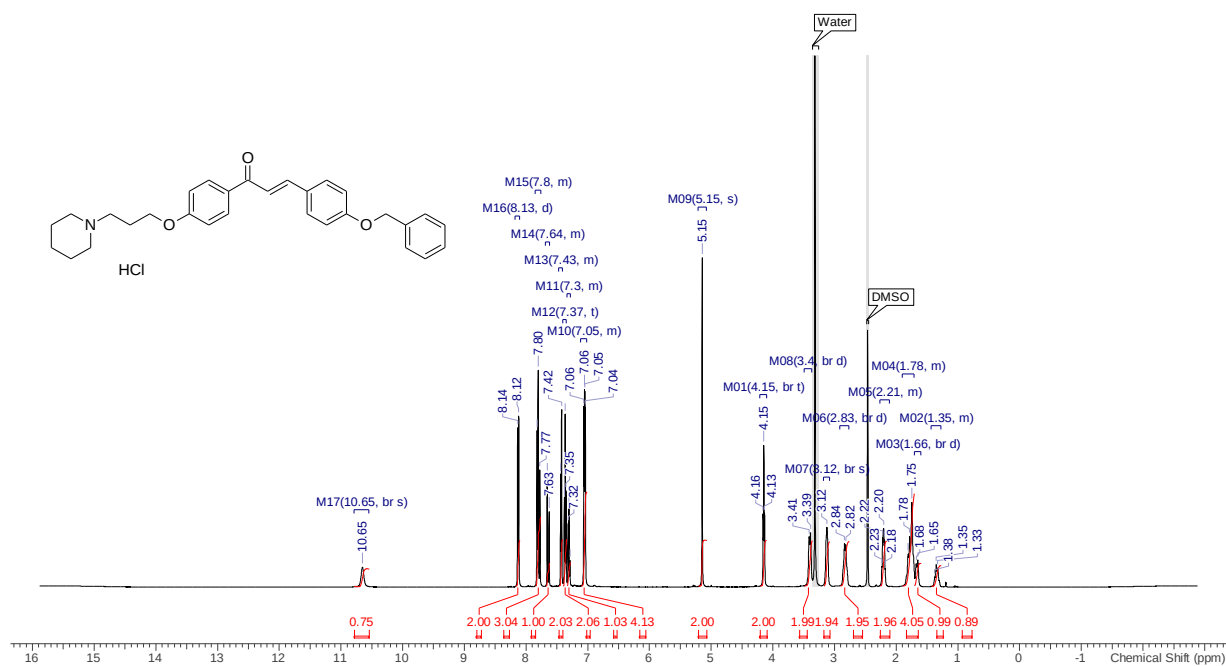


Figure S29. ^1H NMR spectrum of compound 16.

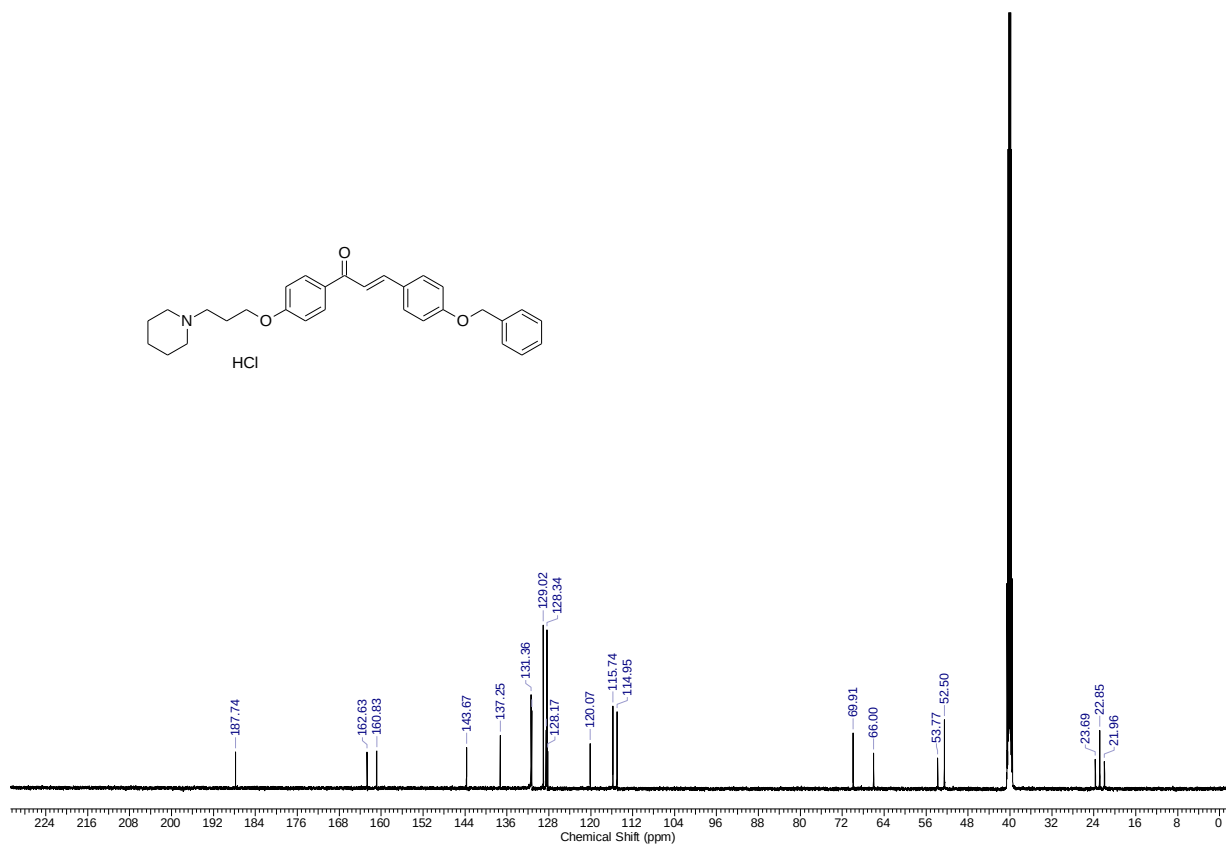


Figure S30. ^{13}C NMR spectrum of compound 16.

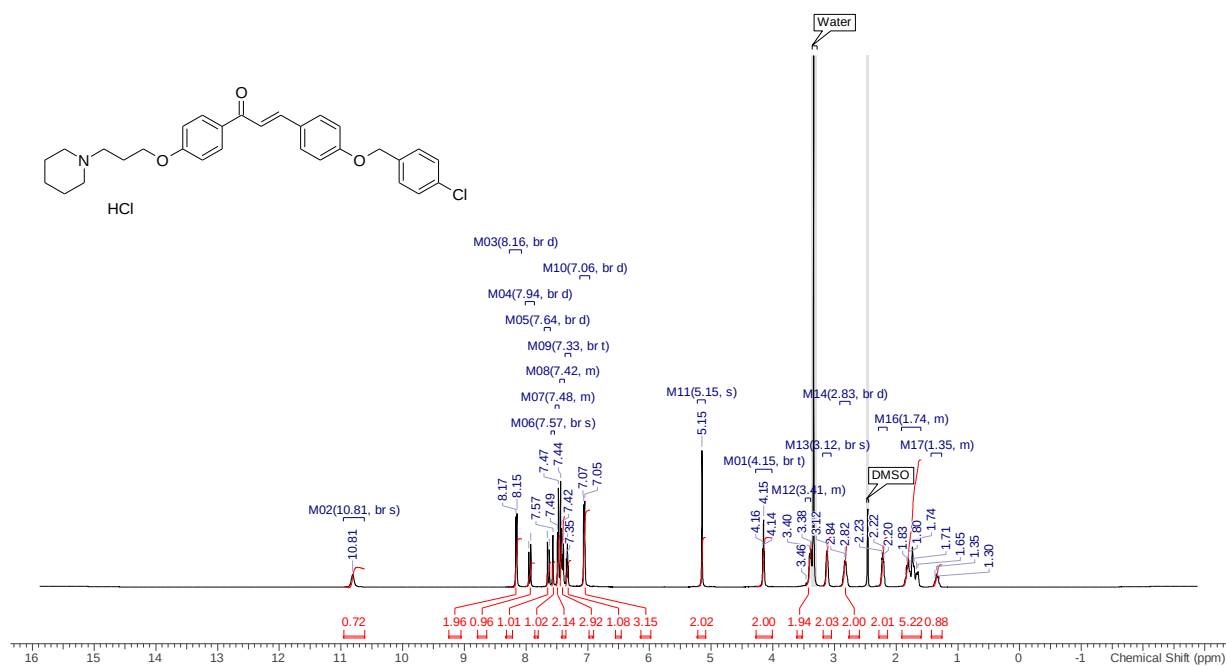


Figure S31. ¹H NMR spectrum of compound 17.

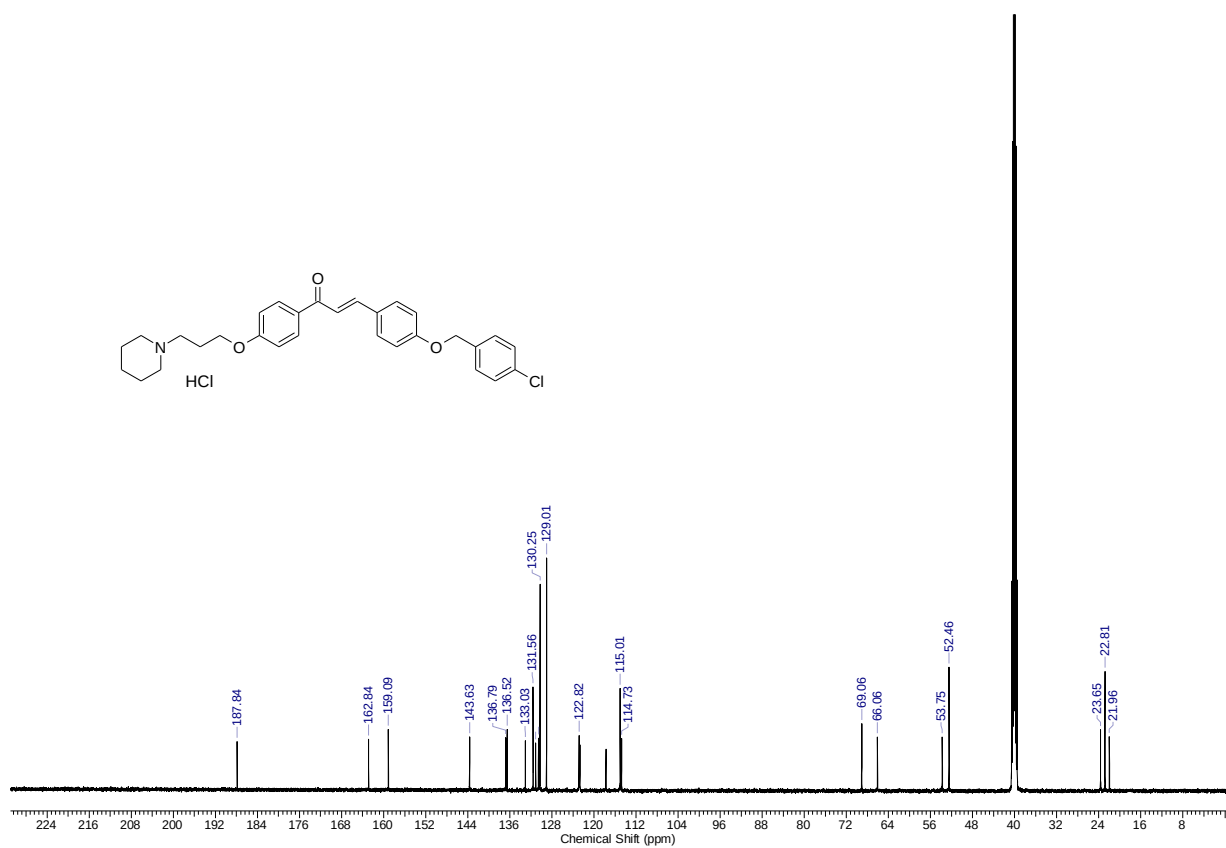


Figure S32. ¹³C NMR spectrum of compound 17.

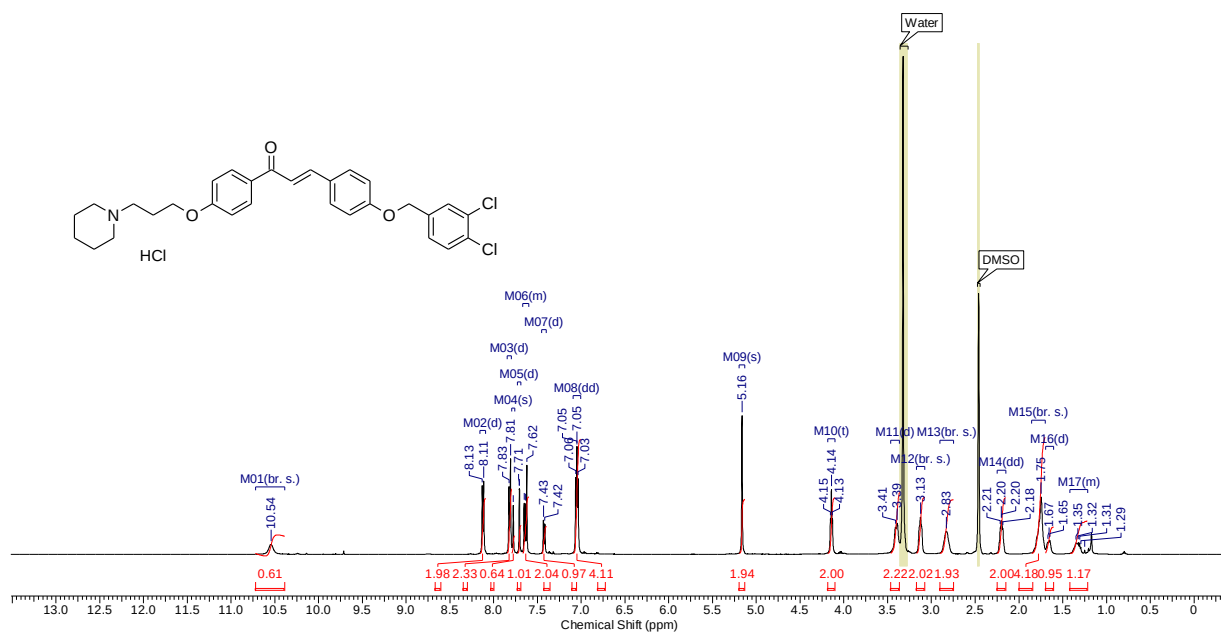


Figure S33. ^1H NMR spectrum of compound 18.

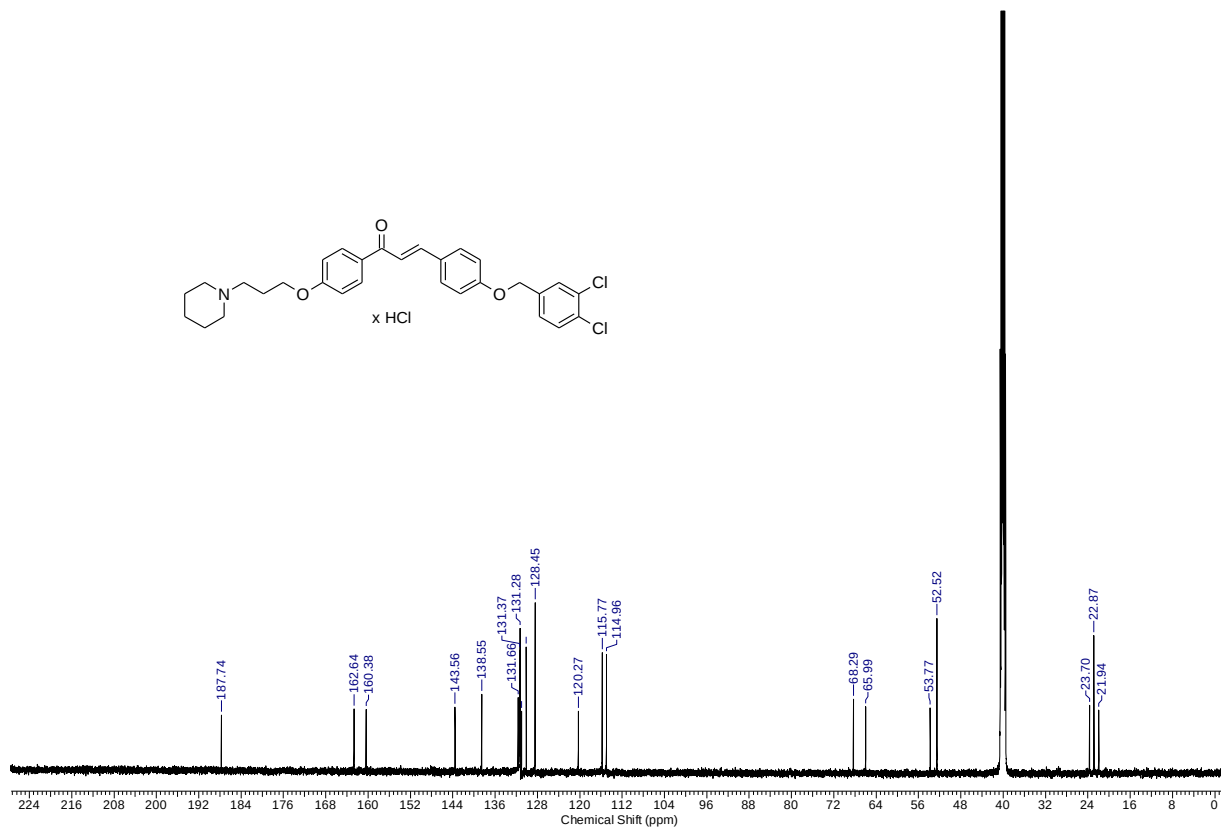


Figure S34. ^{13}C NMR spectrum of compound 18.

Purity assessment of compounds 1-18 determined by LC-MS.

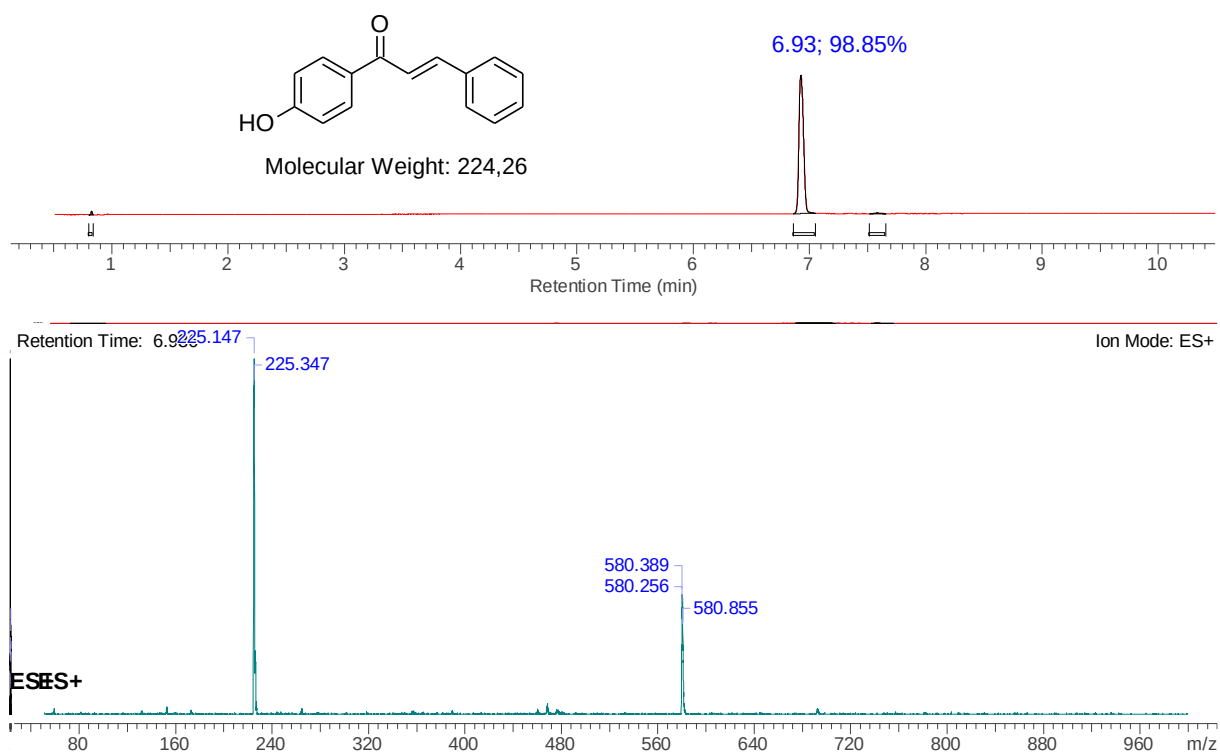


Figure S35. LC-MS assessment of the purity of compound **1**. The purity of compound **1** is 98.85% (retention time: 6.93 min).

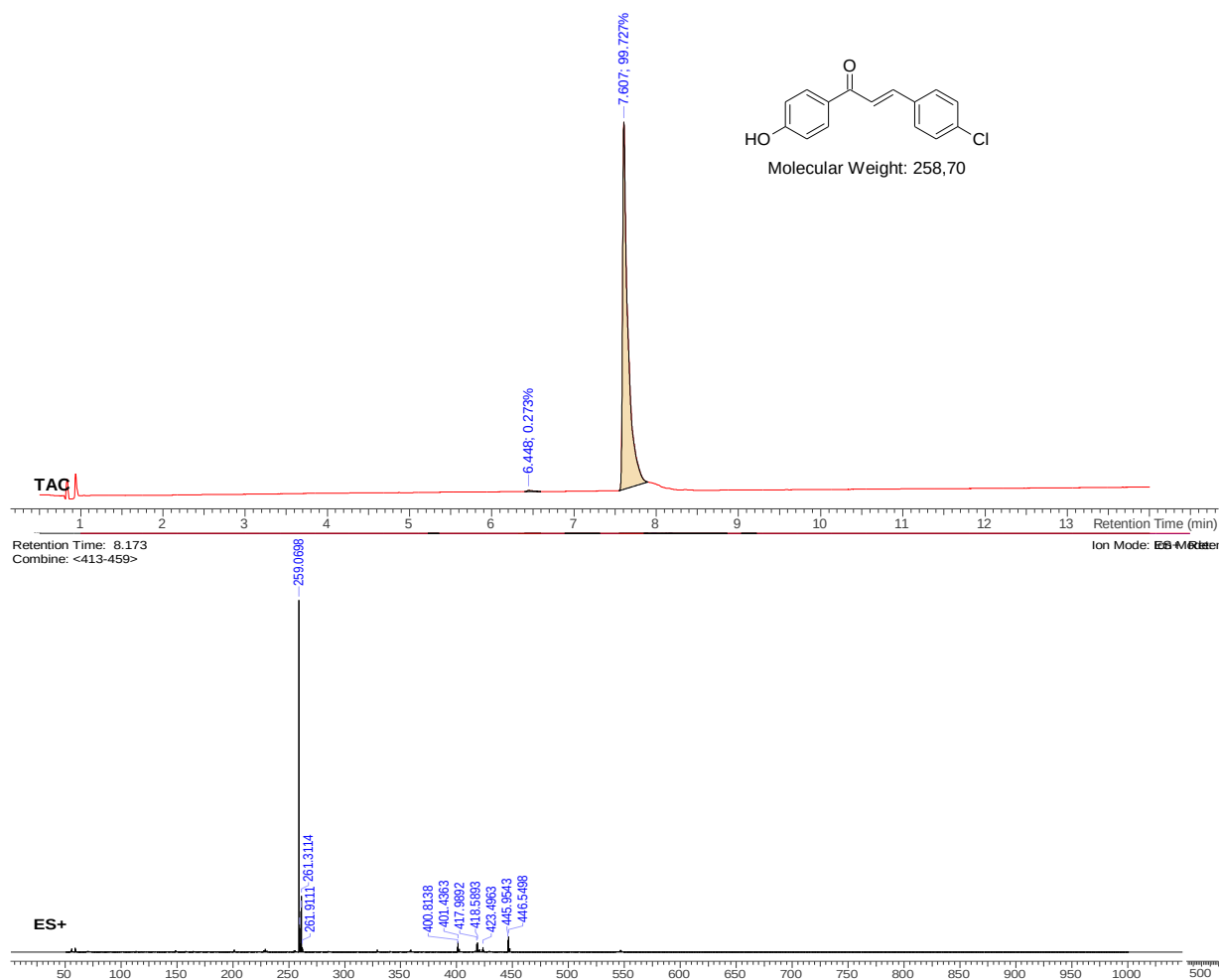


Figure S36. LC-MS assessment of the purity of compound 2. The purity of compound 2 is 99.72% (retention time: 7.61 min).

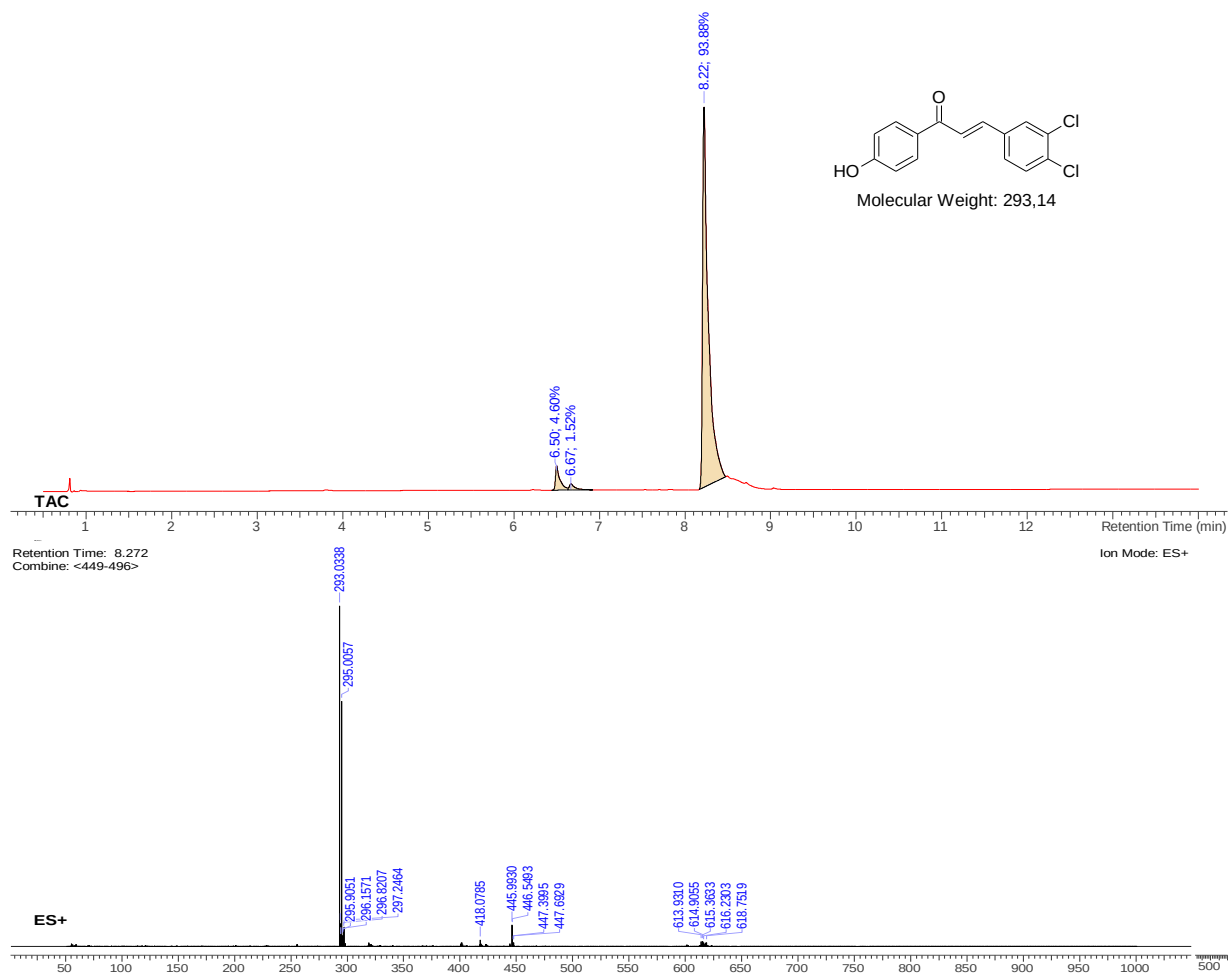


Figure S37. LC-MS assessment of the purity of compound **3**. The purity of compound **3** is 93.88% (retention time: 8.22 min).

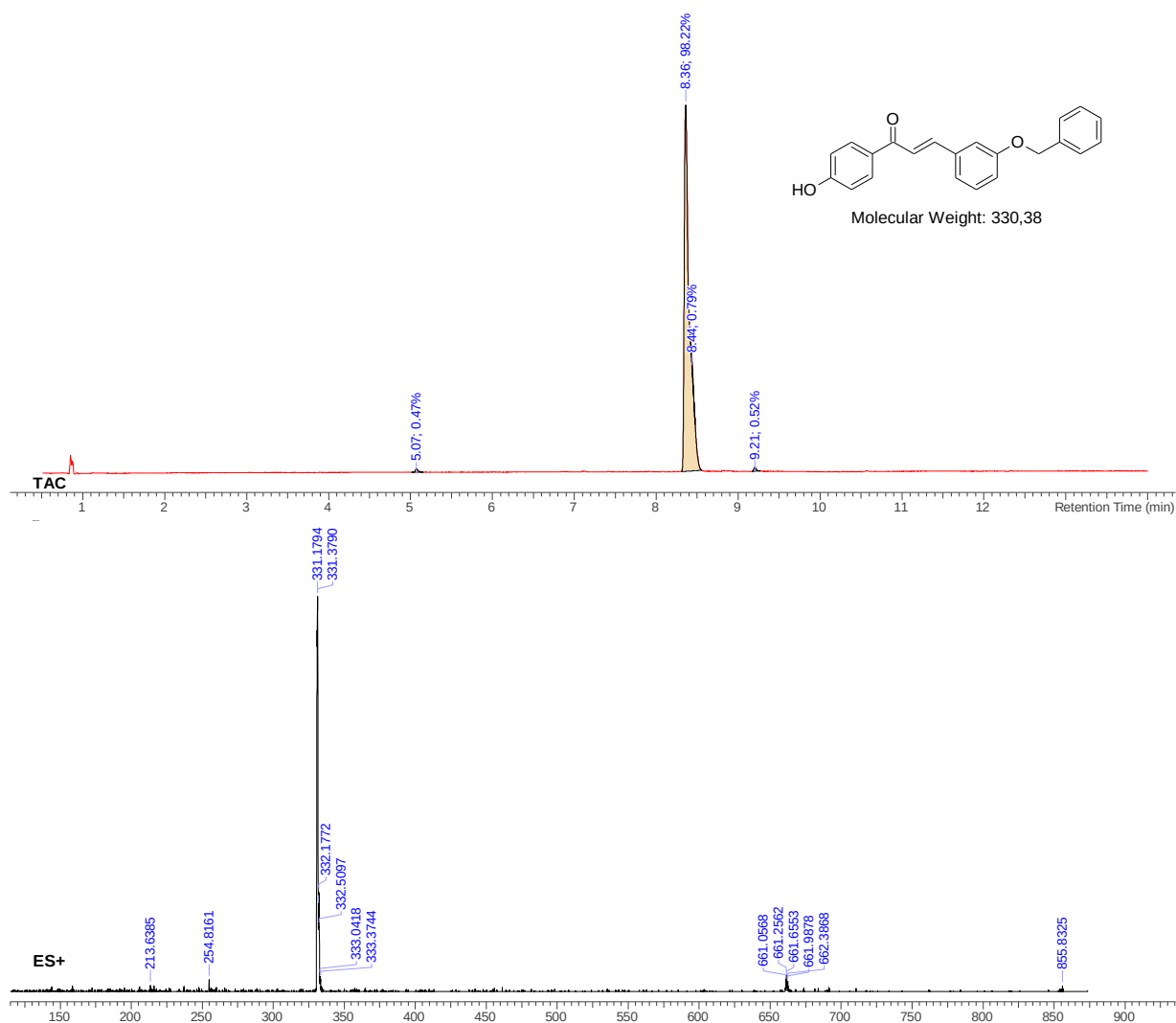


Figure S38. LC-MS assessment of the purity of compound **4**. The purity of compound **4** is 98.22% (retention time: 8.36 min).

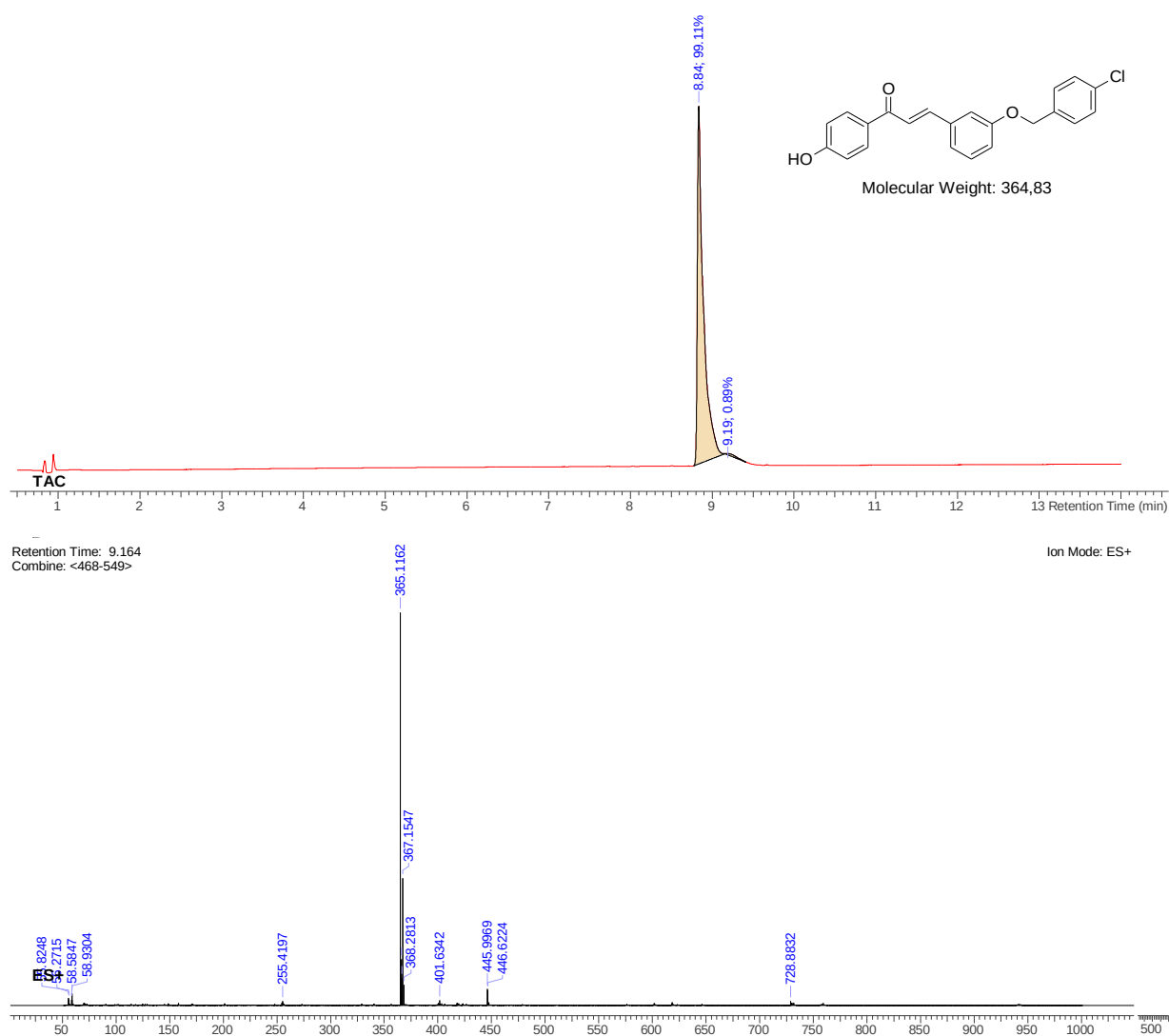


Figure S39. LC-MS assessment of the purity of compound 5. The purity of compound 5 is 99.11% (retention time: 8.84 min).

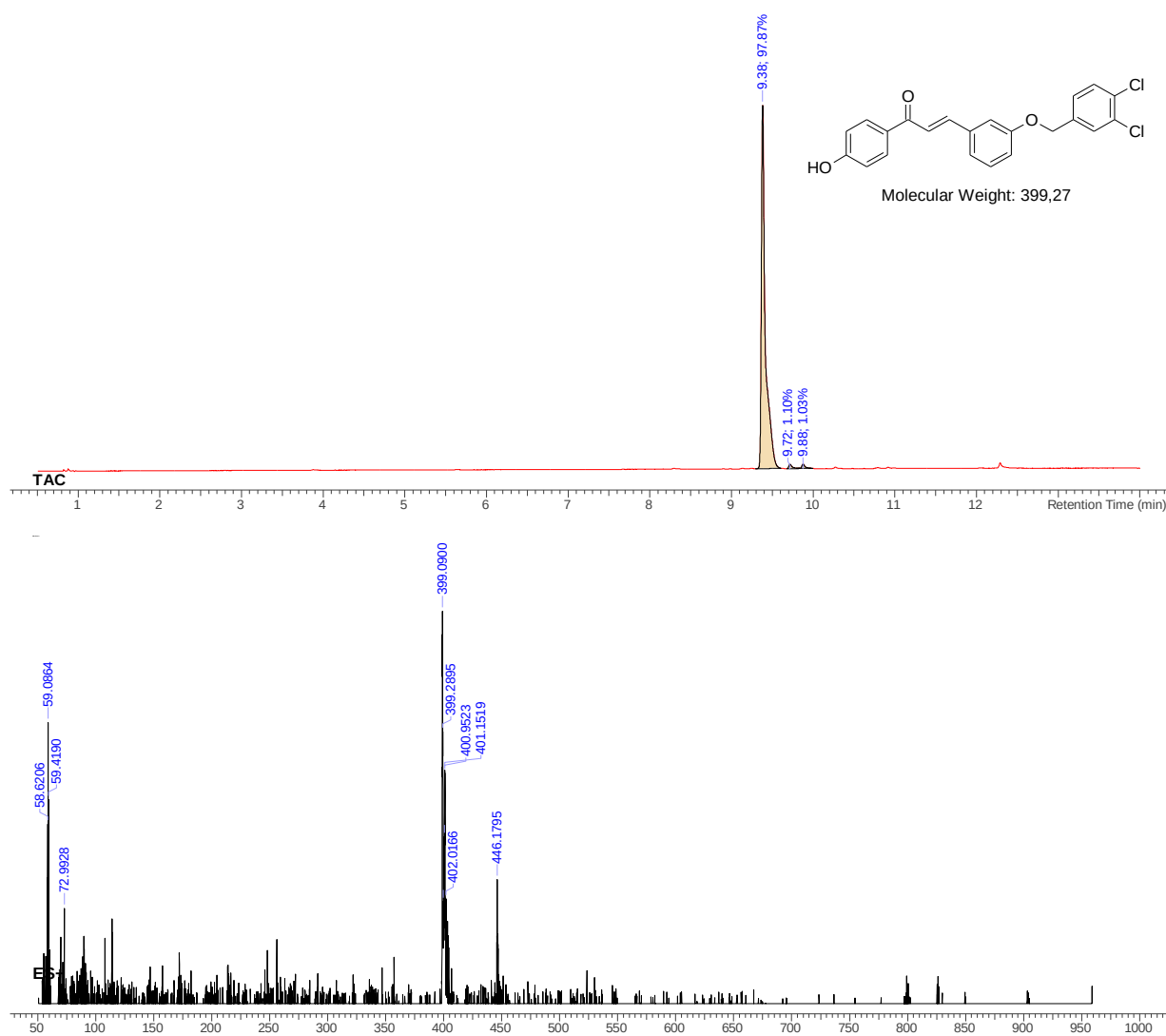


Figure S40. LC-MS assessment of the purity of compound **6**. The purity of compound **6** is 97.98% (retention time: 9.38 min).

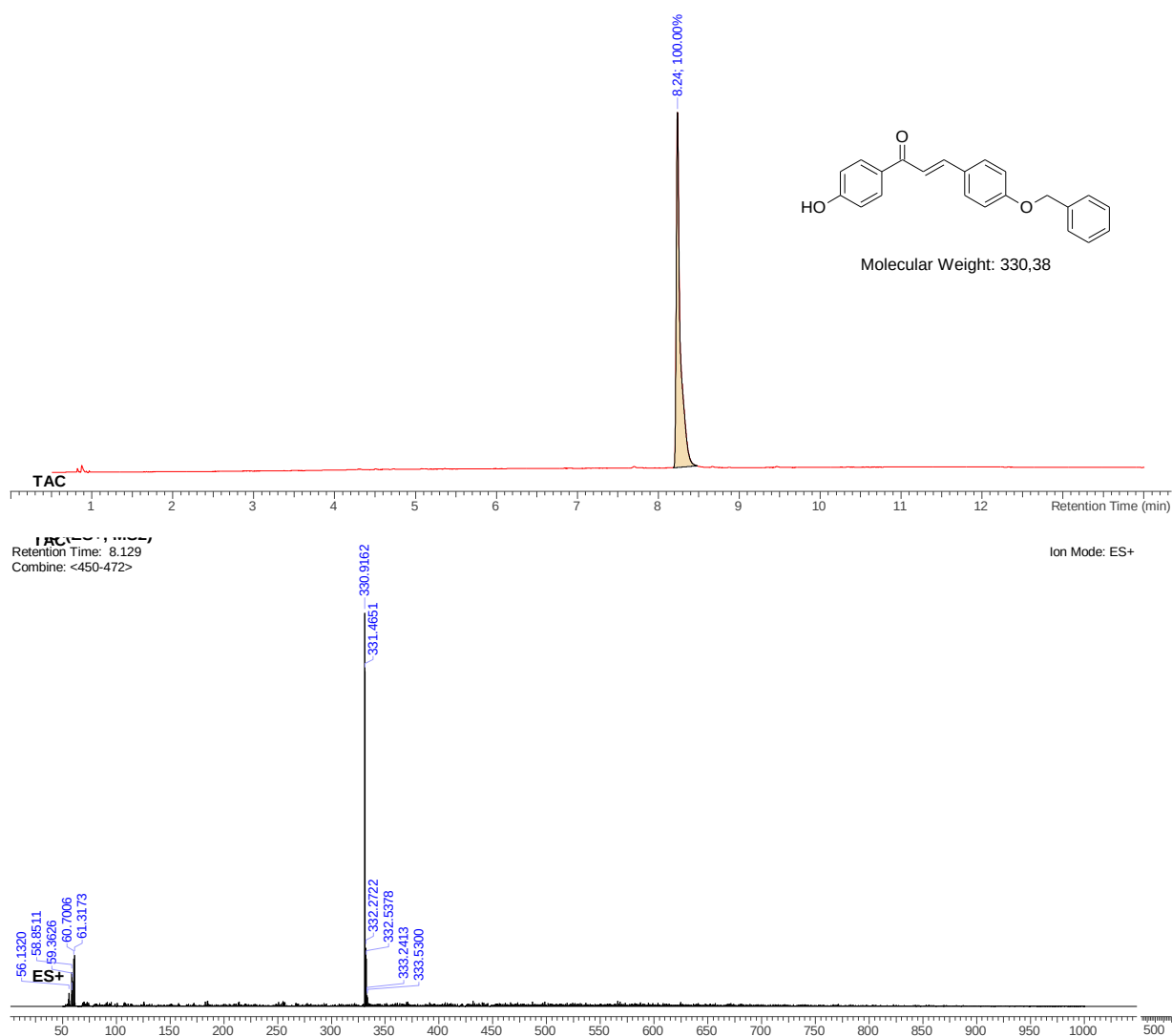


Figure S41. LC-MS assessment of the purity of compound 7. The purity of compound 7 is 100% (retention time: 8.24 min).

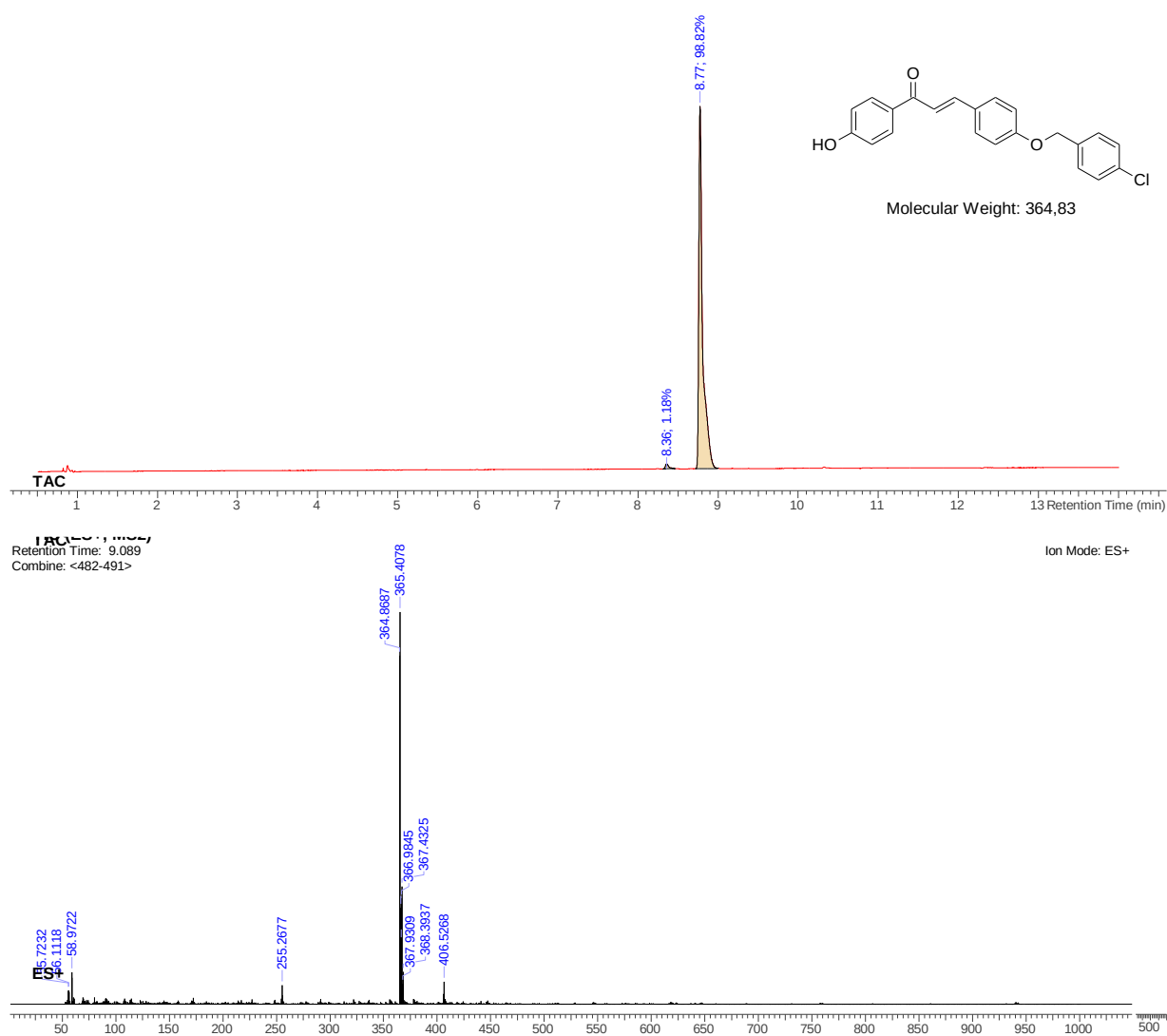


Figure S42. LC-MS assessment of the purity of compound **8**. The purity of compound **8** is 98.82% (retention time: 8.77 min).

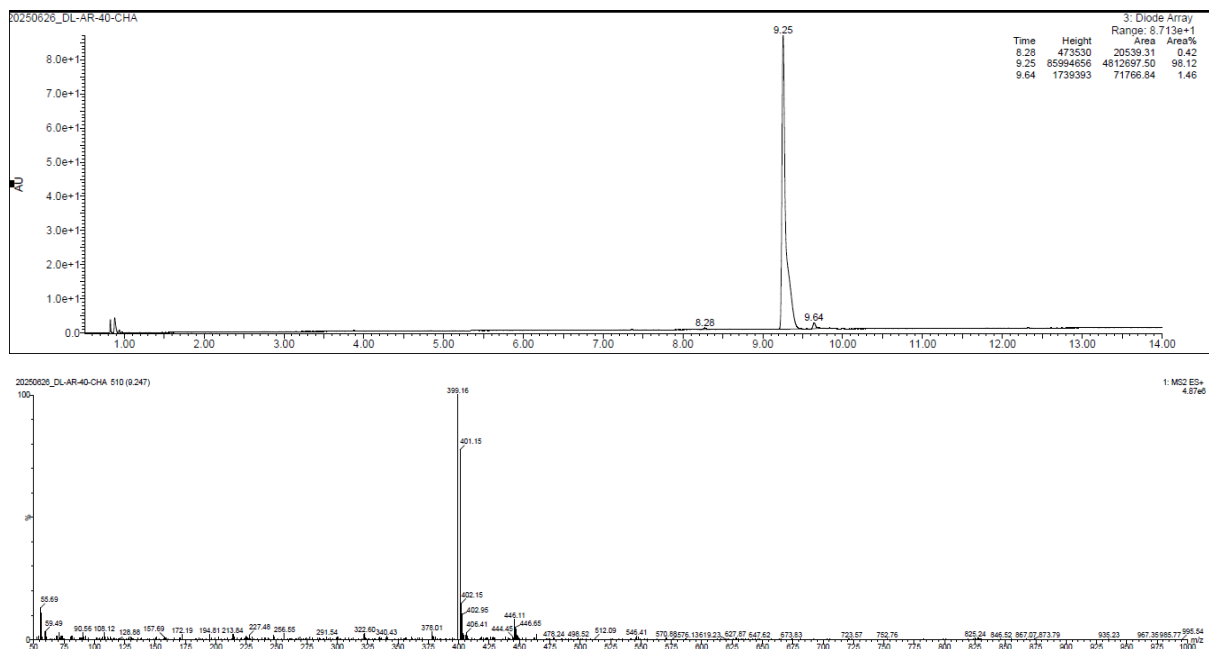
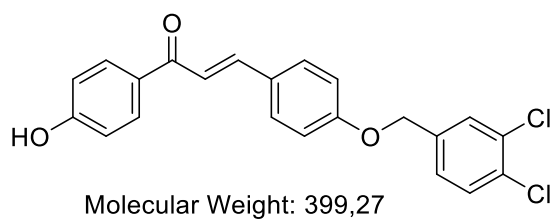


Figure S43. LC-MS assessment of the purity of compound **9**. The purity of compound **9** is 98.12% (retention time: 9.25 min).

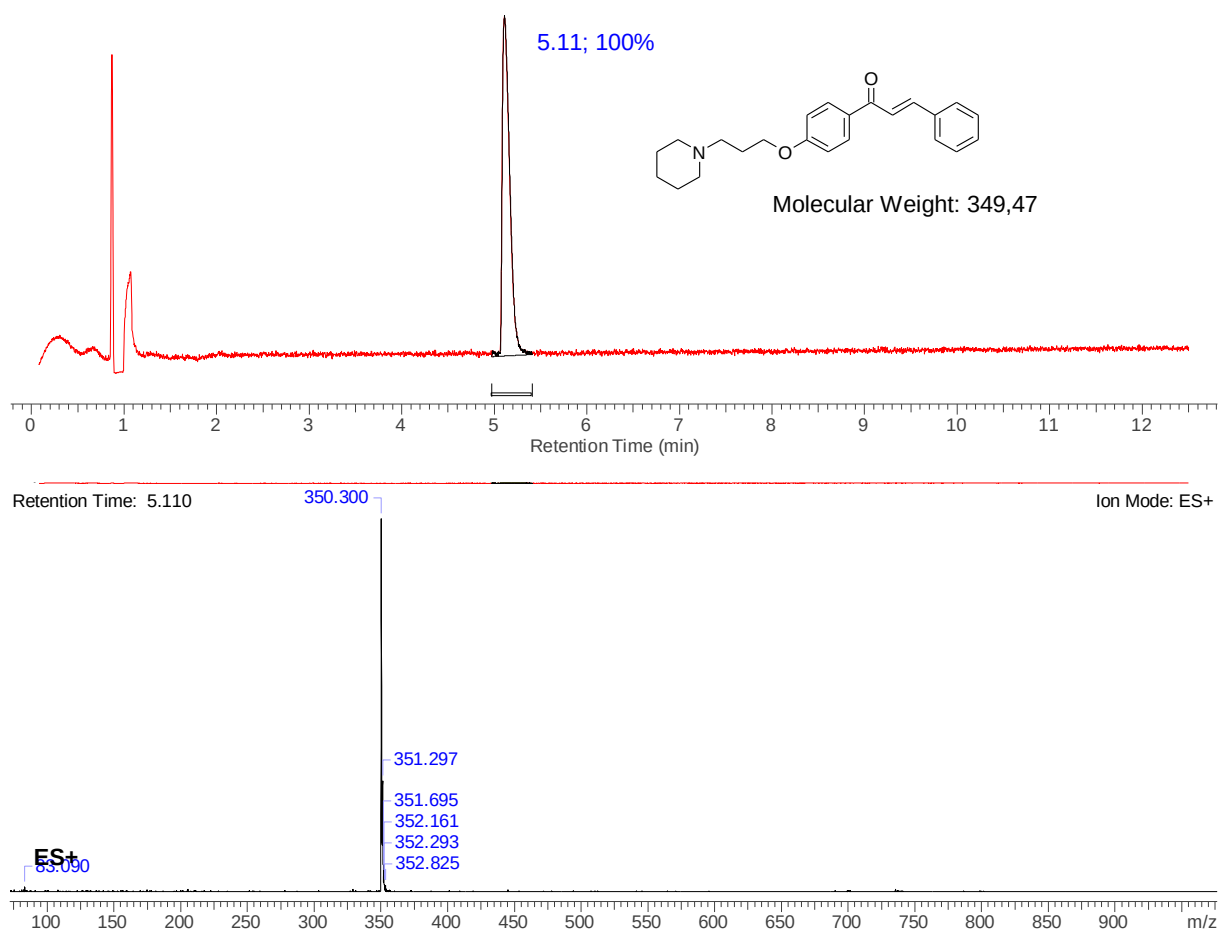


Figure S44. LC-MS assessment of the purity of compound **10**. The purity of compound **10** is 100% (retention time: 5.11 min).

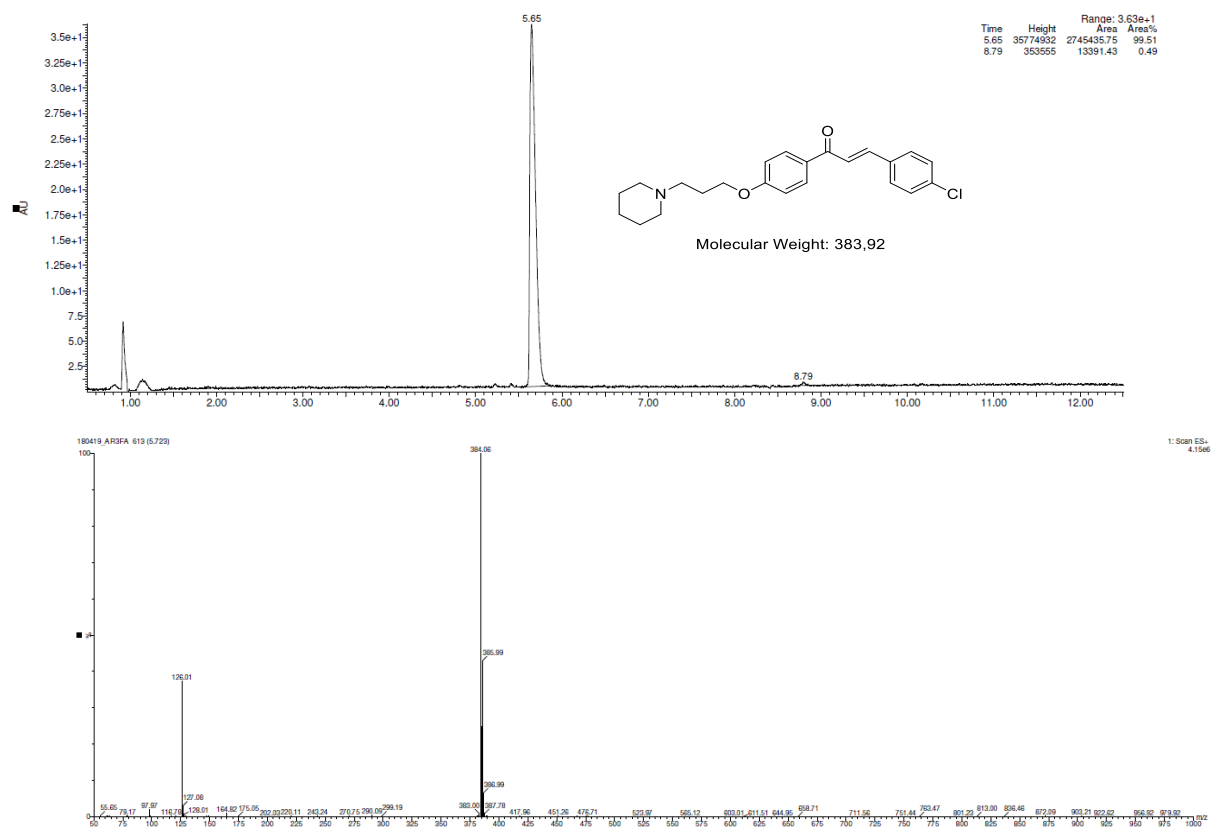


Figure S45. LC-MS assessment of the purity of compound **11**. The purity of compound **11** is 99.51% (retention time: 5.65 min).

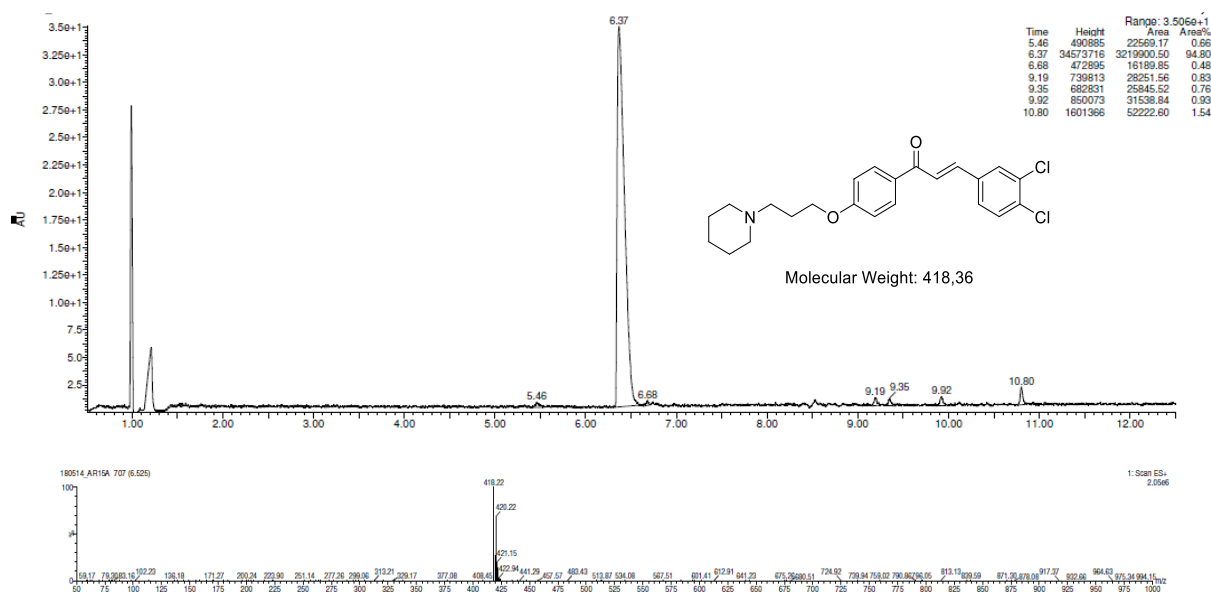


Figure S46. LC-MS assessment of the purity of compound **12**. The purity of compound **12** is 94.80% (retention time: 6.37 min).

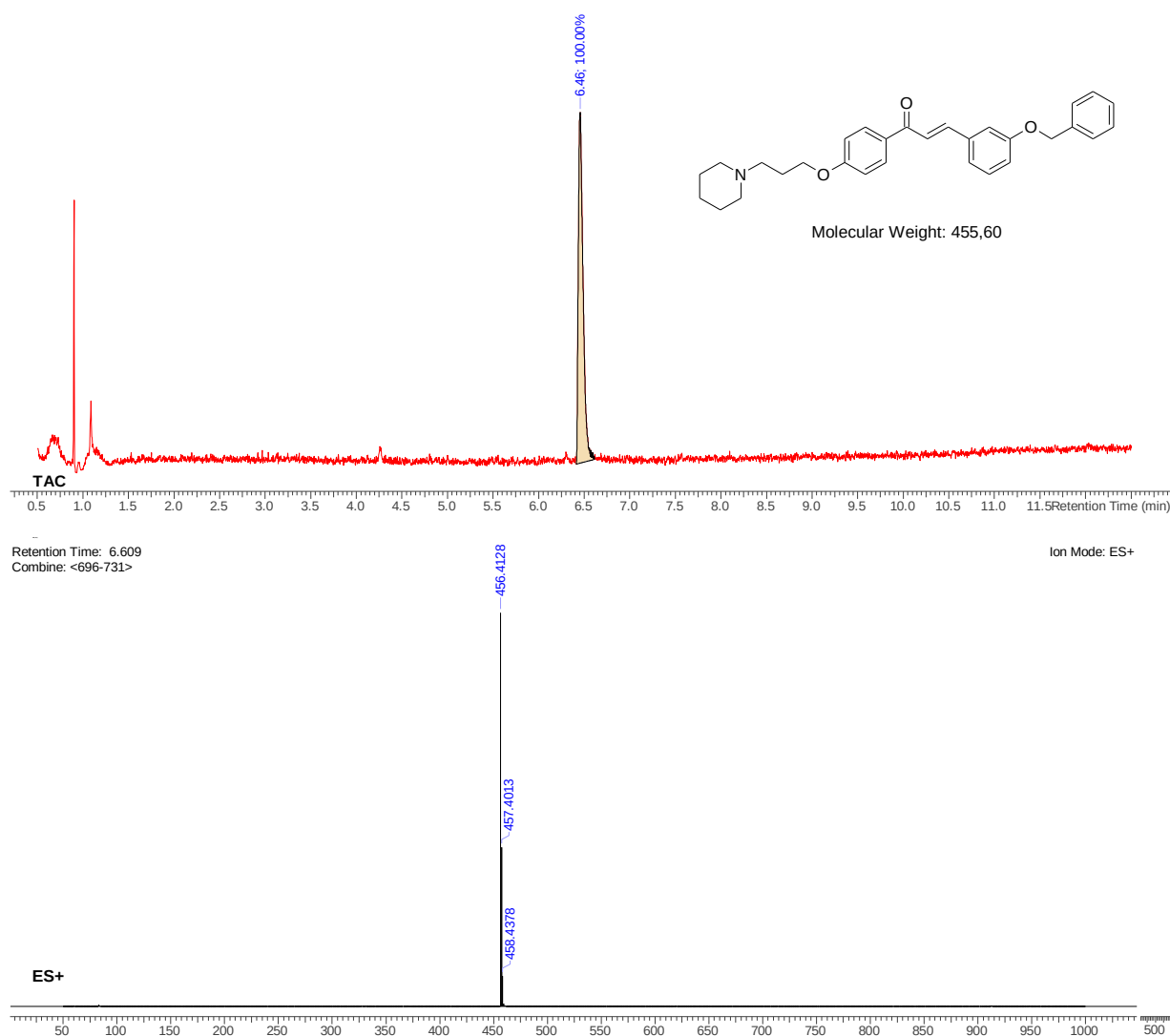


Figure S47. LC-MS assessment of the purity of compound **13**. The purity of compound **13** is 100% (retention time: 6.46 min).

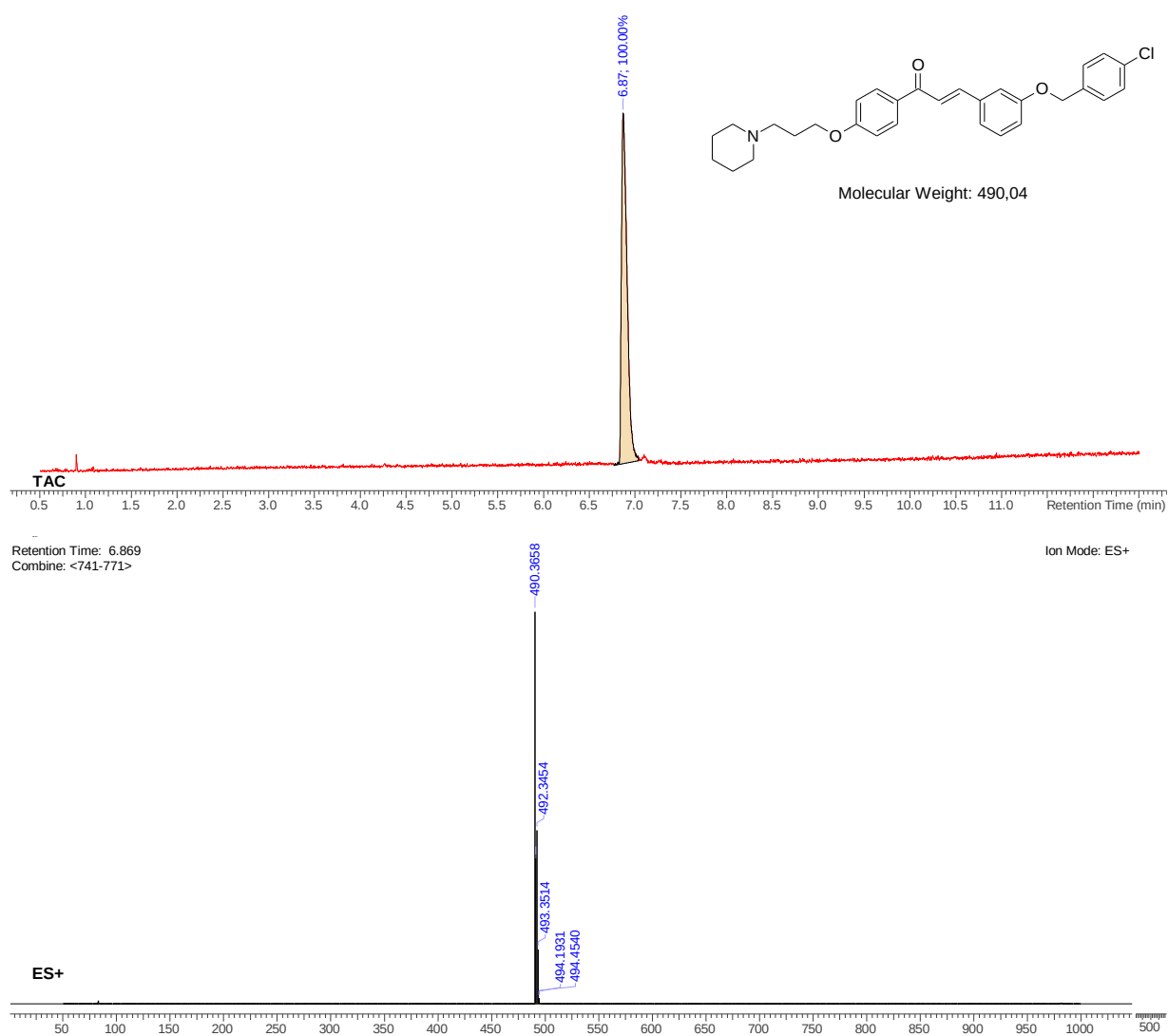


Figure S48. LC-MS assessment of the purity of compound **14**. The purity of compound **14** is 100% (retention time: 6.87 min).

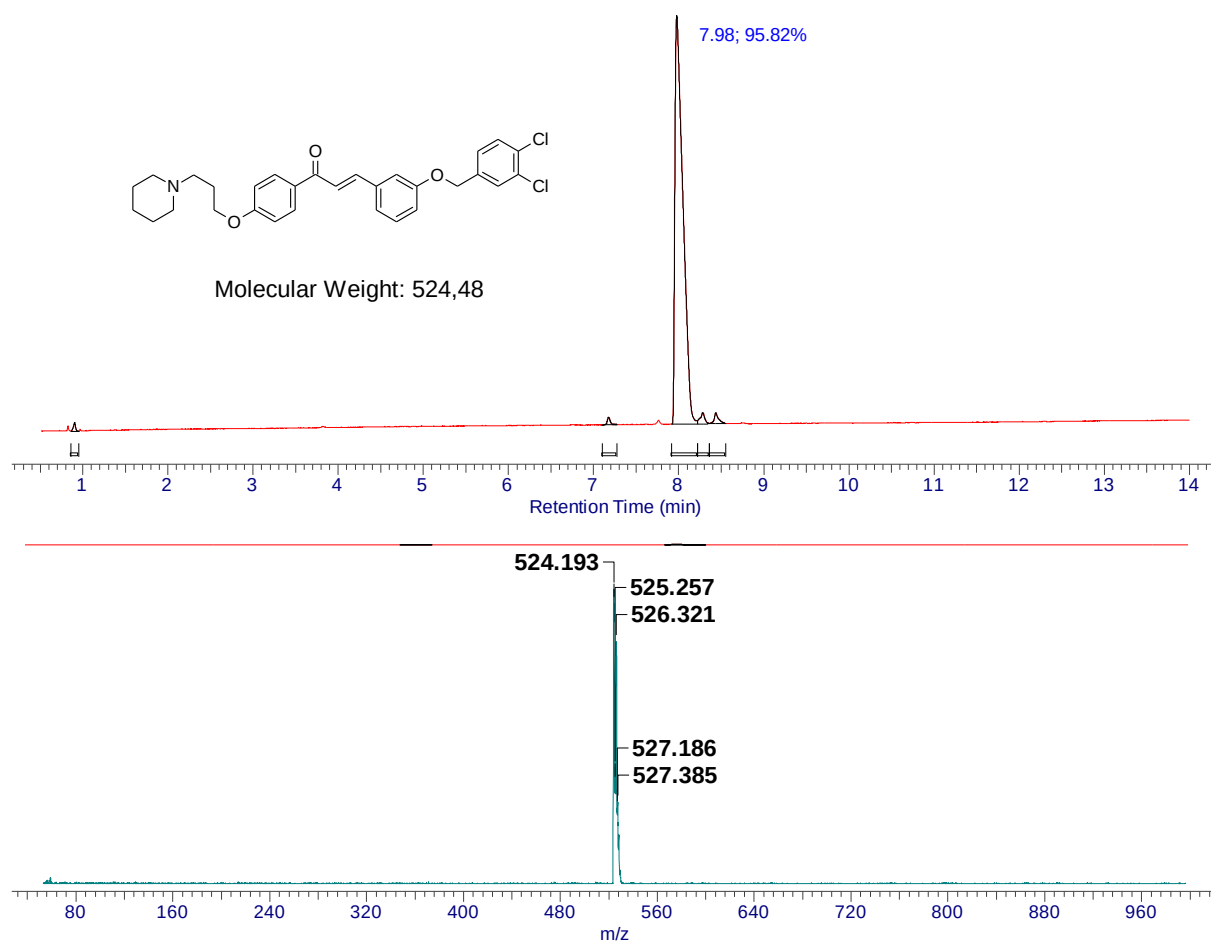


Figure S49. LC-MS assessment of the purity of compound **15**. The purity of compound **15** is 100% (retention time: 6.87 min).

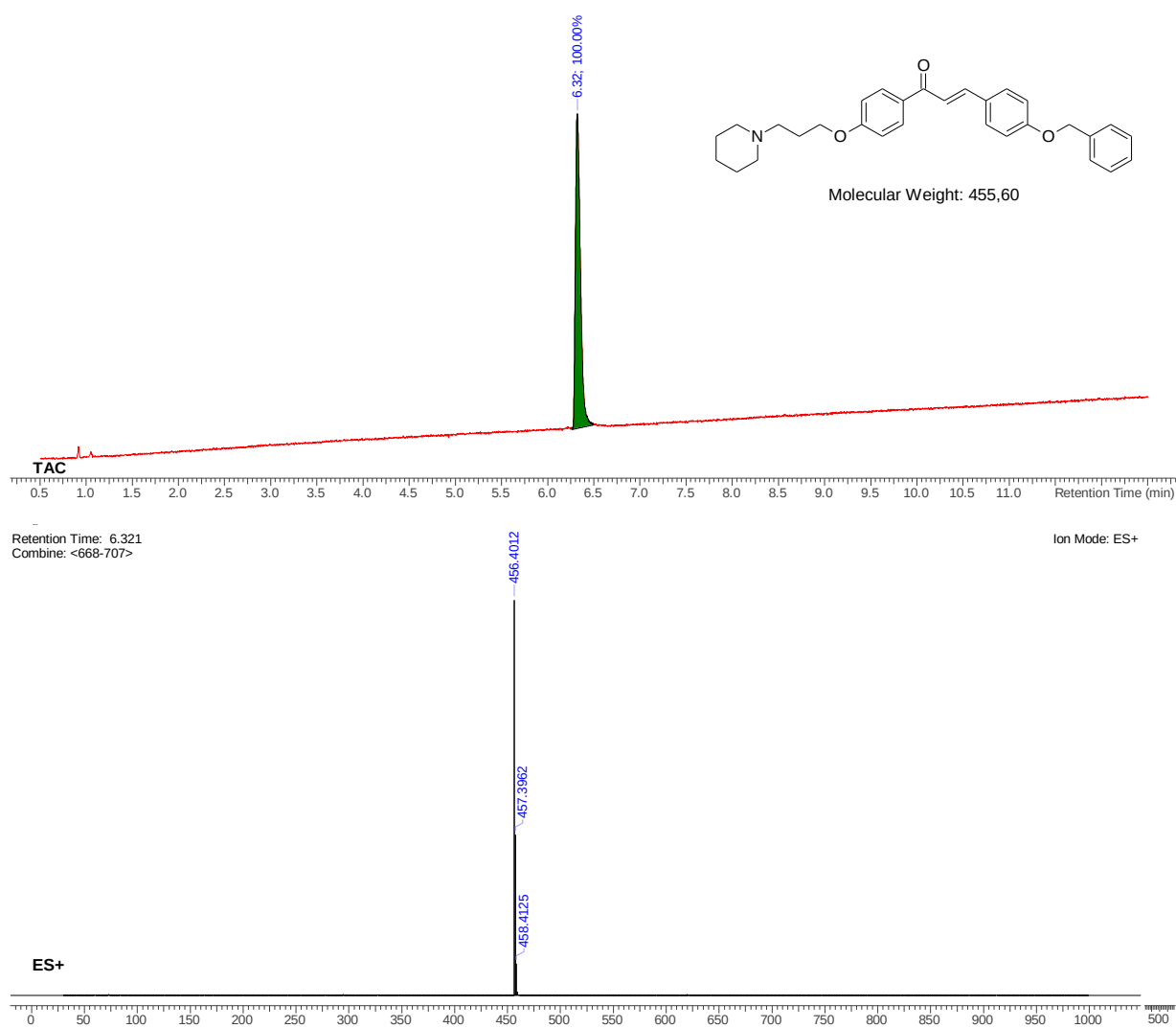


Figure S50. LC-MS assessment of the purity of compound **16**. The purity of compound **16** is 100% (retention time: 6.32 min).

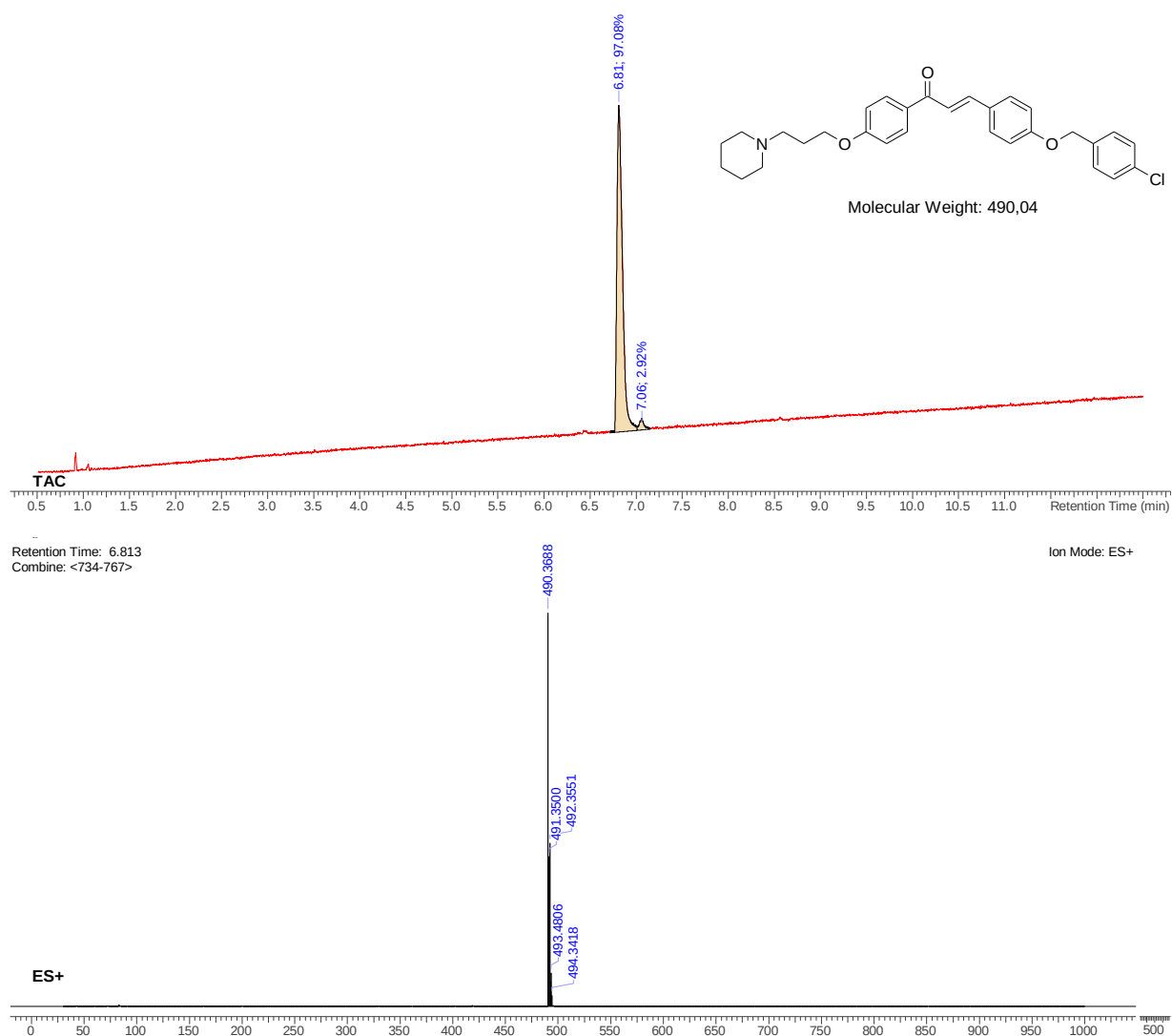


Figure S51. LC-MS assessment of the purity of compound **17**. The purity of compound **17** is 97.08% (retention time: 6.81 min).

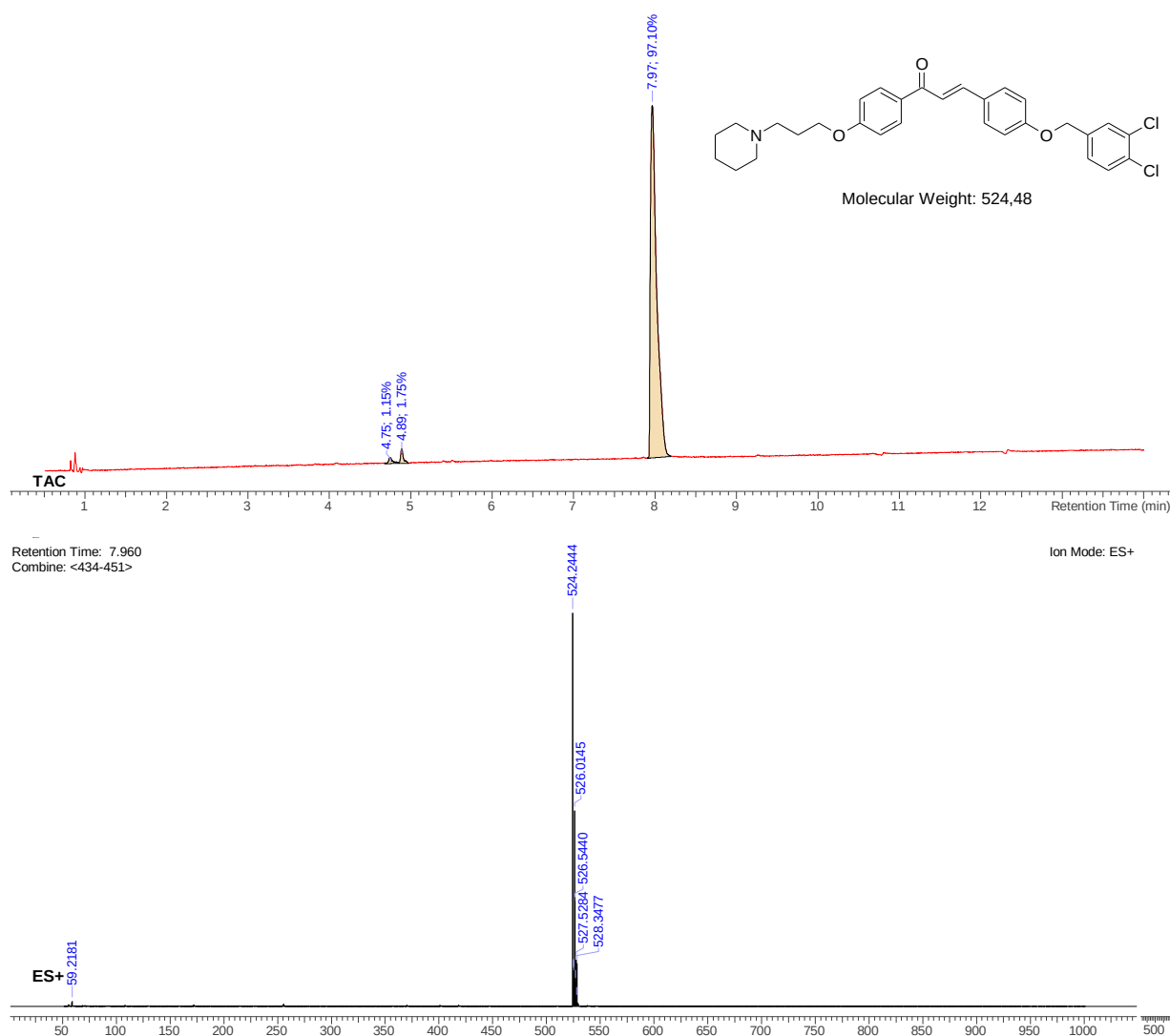


Figure S52. LC-MS assessment of the purity of compound **18**. The purity of compound **18** is 97.10% (retention time: 7.97 min).