Supporting Information

Rational development of a potent 15-lipoxygenase-1 inhibitor with *in vitro* and *ex vivo* anti-inflammatory properties

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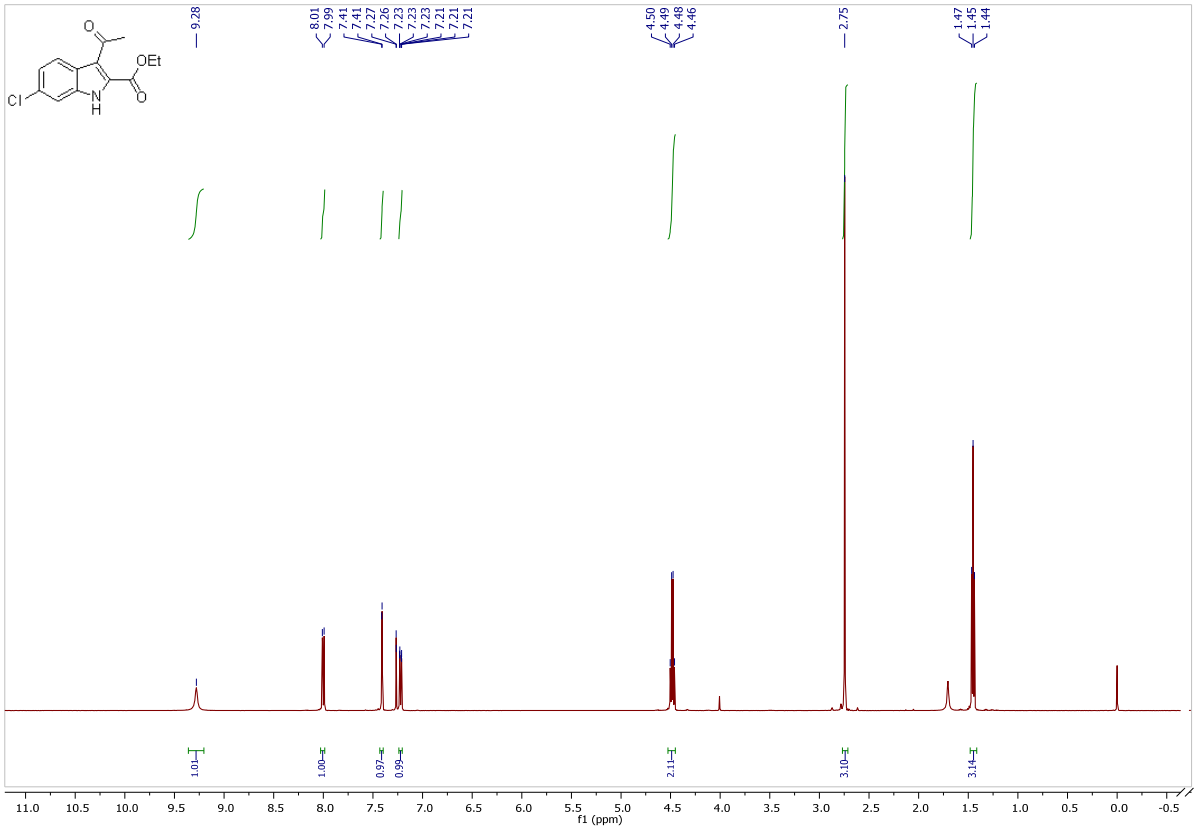
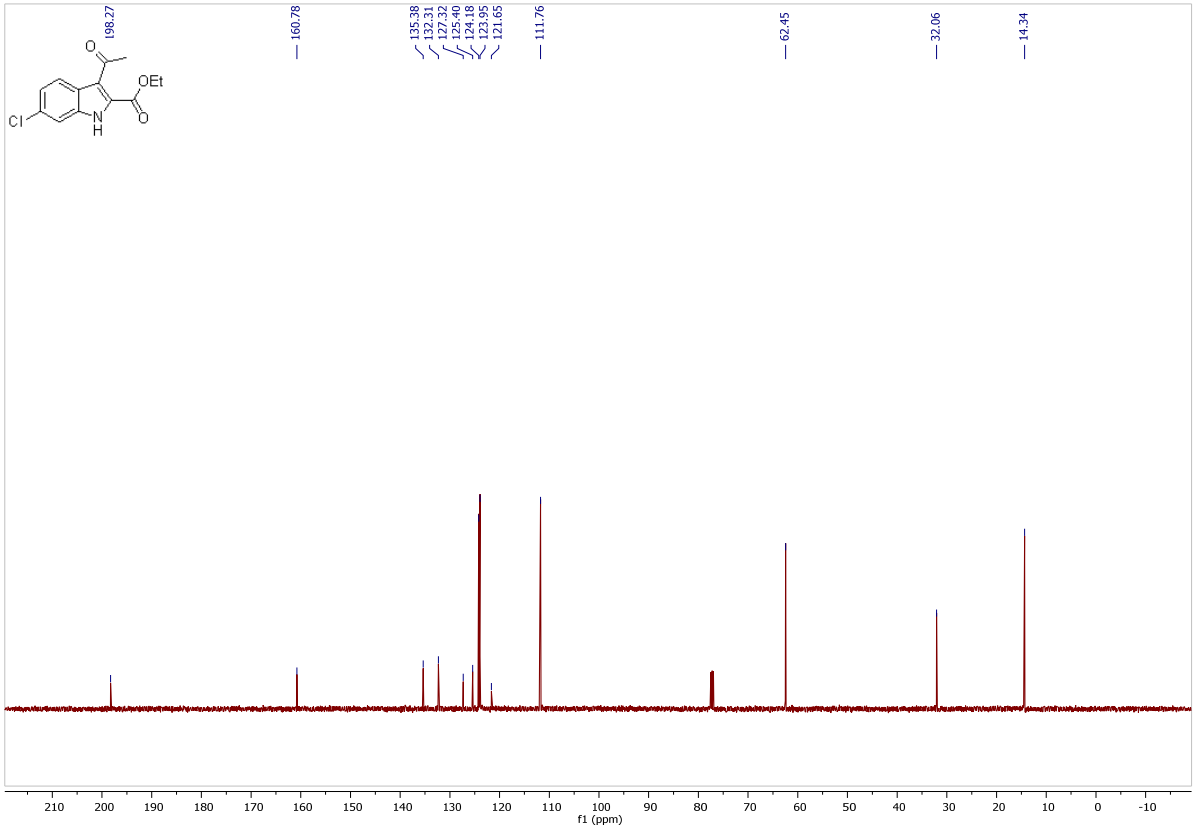
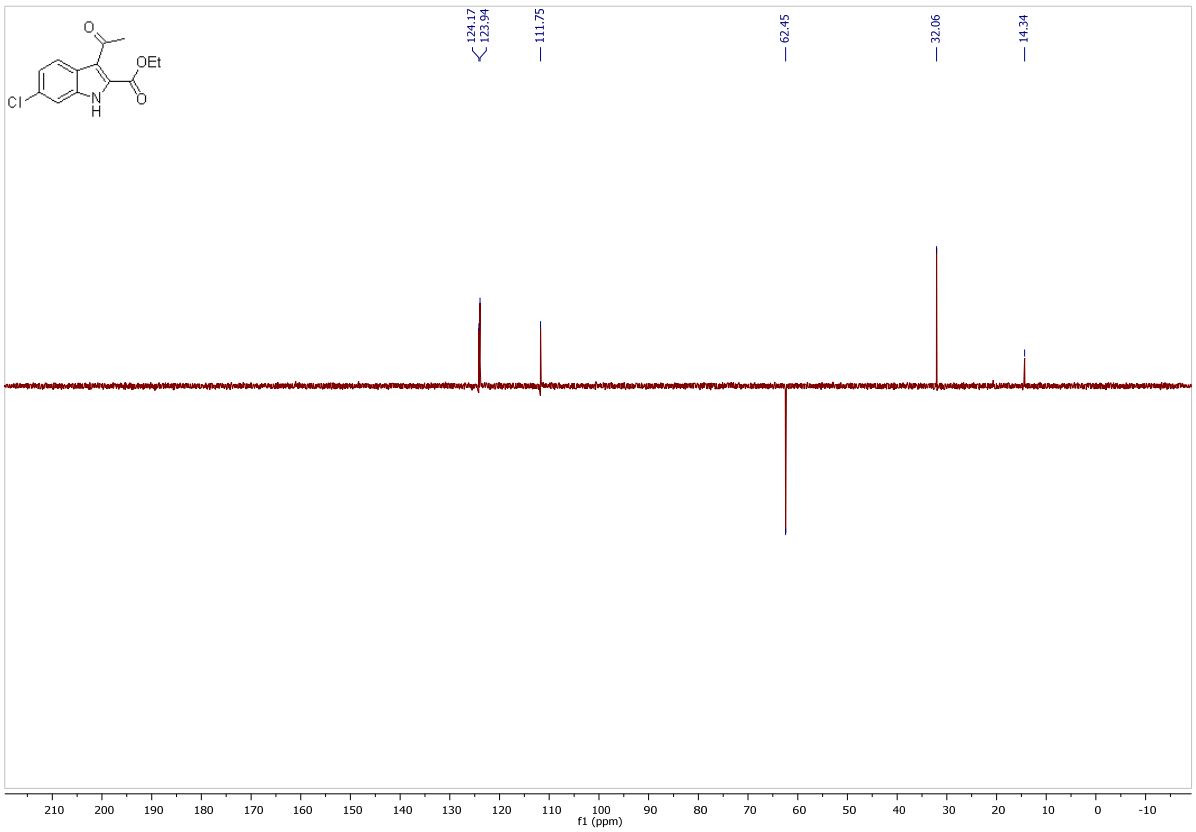
^{1}H NMR spectrum of **14a**, DN397

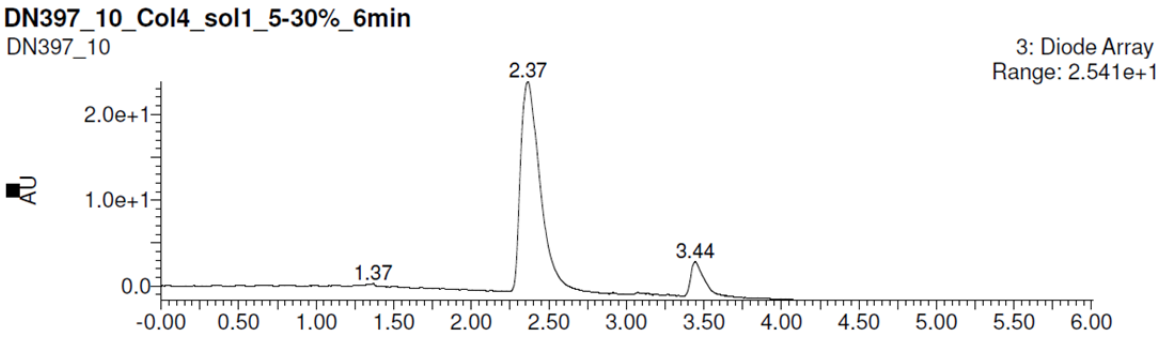


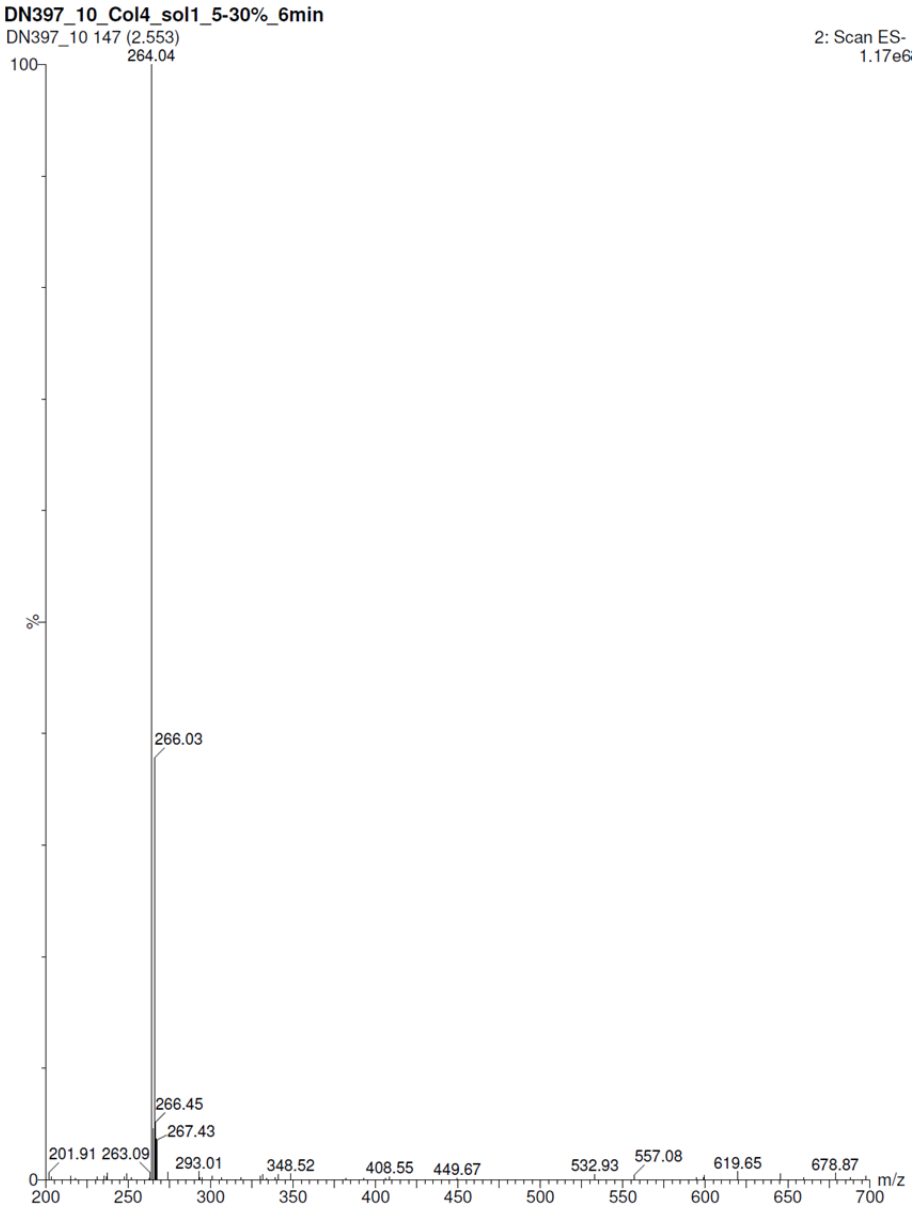
^{13}C NMR and DEPT-135 spectra of **14a**, DN397



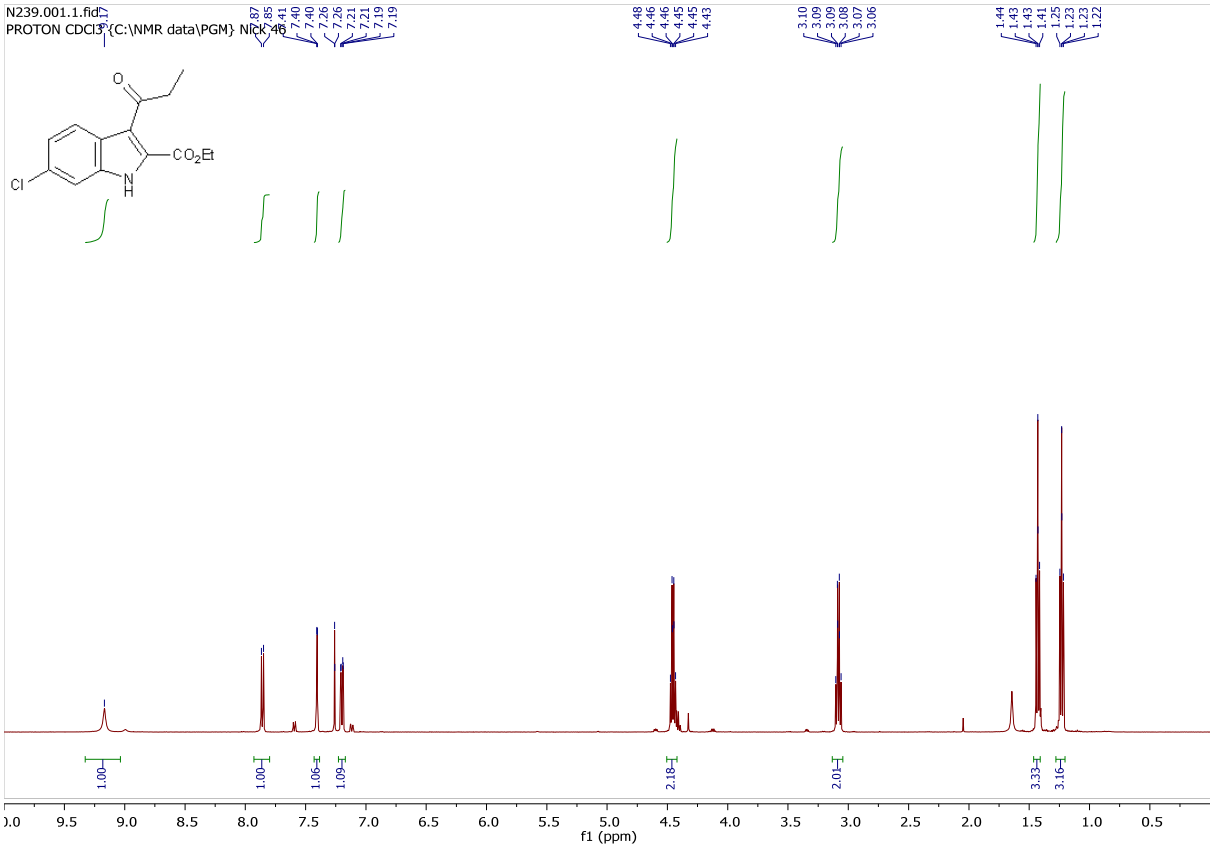


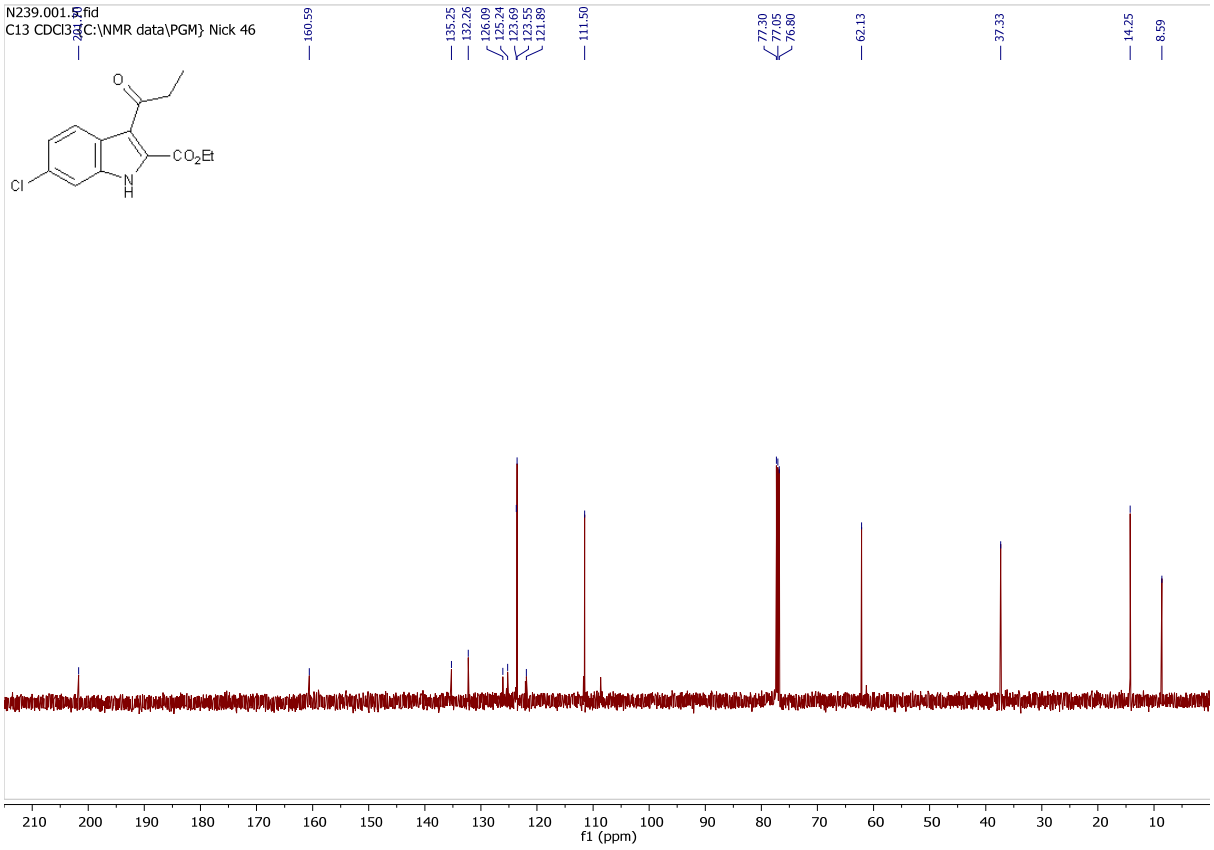
Chromatogram and MS spectra of **14a**, DN397



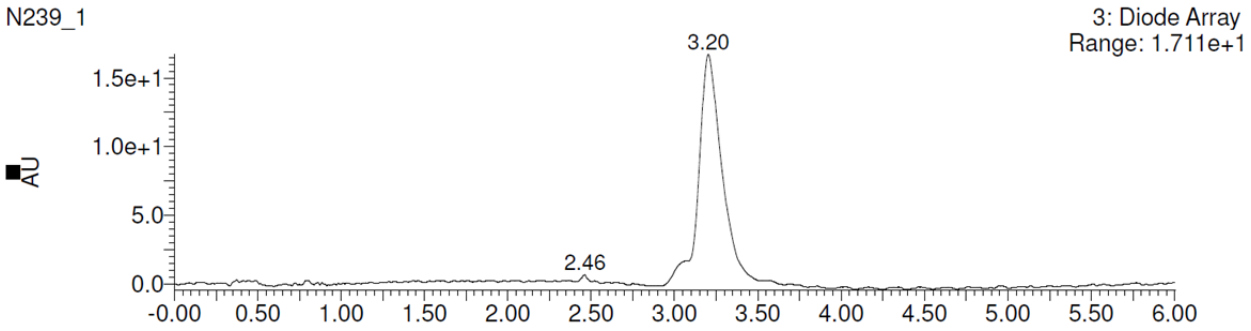


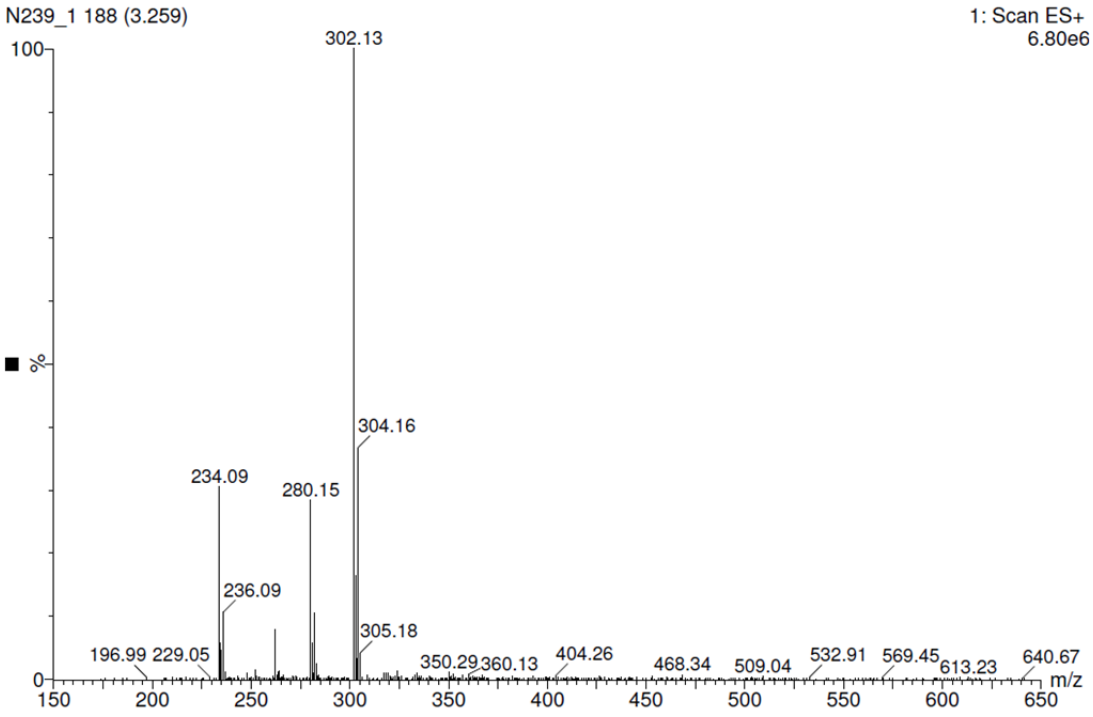
^{1}H NMR and ^{13}C NMR spectra of **14b**, N239



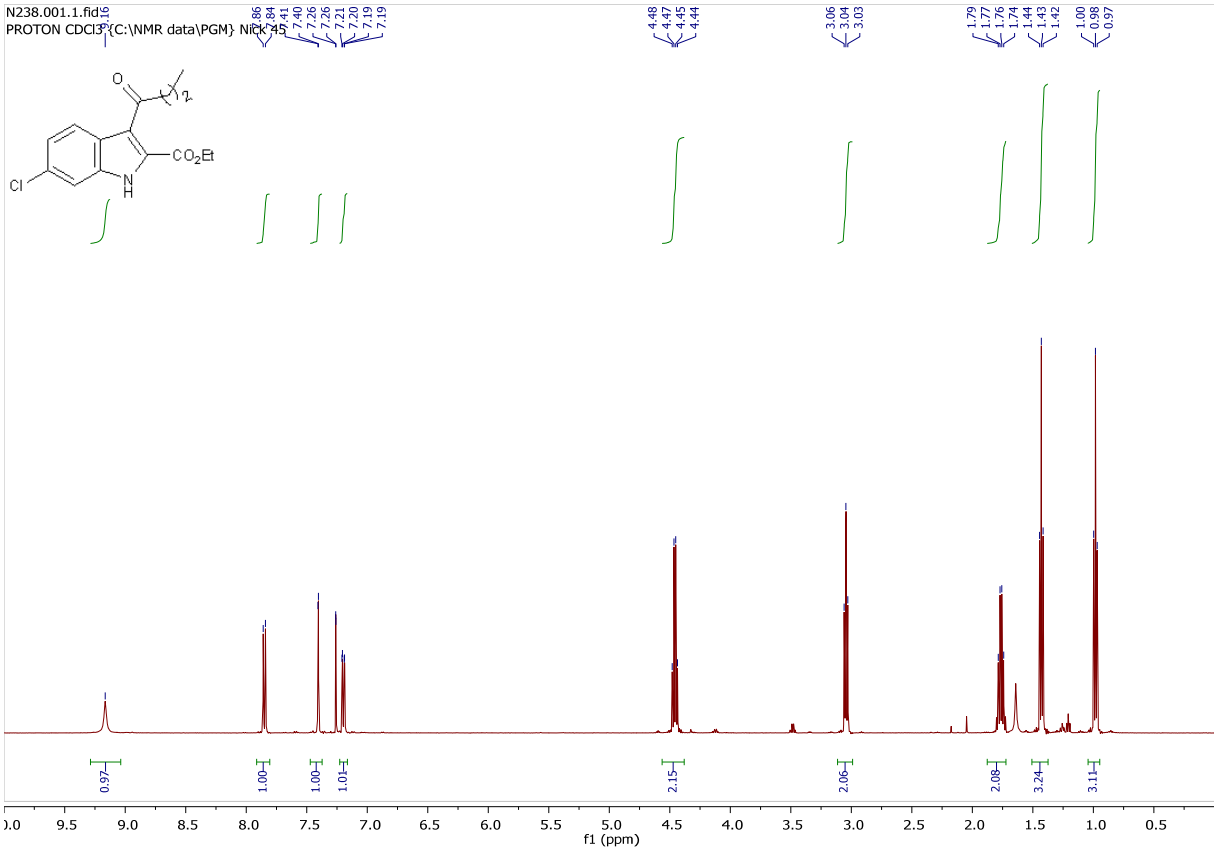


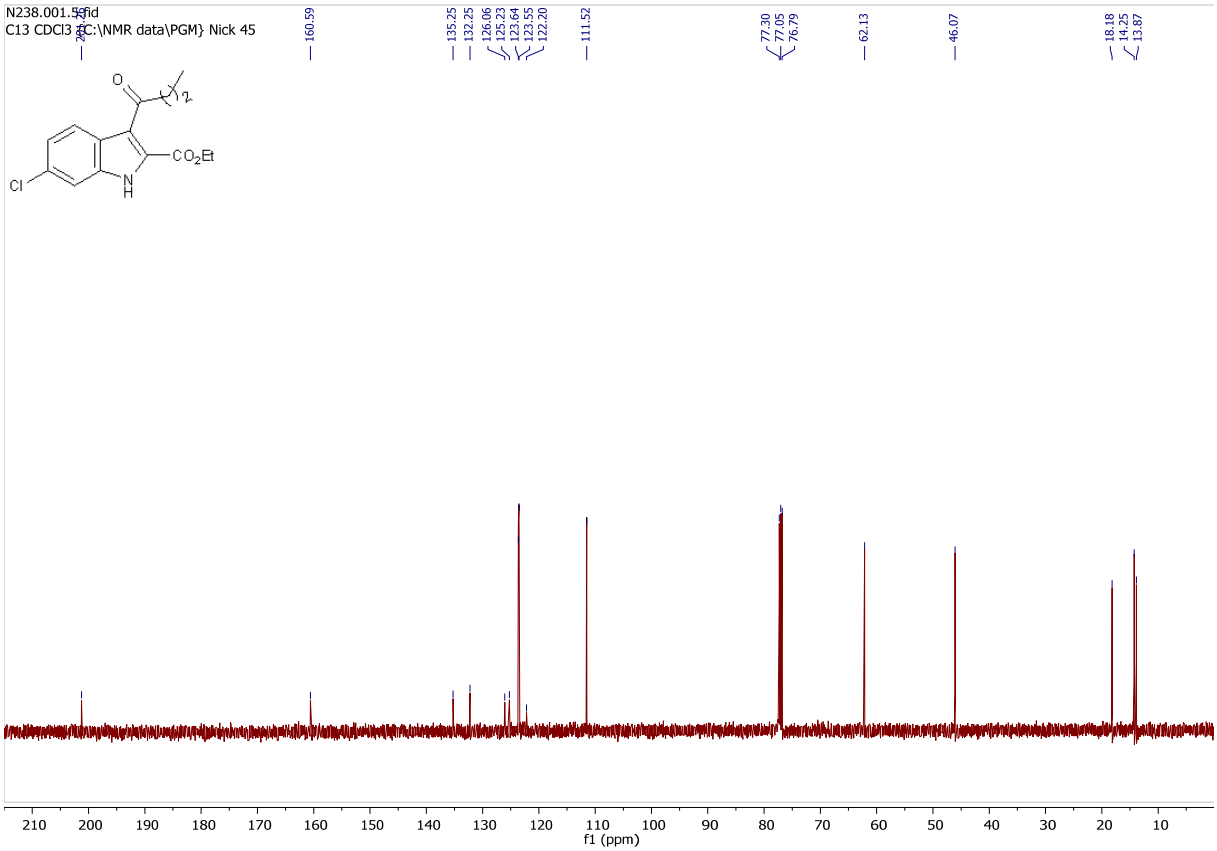
Chromatogram and MS spectra of **14b**, N239



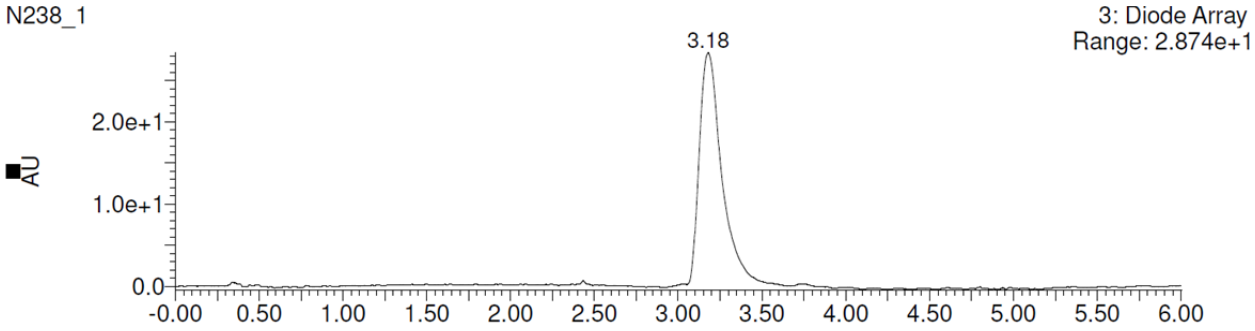


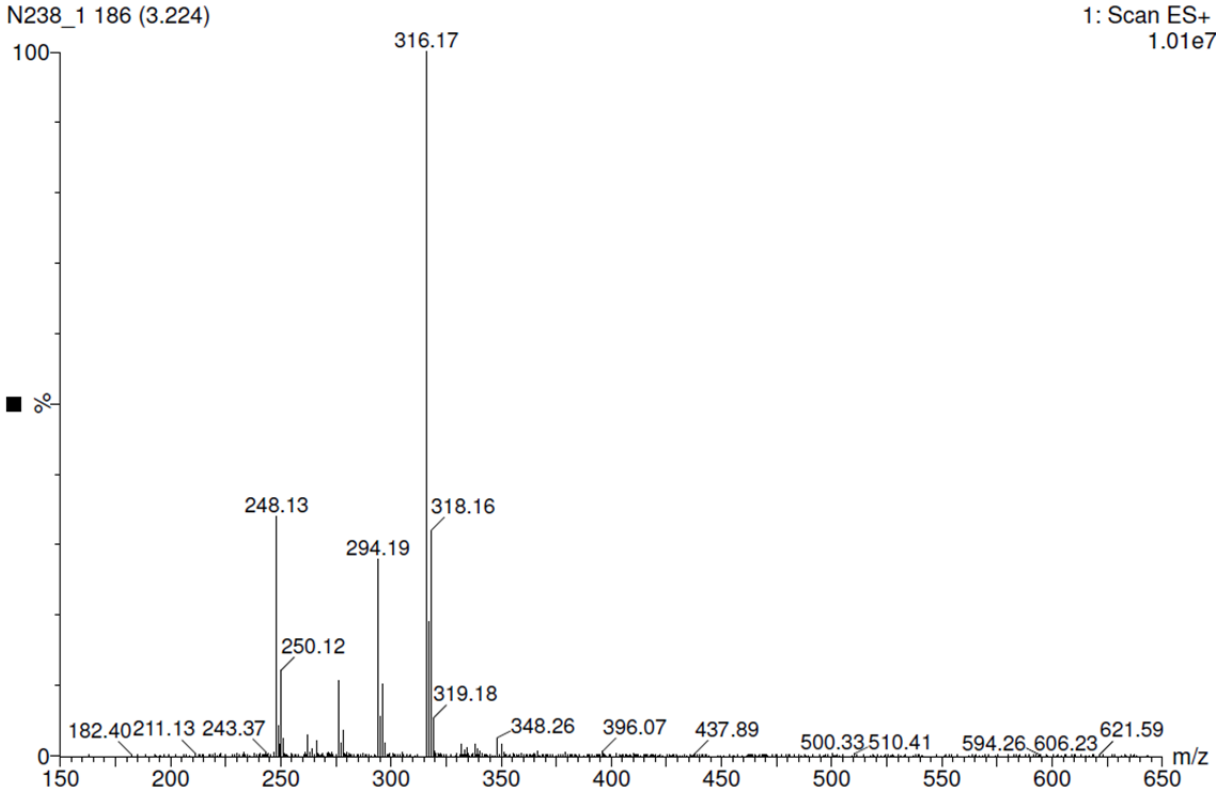
^{1}H NMR and ^{13}C NMR spectra of **14c**, N238



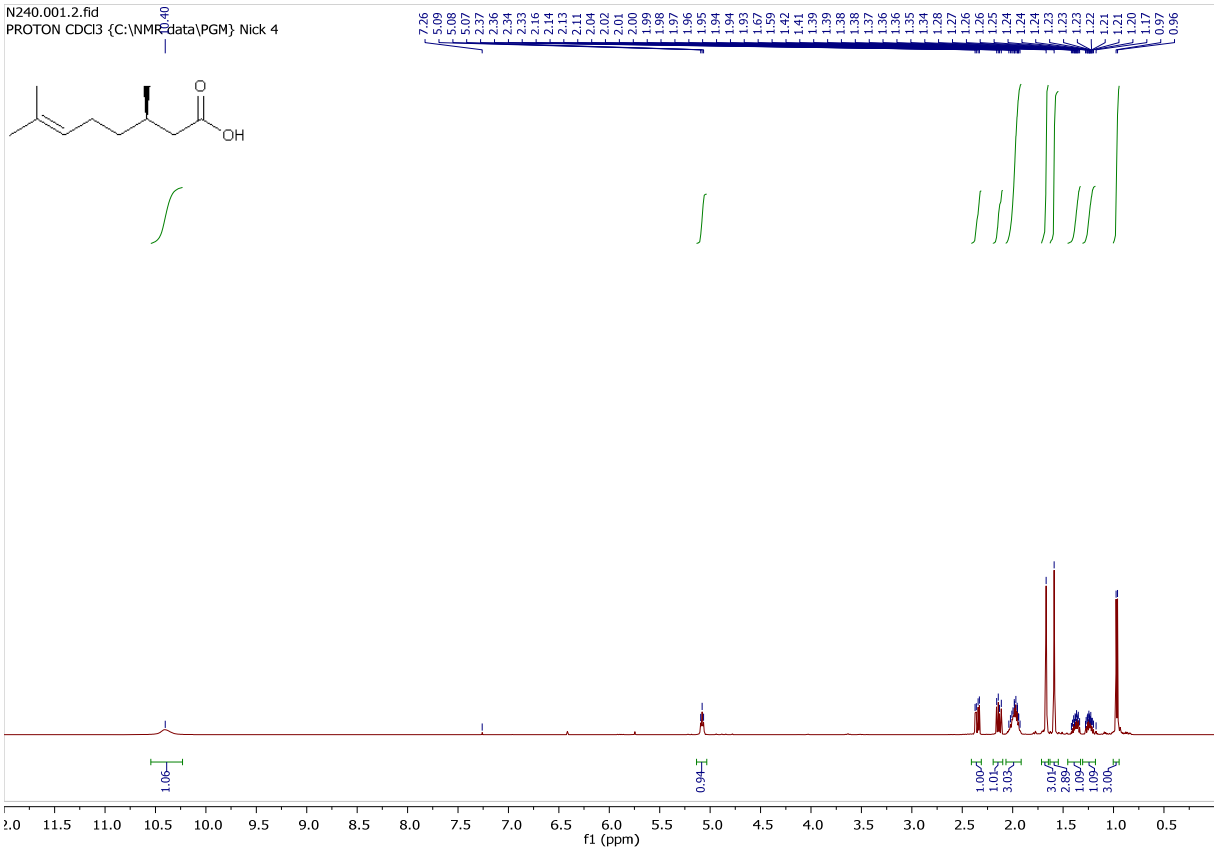


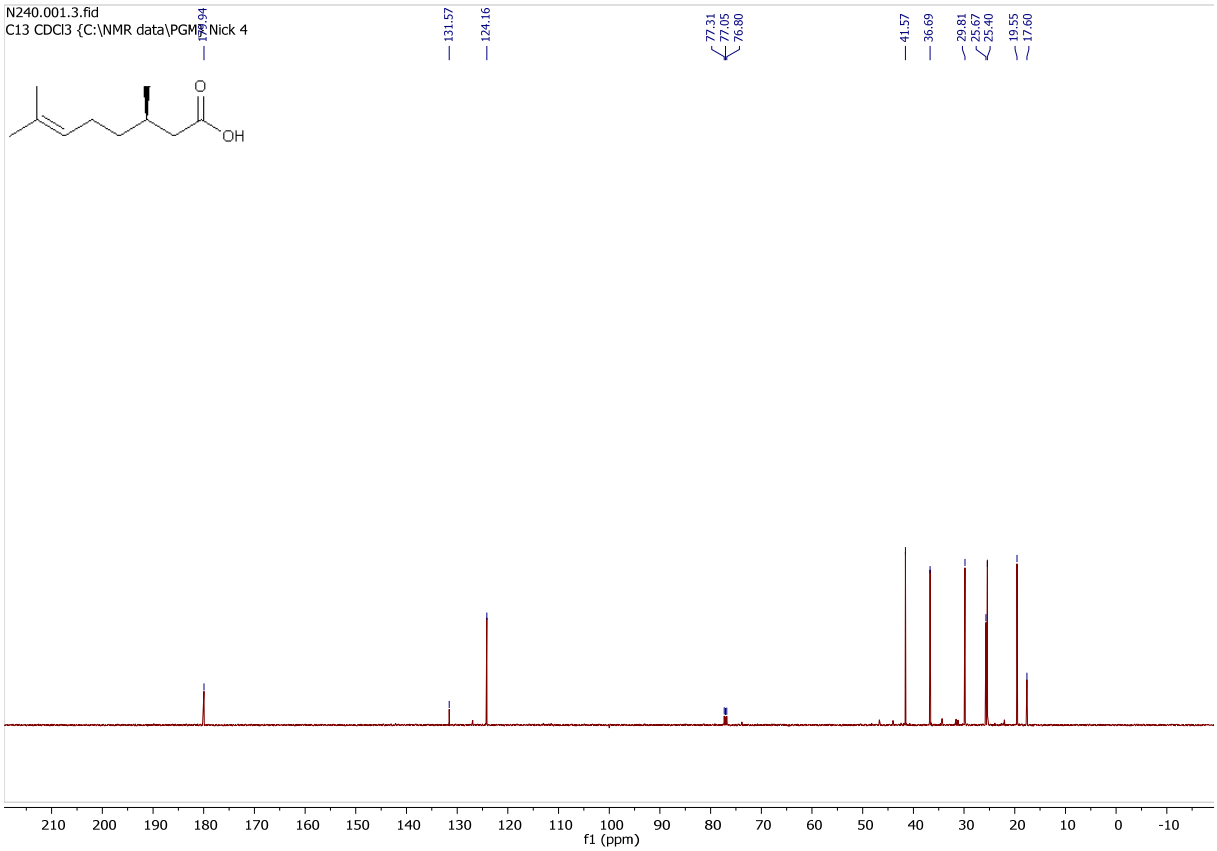
Chromatogram and MS spectra of **14c**, N238





^{1}H NMR and ^{13}C NMR spectra of **11d**, N240





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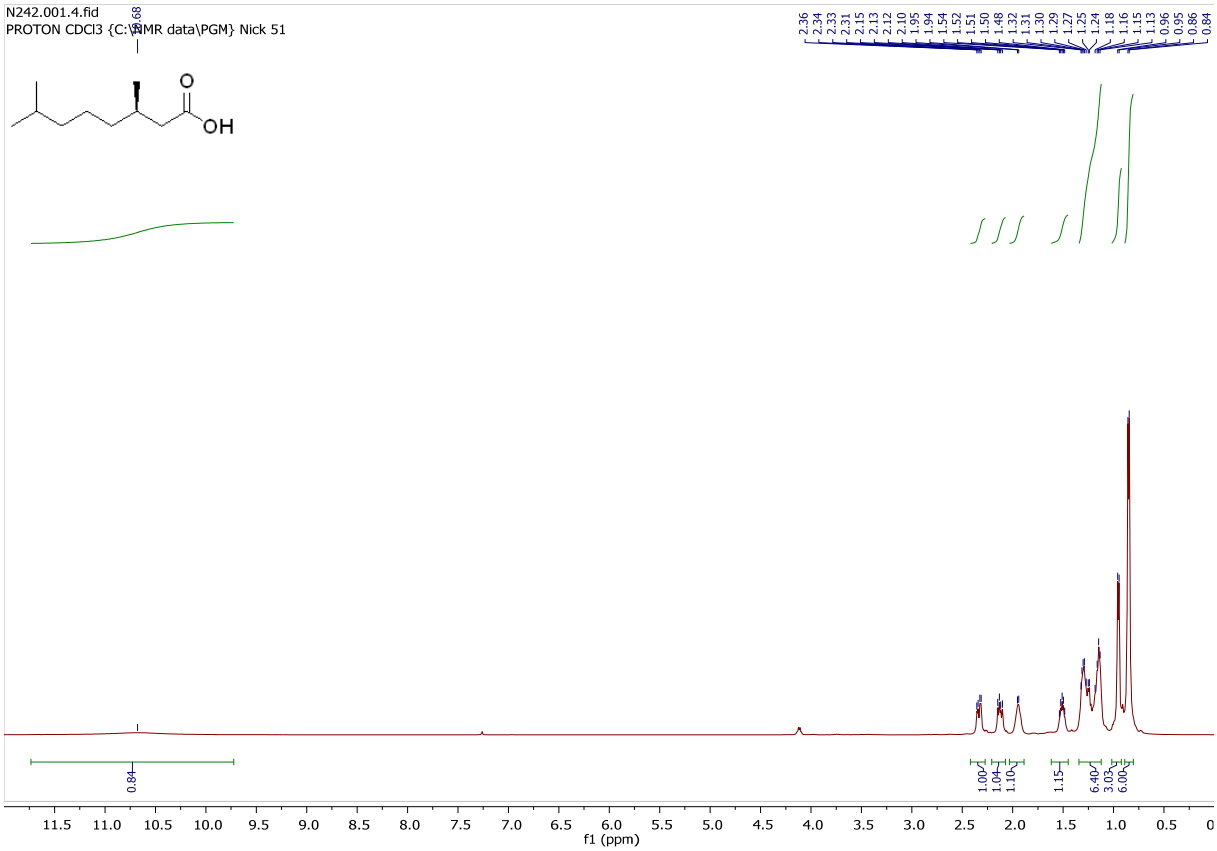
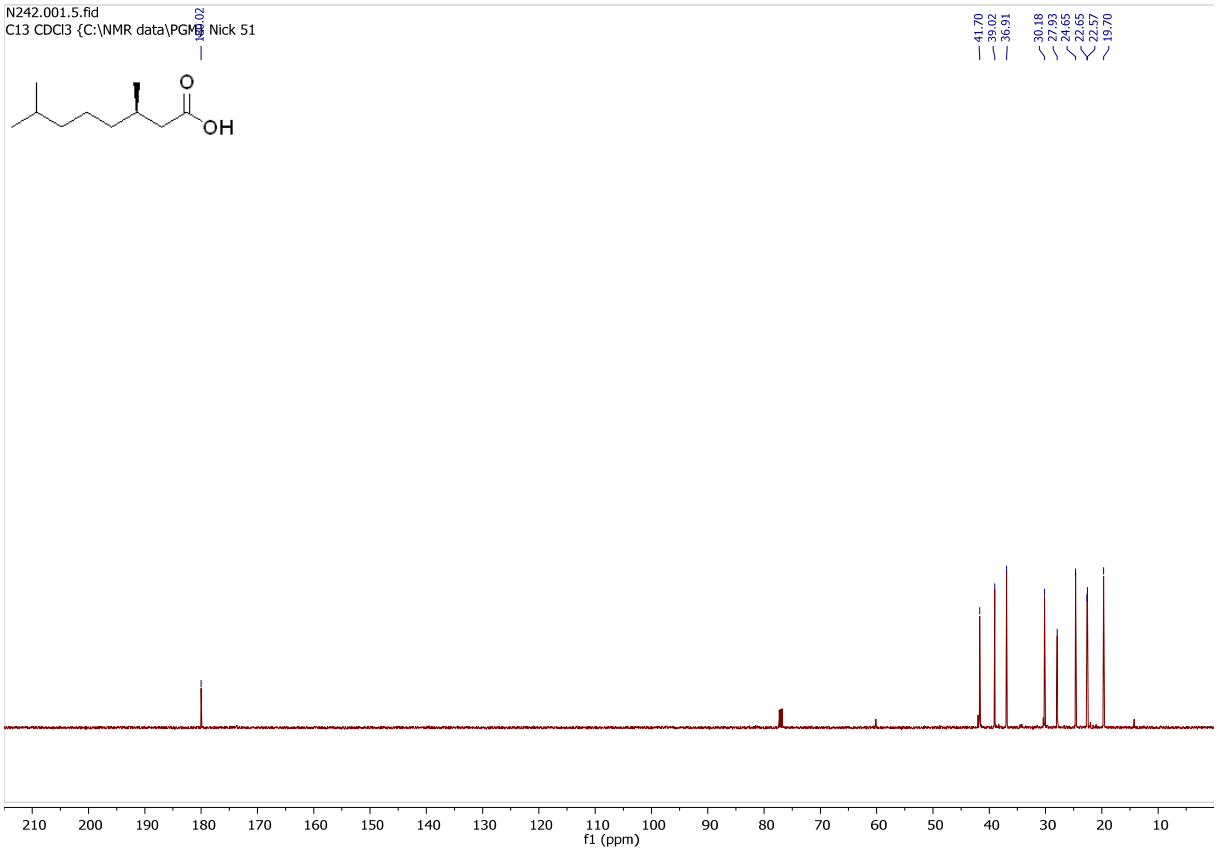
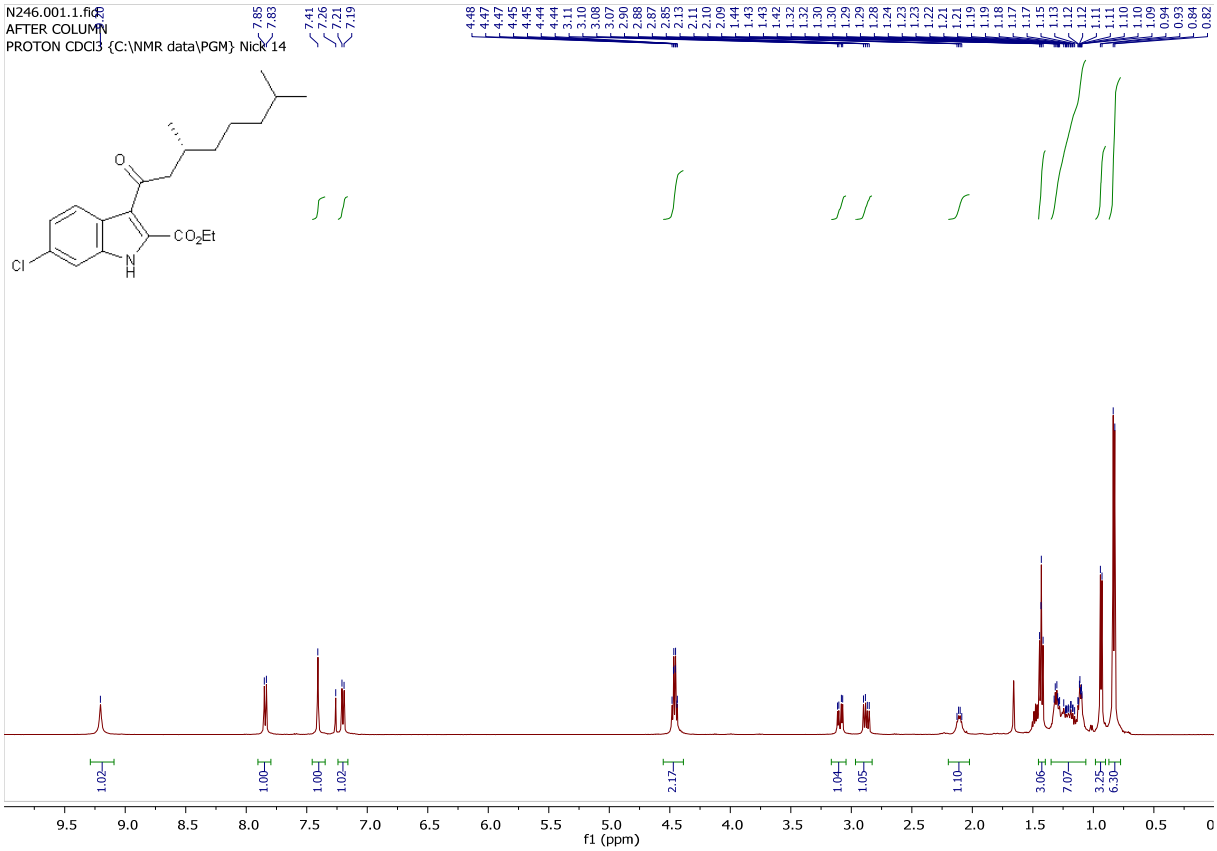
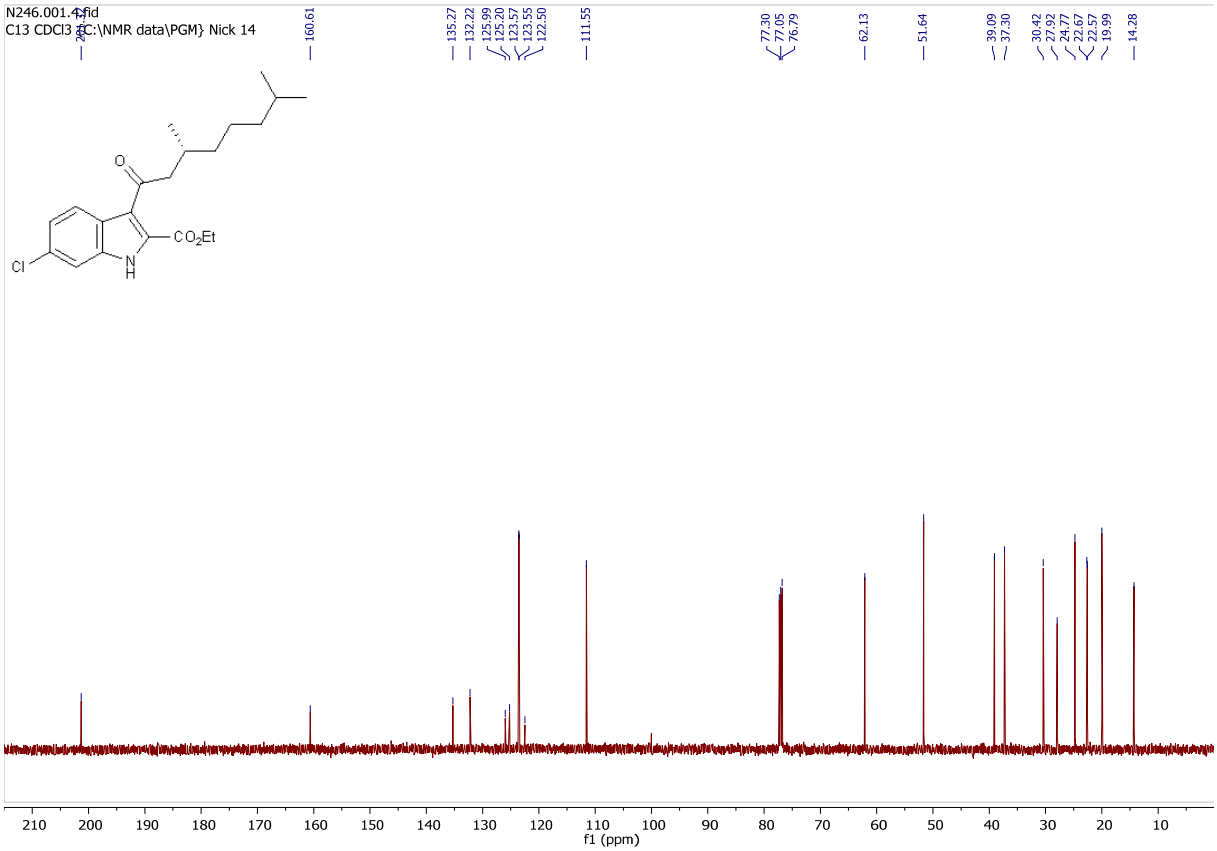


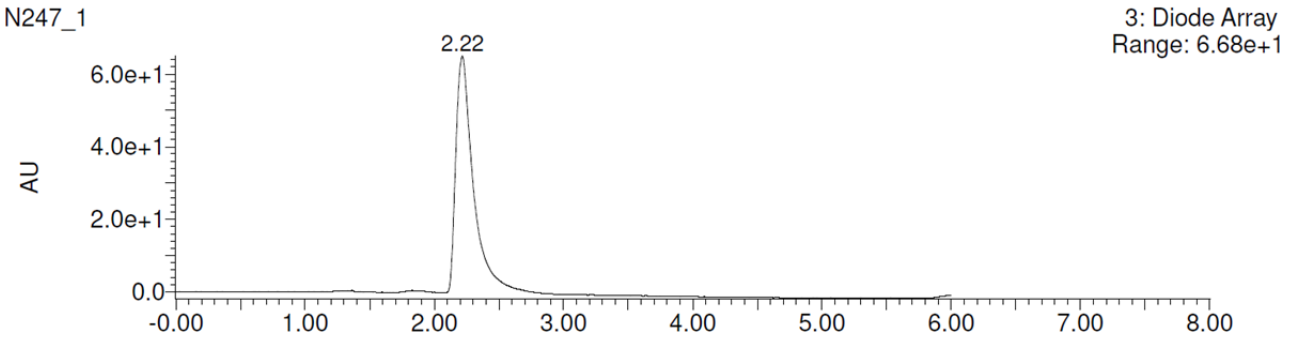


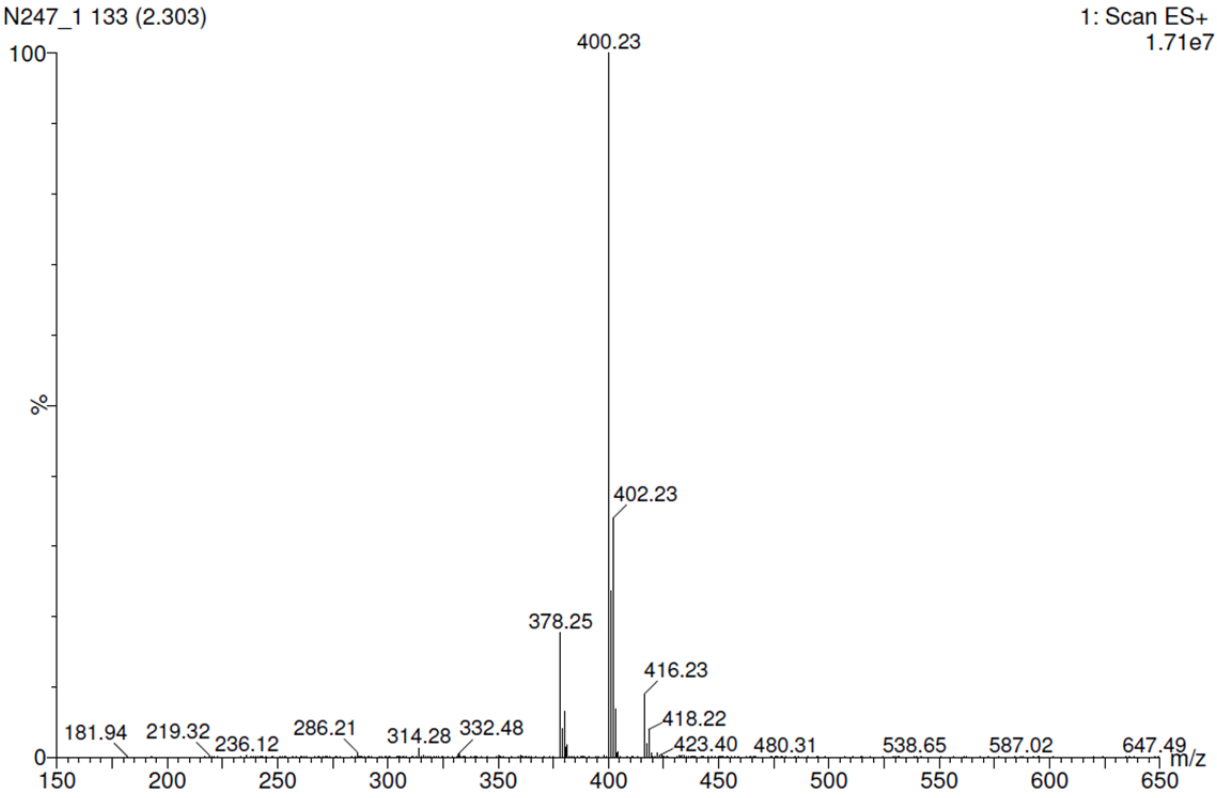
^{1}H NMR and ^{13}C NMR spectra of **14e**, N246



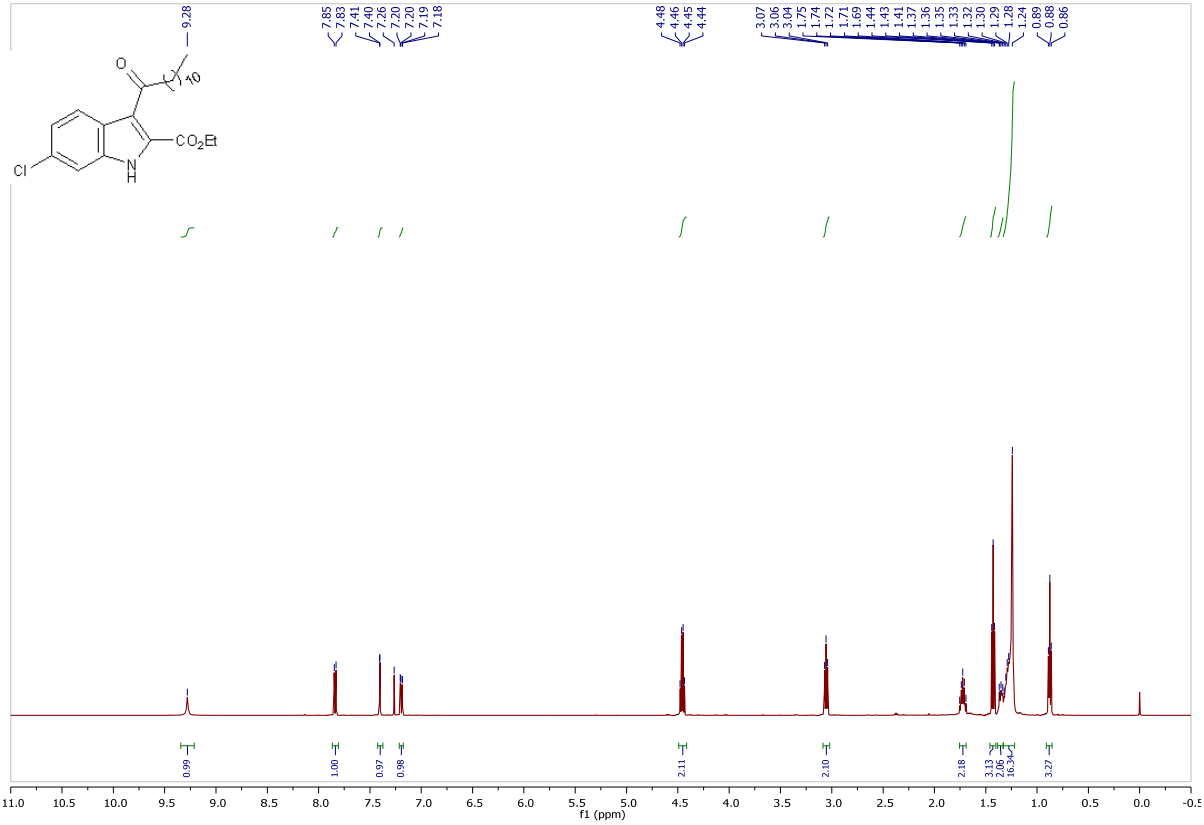


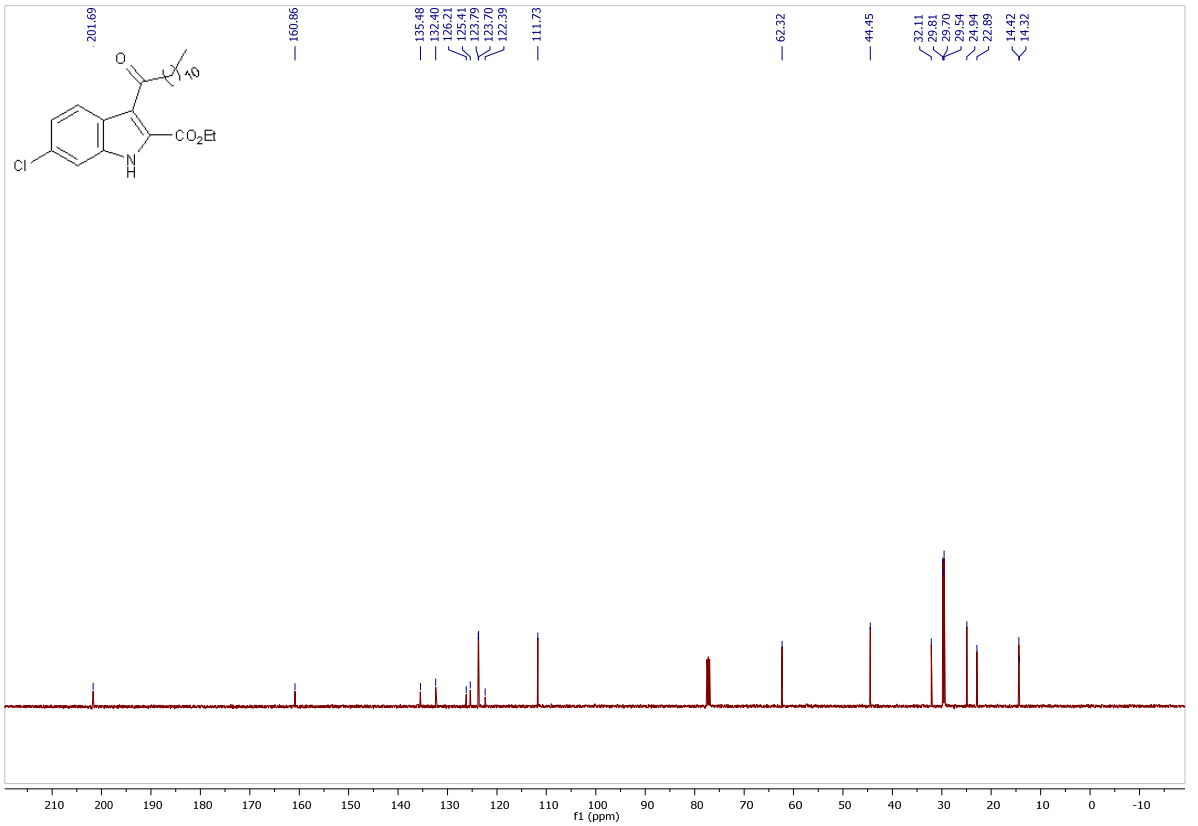
Chromatogram and MS spectra of **14d**, N247



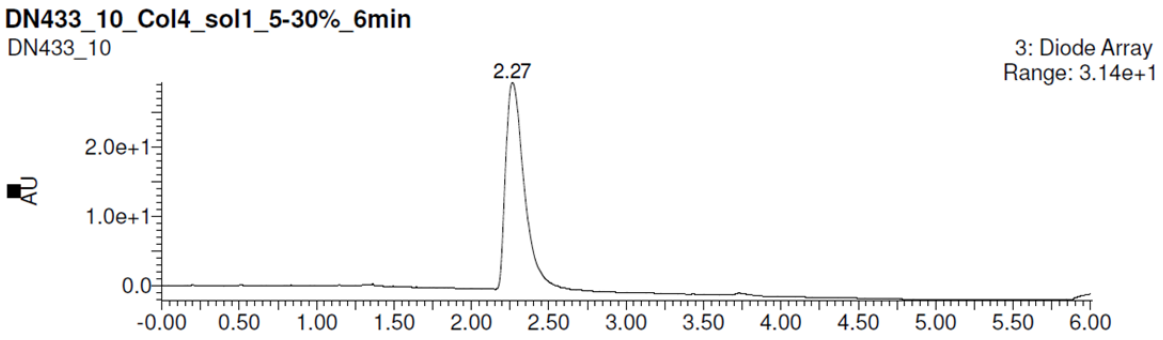


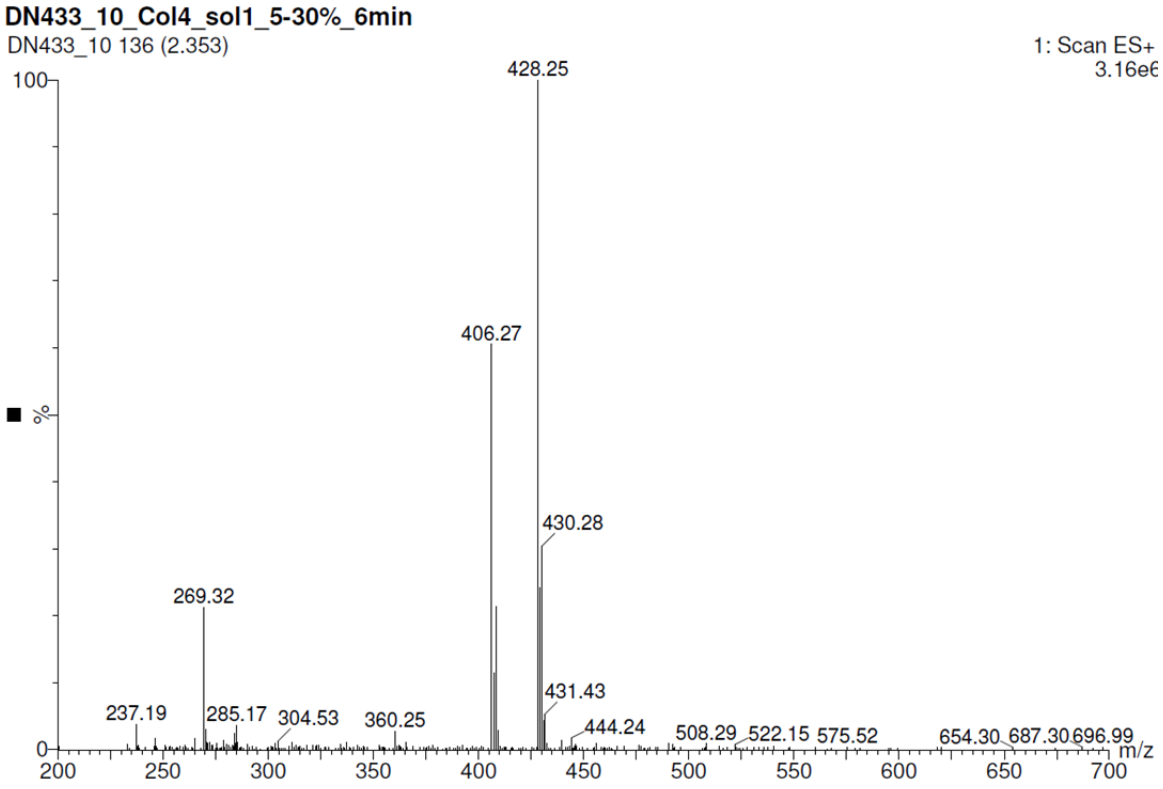
^{1}H NMR and ^{13}C NMR spectra of **14f**, DN433



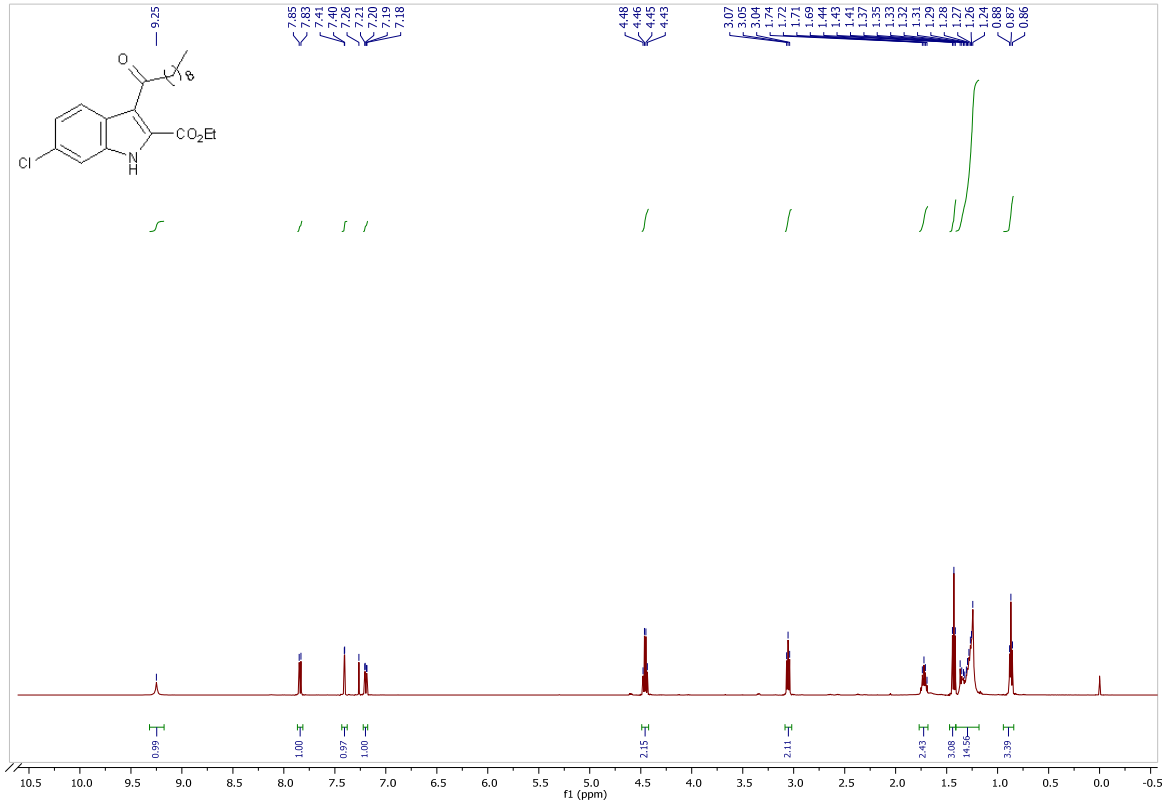


Chromatogram and MS spectra of **14f**, DN433

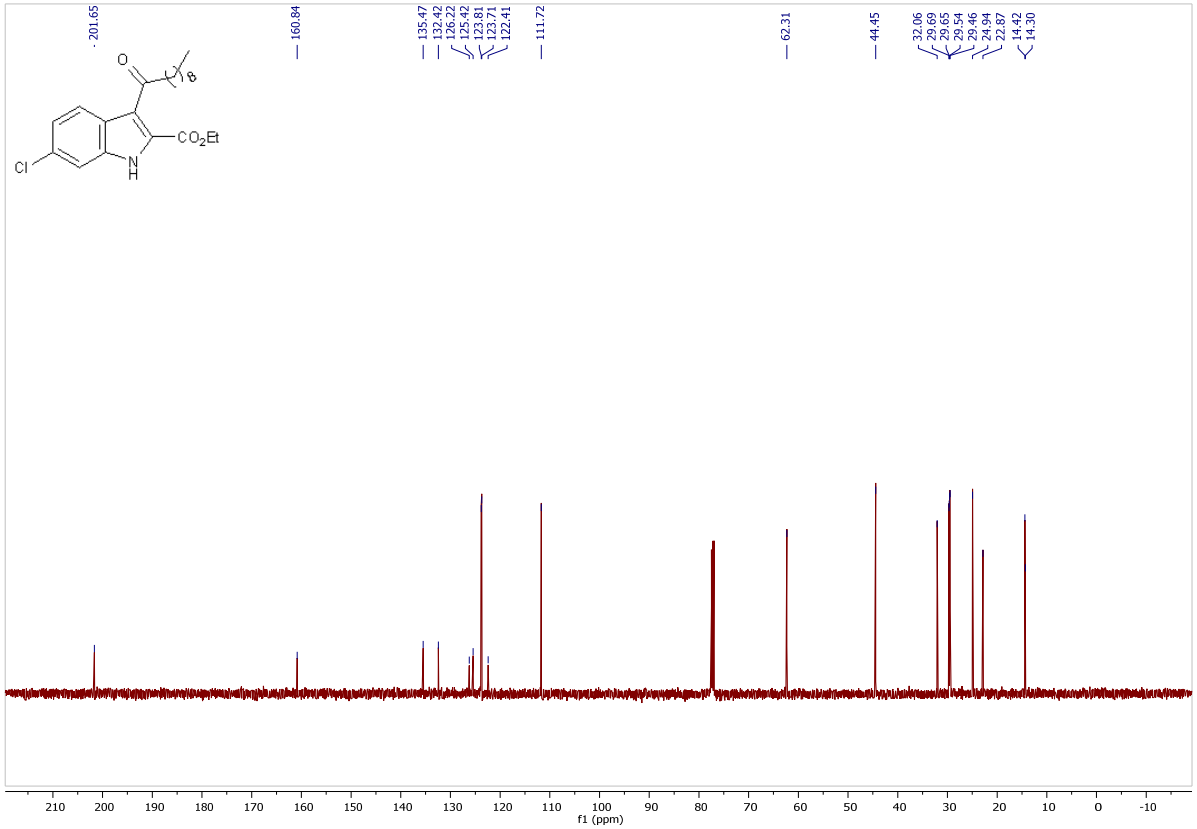


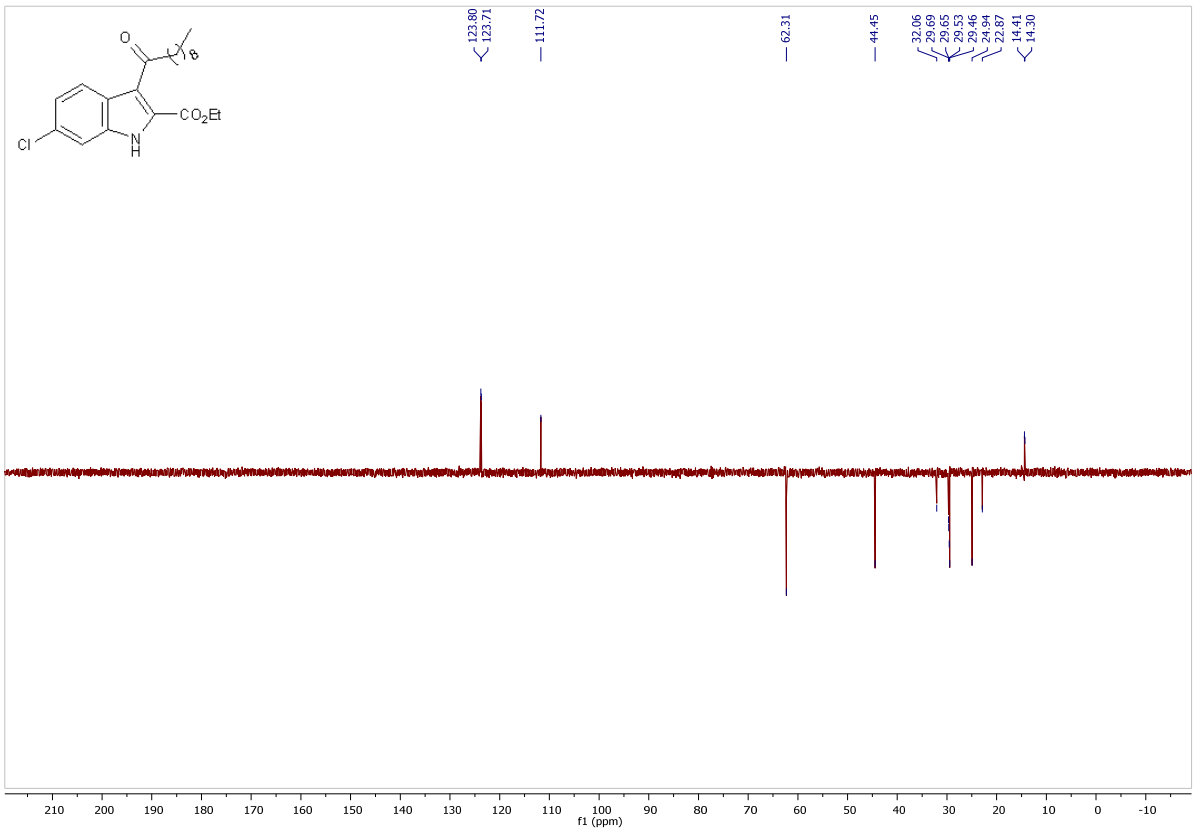


^{1}H NMR spectrum of **14g**, DN432

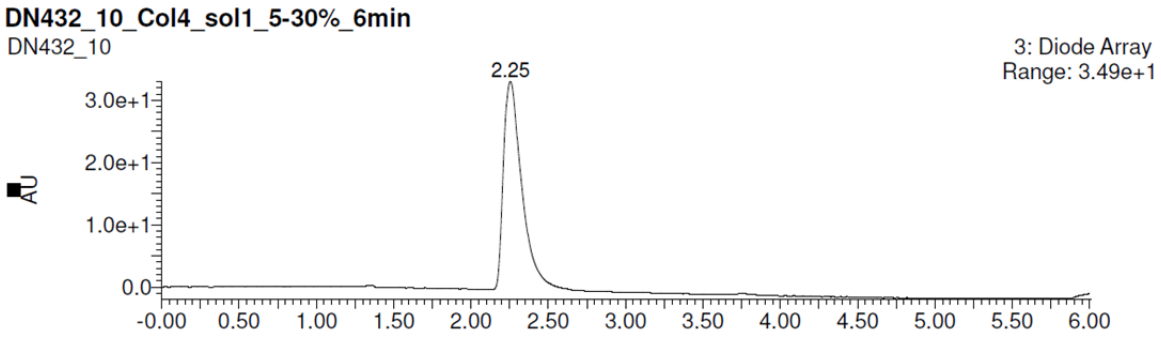


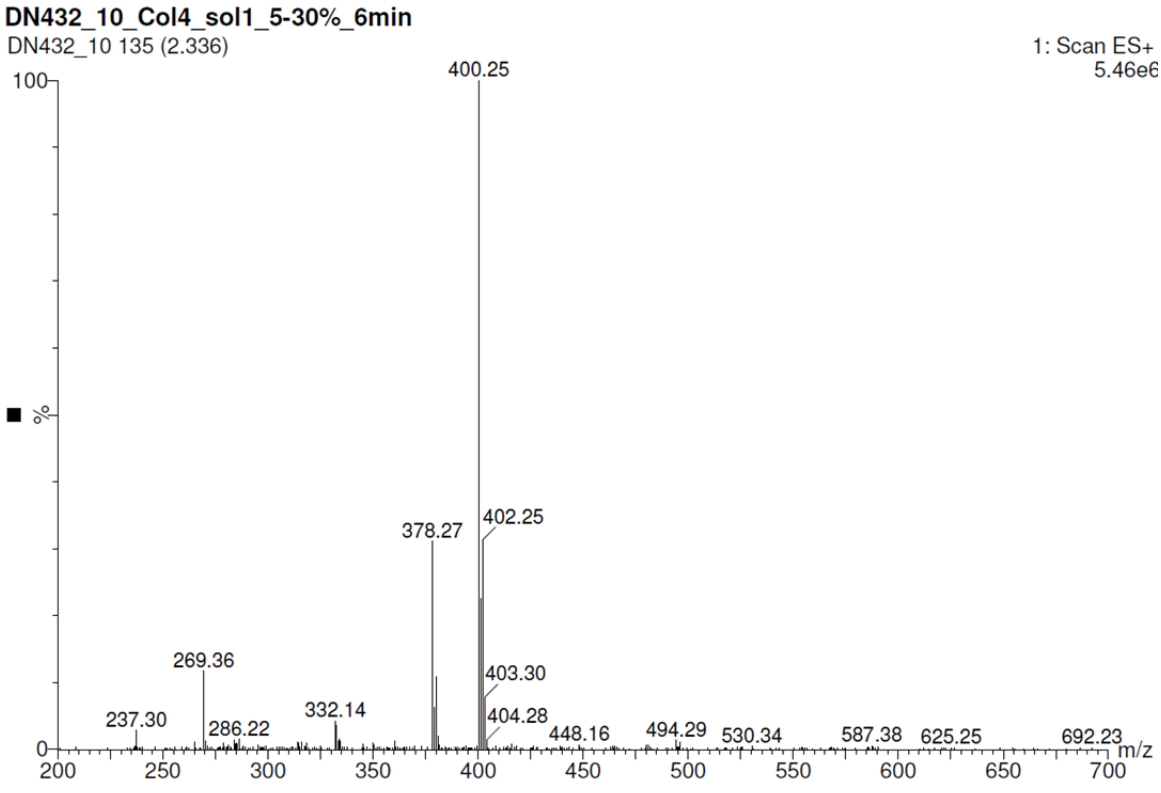
^{13}C NMR and DEPT-135 spectra of **14g**, DN432





Chromatogram and MS spectra of **14g**, DN432





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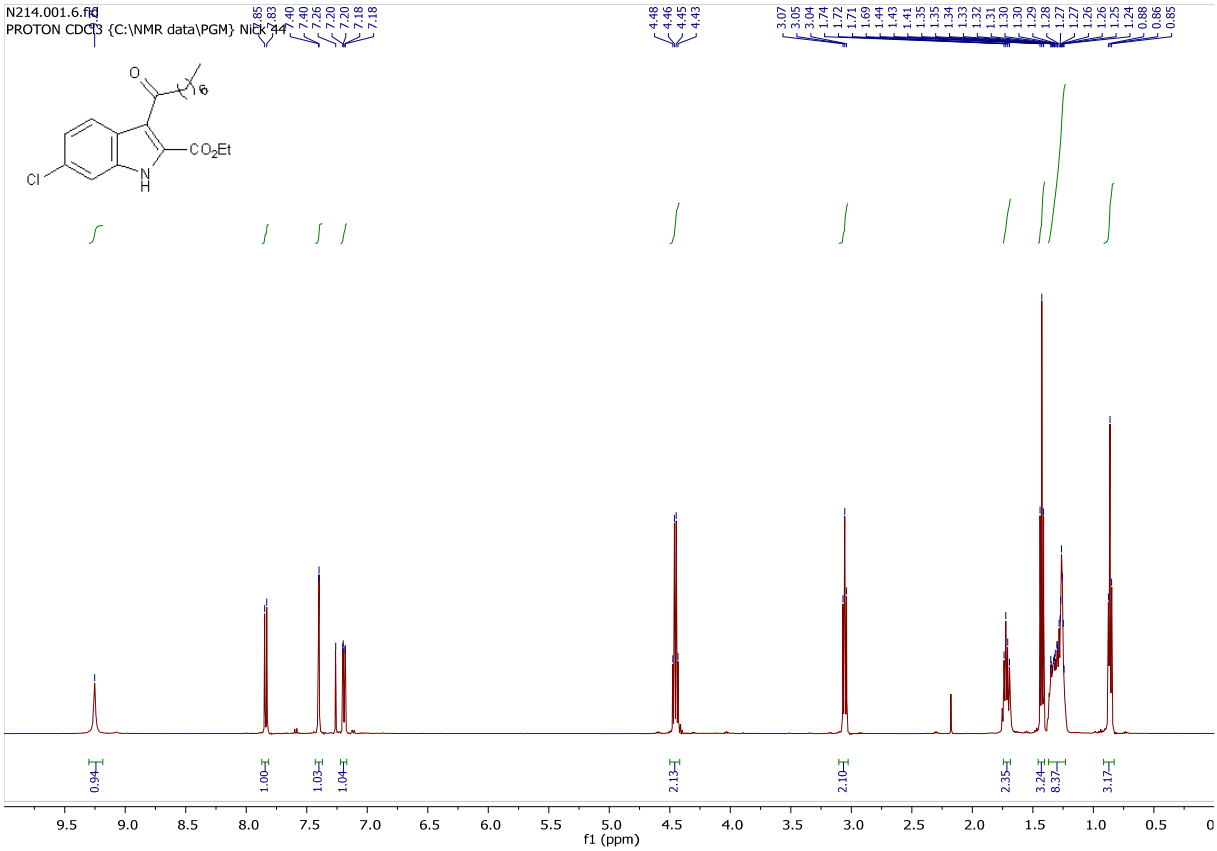
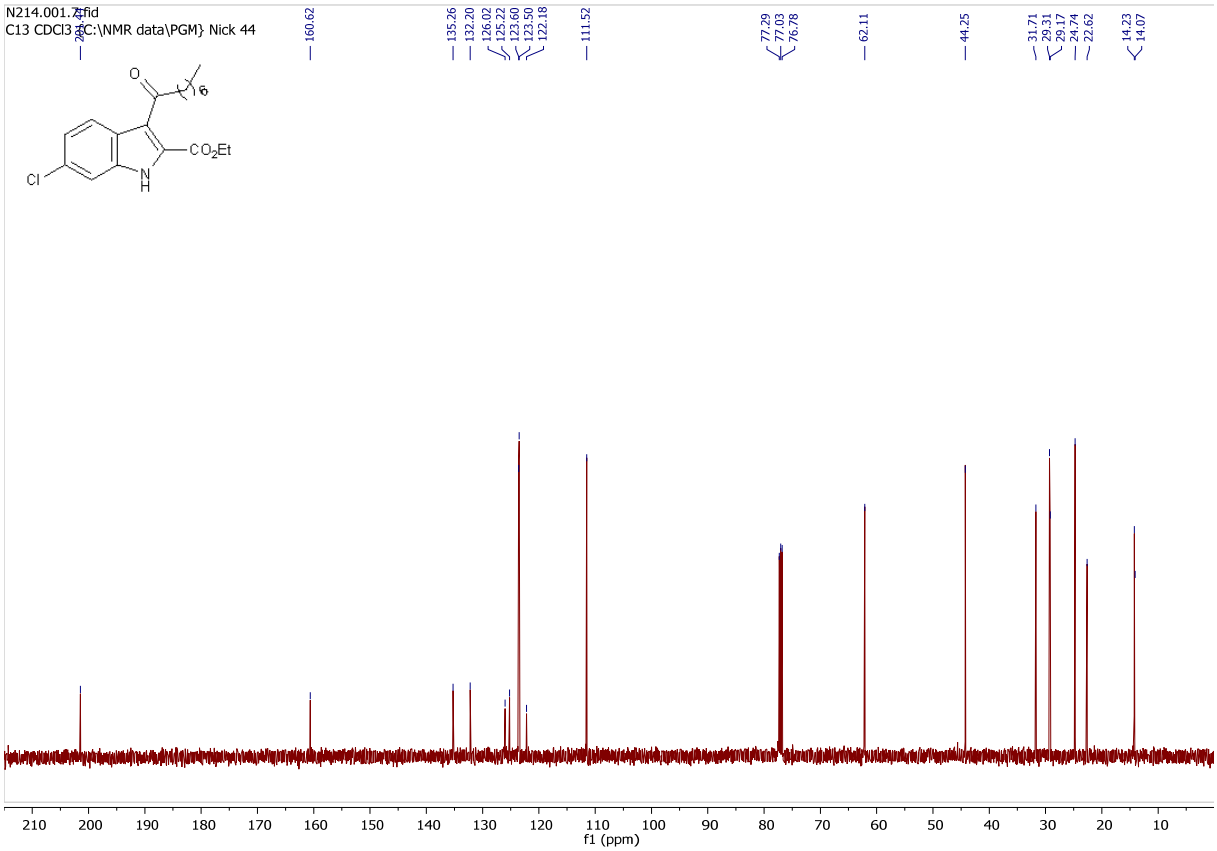
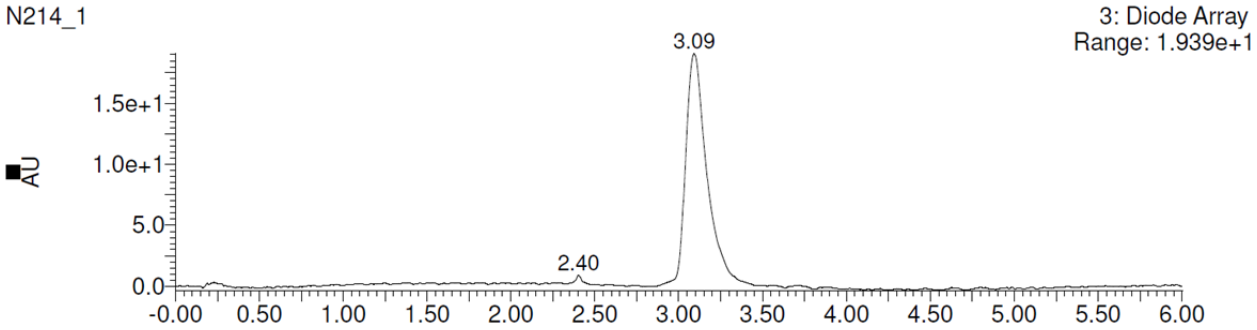
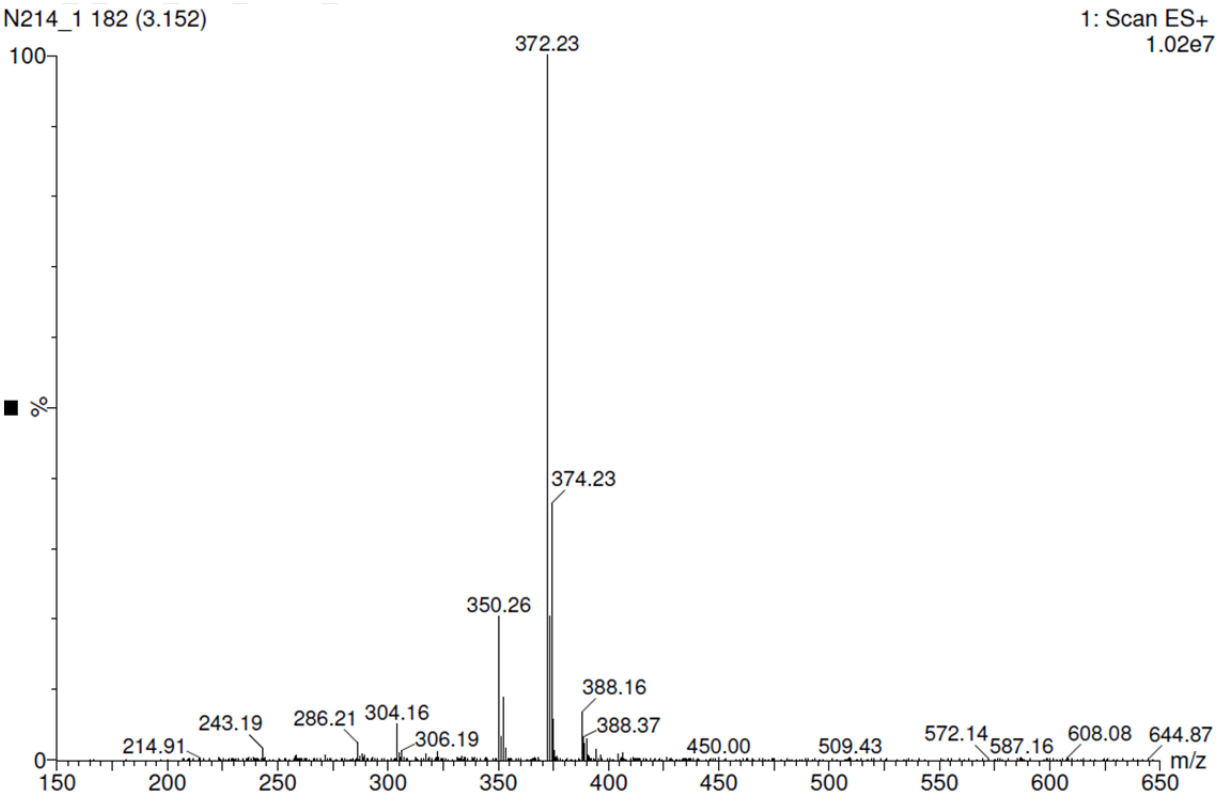


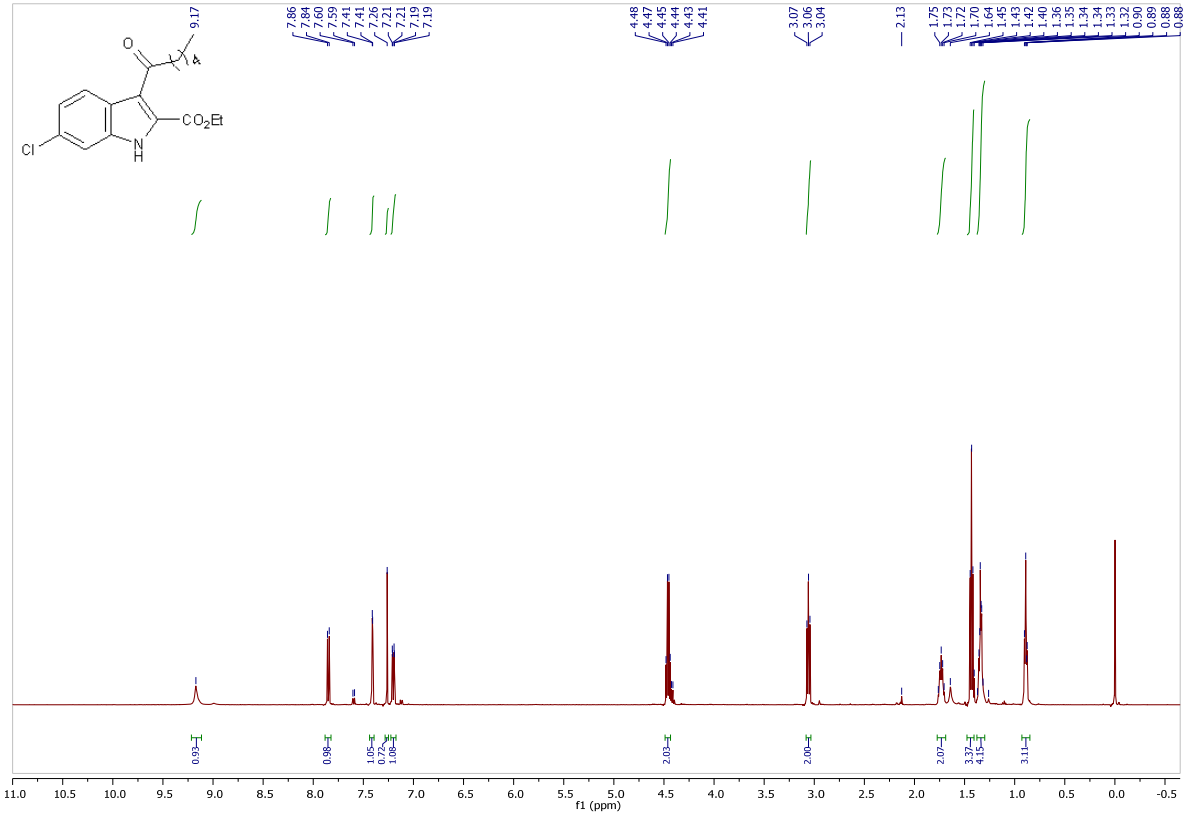


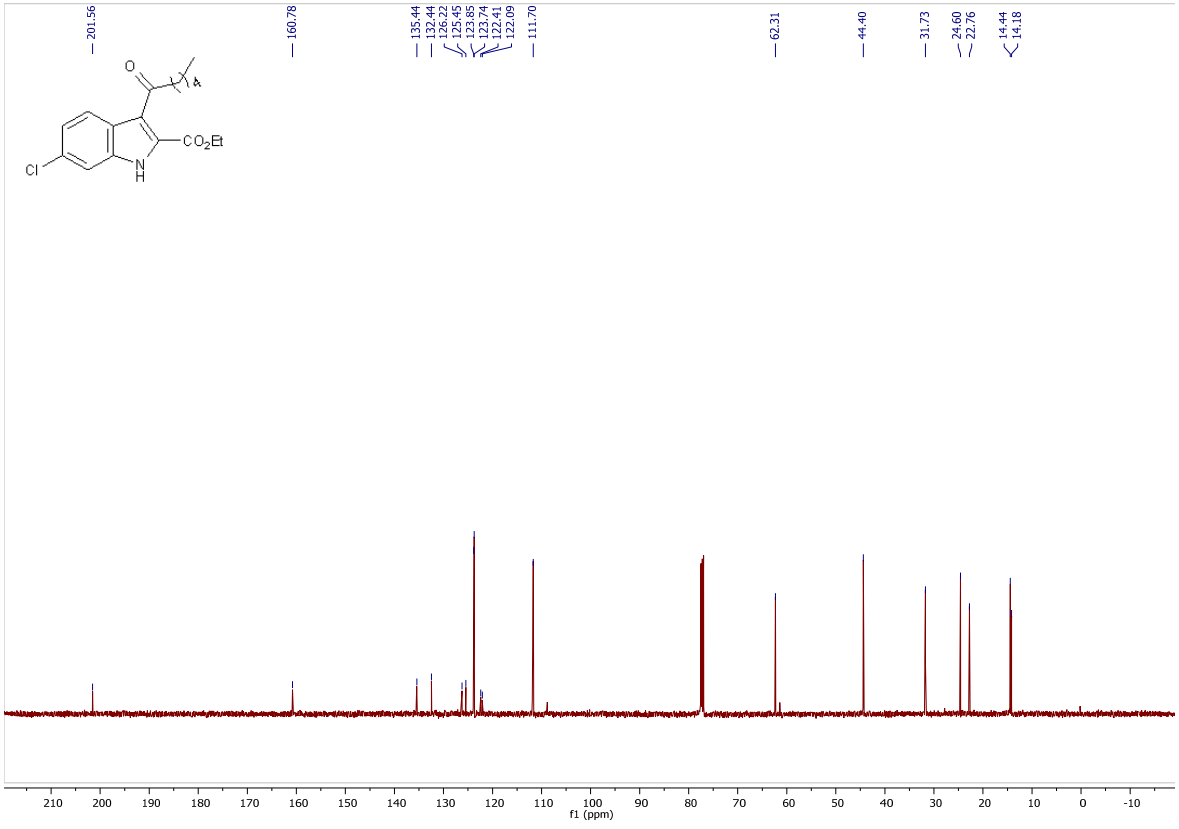
Chromatogram and MS spectra of **14h**, N214



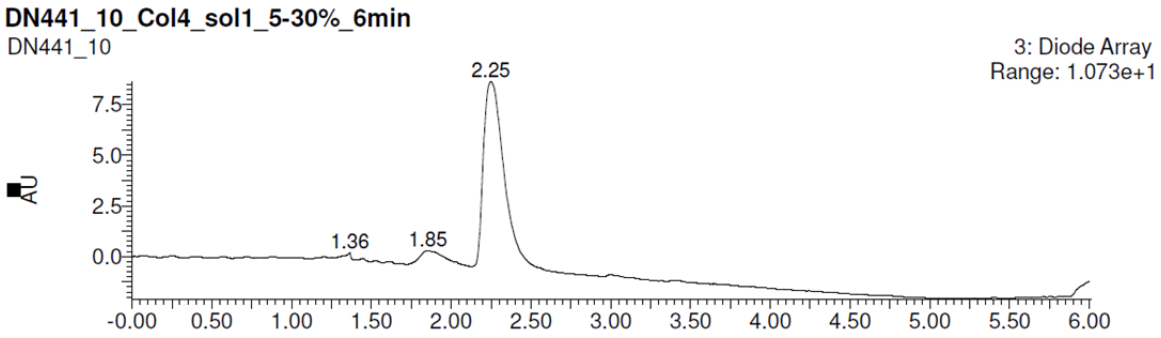


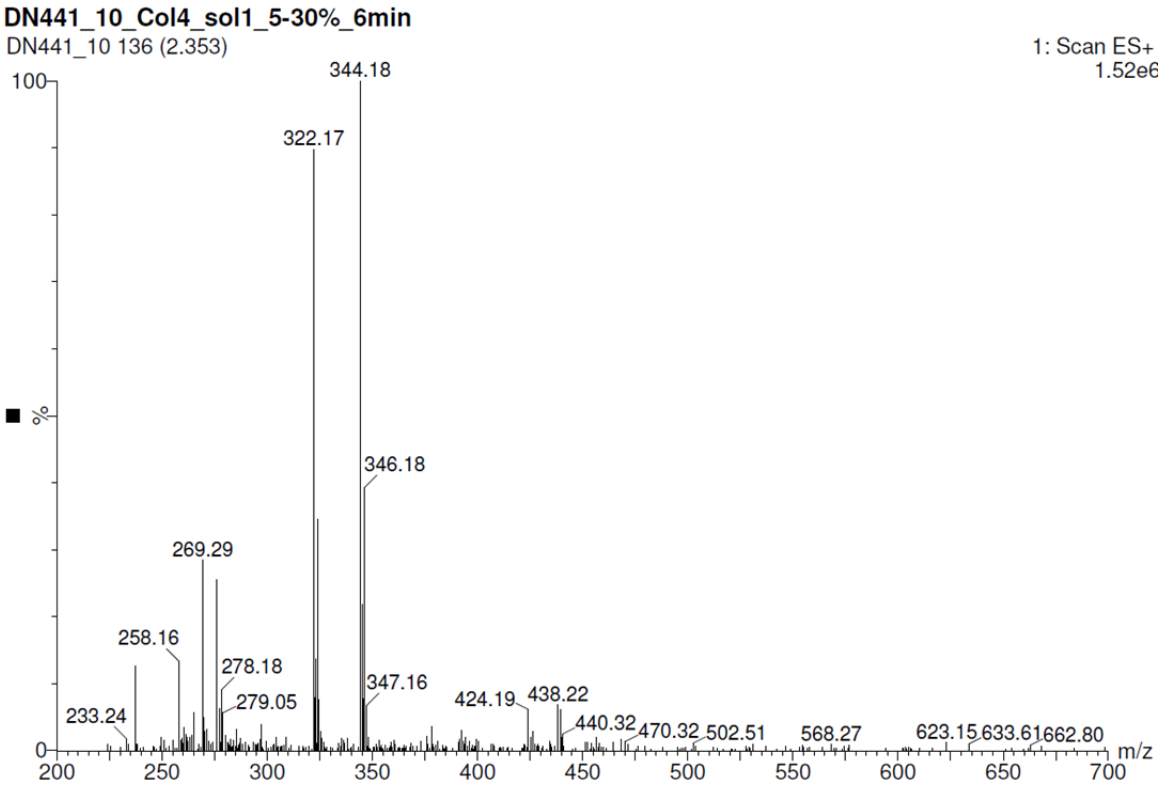
^{1}H NMR and ^{13}C NMR spectra of **14i**, DN441



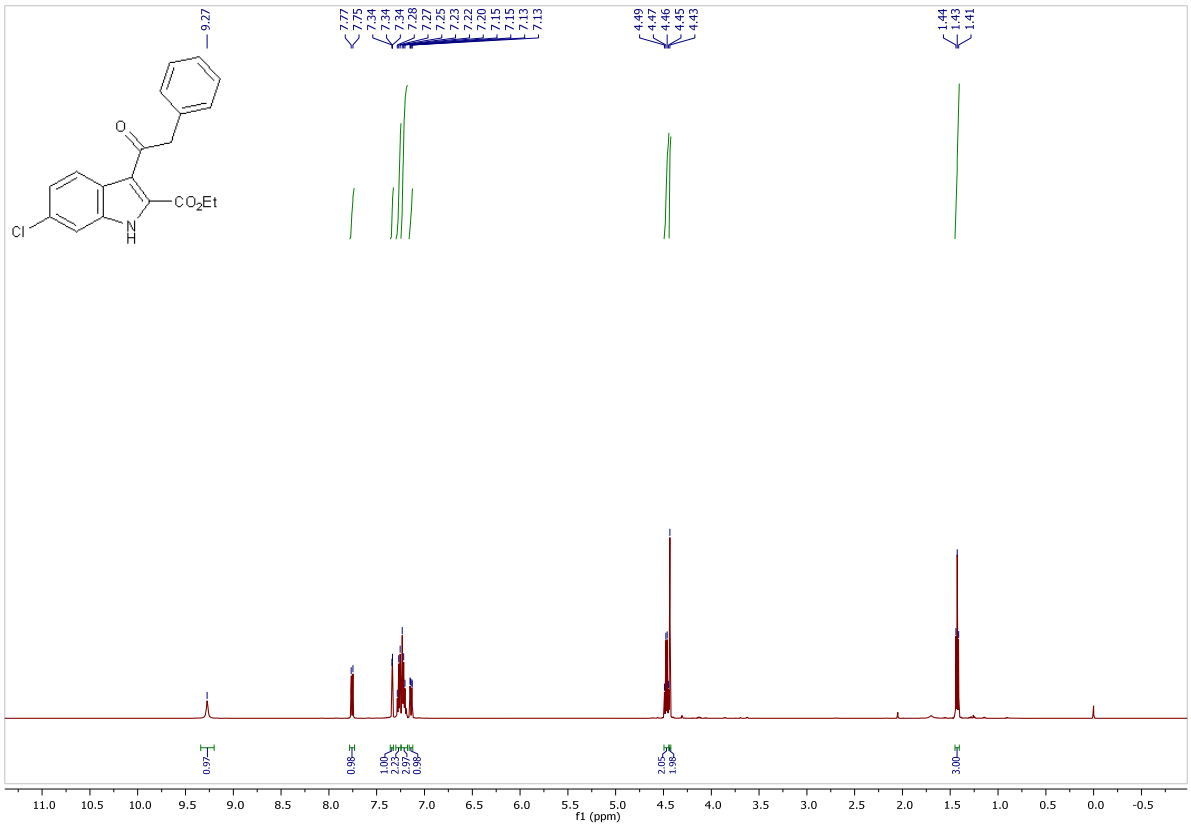


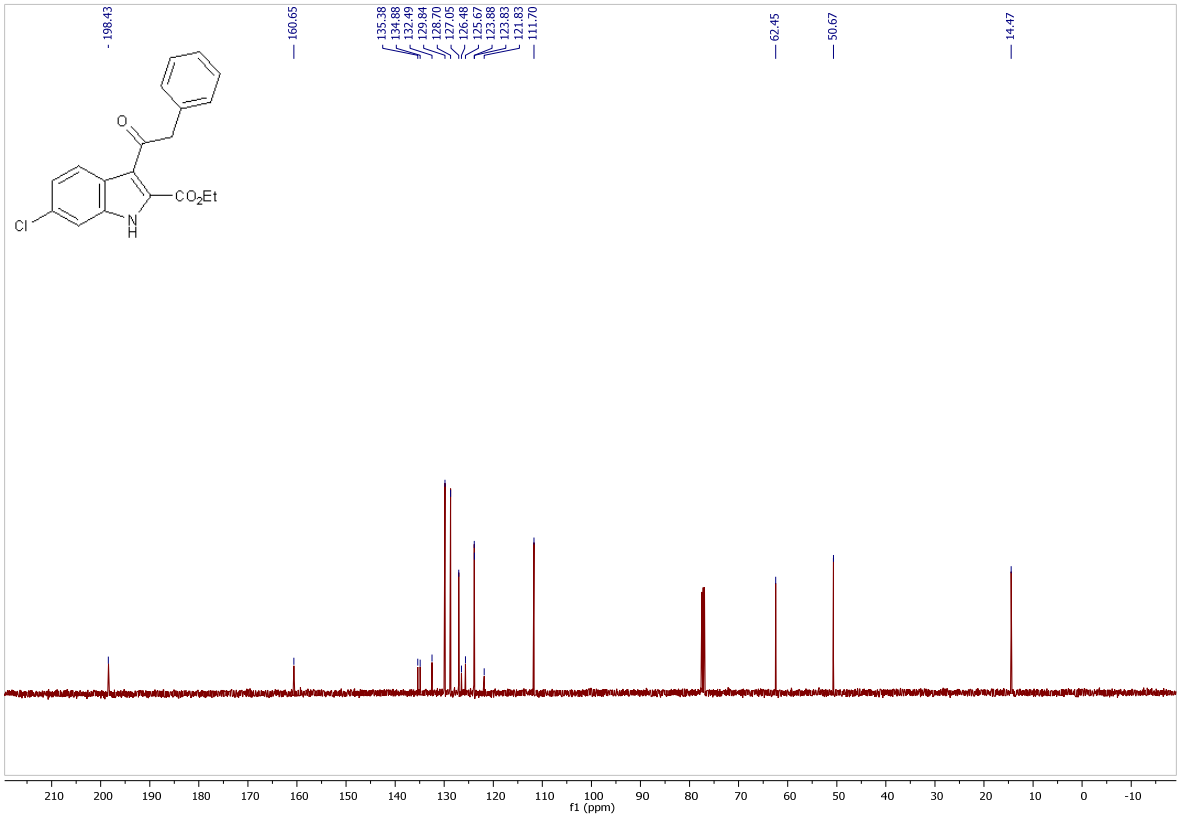
Chromatogram and MS spectra of **14i**, DN441



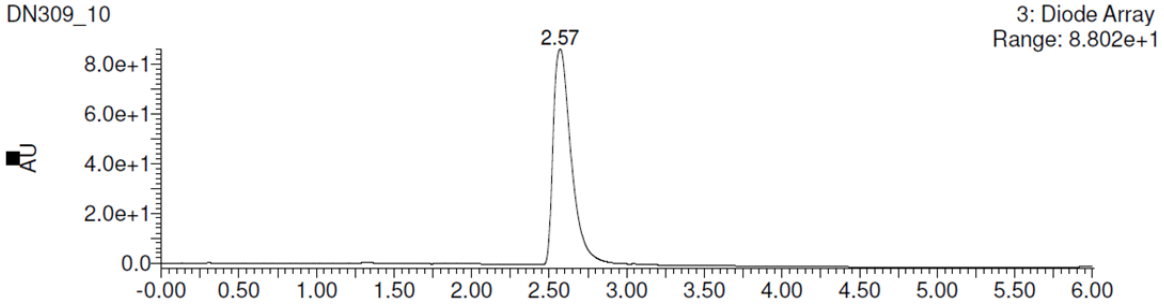


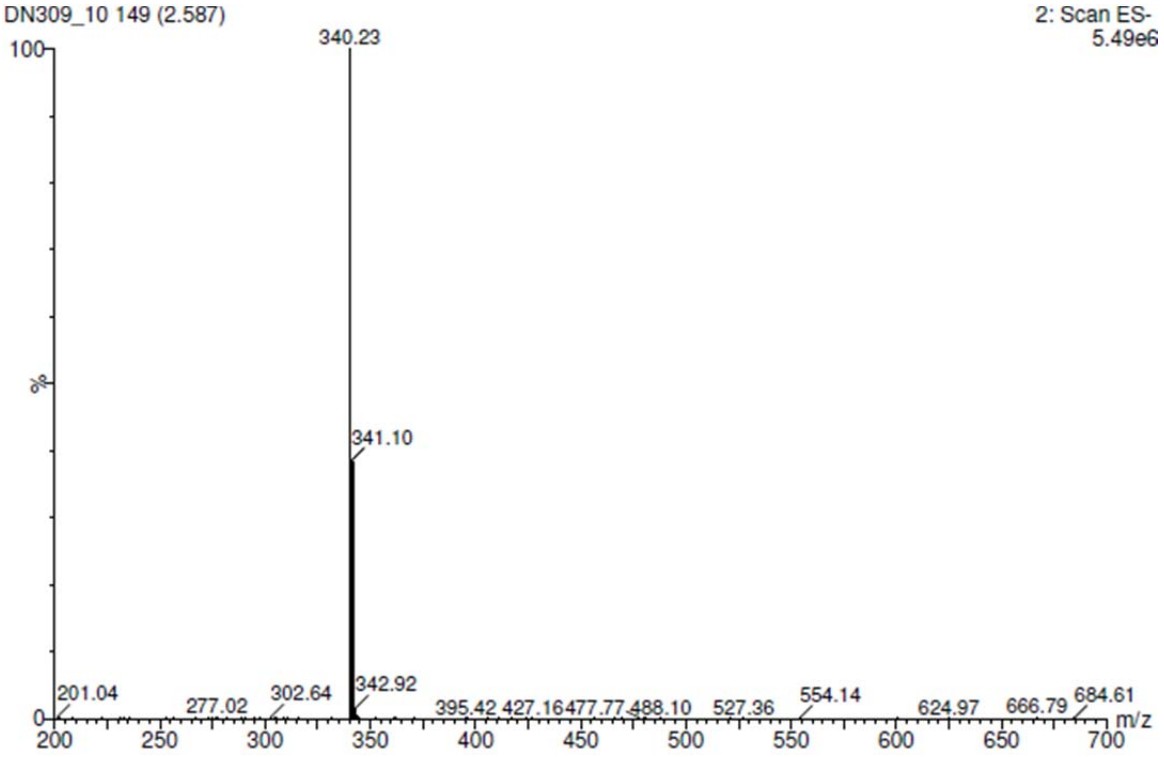
^{1}H NMR and ^{13}C NMR spectra of **14J**, DN309



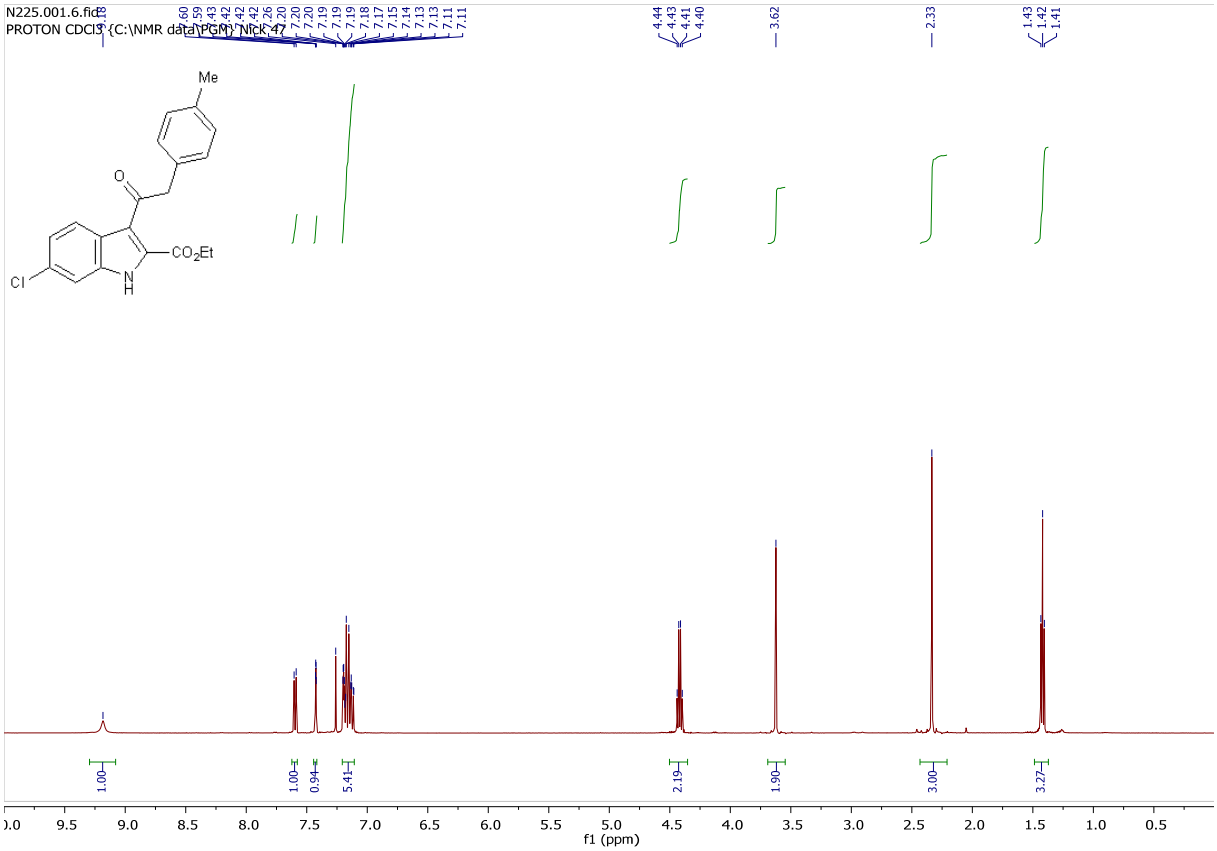


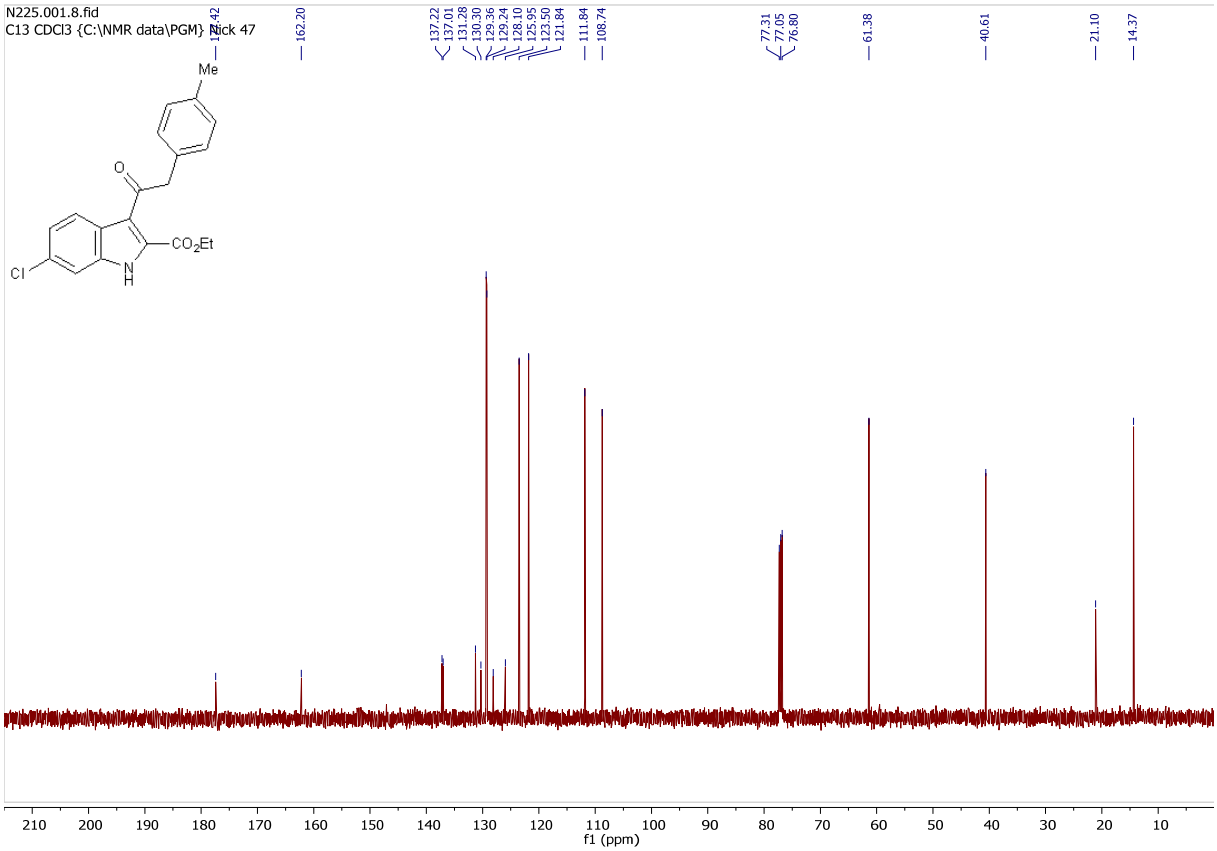
Chromatogram and MS spectra of **14*J***, DN309



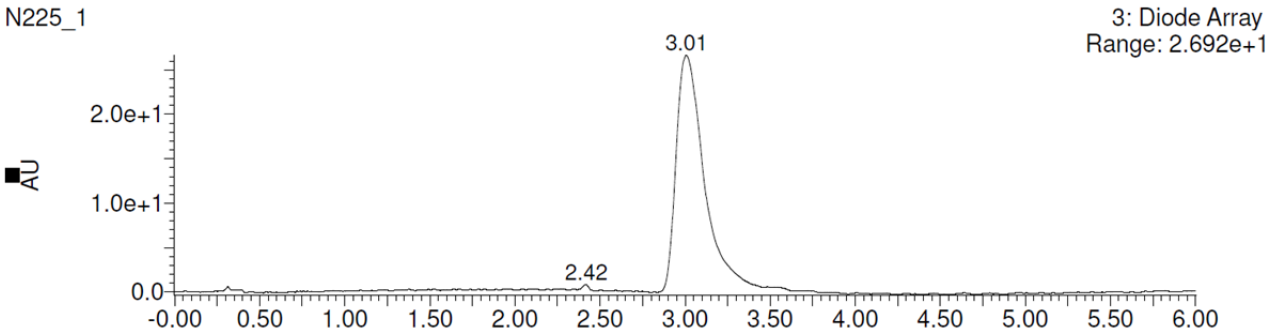


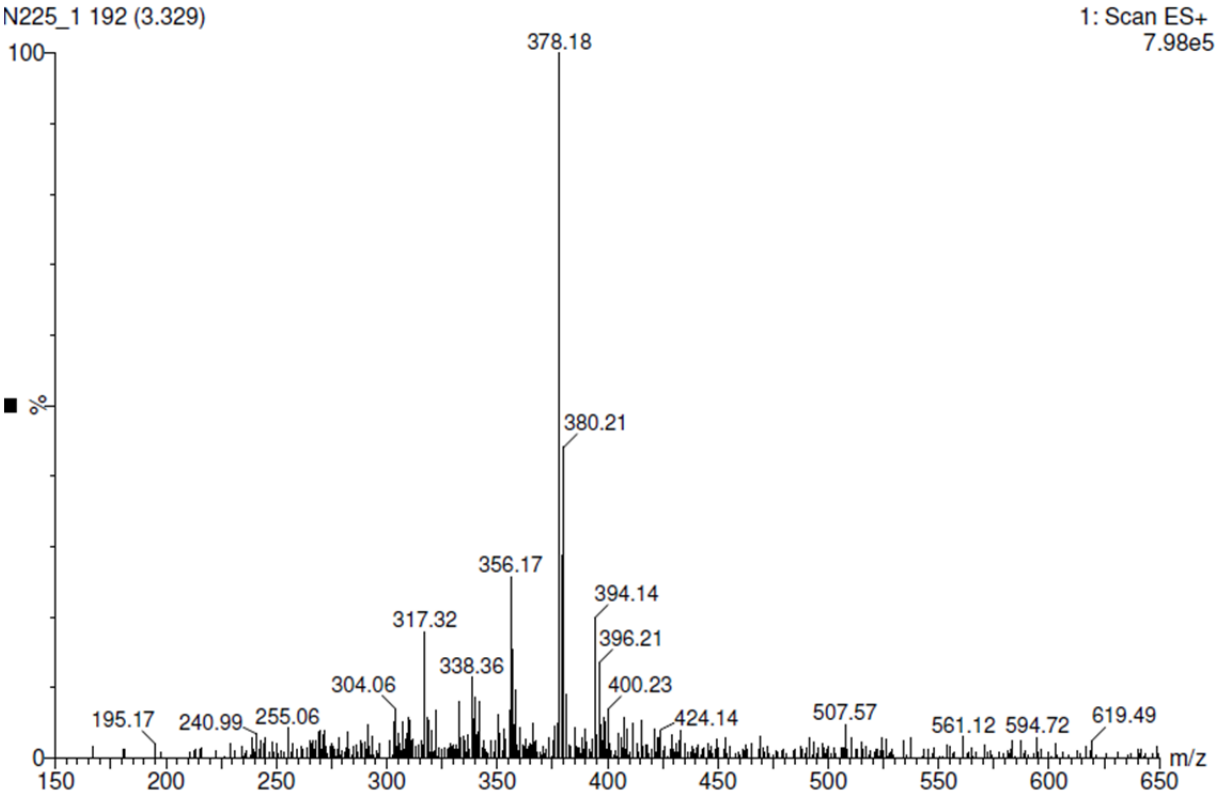
^{1}H NMR and ^{13}C NMR spectra of **14k**, N225



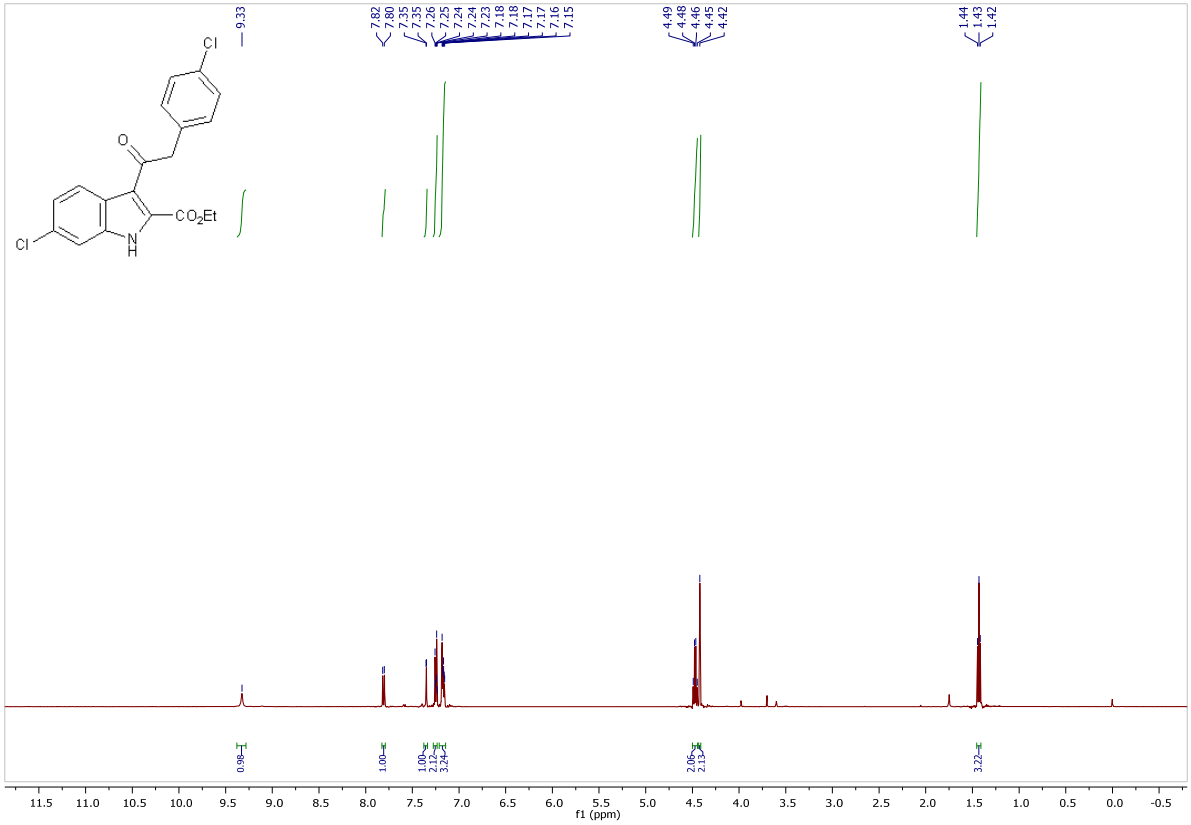


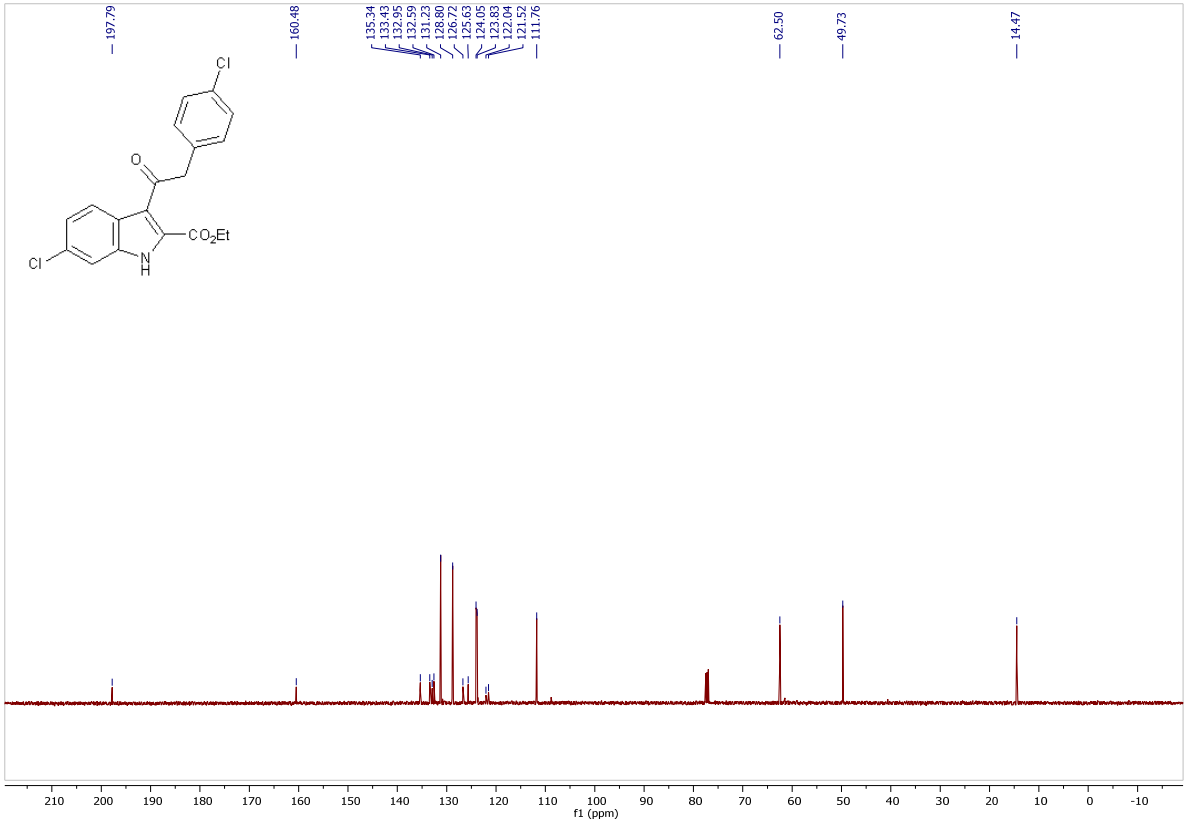
Chromatogram and MS spectra of **14k**, N225



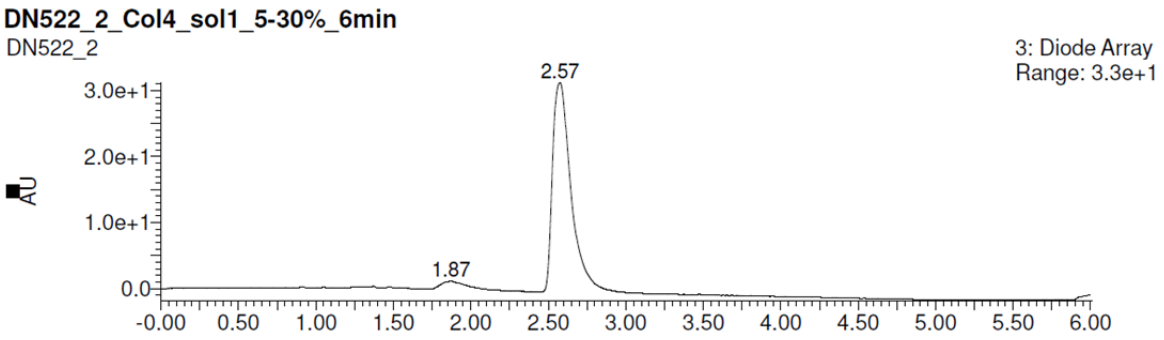


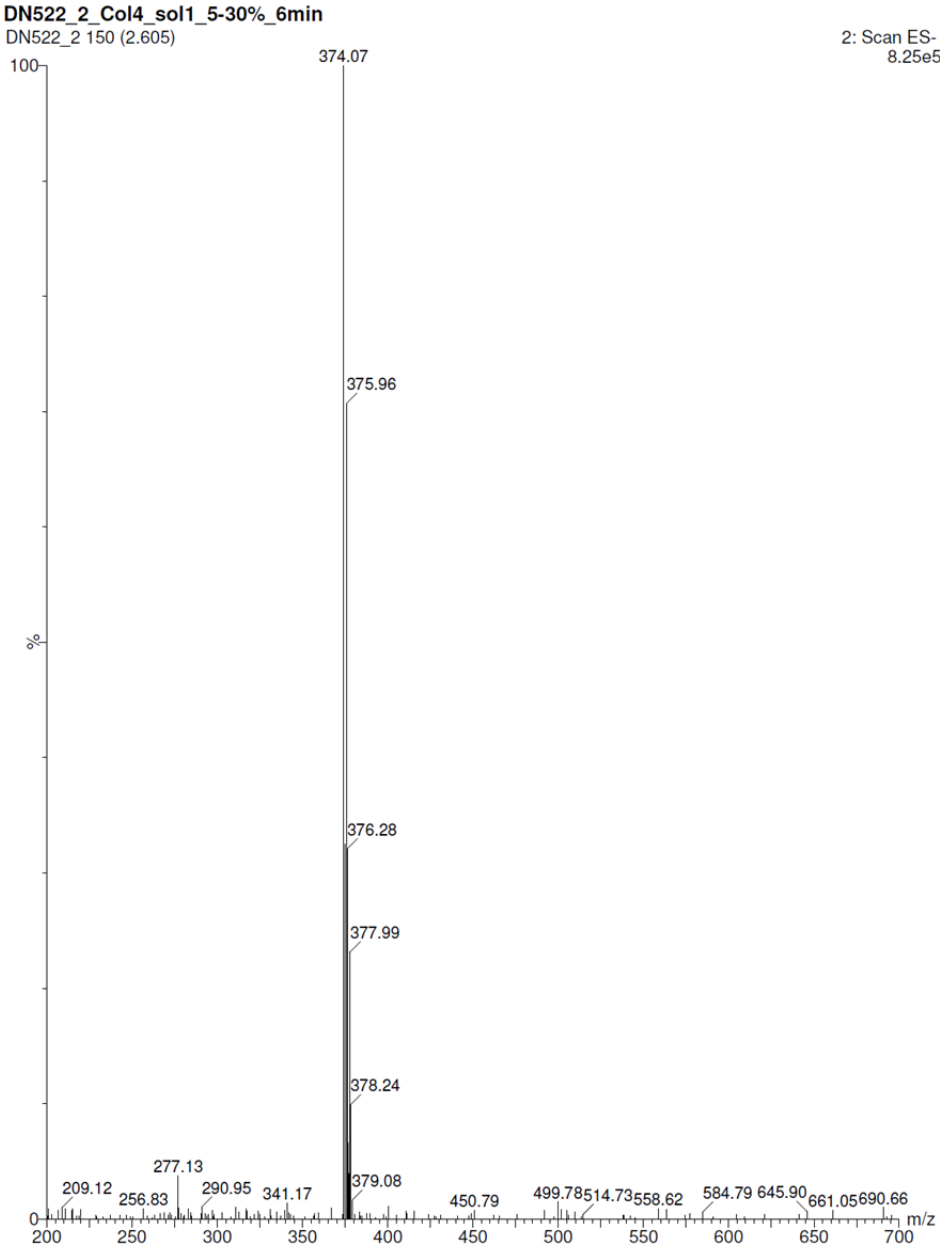
^{1}H NMR and ^{13}C NMR spectra of **14l**, DN522



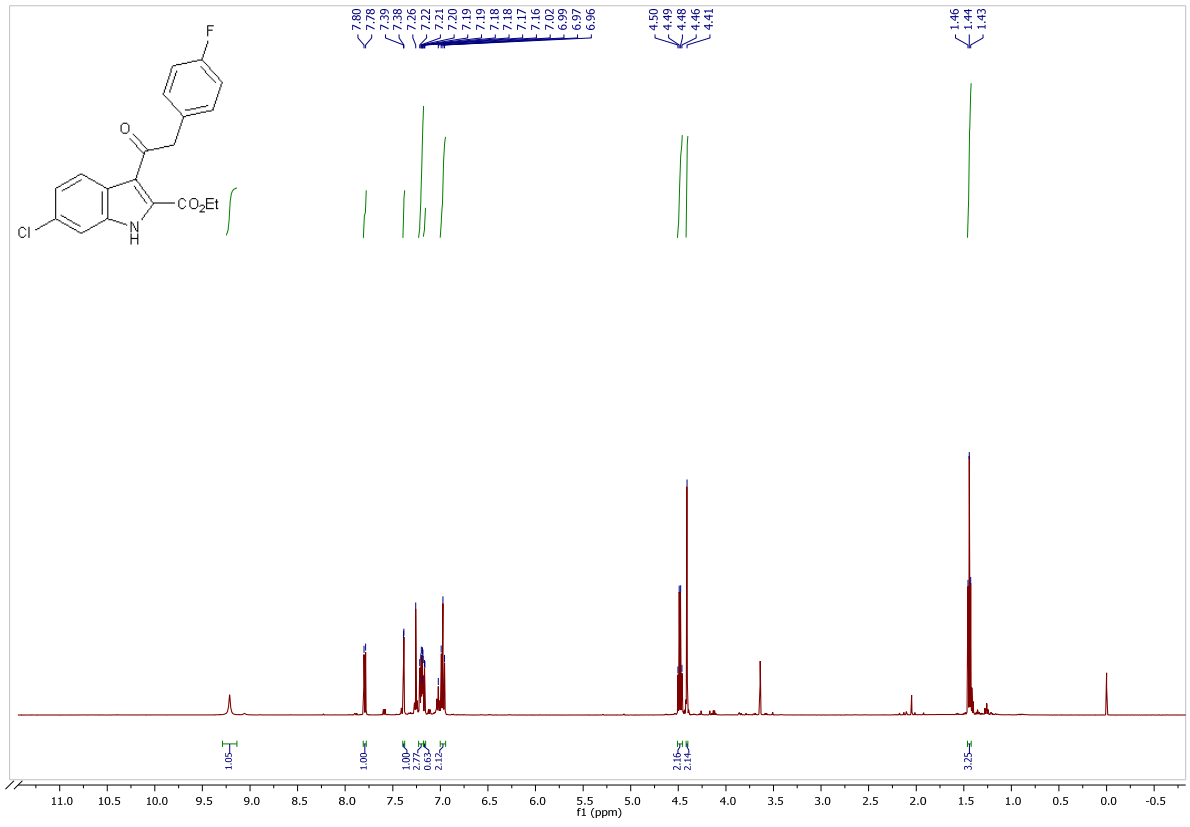


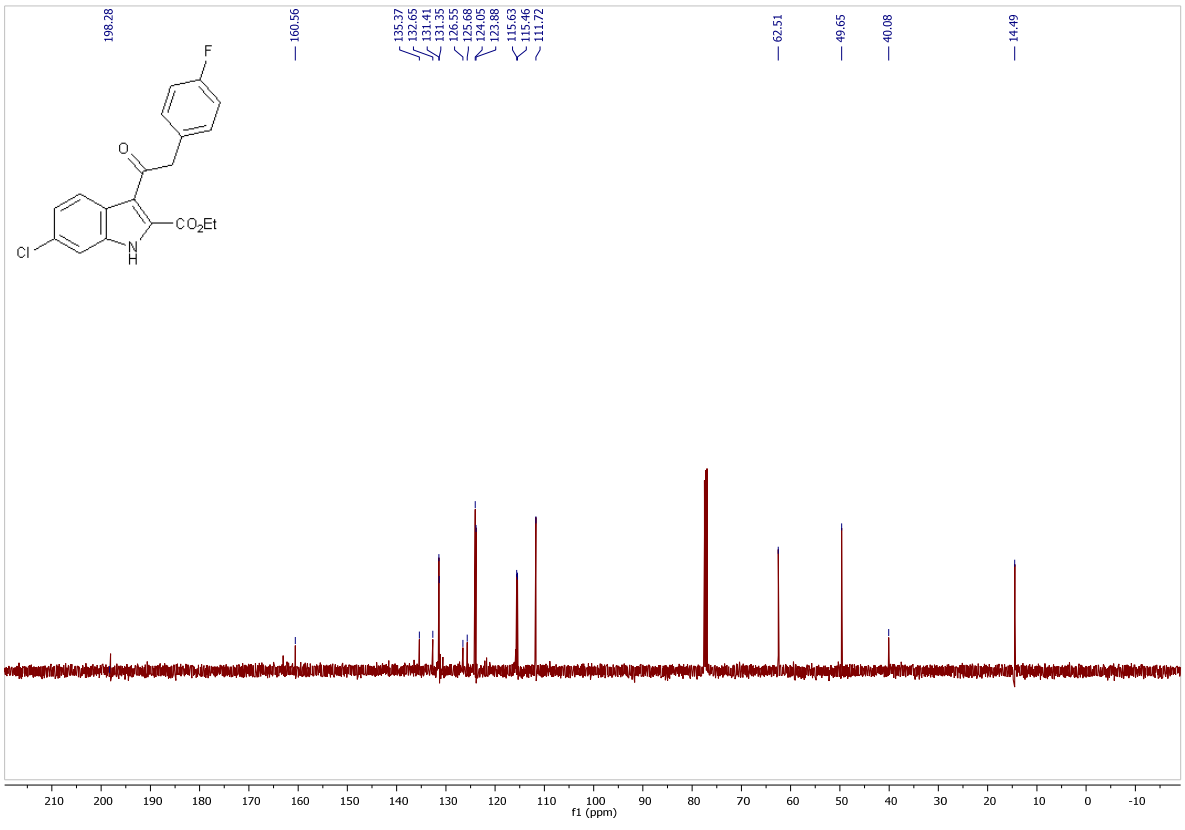
Chromatogram and MS spectra of **14l**, DN522



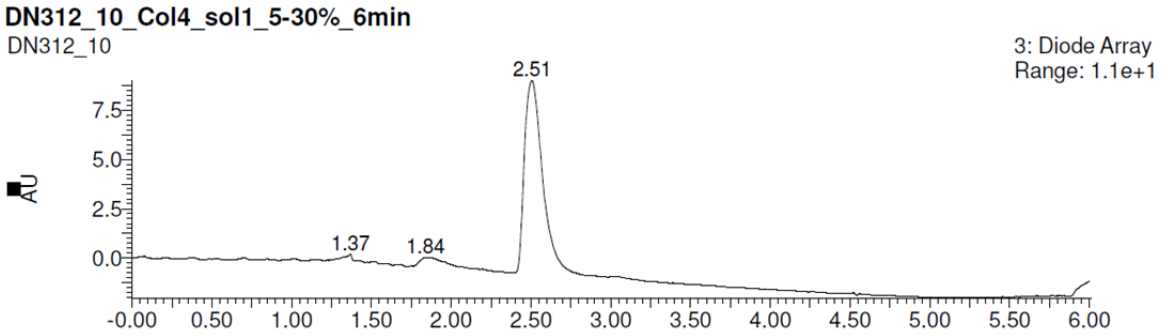


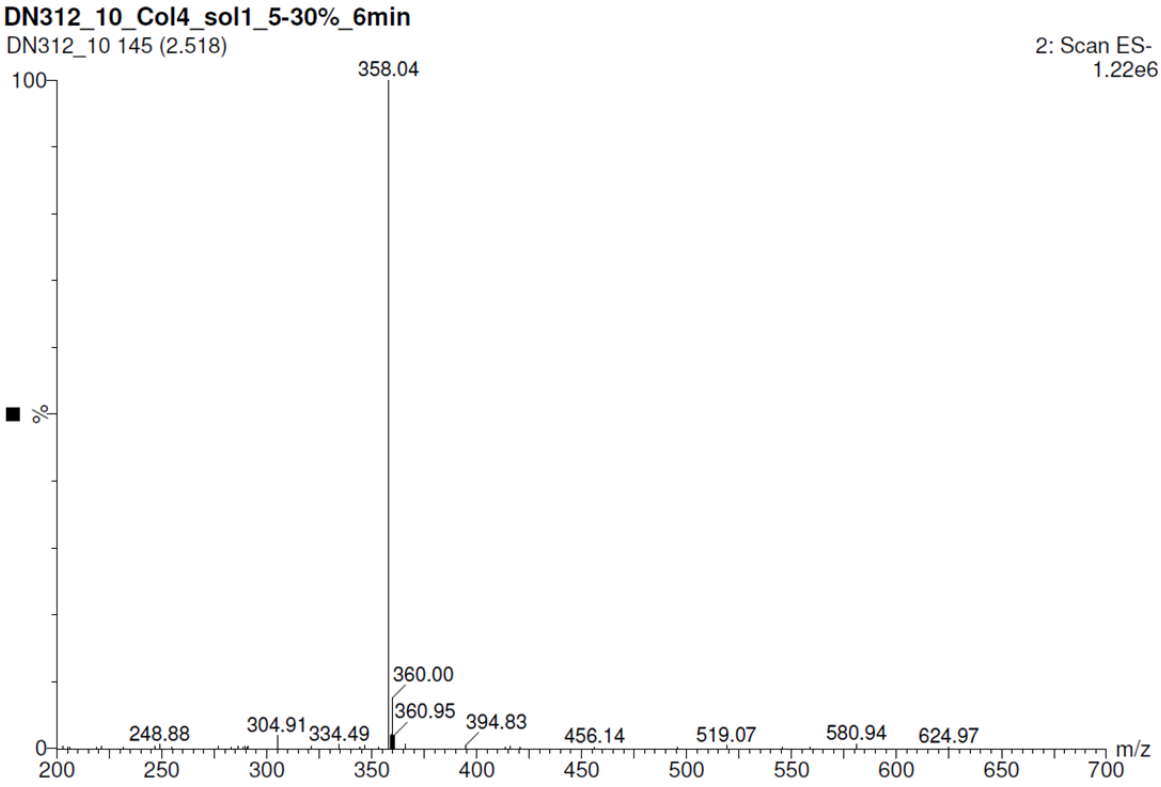
^{1}H NMR and ^{13}C NMR spectra of **14m**, DN312



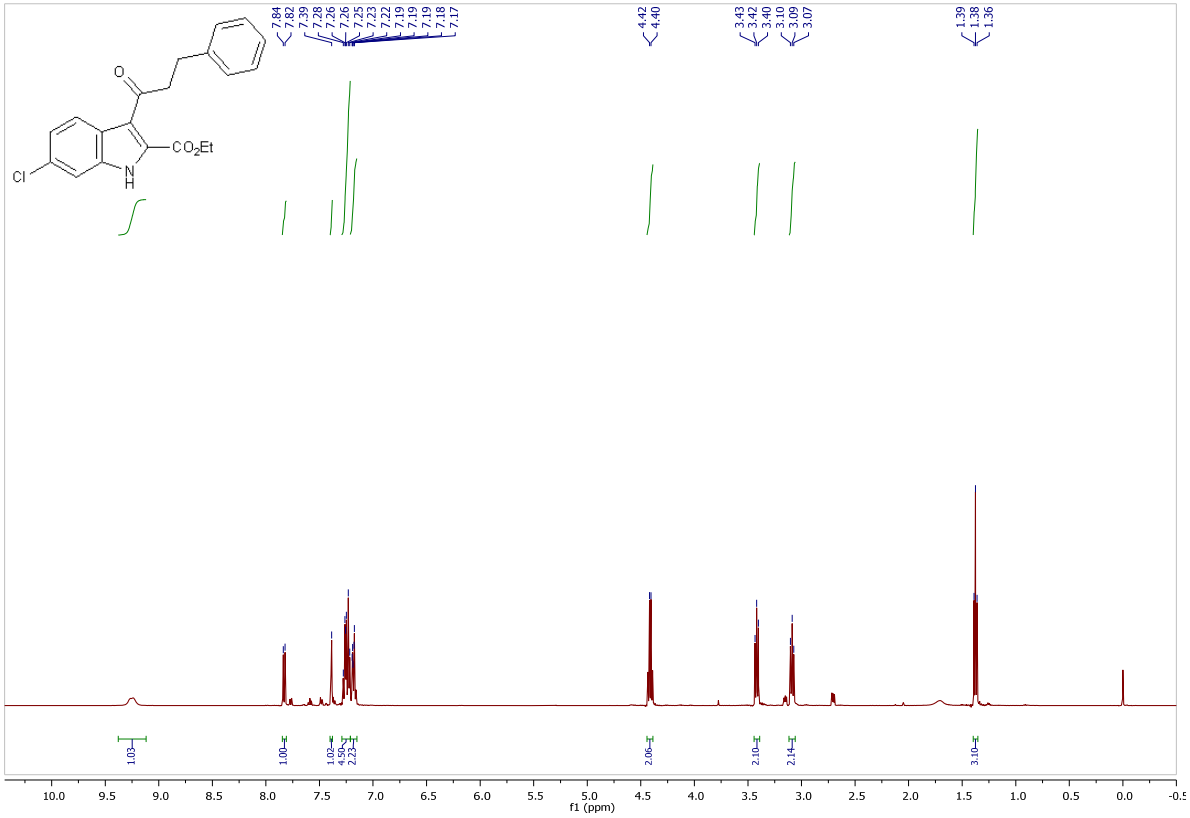


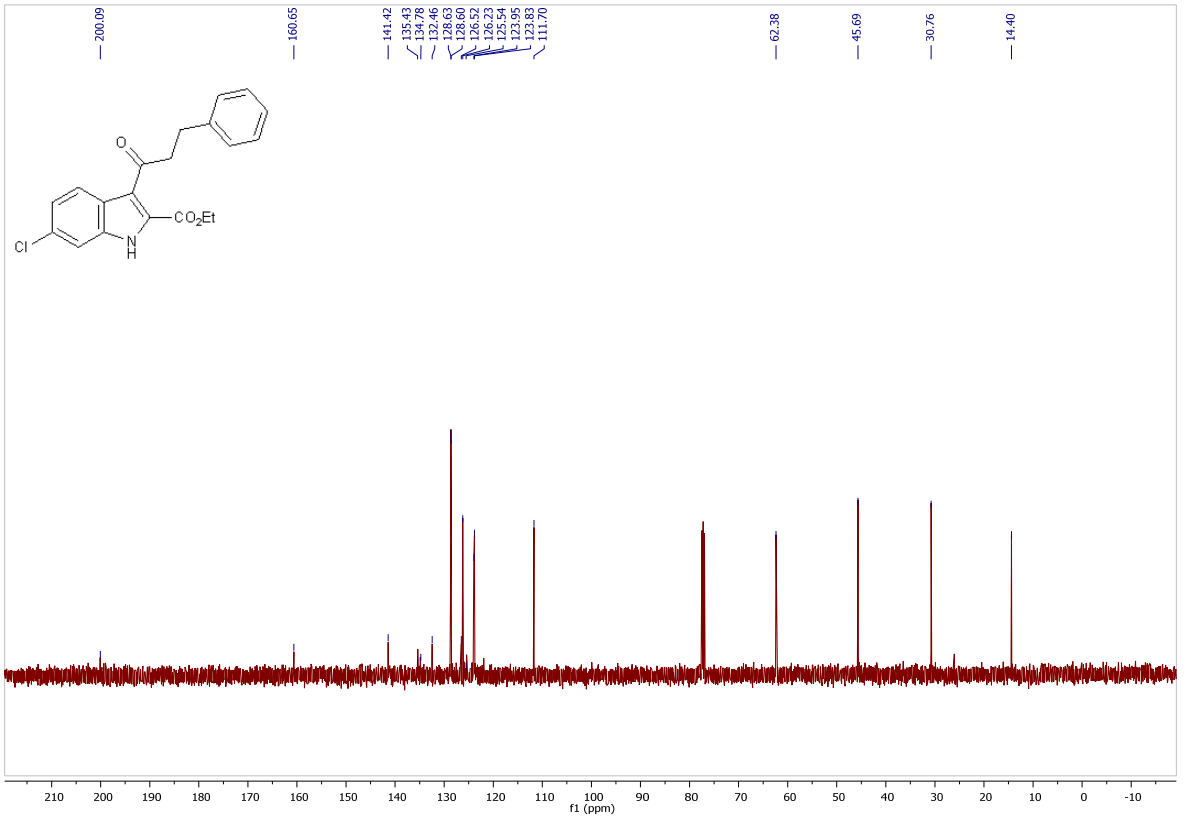
Chromatogram and MS spectra of **14m**, DN312



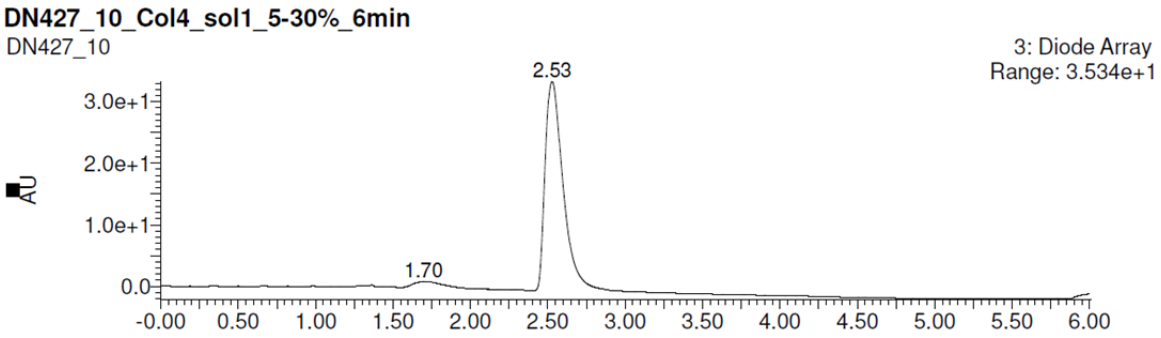


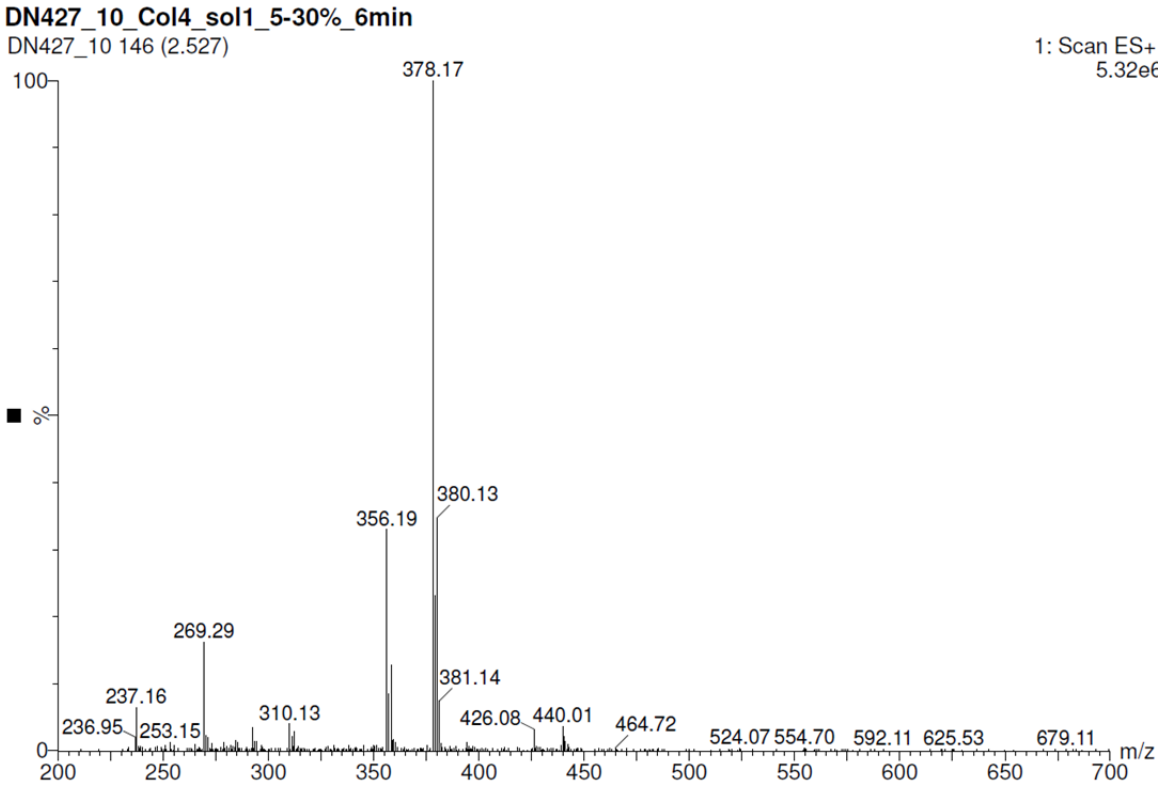
^{1}H NMR and ^{13}C NMR spectra of **14n,** DN427





Chromatogram and MS spectra of **14n,** DN427





Enzyme inhibition studies

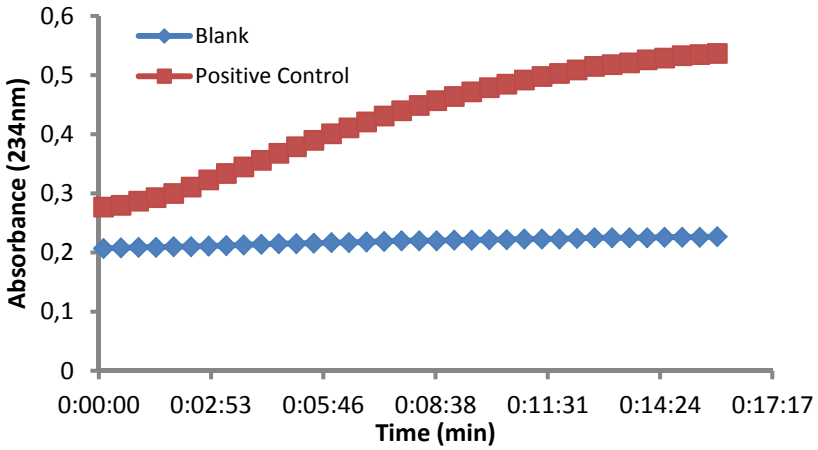


Figure S1: Conversion of linoleic acid in present (Positive Control) or absent of the enzyme (Blank) following with UV-absorbance assay at 234 nm over time.

Focused library

Library of heterocycle nitrogen containing compounds (200). The name of every compound corresponds with the position of the compound in the next table.

Table S1. Screening Compound

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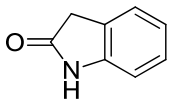
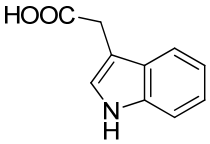
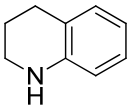
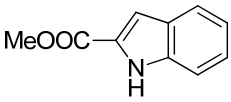
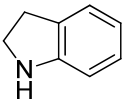
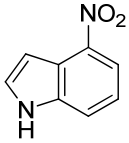
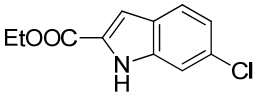
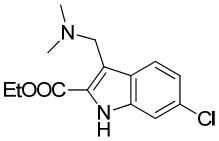
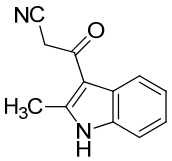
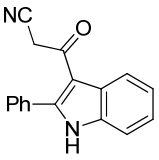
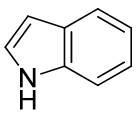
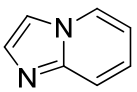
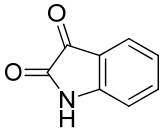
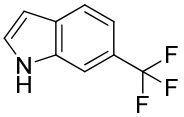
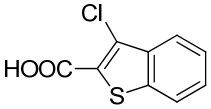
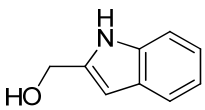
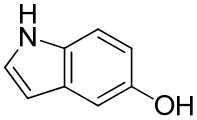
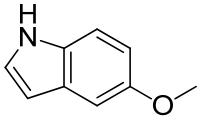
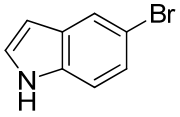
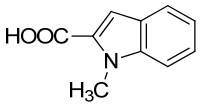
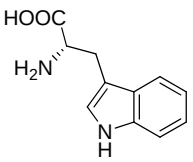
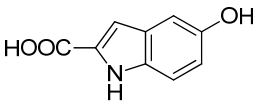
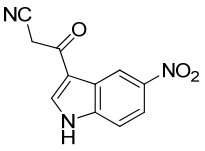
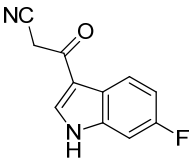
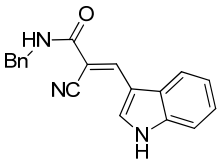
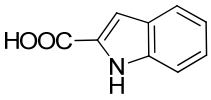
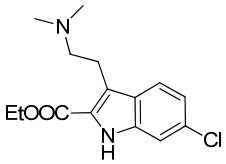
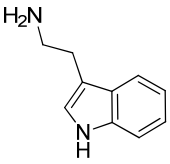
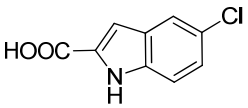
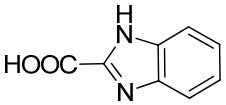
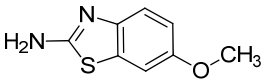
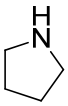
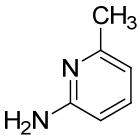
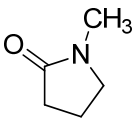
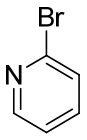
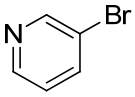
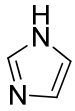
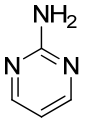
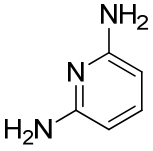
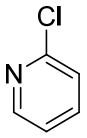
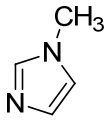
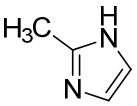
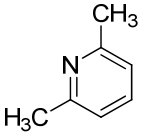
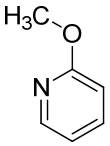
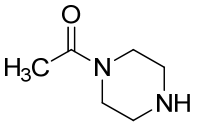
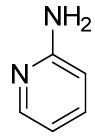
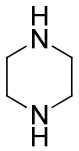
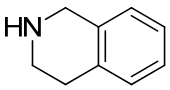
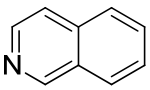
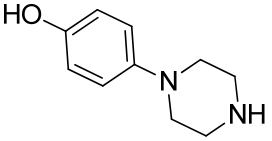
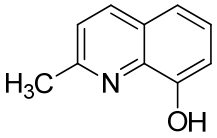
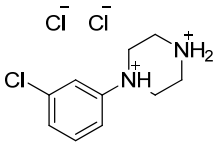
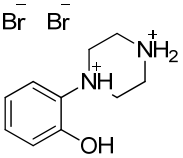
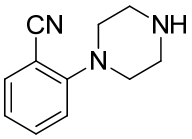
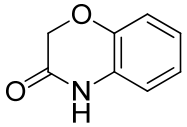
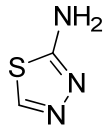
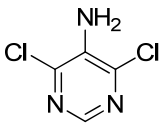
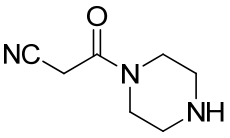
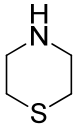
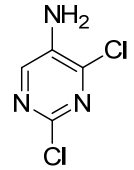
1					
2					
3					
4					

5					
6					
7					
8					
9					
10					
11					
12					
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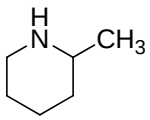
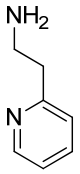
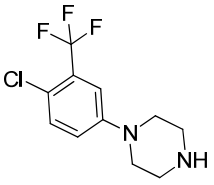
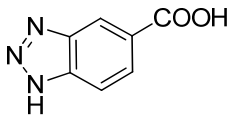
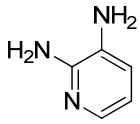
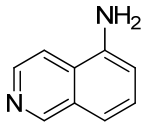
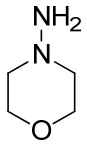
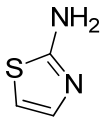
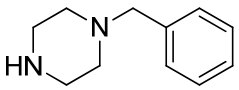
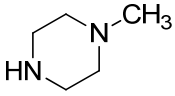
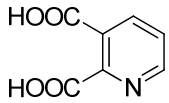
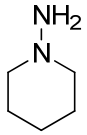
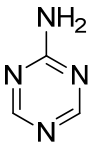
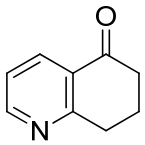
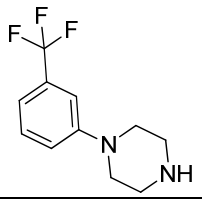
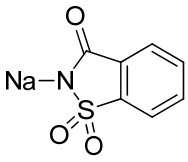
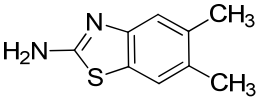
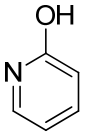
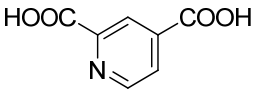
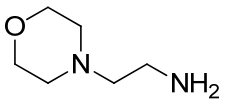
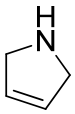
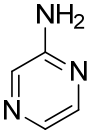
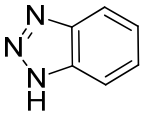
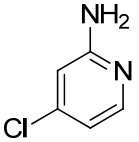
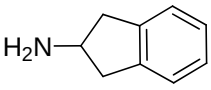
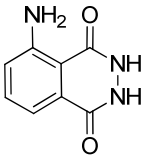
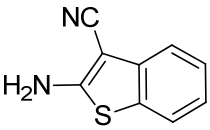
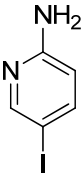
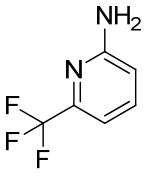
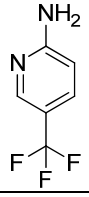
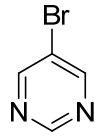
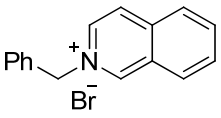
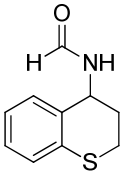
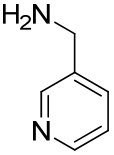
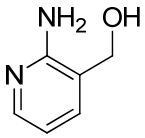
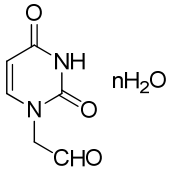
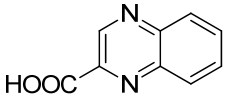
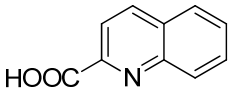
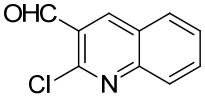
14					
15					
16					
17					
18					
19					
20					
21					
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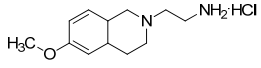
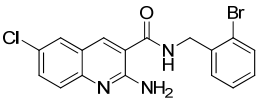
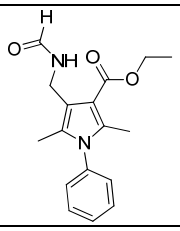
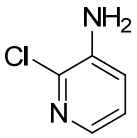
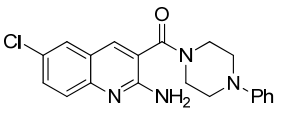
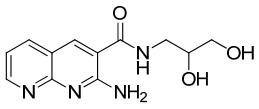
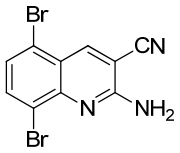
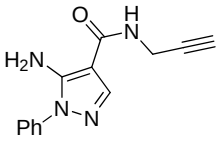
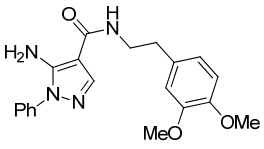
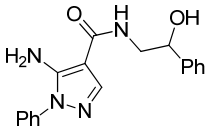
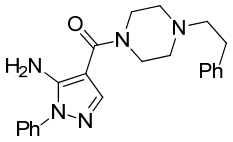
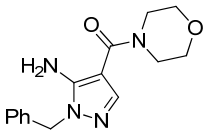
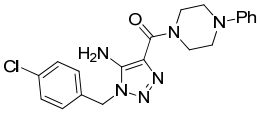
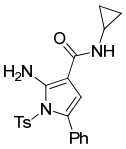
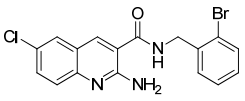
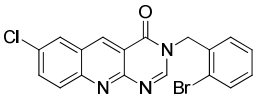
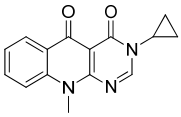
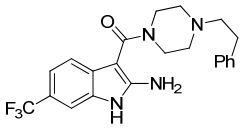
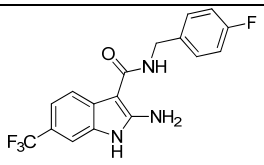
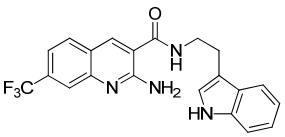
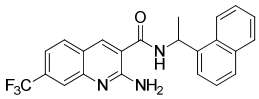
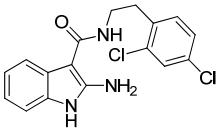
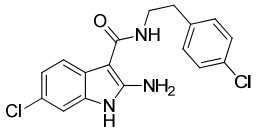
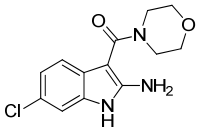
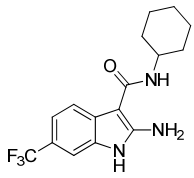
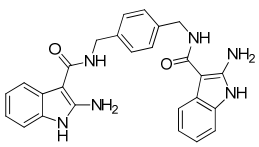
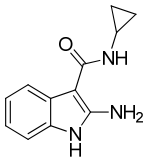
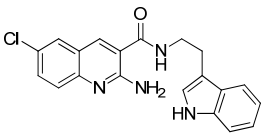
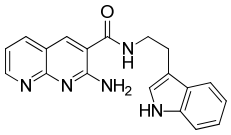
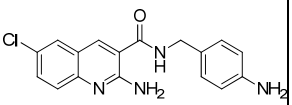
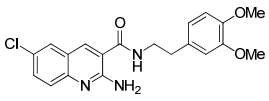
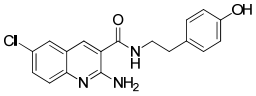
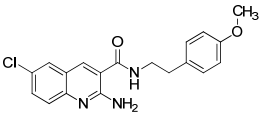
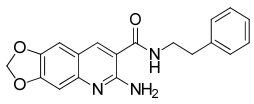
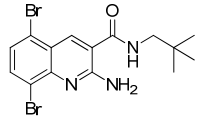
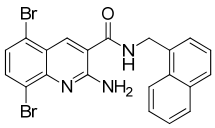
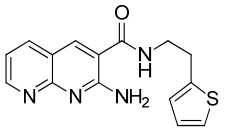
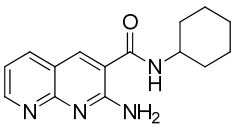
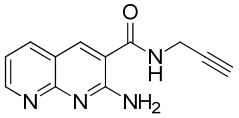
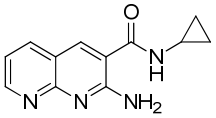
23					
24					
25					
26					
27					
28					
29					
30					
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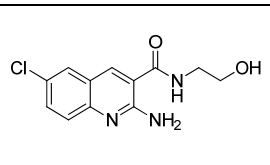
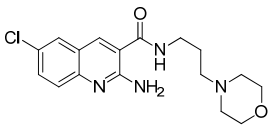
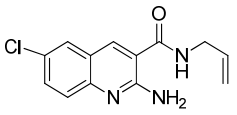
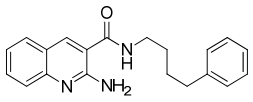
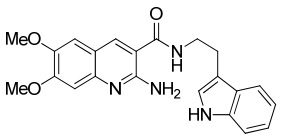
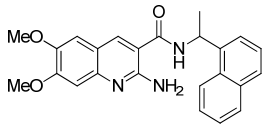
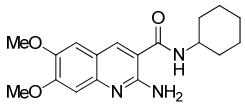
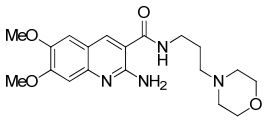
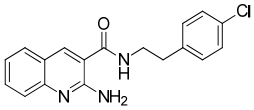
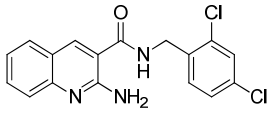
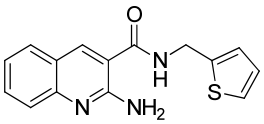
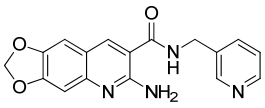
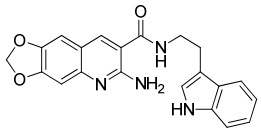
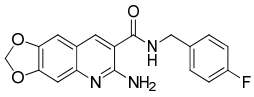
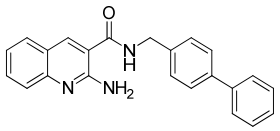
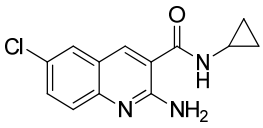
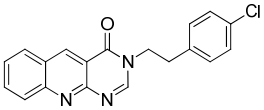
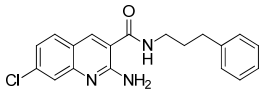
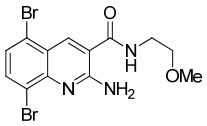
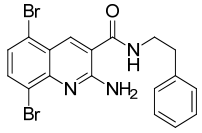
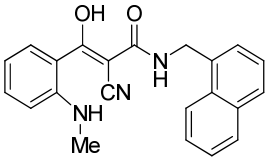
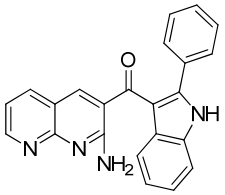
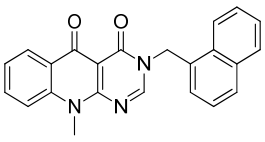
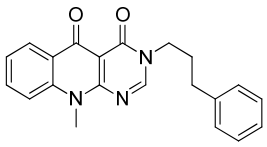
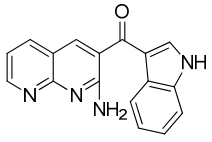
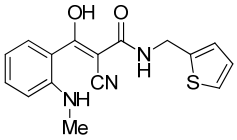
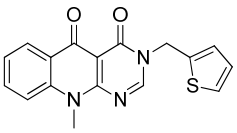
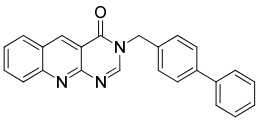
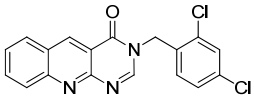
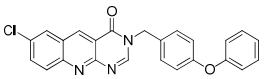
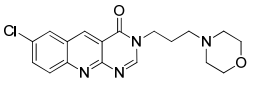
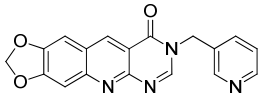
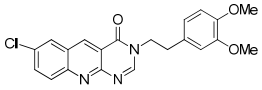
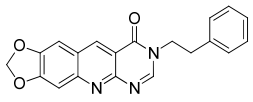
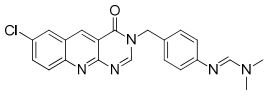
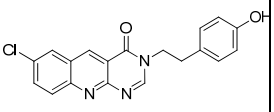
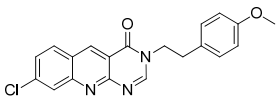
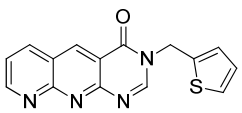
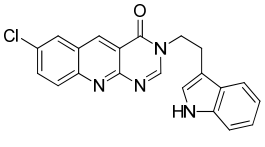
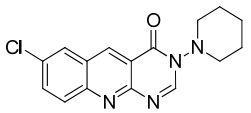
32					
33					
34					
35					
36					
37					
38					
39					
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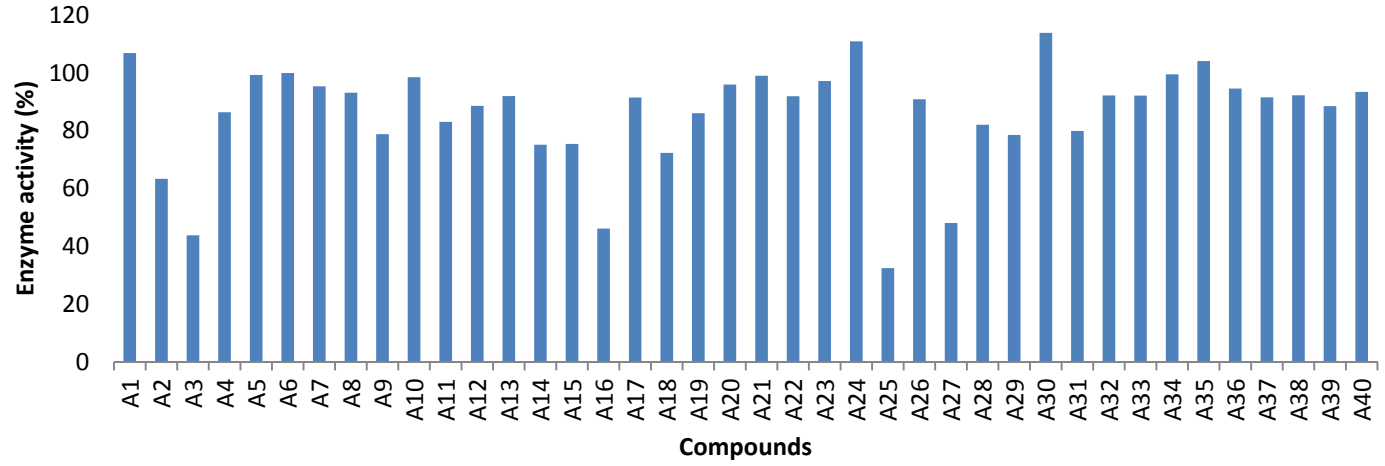
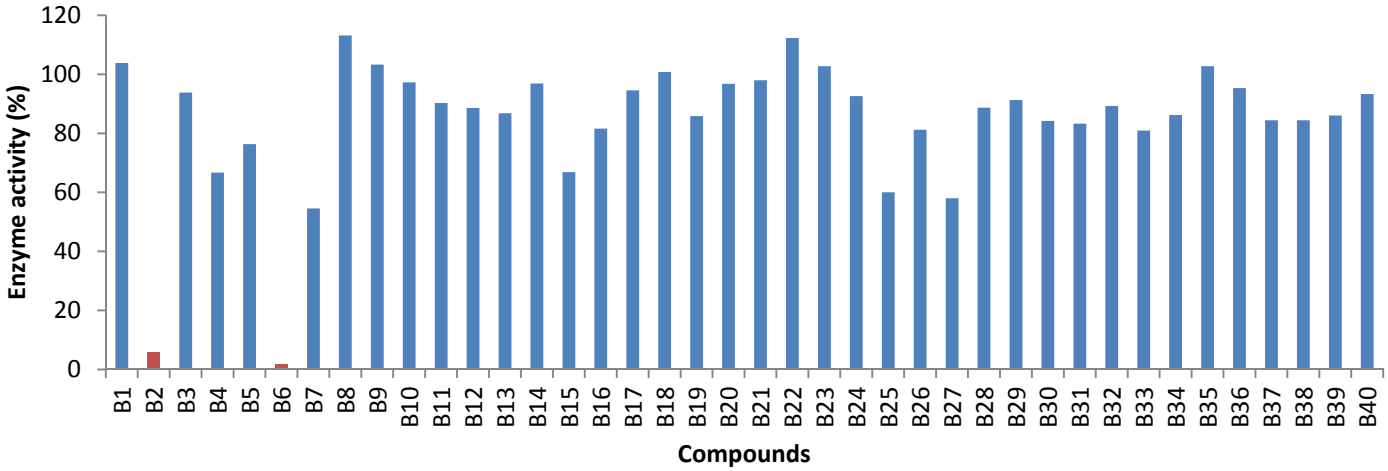
Screening Results

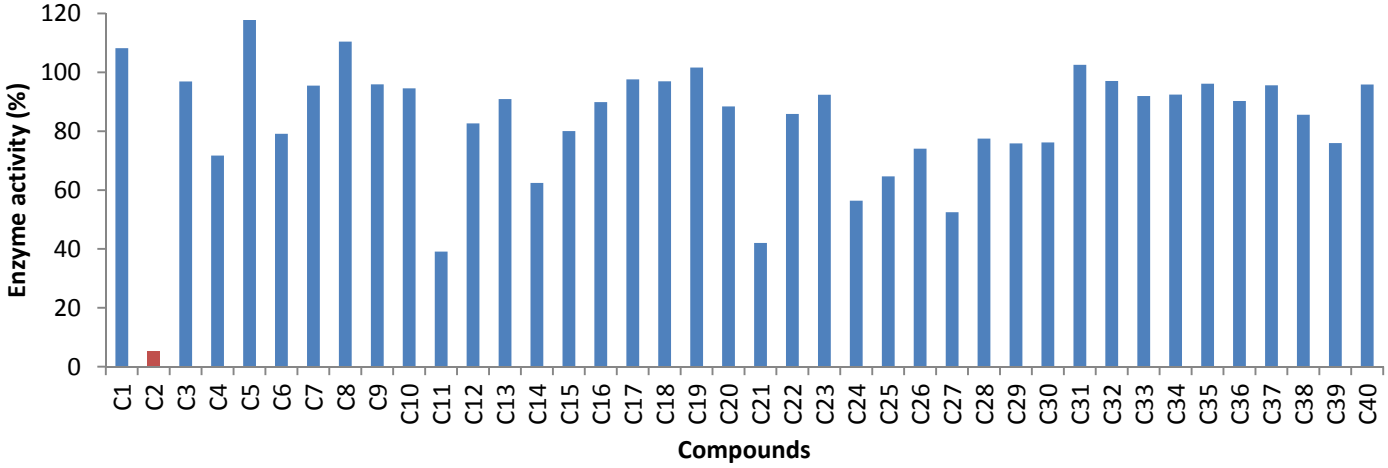
Table S2. Residual enzyme activity (%) that was observed for the screening of a compounds collection for inhibition of h-15-LOX-1 in presence of 50 µM of the respective compounds.

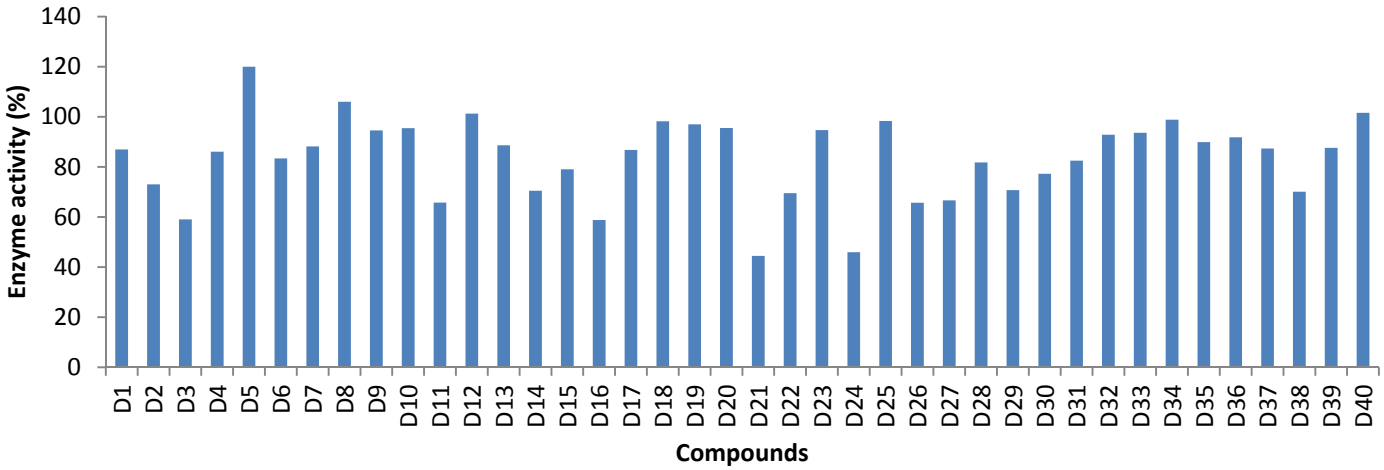
	A	B	C	D	E
1	106,8	103,8	108,2	86,96	102,7
2	63,33	6,060	5,285	73,01	81,14
3	43,78	93,78	96,89	59,06	94,94
4	86,36	66,66	71,71	86,04	75,75
5	99,27	76,36	117,7	120,0	84,33
6	99,95	2,020	79,09	83,38	106,1
7	95,34	54,54	95,45	88,18	94,79
8	93,15	113,1	110,4	106,0	103,0
9	78,75	103,2	95,89	94,54	97,57
10	98,48	97,27	94,54	95,45	27,27
11	83,03	90,30	39,09	65,75	80,60
12	88,60	88,60	82,64	101,2	76,68
13	91,96	86,78	90,93	88,60	92,48
14	75,12	96,89	62,43	70,46	98,78
15	75,38	66,83	80,05	79,01	82,90
16	46,11	81,60	89,89	58,80	64,24
17	91,45	94,55	97,57	86,78	75,64
18	72,27	100,8	96,96	98,18	97,97
19	86,06	85,85	101,6	96,96	98,58
20	95,95	96,76	88,41	95,54	96,96
21	98,98	97,97	42,02	44,44	106,1
22	91,91	112,2	85,85	69,49	116,0
23	97,16	102,7	92,35	94,70	101,0
24	110,9	92,61	56,37	45,95	62,02
25	32,44	60,04	64,62	98,35	71,02
26	90,84	81,25	74,06	65,65	38,45
27	48,04	57,99	52,46	66,64	61,47
28	82,04	88,71	77,46	81,76	80,93
29	78,52	91,32	75,84	70,75	90,33
30	113,8	84,21	76,19	77,26	79,91
31	79,87	83,26	102,5	82,47	89,19
32	92,19	89,23	97,04	92,86	87,92
33	92,11	80,95	91,95	93,58	95,46
34	99,50	86,19	92,44	98,85	89,16
35	104,1	102,7	96,14	89,88	94,03
36	94,56	95,31	90,26	91,80	89,92
37	91,54	84,41	95,61	87,32	91,95
38	92,22	84,42	85,58	70,09	83,96
39	88,49	86,07	75,97	87,62	85,70
40	93,39	93,31	95,83	101,5	92,16

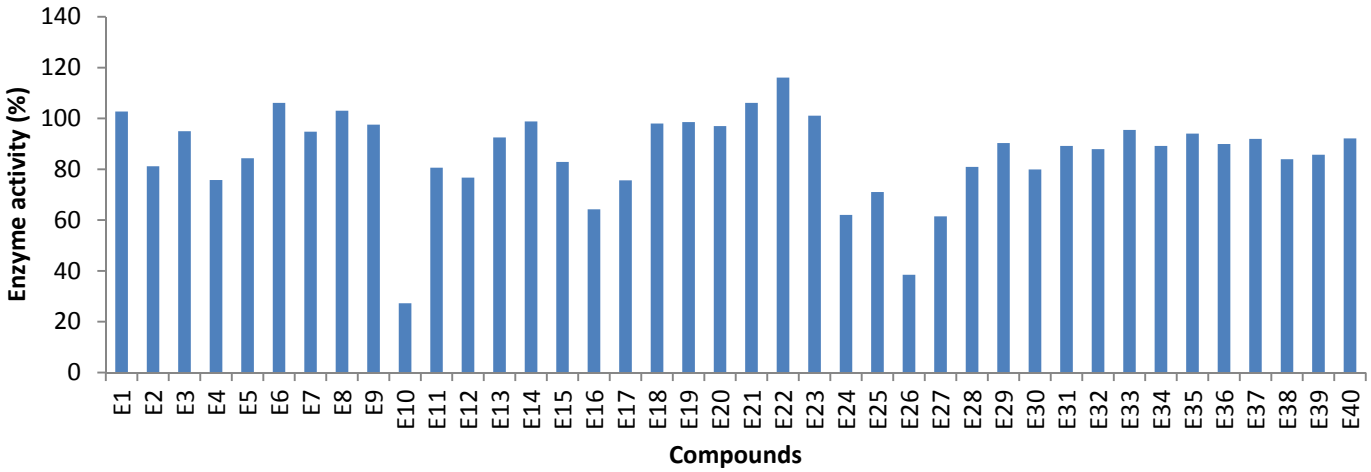
Screening Results (column graphs)





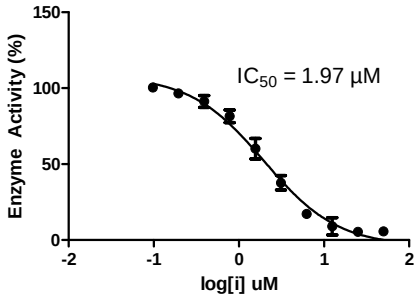



Enzyme activity (%)
140
120
100
80
60
40
20
0
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14 D15 D16 D17 D18 D19 D20 D21 D22 D23 D24 D25 D26 D27 D28 D29 D30 D31 D32 D33 D34 D35 D36 D37 D38 D39 D40
Compounds


Enzyme activity (%)
140
120
100
80
60
40
20
0
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 E13 E14 E15 E16 E17 E18 E19 E20 E21 E22 E23 E24 E25 E26 E27 E28 E29 E30 E31 E32 E33 E34 E35 E36 E37 E38 E39 E40
Compounds

IC_{50} graphs

Compound 1



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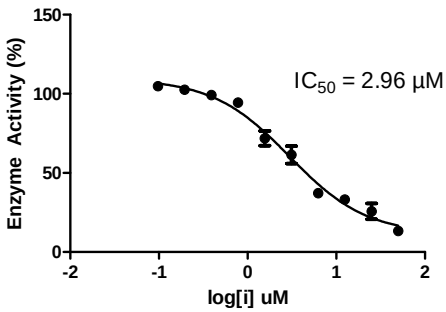
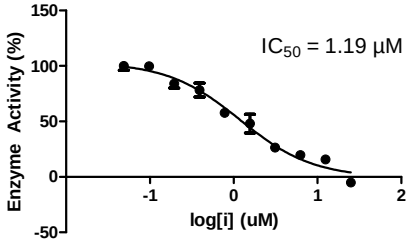
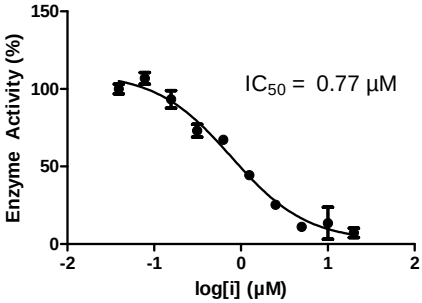


Compound 14l



Compound 14m



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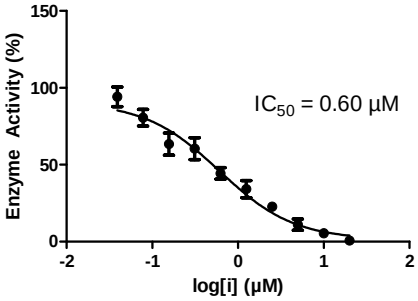
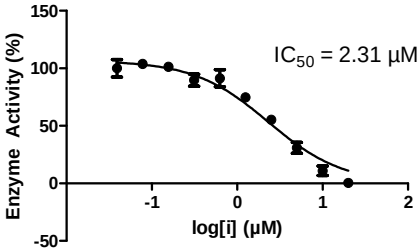
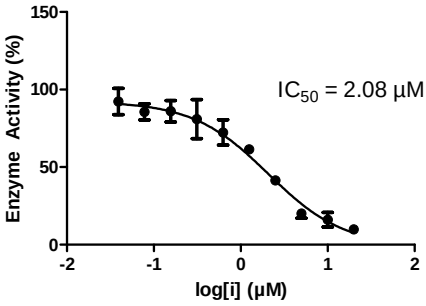


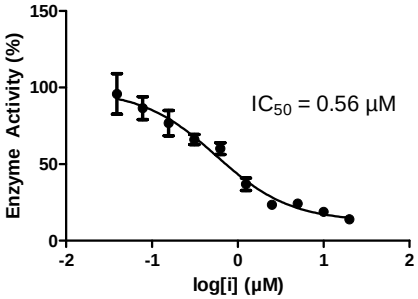
Compound 14a



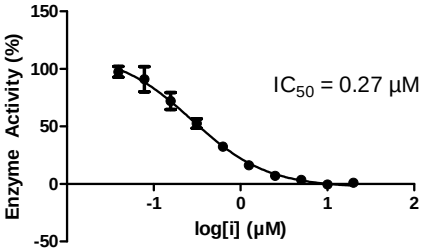
Compound 14k



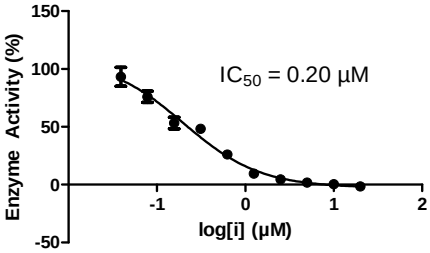
Compound 14n



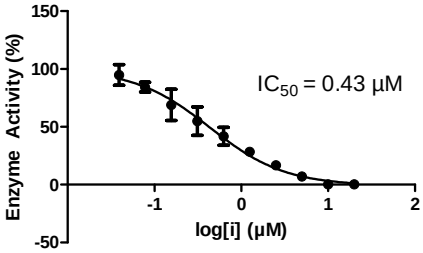
Compound 14h



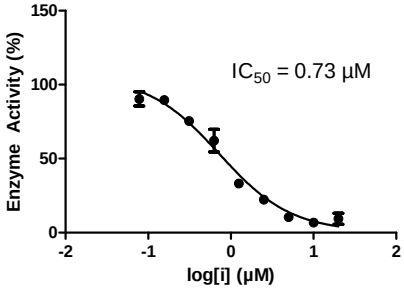
Compound 14g



Compound 14f



Compound 14c



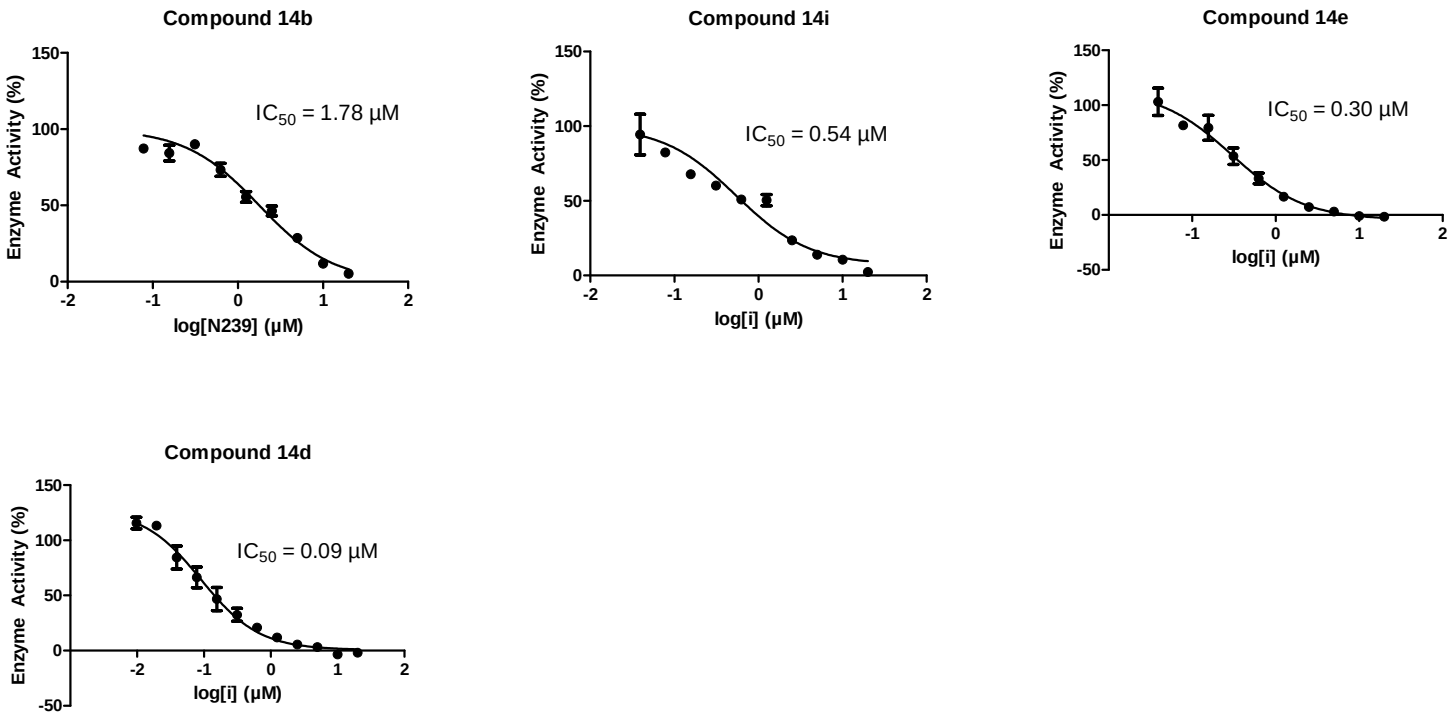


Figure S2. IC_{50} graphs

Enzyme Kinetics

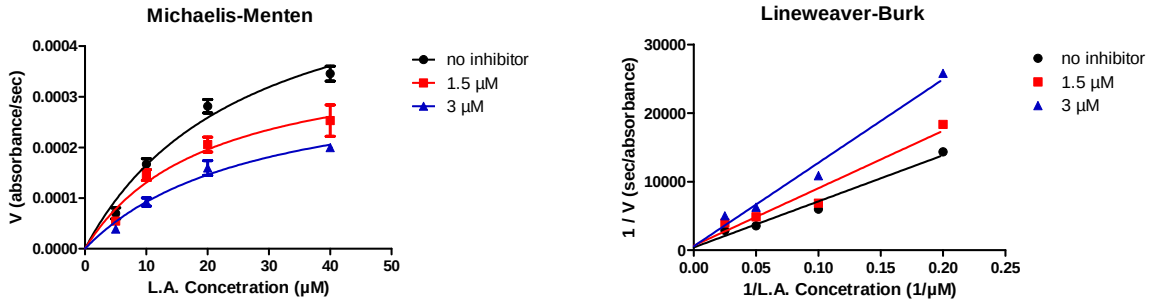


Figure S3. Steady-State kinetic characterization of 15-LOX-1 in the presence of different concentrations of compound 14l: A) Michaelis-Menten representation and B) Lineweaver-Burk representation.

Table S3. Enzyme kinetic parameters for inhibition of 15-LOX-1 by compound 14l.

14l (µM)	K_m^{app} (µM)	V_{max}^{app} (absorbance/s)
0	16.9 ± 9.8	$4.6 \times 10^{-4} \pm 1.1 \times 10^{-4}$
1.5	20.0 ± 15	$3.9 \times 10^{-4} \pm 1.4 \times 10^{-4}$
3	27.5 ± 17	$3.4 \times 10^{-4} \pm 1.1 \times 10^{-4}$

Molecular Modeling

Since competitive inhibition was observed, the inhibitors were docked in the active site of the enzyme. The molecular modeling studies were performed in MOE software (2012.10) and highest scoring docking poses were chosen. The experiments were performed with rescoring model 1 London dG (refinement: forcefield) and rescoring 2: GBVI/WSA dG, followed by minimization energy (forcefield: MMFF94X; eps = r, cutoff {8,10})

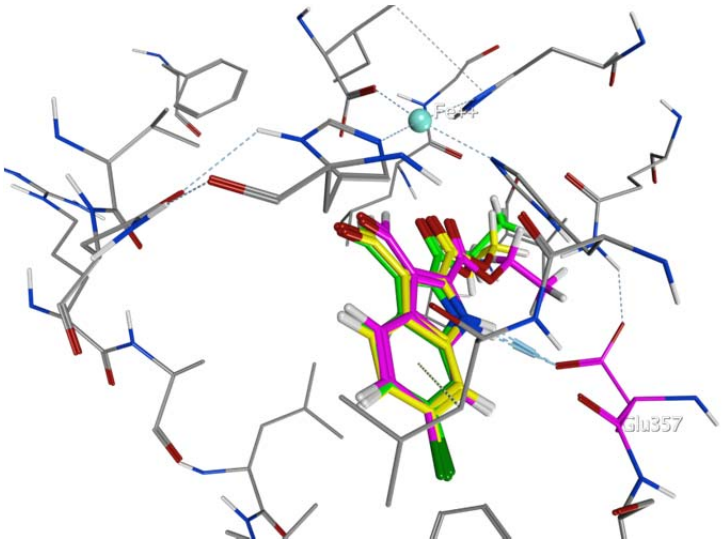


Figure S4. Three of the top five highest scoring poses of the compound 2. The docking score of the pink structure pose is -5.51, from the yellow structure pose is -5.47 and from the green structure pose -5.36.

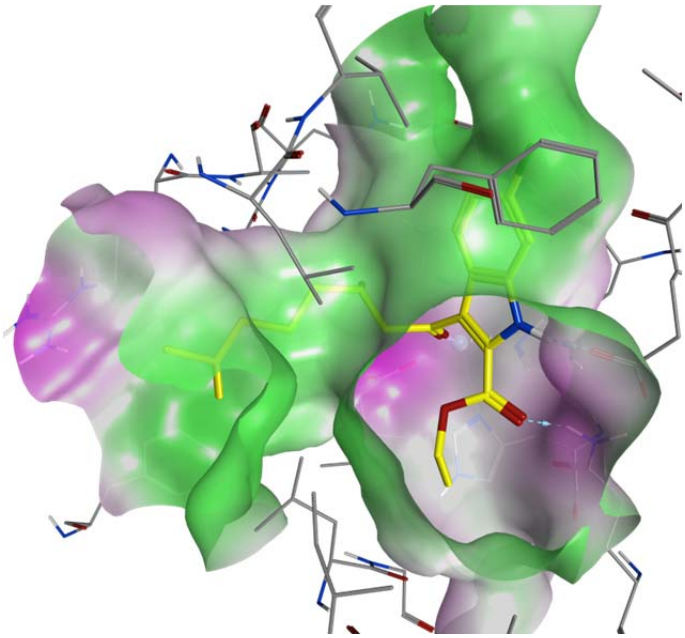


Figure S5. The surface of the active site with the compound 14d in the highest docking score pose. With green is the lipophilic and with red the lipophobic area in the active site of 15-LOX.