Supporting Information

Discovery of a highly potent, selective, and metabolically stable inhibitor of receptor-interacting protein 1 (RIP1) for the treatment of systemic inflammatory response syndrome

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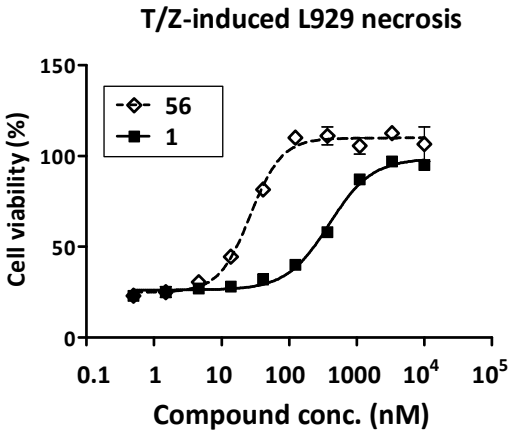


Figure s1. Compound **56** shows efficient protection to TZ-induced murine L929 cells necrosis (Compd **56** EC_{50} = 27 nM, **1** EC_{50} = 391 nM).

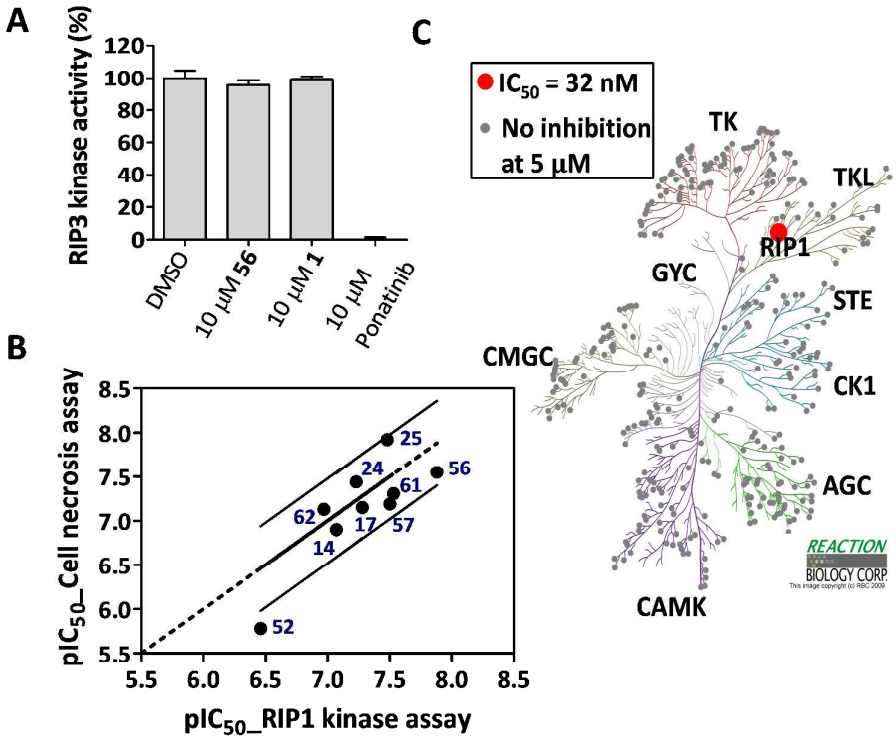


Figure s2. A, 10 μM Compound **56** and **1** showed no inhibition to RIP3 kinase activity, while ponatinib can efficiently inhibit RIP3 activity as reported. B, Inhibition of the new series compounds to RIP1 kinase activity and TSZ-induced HT-29 cell necrosis shows a strong positive correlation (pIC_{50} = -logIC_{50}, M; pEC_{50} = -logEC_{50}, M). C, Compound **57** also shows excellent selectivity to RIP1 kinase according to the kinome panel screening results (conducted by Reaction Biology Corp.)

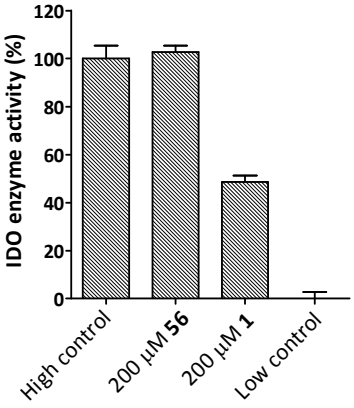


Figure s3. Compound **56** shows no inhibition to IDO enzyme activity at 200 μM, while **1** inhibits 50% IDO activity at 200 μM.

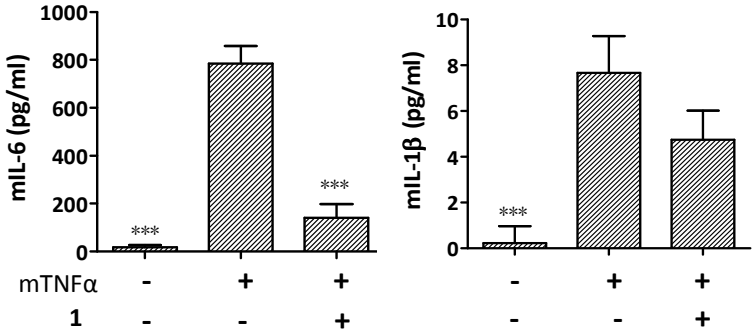


Figure s4. Single dose of 6 mg/kg BW **1** (IV) could partially inhibit mTNFα-induced increase of pro-inflammatory cytokines like mIL-6 and mIL-1ß in C57BL mice. $^{***}P < 0.001$, each compared with TNFα model group.

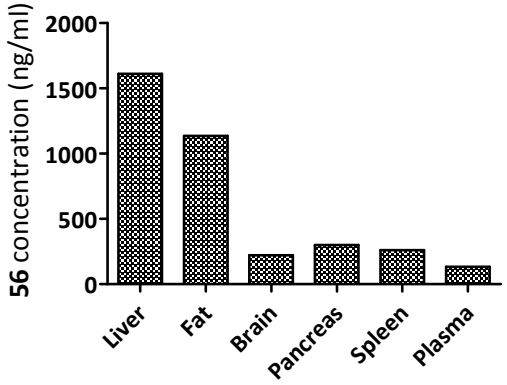


Figure s5. Tissue distribution of **56.** 20 mg/kg **56** was intravenously injected to C57BL/6 mice. The organs were separated two hours later and subjected to concentration determination.

Table s1. Summary of liver microsome stabilty data of the selected compounds

Compd	Human Liver Microsome		Mouse Liver Microsome	
	$t_{1/2}$ (min)	Clint (µl/min/ mg protein)	$t_{1/2}$ (min)	Clint (µl/min/ mg protein)
9	630	1.10	248	2.80
13	10.5	65.9	10.5	65.9
14	4.93	141	0.753	921
25	2.25	308	0.753	921
30	6.08	114	0.829	114
32	4.54	153	0.632	1097
34	9.17	75.6	2.41	288
37	4.34	160	11.9	58.2
42	2.17	319	0.753	921
43	0.753	921	0.753	921
44	0.897	773	0.753	921
49	210	3.3	7.02	98.7
51	> 300	0.06	133	5.2

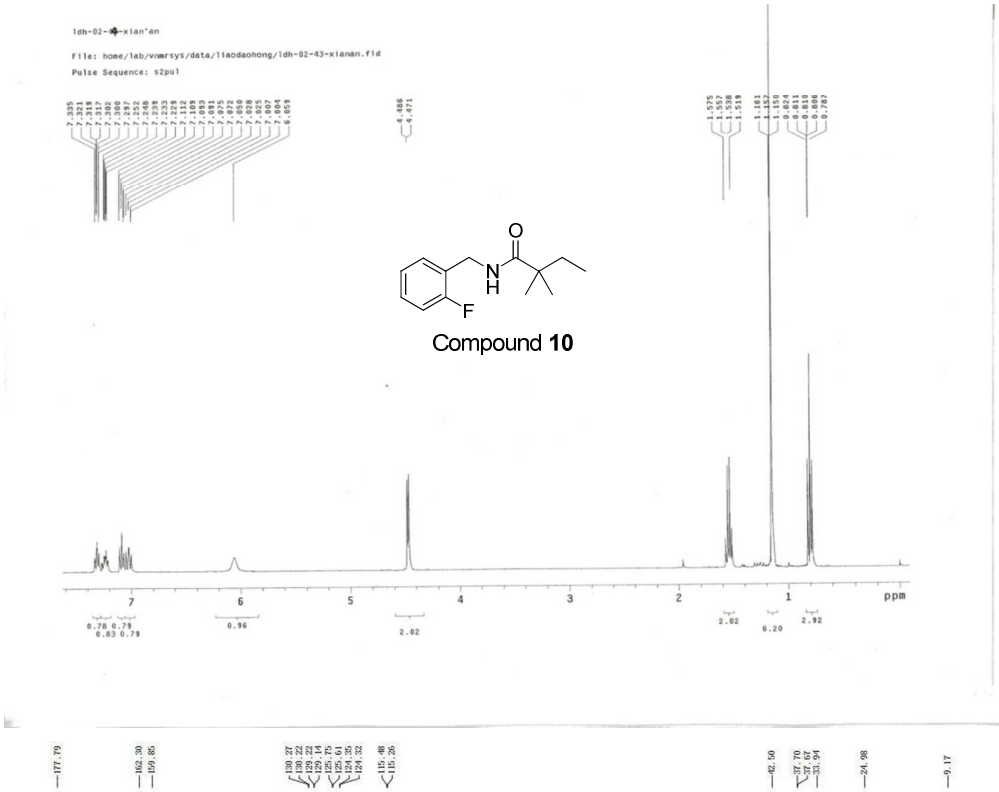
Table s2. Pharmacokinetic parameters of **56** in C57BL/6 mice.

Route	**IV**	**IP**	**PO**
Dose (mg/kg)	2	10	10
C_0 (ng/ml)	1483		
C_{max} (ng/ml)		3499	249
T_{max} (min)		4.98	30
AUC_{tot} (ng·h/ml)	323	2189	349
$t_{1/2}$ (h)	3.1	2.022	1.61
CL_p (ml/min/kg)	103		
V_{ss} (l/kg)	27.8		
F (%)			22

Table s3. Permeability of reference compounds and **56** in MDCK-MDR1 cells (Assayed by ChemPartner Co., Ltd., Shanghai, China).

Test Article		$P_{app}\times10^6$ cm/sec				Efflux Ratio
		Sample - 01	Sample - 02	Mean	RSD	
Metoprolol	A-B	24.17	24.24	**24.21**	0.00	**1.09**
	B-A	25.88	27.13	**26.51**	0.03	
Atenolol	A-B	0.29	0.22	**0.25**	0.20	**1.80**
	B-A	0.47	0.44	**0.46**	0.04	
Quinidine	A-B	5.15	5.09	**5.12**	0.01	**7.18**
	B-A	37.12	36.42	**36.77**	0.01	
56	A-B	35.28	38.11	**36.70**	0.05	**0.87**
	B-A	32.44	31.62	**32.03**	0.02	

^{1}H and ^{13}C NMR spectra



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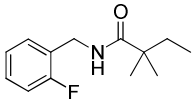

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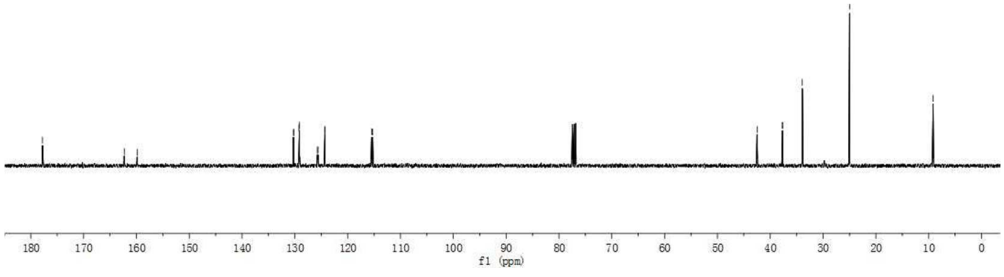

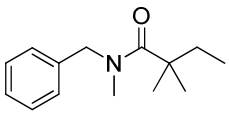

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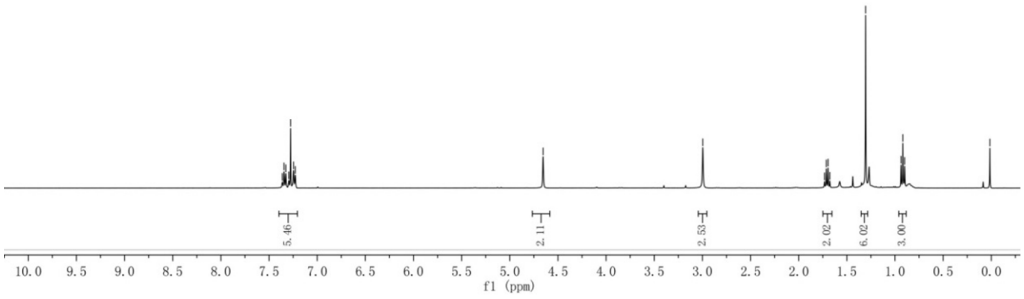

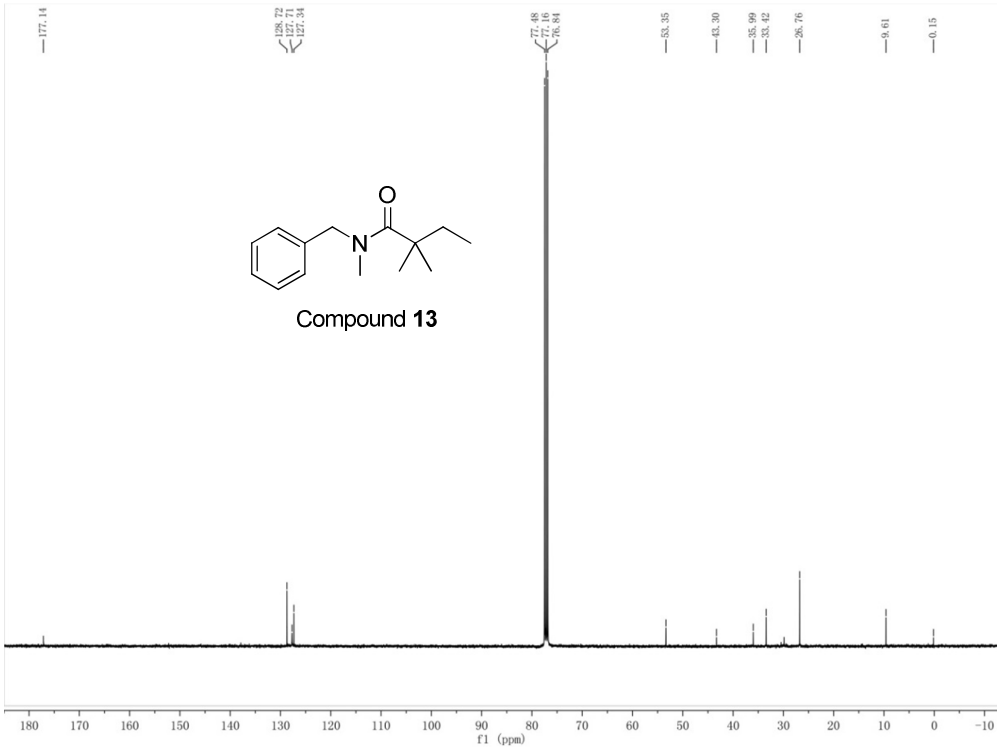

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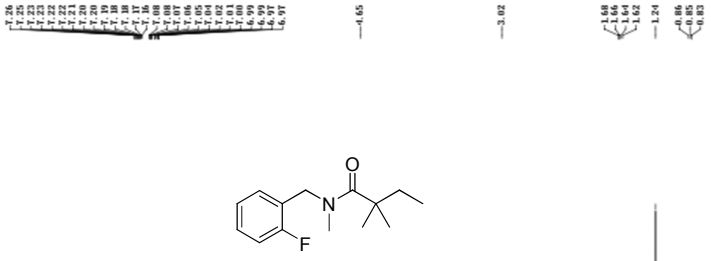

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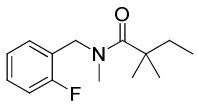

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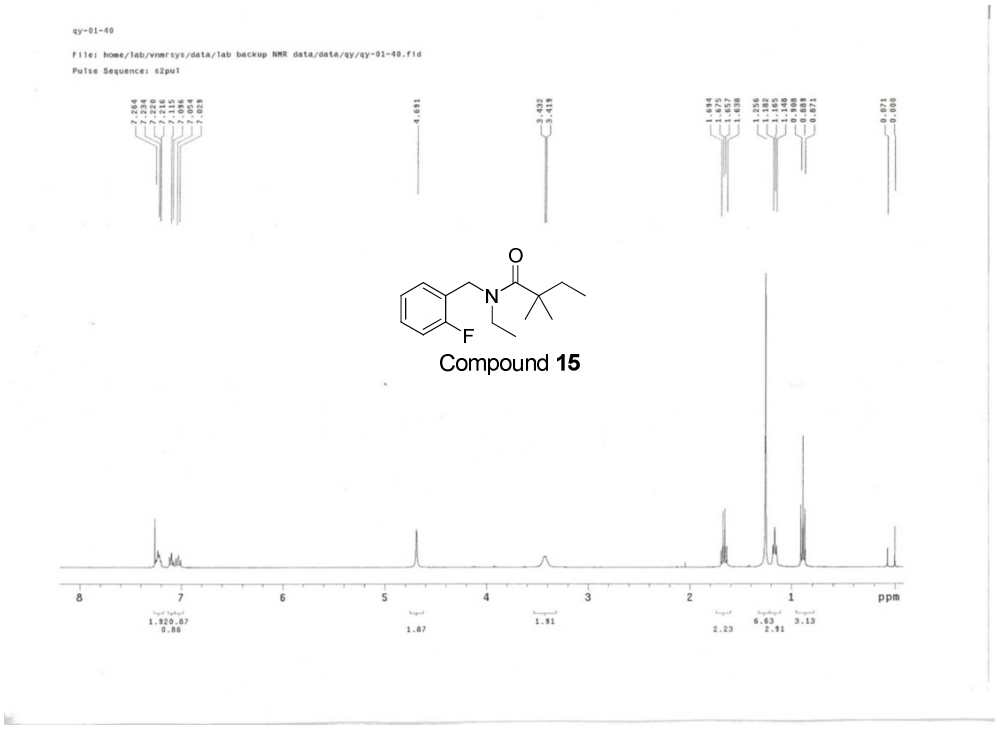

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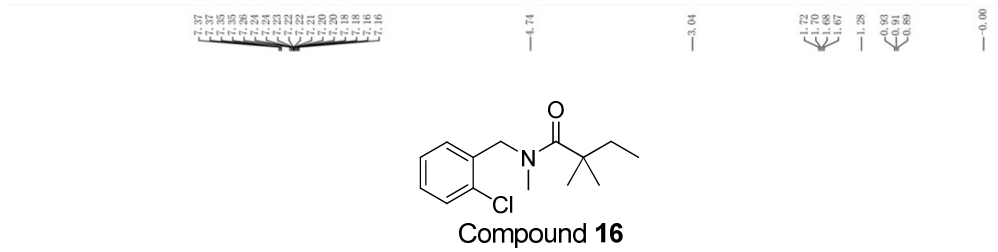

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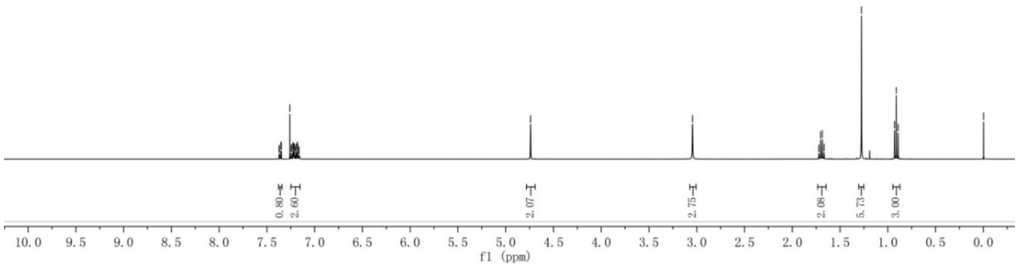

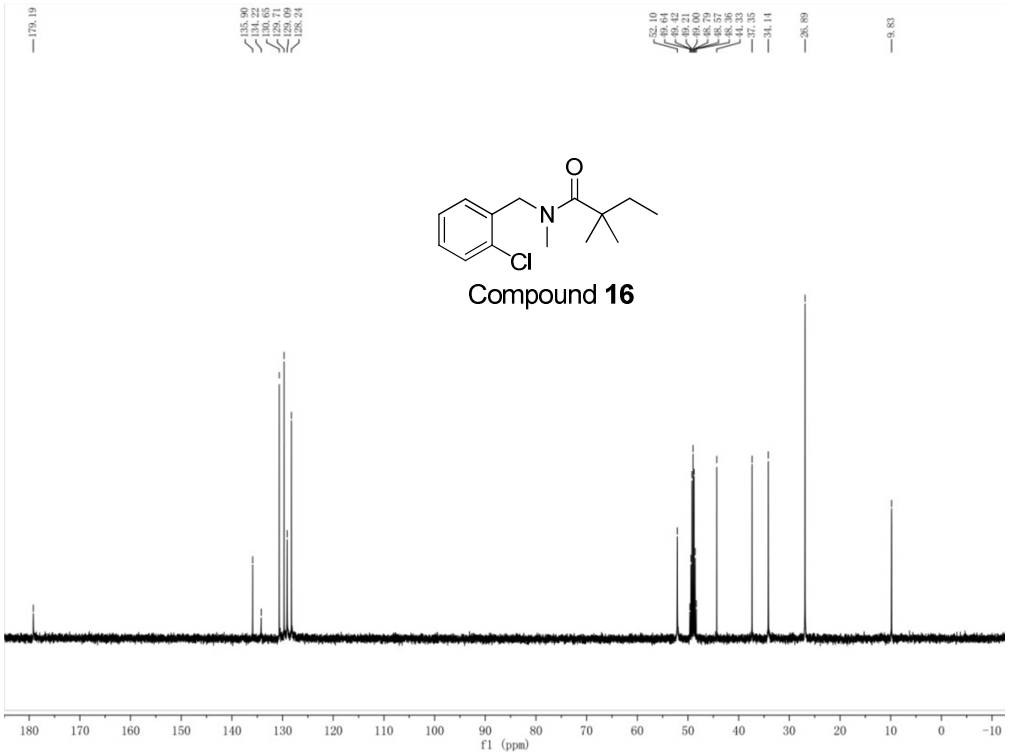

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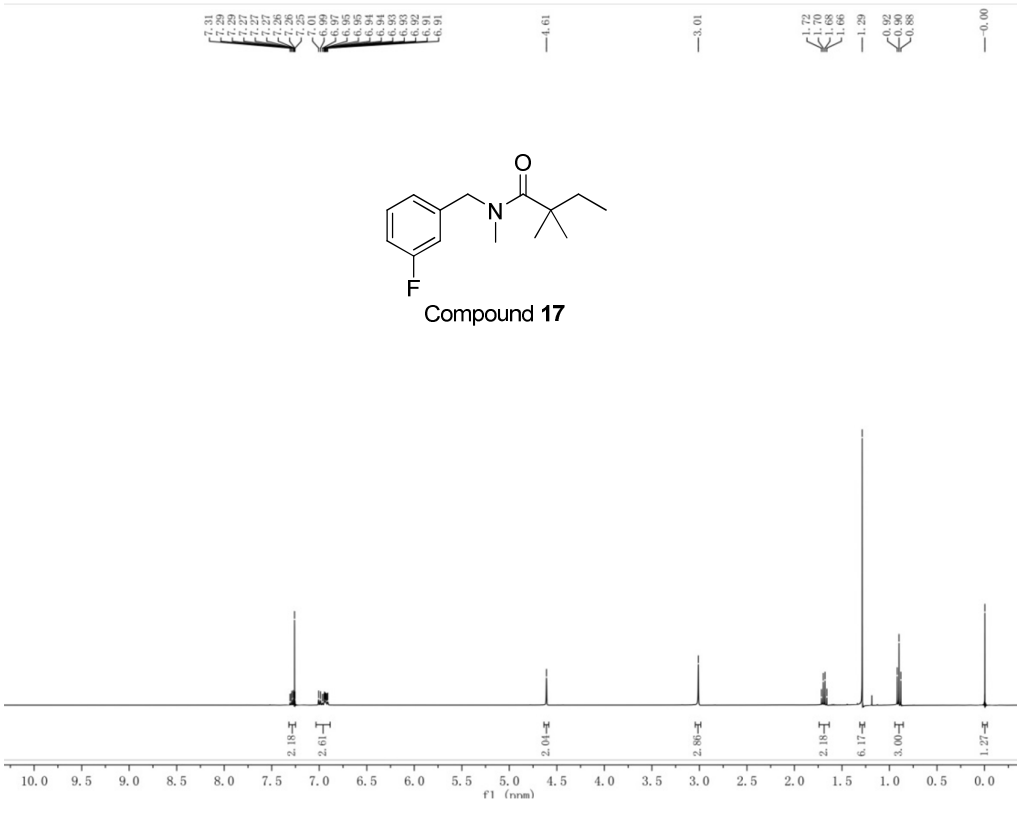

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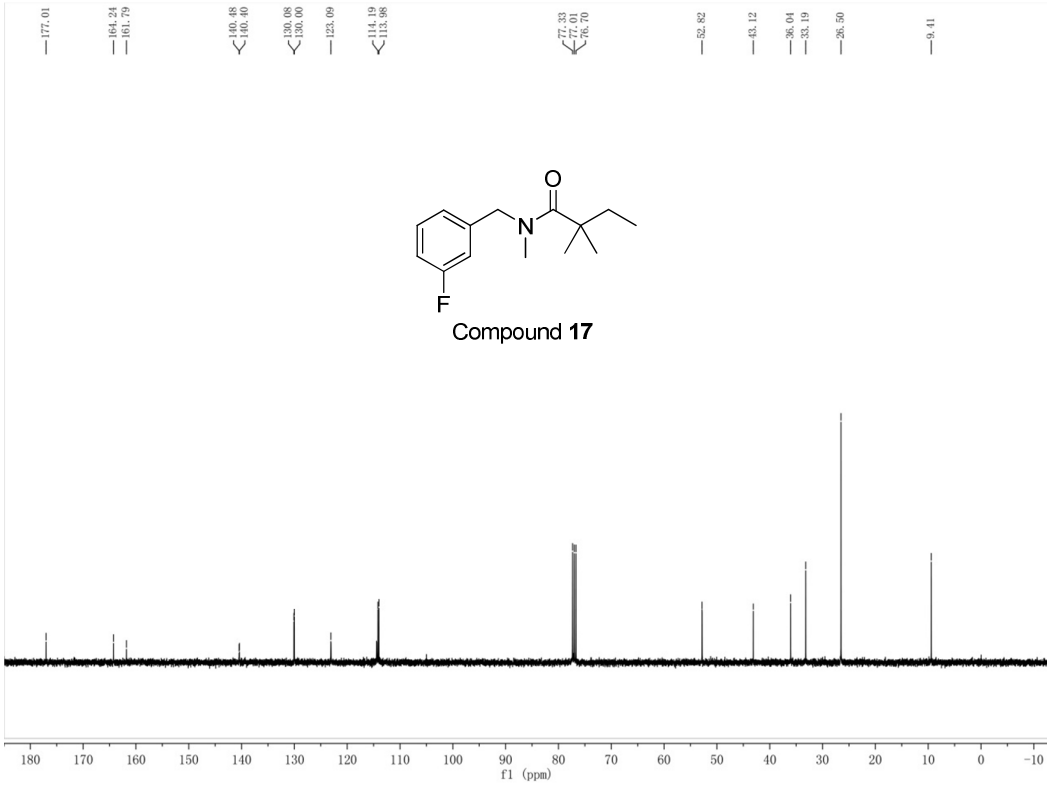

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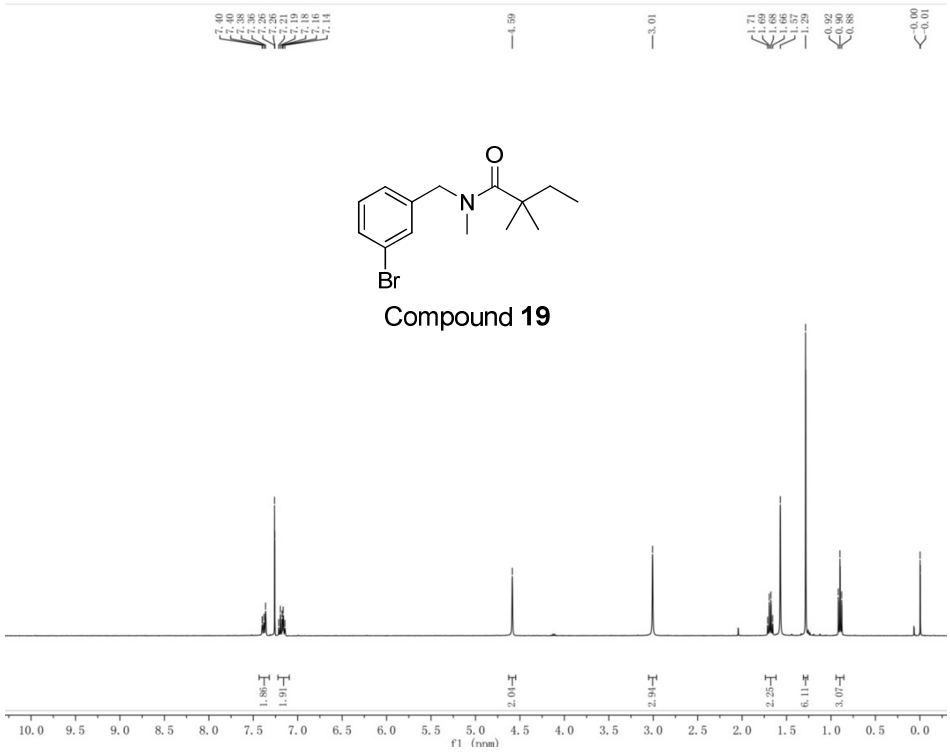

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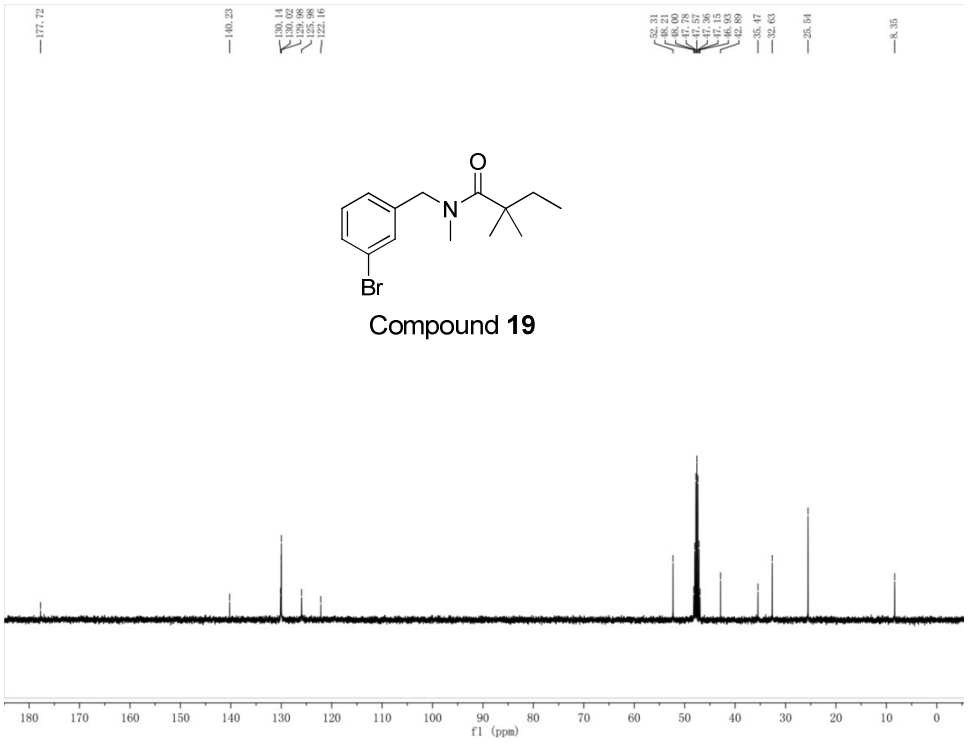

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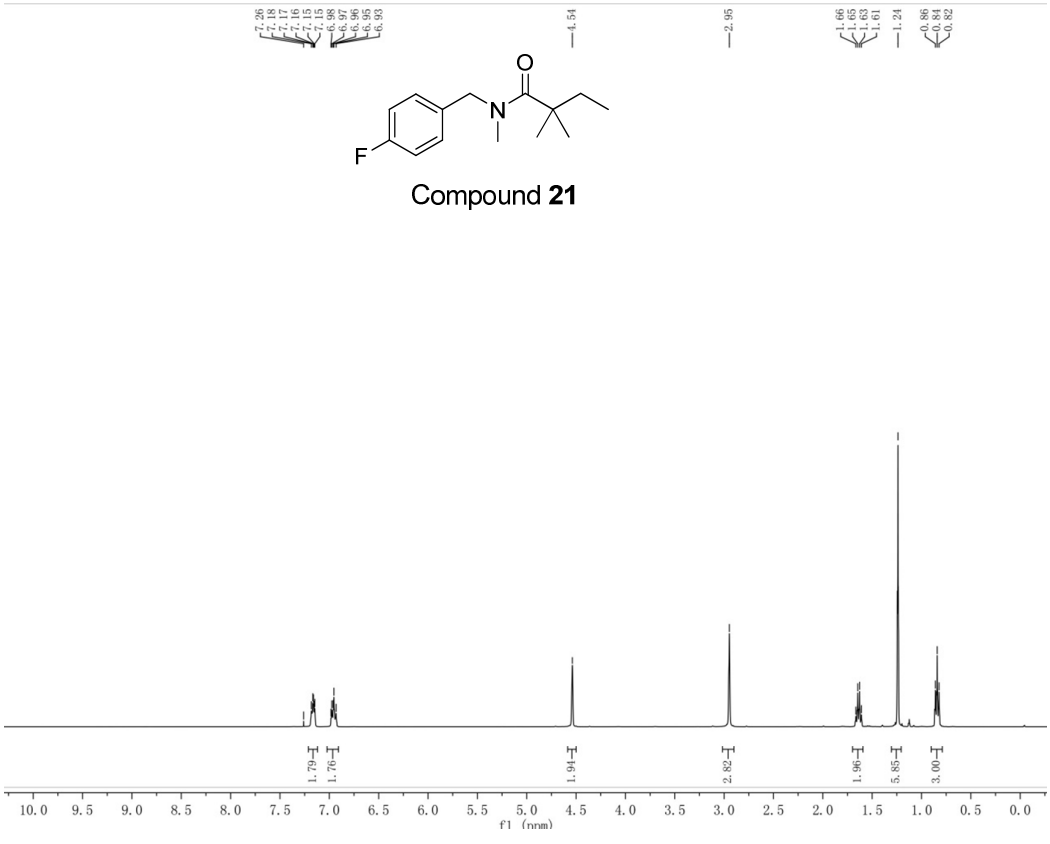

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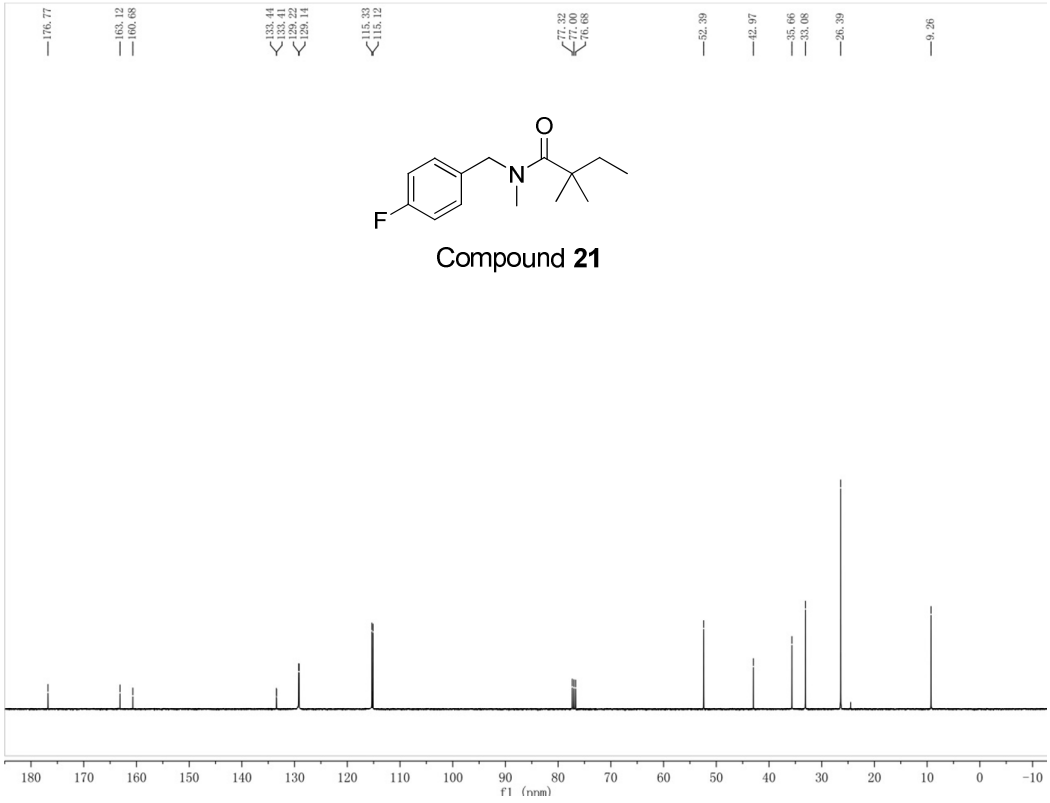

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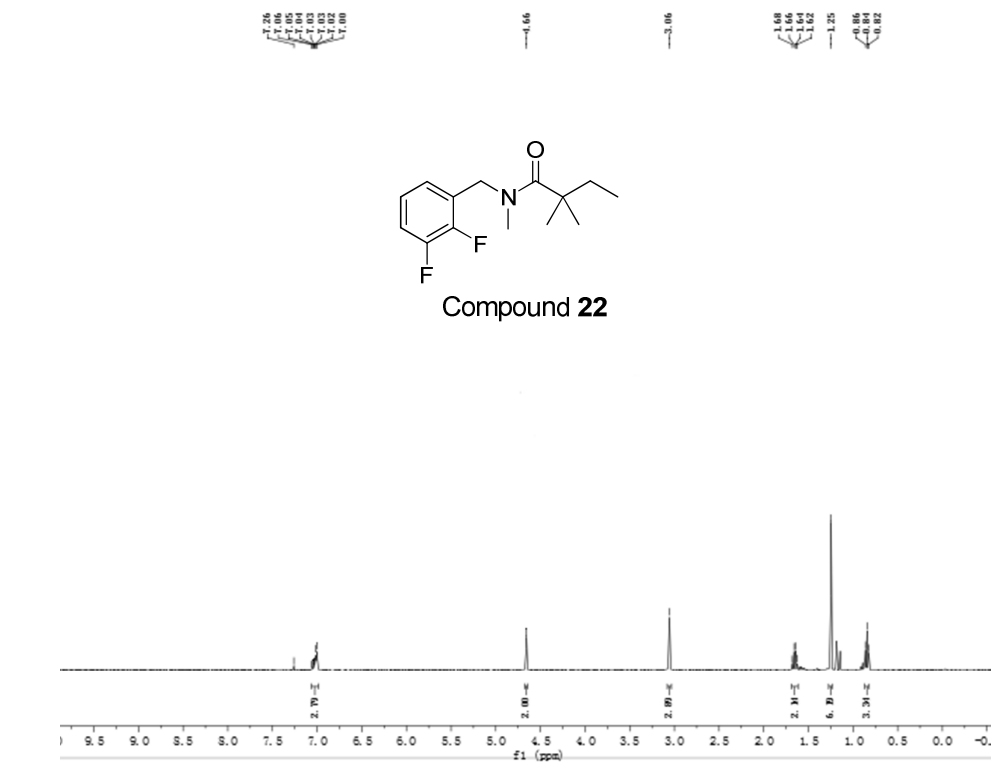

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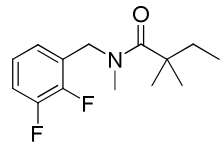

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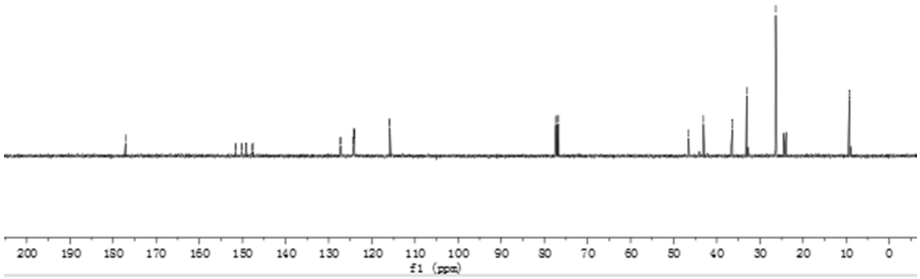

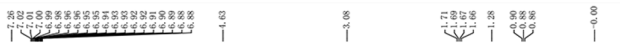

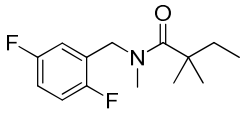

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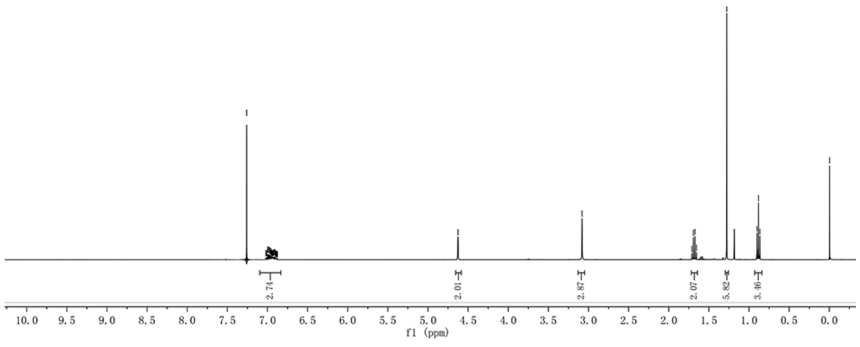

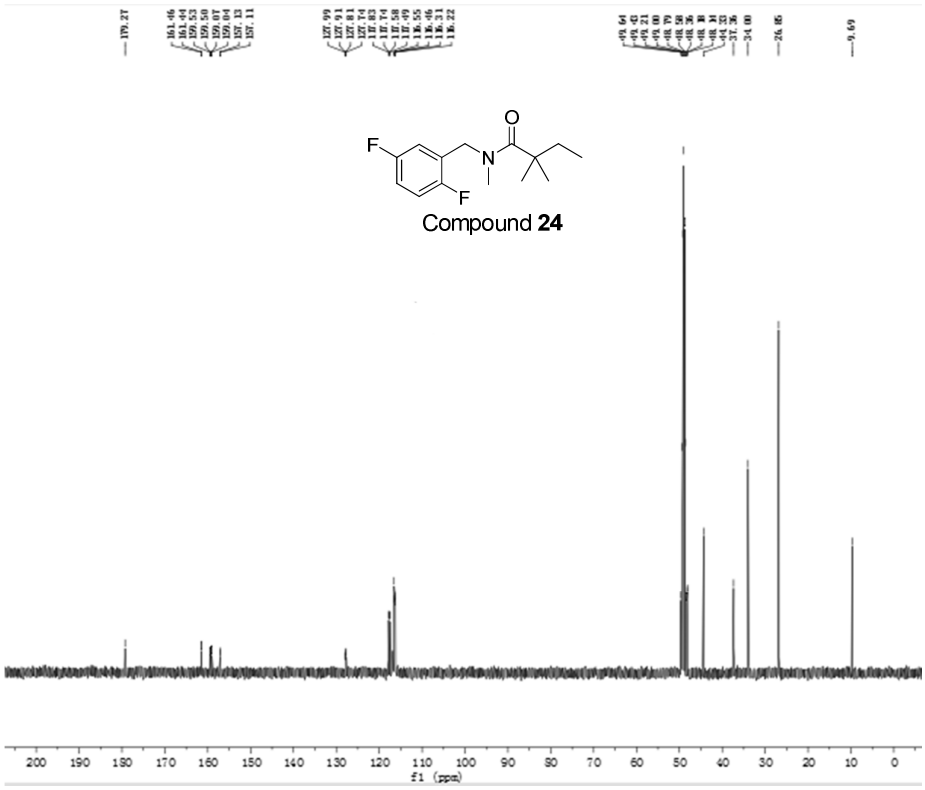
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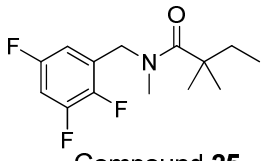
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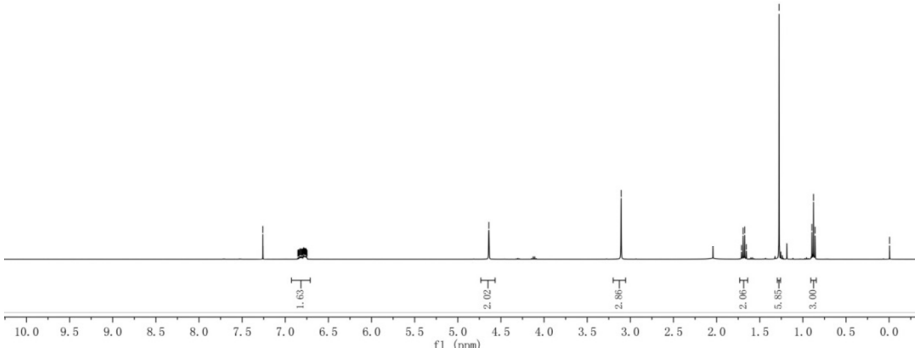
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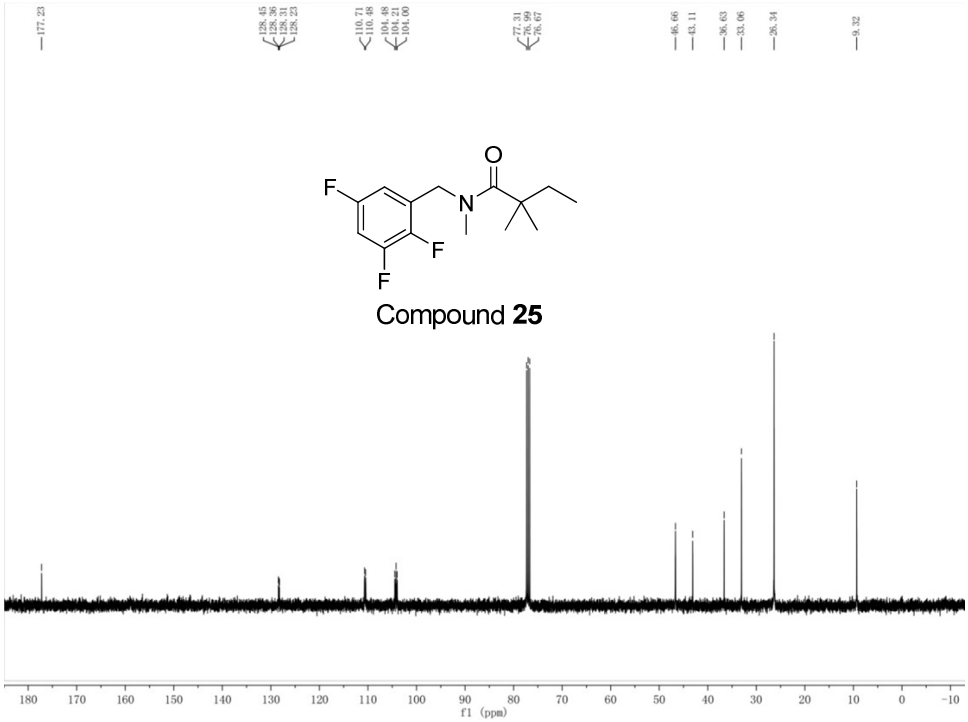

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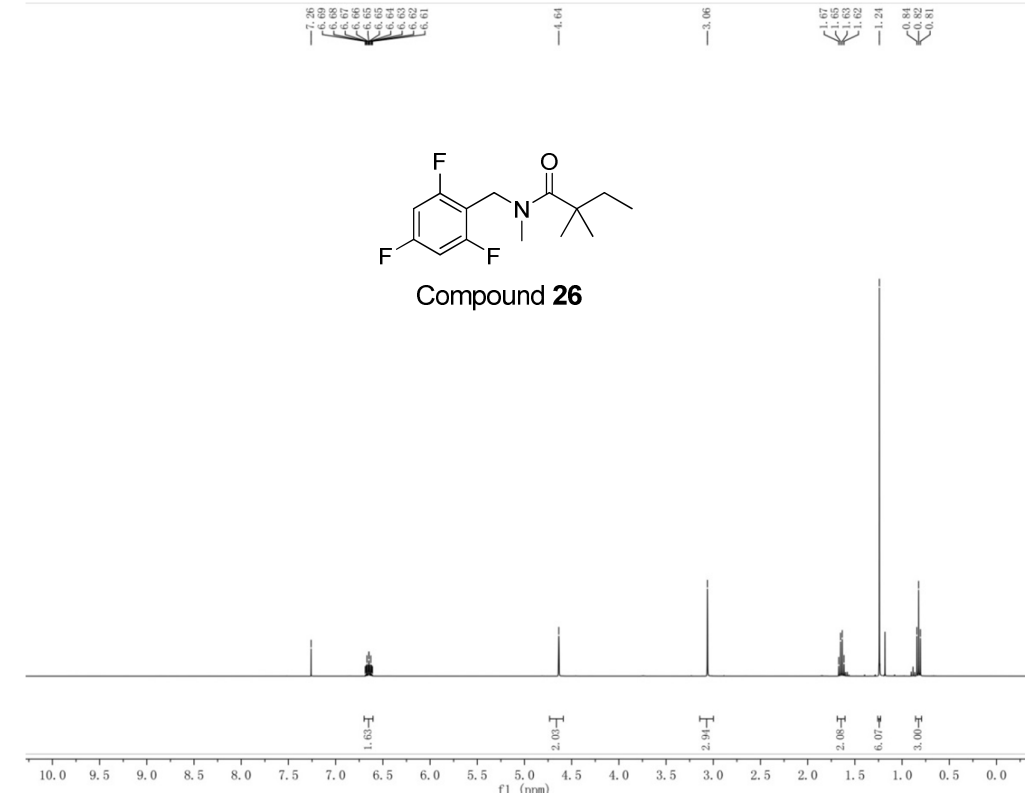

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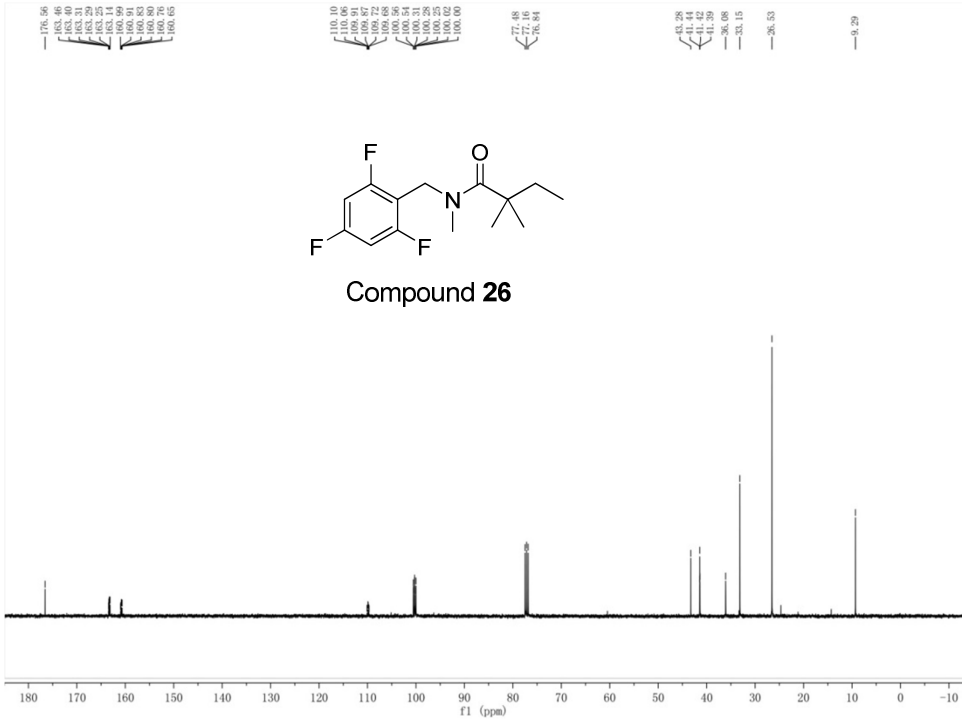

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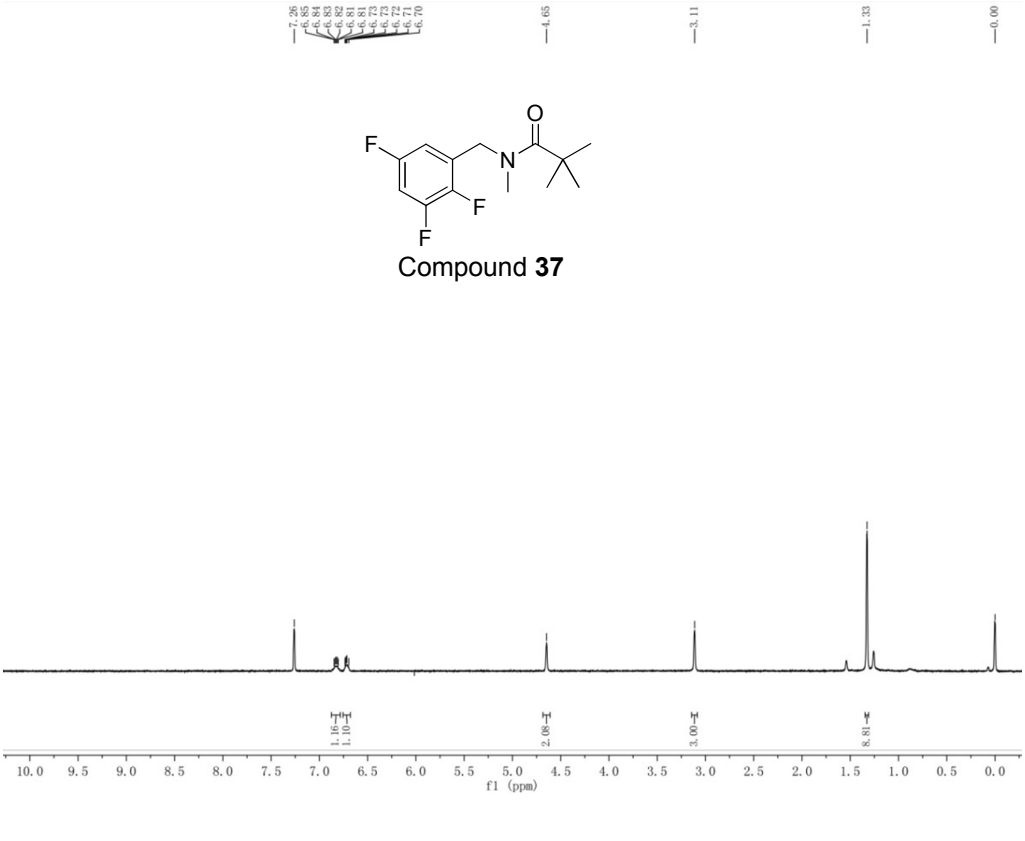

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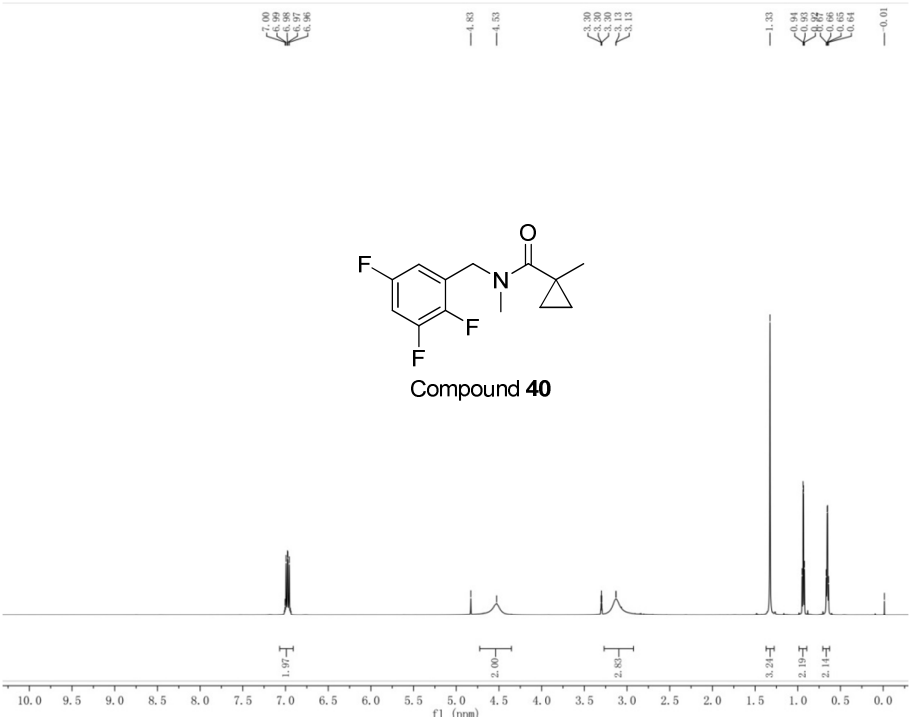
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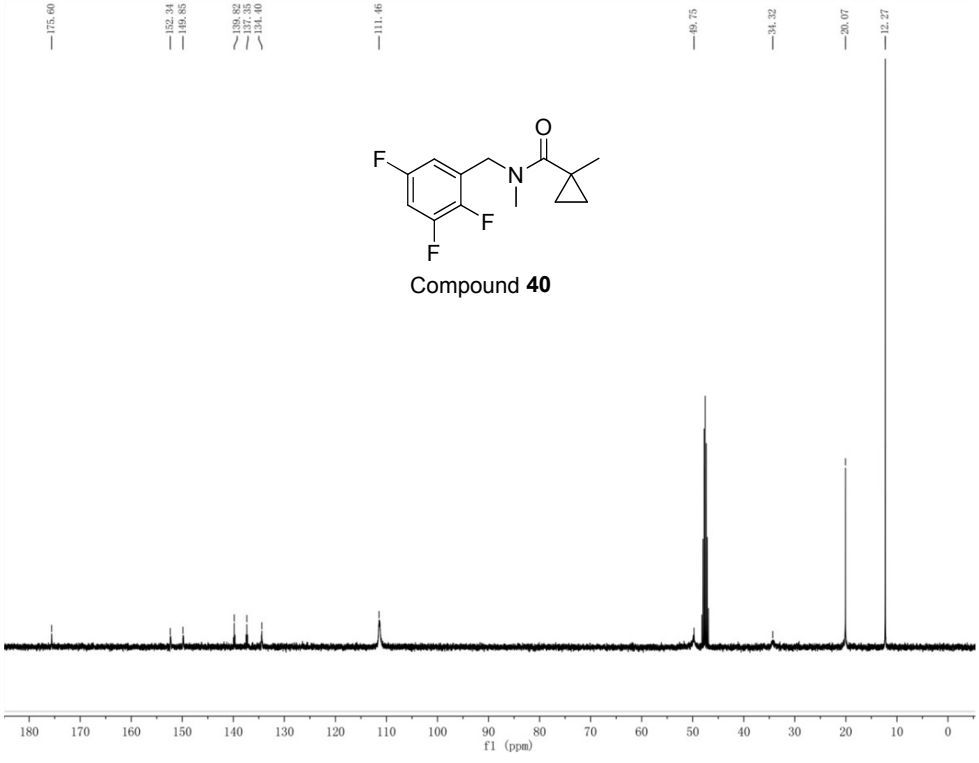
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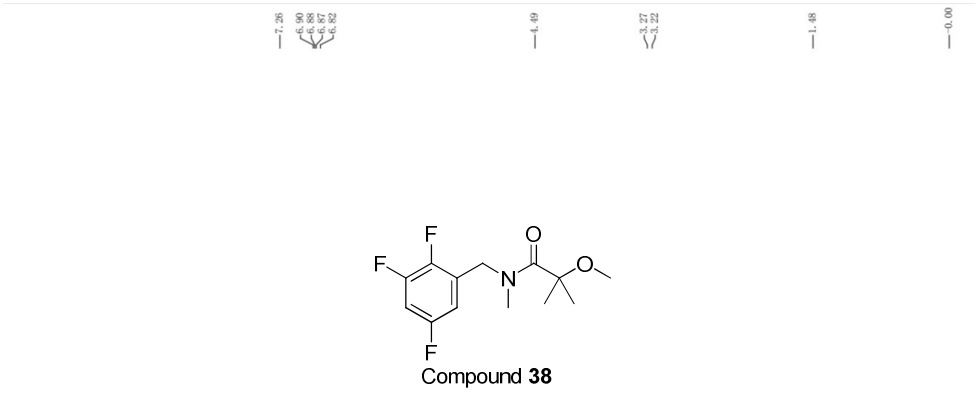

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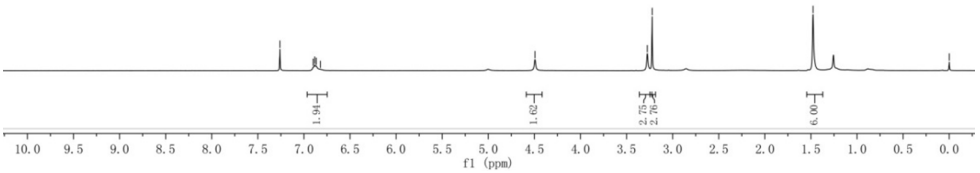

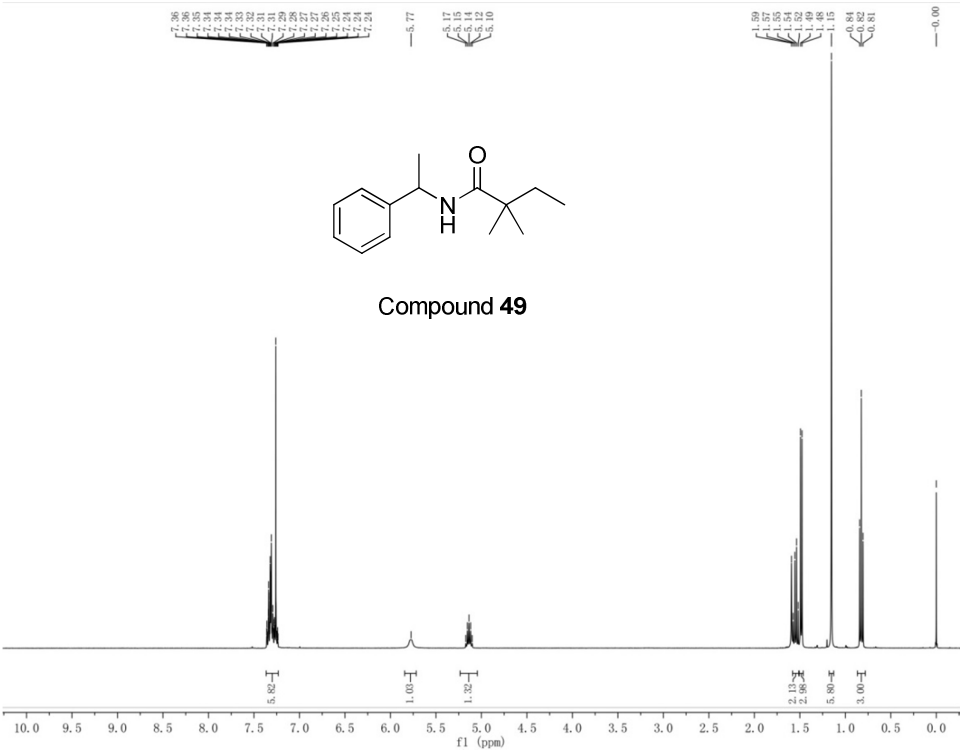

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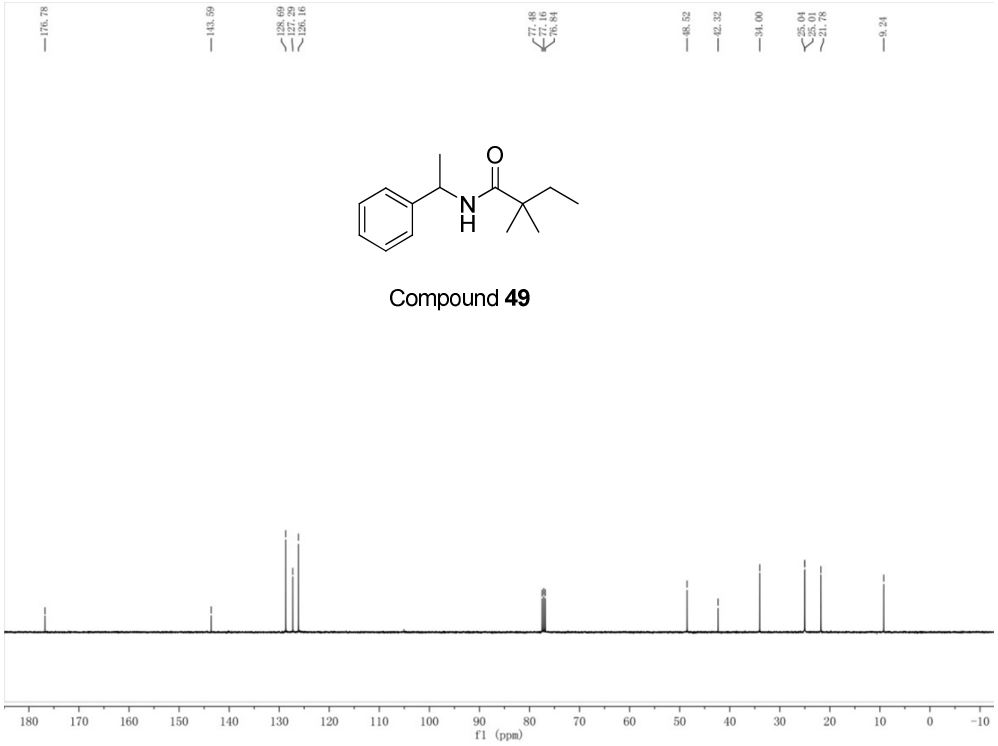

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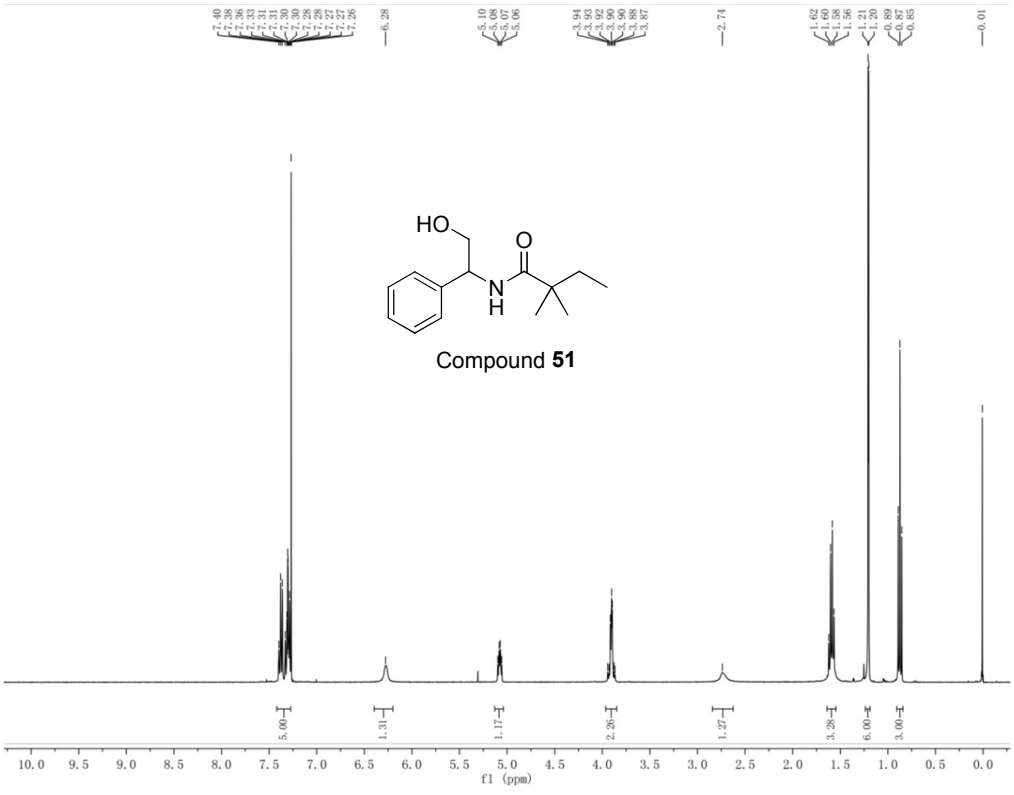

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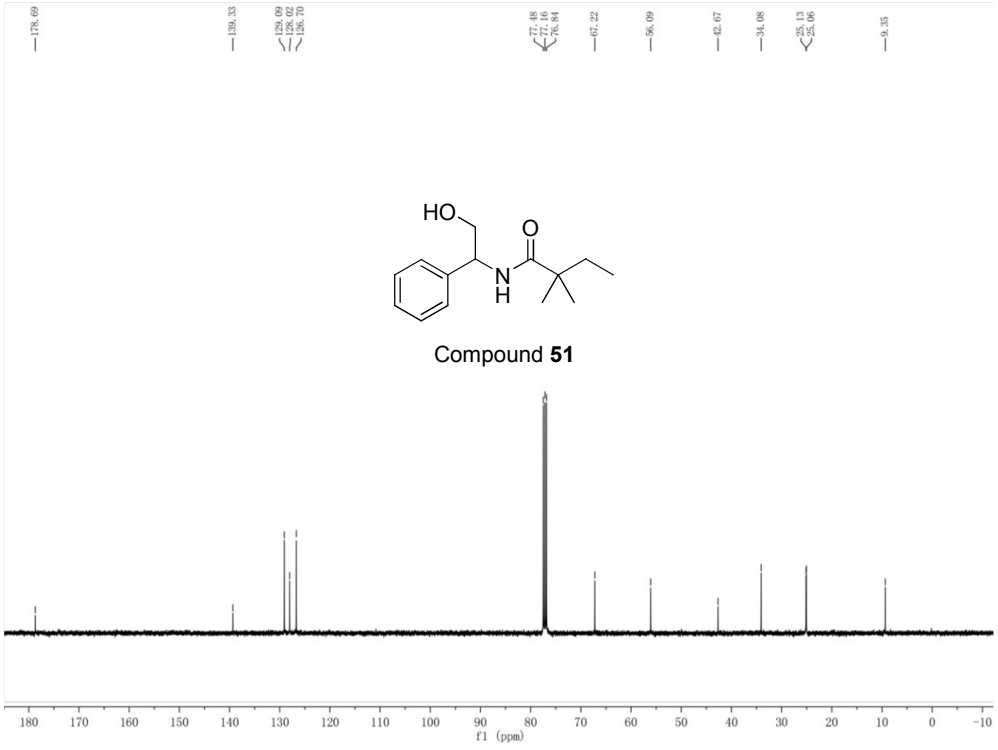

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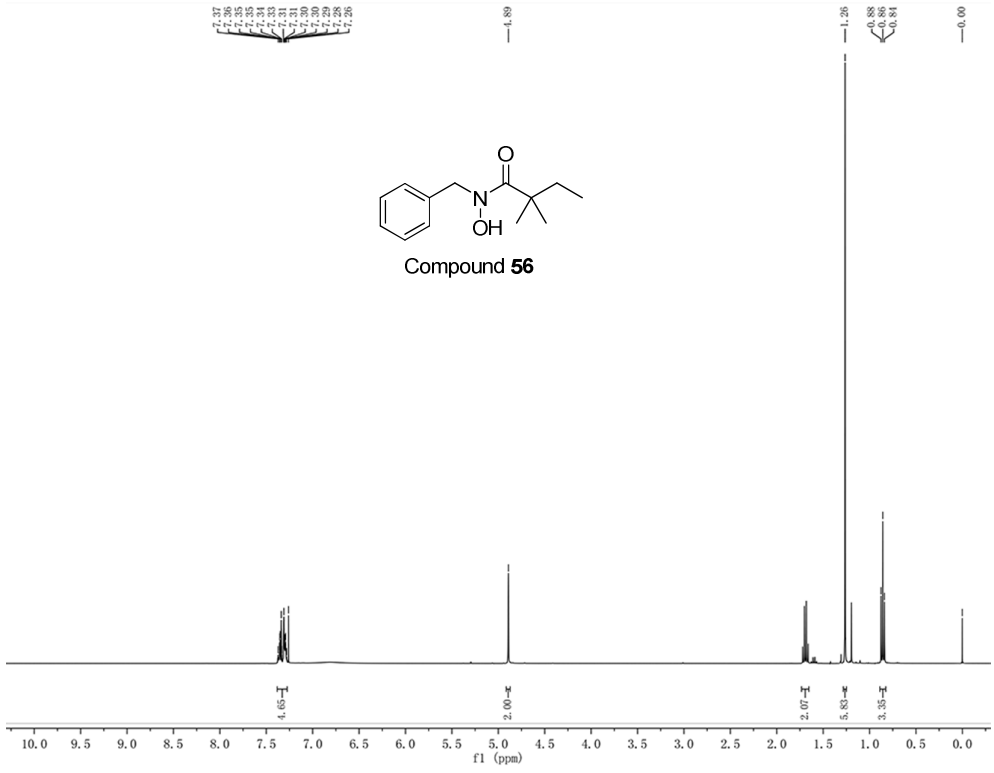

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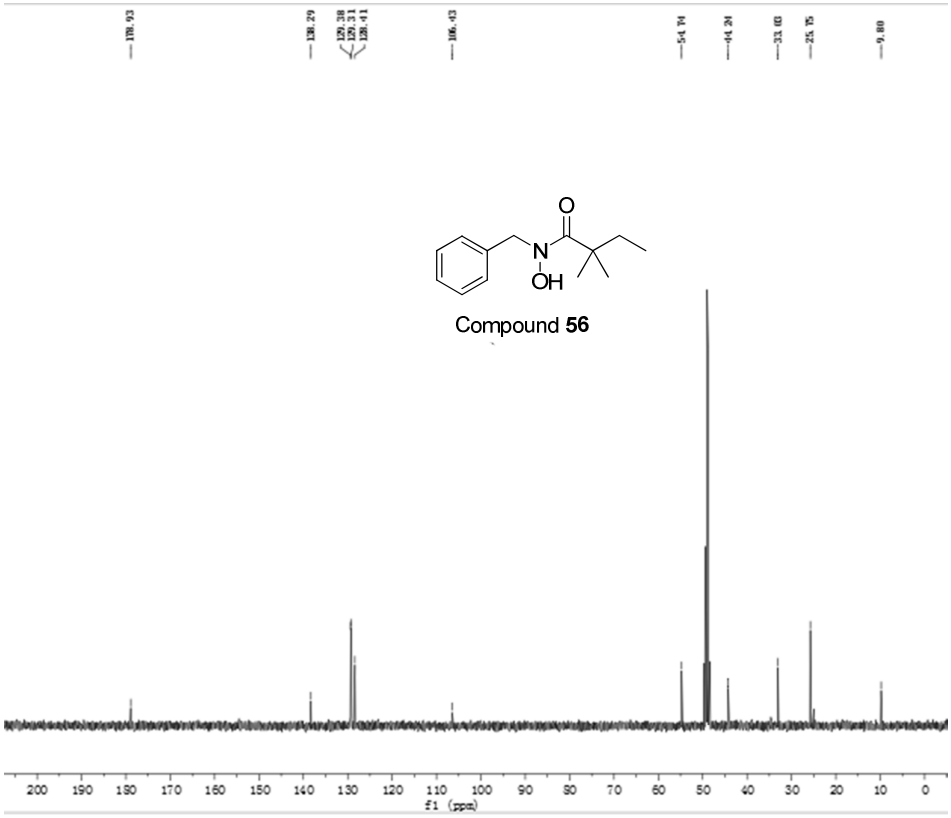

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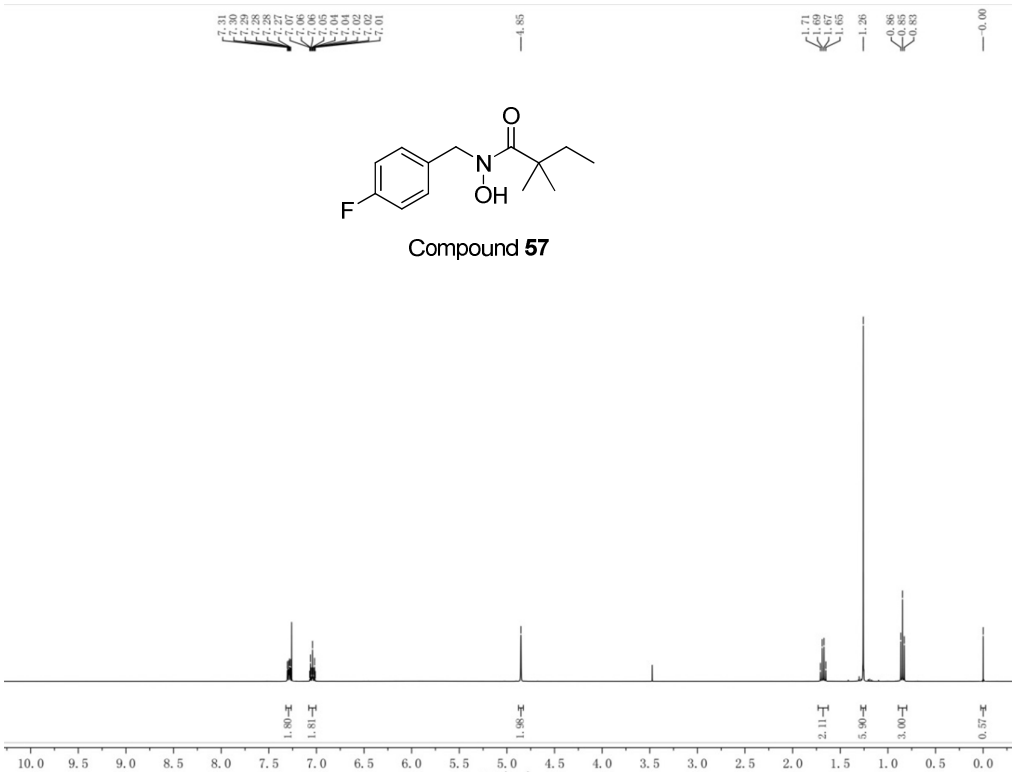

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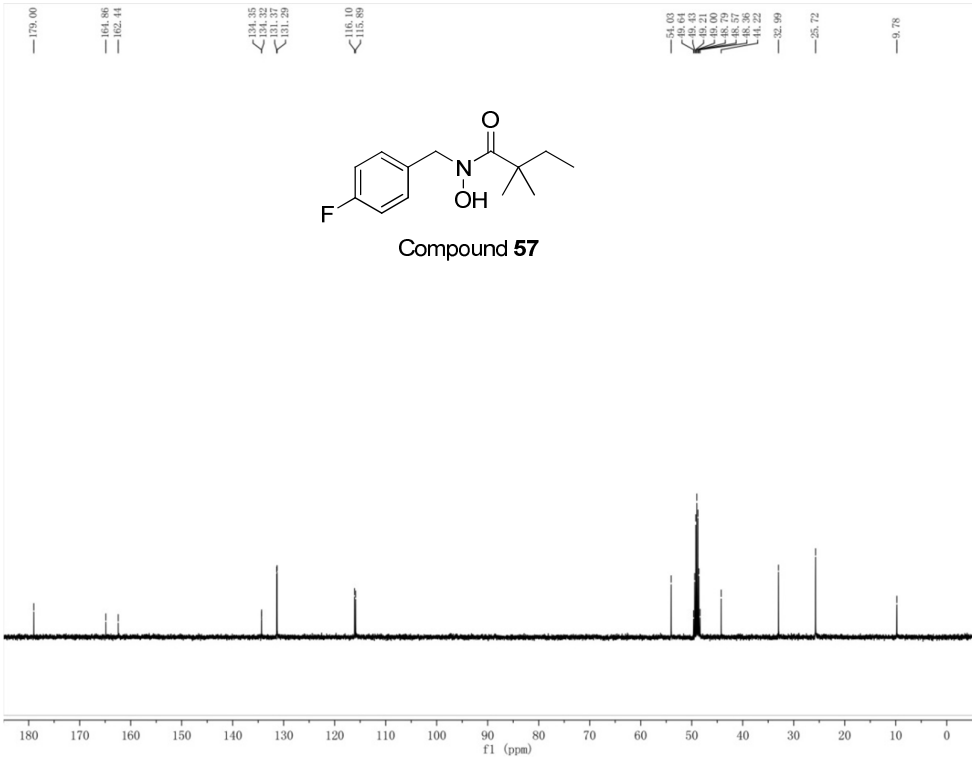

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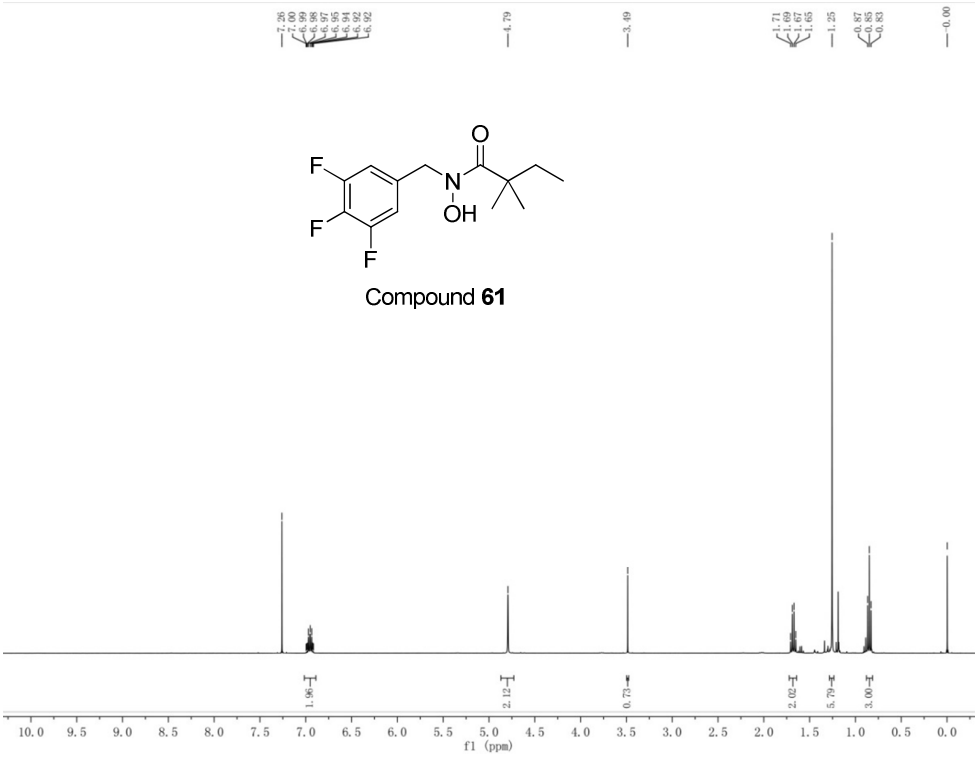
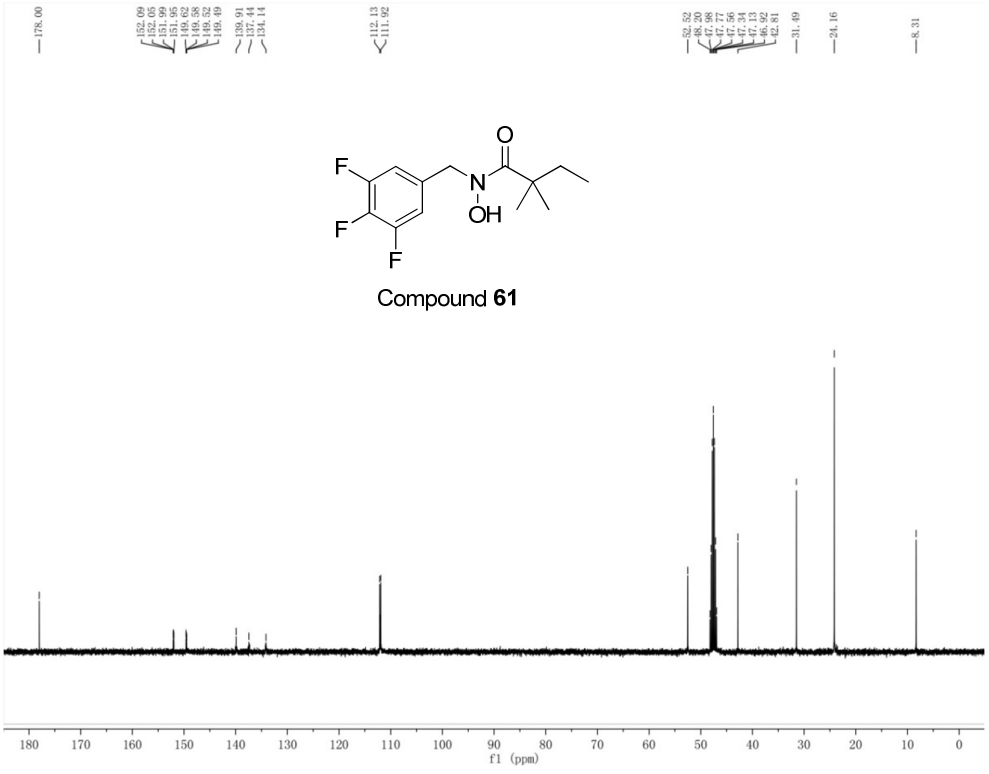
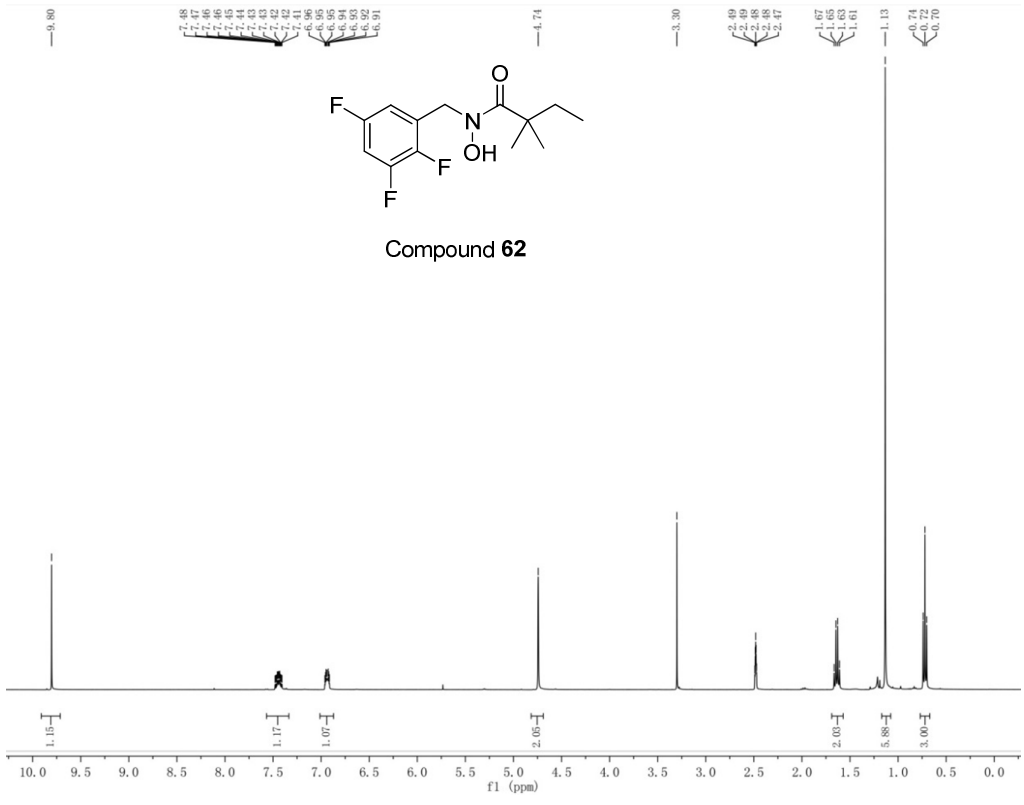


Compound **61**



Compound **61**



Compound **62**