



Article: Supporting Information

1,3-Benzodioxole Derivatives Improve the Anti-Tumor Efficiency of Arsenicals

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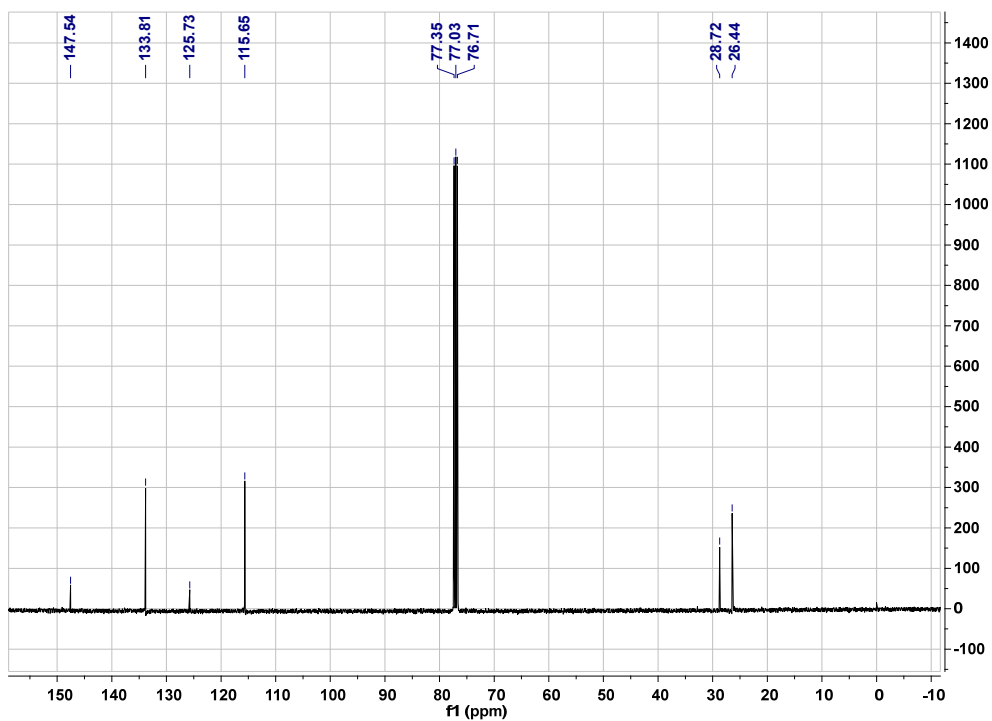
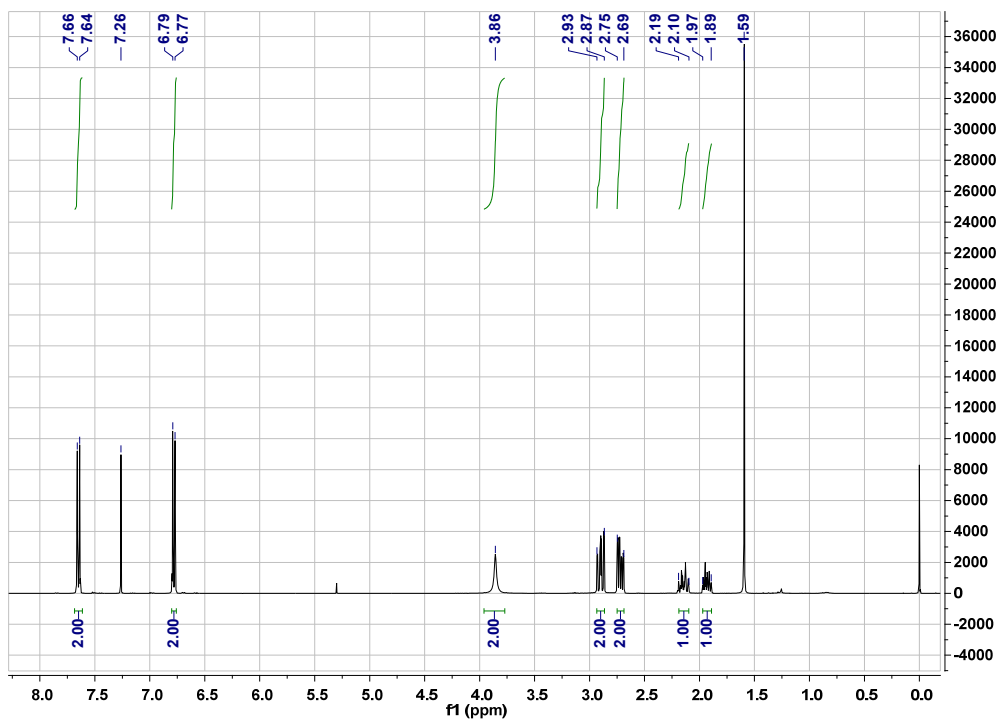
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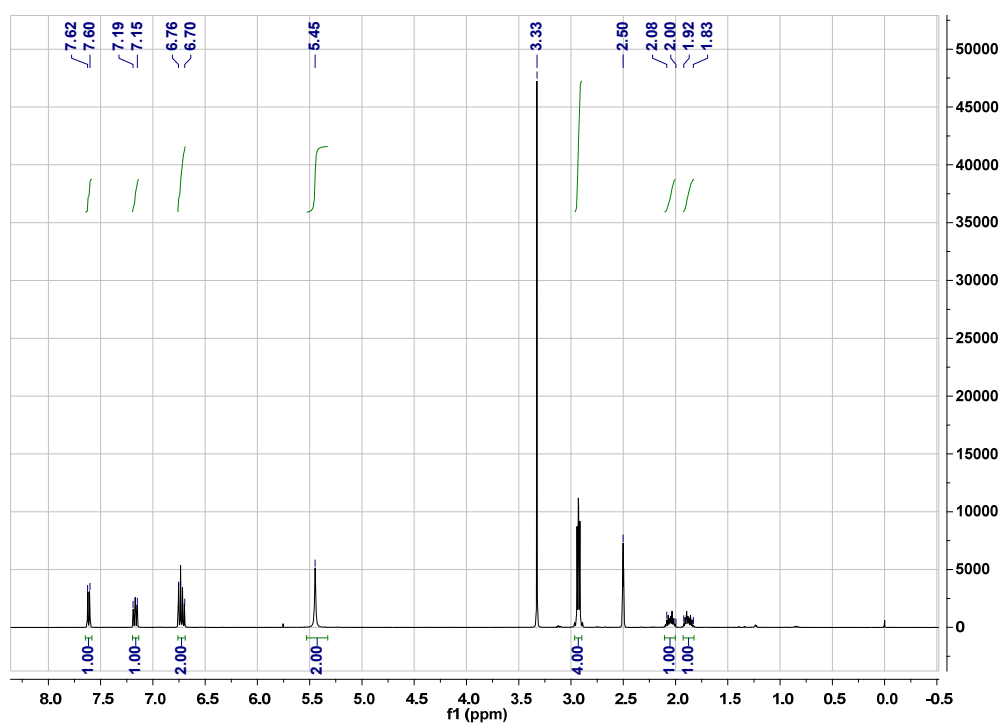
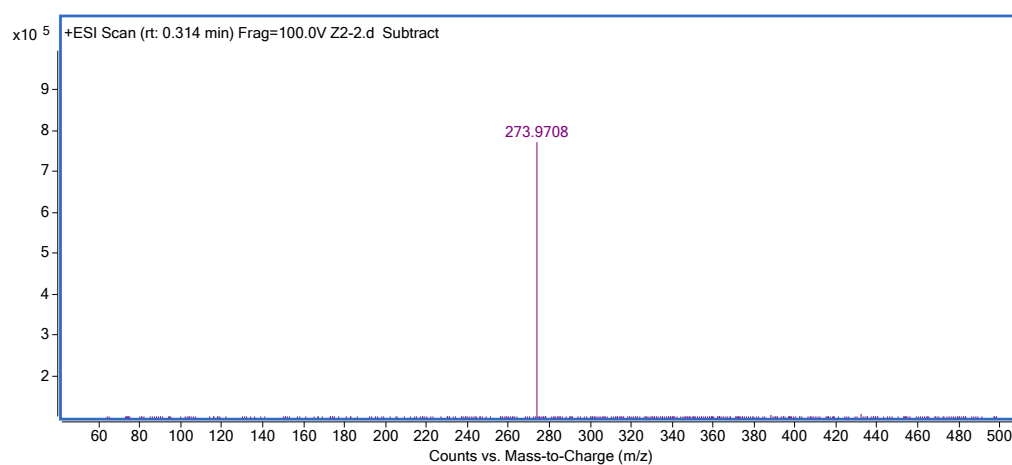
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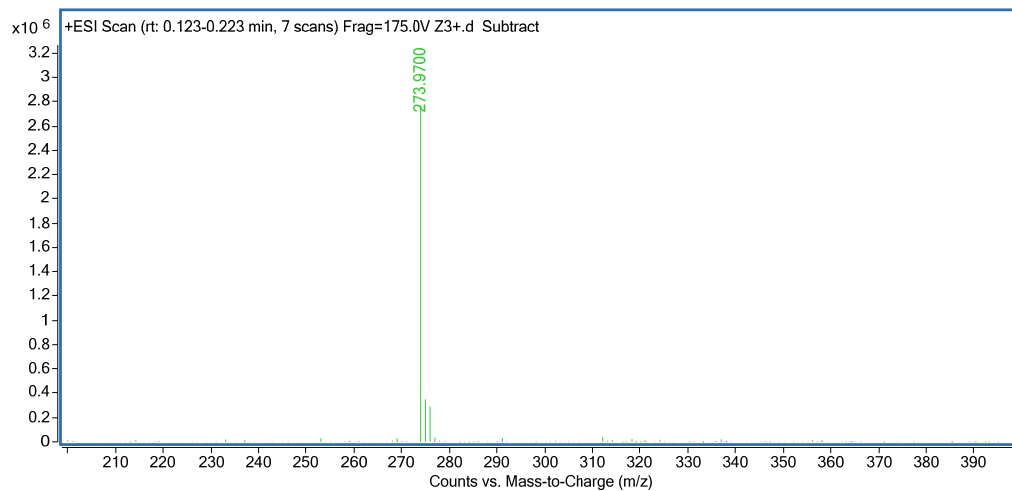
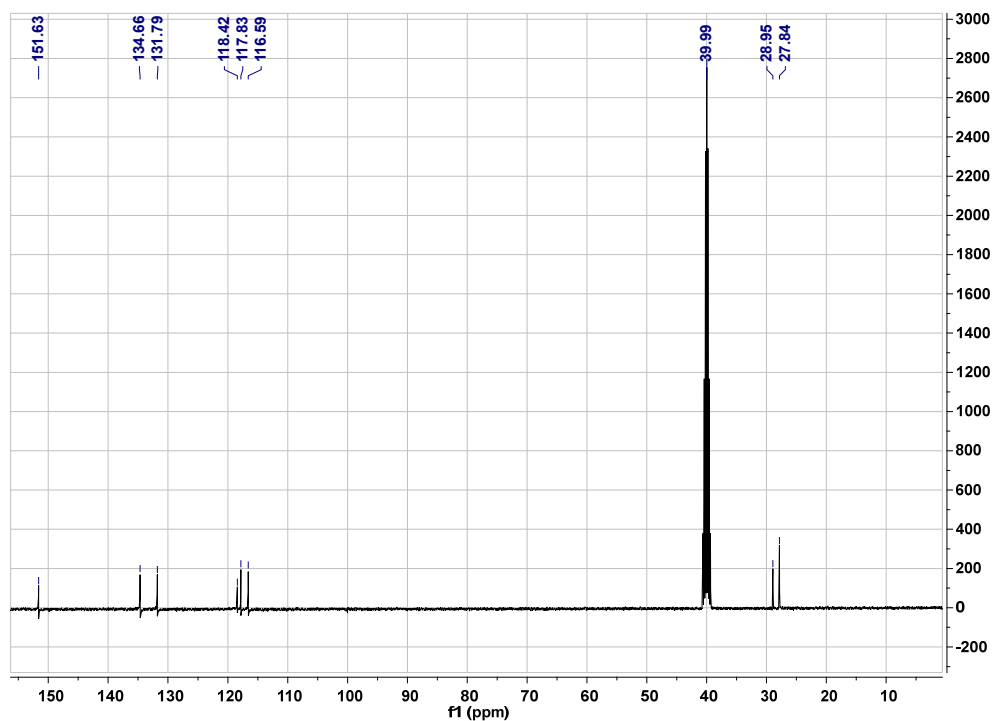
Characterization of chemical structure

Z2

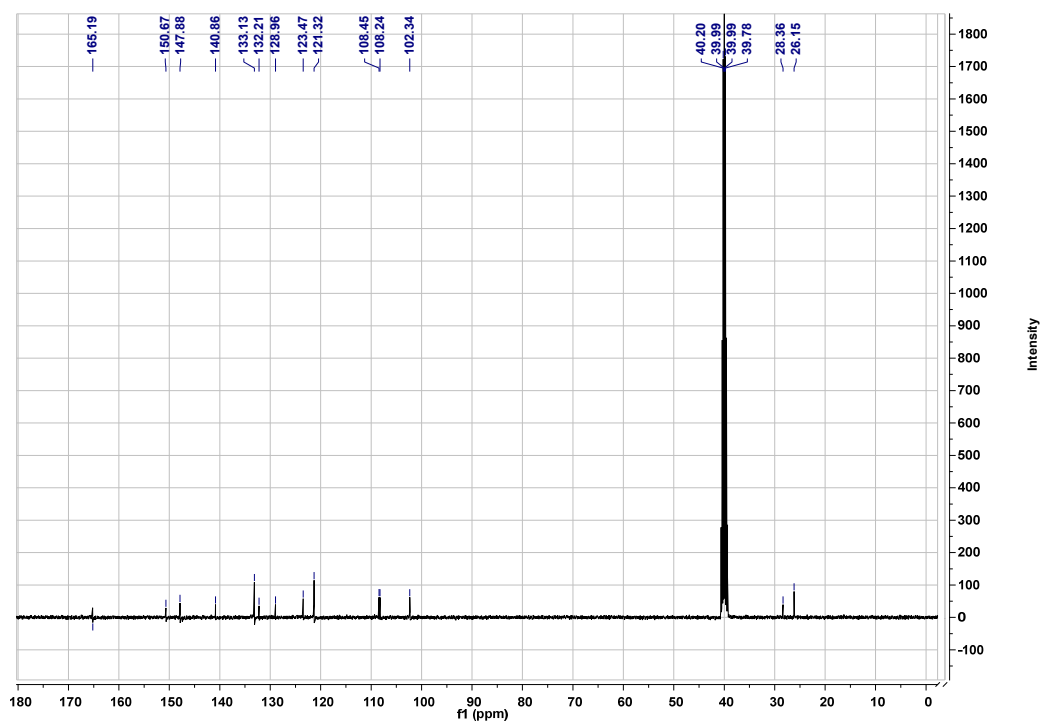
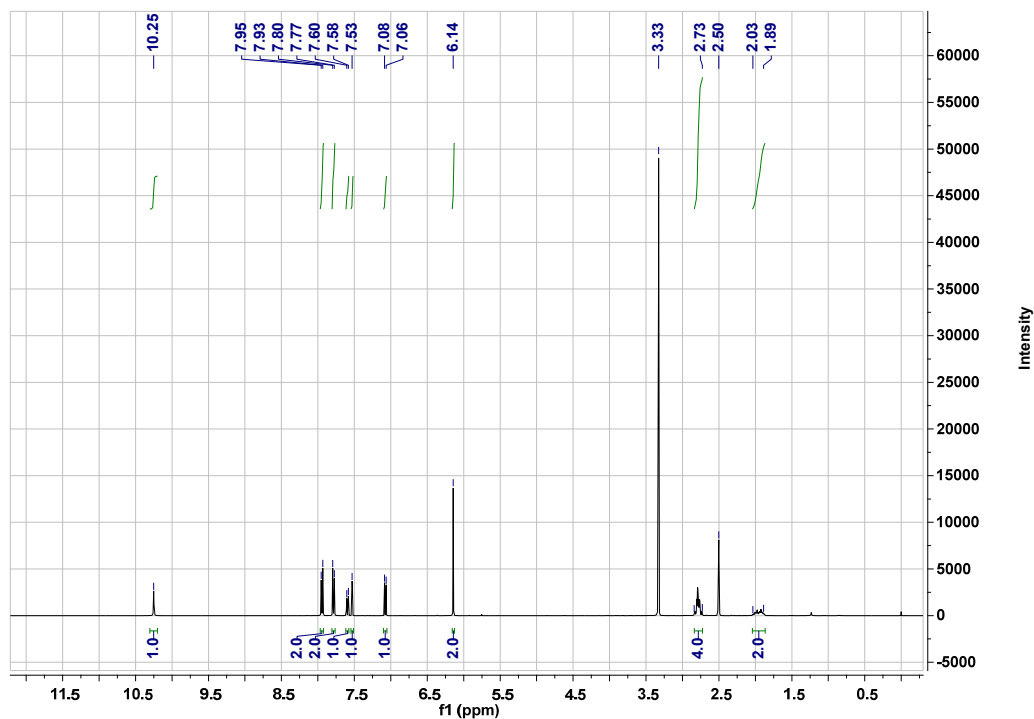


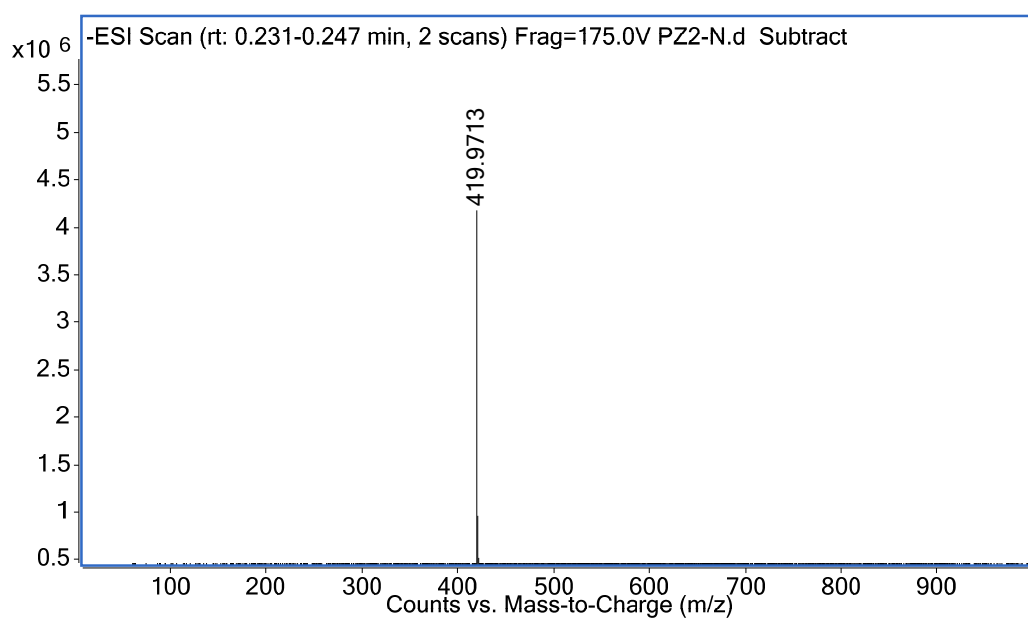


Z5

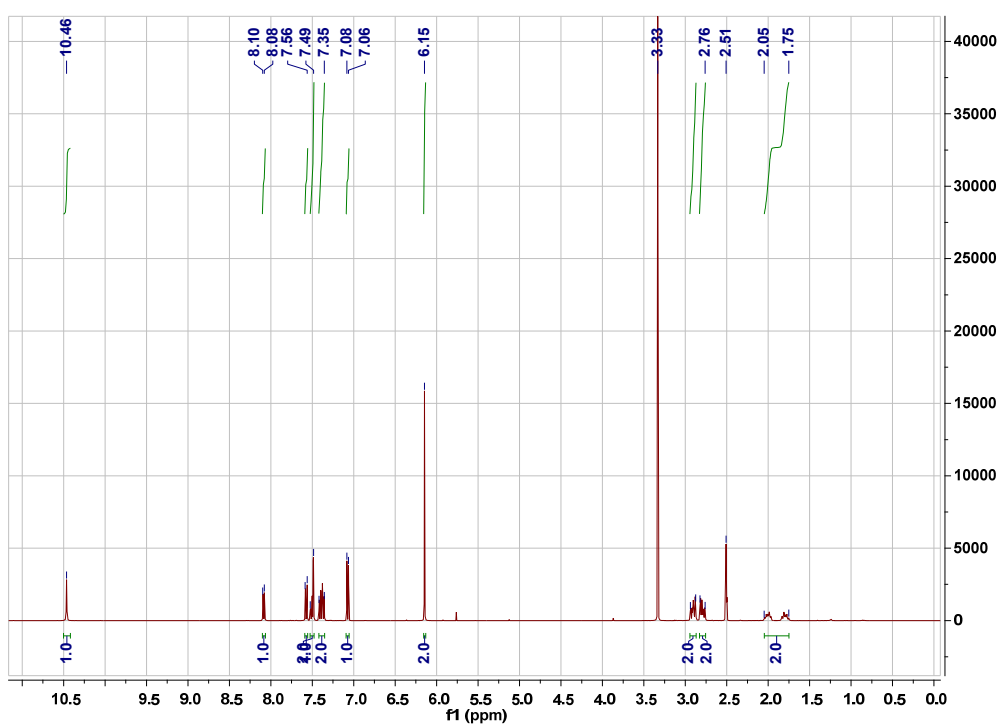


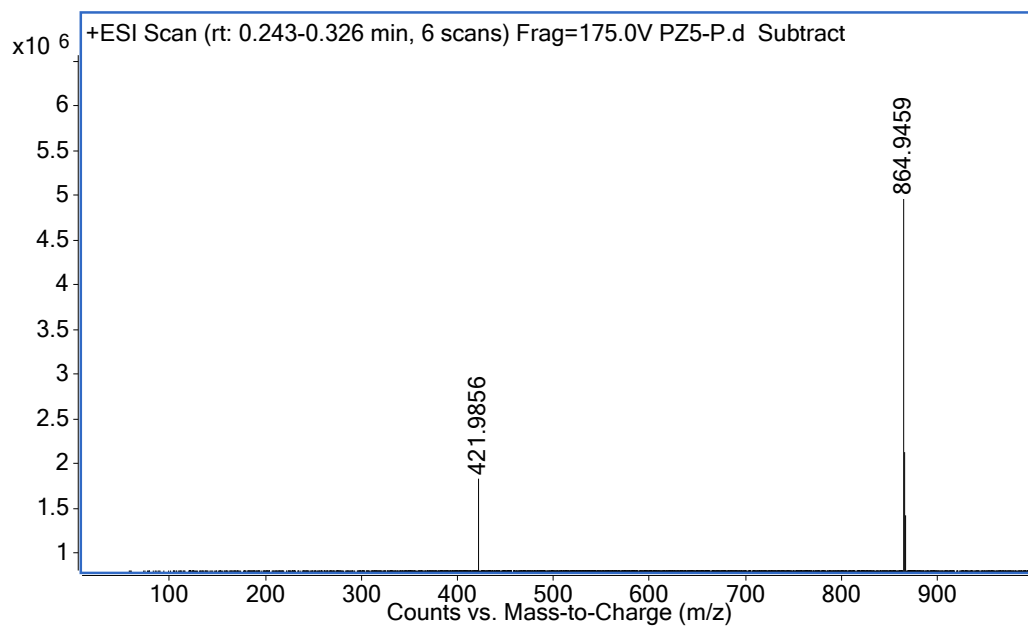
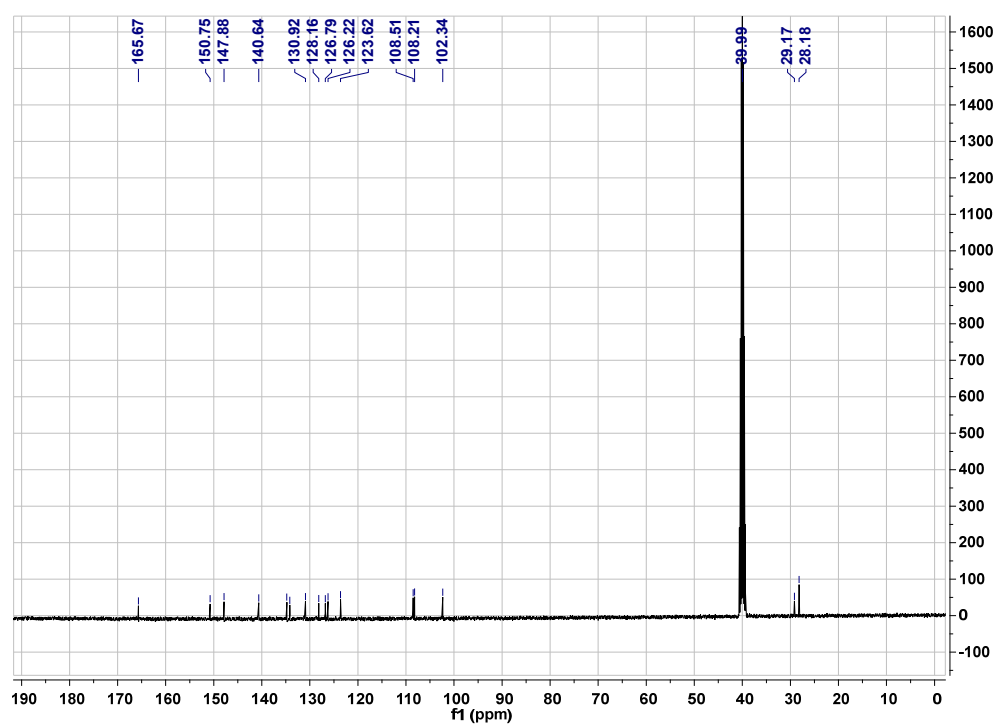
PZ2



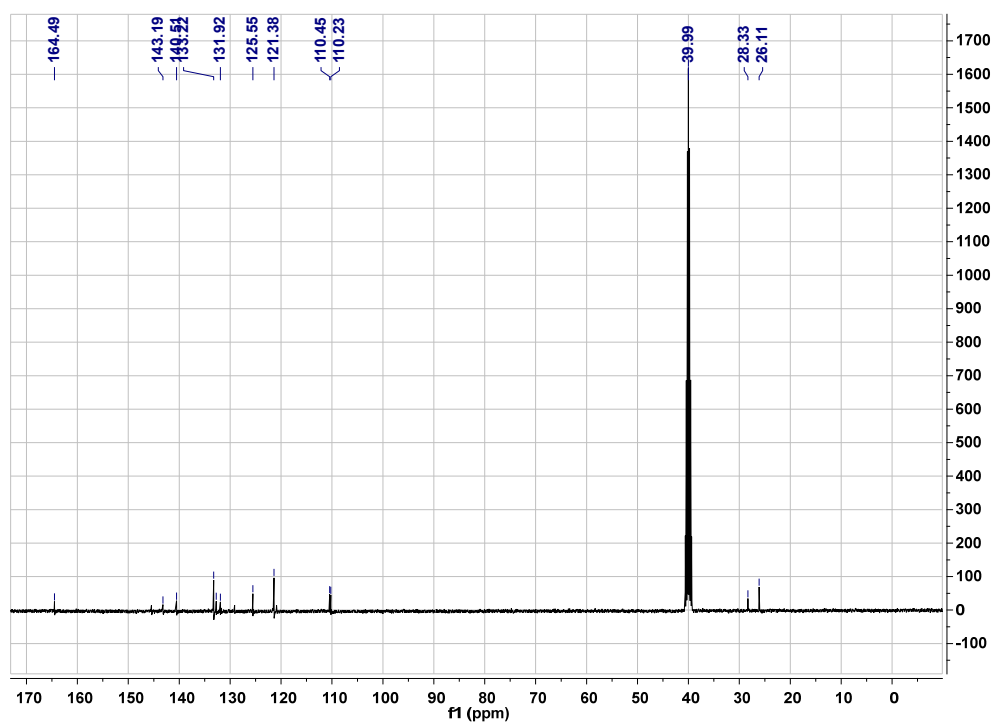
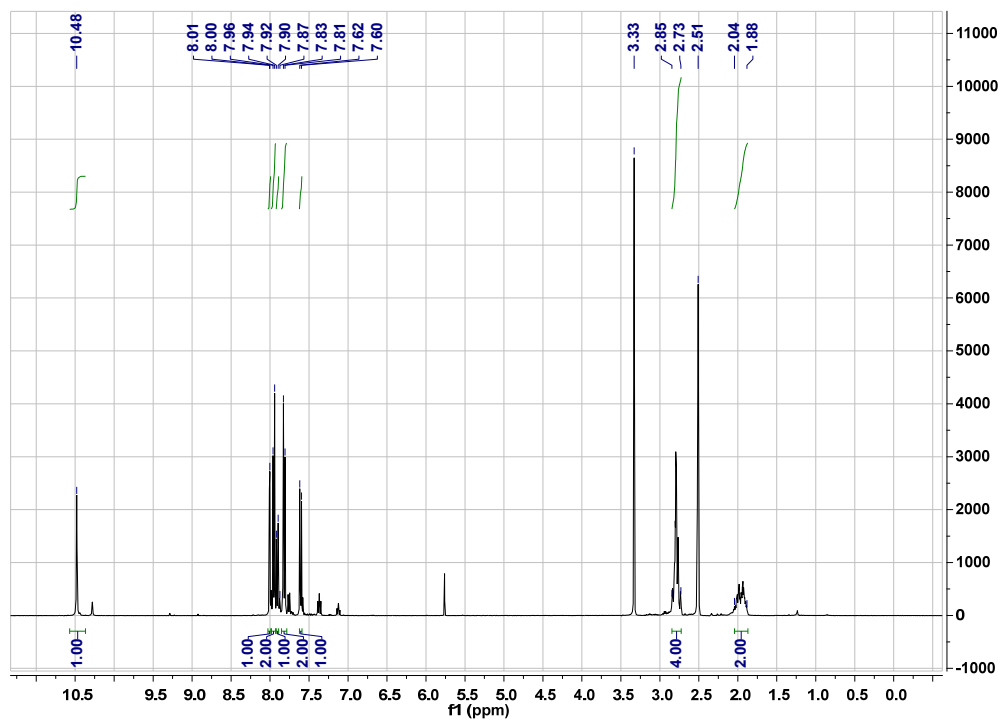


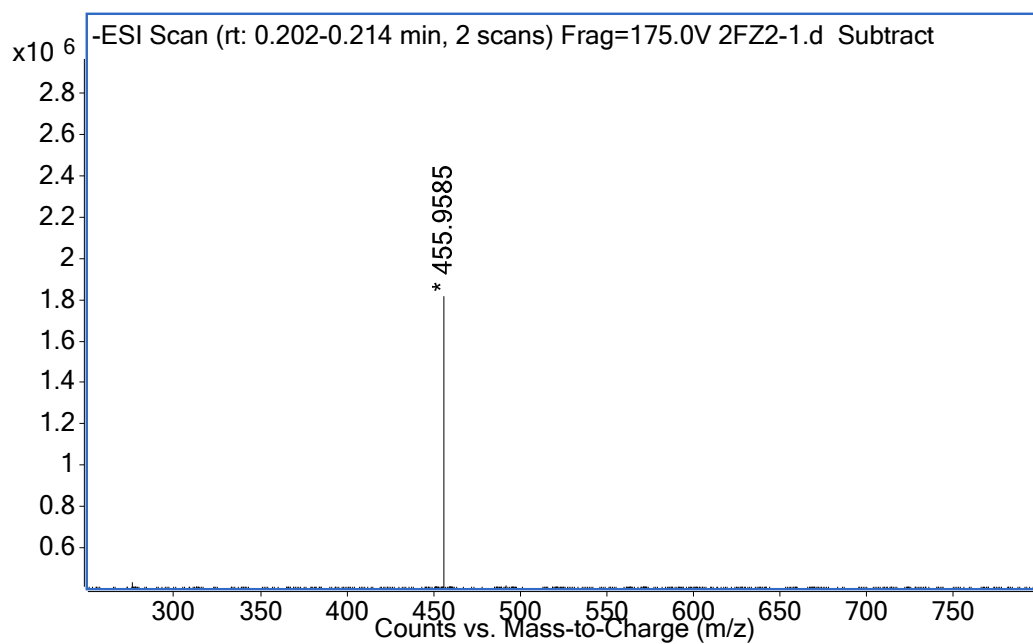
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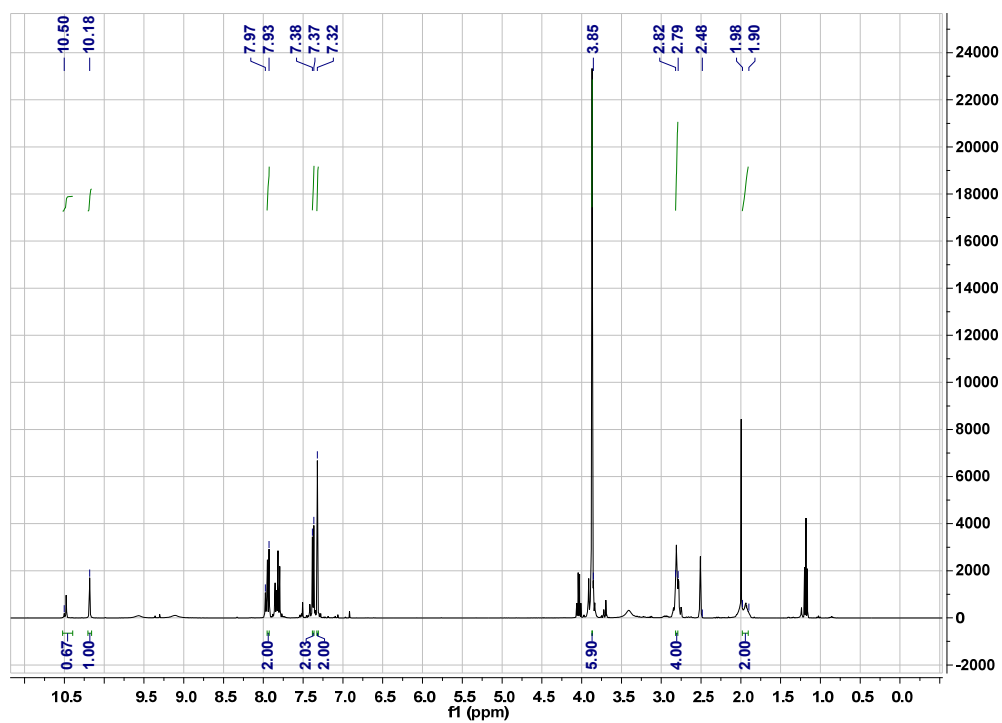


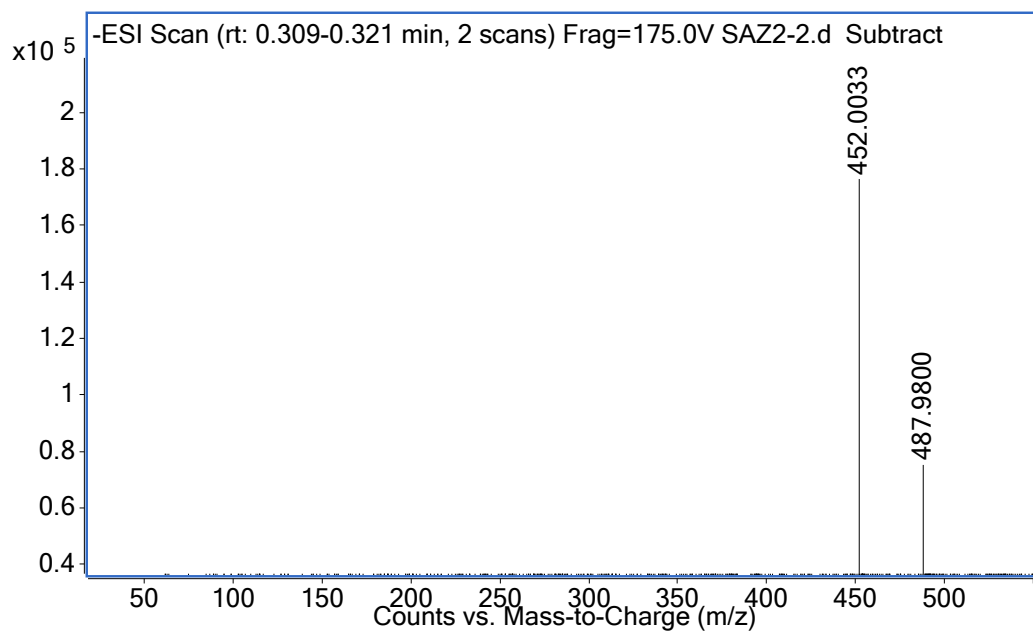
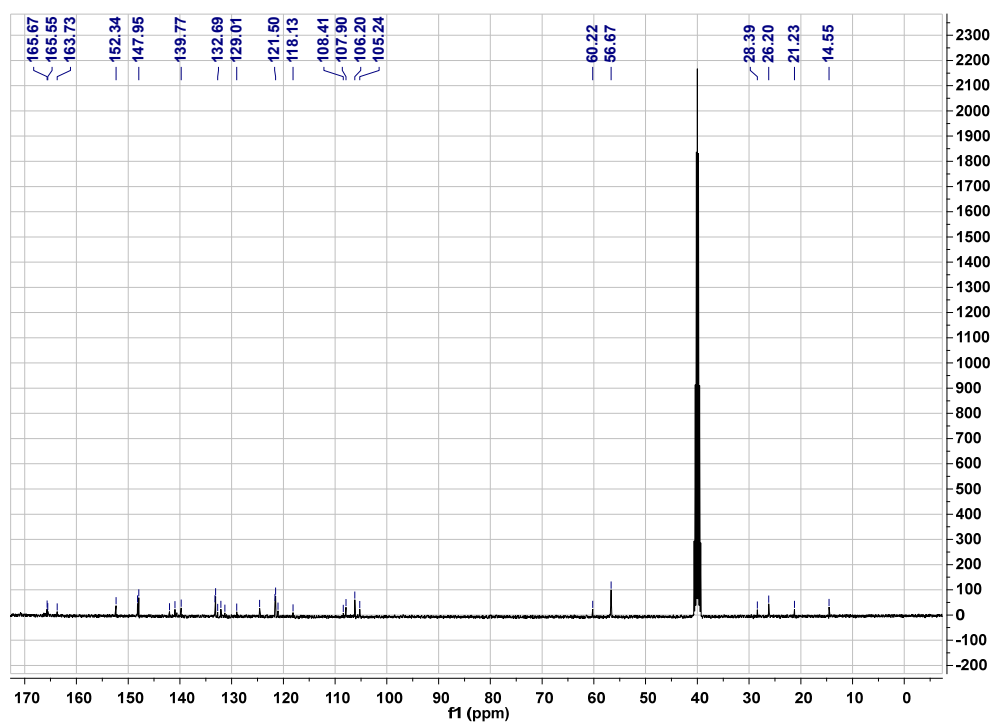
PFZ2



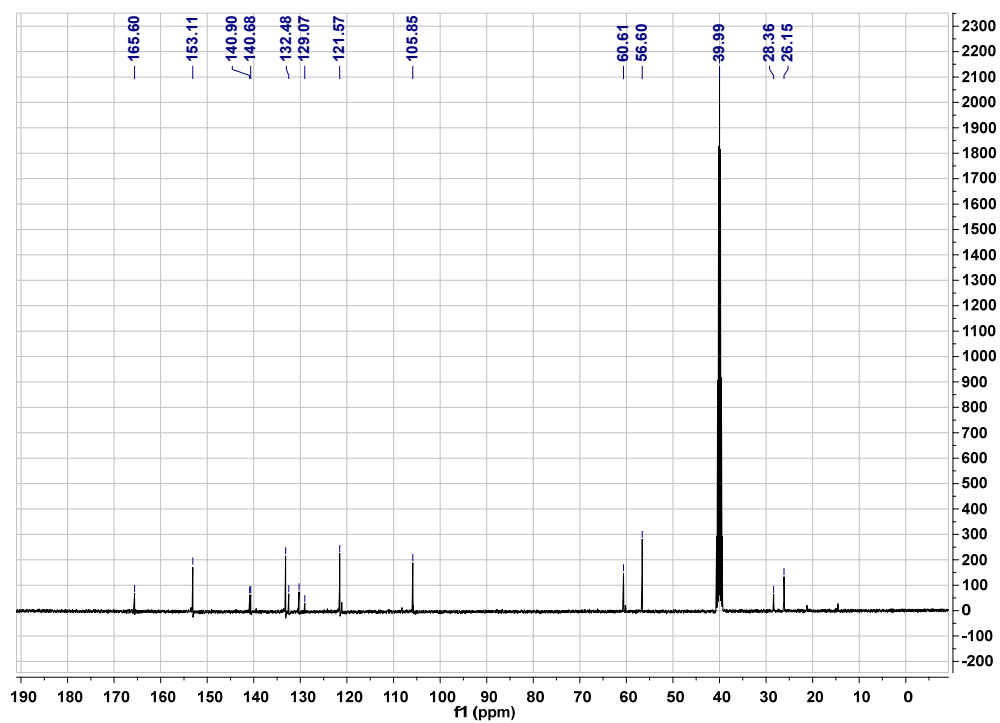
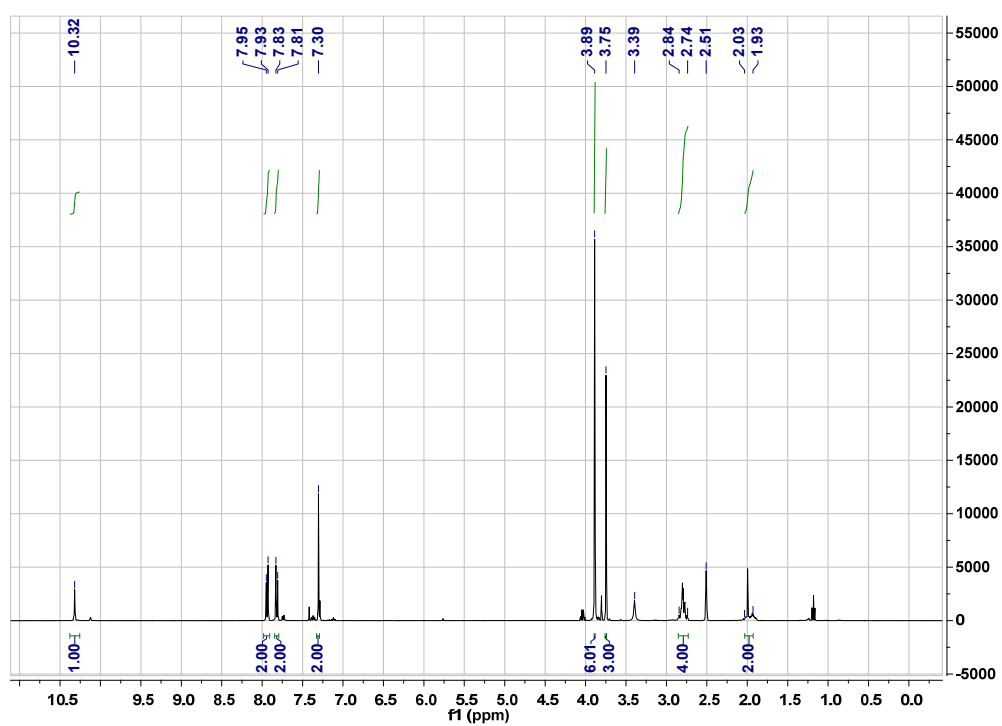


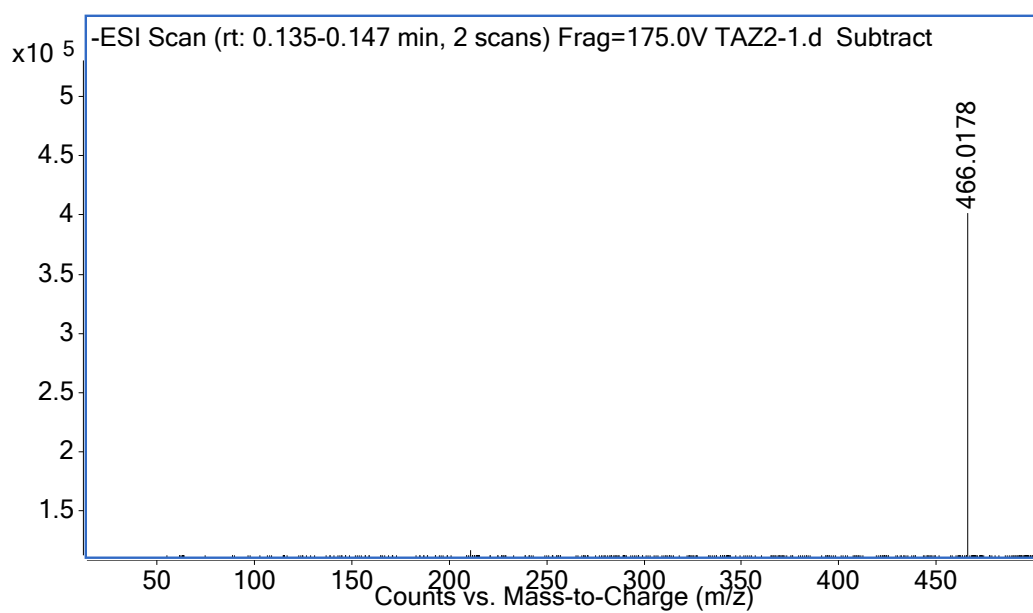
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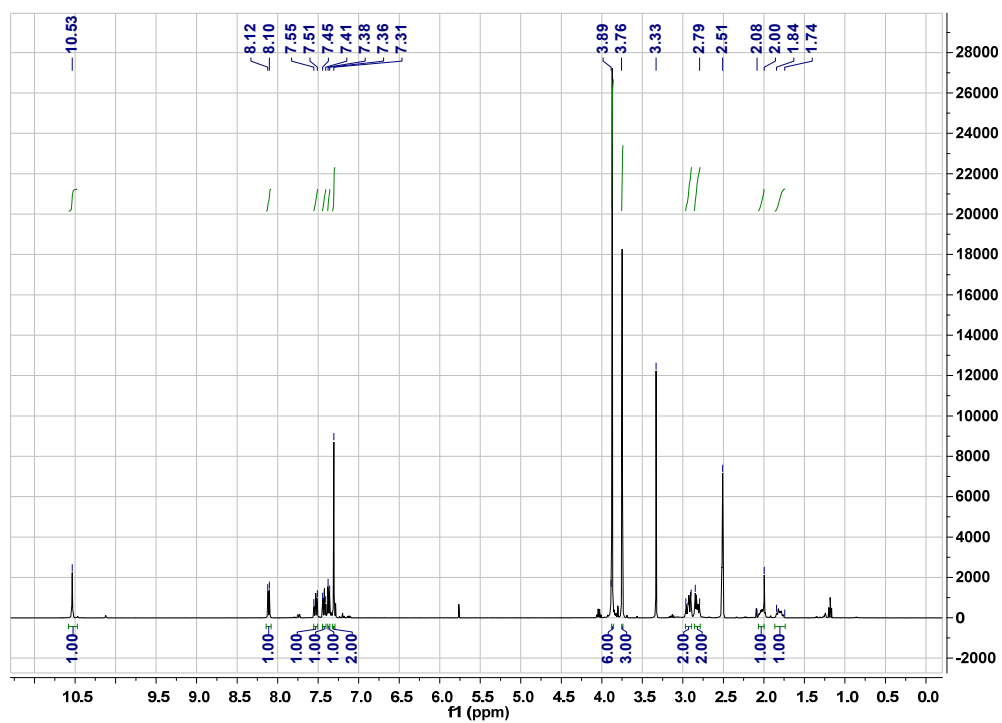


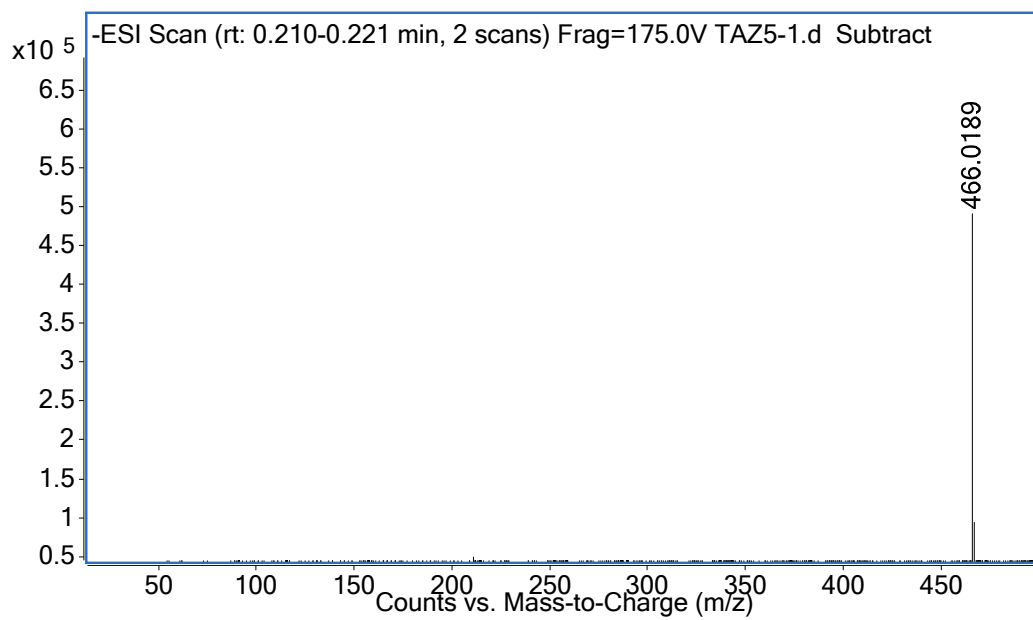
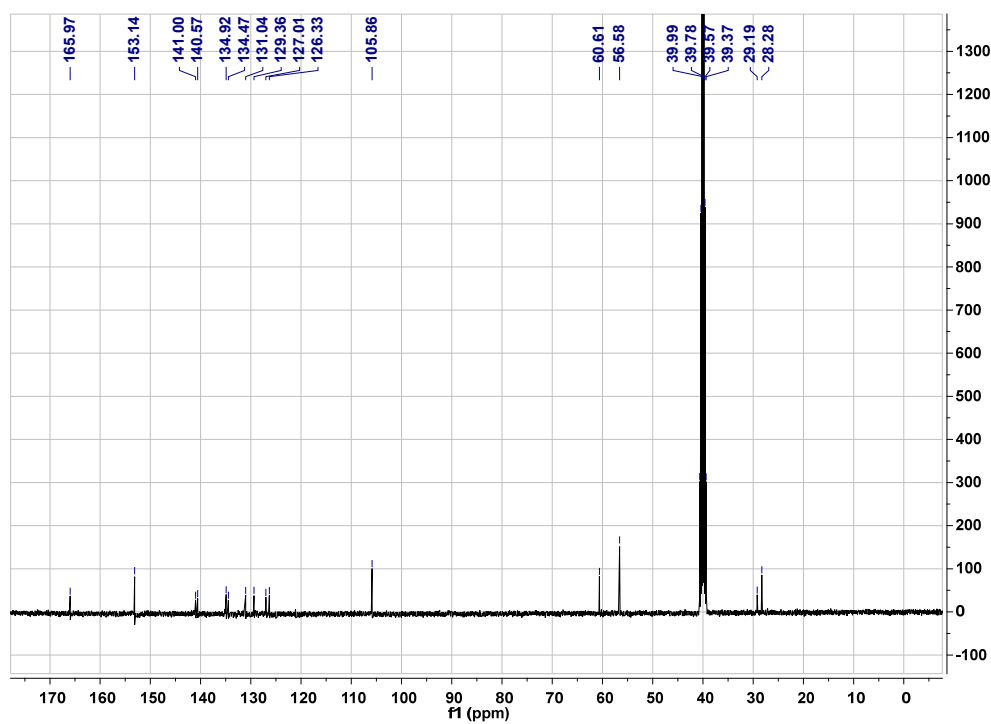
TAZ2



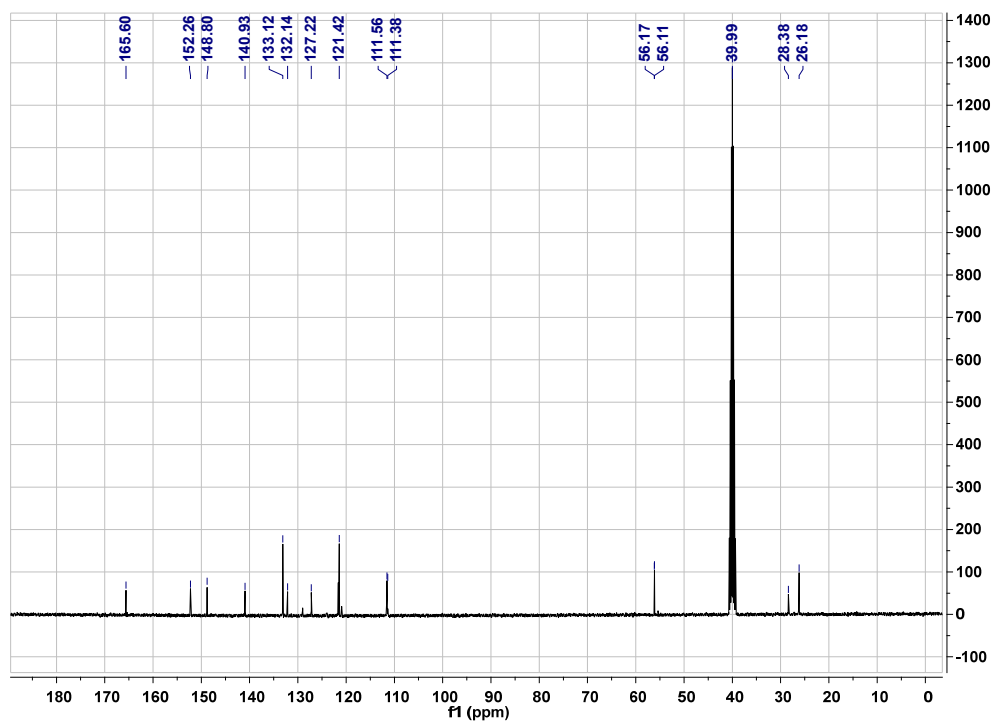
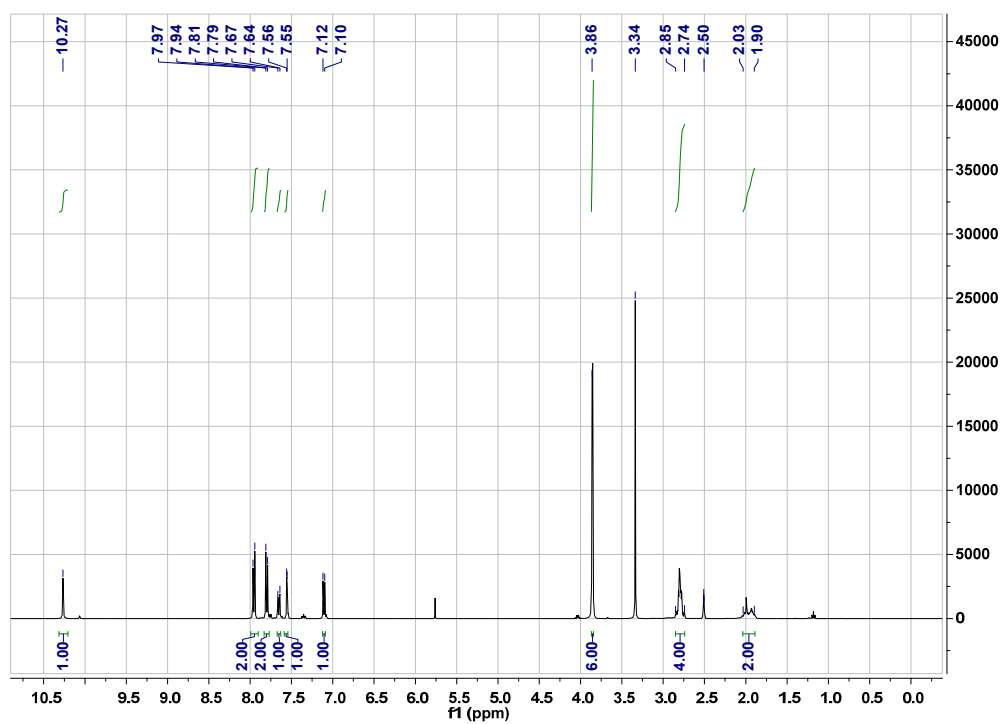


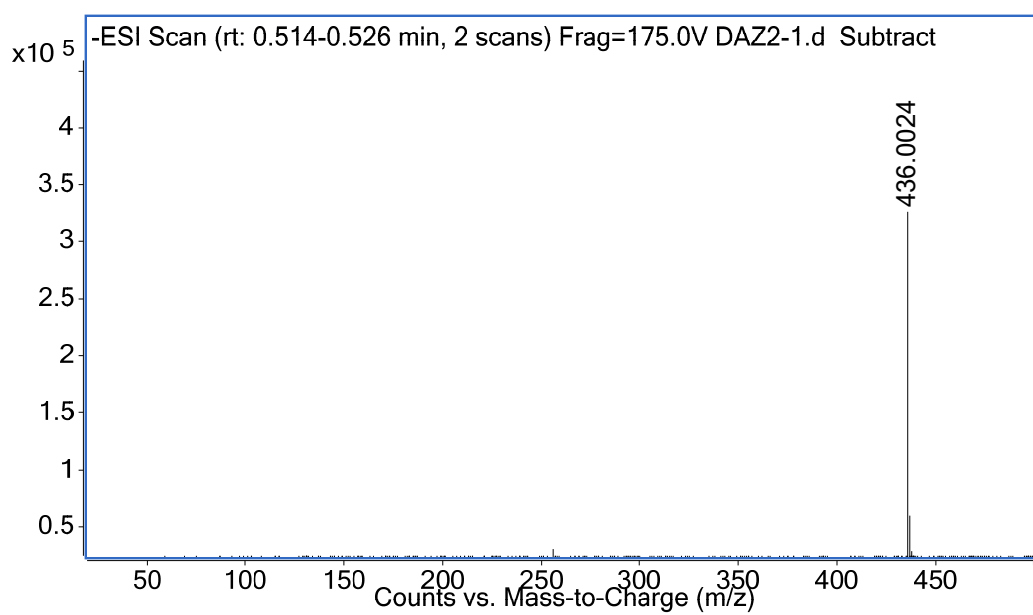
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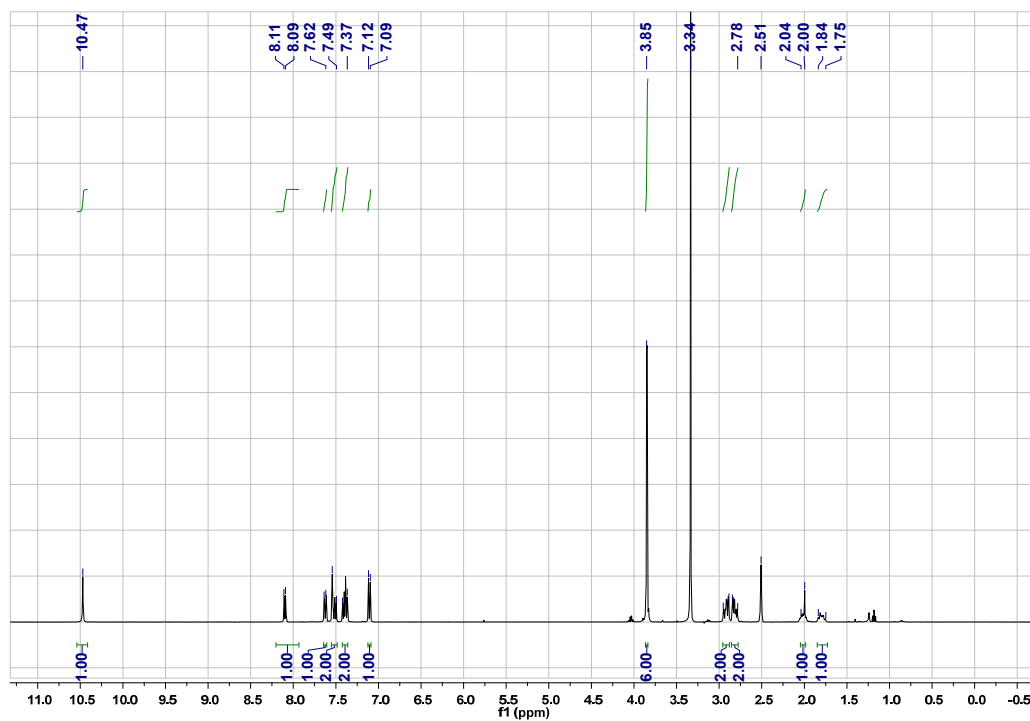


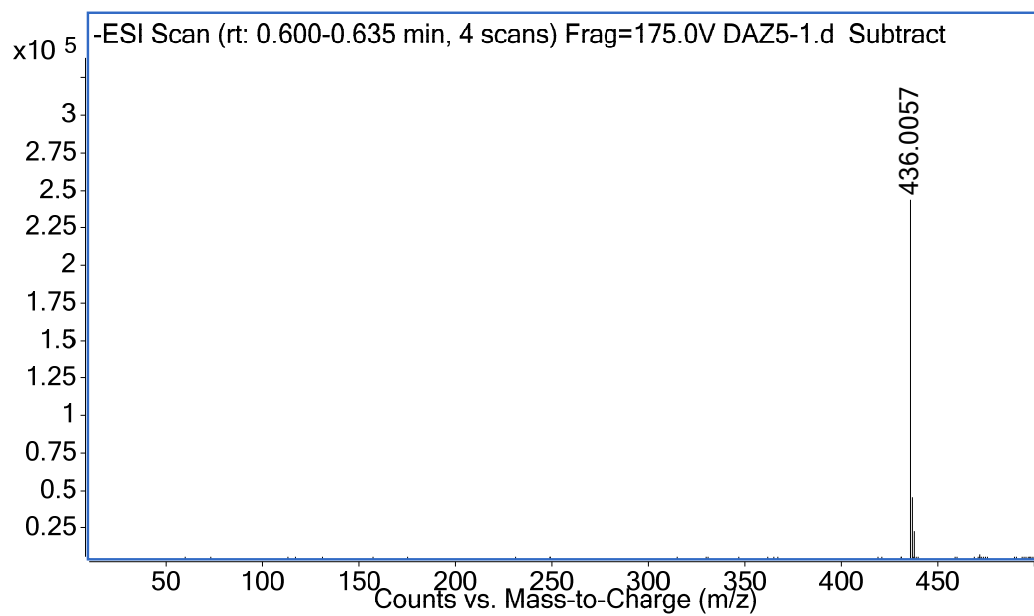
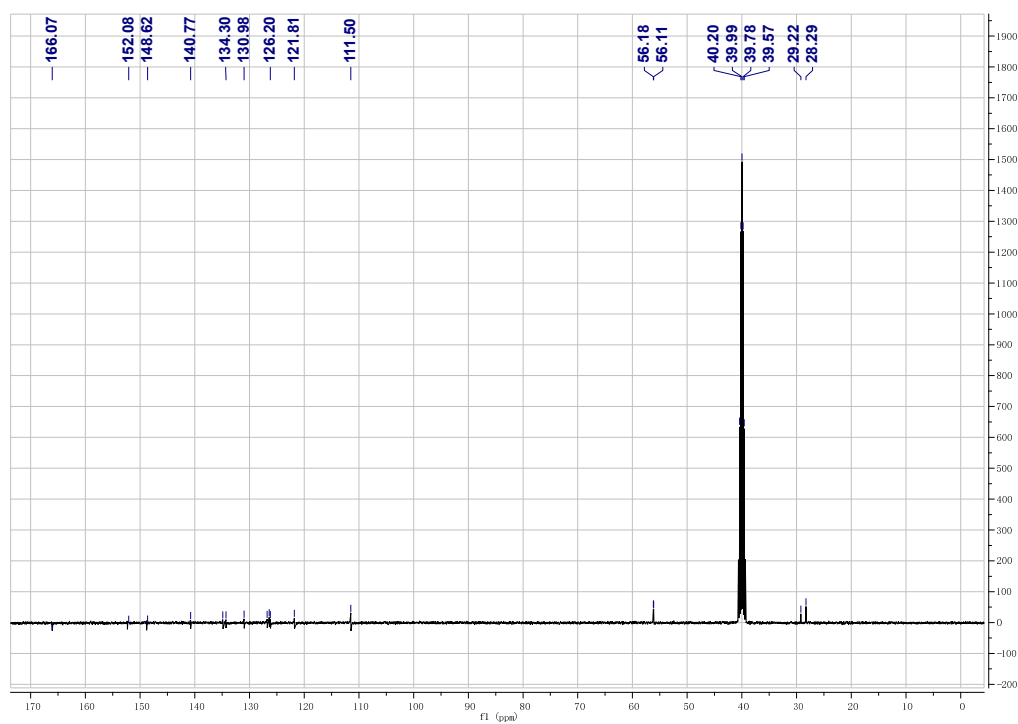
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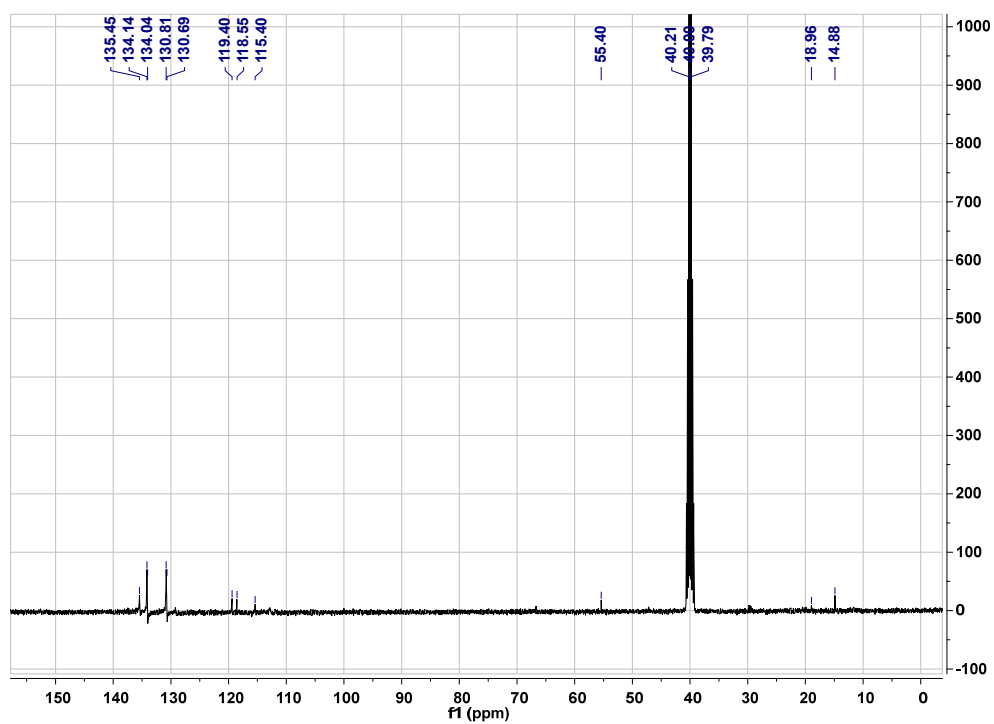
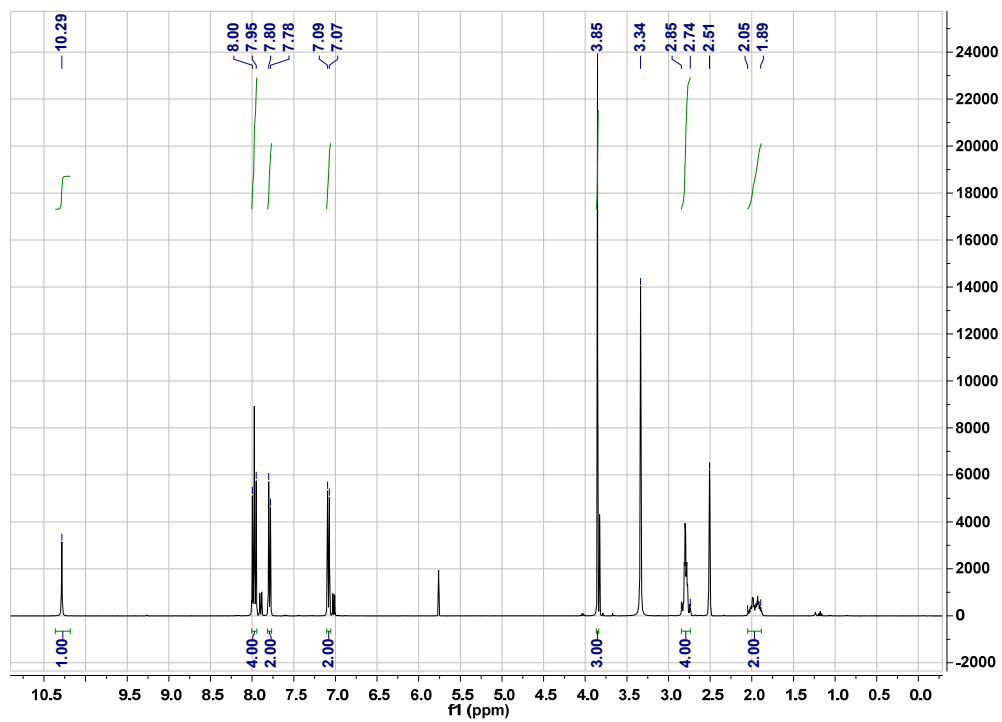


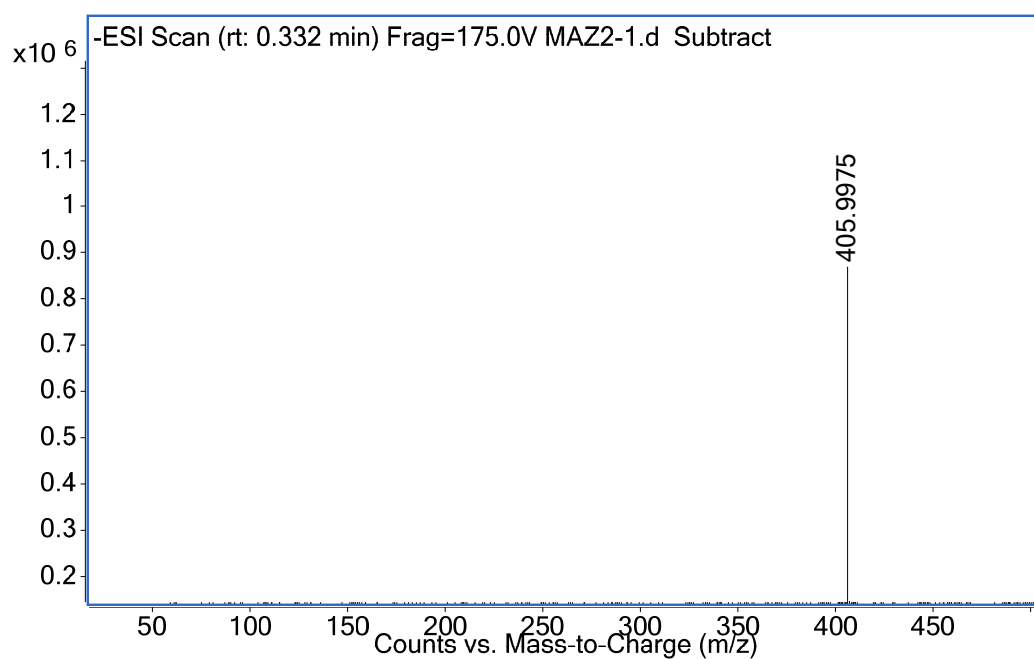
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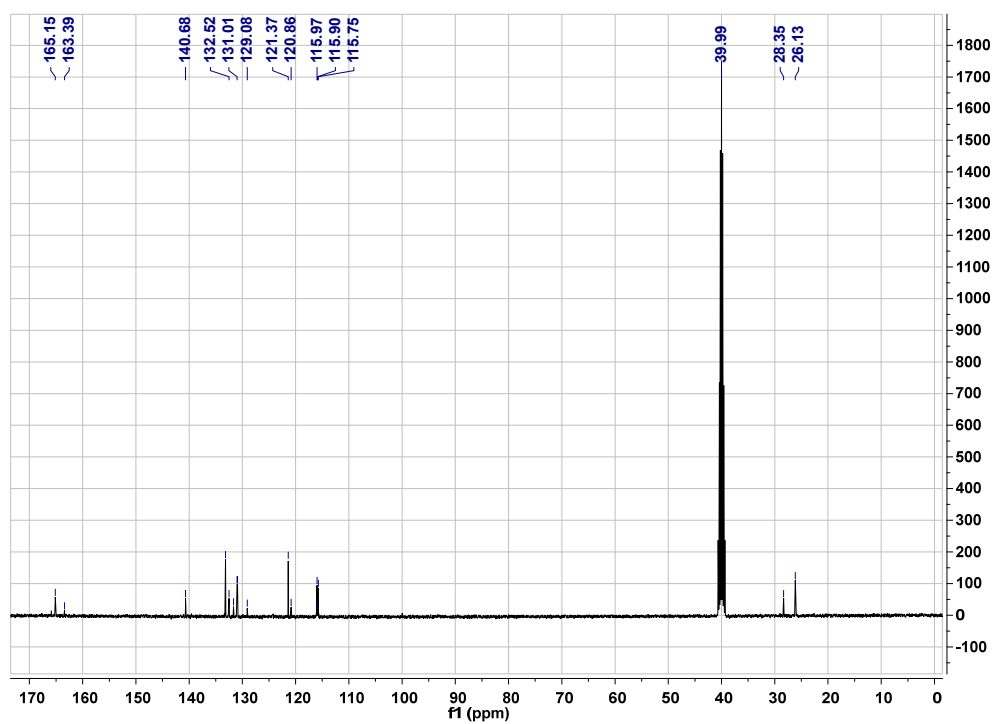
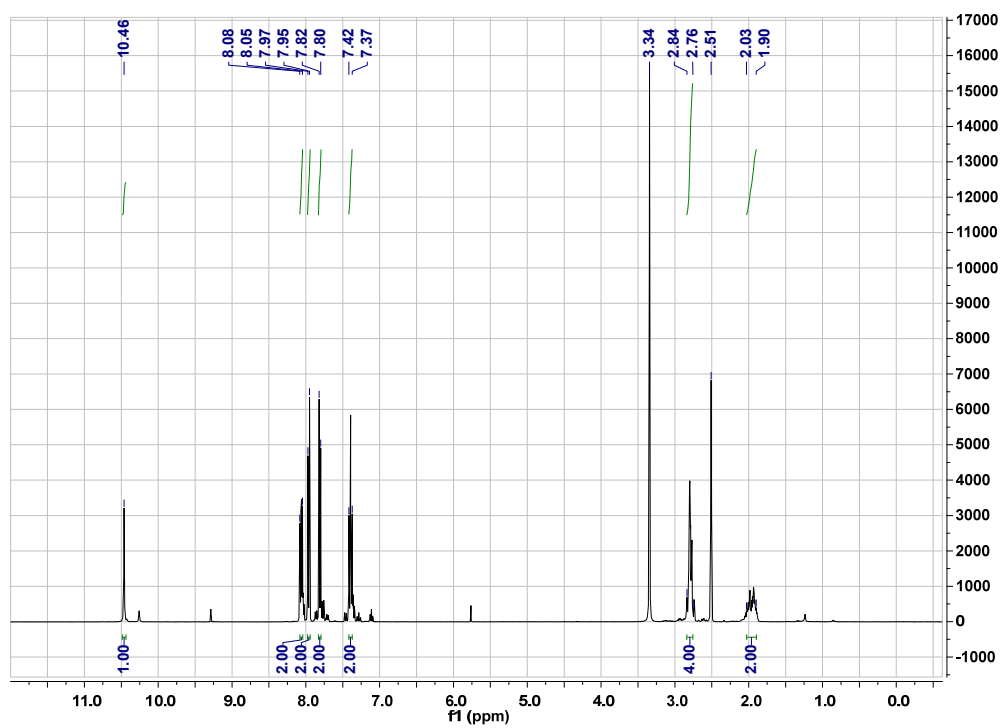


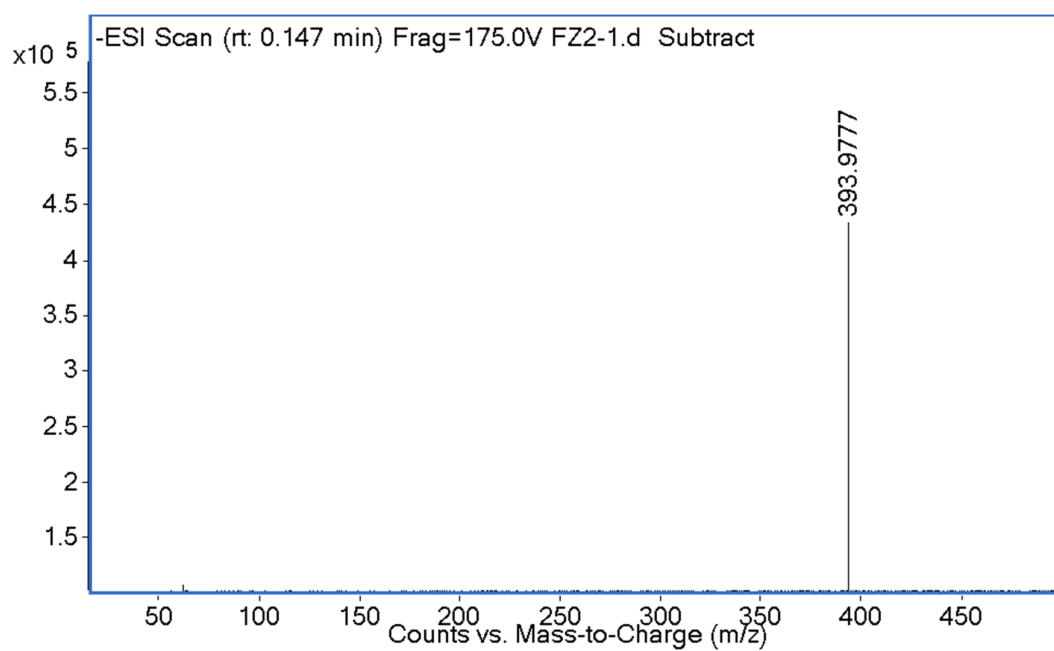
MAZ2



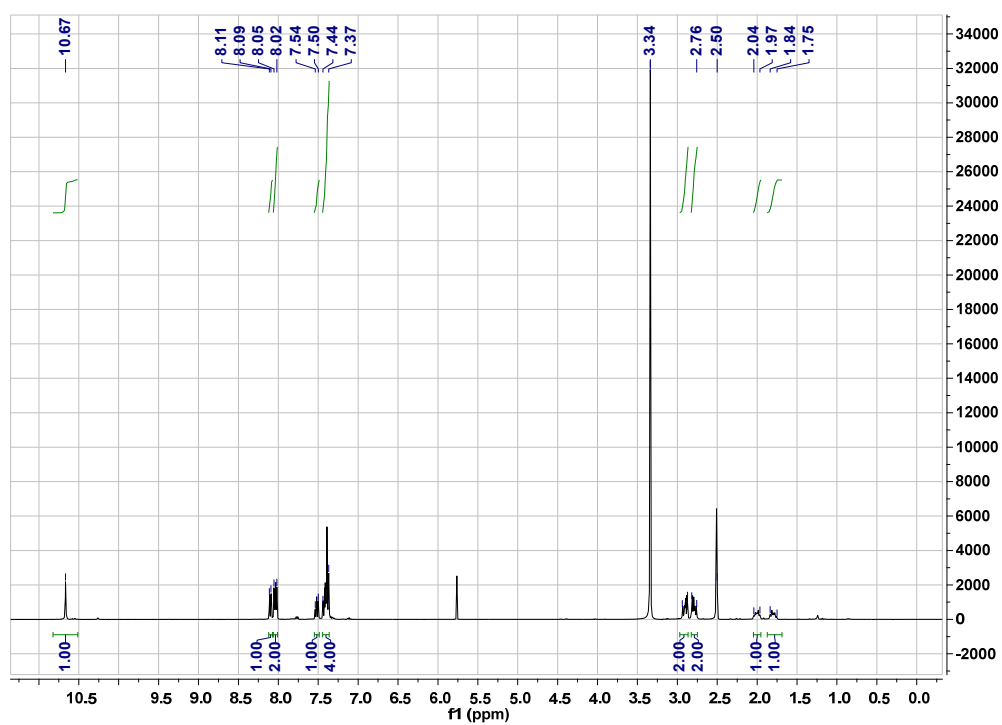


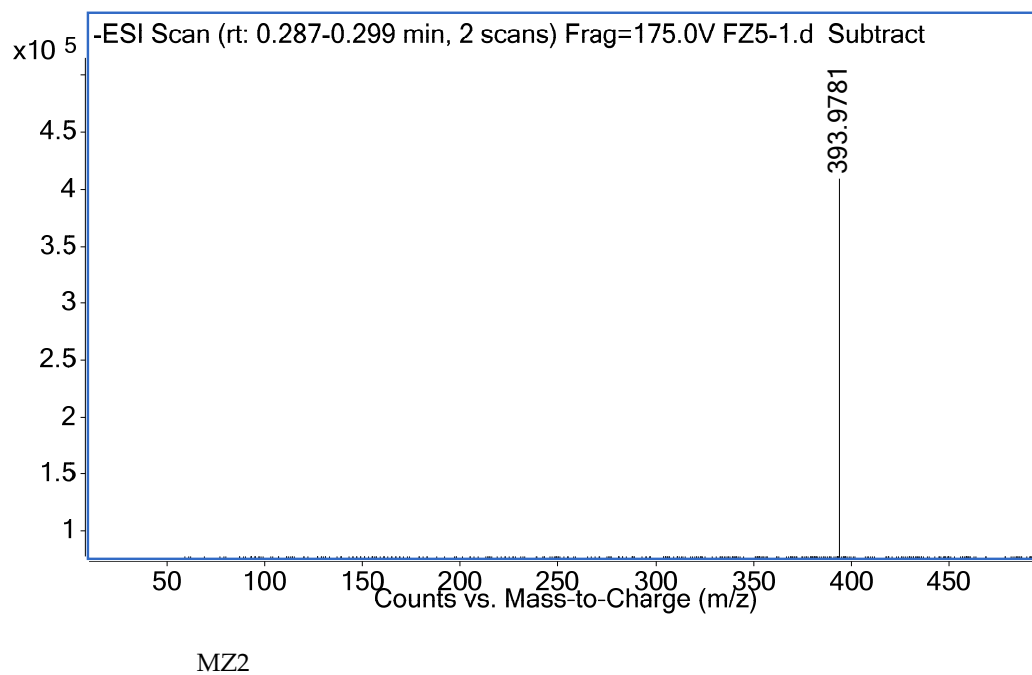
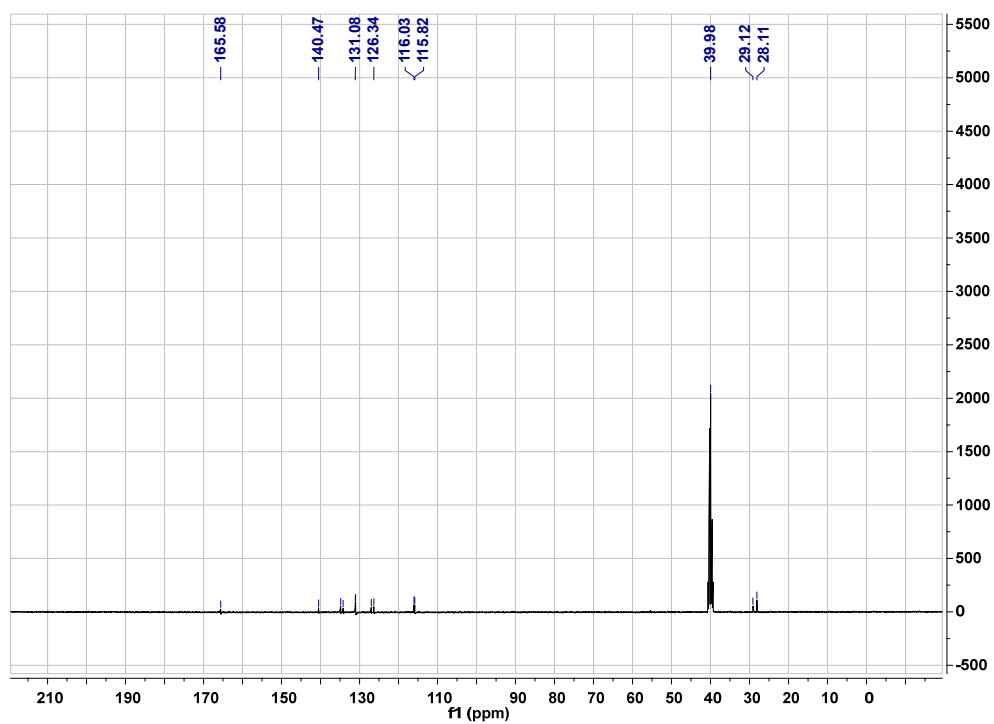
FZ2

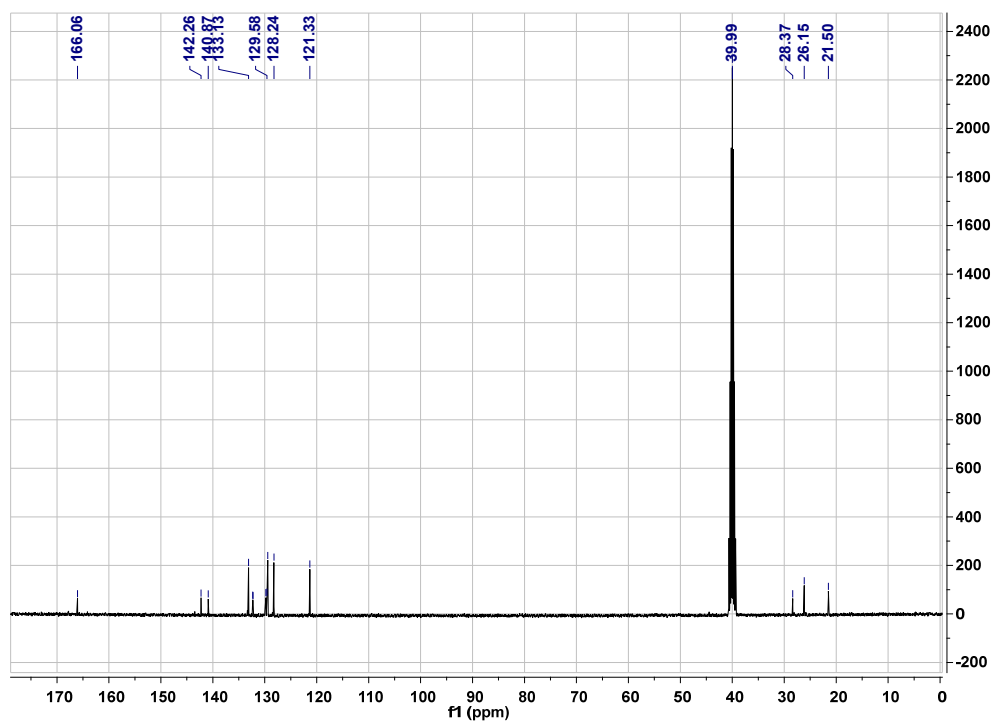
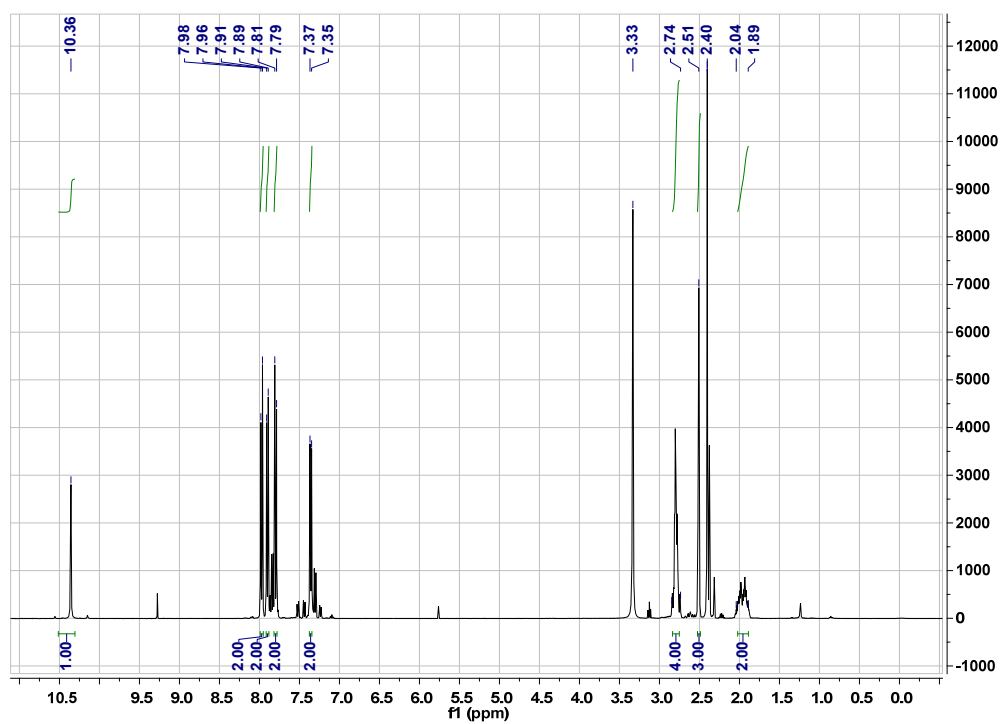


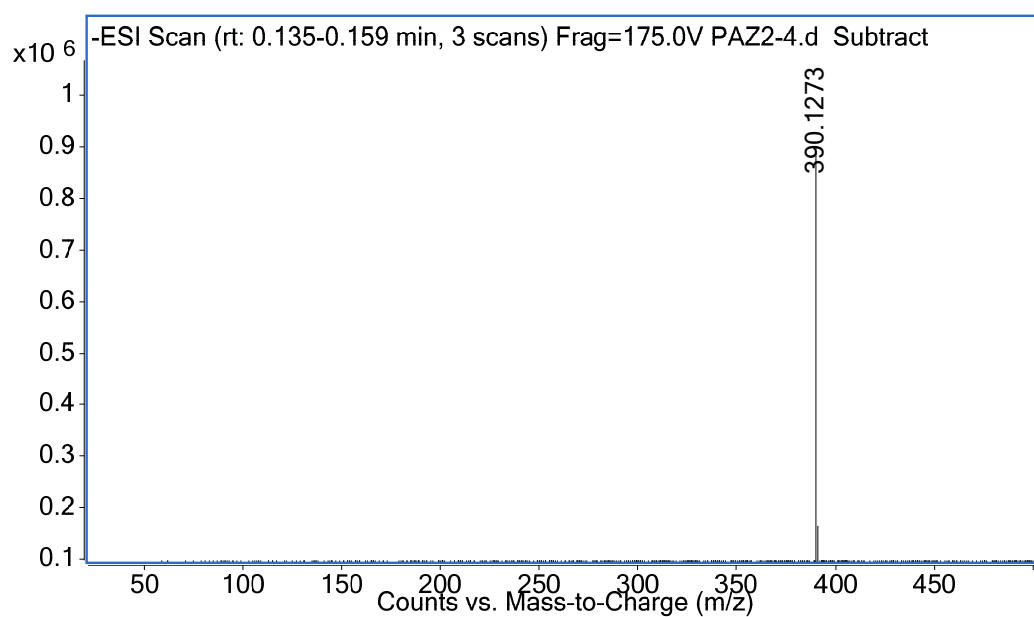


FZ5

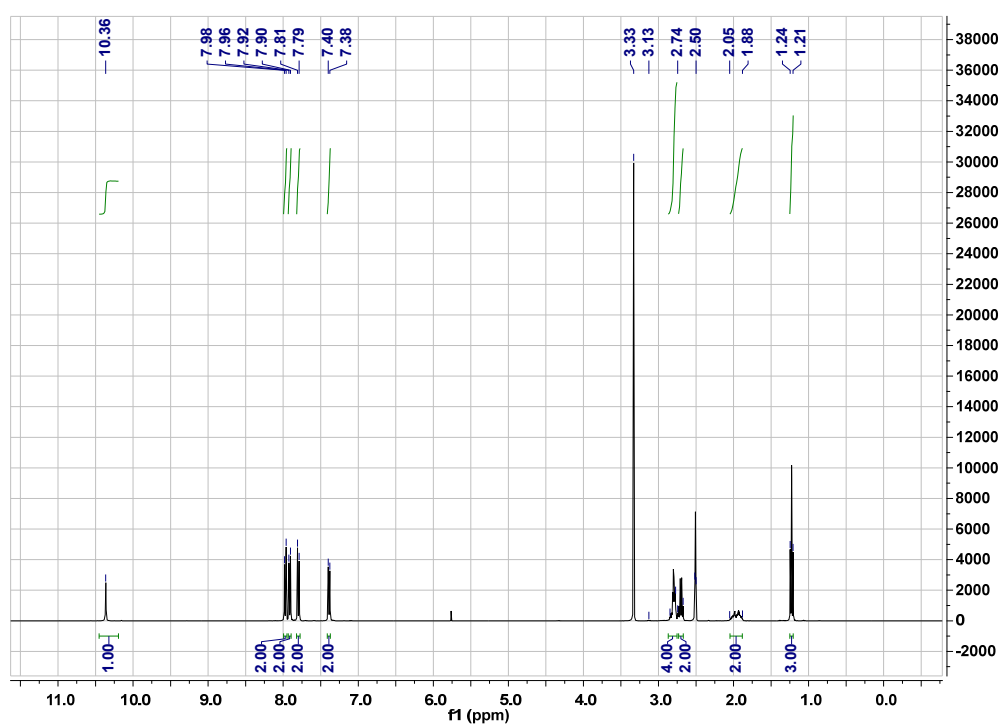


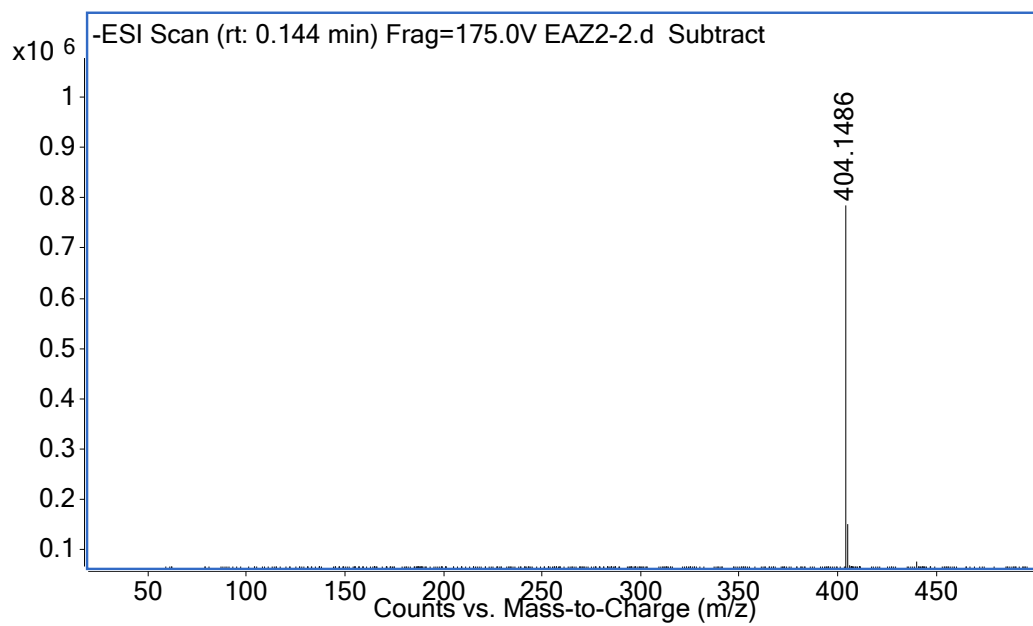
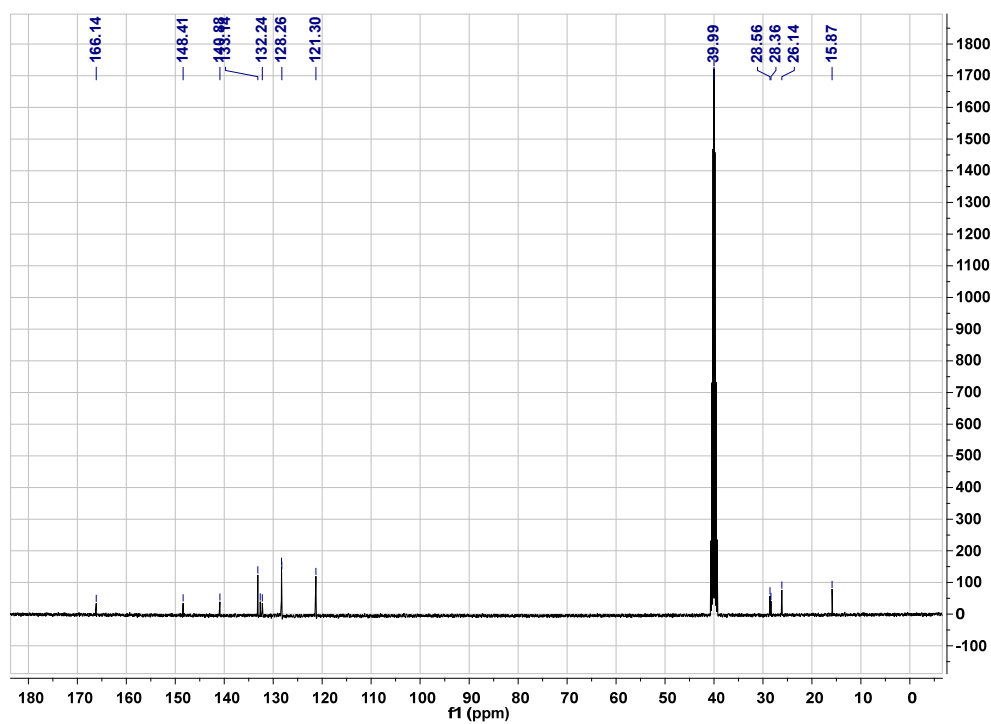




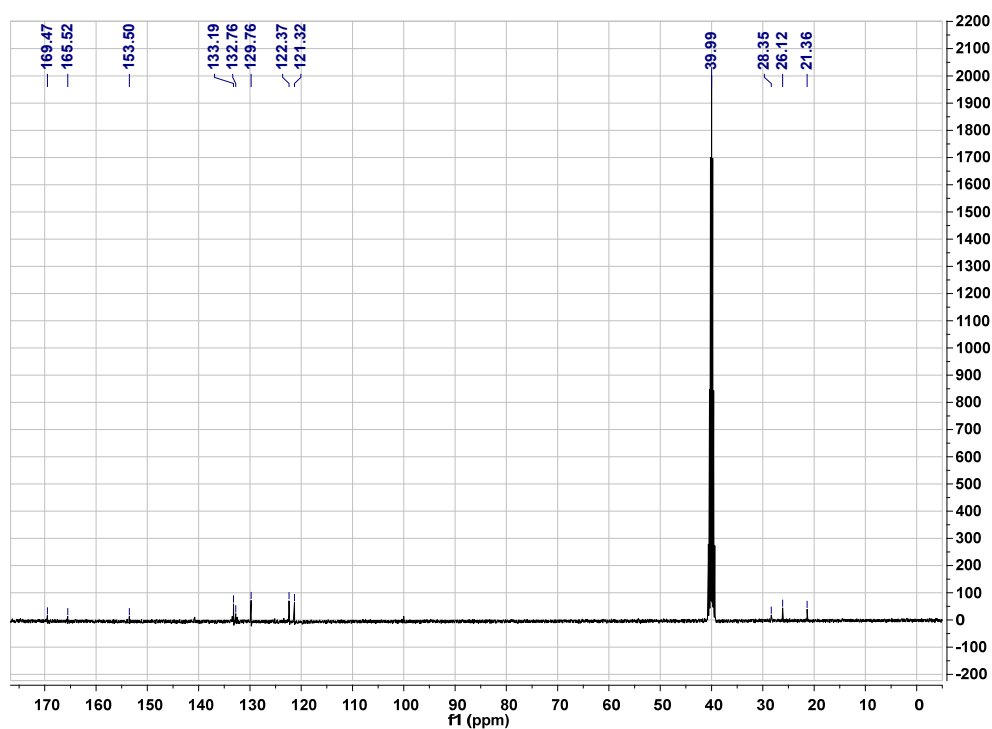
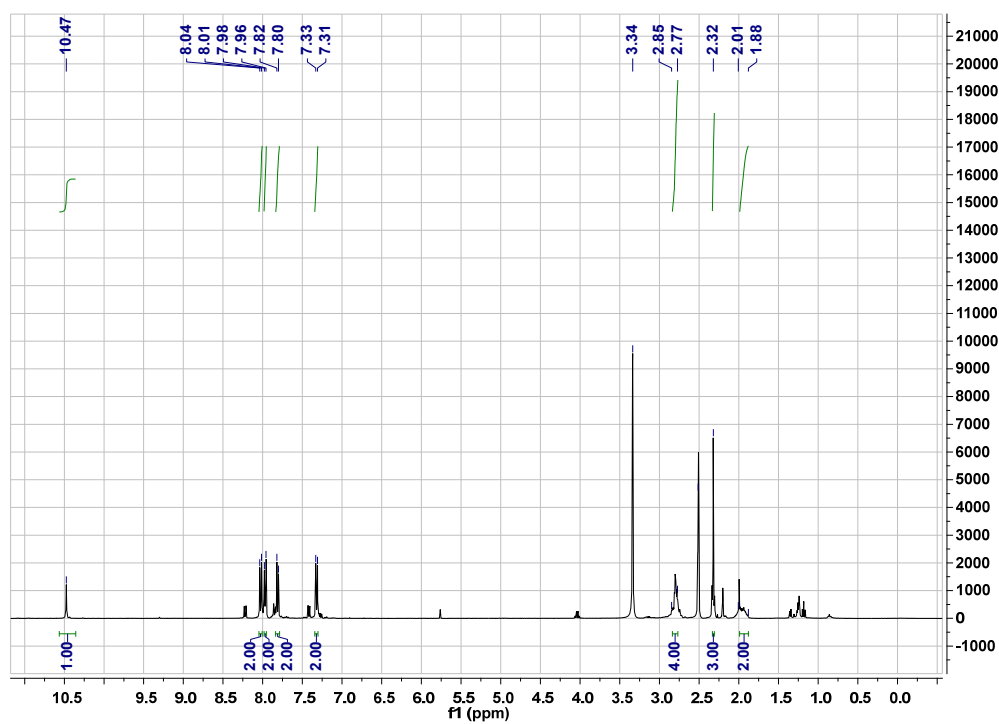


EZ2-003191





AZ2



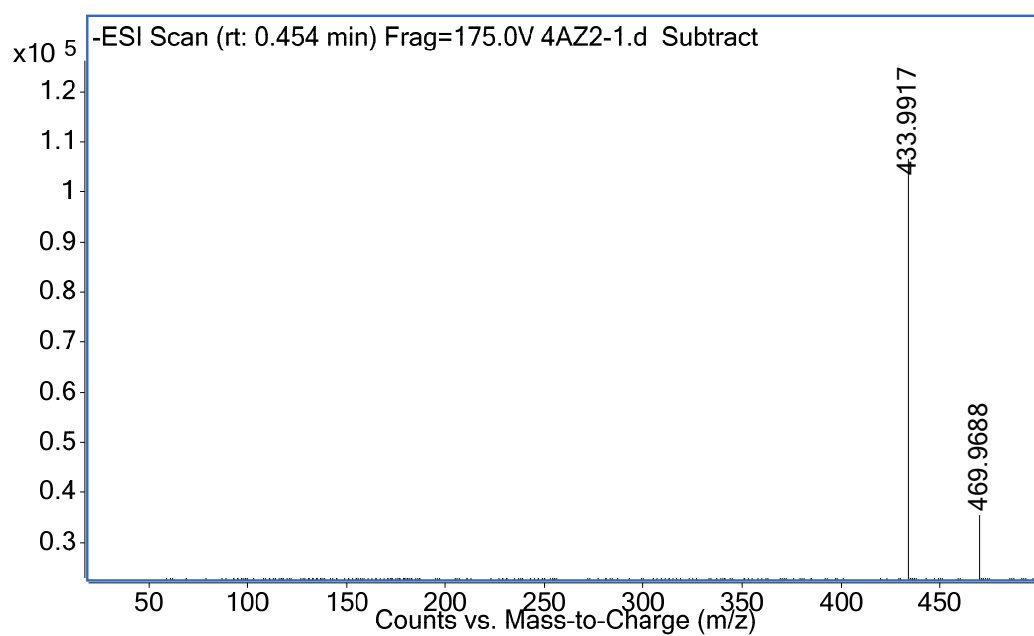


Figure S1. ^1H and ^{13}C NMR and Mass spectra of all the synthesized compounds.

MTT assay of arsenicals to 6 cell lines

Table S1. Inhibition effect of organic arsenicals 24 h incubation.

<i>IC</i> ₅₀ /μM	Molm	NB4	4T1	Hela	COS-7	PBMC
Z2	0.60±0.01	1.50±0.05	1.67±0.14	1.01±0.01	6.1±0.2	4.6±0.3
Z5	0.45±0.01	0.40±0.03	0.87±0.09	0.30±0.13	1.75±0.08	3.5±0.3
PZ2	1.06±0.03	0.85±0.10	2.55±0.22	0.70±0.05	4.1±0.1	6.4±0.2
PZ5	0.27±0.09	0.3±0.03	0.40±0.04	0.26±0.03	1.01±0.02	5.0±0.1
PFZ2	0.80±0.02	0.50±0.02	1.00±0.11	0.50±0.12	6.0±0.1	6.3±0.1
HDZ2	0.60±0.12	0.80±0.11	1.00±0.11	2.0±0.2	9.1±0.2	5.8±0.2
TAZ2	1.60±0.12	1.00±0.11	3.5±0.1	2.0±0.2	9.1±0.15	7.3±0.3
TAZ5	0.71±0.02	0.20±0.03	0.70±0.03	0.80±0.04	2.0±0.1	2.2±0.1
DAZ2	0.90±0.09	1.71±0.12	1.50±0.12	2.3±0.1	7.1±0.1	6.2±0.3
DAZ5	0.45±0.06	0.80±0.01	0.60±0.10	0.81±0.03	1.61±0.09	4.1±0.3
MAZ2	0.20±0.09	0.40±0.12	0.80±0.12	0.40±0.06	5.2±0.1	7.6±0.2
FZ2	1.20±0.11	0.40±0.02	2.3±0.2	1.20±0.10	3.5±0.1	5.5±0.2
FZ5	0.50±0.01	0.15±0.1	0.70±0.02	0.35±0.10	1.10±0.21	2.4±0.2
MZ2	0.20±0.09	0.40±0.12	0.80±0.12	0.40±0.06	5.2±0.1	3.4±0.2
EZ2	1.10±0.10	0.41±0.05	1.40±0.12	1.01±0.12	4.2±0.1	3.7±0.2
AZ2	1.11±0.10	0.60±0.05	2.0±0.1	0.80±0.12	2.2±0.1	5.2±0.3

Table S2. Inhibition effect of organic arsenicals 48 h incubation.

<i>IC</i> ₅₀ /μM	Molm	NB4	4T1	Hela	COS-7	PBMC
Z2	0.53±0.02	1.20±0.10	1.89±0.22	0.70±0.03	3.2±0.1	4.0±0.2
Z5	0.41±0.02	0.30±0.01	0.84±0.12	0.25±0.20	0.81±0.05	2.8±0.2
PZ2	0.75±0.03	0.70±0.06	3.35±0.42	0.44±0.05	1.75±0.11	5.3±0.3
PZ5	0.24±0.08	0.2±0.02	0.83±0.06	0.14±0.01	0.82±0.04	4.5±0.2
PFZ2	0.70±0.03	0.30±0.01	0.80±0.11	0.15±0.03	3.5±0.1	5.7±0.2
HDZ2	0.60±0.12	0.50±0.05	1.01±0.12	1.01±0.11	3.5±0.1	4.9±0.1
TAZ2	1.50±0.12	0.60±0.05	2.2±0.1	1.04±0.11	5.2±0.11	6.5±0.2
TAZ5	0.70±0.04	0.15±0.01	0.70±0.03	0.50±0.10	1.80±0.12	1.54±0.32
DAZ2	0.75±0.11	1.14±0.09	1.20±0.11	1.06±0.05	3.3±0.2	5.7±0.1
DAZ5	0.32±0.03	0.51±0.08	0.50±0.11	0.55±0.13	1.27±0.15	3.5±0.2
MAZ2	0.10±0.11	0.30±0.09	0.40±0.11	0.30±0.05	3.3±0.2	6.9±0.1
FZ2	1.00±0.11	0.30±0.1	2.2±0.1	0.40±0.05	2.2±0.1	4.7±0.2
FZ5	0.50±0.02	0.15±0.02	0.60±0.01	0.15±0.05	0.80±0.05	1.82±0.12
MZ2	0.10±0.11	0.30±0.09	0.40±0.11	0.30±0.05	3.3±0.2	2.7±0.3
EZ2	1.02±0.02	0.30±0.03	1.40±0.12	0.81±0.11	2.1±0.1	2.9±0.3
AZ2	1.01±0.02	0.60±0.03	1.00±0.12	0.50±0.11	2.1±0.1	4.9±0.3

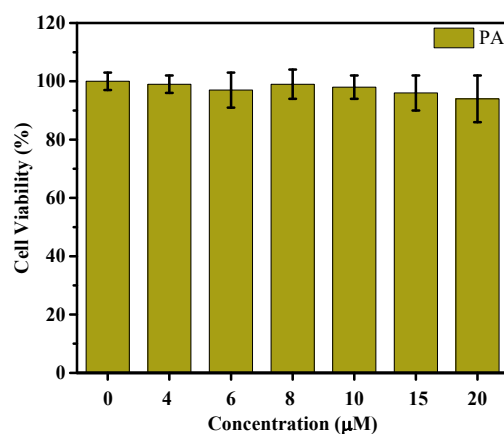


Figure S2. MTT assay of piperonylic acid (PA), precursor of PZ2.

MAZ2 inhibited TrxR activity by binding to the C-terminal Sec/Cys pair

Table S3. The distance of arsenic and active sites of TrxR or Trx, and the binding energy.

			Z2	MAZ2
distance (Å)	TrxR	Cys497	10.6	7.4
		Sec498	6.0	4.7
	Trx	Cys32	9.3	10.1
		Cys35	7.6	8.6
binding energy (kcal/mol)	TrxR		-3.90	-5.20
	Trx		-3.83	-4.12

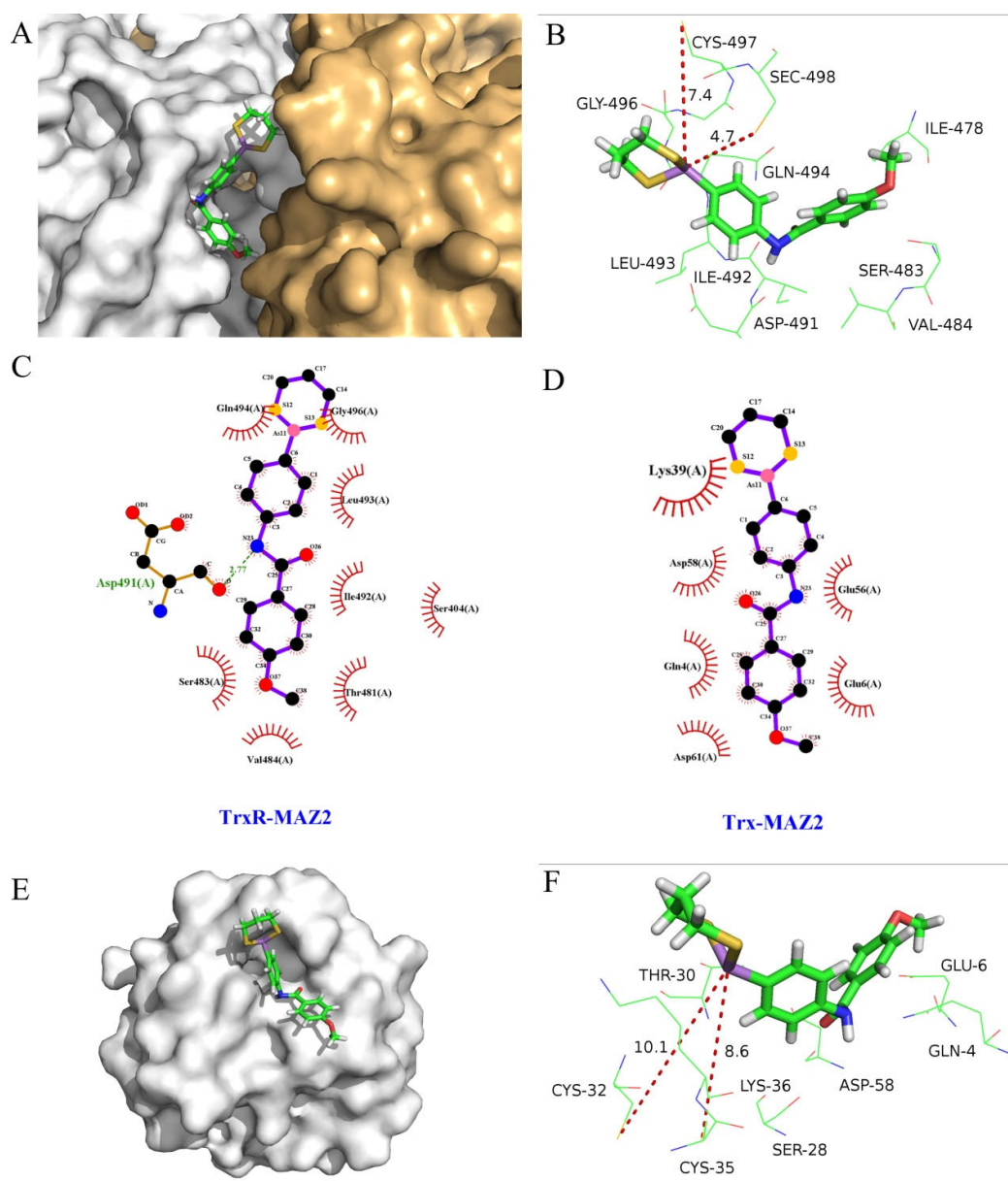


Figure S3. Binding site of MAZ2 in TrxR1 (PDB: 3EAN) and Trx1(PDB: 1ERT).

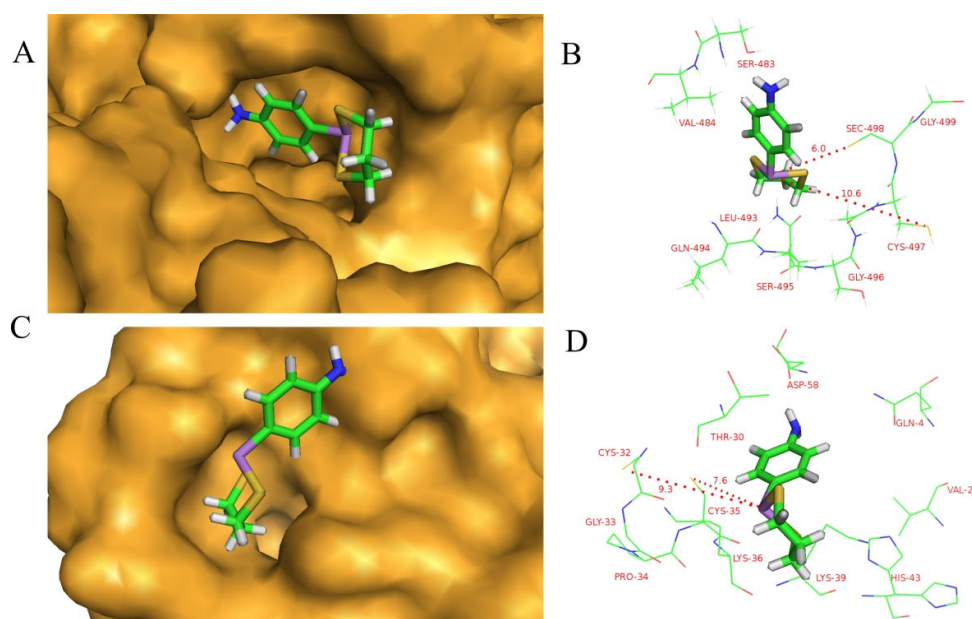


Figure S4. Binding site of Z2 in TrxR1 (PDB: 3EAN) and Trx1(PDB: 1ERT).

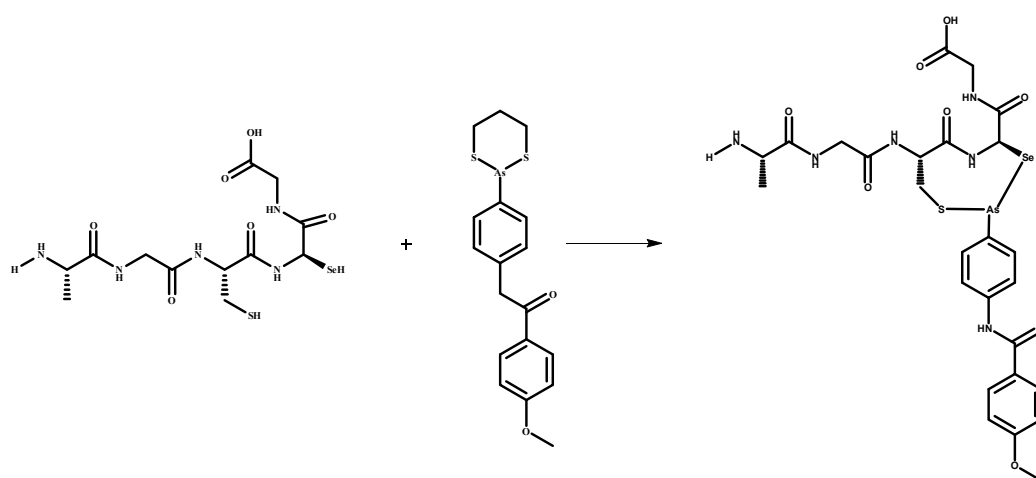


Figure S5. Reaction of AGCUG and MAZ2.

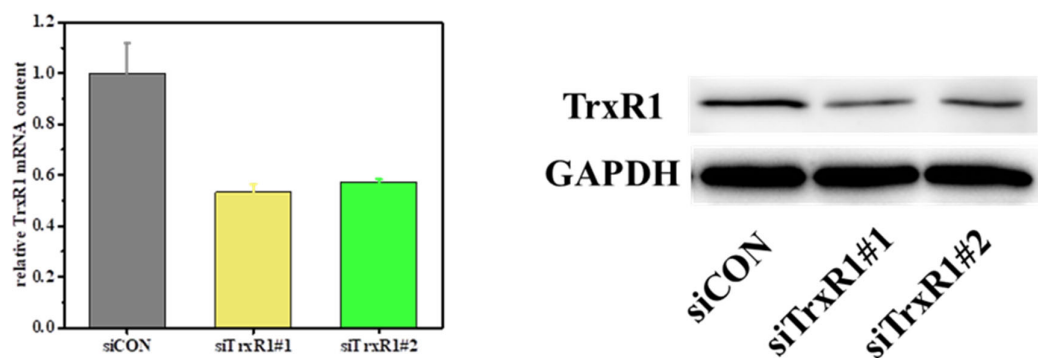


Figure S6. TrxR was knocked down by two siRNA, proved by PCR and WB.

MAZ2 administration had no obvious side effects

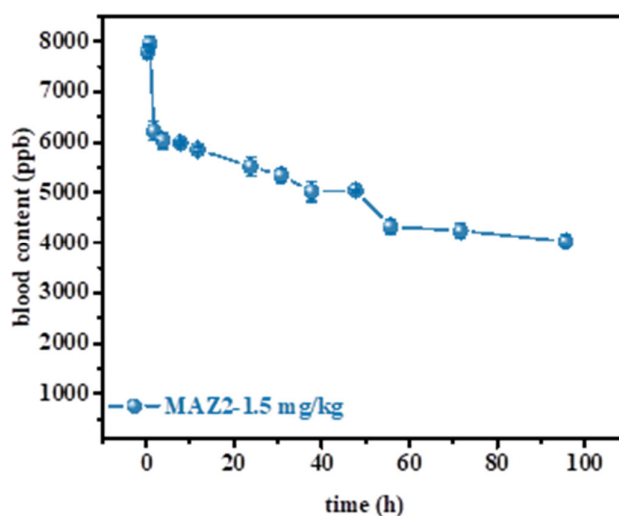


Figure S7. Arsenic content of rat blood administrated with 1.5 mg/kg MAZ2.

siRNA

Table S4. sequence of siRNA.

	sense (5'-3')	antisense (5'-3')
TrxR1-#1	CAACCUUAAAGACGAUGAATT	UUCAUCGUCUUUAAGGUUGTT
TrxR1-#2	GGAAGAGAUUCUUGUACGATT	UCGUACAAGAAUCUCUUCCTT

PCR

Table S5. sequence of primer.

Gene	sequences
<i>TrxR1</i>	Forward 5'-TCCACAAACAGCGAGGAGAC -3'
	Reverse 5'-TGTCACCGATGGCGTAGATG -3'
<i>TrxR2</i>	Forward 5'-AATCTGGAGAAGGCTGGCATC-3'
	Reverse 5'-TTCCTGCCTTGATAGCTGTGG -3'
<i>Trx1</i>	Forward 5'-ACTTCTCTGCTACGTGGTGTG -3'
	Reverse 5'-CGGCATGCATTTGACTTCACA -3'
<i>Trx2</i>	Forward 5'-TAACAGTAATGCCCAGCCCAG -3'
	Reverse 5'-ACCACTGTGCATGAAAGTCCA -3'
<i>β-actin</i>	Forward 5'-GGACTCCTATGTGGGTGACG -3'
	Reverse 5'-CTTCTCCATGTCGTCCCAGT -3'