Synthesis, Molecular Modelling, and Evaluation of New Mono- and Bis-isoxazoline as Antibacterial Agents: *In vitro* and *In silico* Analysis

Ali Altharawi[1], Bharath Kumar Chagaleti[2,*], Mubarak A. Alamri[1], Yassine Riadi[1], Taibah Aldakhil[1], Ait Itto Moulay Youssef [3], Ali Oubella[4], Reda A. Haggam[5*]

[1] Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
[2] Department of Pharmaceutical Chemistry, SRM College of Pharmacy, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Chennai 603203, India
[3] Laboratory of Organic Synthesis and Physico-Molecular Chemistry, Department of Chemistry, Faculty of Sciences Semlalia, Université Cadi Ayyad, BP PO Box 2390, Marrakech 40001, Morocco
[4] Laboratory of Organic & Physical Chemistry, Applied Bioorganic Chemistry Team, Faculty of Sciences, Ibn Zohr University. Agadir, Morocco
[5] Department of Chemistry, Faculty of Science, Islamic University of Madinah, 42351 Madinah, Saudi Arabia

⁎ ***Corresponding authors.***

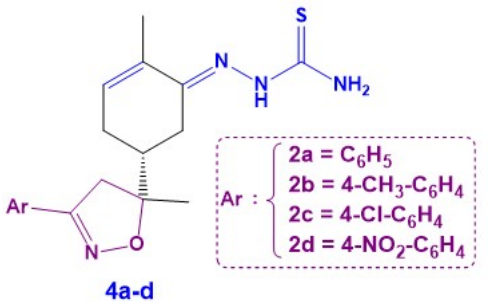
E-mail addresses : chagalek@srmist.edu.in (Bharath Kumar Chagaleti); relhaggan@iu.edu.sa (Reda A. Haggam)

Abstract

This study aimed to design, synthesize, and evaluate a novel series of isoxazoline-thiosemicarbazone derivatives as potential antibacterial agents, supported by computational investigations. Compounds were synthesized and characterized using ^{1}H and ^{13}C NMR and HRMS. Compounds **4a-d** and **6** were evaluated for their *in vitro* antibacterial activity against Gram-positive and Gram-negative bacterial strains by determining their minimum inhibitory concentrations (MICs). Molecular docking studies were conducted to assess binding interactions with the PTGS2 protein, followed by molecular dynamics simulations to evaluate the stability of the ligand-protein complexes. MM-GBSA calculations were performed to estimate binding free energies, and *in silico* ADMET predictions were carried out to assess drug-likeness. Compounds **4a-d** and **6** exhibited potent antibacterial activity, with MIC values ranging from 0.31 to 0.5 µg/mL. Molecular docking results revealed strong binding affinities of compound **4a** toward the active site of PTGS2. Molecular dynamics confirmed the structural stability of the PTGS2-4a complex. MM-GBSA analysis demonstrated that PTGS2-4a displayed the most favorable binding free energy, showing superior or comparable performance to the reference inhibitor Resatorvid. *In silico* ADME-T analysis indicated acceptable pharmacokinetic properties. These results highlight compounds **4a-d** as promising

antibacterial candidates, providing a strong rationale for the development of novel isoxazoline-thiosemicarbazone-based antibacterial agents.

Keywords: Synthesis, Thiosemicarbazone, Isoxazoline, Antibacterial activity, Docking, ADME-T



Compound 4a-d

Compound 4a

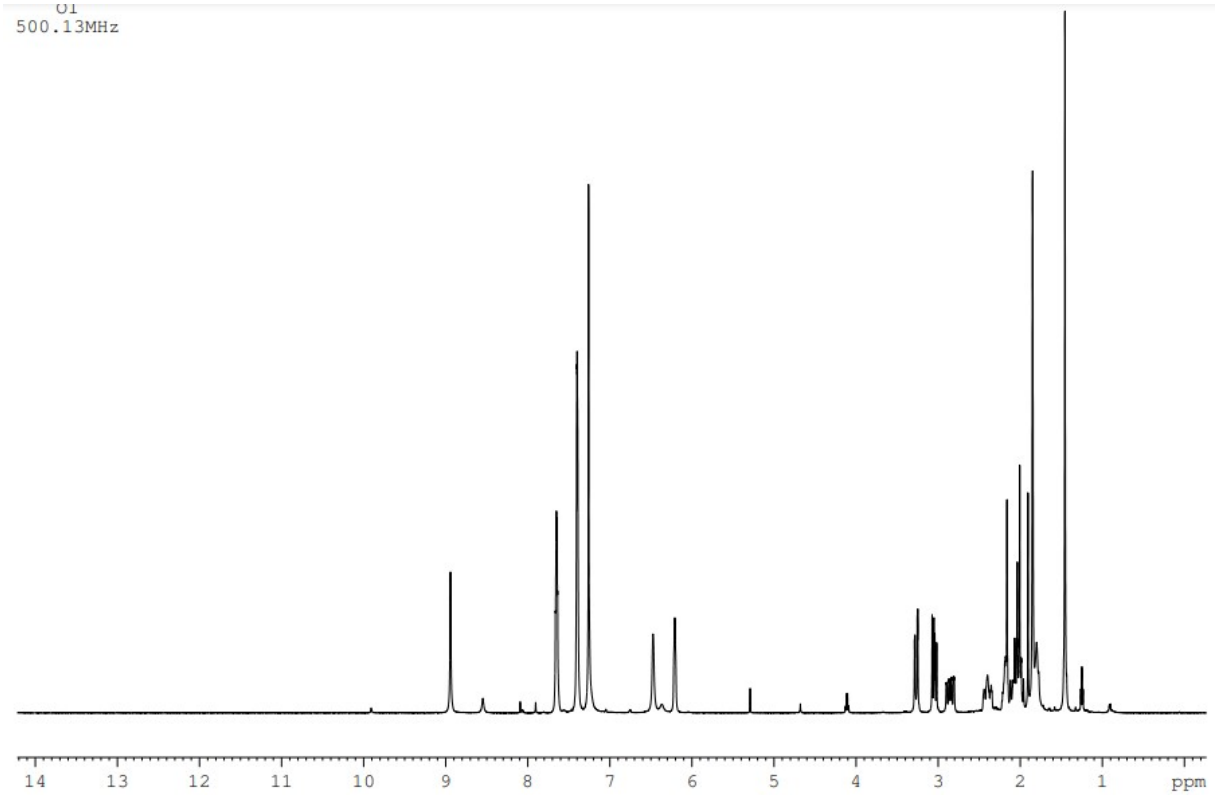


Fig. 1S. ^{1}H-NMR spectrum of compound **4a** (500 MHz, $CDCl_3$)

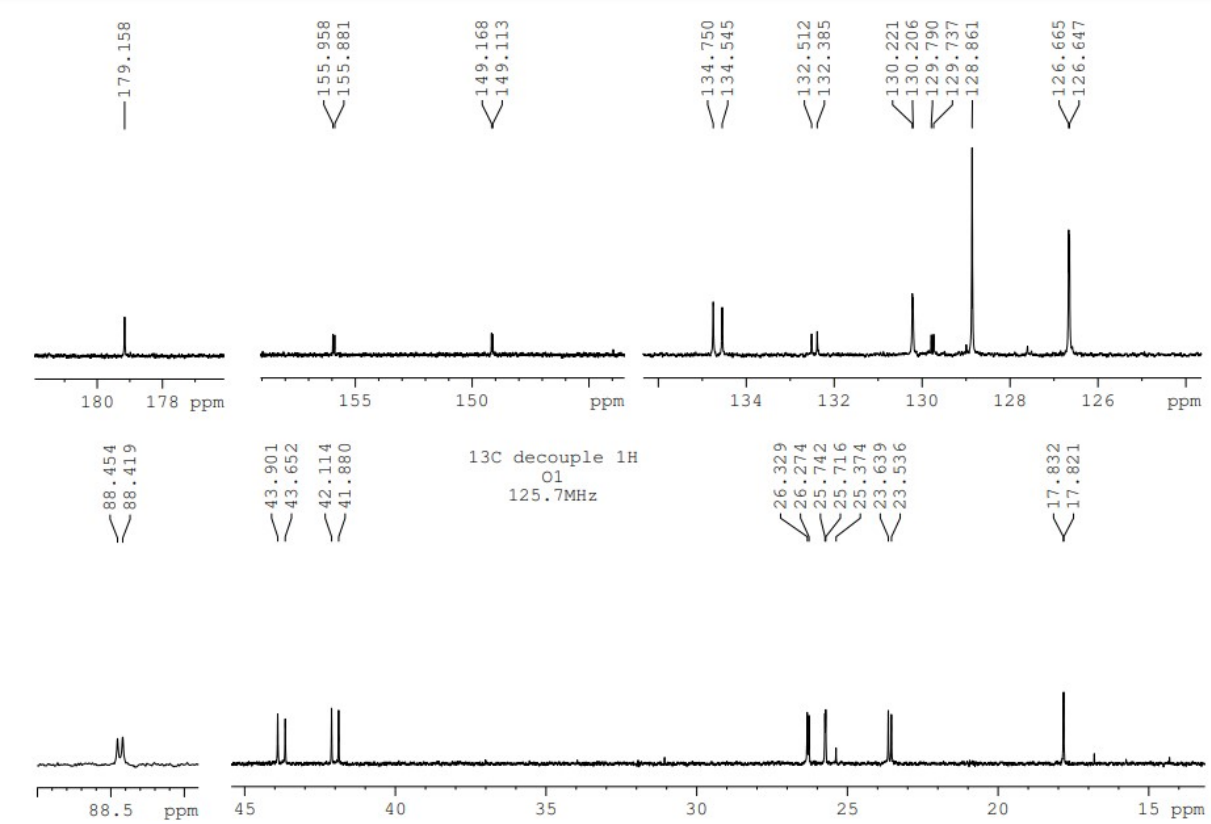


Fig. 2S. ^{1}H-NMR spectrum of compound **4a** (500 MHz, $CDCl_3$, ''Zoom'')

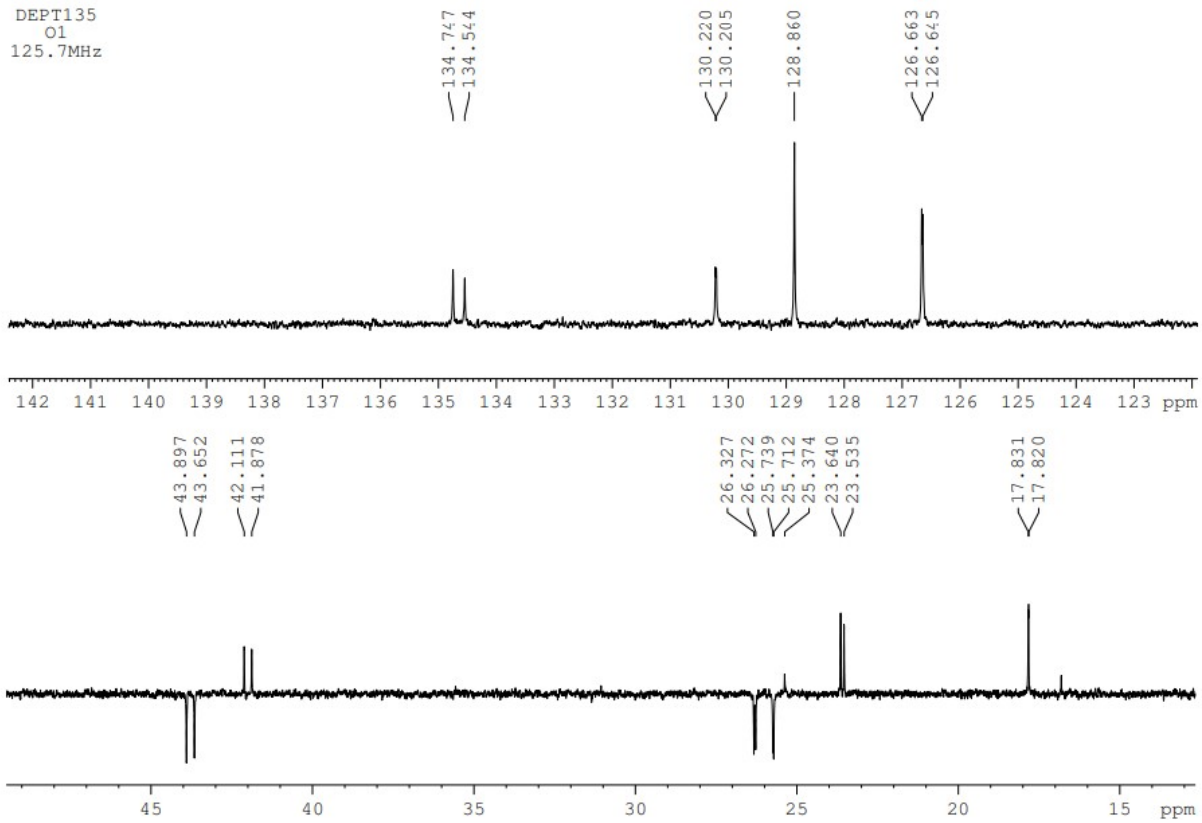
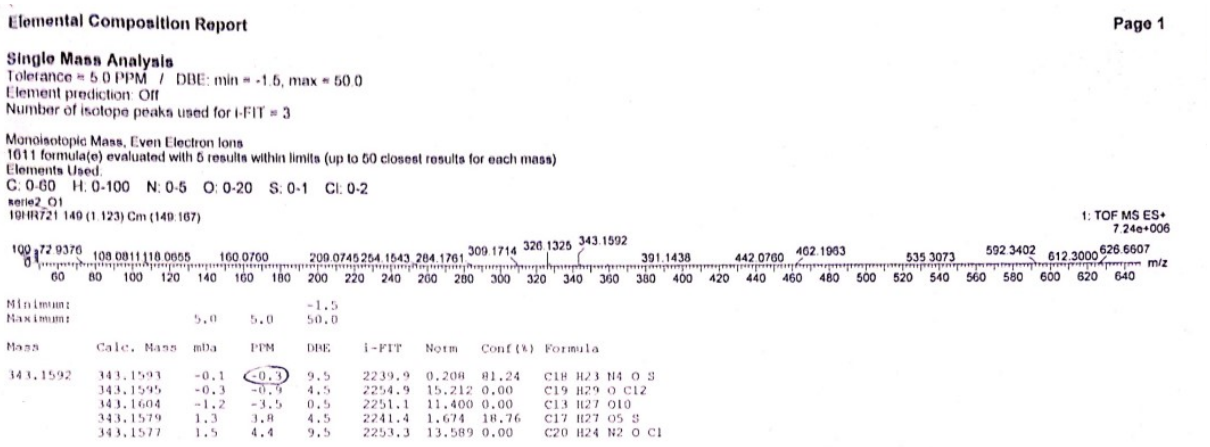


Fig. 3S. ^{13}C Decoupled ^{1}H-NMR spectrum DEPT 135 Mode (500 MHz, $CDCl_3$, ''Zoom'')



Elemental Composition Report — Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1611 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-60 H: 0-100 N: 0-5 O: 0-20 S: 0-1 Cl: 0-2

serie2_01
19HR721 149 (1.123) Cm (149:167)

1: TOF MS ES+
7.24e+006

100 %
72.9376 108.0811 118.0655 160.0760 209.0745 254.1543 284.1761 309.1714 326.1325 343.1592 391.1438 442.0760 462.1963 535.3073 592.3402 612.3000 626.6607
m/z

Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
343.1592	343.1593	-0.1	-0.3	9.5	2239.9	0.208	81.24	C18 H23 N4 O S
	343.1595	-0.3	-0.9	4.5	2254.9	15.212	0.00	C19 H29 O Cl2
	343.1604	-1.2	-3.5	0.5	2251.1	11.400	0.00	C13 H27 O10
	343.1579	1.3	3.8	4.5	2241.4	1.674	18.76	C17 H27 O5 S
	343.1577	1.5	4.4	9.5	2253.3	13.589	0.00	C20 H24 N2 O Cl

Fig. 4S. HRMS spectrum of compound **4a**

Compound 4b

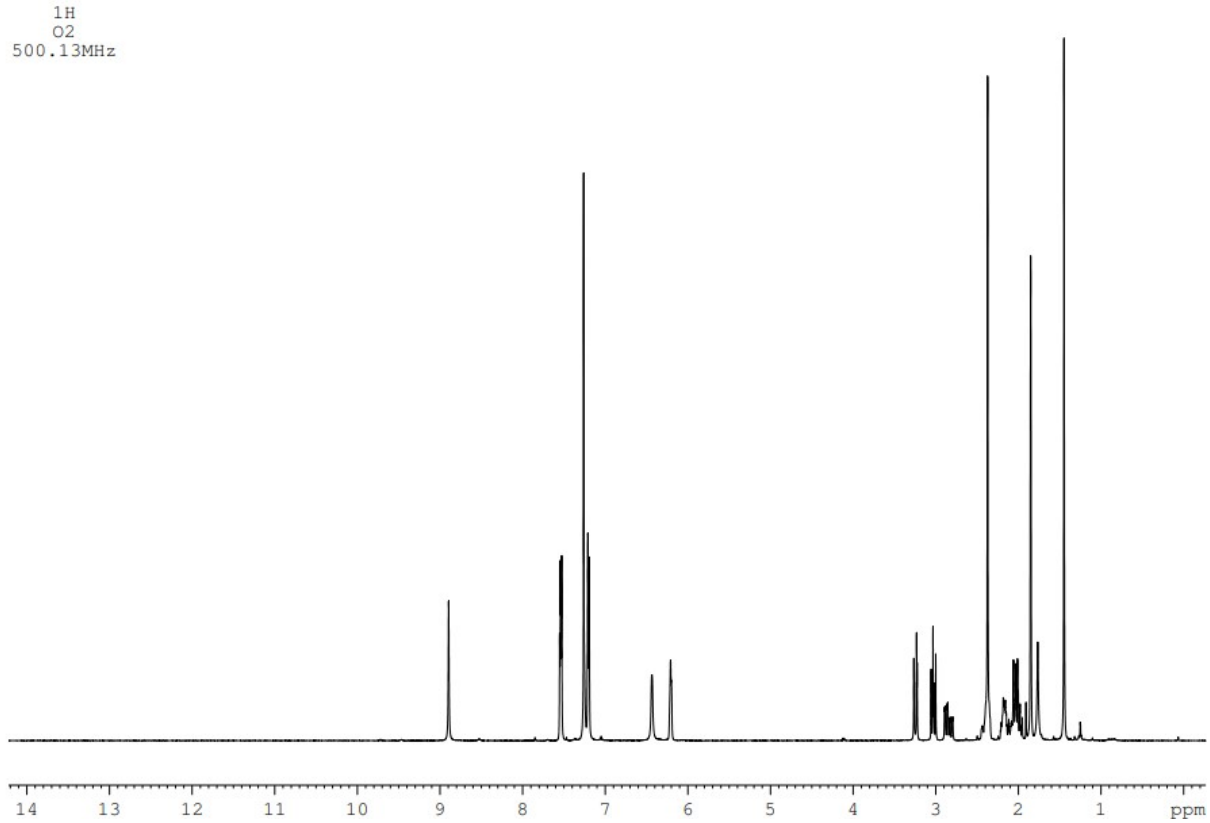


Fig. 5S. ^{1}H-NMR spectrum of compound **4b** (500 MHz, $CDCl_3$)

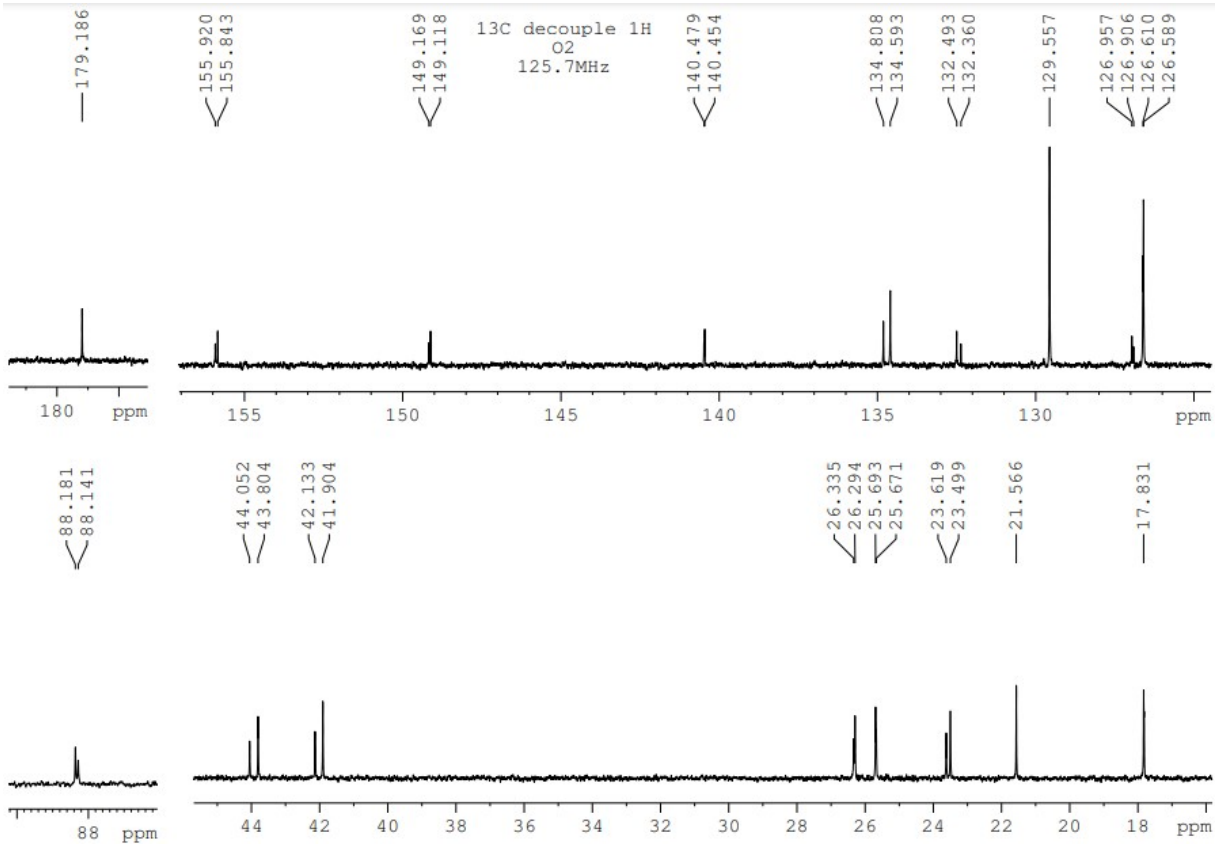


Fig. 6S. ^{1}H-NMR spectrum of compound **4b** (500 MHz, $CDCl_3$ ''Zoom'')

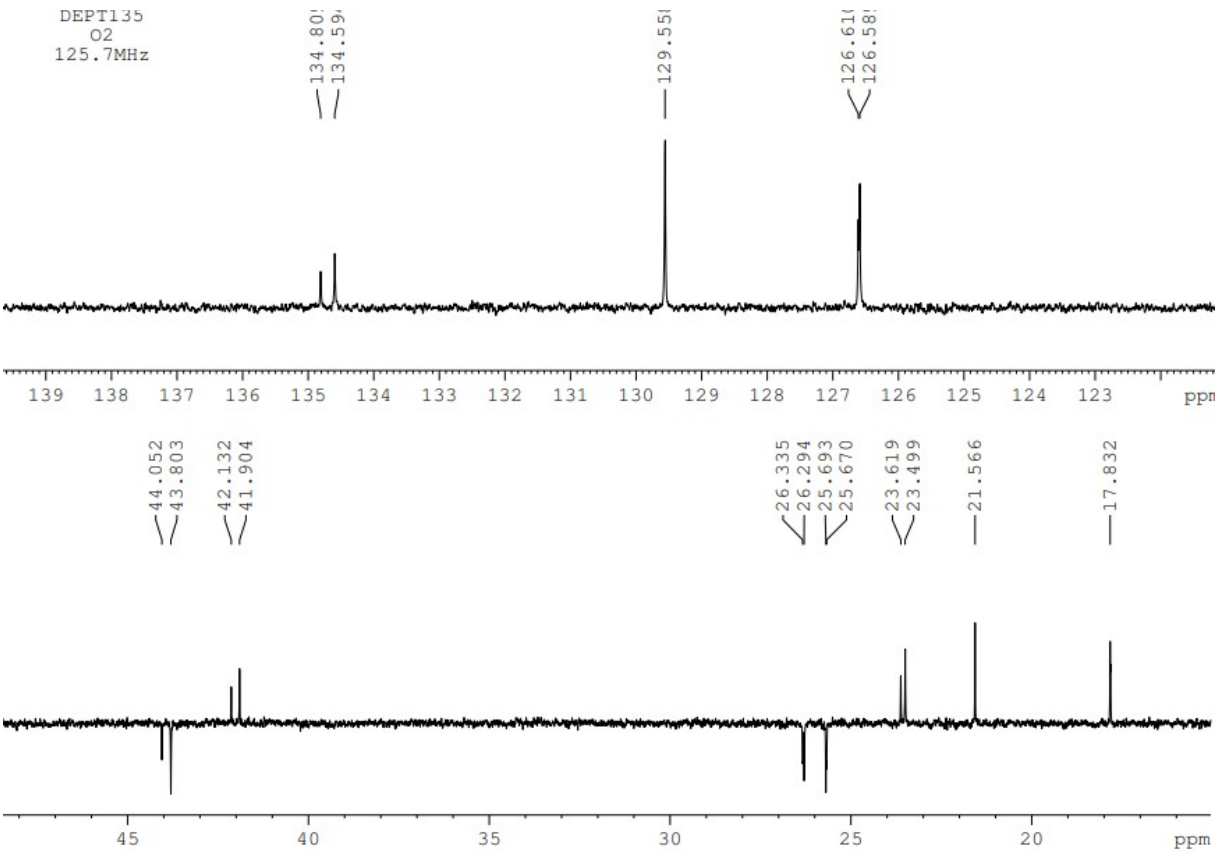


Fig. 7S. ^{13}C Decoupled ^{1}H-NMR spectrum of compound **4b** (DEPT 135 Mode)

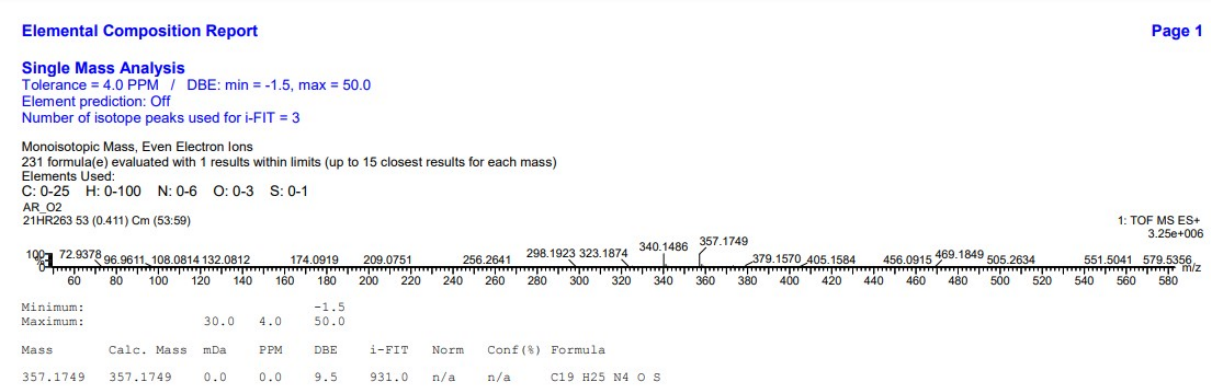


Fig. 8S. HRMS spectrum of compound **4b**

Compound 4c

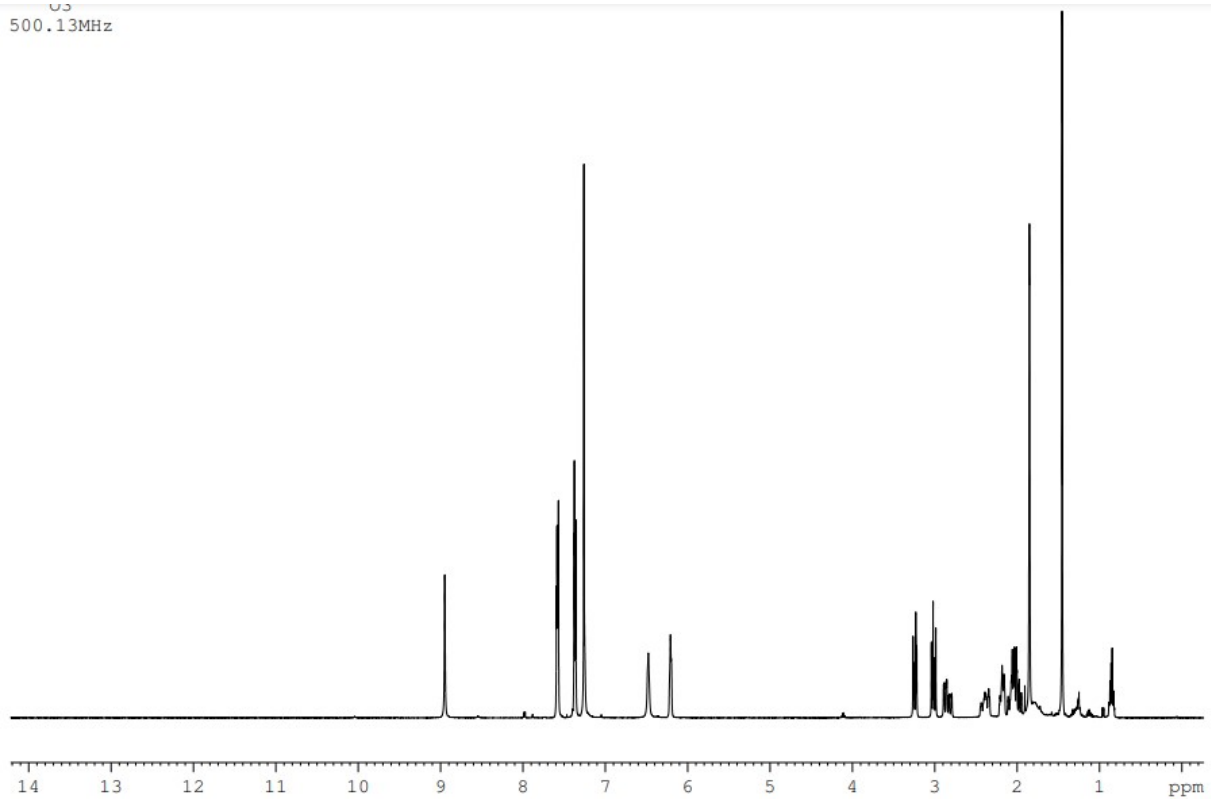


Fig. 9S. ^{1}H-NMR spectrum of compound **4c** (500 MHz, $CDCl_3$)

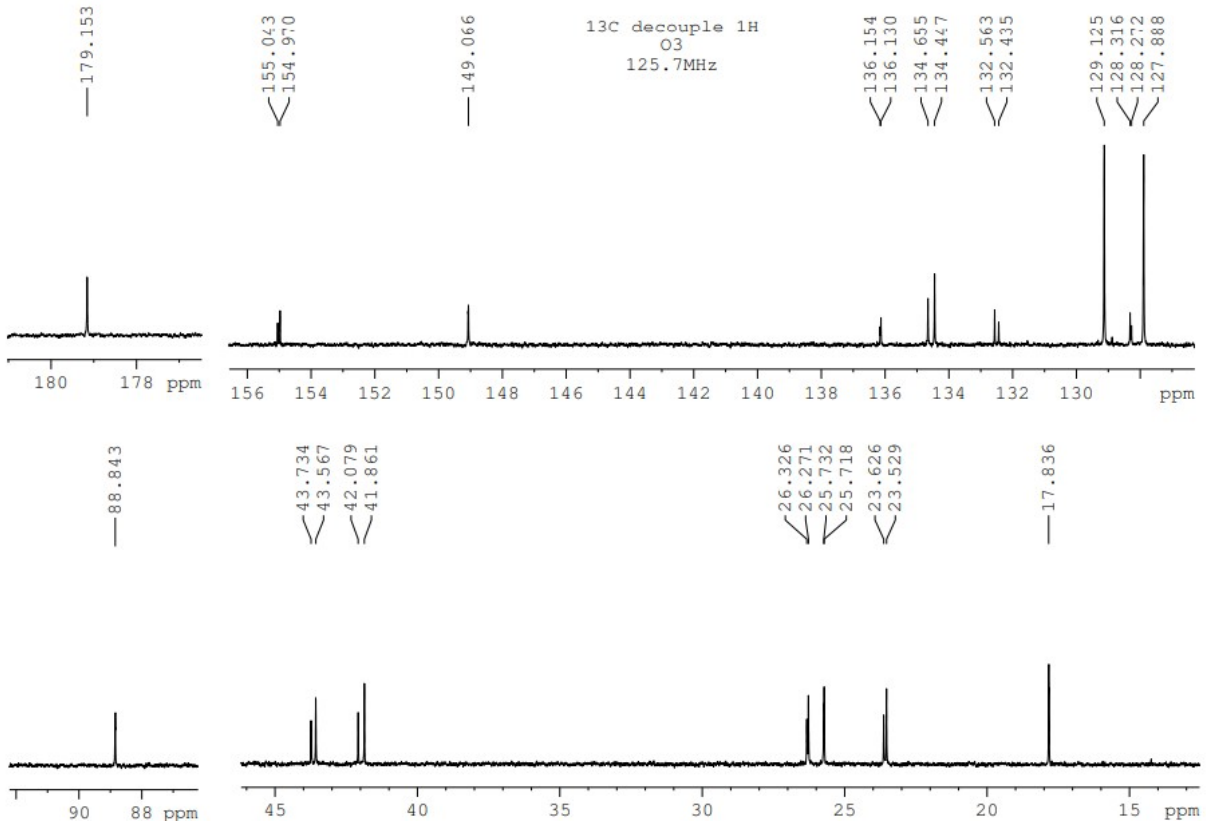


Fig. 10S. ^{13}C Decoupled ^{1}H-NMR spectrum of compound **4c**

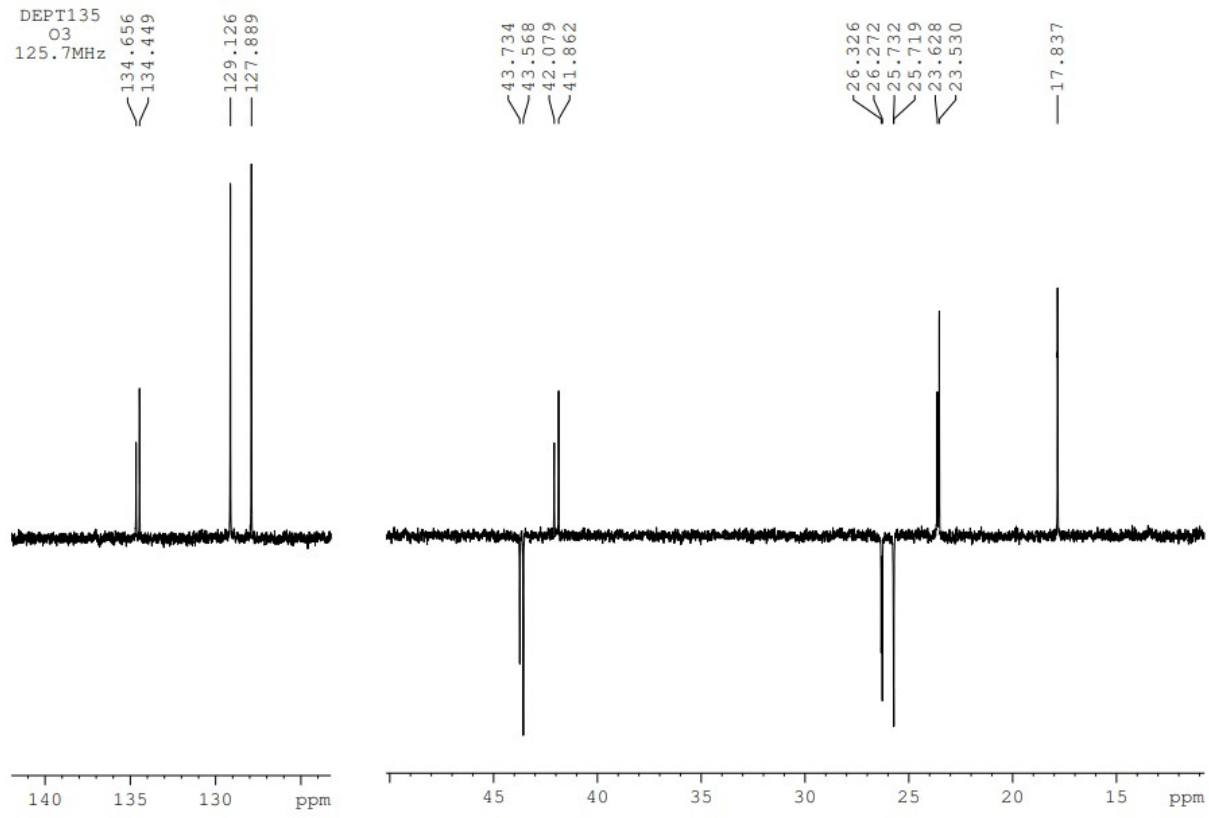
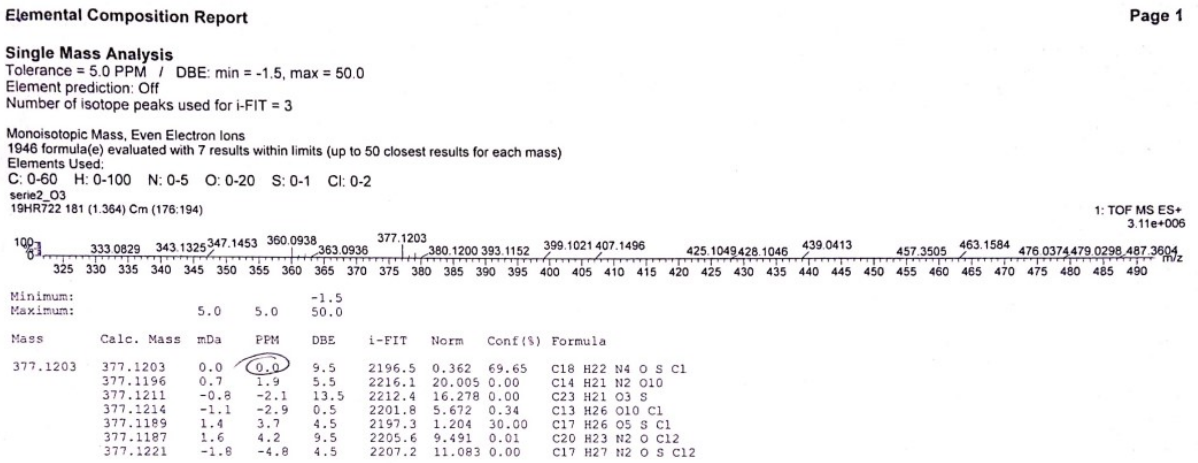


Fig. 11S. ^{13}C Decoupled ^{1}H-NMR spectrum of compound **4c** (DEPT 135 Mode)



Elemental Composition Report — Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1946 formula(e) evaluated with 7 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-60 H: 0-100 N: 0-5 O: 0-20 S: 0-1 Cl: 0-2

serie2_O3
19HR722 181 (1.364) Cm (176:194)

1: TOF MS ES+
3.11e+006

Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
377.1203	377.1203	0.0	0.0	9.5	2196.5	0.362	69.65	C18 H22 N4 O S Cl
	377.1196	0.7	1.9	5.5	2216.1	20.005	0.00	C14 H21 N2 O10
	377.1211	-0.8	-2.1	13.5	2212.4	16.278	0.00	C23 H21 O3 S
	377.1214	-1.1	-2.9	0.5	2201.8	5.672	0.34	C13 H26 O10 Cl
	377.1189	1.4	3.7	4.5	2197.3	1.204	30.00	C17 H26 O5 S Cl
	377.1187	1.6	4.2	9.5	2205.6	9.491	0.01	C20 H23 N2 O Cl2
	377.1221	-1.8	-4.8	4.5	2207.2	11.083	0.00	C17 H27 N2 O S Cl2

Fig. 12S. HRMS spectrum of compound **4c**

Compound 4d

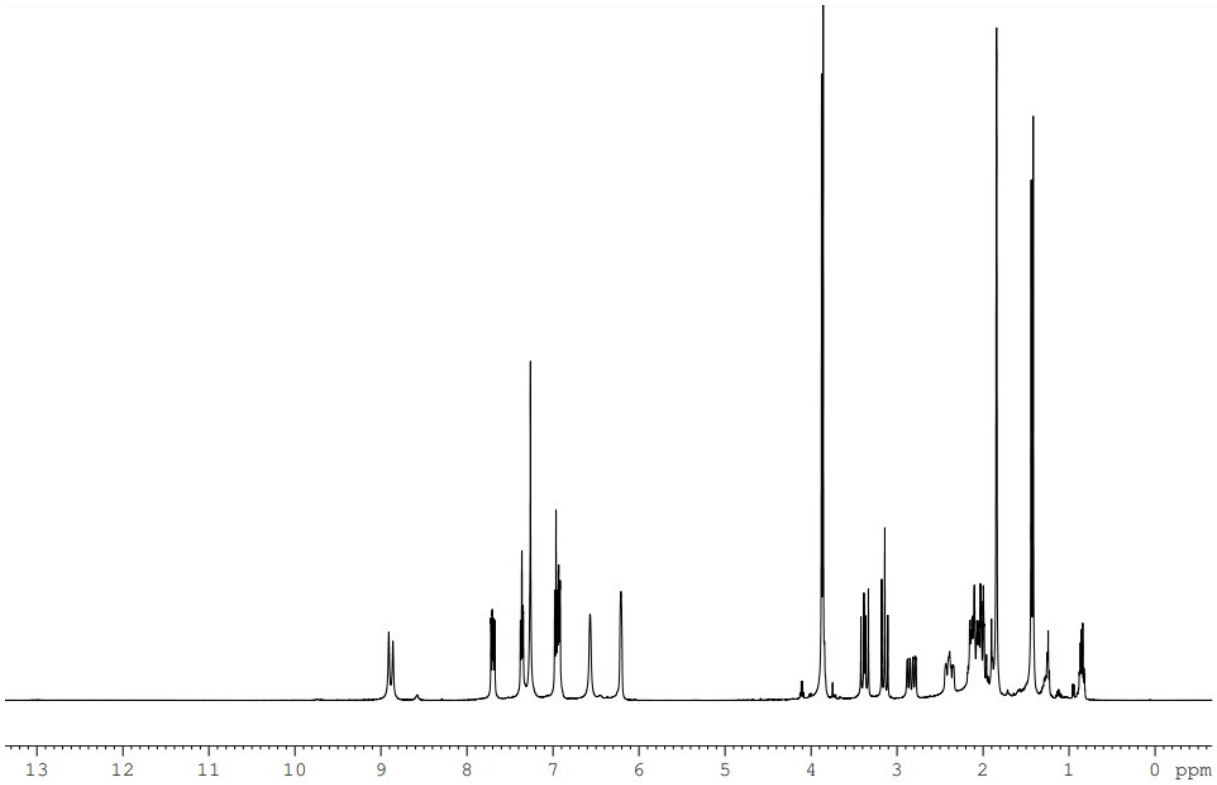


Fig. 13S. ^{1}H-NMR spectrum of compound **4d** (500 MHz, $CDCl_3$)

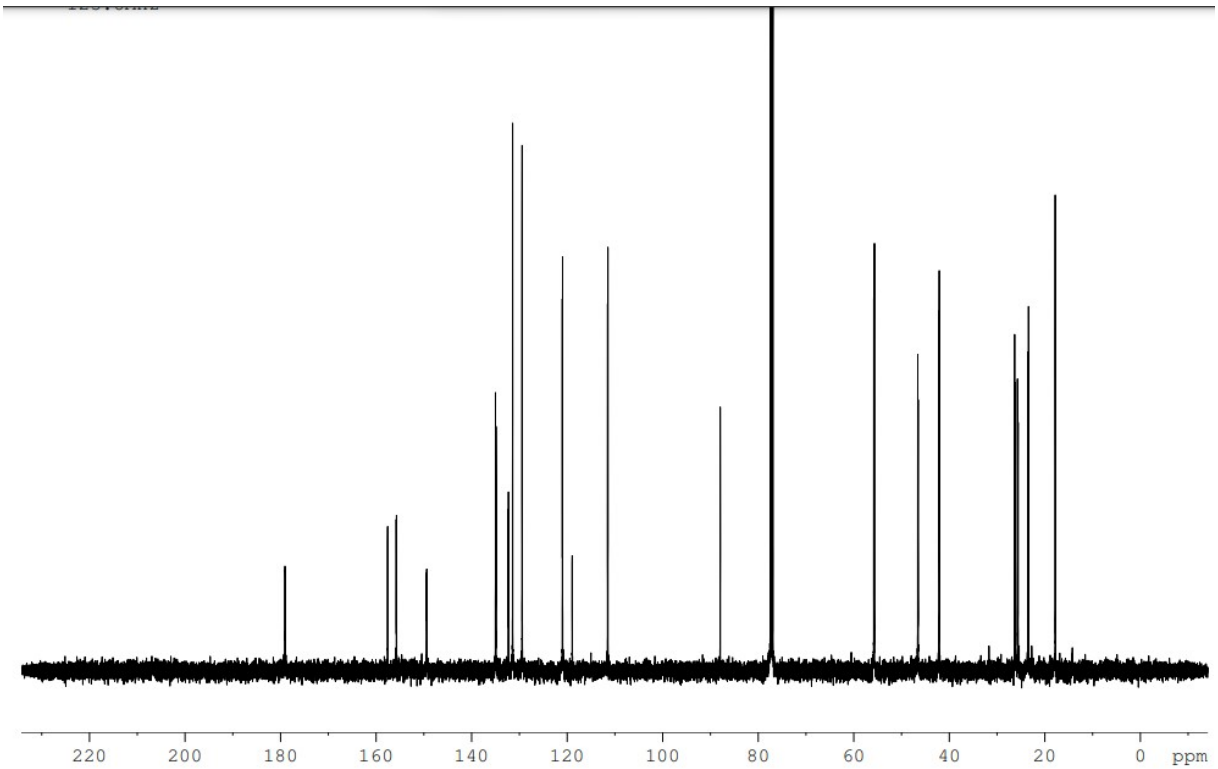


Fig. 14S. ^{13}C Decoupled ^{1}H-NMR spectrum of compound **4d**

Elemental Composition Report **Page 1**

Single Mass Analysis
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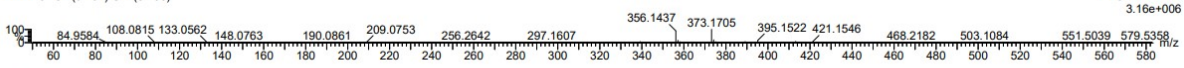
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
217 formula(e) evaluated with 1 results within limits (up to 15 closest results for each mass)
Elements Used:
C: 0-25 H: 0-100 N: 0-6 O: 0-3 S: 0-1
AR_O5
21HR264 52 (0.404) Cm (52:56)

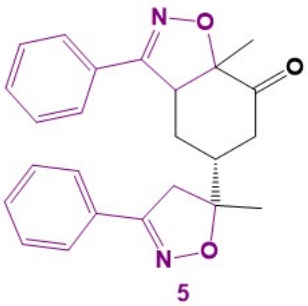
1: TOF MS ES+
3.16e+006



Minimum:				-1.5				
Maximum:		30.0	4.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
373.1705	373.1698	0.7	1.9	9.5	945.1	n/a	n/a	C19 H25 N4 O2 S

Fig. 15S. HRMS spectrum of compound **4d**

Compound 5



5

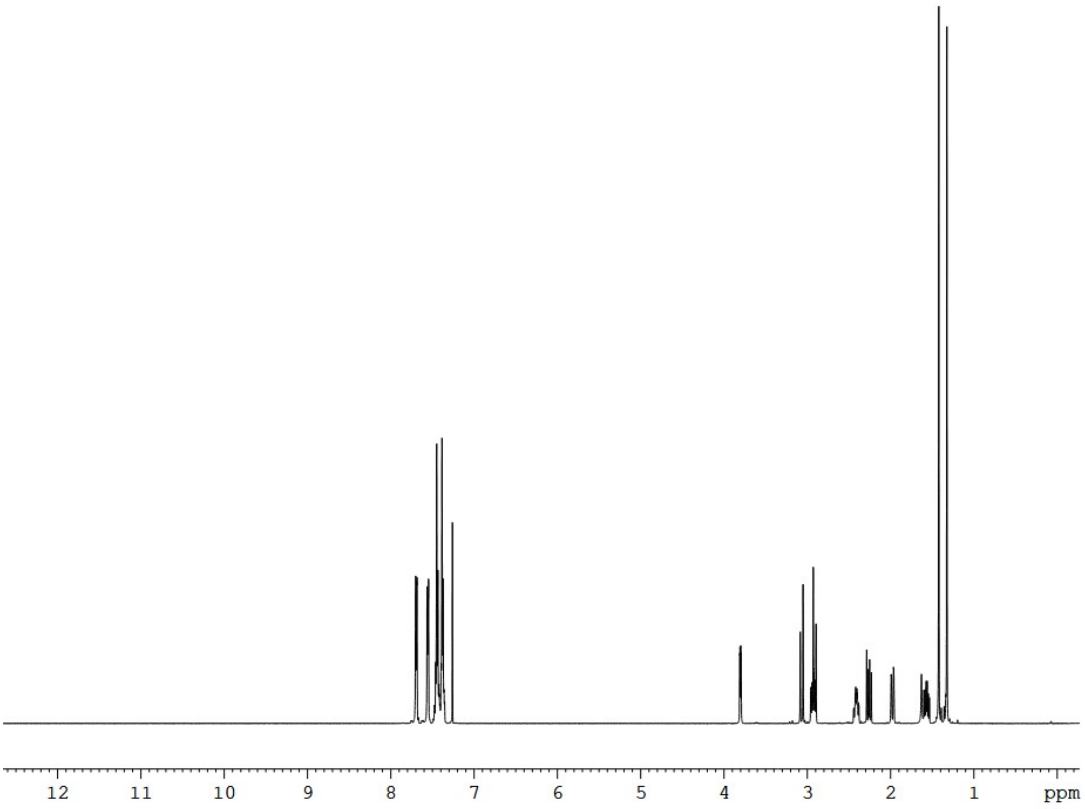


Fig. 16S. ^{1}H-NMR spectrum of compound **5**

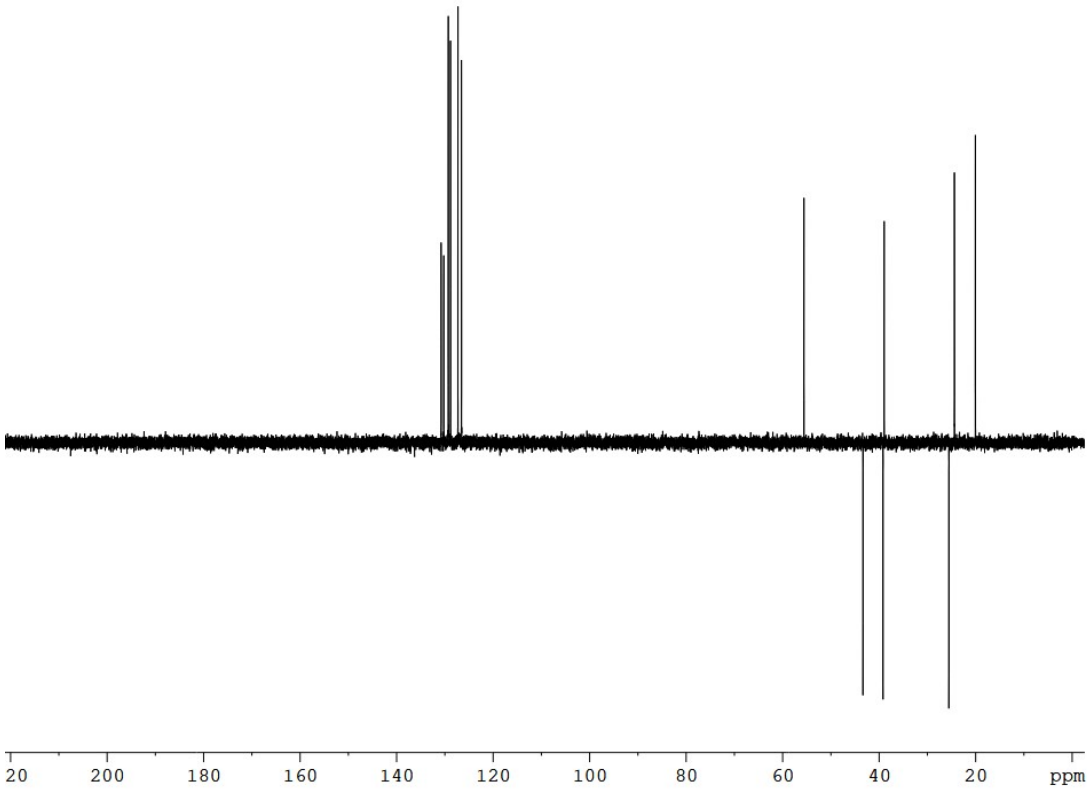


Fig. 17S. ^{13}C NMR spectrum of compound **5**

Elemental Composition Report **Page 1**

Single Mass Analysis
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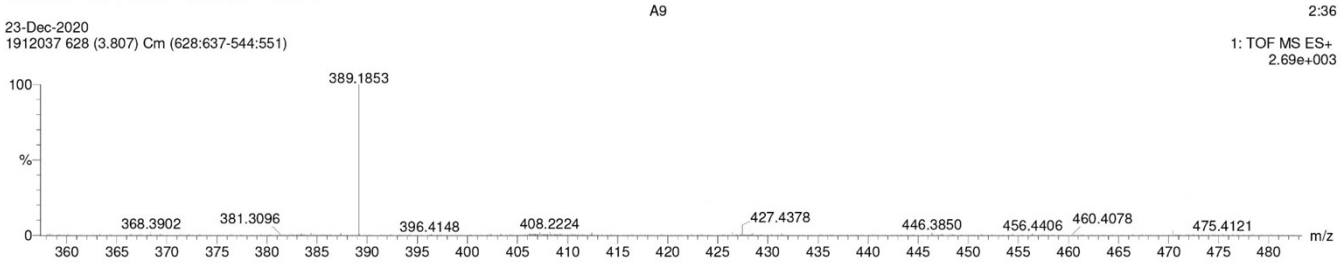
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

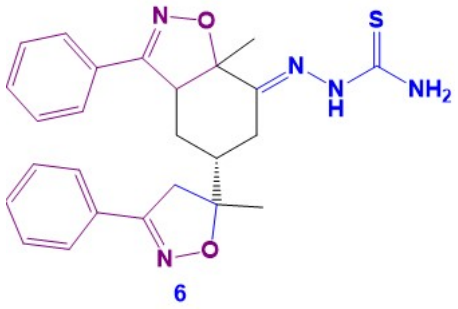
Monoisotopic Mass, Even Electron Ions
632 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-10 O: 0-4



Minimum:				0.0			
Maximum:		5.0	5.0	18.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
389.1853	389.1849	-0.4	-0.8	14.5	298.2	0.0	C24 H25 N2 O3

Fig. 18S. HRMS spectrum of compound **5**

Compound 6



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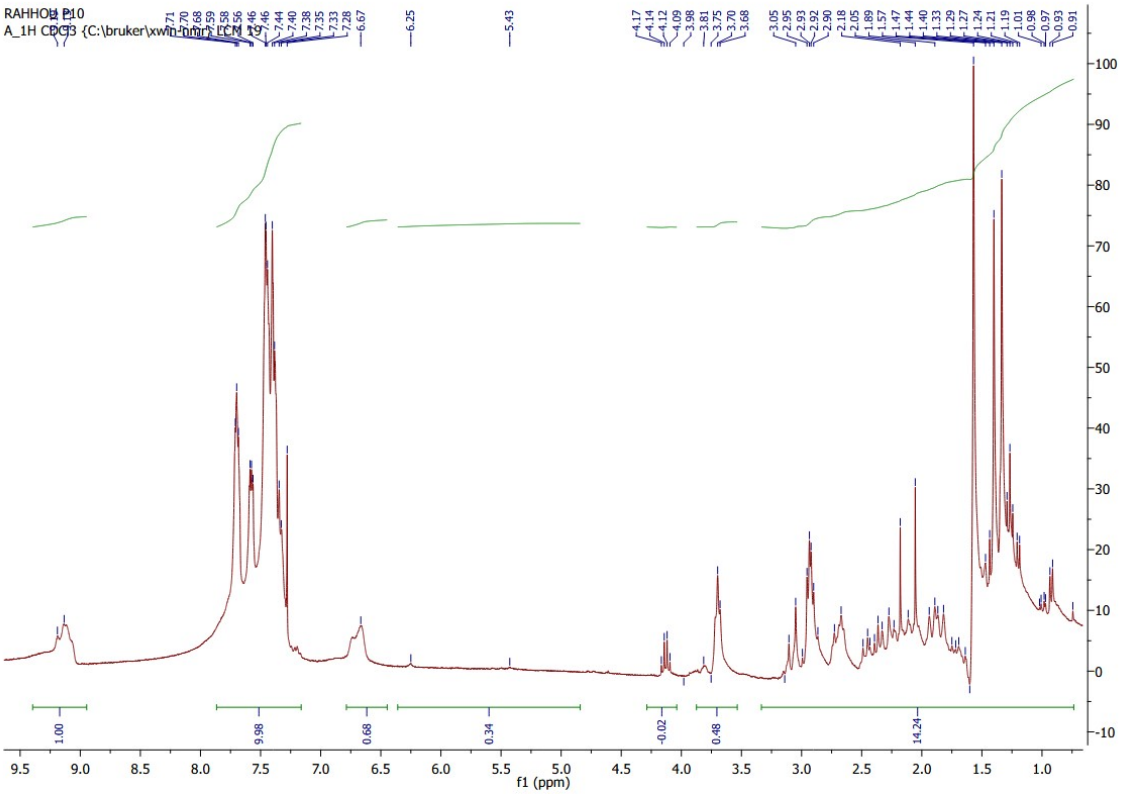

Fig. 19S. ^{1}H NMR spectrum of compound **6**

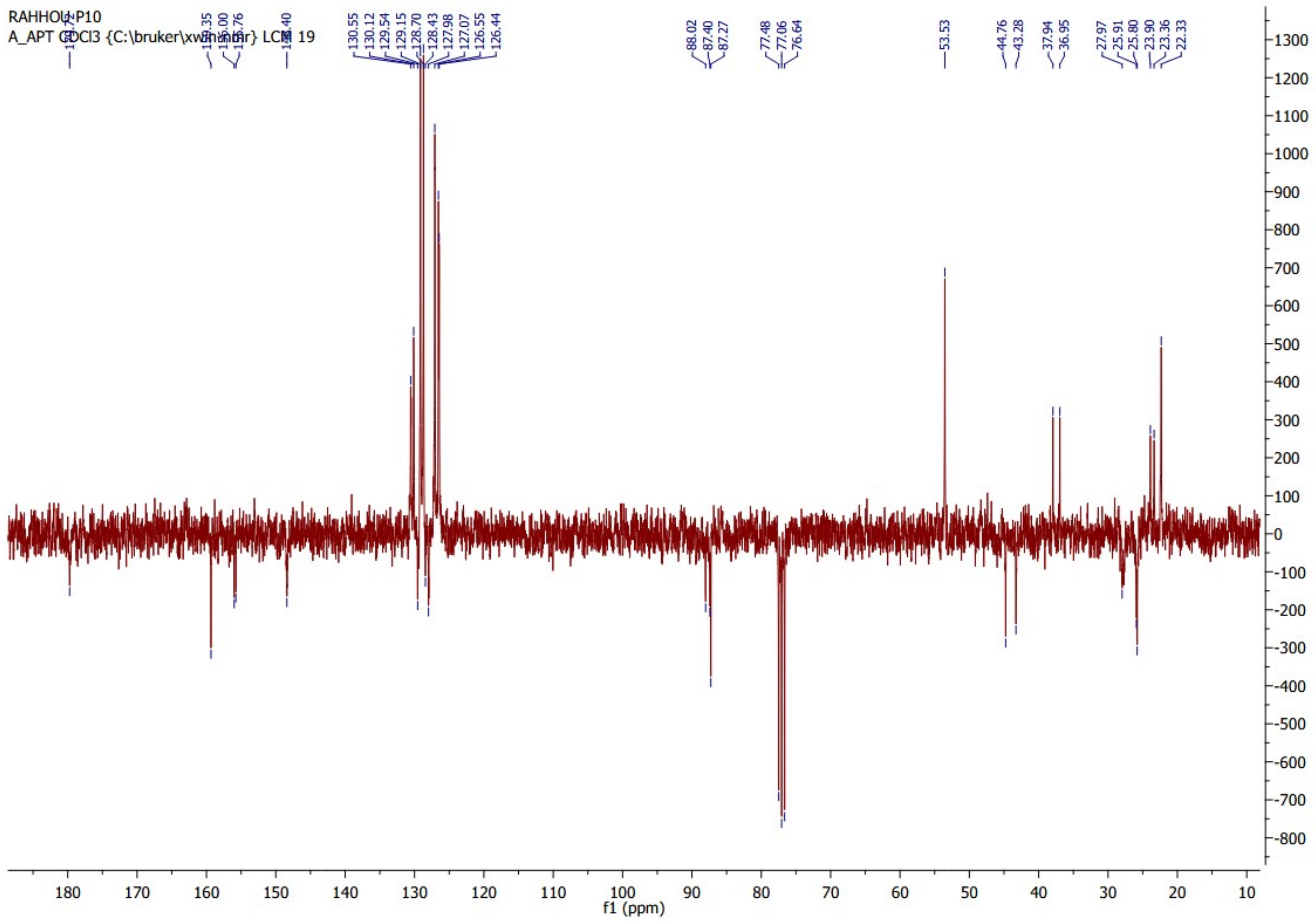

Fig. 20S. ^{13}C NMR spectrum of compound **6**

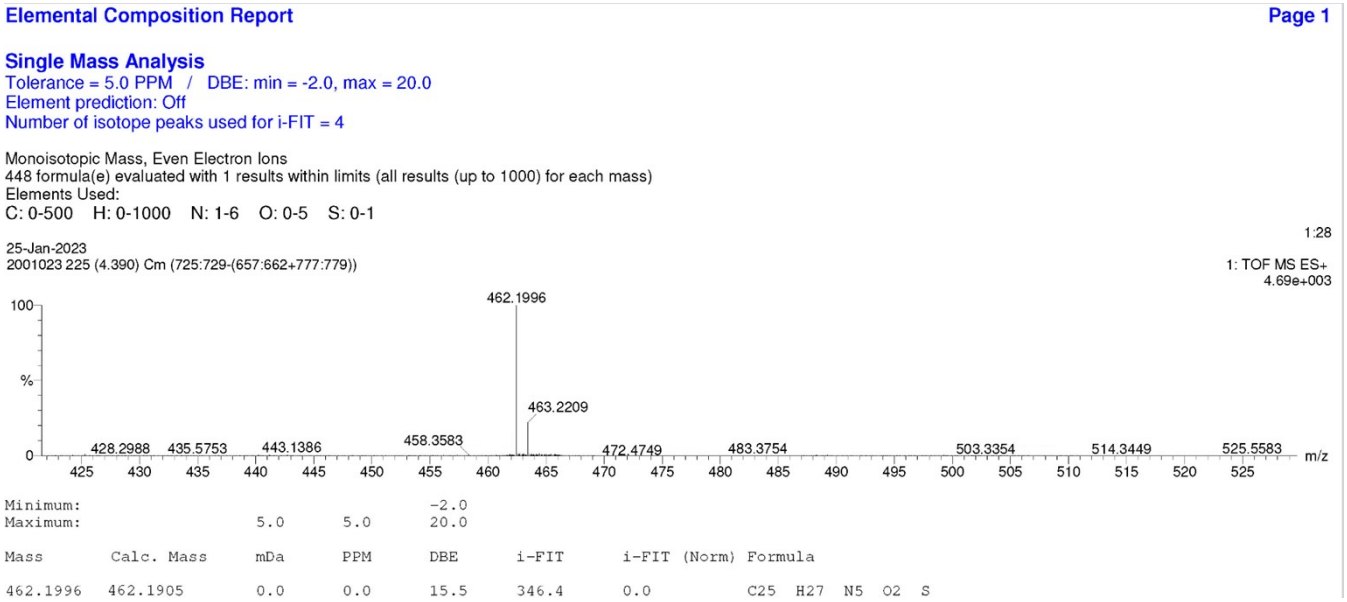

Fig. 21S. HRMS spectrum of compound **6**