

***In Vitro* and *In Silico* Evaluation of Anticancer Activity of New Indole-based 1,3,4-Oxadiazoles as EGFR and COX-2 Inhibitors**

Belgin Sever ^{1,*}, Mehlika Dilek Altıntop ¹, Ahmet Özdemir ¹, Gülşen Akalın Çiftçi ², Doha E. Ellakwa ³, Hiroshi Tateishi ⁴, Mohamed O. Radwan ^{4,5,6}, Mahmoud A. A. Ibrahim ⁷, Masami Otsuka ^{4,5}, Mikako Fujita ^{4,*}, Halil I. Ciftci ^{4,5,*}, Taha F.S. Ali ^{4,8}

¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir 26470, Turkey; mdaltintop@anadolu.edu.tr (M.D.A.); ahmeto@anadolu.edu.tr (A.Ö.)

² Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir 26470, Turkey; gakalin@anadolu.edu.tr (G.A.Ç.)

³ Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Al-Azhar University, Cairo 11765, Egypt; profdoha@gmail.com (D.E.E.)

⁴ Medicinal and Biological Chemistry Science Farm Joint Research Laboratory, School of Pharmacy, Kumamoto University, Kumamoto 862-0973, Japan; htateishi@kumamoto-u.ac.jp (H.T.); mohamedradwan@kumamoto-u.ac.jp (M.O.R.); motsuka@gpo.kumamoto-u.ac.jp (M.O.); tahafaroukali@gmail.com (T.F.S.A.)

⁵ Department of Drug Discovery, Science Farm Ltd., Kumamoto 862-0976, Japan

⁶ Chemistry of Natural Compounds Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Cairo 12622, Egypt

⁷ Computational Chemistry Laboratory, Chemistry Department, Faculty of Science, Minia University, Minia 61519, Egypt; m.ibrahim@compchem.net (M.A.A.I.)

⁸ Medicinal Chemistry Department, Faculty of Pharmacy, Minia University, Minia 61519, Egypt

* Correspondence: belginsever@anadolu.edu.tr (B.S.); mfujita@kumamoto-u.ac.jp (M.F.); hiciftci@kumamoto-u.ac.jp (H.I.C.); Tel.: +90-222-335-0580 (ext. 3807)

Table of Contents

Figure S1.	IR spectrum of compound 2a .
Figure S2.	^1H NMR spectrum of compound 2a .
Figure S3.	^{13}C NMR spectrum of compound 2a .
Figure S4.	HRMS spectrum of compound 2a .
Figure S5.	IR spectrum of compound 2b .
Figure S6.	^1H NMR spectrum of compound 2b .
Figure S7.	^{13}C NMR spectrum of compound 2b .
Figure S8.	HRMS spectrum of compound 2b .
Figure S9.	IR spectrum of compound 2c .
Figure S10.	^1H NMR spectrum of compound 2c .
Figure S11.	^{13}C NMR spectrum of compound 2c .
Figure S12.	HRMS spectrum of compound 2c .
Figure S13.	IR spectrum of compound 2d .
Figure S14.	^1H NMR spectrum of compound 2d .
Figure S15.	^{13}C NMR spectrum of compound 2d .
Figure S16.	HRMS spectrum of compound 2d .
Figure S17.	IR spectrum of compound 2e .
Figure S18.	^1H NMR spectrum of compound 2e .
Figure S19.	^{13}C NMR spectrum of compound 2e .
Figure S20.	HRMS spectrum of compound 2e .
Figure S21.	IR spectrum of compound 2f .
Figure S22.	^1H NMR spectrum of compound 2f .
Figure S23.	^{13}C NMR spectrum of compound 2f .
Figure S24.	HRMS spectrum of compound 2f .
Figure S25.	IR spectrum of compound 2g .
Figure S26.	^1H NMR spectrum of compound 2g .
Figure S27.	^{13}C NMR spectrum of compound 2g .
Figure S28.	HRMS spectrum of compound 2g .
Figure S29.	IR spectrum of compound 2h .
Figure S30.	^1H NMR spectrum of compound 2h .
Figure S31.	^{13}C NMR spectrum of compound 2h .
Figure S32.	HRMS spectrum of compound 2h .
Figure S33.	IR spectrum of compound 2i .

Figure S34. ^1H NMR spectrum of compound **2i**.

Figure S35. ^{13}C NMR spectrum of compound **2i**.

Figure S36. HRMS spectrum of compound **2i**.

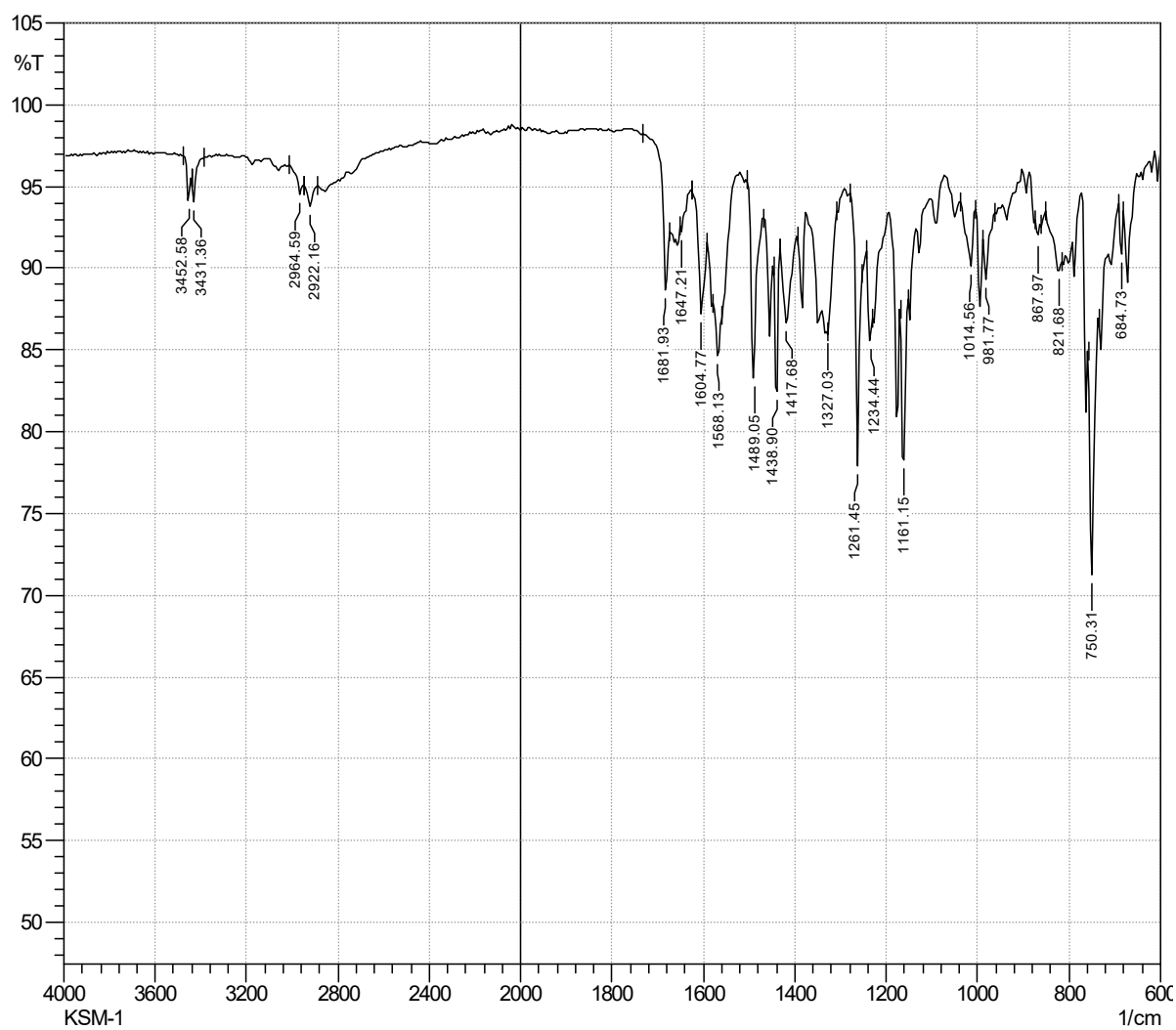


Figure S1. IR spectrum of compound 2a.

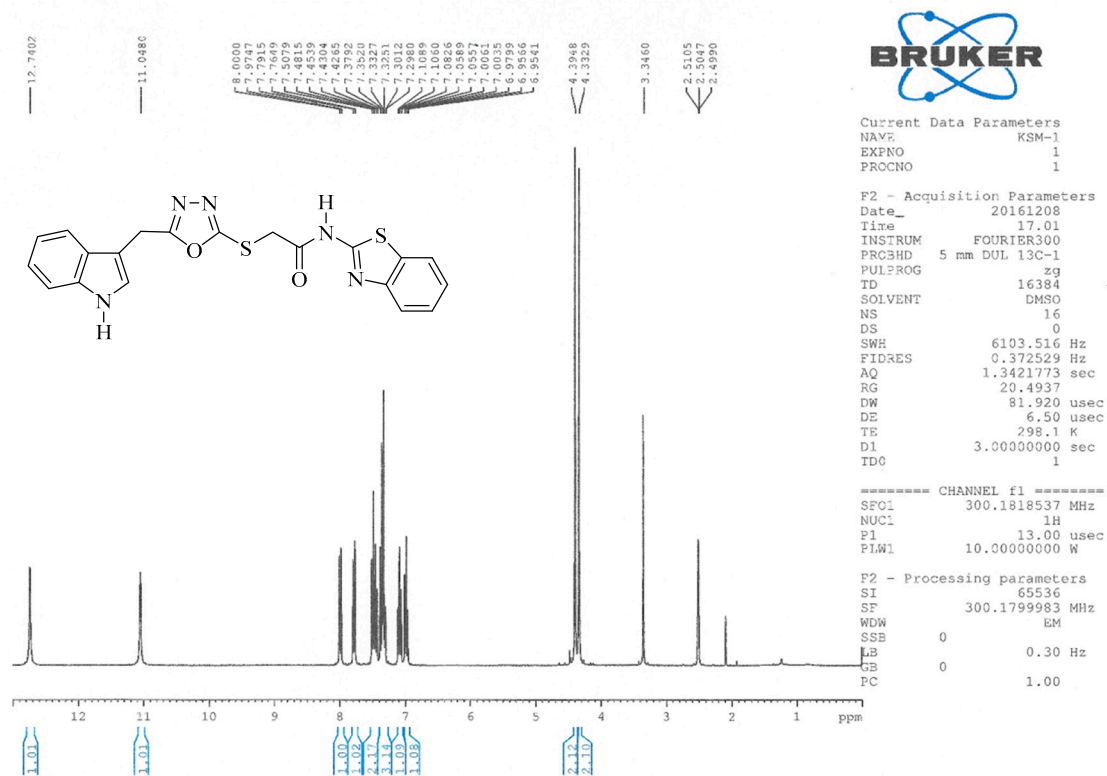


Figure S2. ¹H NMR spectrum of compound **2a**.

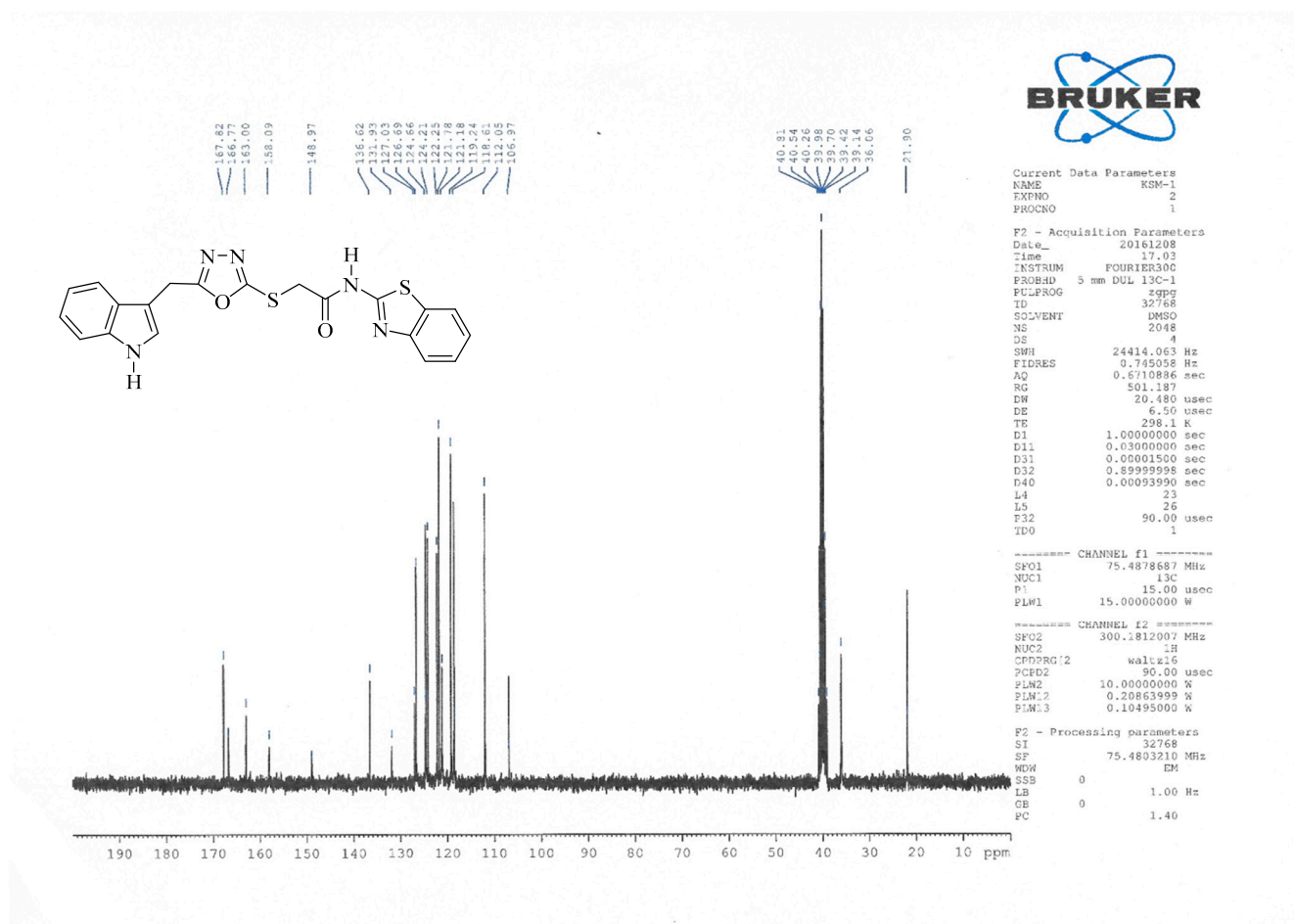


Figure S3. ¹³C NMR spectrum of compound 2a.

Data File: C:\LabSolutions\Data\Analiz\mdalt\intop\KSM-1_1.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	0	I	3	0	0	H
C	4	18	30	F	1	0	0	Br	1	0	0					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: yes

Isotope RI (%): 1.00

MSn Logic Mode: AND

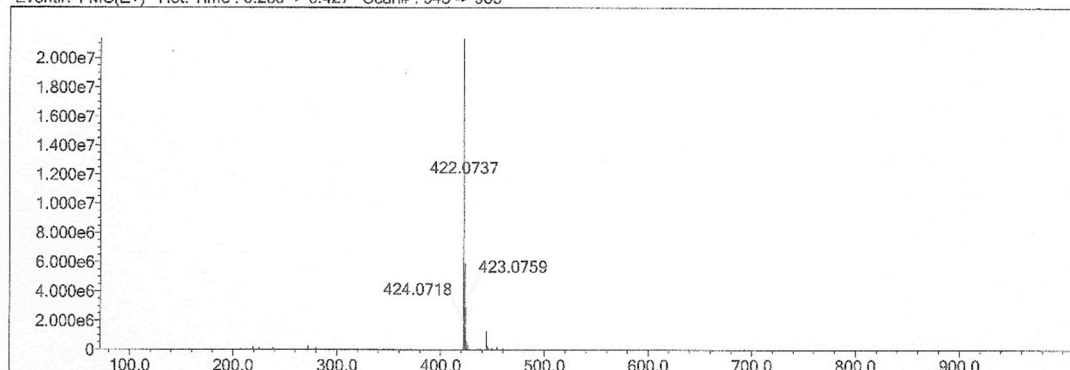
Electron Ions: both

Use MSn Info: no

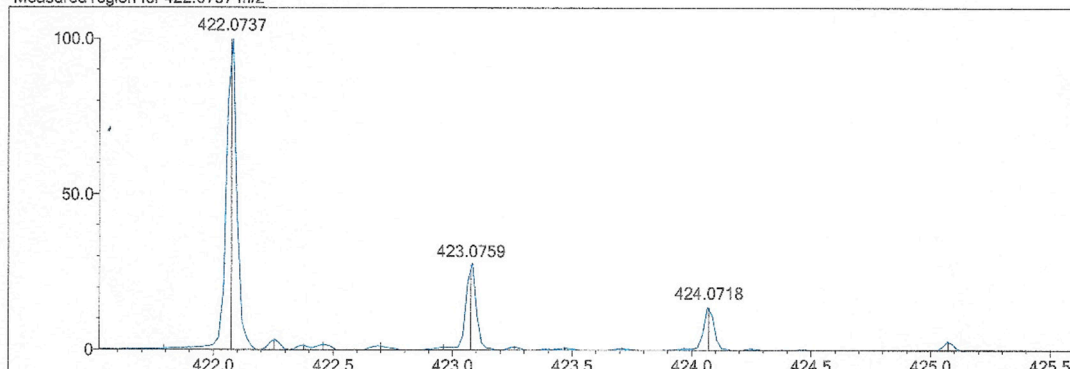
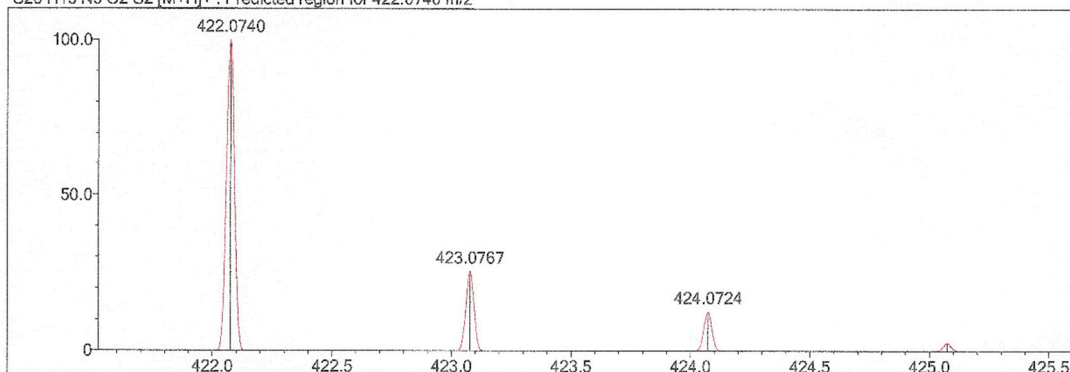
Isotope Res: 10000

Max Results: 500

Event#: 1 MS(E+) Ret. Time : 6.280 -> 6.427 Scan#: 943 -> 965



Measured region for 422.0737 m/z

C20 H15 N5 O2 S2 [M+H]⁺ : Predicted region for 422.0740 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	93.51	C20 H15 N5 O2 S2	[M+H] ⁺	422.0737	422.0740	-0.3	-0.71	93.51	16.0

Figure S4. HRMS spectrum of compound 2a.

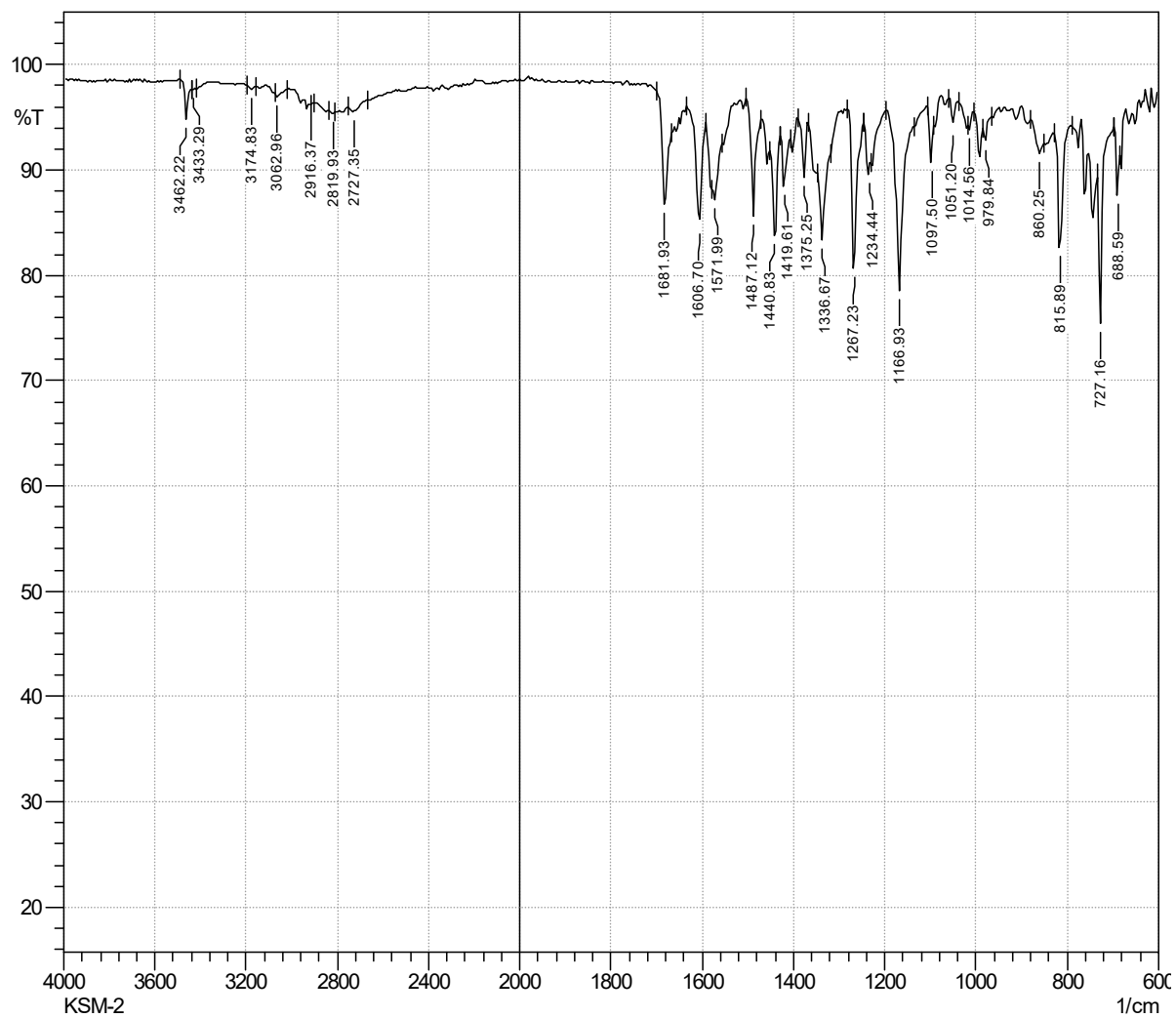


Figure S5. IR spectrum of compound 2b.

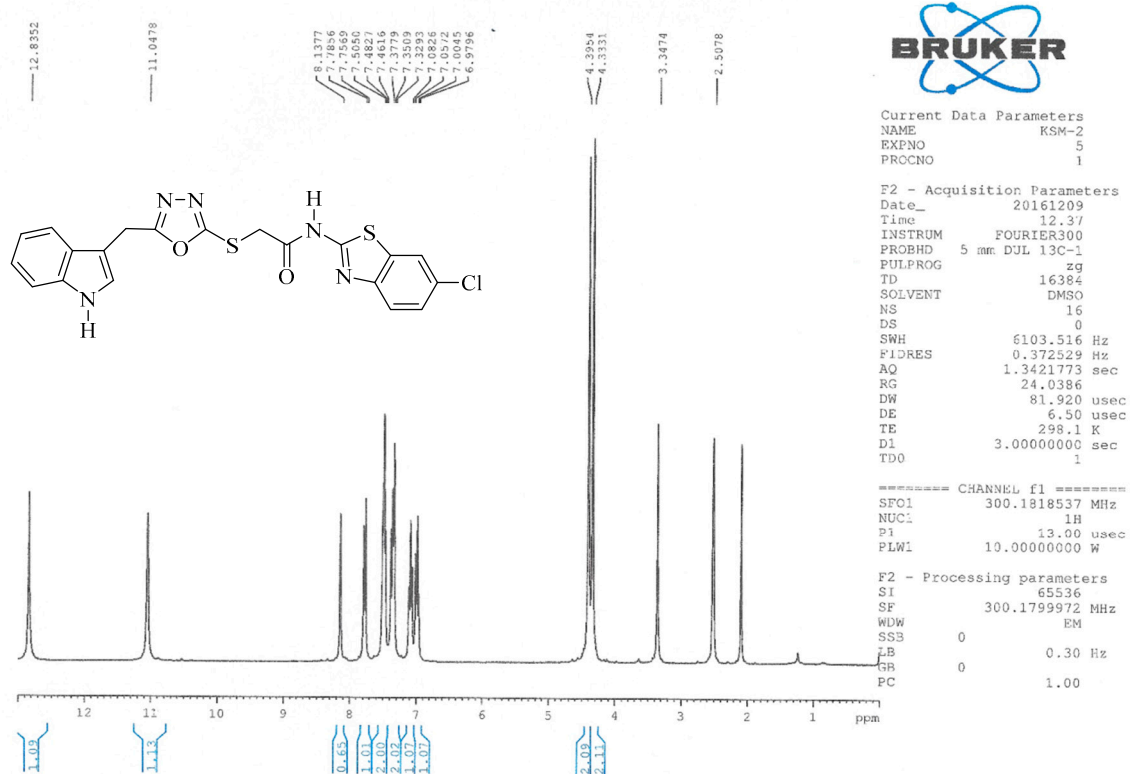


Figure S6. ¹H NMR spectrum of compound 2b.

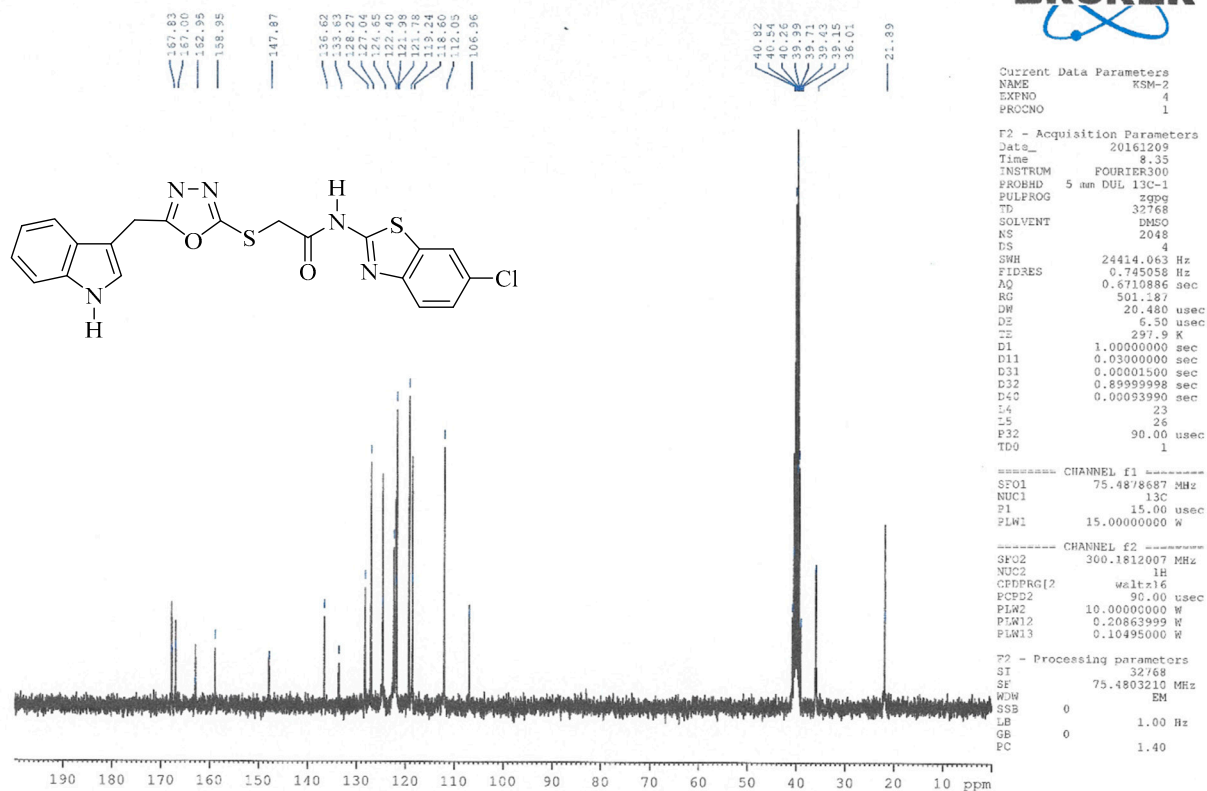


Figure S7. ^{13}C NMR spectrum of compound 2b.

Data File: C:\LabSolutions\Data\Analiz\mdaltintop\KSM-2_2.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	18	30	F	1	0	0	Br	1	0	0					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: yes

Isotope RI (%): 1.00

MSn Logic Mode: AND

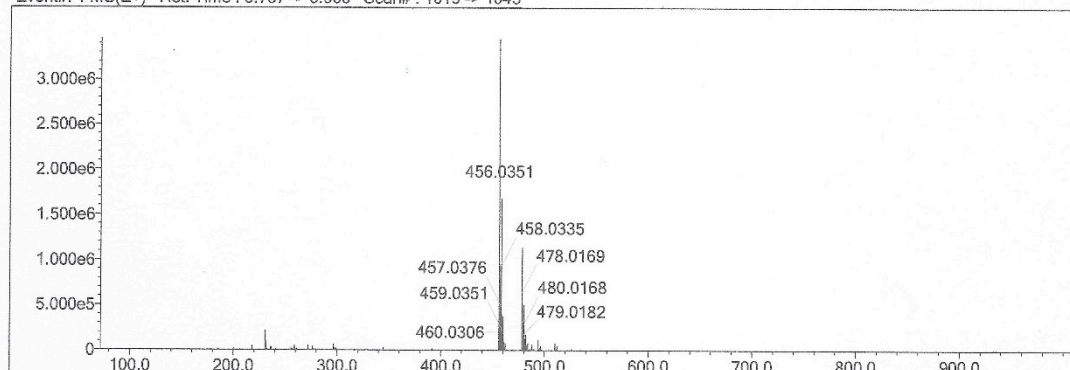
Electron Ions: both

Use MSn Info: no

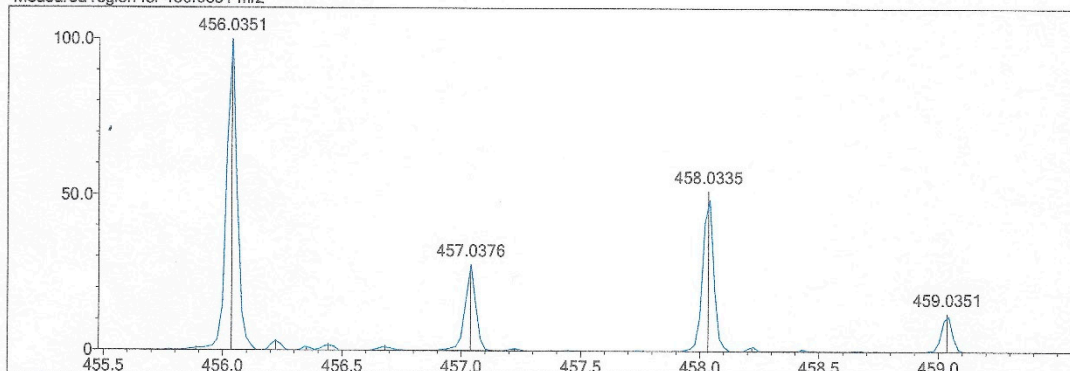
Isotope Res: 10000

Max Results: 500

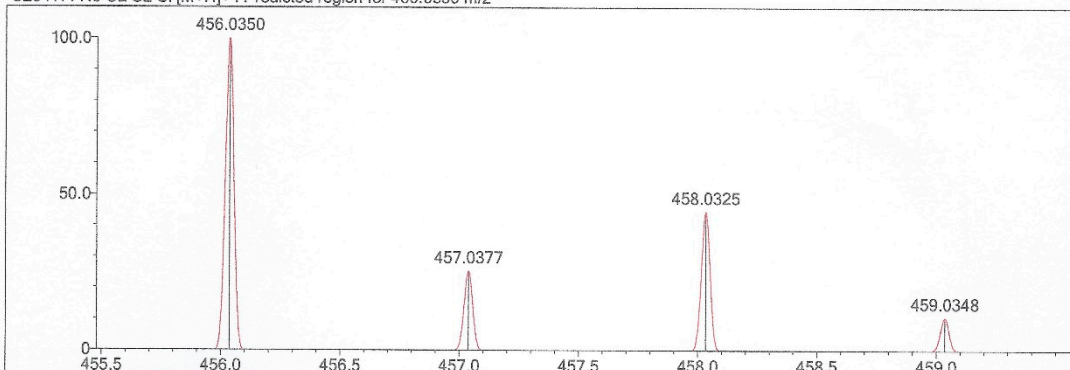
Event#: 1 MS(E+) Ret. Time : 6.787 -> 6.960 Scan#: 1019 -> 1045



Measured region for 456.0351 m/z



C20 H14 N5 O2 S2 Cl [M+H]+ : Predicted region for 456.0350 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	96.94	C20 H14 N5 O2 S2 Cl	[M+H] ⁺	456.0351	456.0350	0.1	0.22	96.94	16.0

Figure S8. HRMS spectrum of compound 2b.

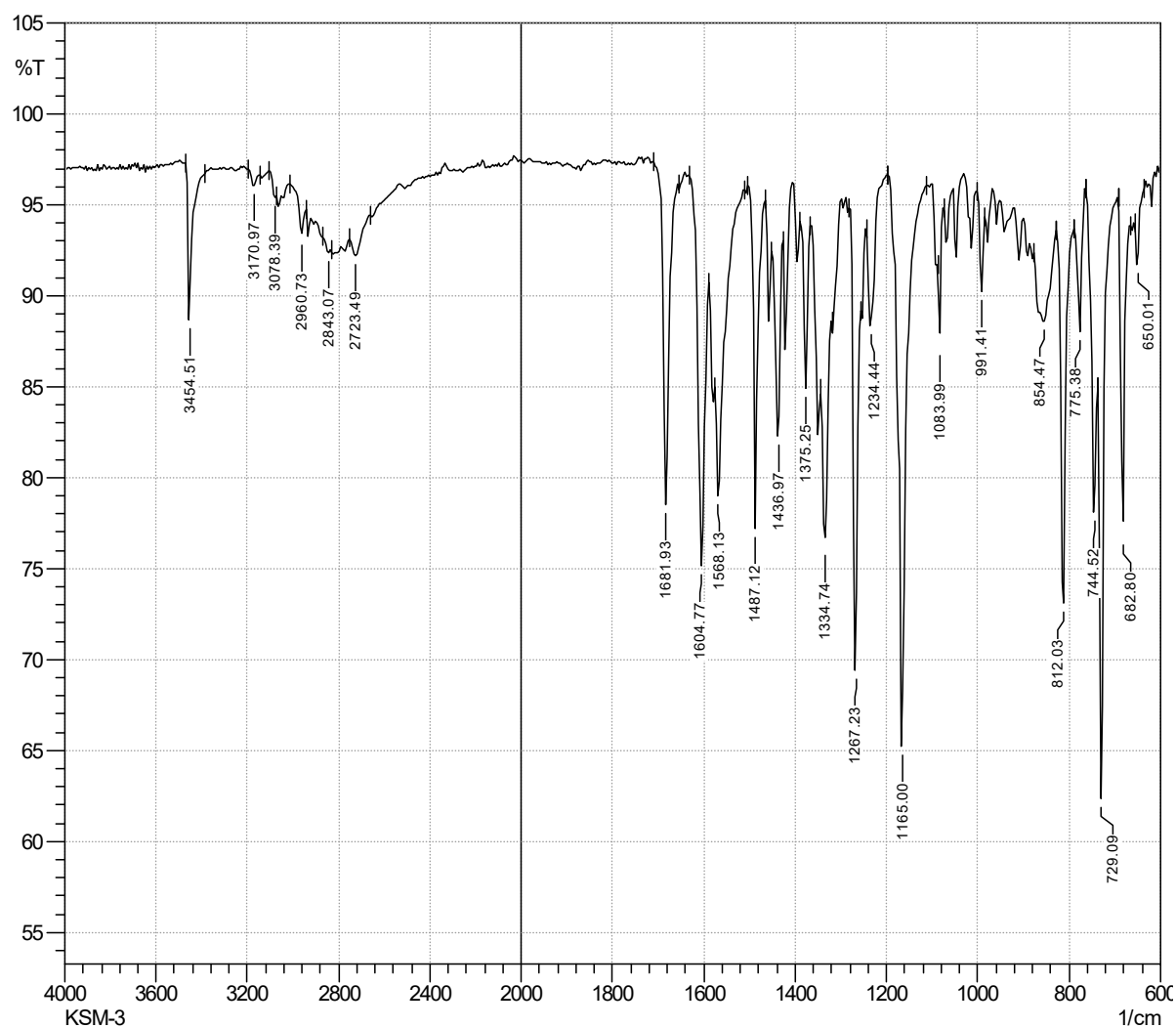


Figure S9. IR spectrum of compound 2c.

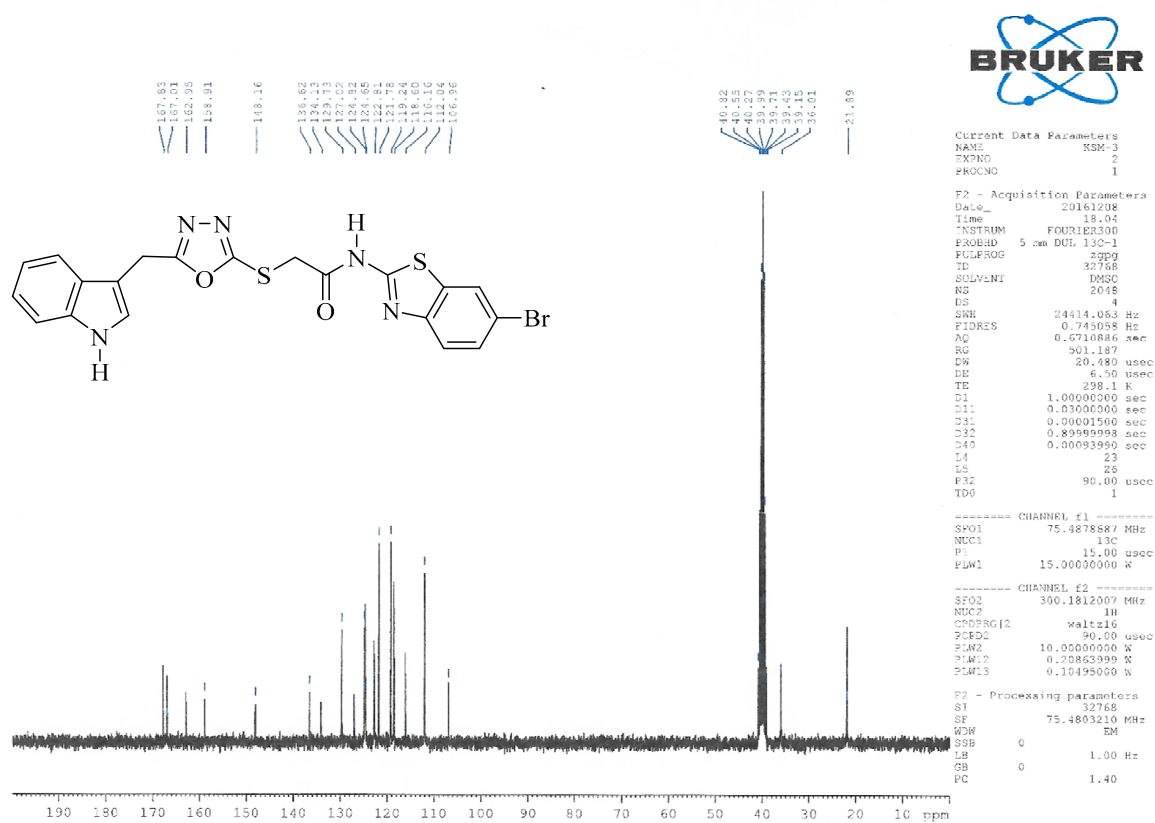


Figure S11. ¹³C NMR spectrum of compound 2c.

Data File: C:\LabSolutions\Data\Analiz\mdallintop\KSM-3_3.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1		3	0	0	H
C	4	18	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

DBE Range: 0.0 - 30.0

Electron Ions: both

HC Ratio: unlimited

Apply N Rule: yes

Use MSn Info: no

Max Isotopes: 3

Isotope RI (%): 1.00

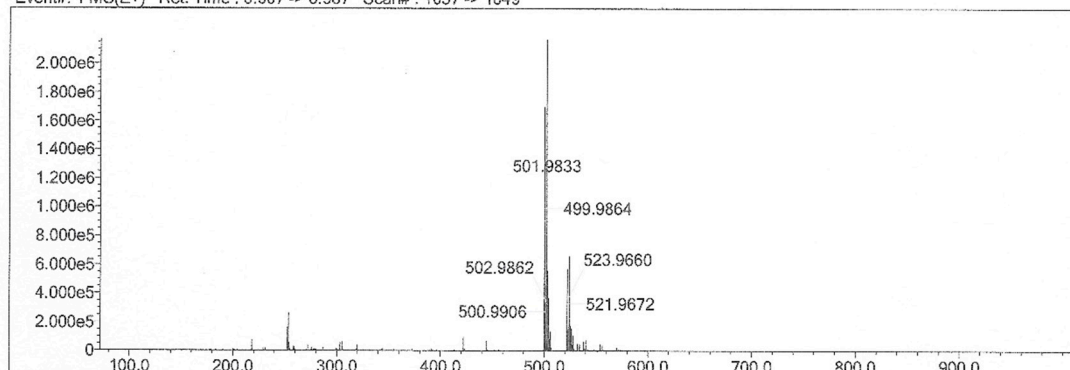
Isotope Res: 10000

MSn Iso RI (%): 10.00

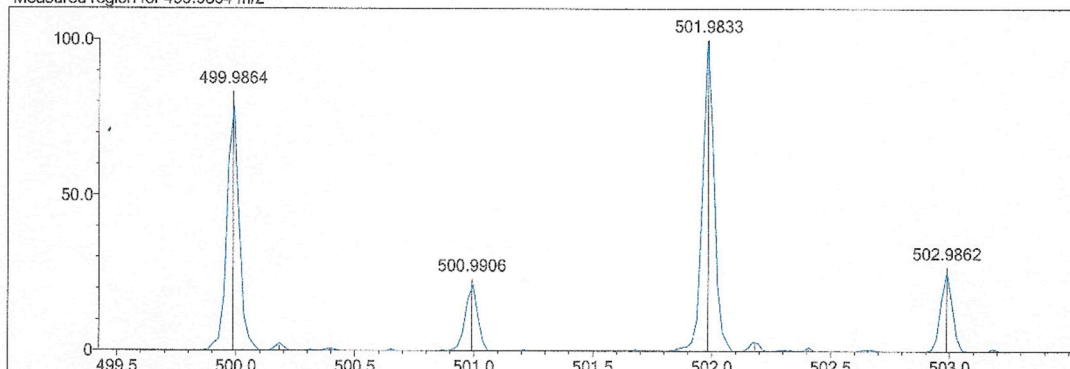
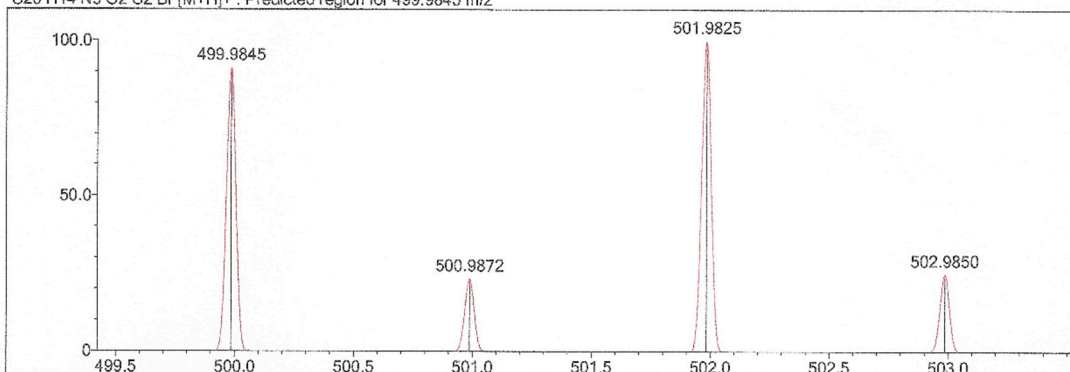
MSn Logic Mode: AND

Max Results: 500

Event#: 1 MS(E+) Ret. Time: 6.907 -> 6.987 Scan#: 1037 -> 1049



Measured region for 499.9864 m/z

C20 H14 N5 O2 S2 Br [M+H]⁺: Predicted region for 499.9845 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	63.43	C20 H14 N5 O2 S2 Br	[M+H] ⁺	499.9864	499.9845	1.9	3.80	68.20	16.0

Figure S12. HRMS spectrum of compound 2c.

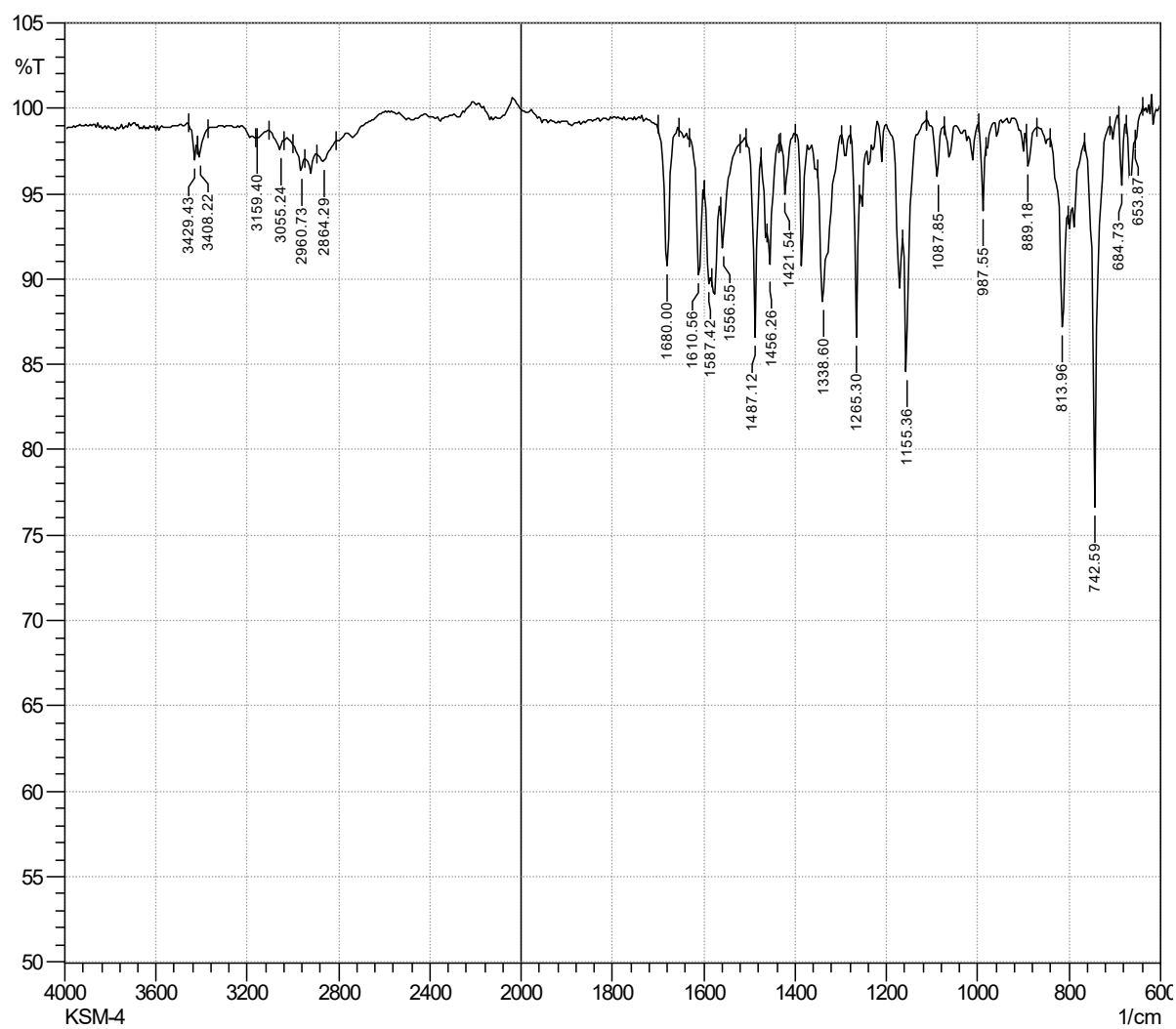


Figure S13. IR spectrum of compound 2d.

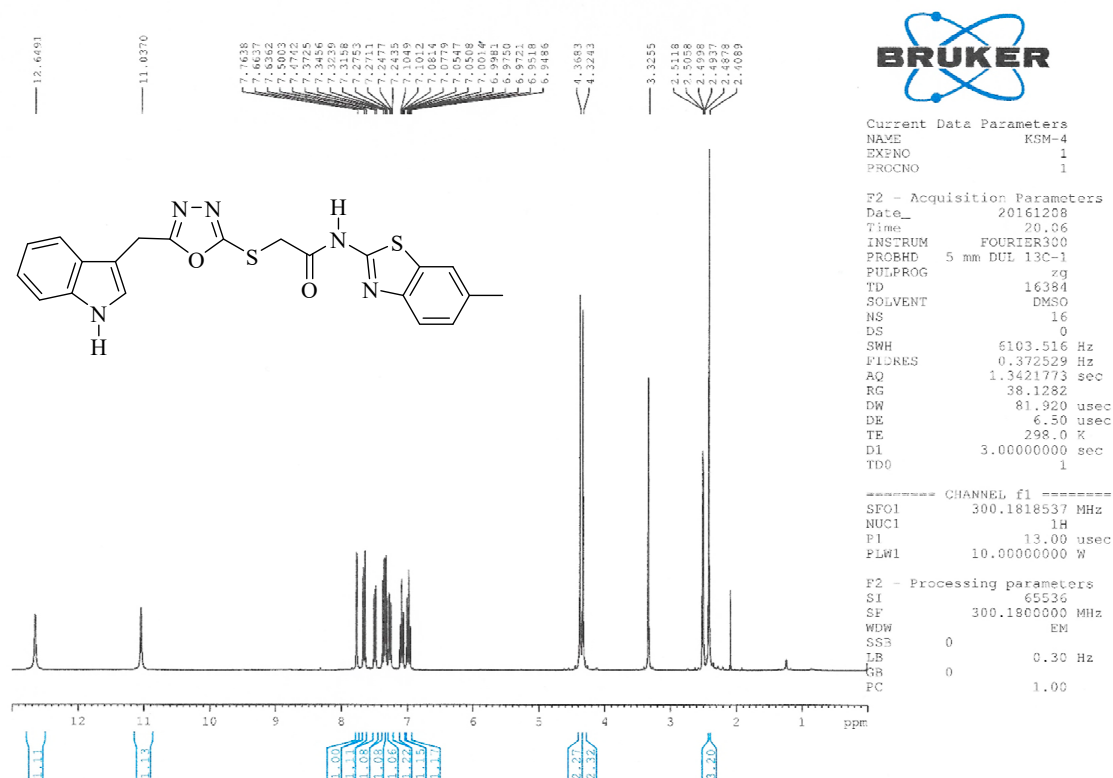


Figure S14. ¹H NMR spectrum of compound 2d.

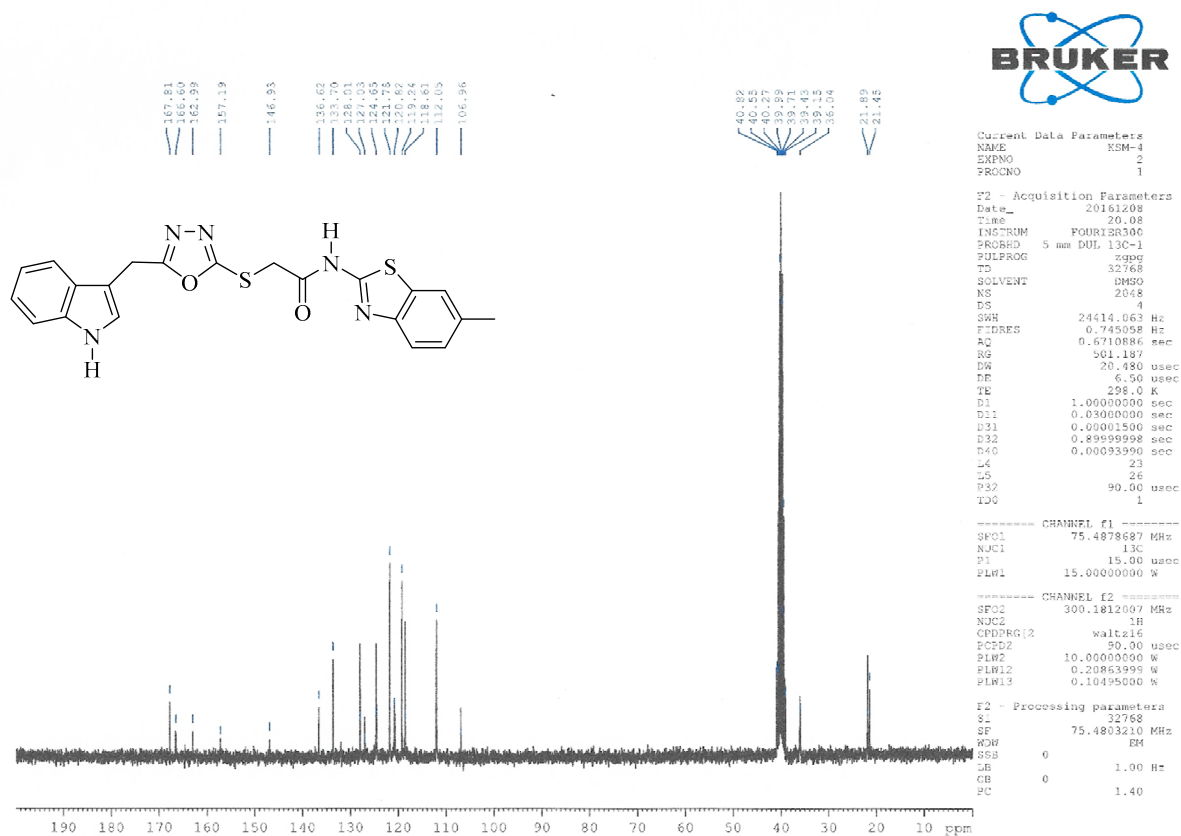


Figure S15. ^{13}C NMR spectrum of compound 2d.

Data File: C:\LabSolutions\Data\Analiz\mdallintop\KSM-4_4.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	18	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

DBE Range: 0.0 - 30.0

Electron Ions: both

HC Ratio: unlimited

Apply N Rule: yes

Use MSn Info: no

Max Isotopes: 3

Isotope RI (%): 1.00

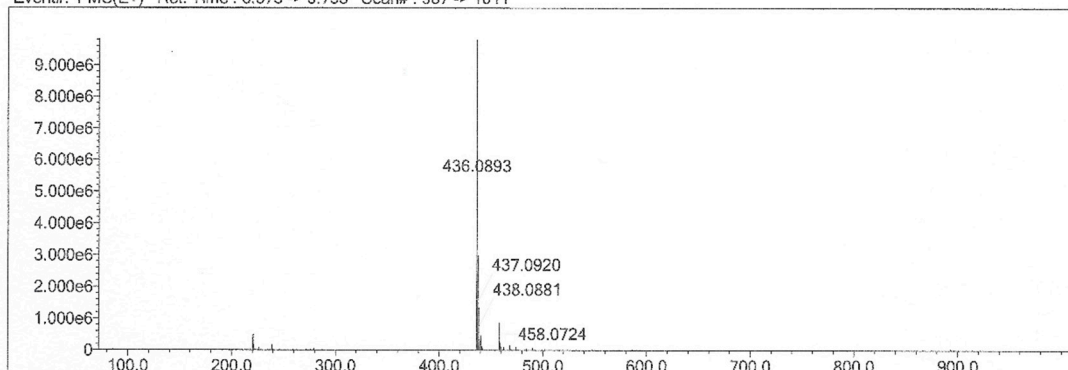
Isotope Res: 10000

MSn Iso RI (%): 10.00

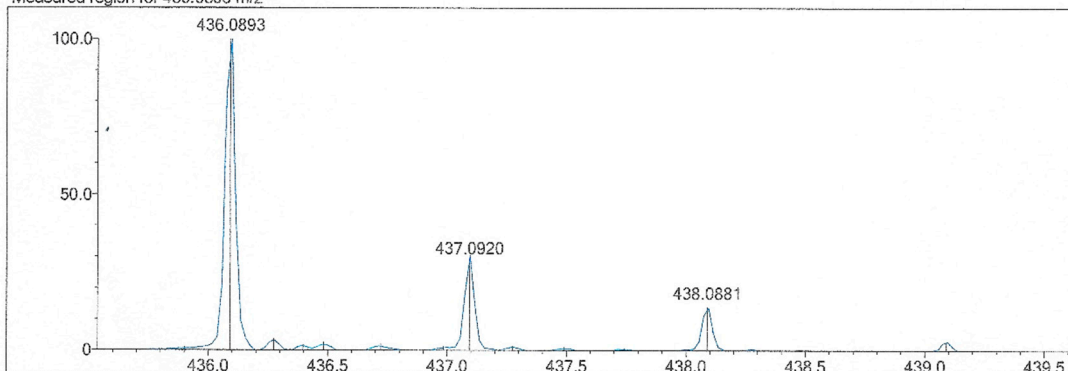
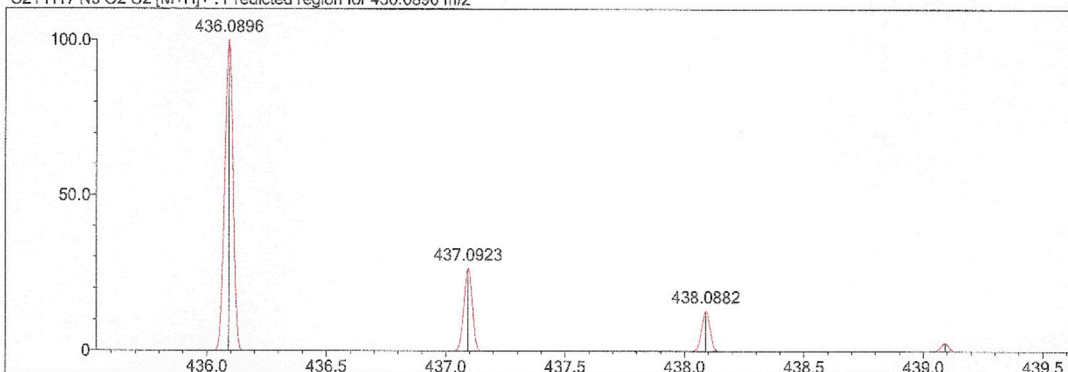
MSn Logic Mode: AND

Max Results: 500

Event#: 1 MS(E+) Ret. Time : 6.573 -> 6.733 Scan#: 987 -> 1011



Measured region for 436.0893 m/z

C21 H17 N5 O2 S2 [M+H]⁺ : Predicted region for 436.0893 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	92.79	C21 H17 N5 O2 S2	[M+H] ⁺	436.0893	436.0896	-0.3	-0.69	92.79	16.0

Figure S16. HRMS spectrum of compound 2d.

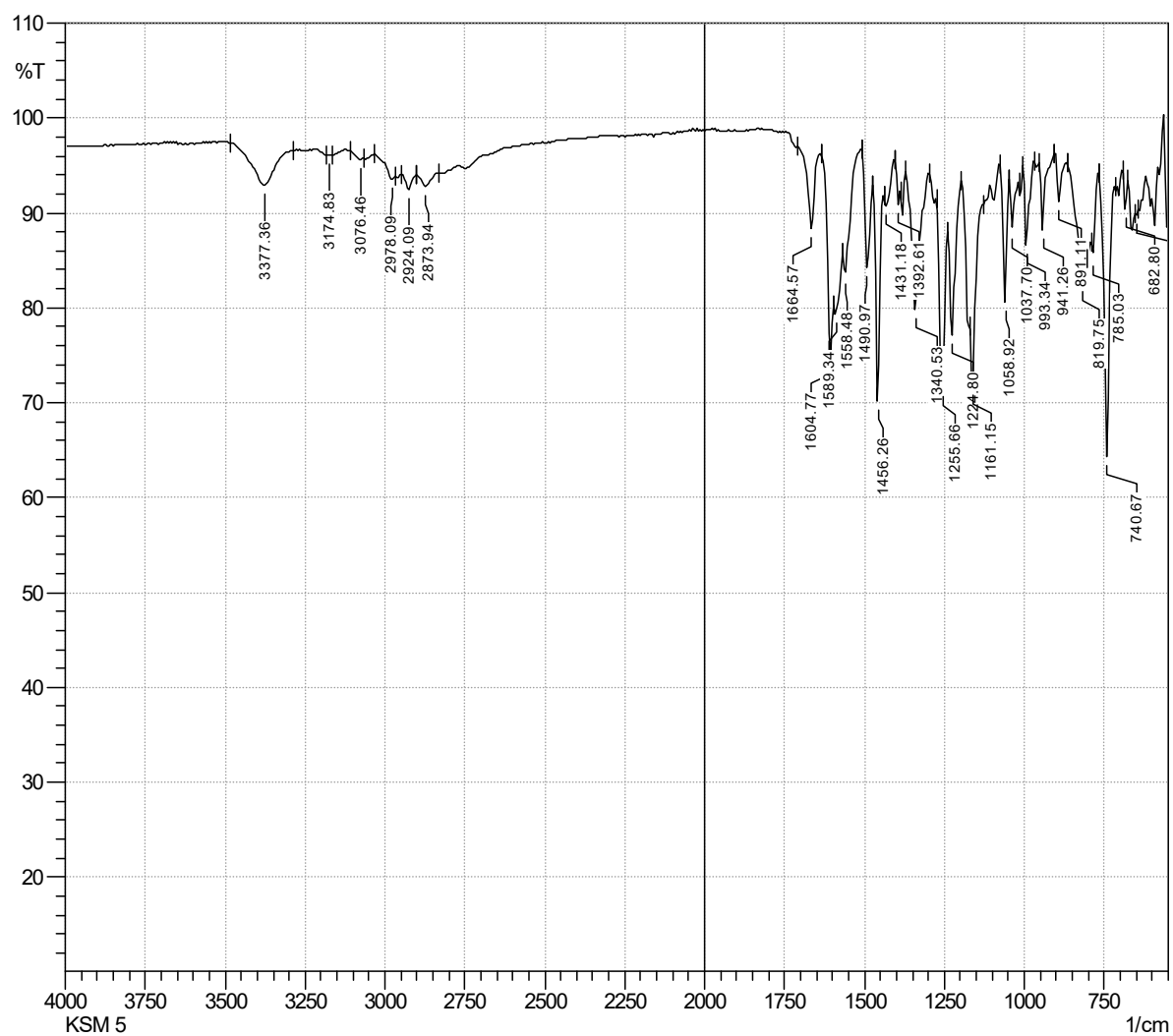


Figure S17. IR spectrum of compound 2e.

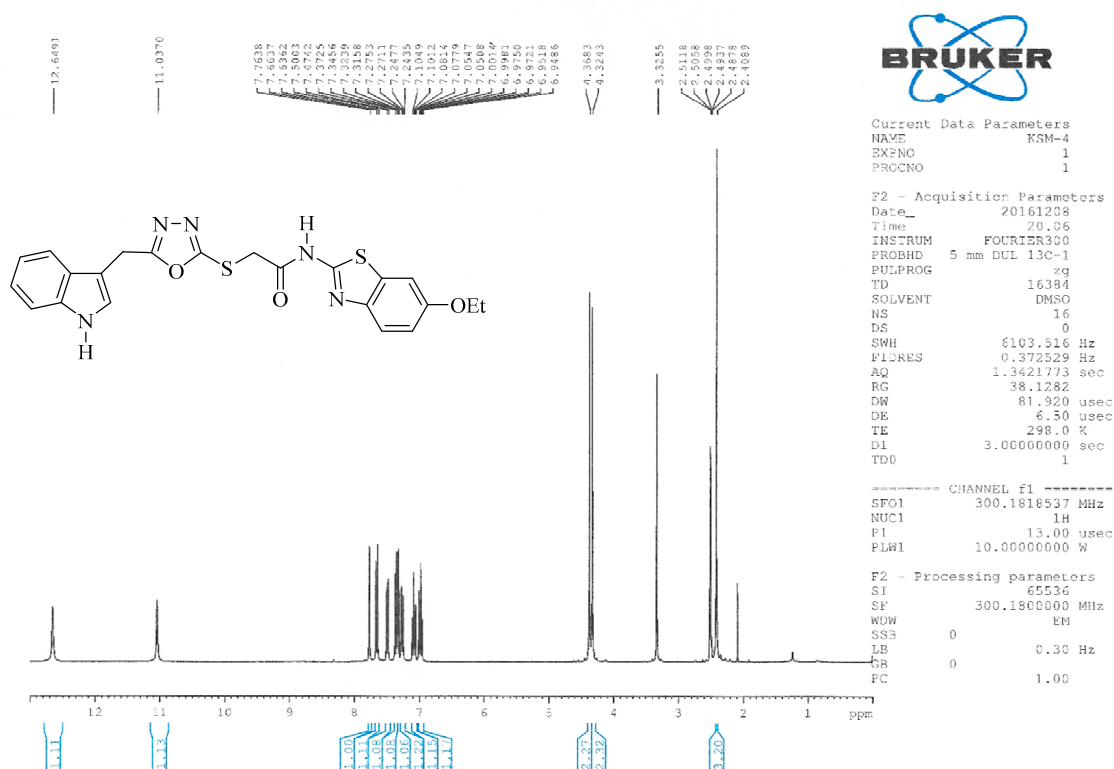


Figure S18. ¹H NMR spectrum of compound 2e.

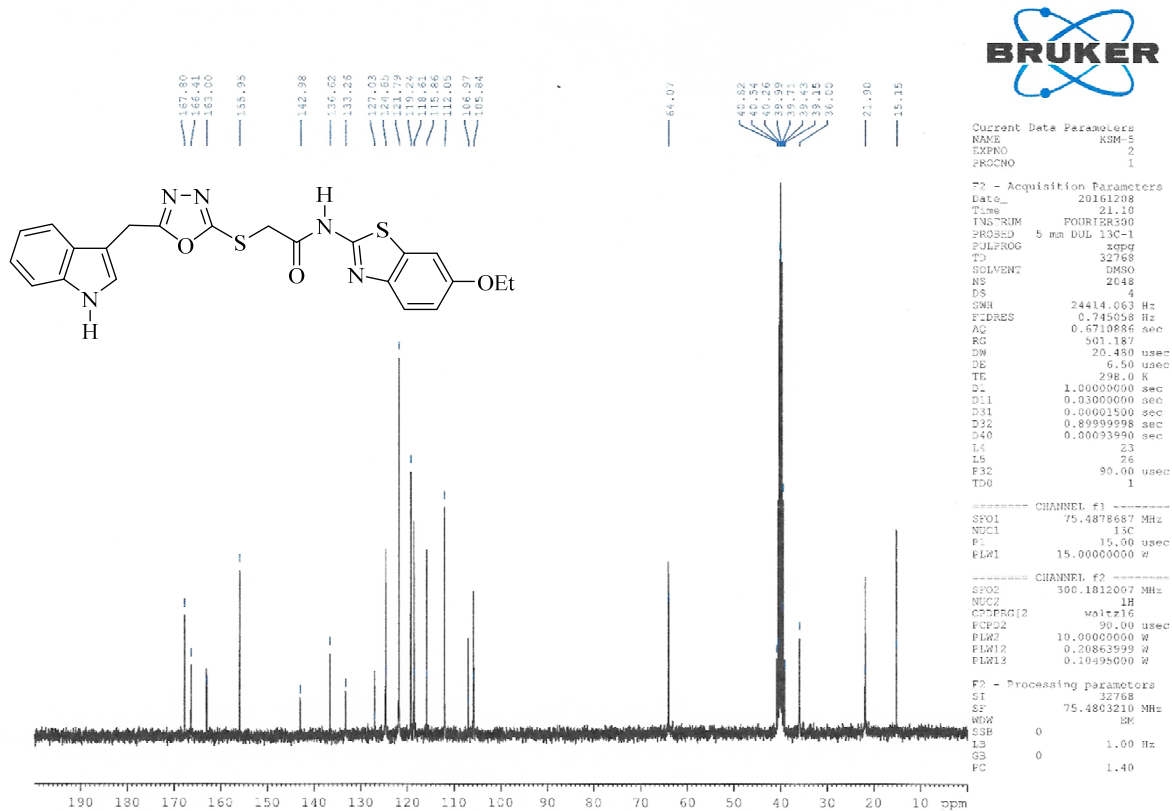


Figure S19. ¹³C NMR spectrum of compound 2e.

Data File: C:\LabSolutions\Data\Analiz\mdaltintop\KSM-5_5.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	18	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: yes

Isotope RI (%): 1.00

MSn Logic Mode: AND

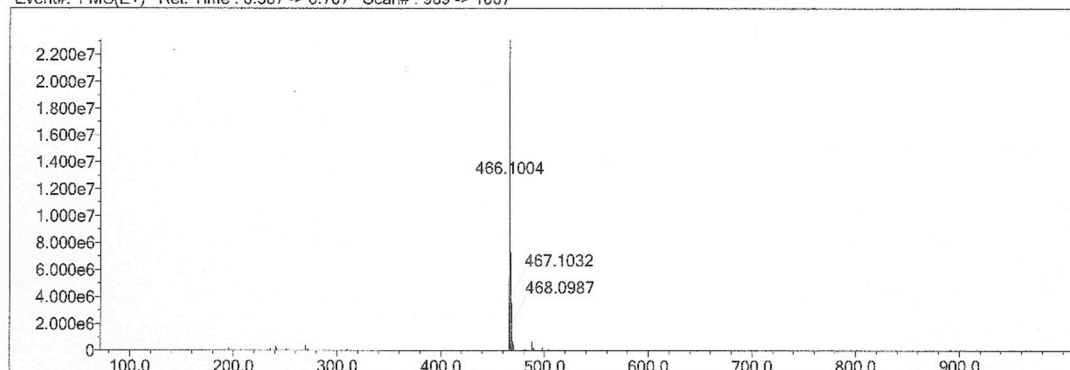
Electron Ions: both

Use MSn Info: no

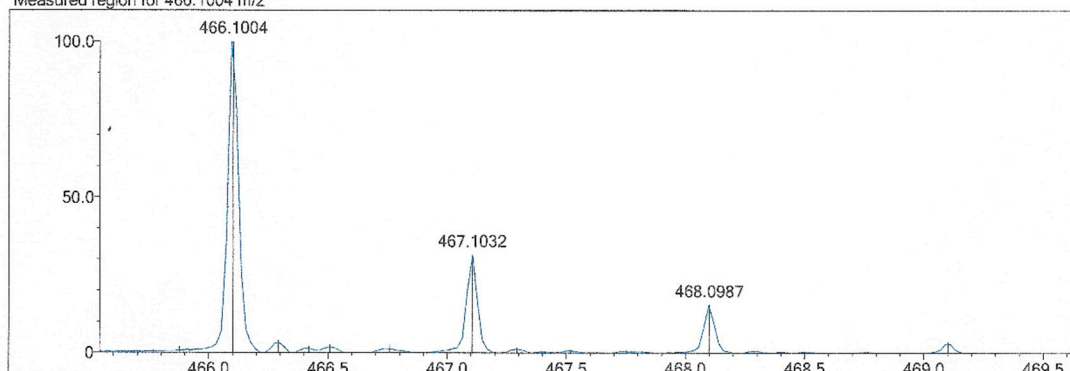
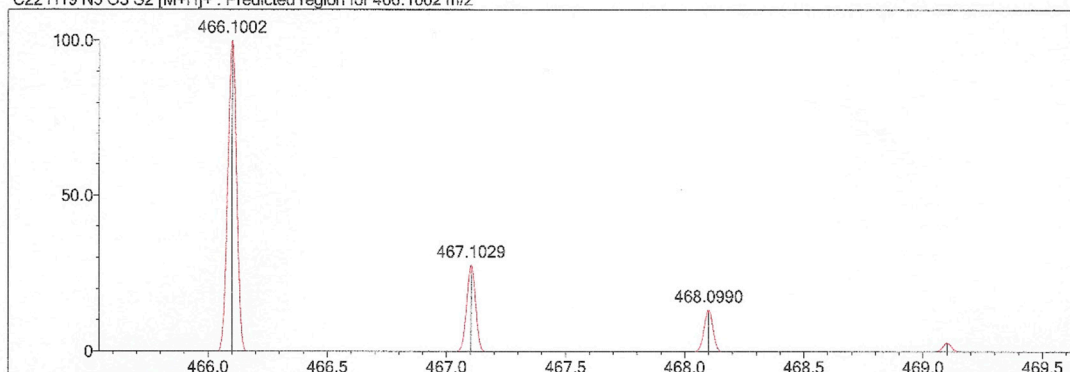
Isotope Res: 10000

Max Results: 500

Event#: 1 MS(E+) Ret. Time : 6.587 -> 6.707 Scan#: 989 -> 1007



Measured region for 466.1004 m/z

C22 H19 N5 O3 S2 [M+H]⁺ : Predicted region for 466.1002 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	86.85	C22 H19 N5 O3 S2	[M+H] ⁺	466.1004	466.1002	0.2	0.43	86.85	16.0

Figure S20. HRMS spectrum of compound 2e.

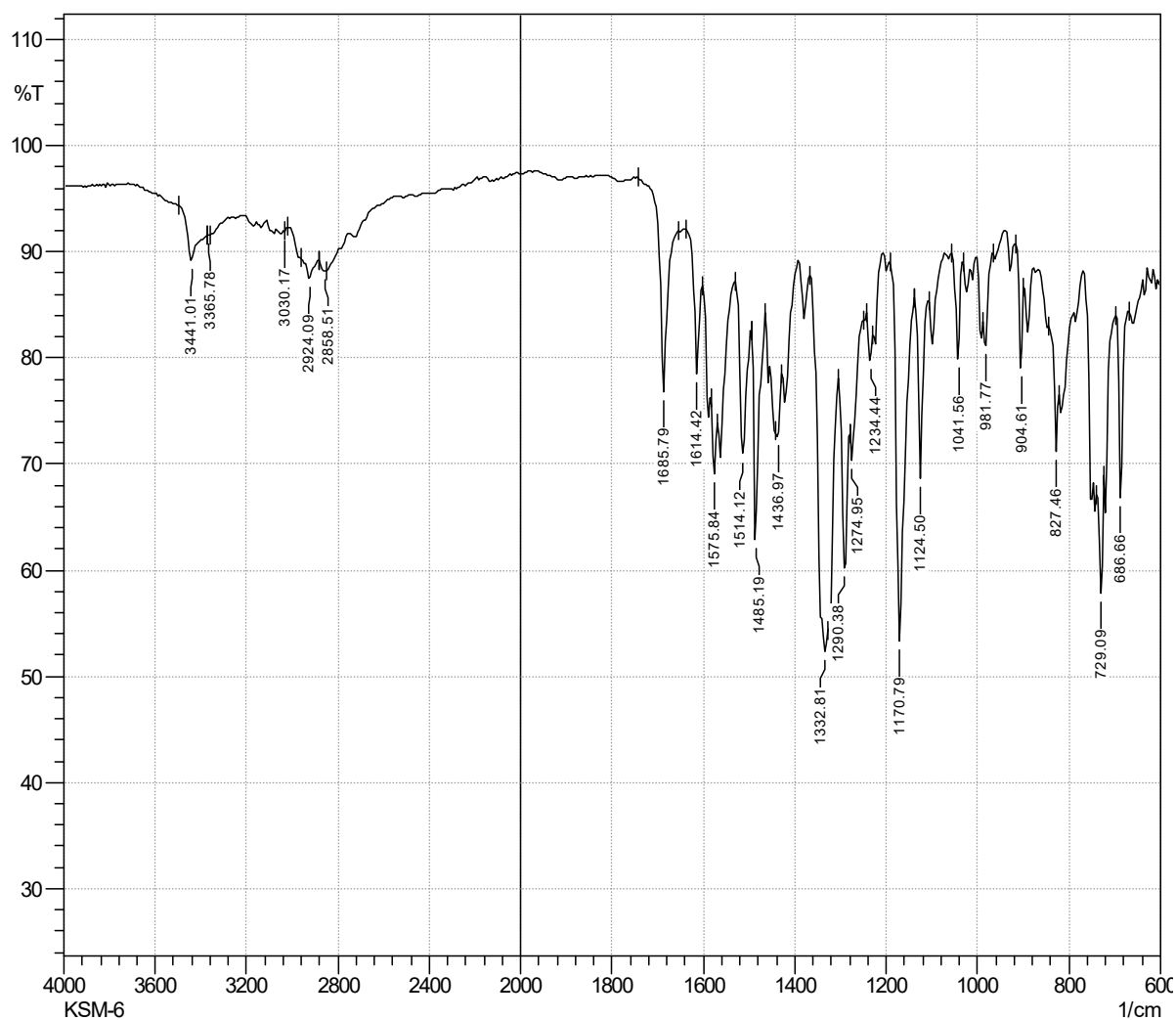


Figure S21. IR spectrum of compound 2f.

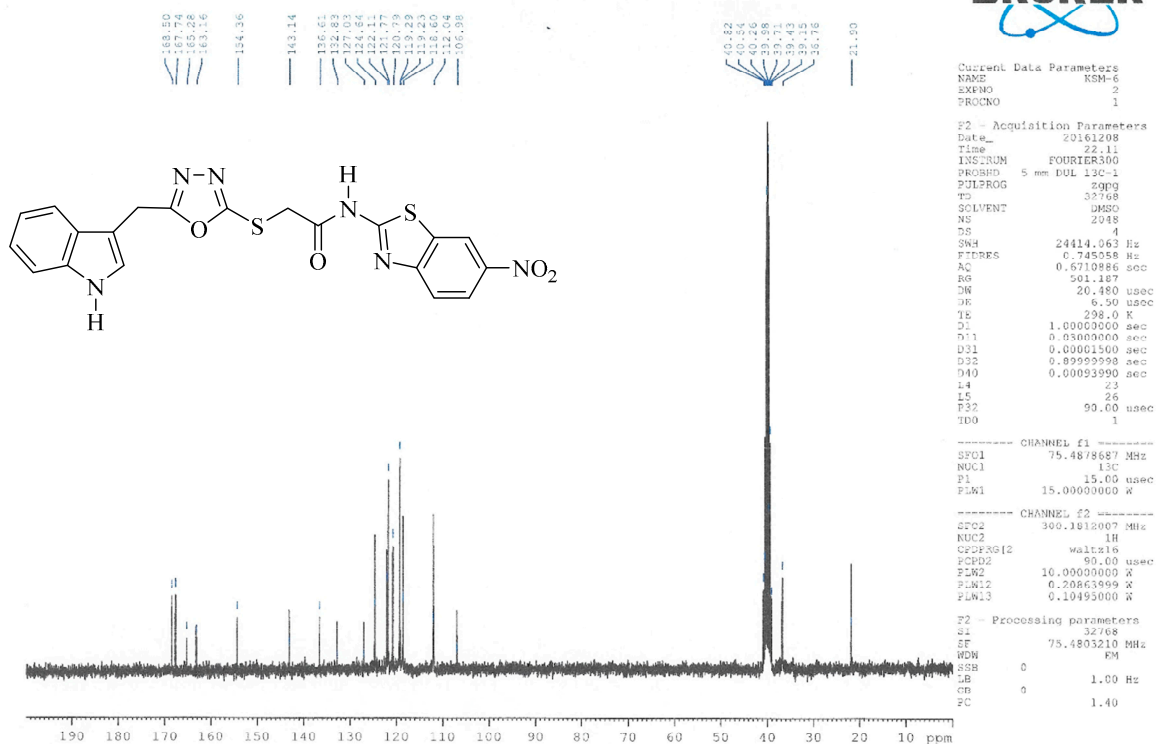


Figure S23. ¹³C NMR spectrum of compound 2f.

Data File: C:\LabSolutions\Data\Analiz\mdalt\intop\KSM-6_6.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	18	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

DBE Range: 0.0 - 30.0

Electron Ions: both

HC Ratio: unlimited

Apply N Rule: yes

Use MSn Info: no

Max Isotopes: 3

Isotope RI (%): 1.00

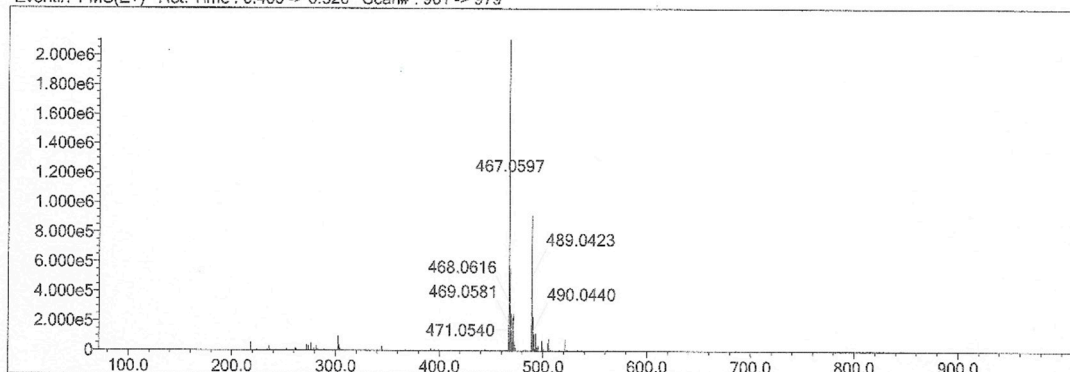
Isotope Res: 10000

MSn Iso RI (%): 10.00

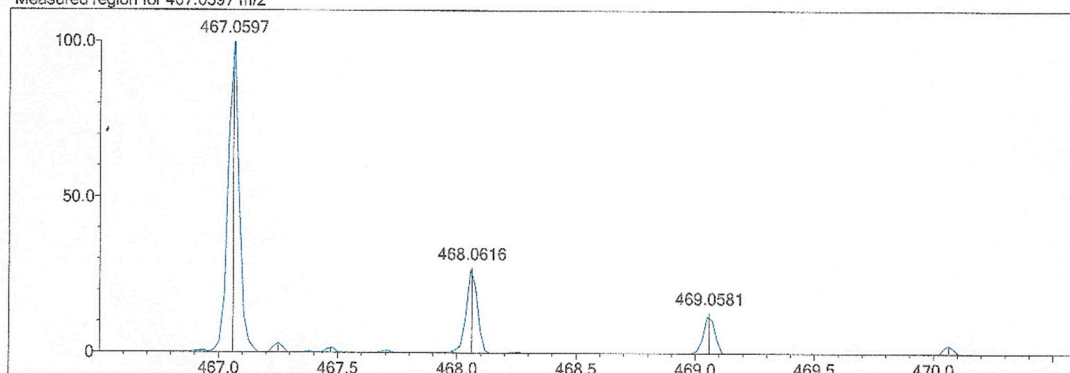
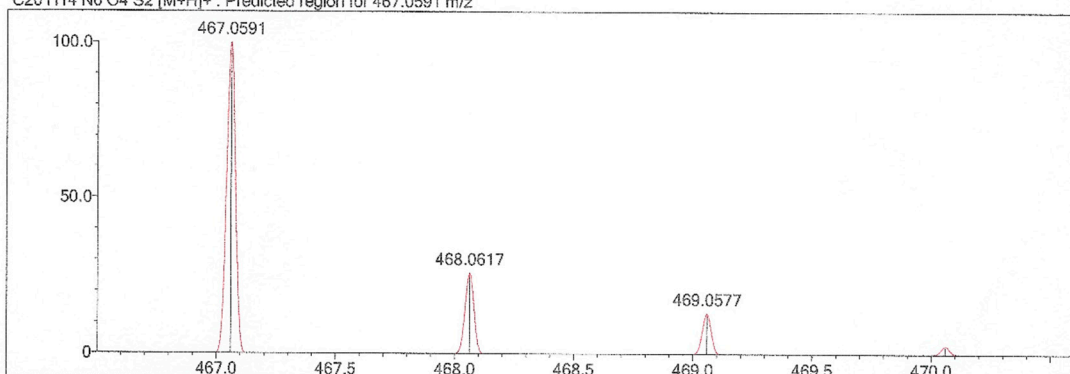
MSn Logic Mode: AND

Max Results: 500

Event#: 1 MS(E+) Ret. Time : 6.400 -> 6.520 Scan# : 961 -> 979



Measured region for 467.0597 m/z

C20 H14 N6 O4 S2 [M+H]⁺ : Predicted region for 467.0591 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	92.22	C20 H14 N6 O4 S2	[M+H] ⁺	467.0597	467.0591	0.6	1.28	92.87	17.0

Figure S24. HRMS spectrum of compound 2f.

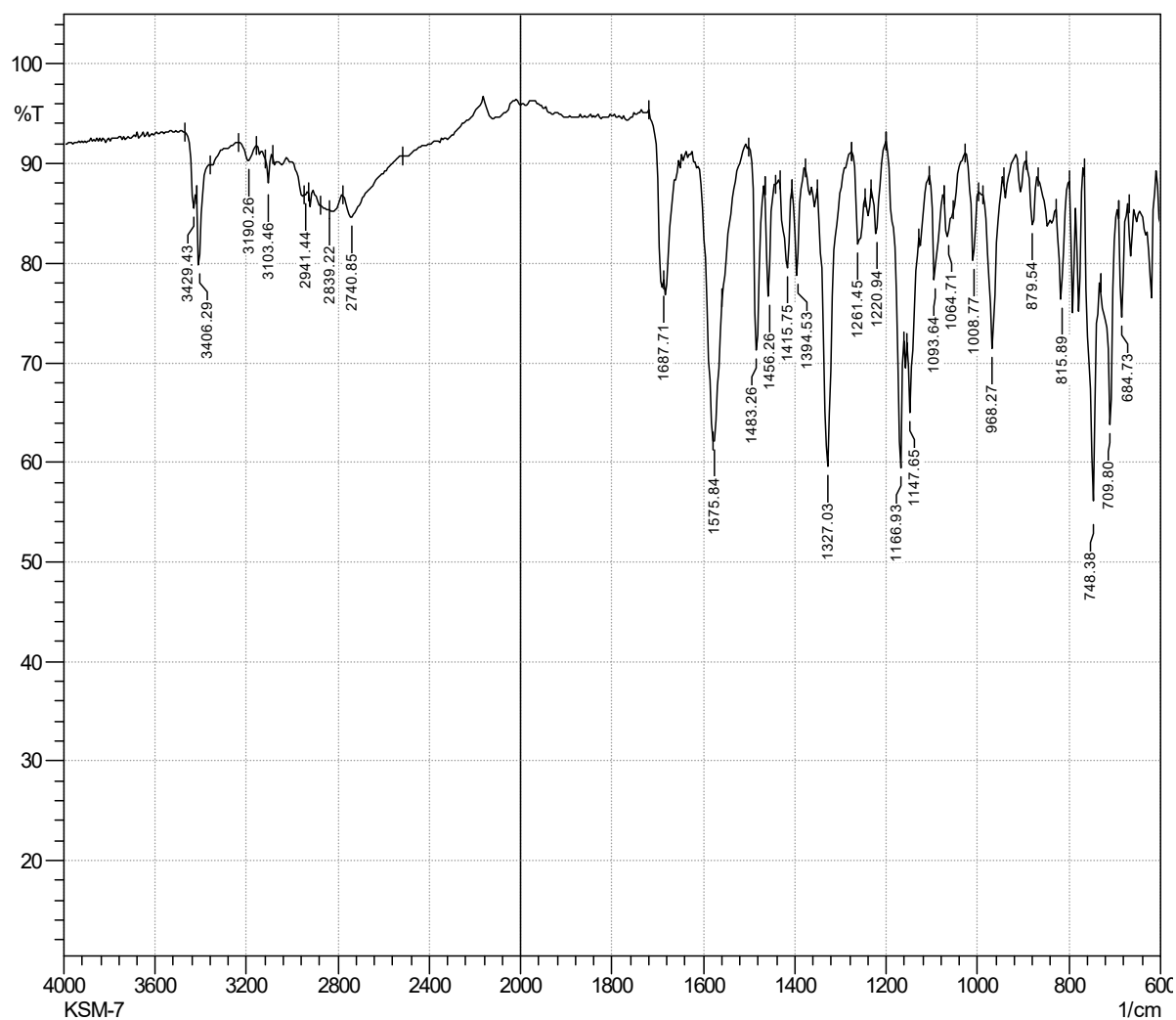


Figure S25. IR spectrum of compound 2g.

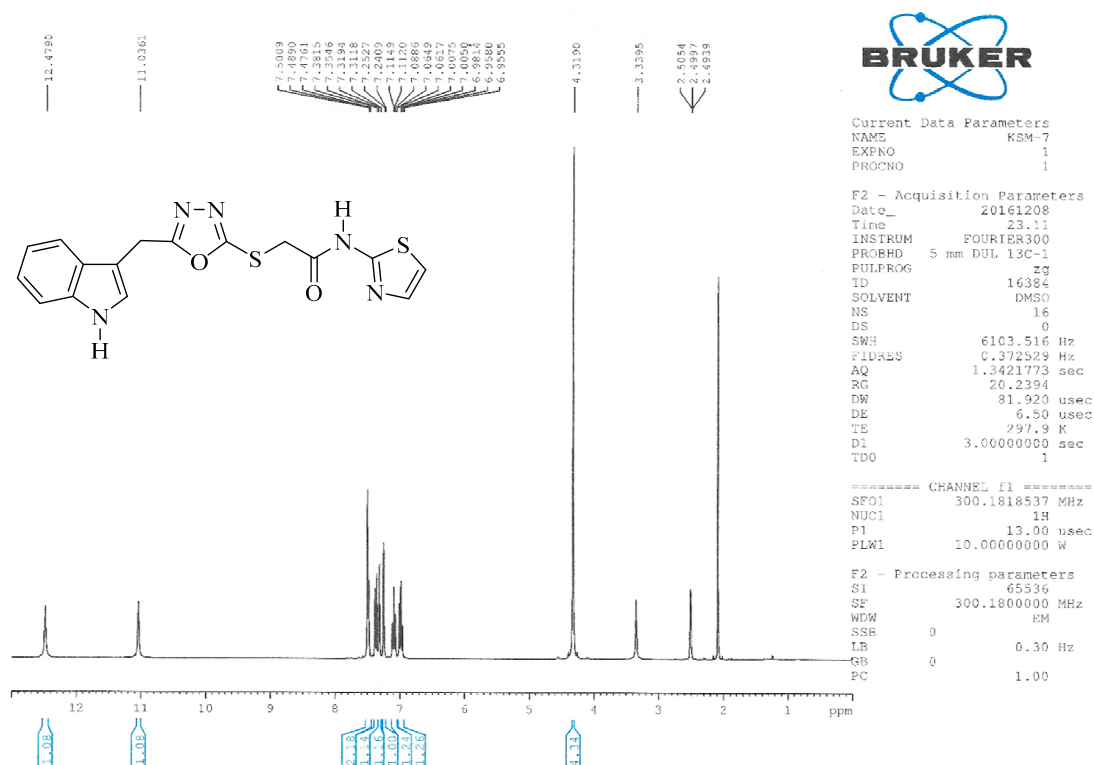


Figure S26. ¹H NMR spectrum of compound 2g.

Data File: C:\LabSolutions\Data\Analiz\mdaltintop\KSM-7_7.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	15	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: yes

Isotope RI (%): 1.00

MSn Logic Mode: AND

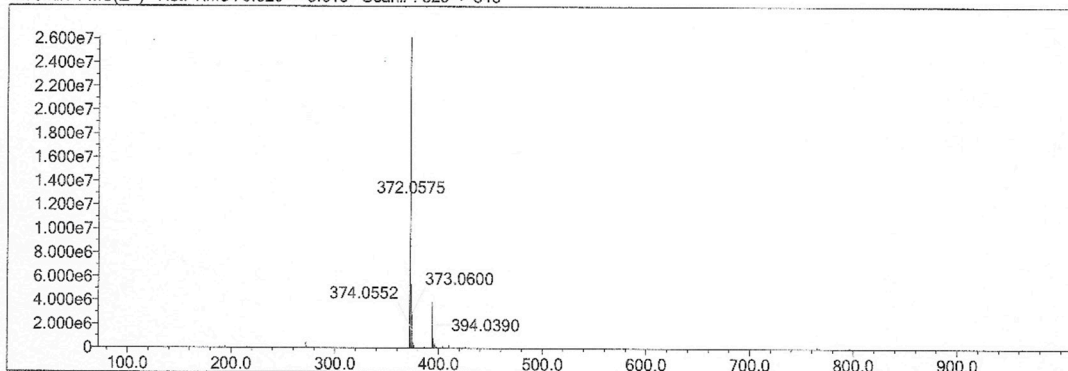
Electron Ions: both

Use MSn Info: no

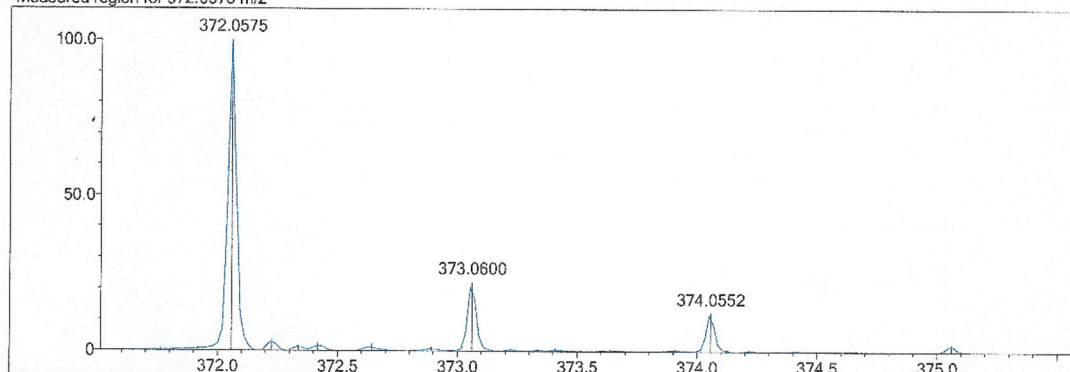
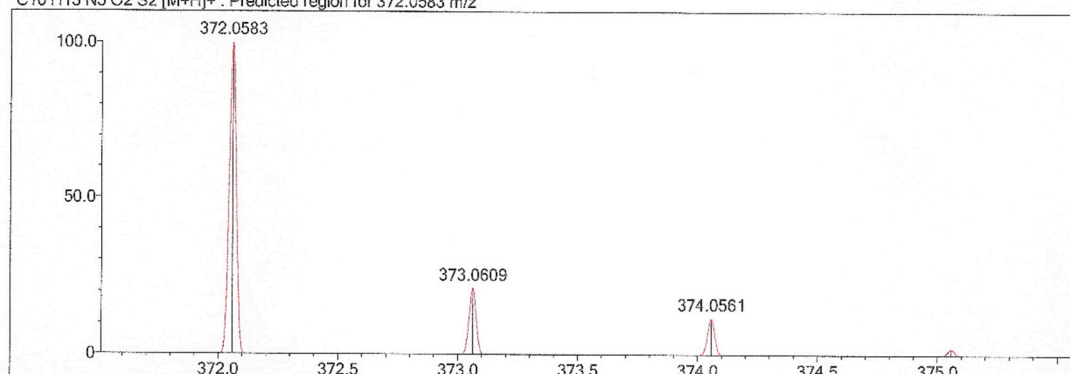
Isotope Res: 10000

Max Results: 500

Event#: 1 MS(E+) Ret. Time: 5.520 -> 5.613 Scan#: 829 -> 843



Measured region for 372.0575 m/z

C16 H13 N5 O2 S2 [M+H]⁺: Predicted region for 372.0583 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	87.85	C16 H13 N5 O2 S2	[M+H] ⁺	372.0575	372.0583	-0.8	-2.15	90.45	13.0

Figure S28. HRMS spectrum of compound **2g**.

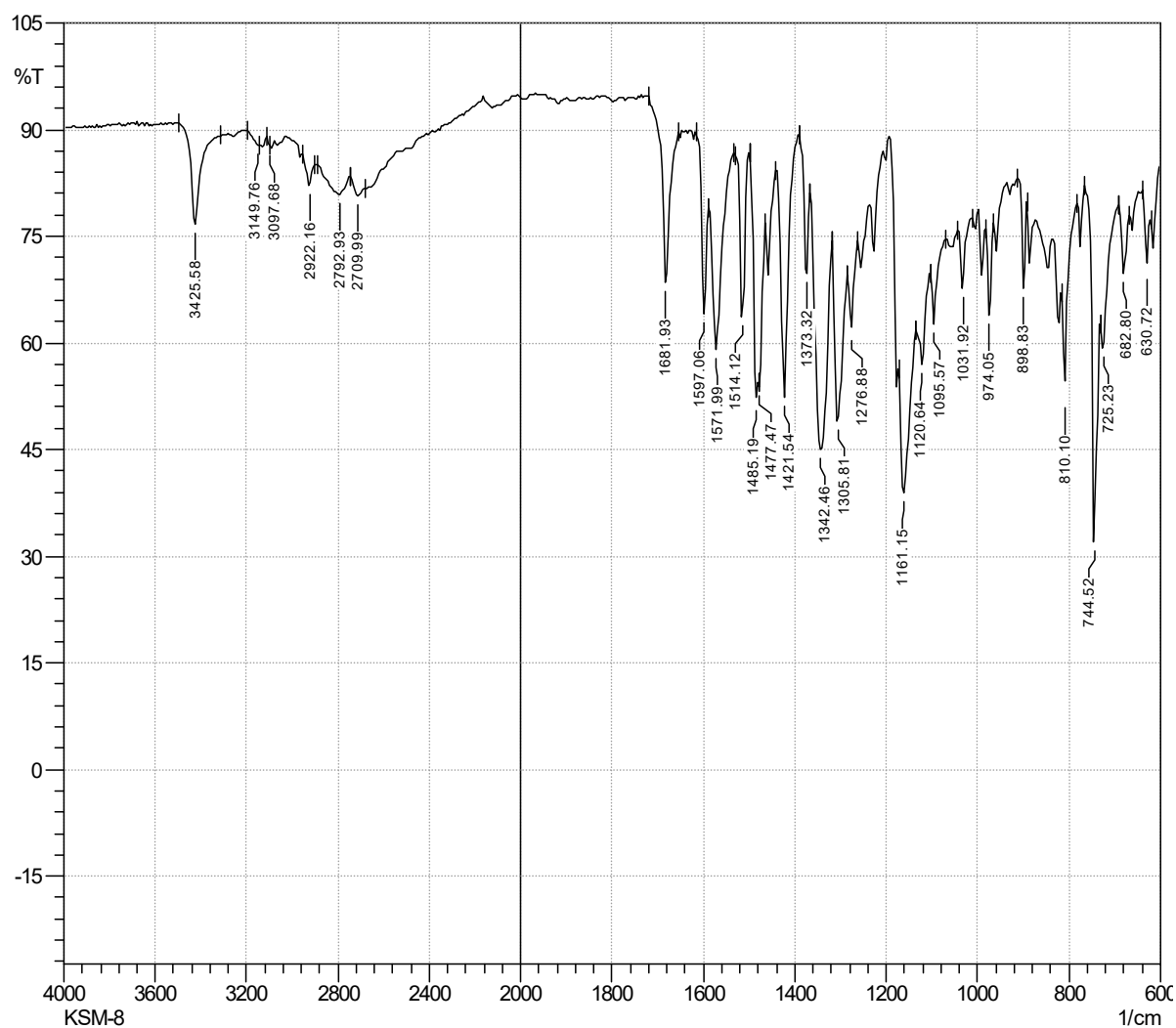


Figure S29. IR spectrum of compound 2h.

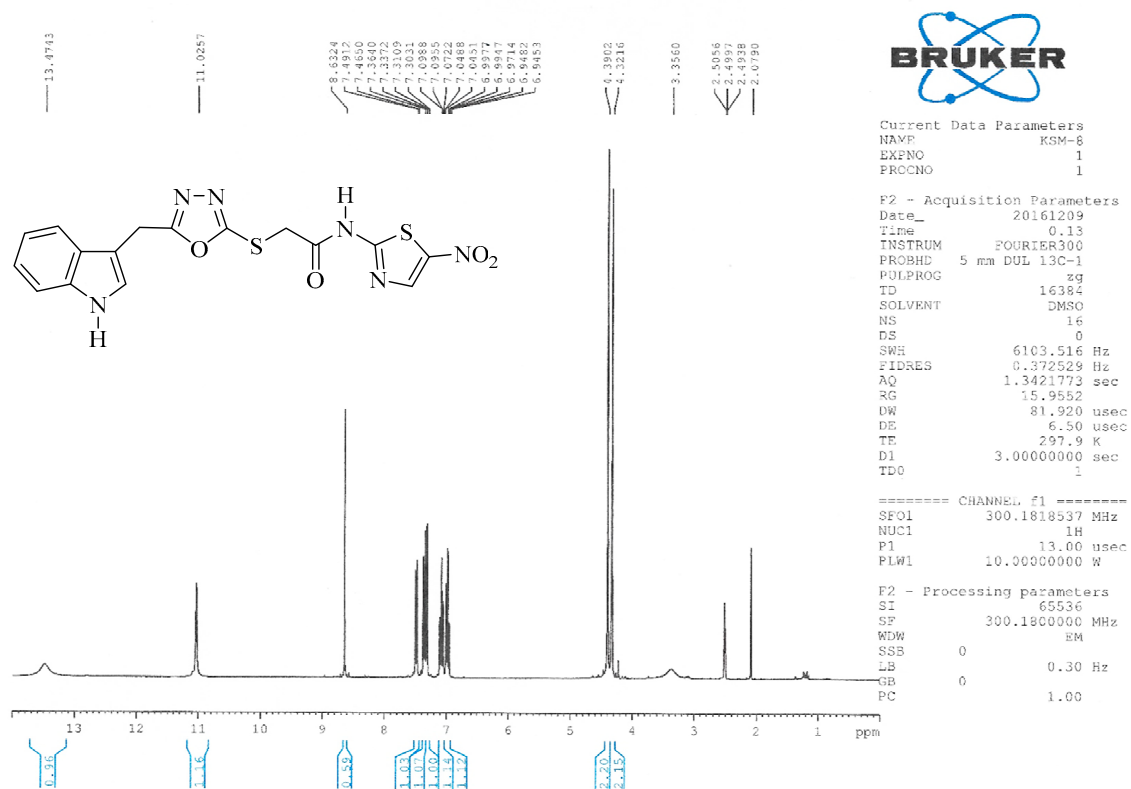


Figure S30. ¹H NMR spectrum of compound 2h.

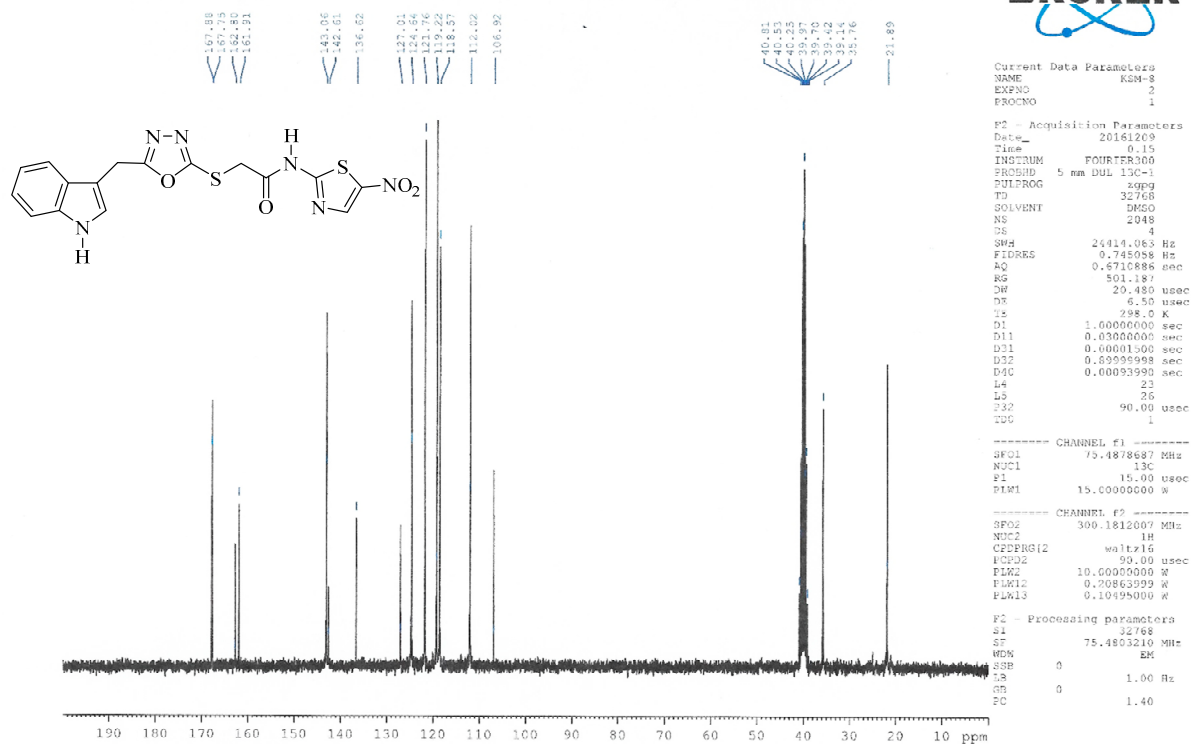


Figure S31. ^{13}C NMR spectrum of compound 2h.

Data File: C:\LabSolutions\Data\Analyze\mdeltintop\KSM-8_8.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	15	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

DBE Range: 0.0 - 30.0

Electron Ions: both

HC Ratio: unlimited

Apply N Rule: yes

Use MSn Info: no

Max Isotopes: 3

Isotope RI (%): 1.00

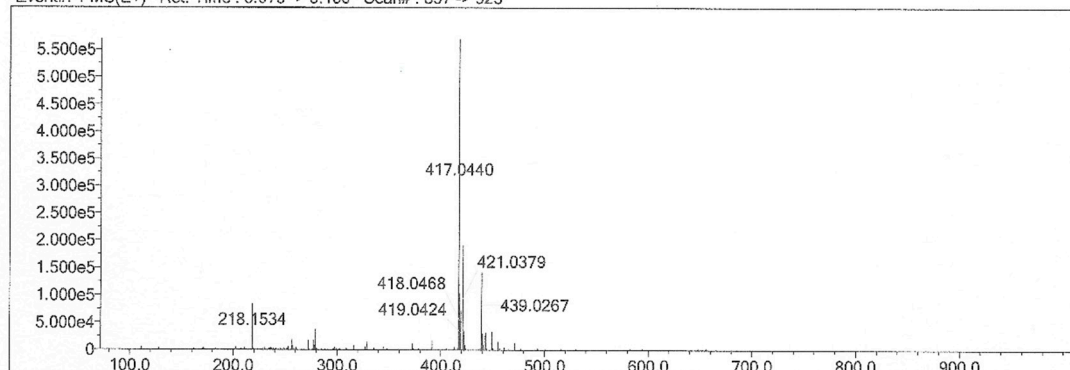
Isotope Res: 10000

MSn Iso RI (%): 10.00

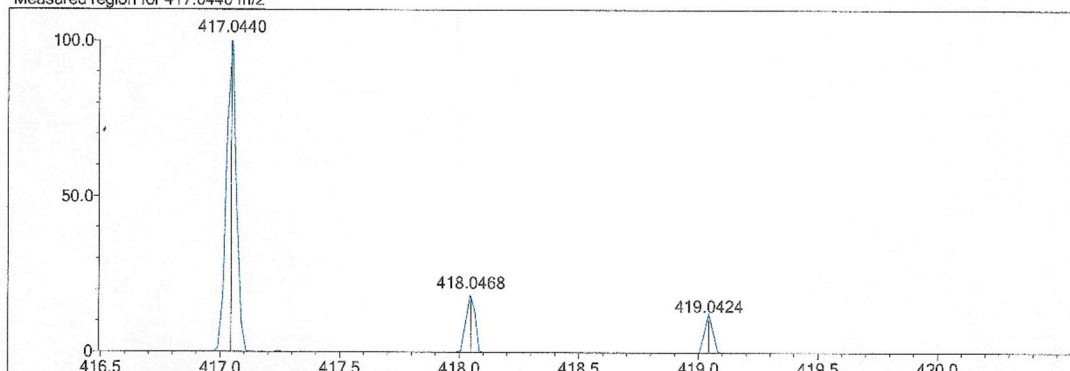
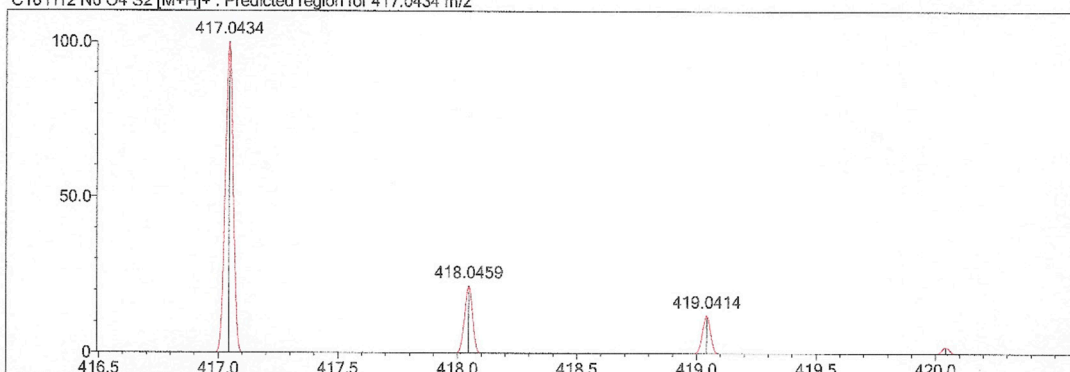
MSn Logic Mode: AND

Max Results: 500

Event#: 1 MS(E+) Ret. Time : 5.973 -> 6.160 Scan# : 897 -> 925



Measured region for 417.0440 m/z

C16 H12 N6 O4 S2 [M+H]⁺ : Predicted region for 417.0434 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	77.58	C16 H12 N6 O4 S2	[M+H] ⁺	417.0440	417.0434	0.6	1.44	78.45	14.0

Figure S32. HRMS spectrum of compound 2h.

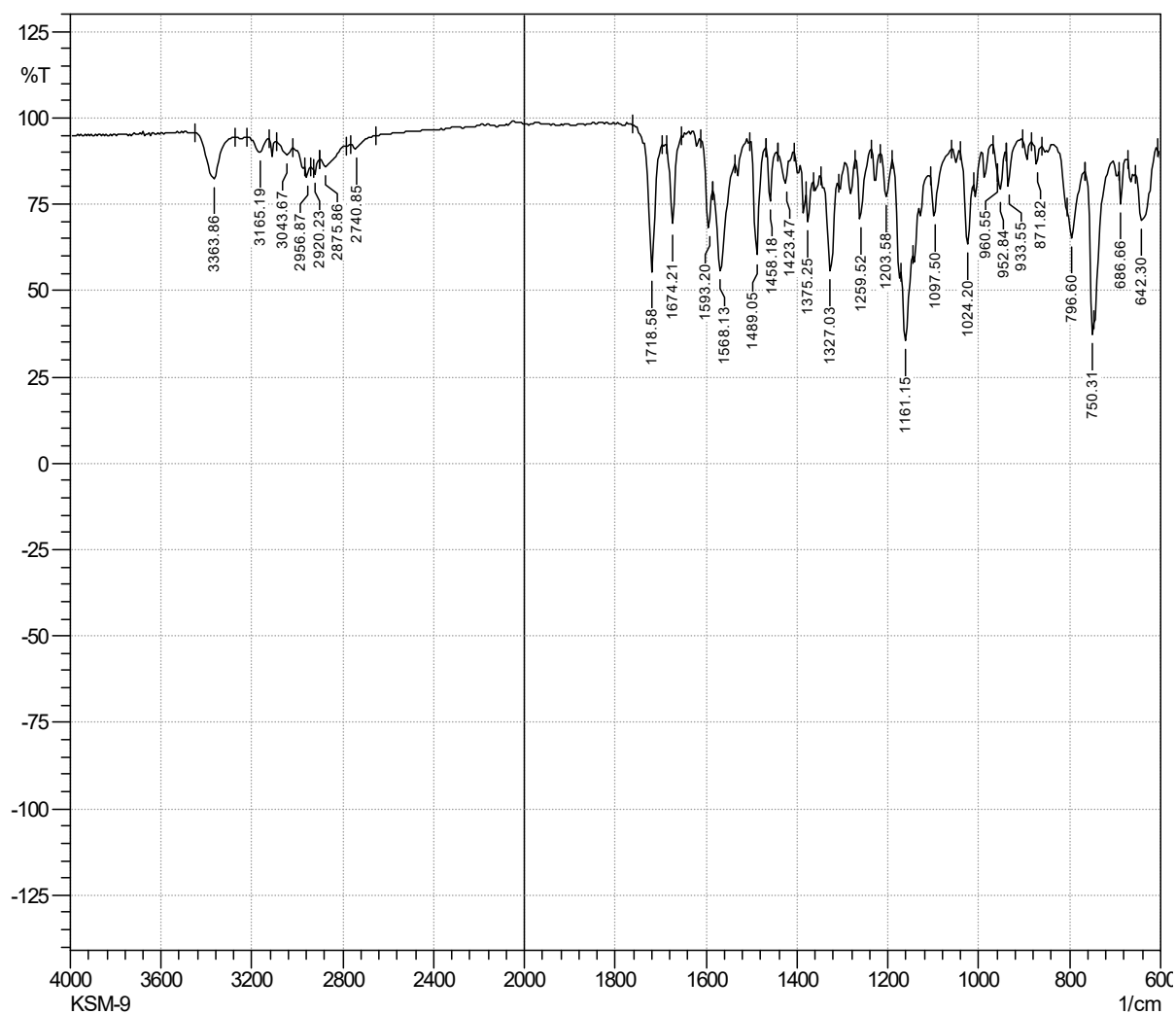


Figure S33. IR spectrum of compound 2i.

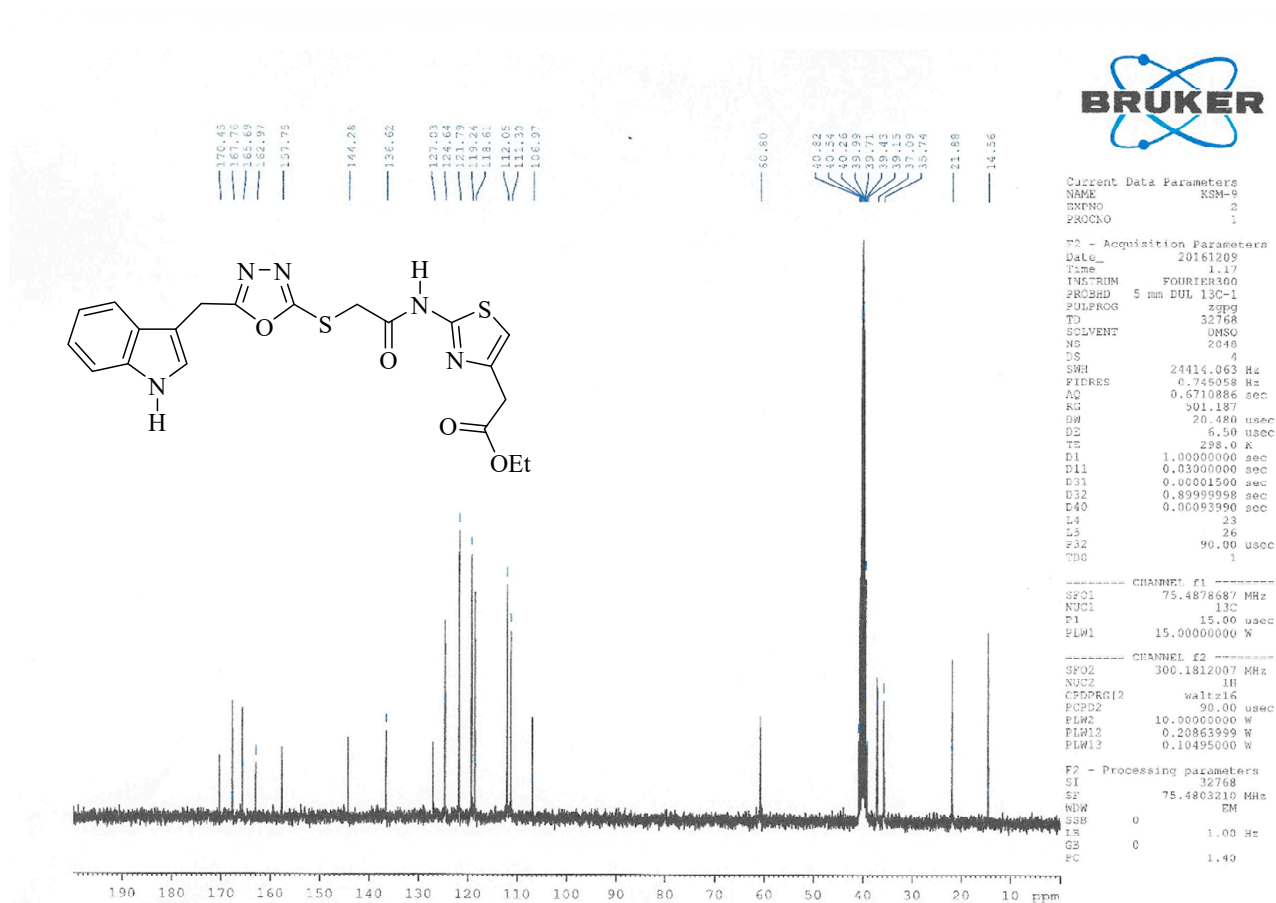


Figure S35. ^{13}C NMR spectrum of compound **2i**.

Data File: C:\LabSolutions\Data\Analiz\mdaltintop\KSM-9_9.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	20	O	2	2	4	Cl	1	0	1	I	3	0	0	H
C	4	15	30	F	1	0	0	Br	1	0	1					
N	3	5	6	S	2	2	2	Ru	2	0	0					

Error Margin (ppm): 5

DBE Range: 0.0 - 30.0

Electron Ions: both

HC Ratio: unlimited

Apply N Rule: yes

Use MSn Info: no

Max Isotopes: 3

Isotope RI (%): 1.00

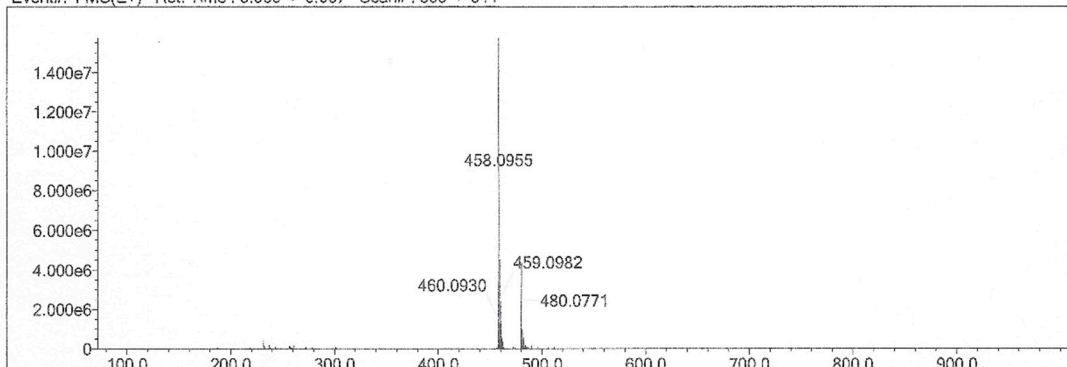
Isotope Res: 10000

MSn Iso RI (%): 10.00

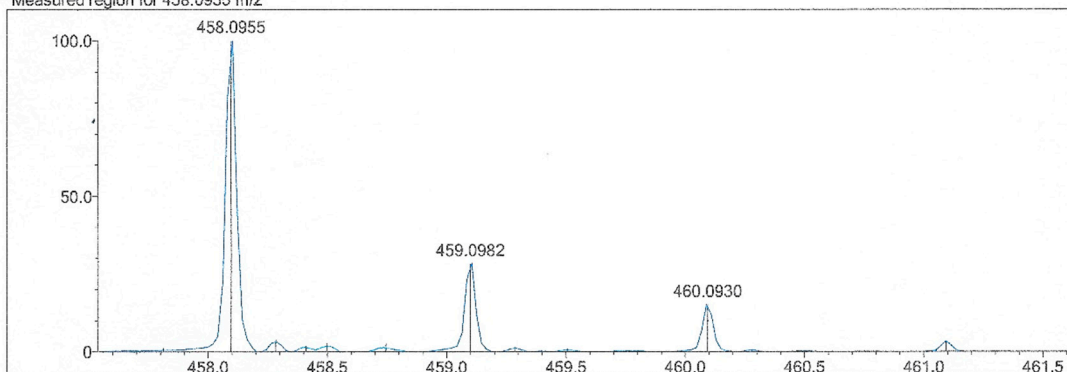
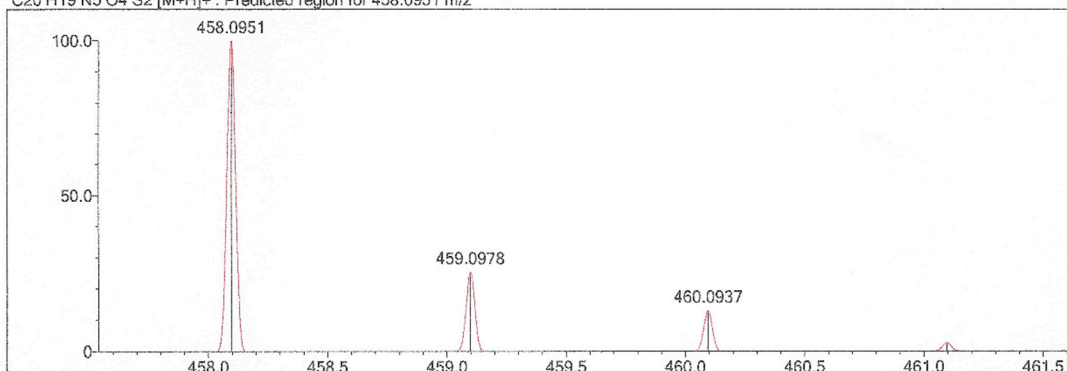
MSn Logic Mode: AND

Max Results: 500

Event#: 1 MS(E+) Ret. Time : 5.960 -> 6.067 Scan# : 895 -> 911



Measured region for 458.0955 m/z

C20 H19 N5 O4 S2 [M+H]⁺ : Predicted region for 458.0951 m/z

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	93.24	C20 H19 N5 O4 S2	[M+H] ⁺	458.0955	458.0951	0.4	0.87	93.24	14.0

Figure S36. HRMS spectrum of compound 2i.