ARTICLE

Design, synthesis, electrochemistry and anti-trypanosomatid hit/lead identification of nitrofuranylazines

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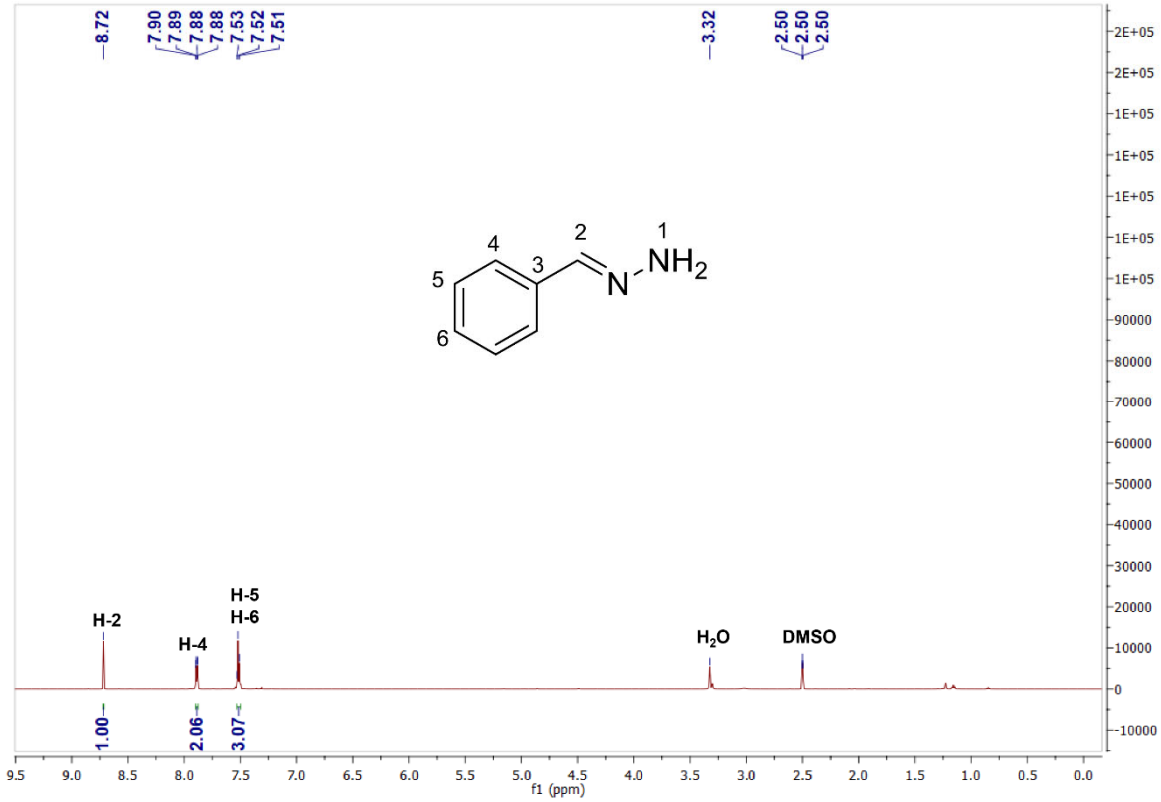
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

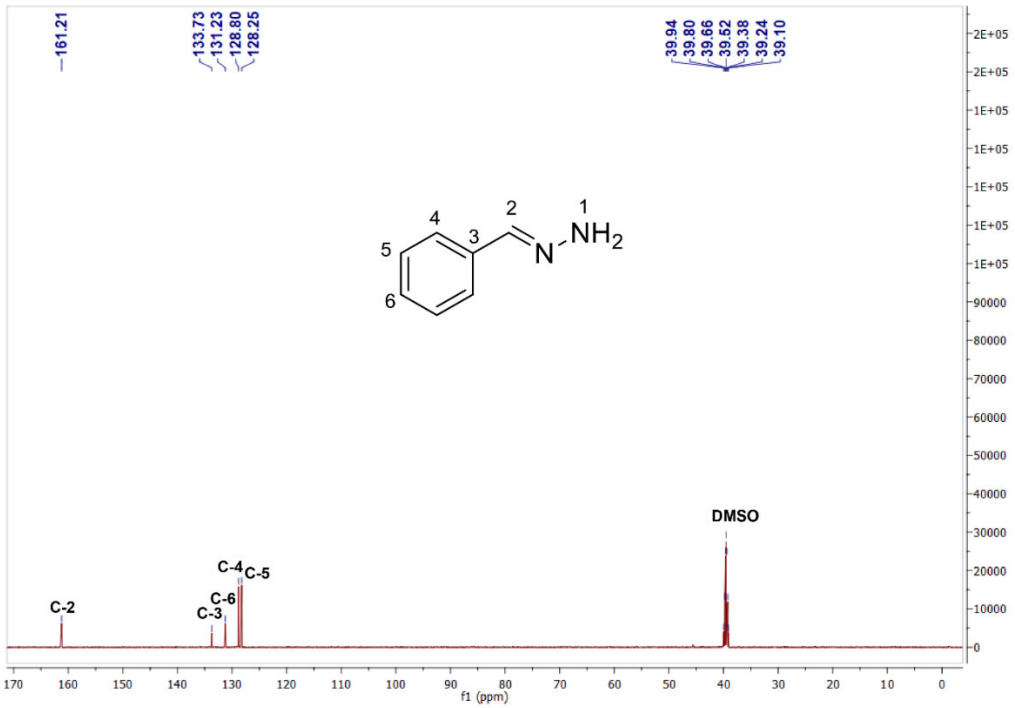
APPENDIX A: CHEMICAL CHARACTERIZATION

(*E*)-Benzylidenehydrazine (1)

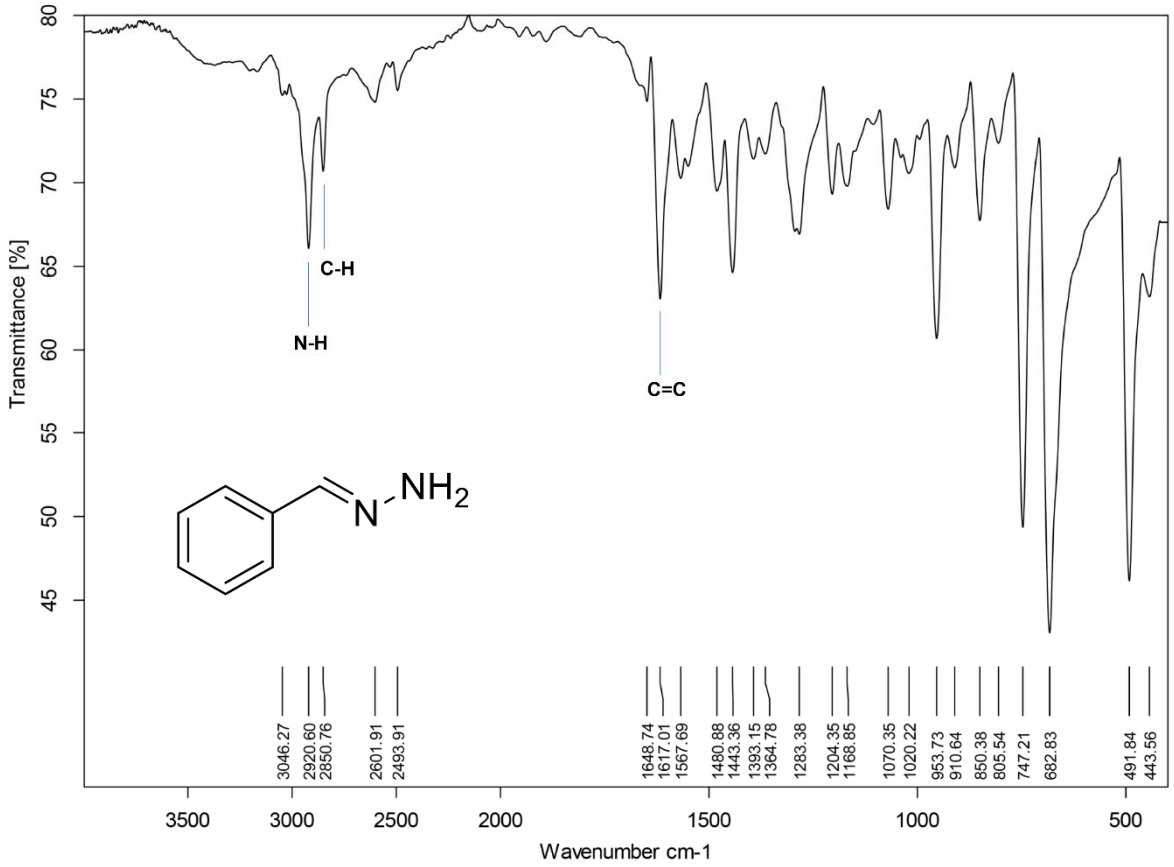
^{1}H NMR in DMSO



^{13}C NMR in DMSO



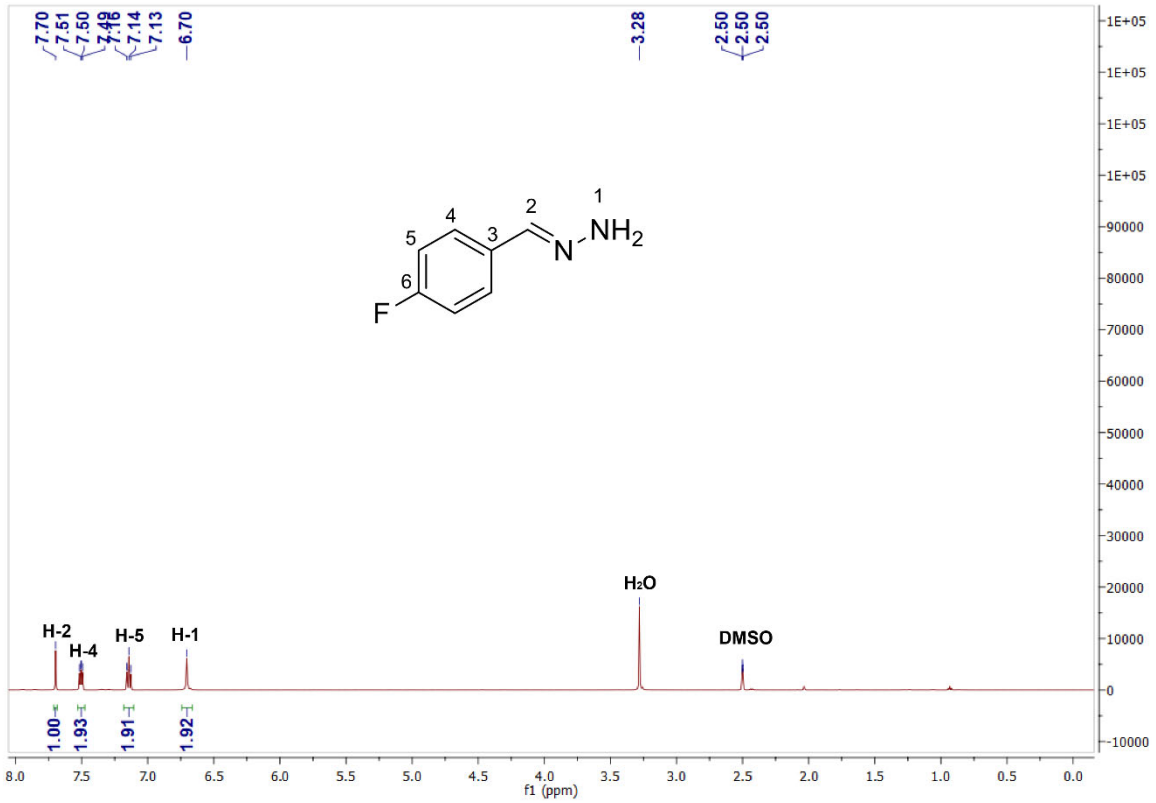
IR Spectrum



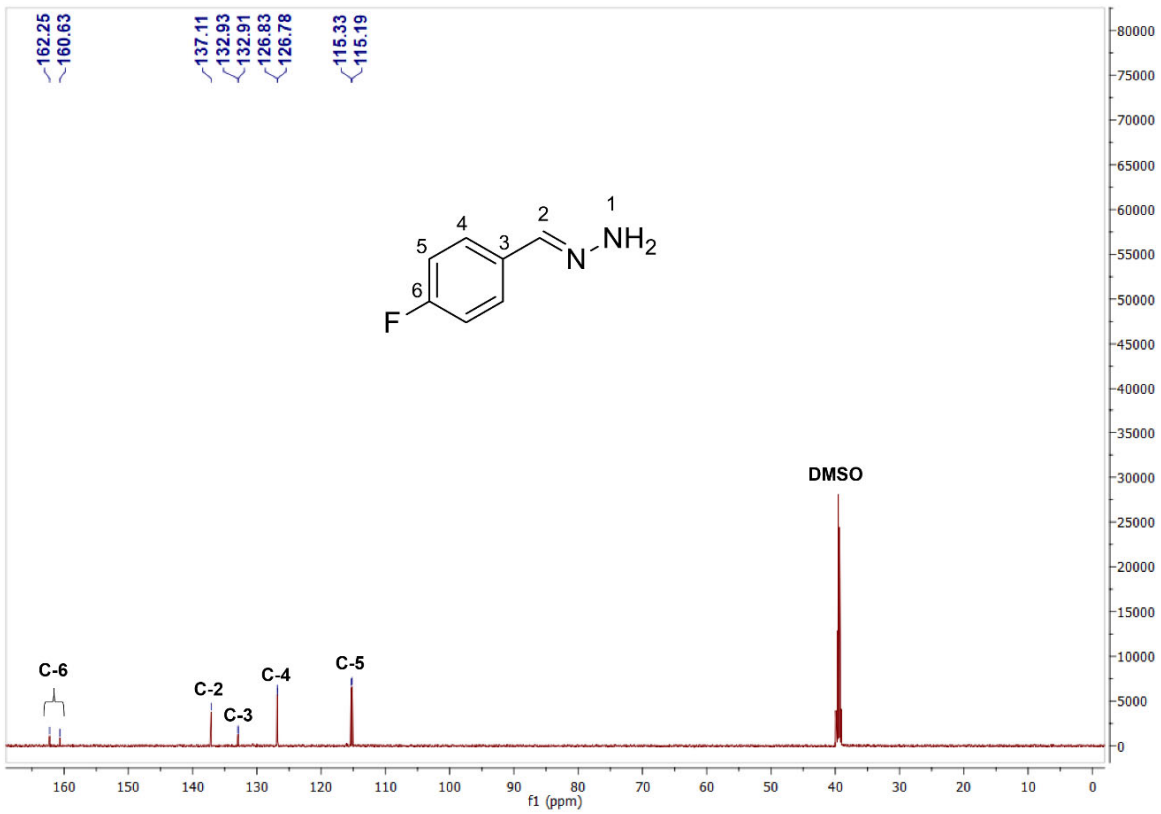
Dark yellow powder; yield: 565 mg (47%); mp 88-90 °C; IR $_{Vmax}$ (cm^{-1}): 2921 (N-H), 2851 (C-H), 1649 (C=C); ^{1}H NMR (600 MHz, DMSO) δ 8.72 (s, 1H, H-2), 8.89 (dd, *J* = 7.6, 1.8 Hz, 2H, H-4), 7.53 (dd, *J* = 13.1, 5.8 Hz, 3H, H-5/6), 6.62 (s, 2H, H-1). ^{13}C NMR (151 MHz, DMSO) δ 161.21 (C-2), 133.73 (C-3), 131.23 (C-6), 128.80 (C-4), 128.25 (C-5).

(*E*)-(4-Fluorobenzylidene)hydrazine (2)

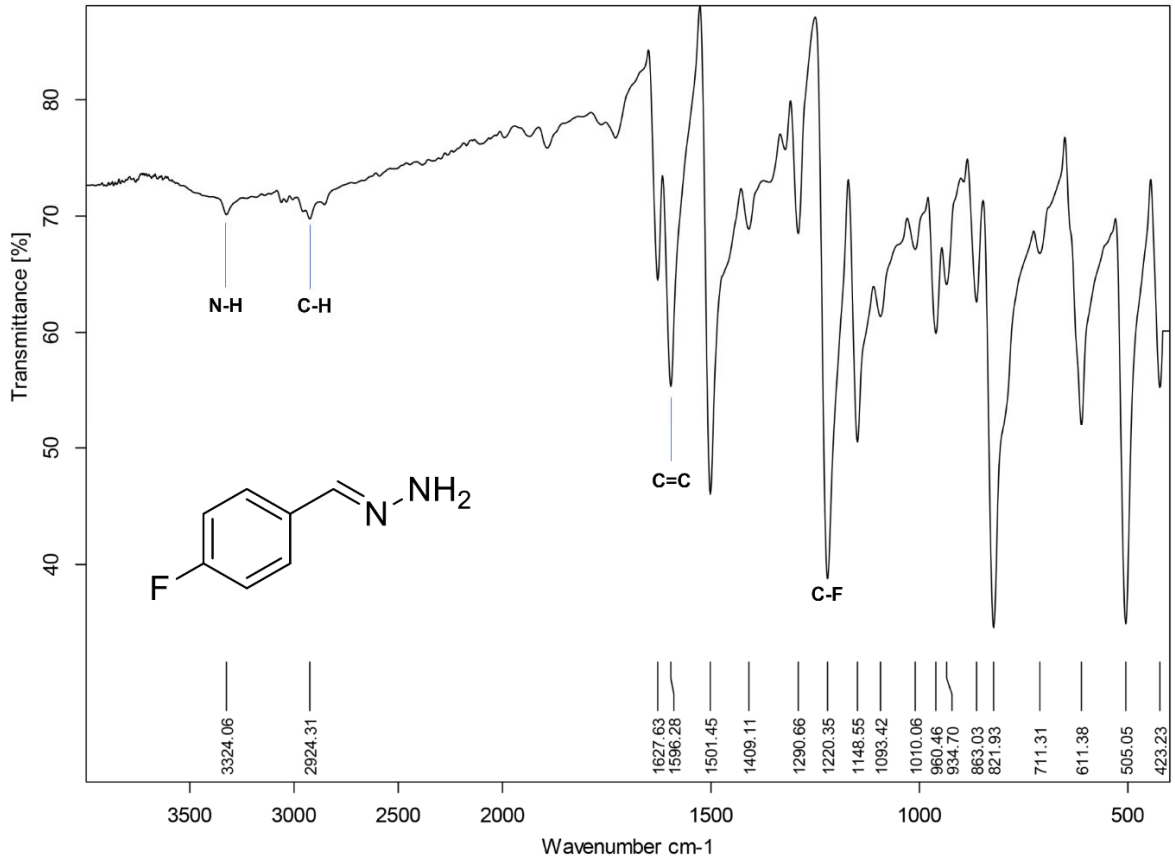
^{1}H NMR in DMSO



^{13}C NMR in DMSO



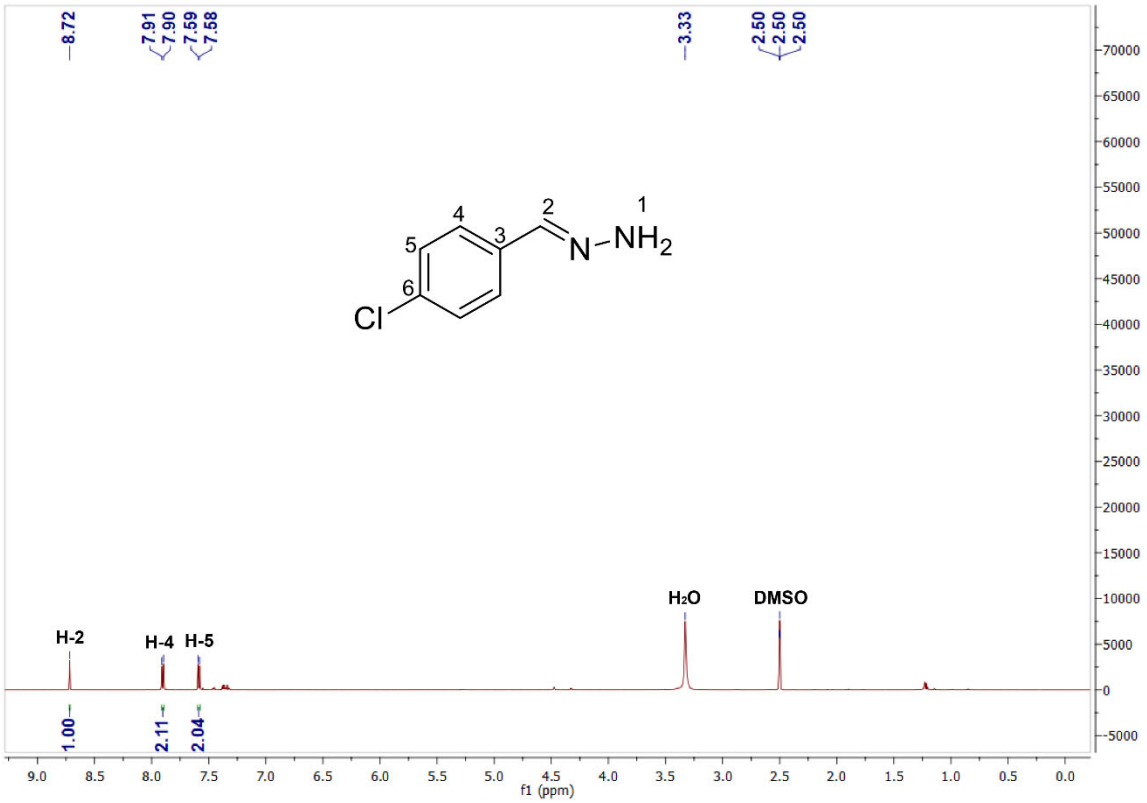
IR Spectrum



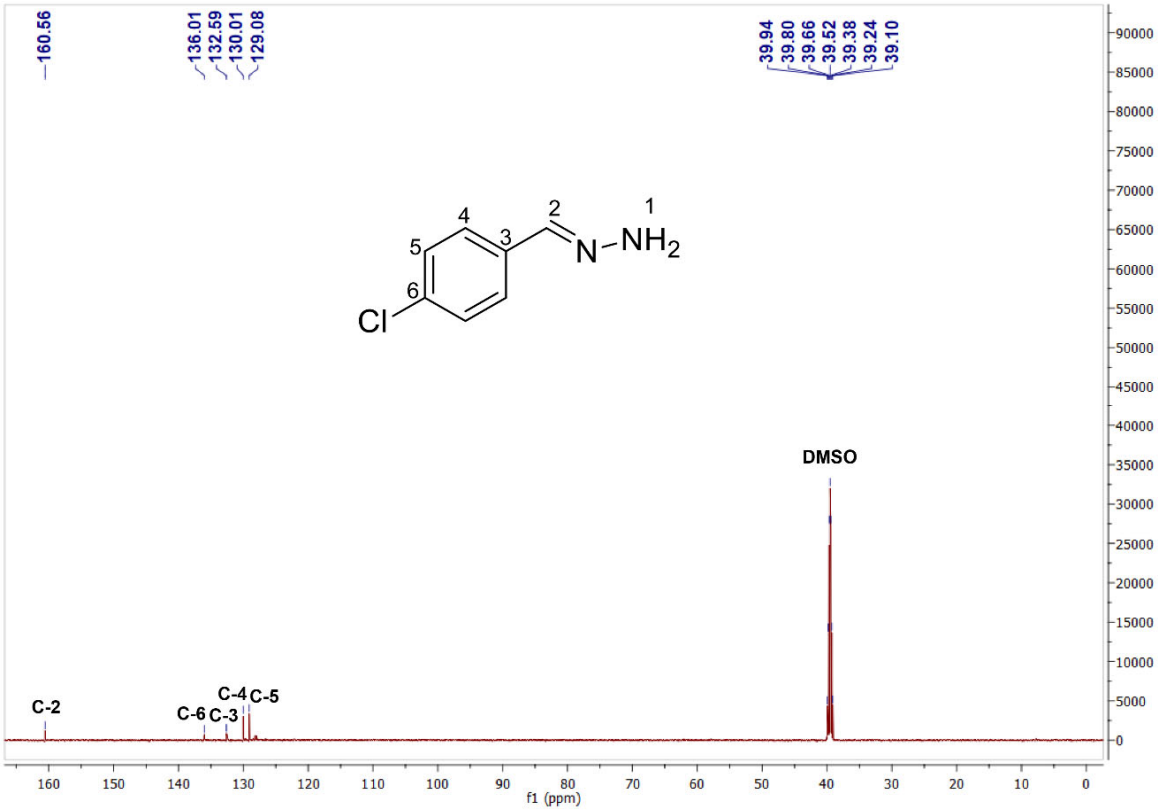
Light yellow powder; yield: 857 mg (62%); mp 181-183 °C; IR $_{Vmax}$ (cm^{-1}): 3324 (N-H), 2924 (C-H), 1596 (C=C), 1220 (C-F); ^{1}H NMR (600 MHz, DMSO) δ 7.70 (s, 1H, H-2), 7.50 (dd, J_{H-F} = 8.6, 5.8 Hz, 2H, H-4), 7.14 (t, J_{H-F} = 8.6 Hz, 2H, H-5), 6.70 (s, 2H, H-1). ^{13}C NMR (151 MHz, DMSO) δ 161.44 (d, $^{1}J_{C-F}$ = 244.0 Hz, C-6), 137.11 (C-2), 132.92 (d, $^{2}J_{C-F}$ = 3.0 Hz, C-3), 126.81 (d, $^{3}J_{C-F}$ = 8.0 Hz, C-4), 115.26 (d, $^{4}J_{C-F}$ = 21.7 Hz, C-5).

(*E*)-(4-Chlorobenzylidene)hydrazine (3)

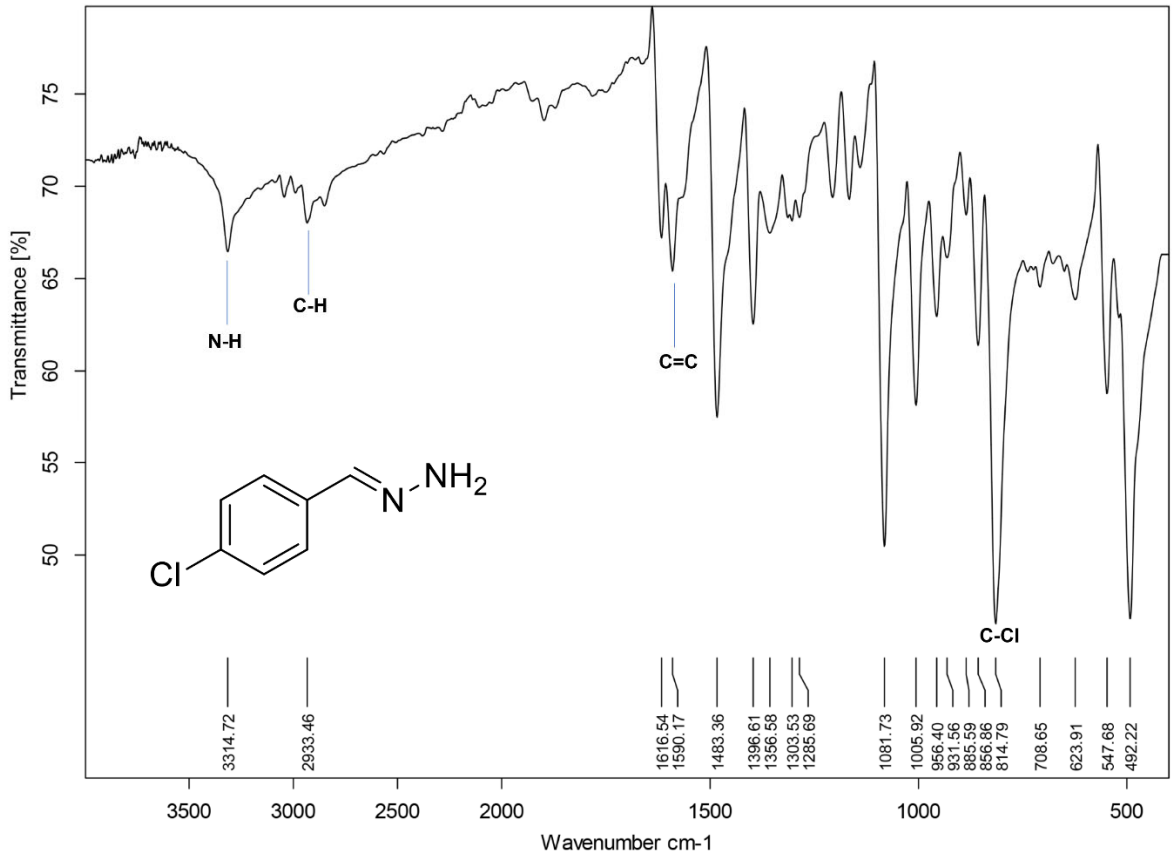
^{1}H NMR in DMSO



^{13}C NMR in DMSO



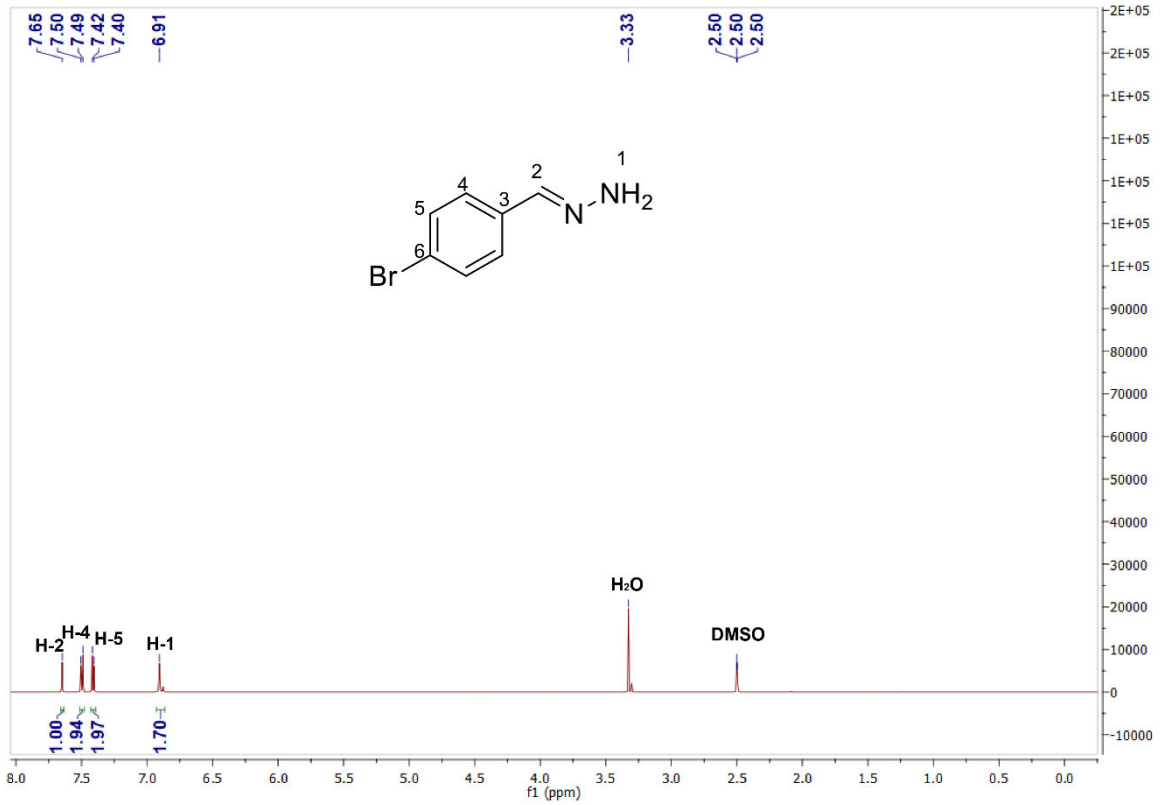
IR Spectrum



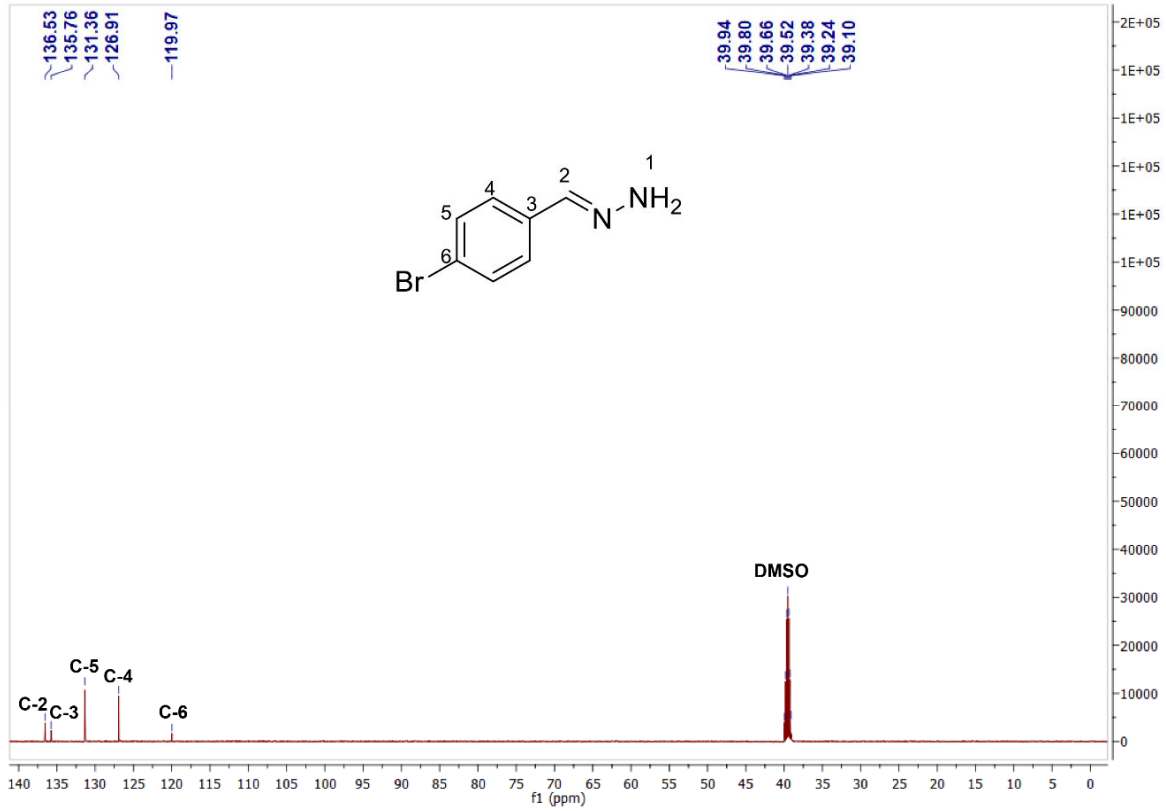
Yellow powder; yield: 1.0 g (65%); mp 196-198 °C; IR $_{Vmax}$ (cm^{-1}): 3315 (N-H), 2933 (C-H), 1590 (C=C), 815 (C-Cl); ^{1}H NMR (600 MHz, DMSO) δ 8.72 (s, 1H, H-2), 7.90 (d, *J* = 8.5 Hz, 2H, H-4), 7.59 (d, *J* = 8.5 Hz, 2H, H-5), 6.62 (s, 2H, H-1). ^{13}C NMR (151 MHz, DMSO) δ 160.56 (C-2), 136.01 (C-6), 132.59 (C-3), 130.01 (C-4), 129.08 (C-5).

(*E*)-(4-Bromobenzylidene)hydrazine (4)

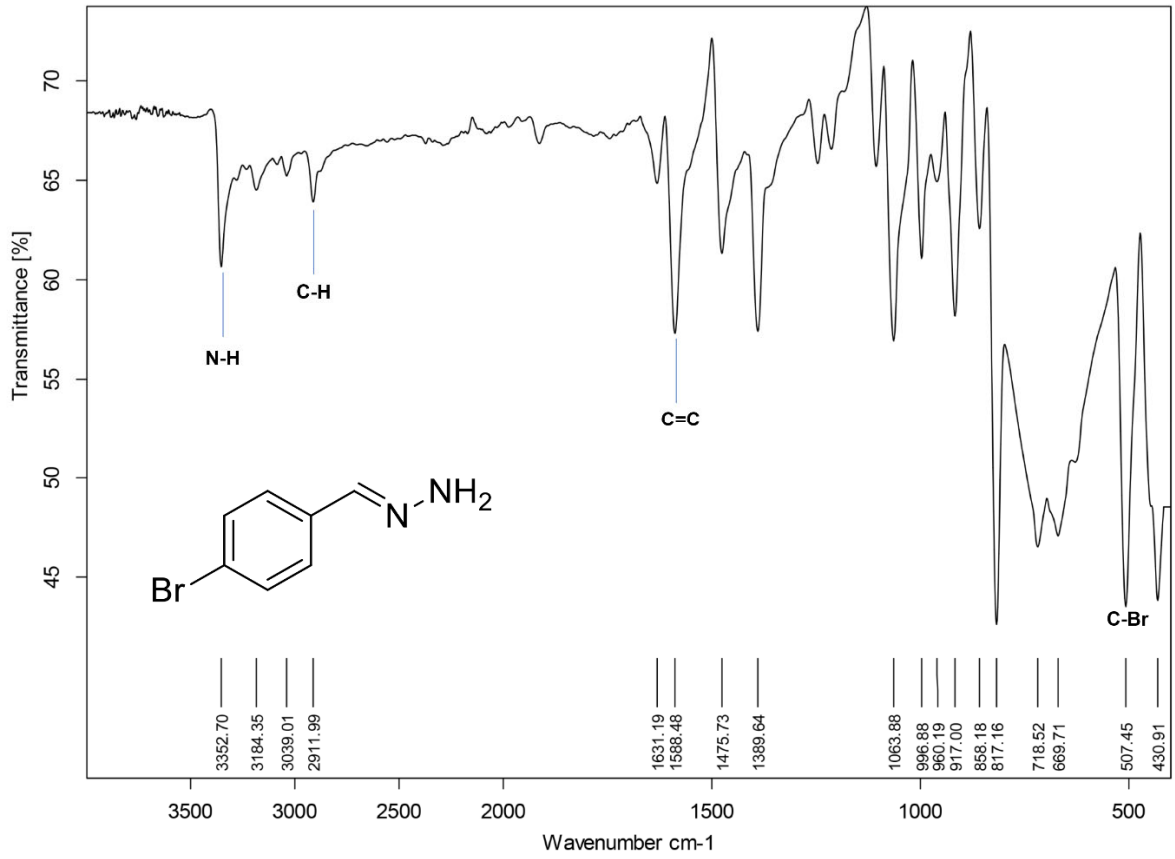
^{1}H NMR in DMSO



^{13}C NMR in DMSO



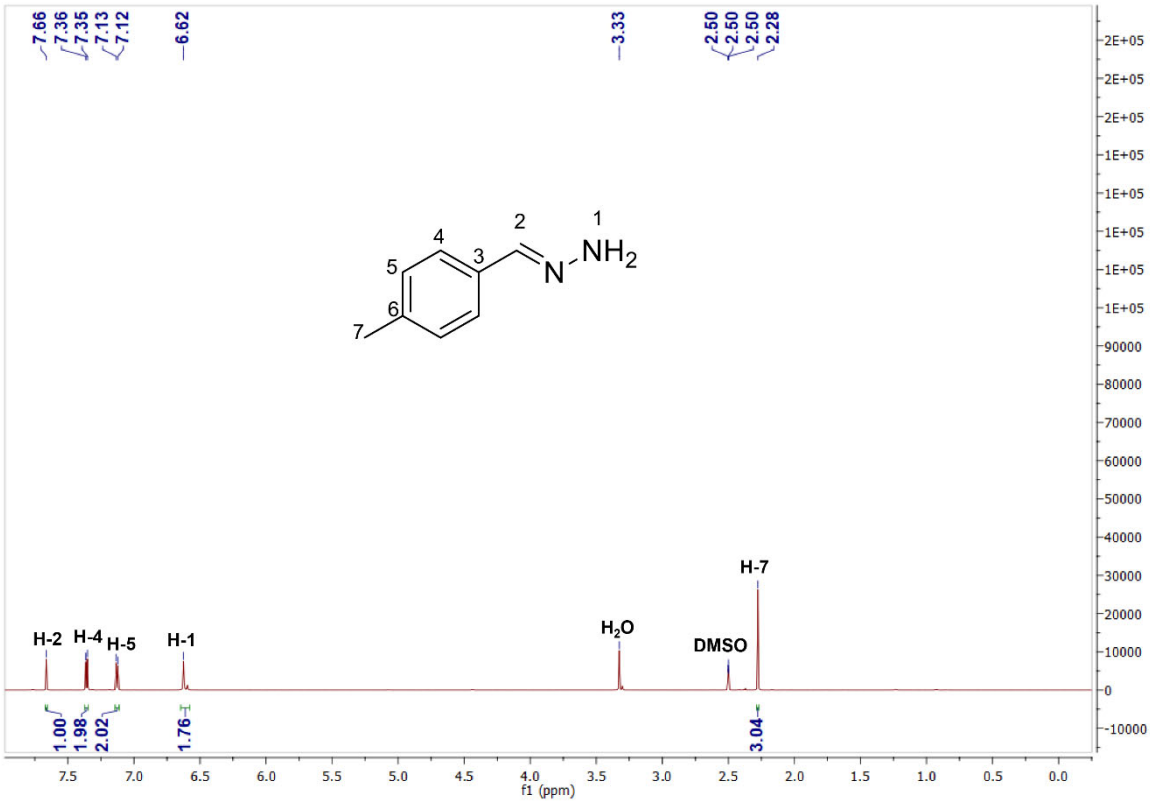
IR Spectrum



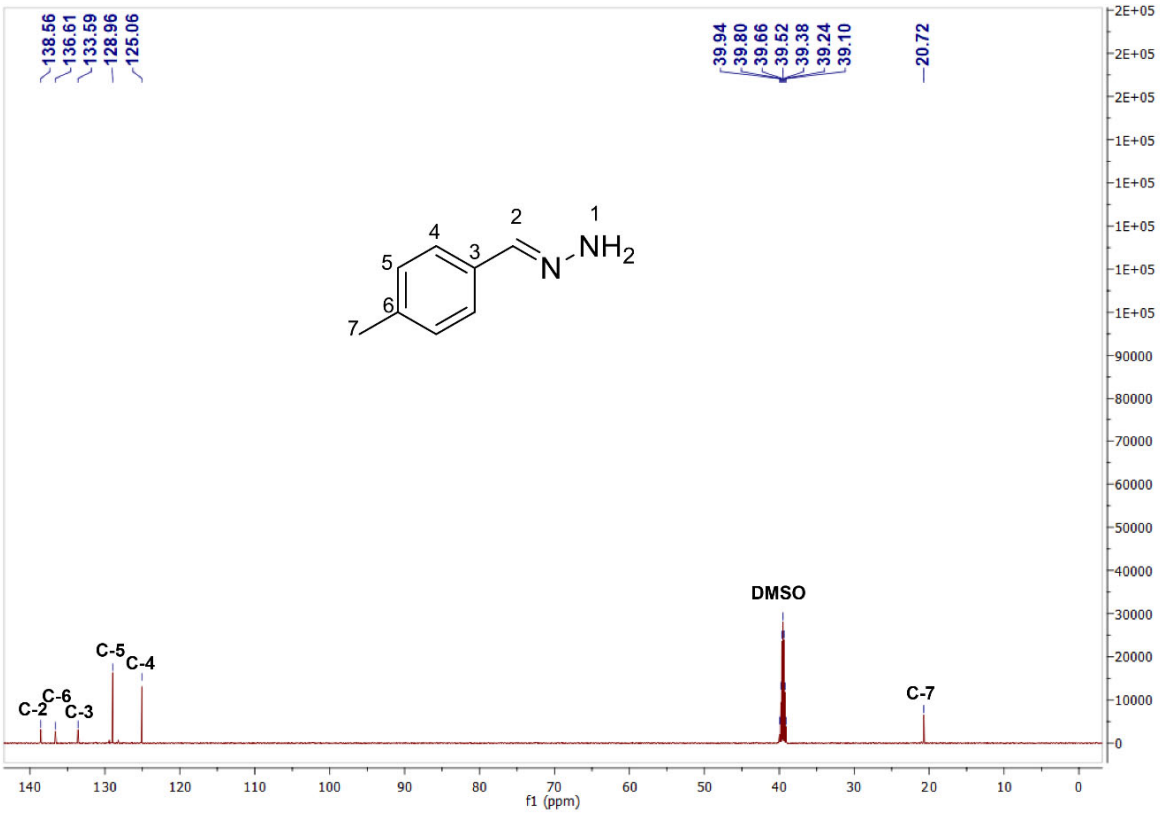
White crystal-like powder; yield: 1.2 g (61%); mp 72-74 °C; IR ν_{max} (cm^{-1}): 3353 (N-H), 2912 (C-H), 1588 (C=C), 507 (C-Br); ^{1}H NMR (600 MHz, DMSO) δ 7.65 (s, 1H, H-2), 7.50 (d, *J* = 8.5 Hz, 2H, H-4), 7.41 (d, *J* = 8.5 Hz, 2H, H-5), 6.91 (s, 2H, H-1). ^{13}C NMR (151 MHz, DMSO) δ 136.53 (C-2), 135.76 (C-3), 131.36 (C-5), 126.91 (C-4), 119.97 (C-6).

(*E*)-(4-Methylbenzylidene)hydrazine (5)

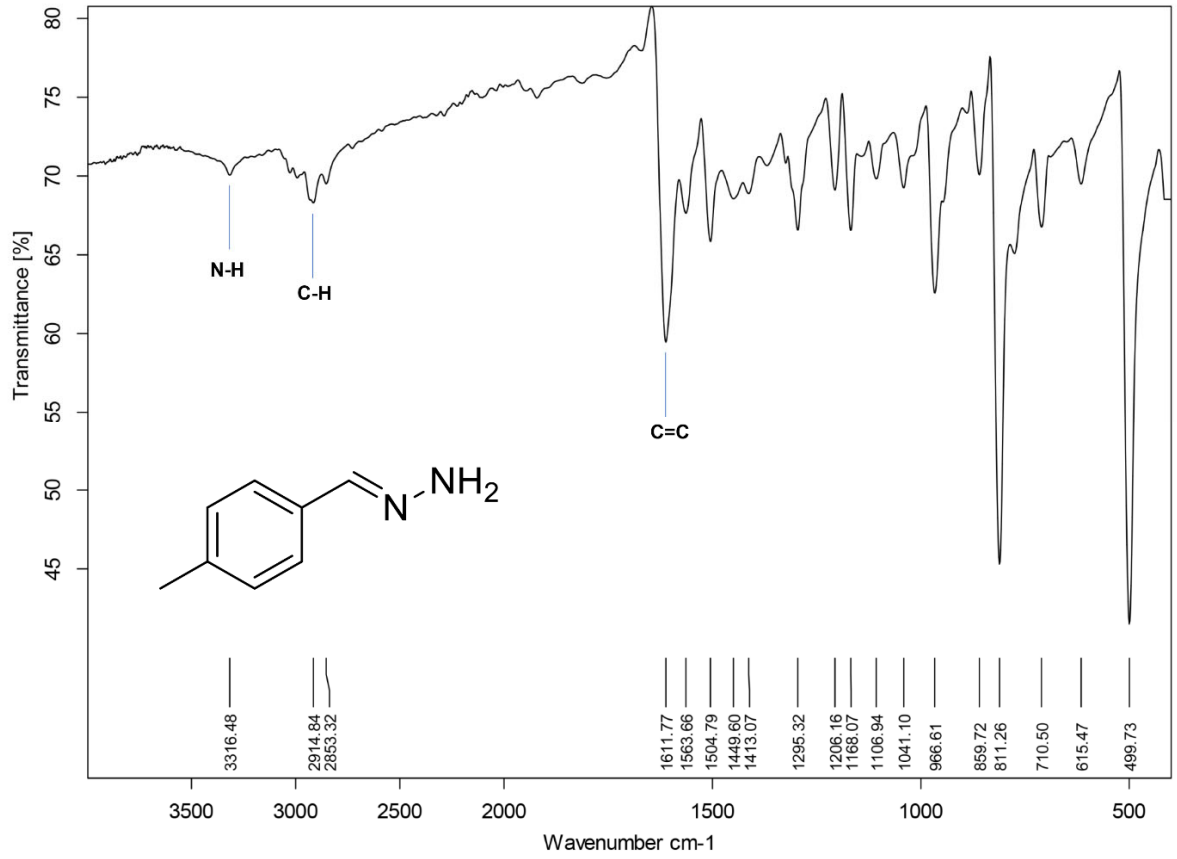
^{1}H NMR in DMSO



^{13}C NMR in DMSO



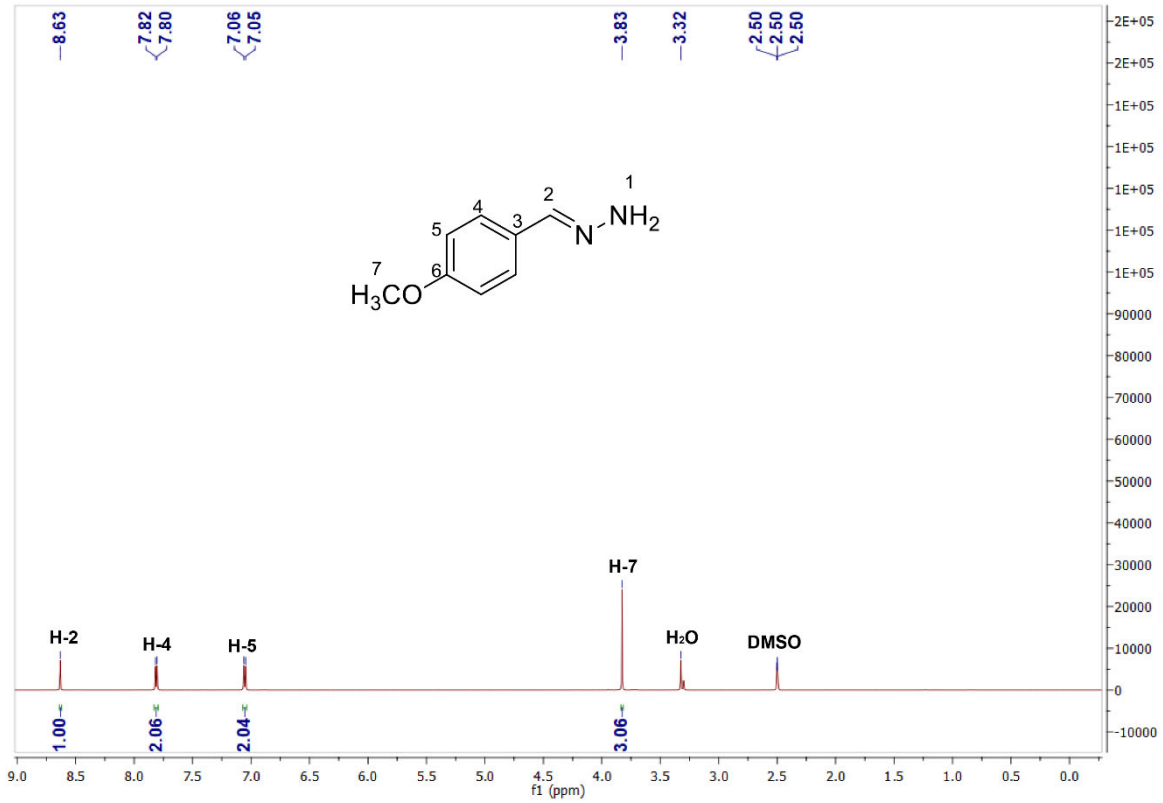
IR Spectrum



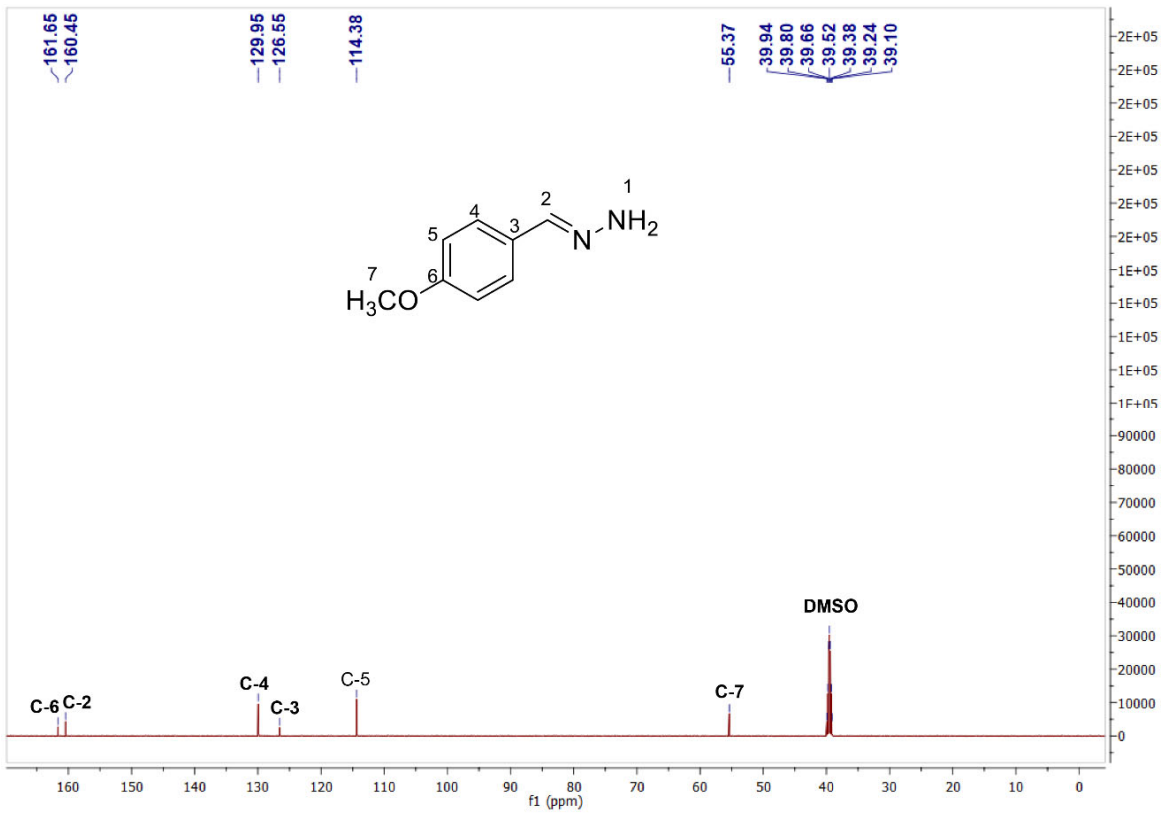
Yellow powder; yield: 1.14 g (85%); mp 150-152 °C; IR $_{Vmax}$ (cm^{-1}): 3316 (N-H), 2915 (C-H), 1612 (C=C); ^{1}H NMR (600 MHz, DMSO) δ 7.66 (s, 1H, H-2), 7.36 (d, *J* = 8.0 Hz, 2H, H-4), 7.13 (d, *J* = 8.0 Hz, 2H, H-5), 6.62 (s, 2H, H-1), 2.28 (s, 3H, H-7). ^{13}C NMR (151 MHz, DMSO) δ 138.56 (C-2), 136.61 (C-6), 133.59 (C-3), 128.96 (C-5), 125.06 (C-4), 20.72 (C-7).

(*E*)-(4-Methoxybenzylidene)hydrazine (6)

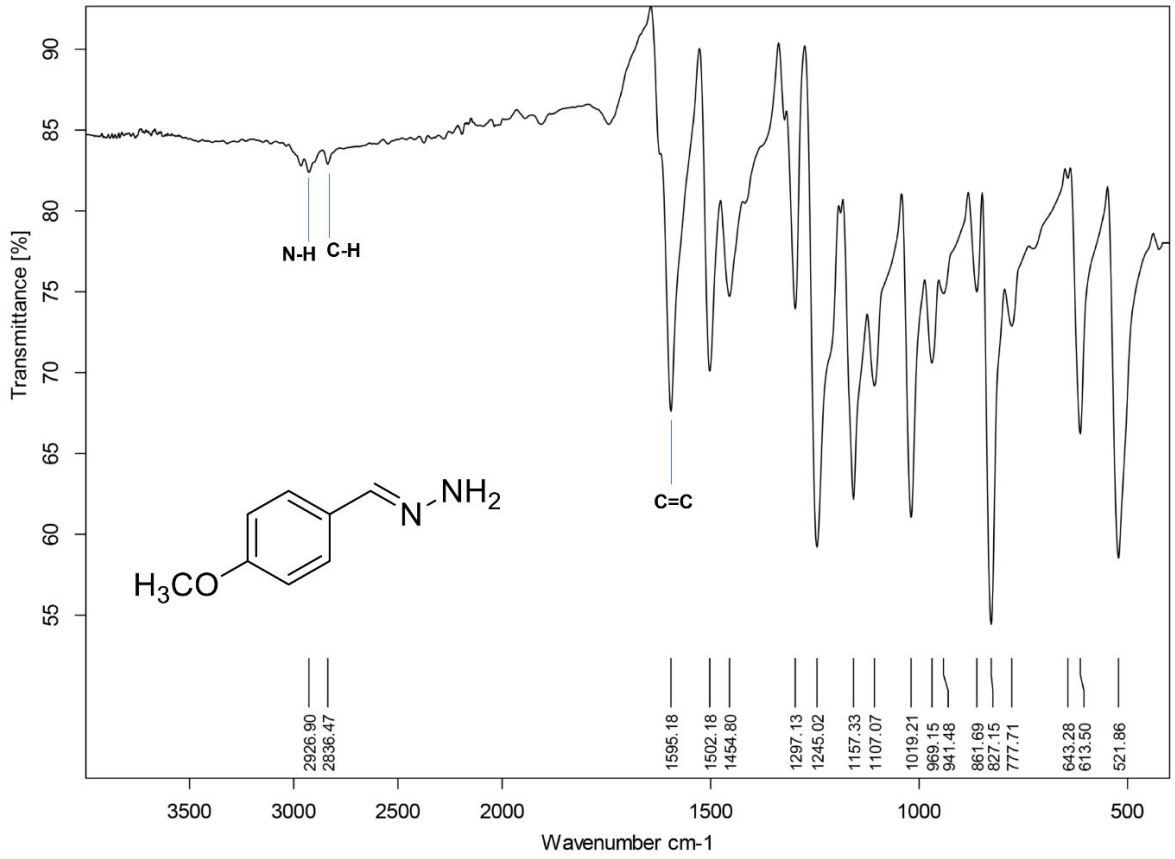
^{1}H NMR in DMSO



^{13}C NMR in DMSO



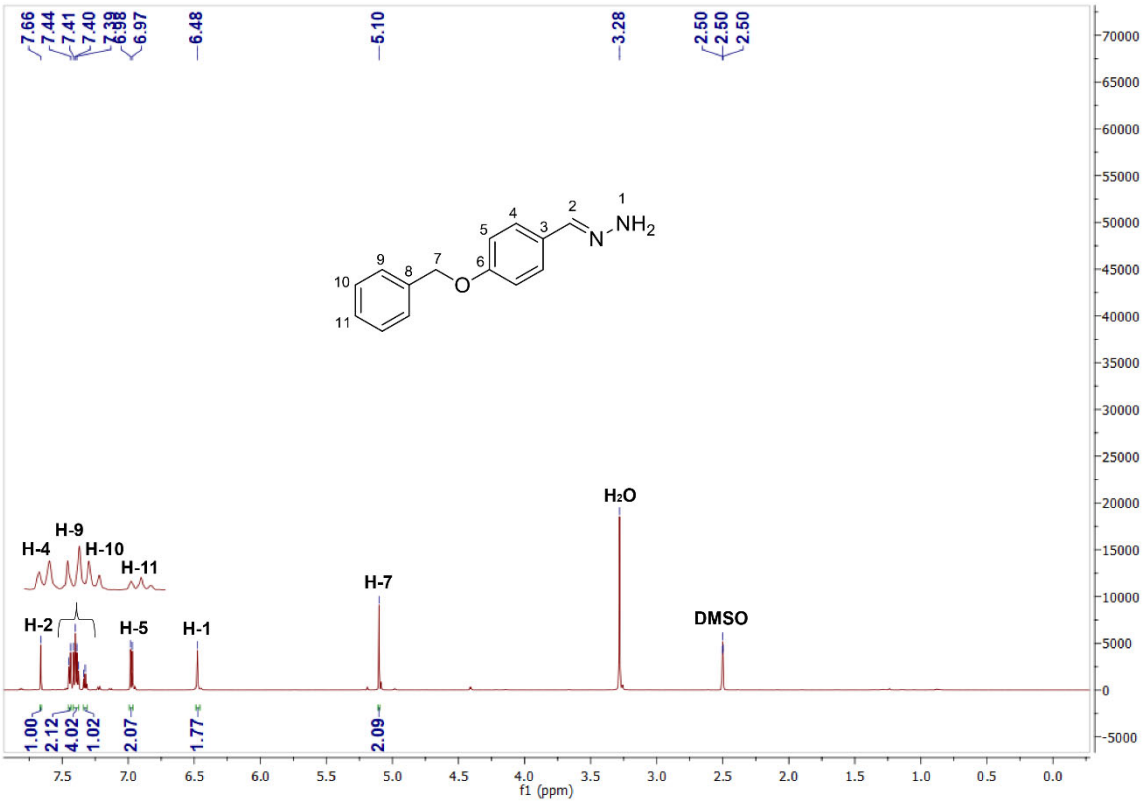
IR Spectrum



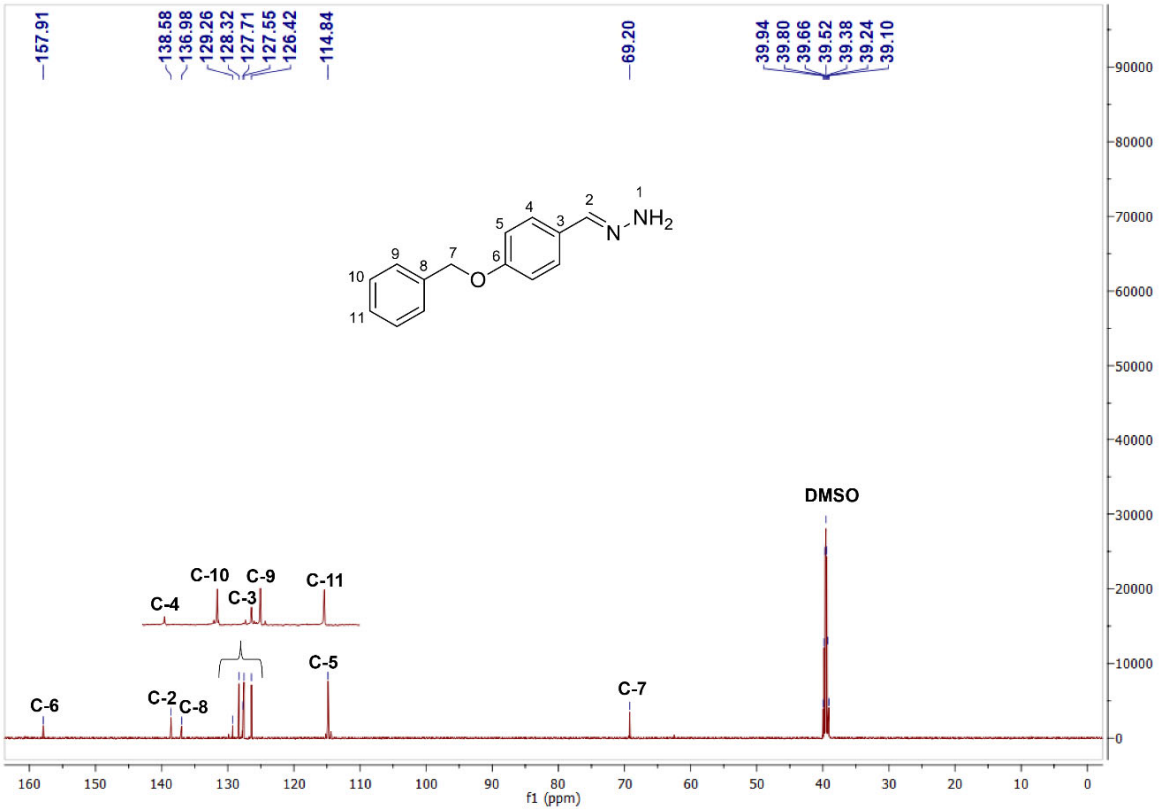
Light yellow powder; yield: 1.0 g (67%); mp 170-172 °C; IR $_{Vmax}$ (cm^{-1}): 2927 (N-H), 2836 (C-H), 1595 (C=C); ^{1}H NMR (600 MHz, DMSO) δ 8.63 (s, 1H, H-2), 7.81 (d, *J* = 8.7 Hz, 2H, H-4), 7.05 (d, *J* = 8.7 Hz, 2H, H-5), 6.62 (s, 2H, H-1), 3.83 (s, 3H, H-7). ^{13}C NMR (151 MHz, DMSO) δ 161.65 (C-6), 160.45 (C-2), 129.95 (C-4), 126.55 (C-3), 114.38 (C-5), 55.37 (C-7).

(*E*)-(4-(Benzyloxy)benzylidene)hydrazine (7)

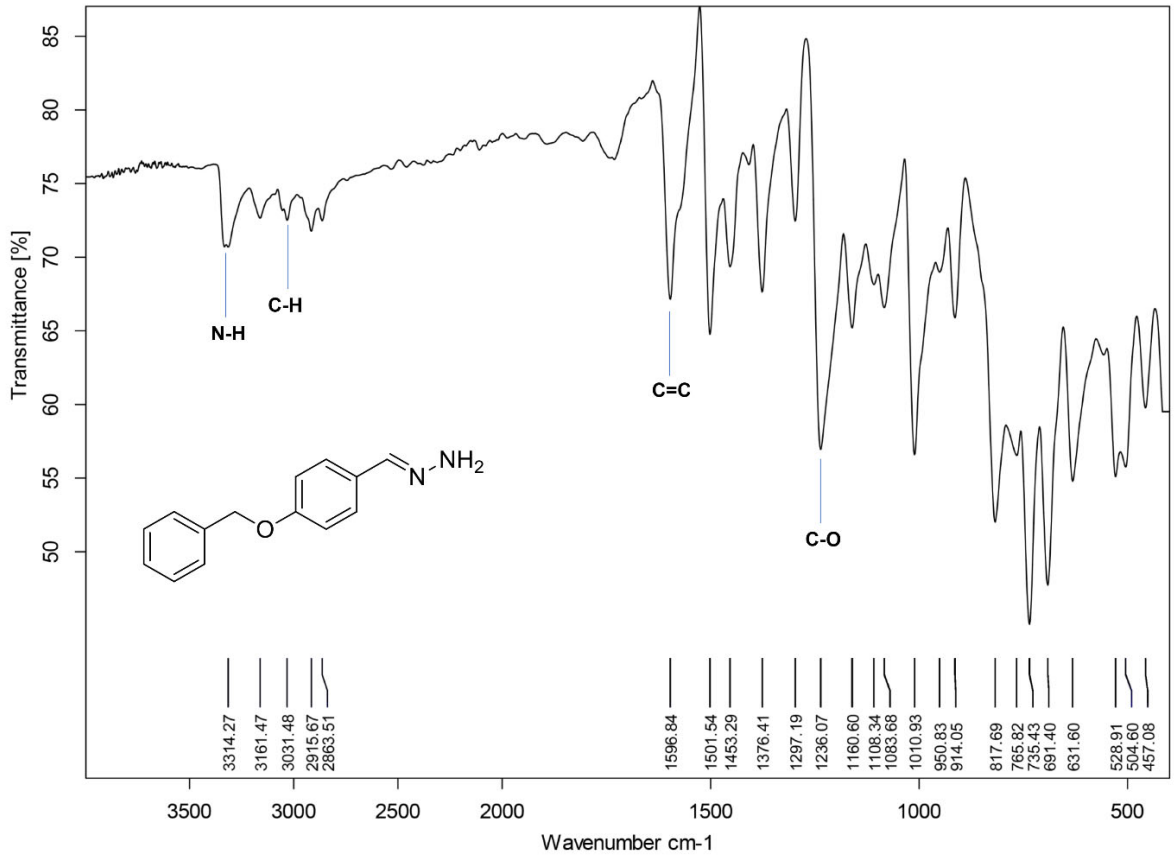
^{1}H NMR in DMSO



^{13}C NMR in DMSO



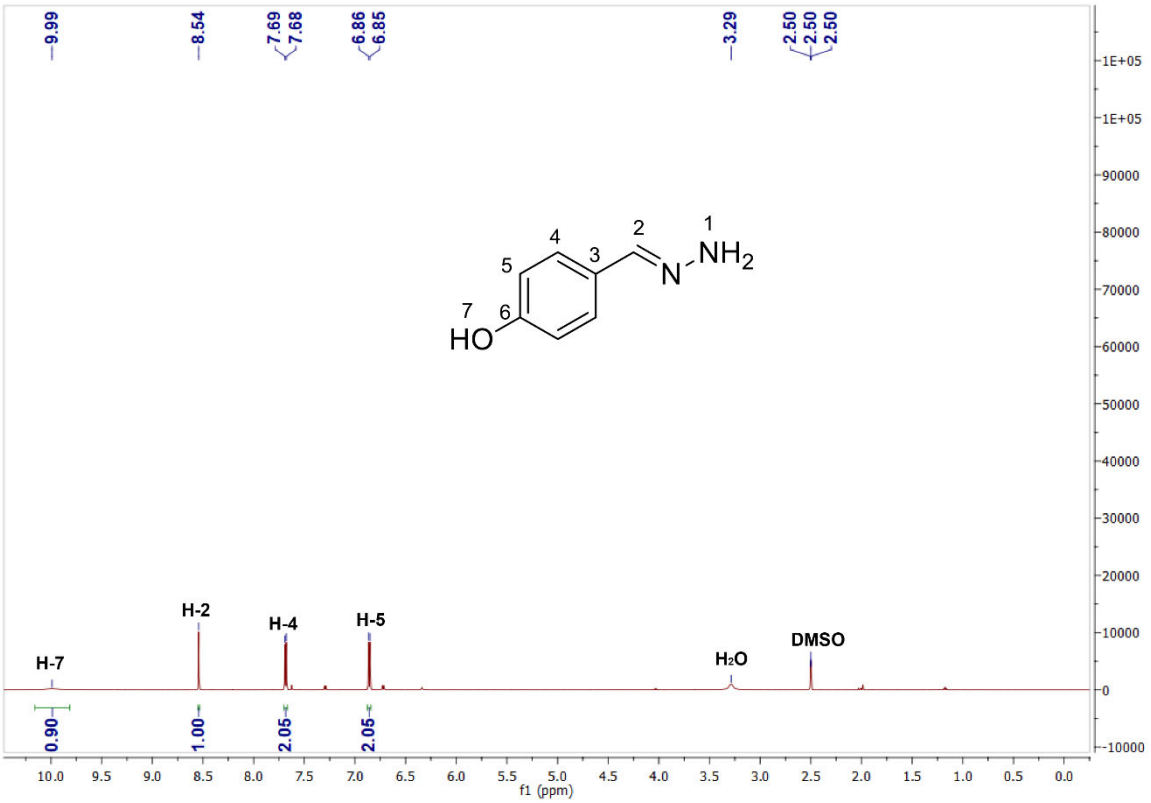
IR Spectrum



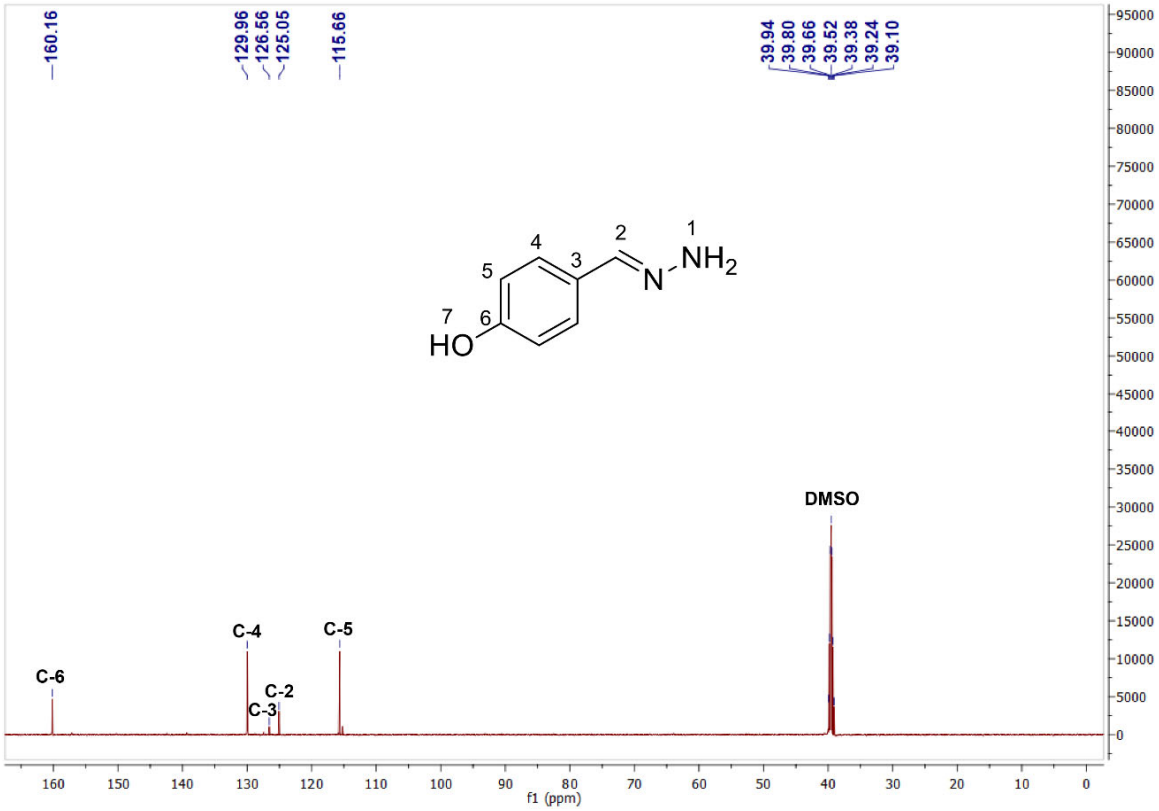
White powder; yield: 1.9 g (84%); mp.125-127 °C; IR $_{Vmax}$ (cm^{-1}): 3314 (N-H), 3031 (C-H), 1597 (C=C), 1236 (C-O); ^{1}H NMR (600 MHz, DMSO) δ 7.66 (s, 1H, H-2), 7.44 (d, *J* = 8.7 Hz, 2H, H-4), 7.41 (d, *J* = 8.5 Hz, 2H, H-9), 7.38 (d, *J* = 8.5 Hz, 2H, H-10), 7.32 (t, *J* = 7.3 Hz, 1H, H-11), 6.98 (d, *J* = 8.7 Hz, 2H, H-5), 6.48 (s, 2H, H-1), 5.10 (s, 2H, H-7). ^{13}C NMR (151 MHz, DMSO) δ 157.91 (C-6), 138.58 (C-2), 136.98 (C-8), 129.26 (C-4), 128.32 (C-10), 127.71 (C-3), 127.55 (C-9), 126.42 (C-11), 114.84 (C-5), 69.20 (C-7).

(*E*)-4-(Hydrazonomethyl)phenol (8)

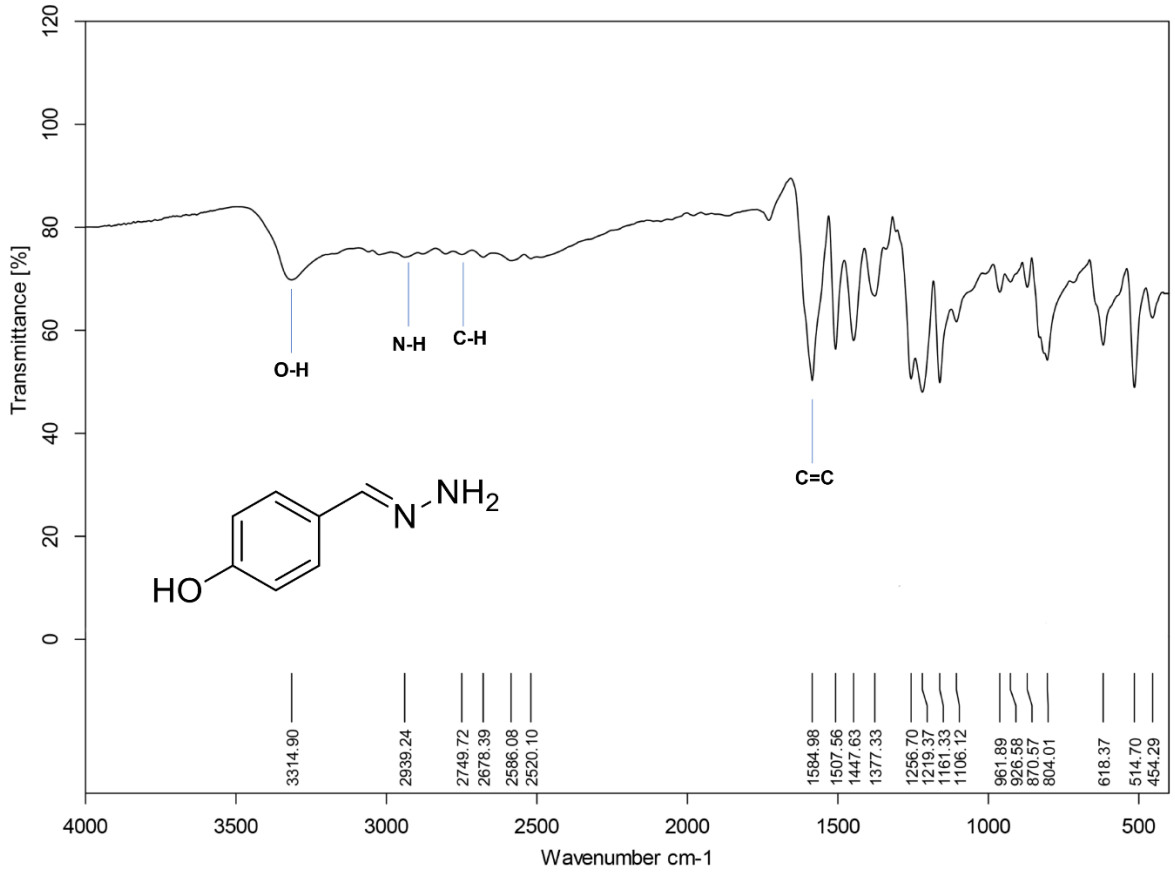
^{1}H NMR in DMSO



^{13}C NMR in DMSO



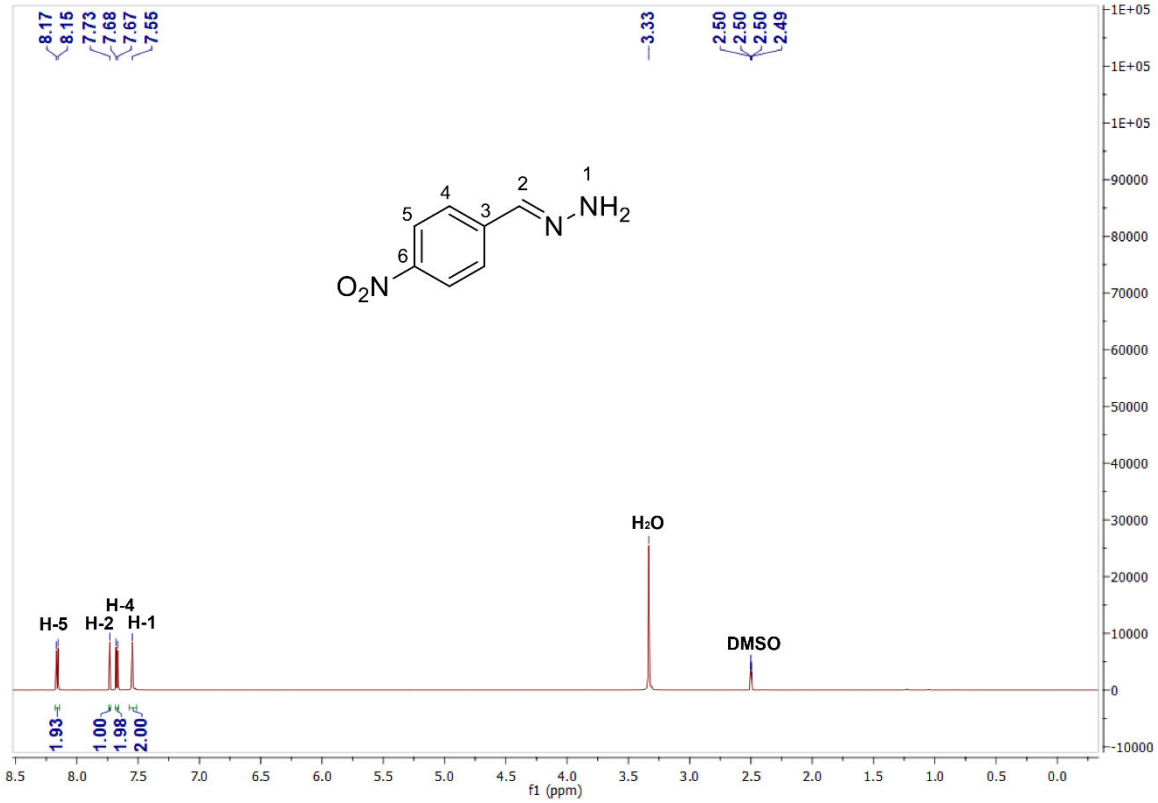
IR Spectrum



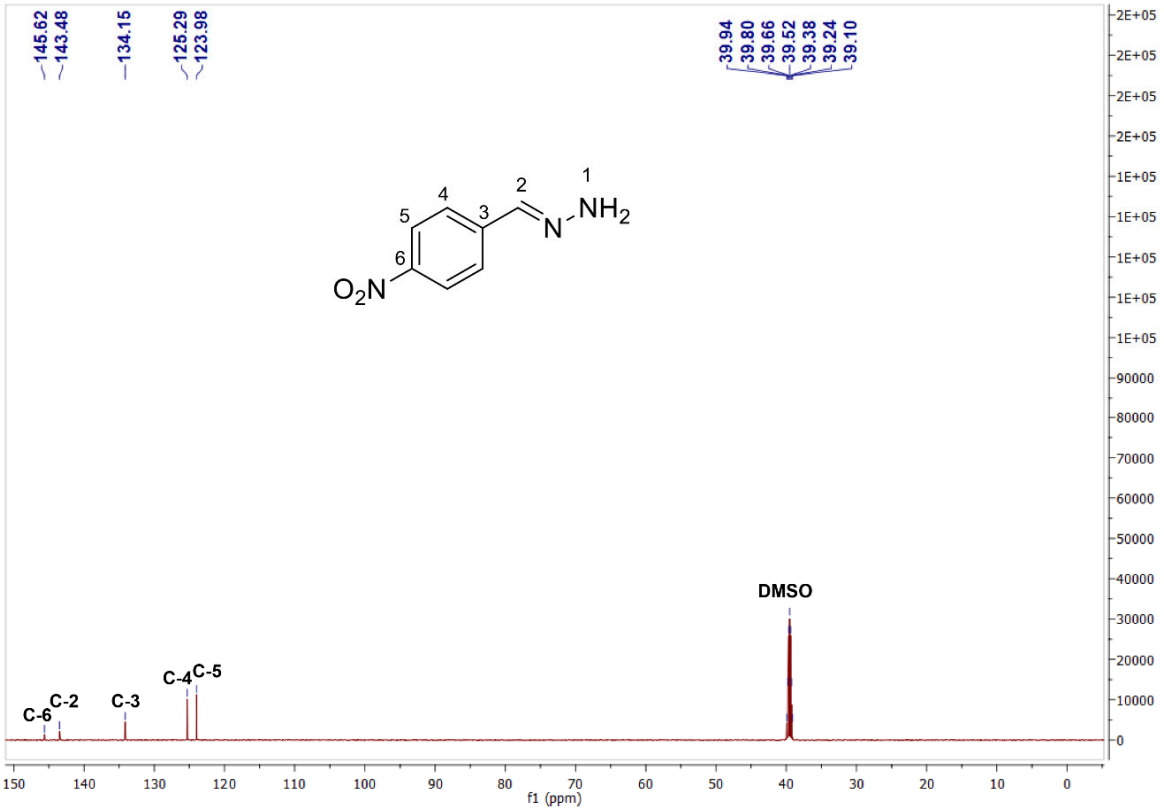
Yellow powder; yield: 967 mg (71%); mp 275-277 °C; IR ν_{max} (cm^{-1}): 3315 (O-H), 2939 (N-H), 2750 (C-H), 1585 (C=C); ^{1}H NMR (600 MHz, DMSO) δ 9.99 (s, 1H, H-7), 8.54 (s, 1H, H-2), 7.68 (d, *J* = 8.5 Hz, 2H, H-4), 6.86 (d, *J* = 8.5 Hz, 2H, H-5), 6.62 (s, 2H, H-1). ^{13}C NMR (151 MHz, DMSO) δ 160.16 (C-6), 129.96 (C-4), 126.56 (C-3), 125.05 (C-2), 115.66 (C-5).

(*E*)-(4-Nitrobenzylidene)hydrazine (9)

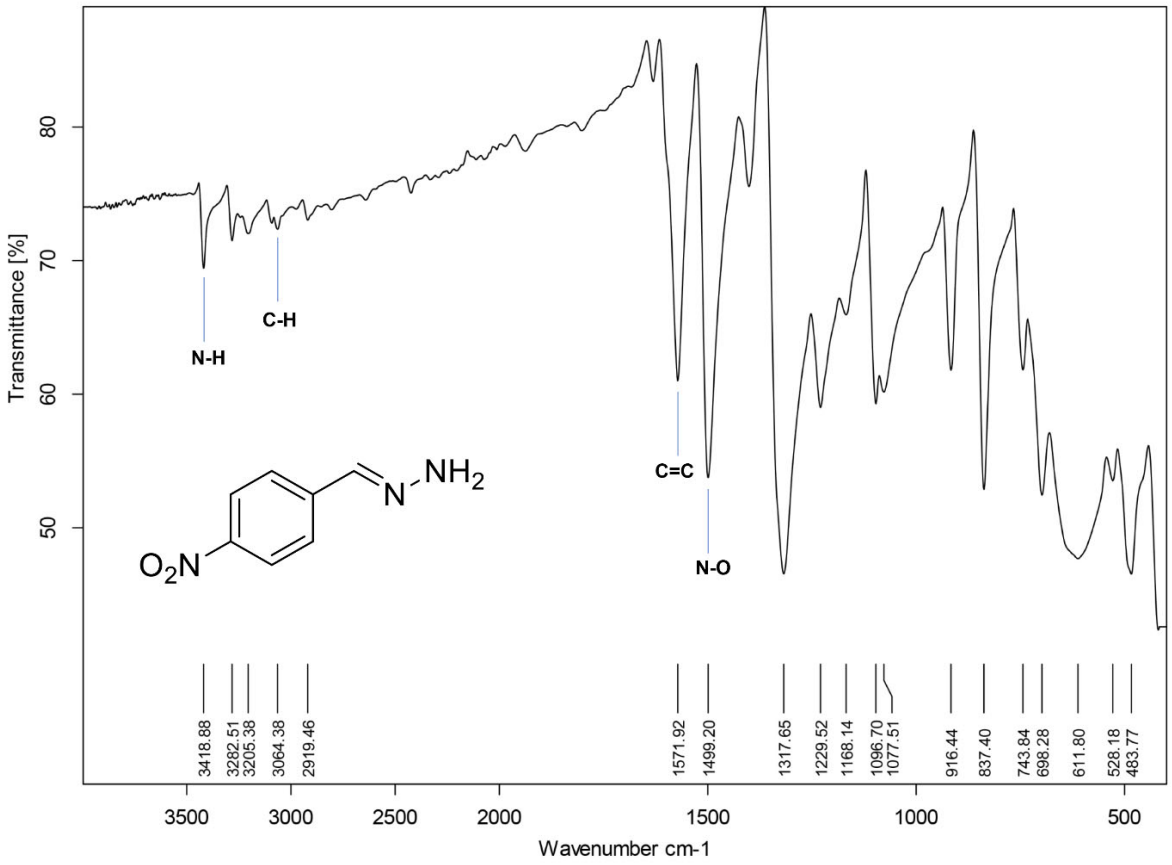
^{1}H NMR in DMSO



^{13}C NMR in DMSO



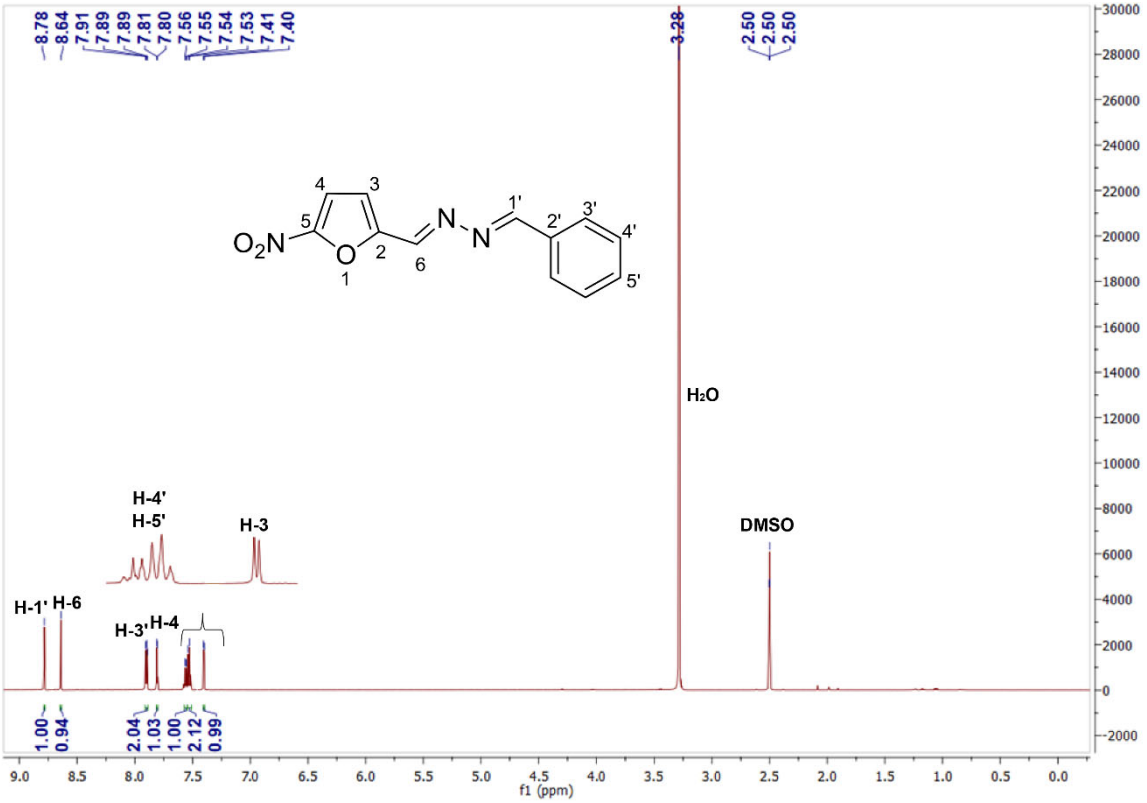
IR Spectrum



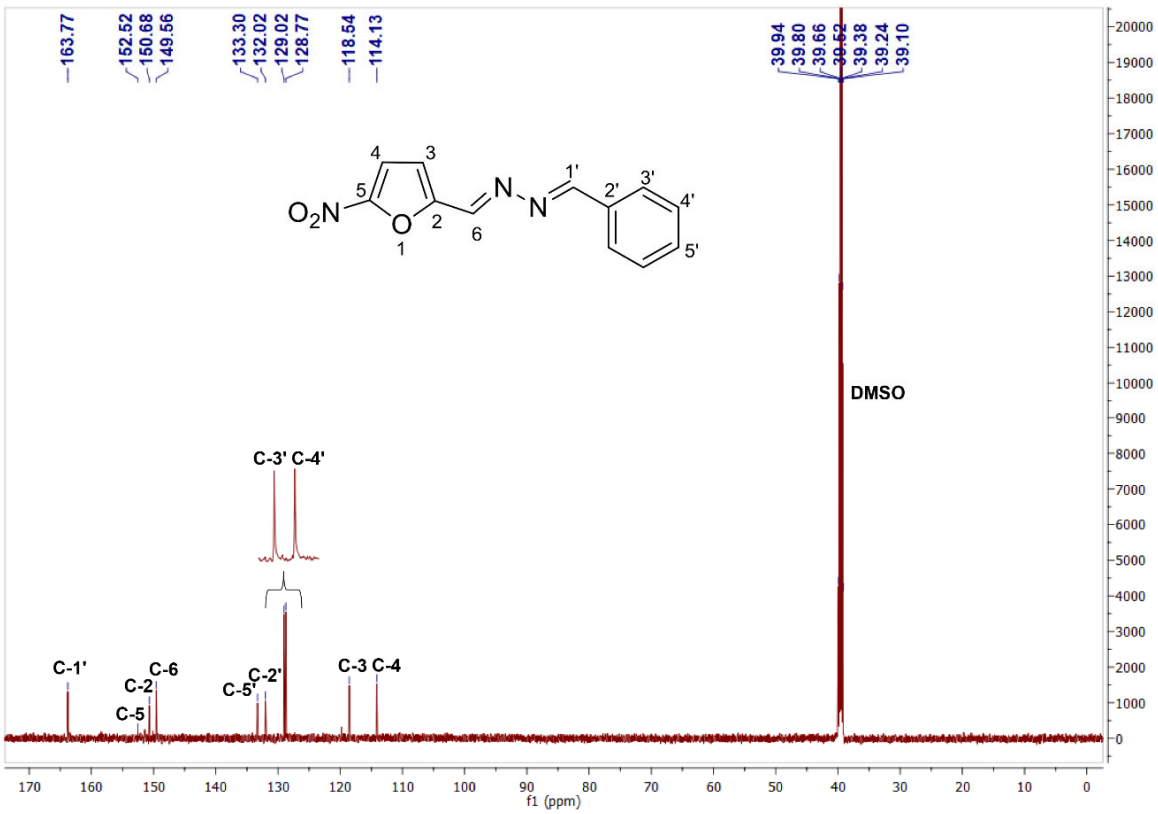
Bright yellow powder; yield: 1.5 g (91%); mp 134-136 °C; IR $_{Vmax}$ (cm^{-1}): 3419 (N-H), 3064 (C-H), 1572 (C=C), 1499 (N-O); ^{1}H NMR (600 MHz, DMSO) δ 8.16 (d, *J* = 8.9 Hz, 2H, H-5), 7.73 (s, 1H, H-2), 7.67 (d, *J* = 8.9 Hz, 2H, H-4), 7.55 (s, 2H, H-1). ^{13}C NMR (151 MHz, DMSO) δ 145.62 (C-6), 143.48 (C-2), 134.15 (C-3), 125.29 (C-4), 123.98 (C-5).

(*1E,2E*)-1-Benzylidene-2-[(5-nitrofuran-2-yl)methylene]hydrazine (1a)

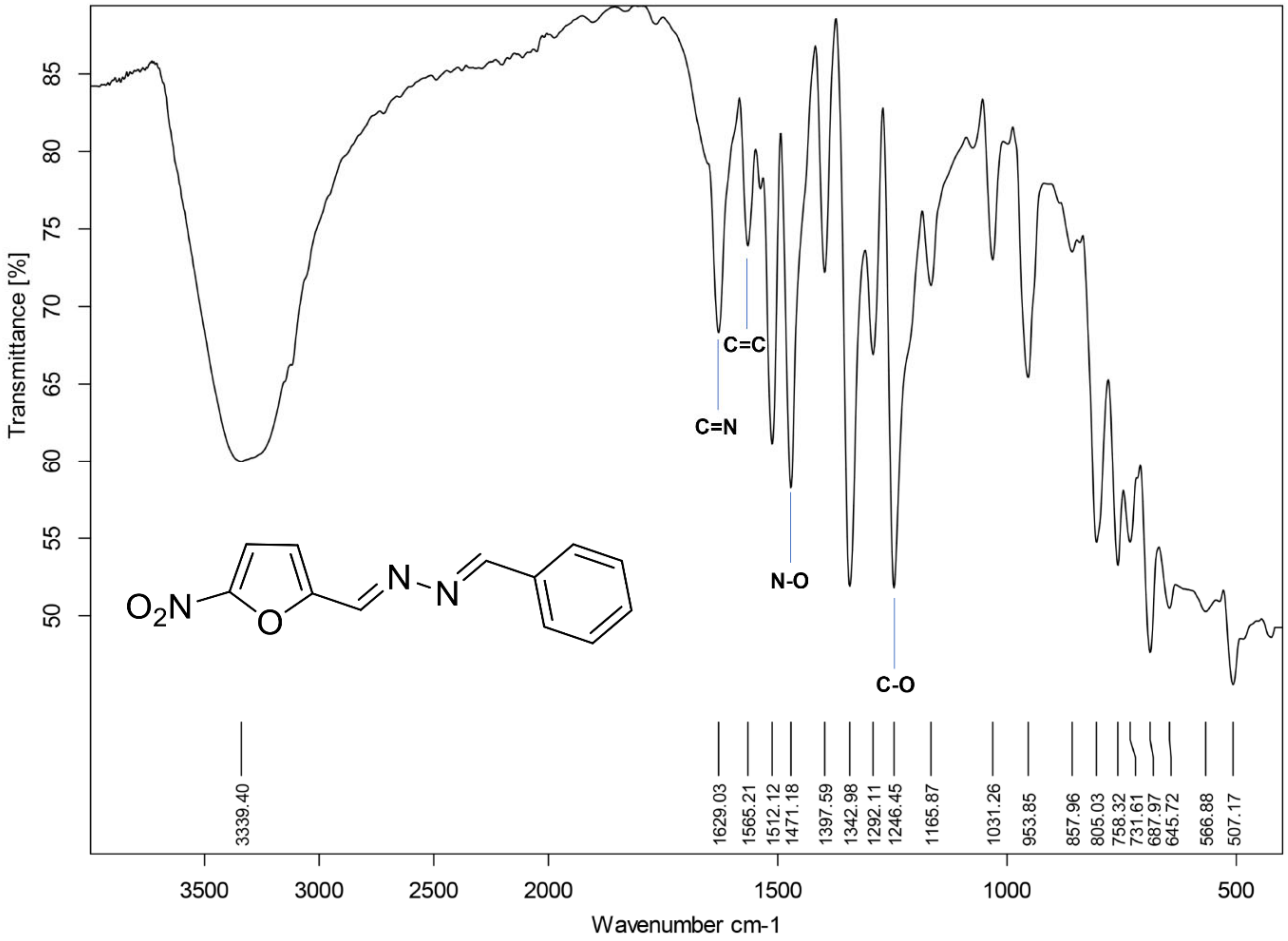
^{1}H NMR in DMSO



^{13}C NMR in DMSO



IR Spectrum



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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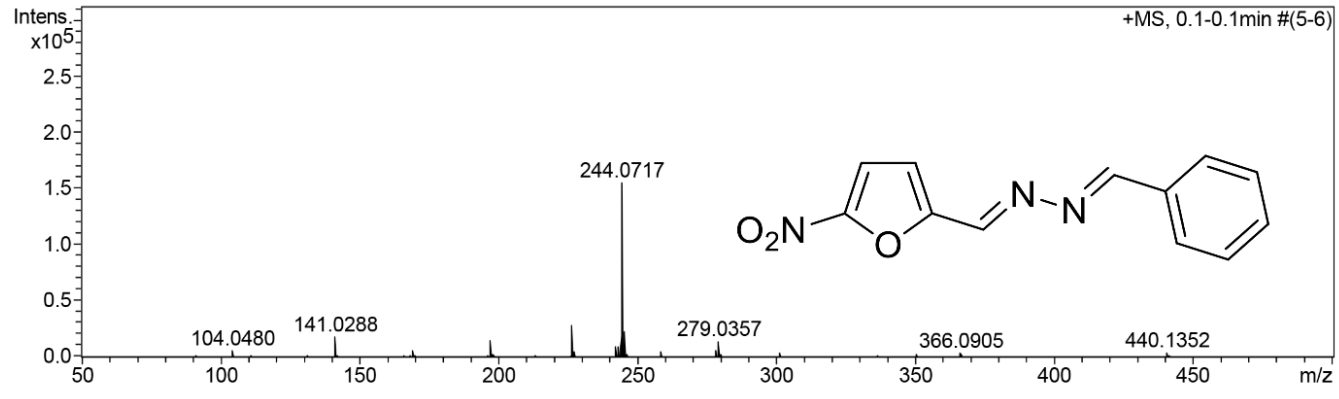

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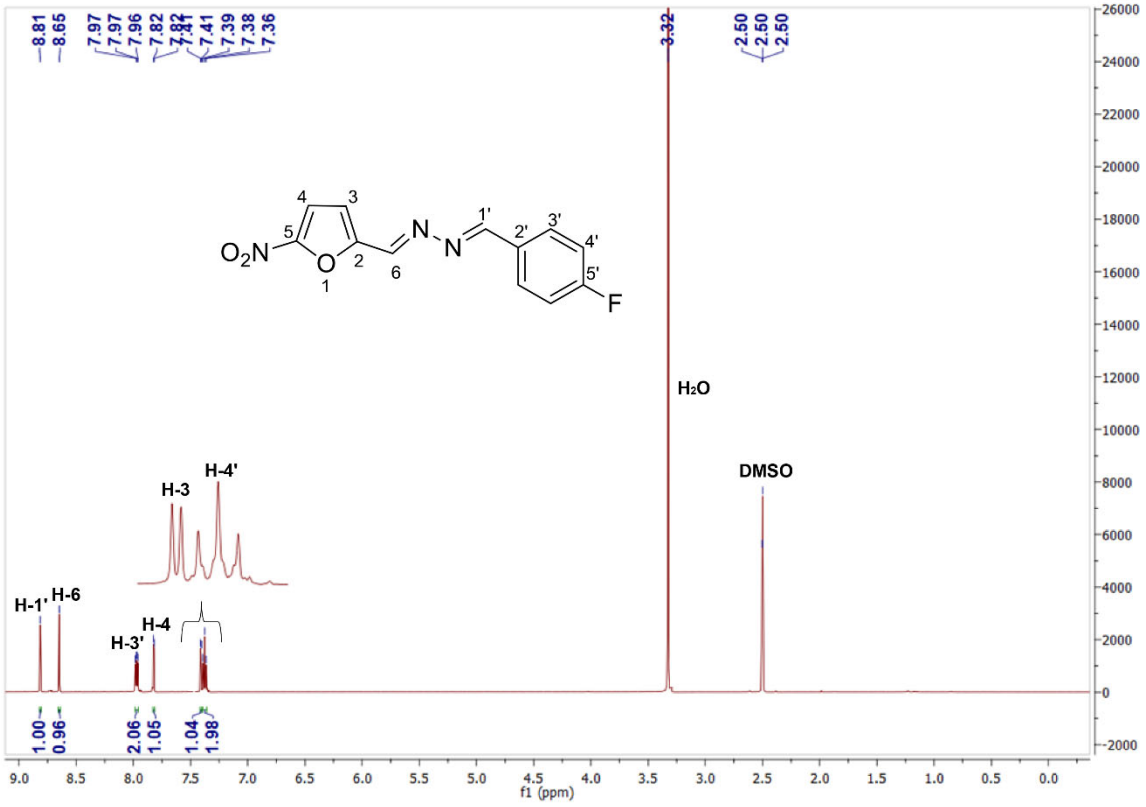
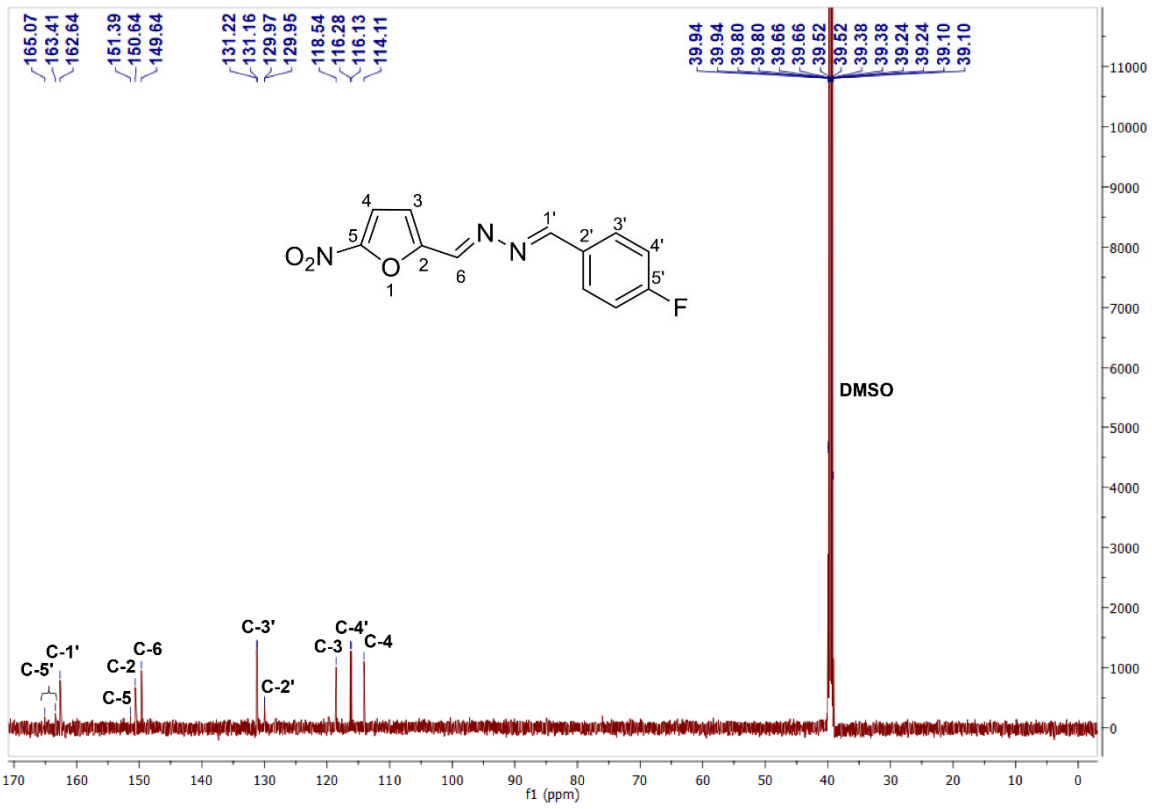
(*1E,2E*)-1-(4-Fluorobenzylidene)-2-[(5-nitrofuran-2-yl)methylene]hydrazine (2a)

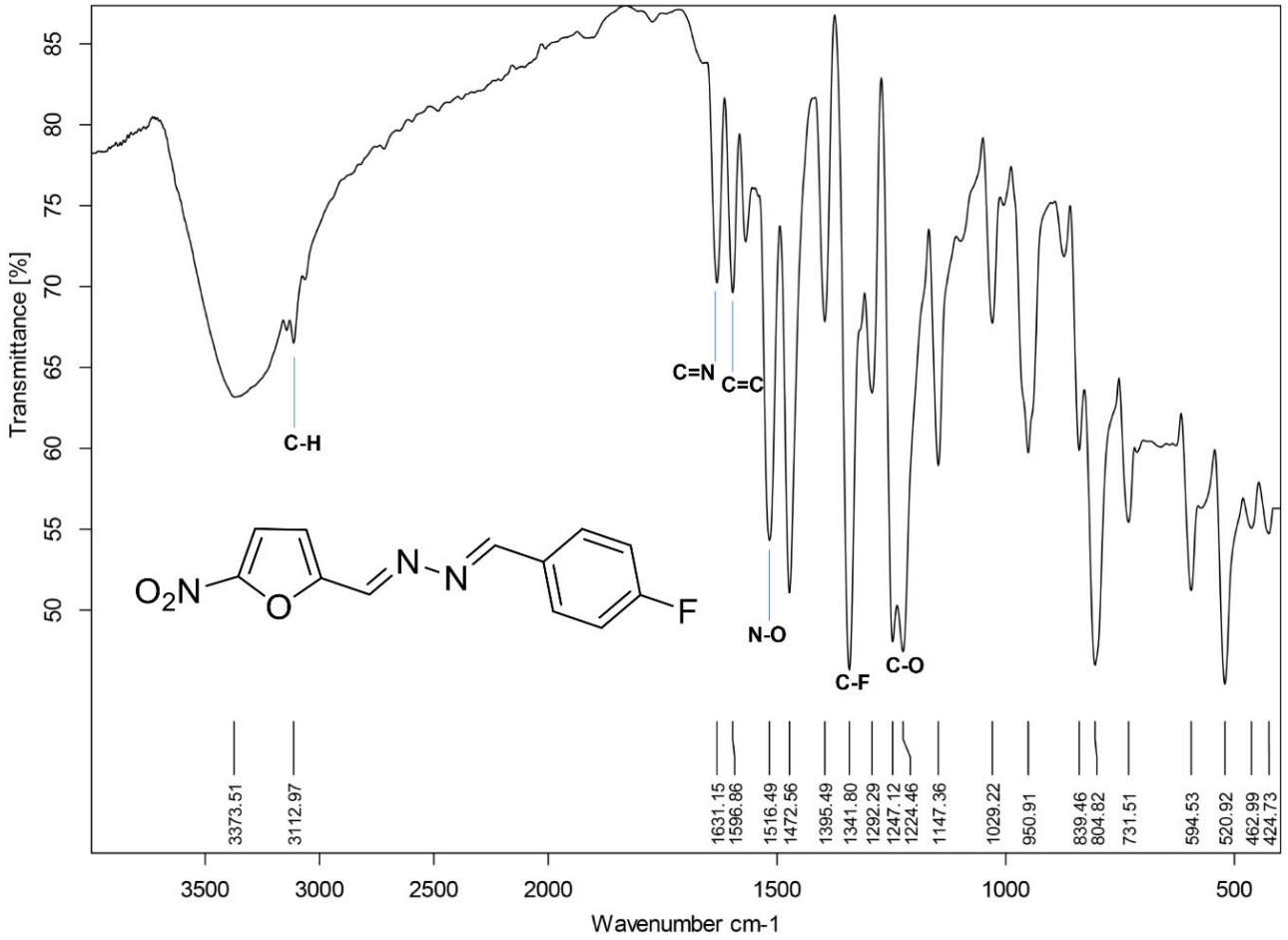
^{1}H NMR in DMSO



^{13}C NMR in DMSO



IR Spectrum



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
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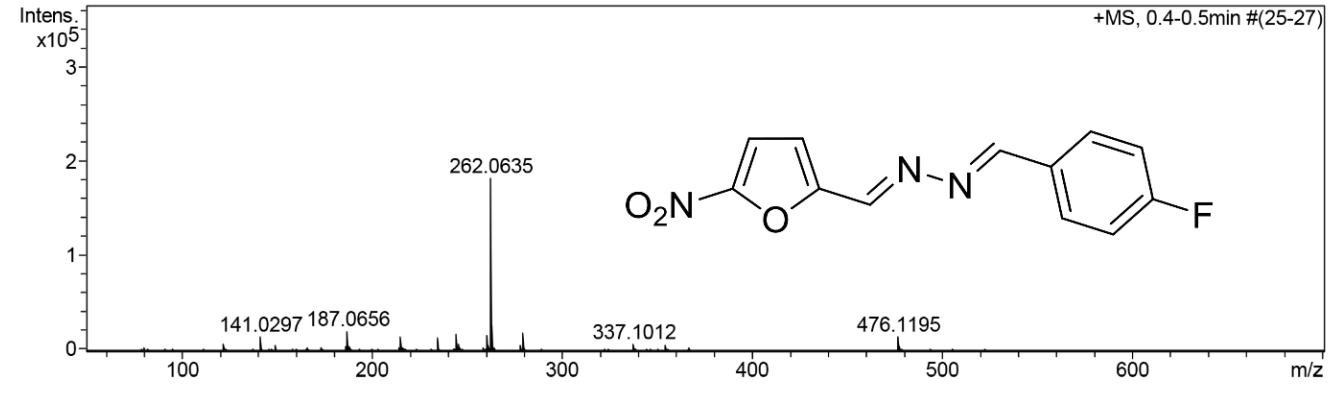
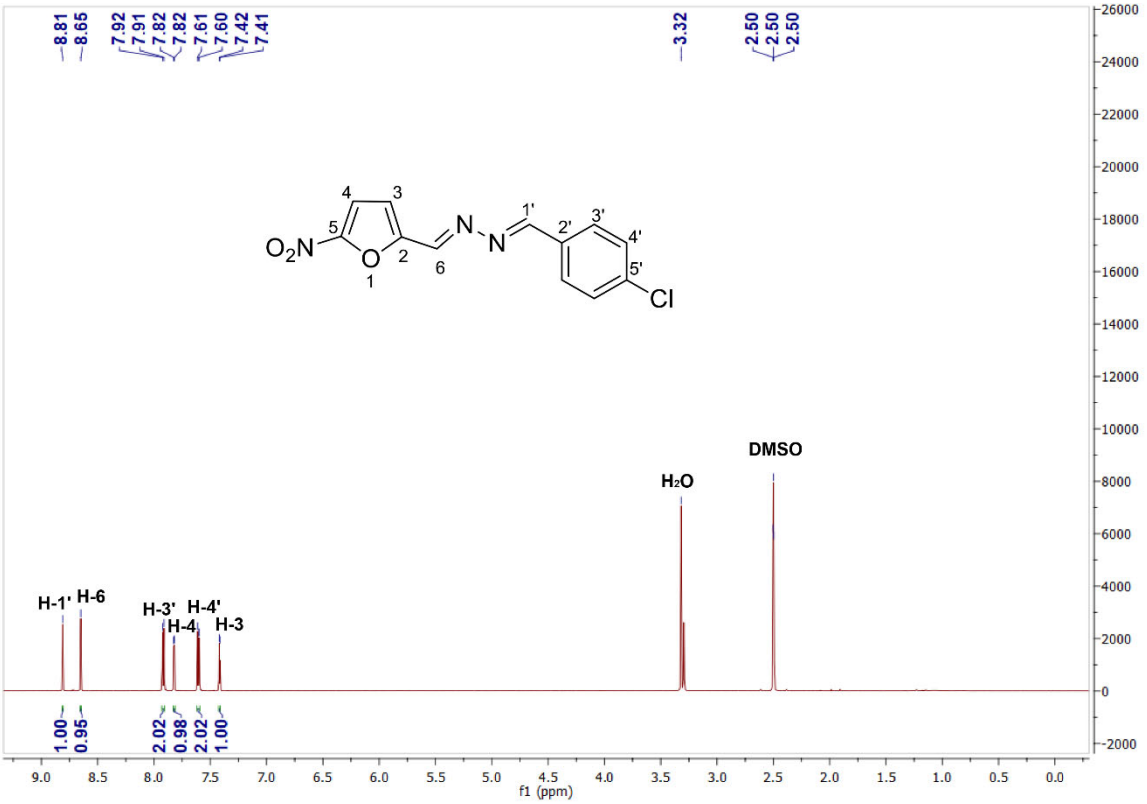
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



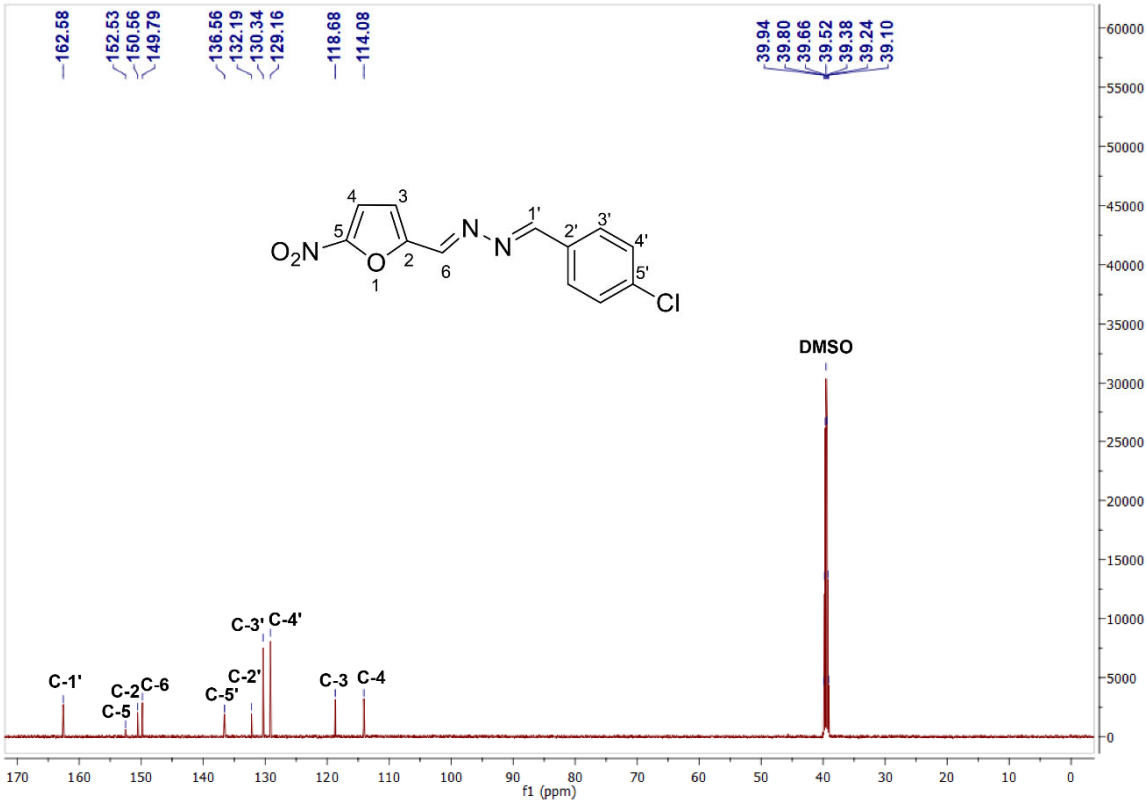
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
262.0635	1	C 12 H 9 F N 3 O 3	100.00	262.0622	-1.3	-5.0	1.2	9.5	even	ok

(*1E,2E*)-1-(4-Chlorobenzylidene)-2-[(5-nitrofuran-2-yl)methylene]hydrazine (3a)

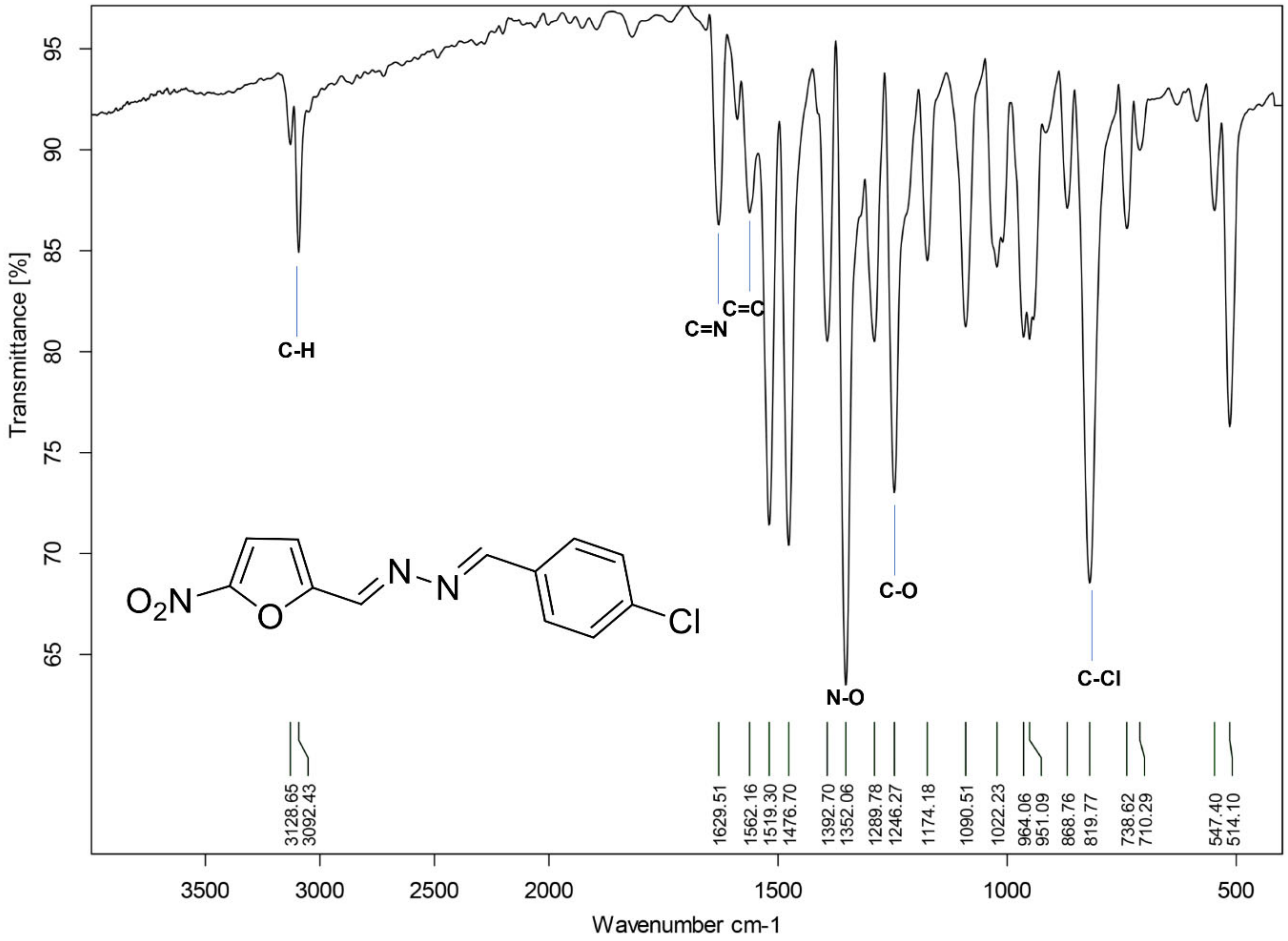
^{1}H NMR in DMSO



^{13}C NMR in DMSO



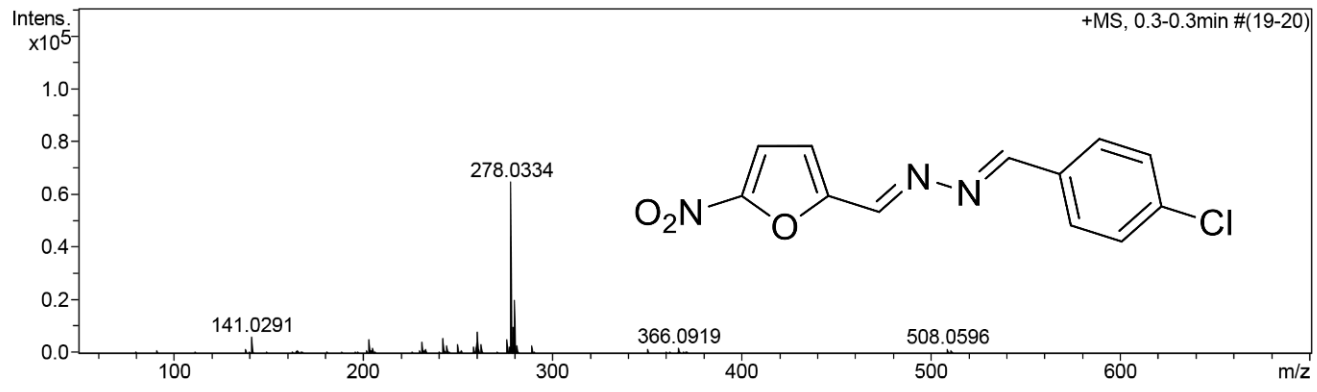
IR Spectrum



HRMS

Acquisition Parameter

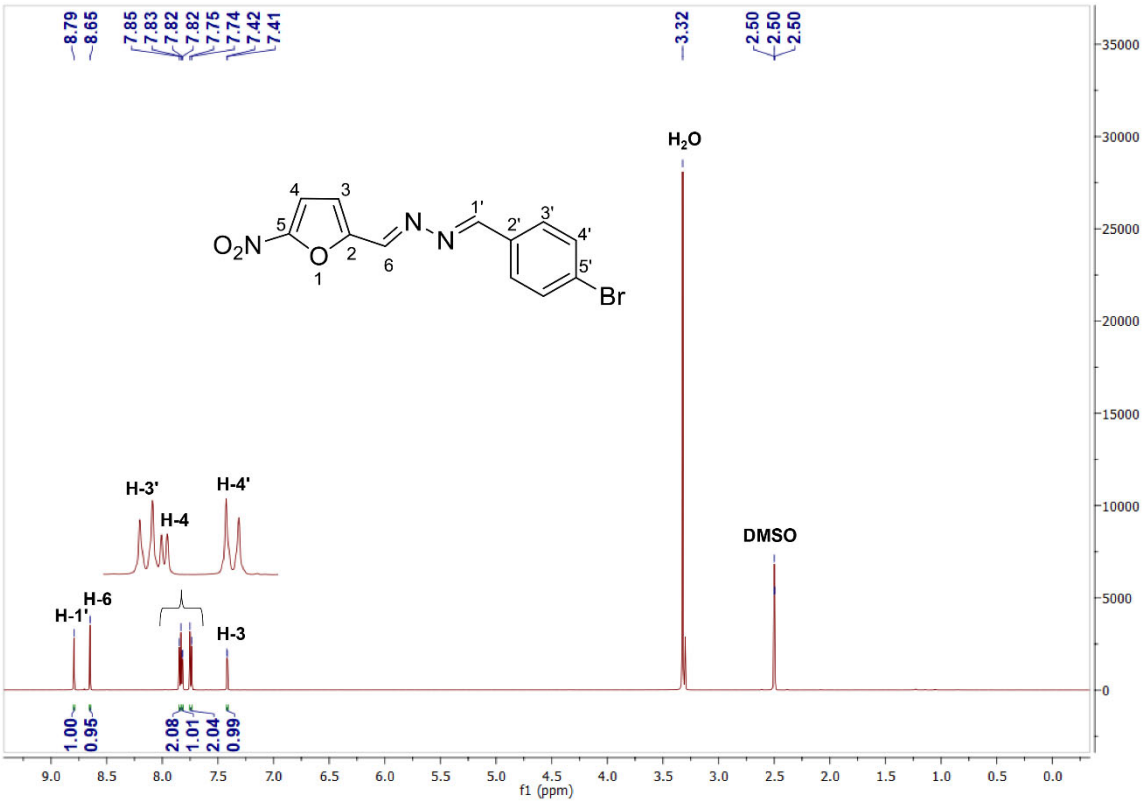
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



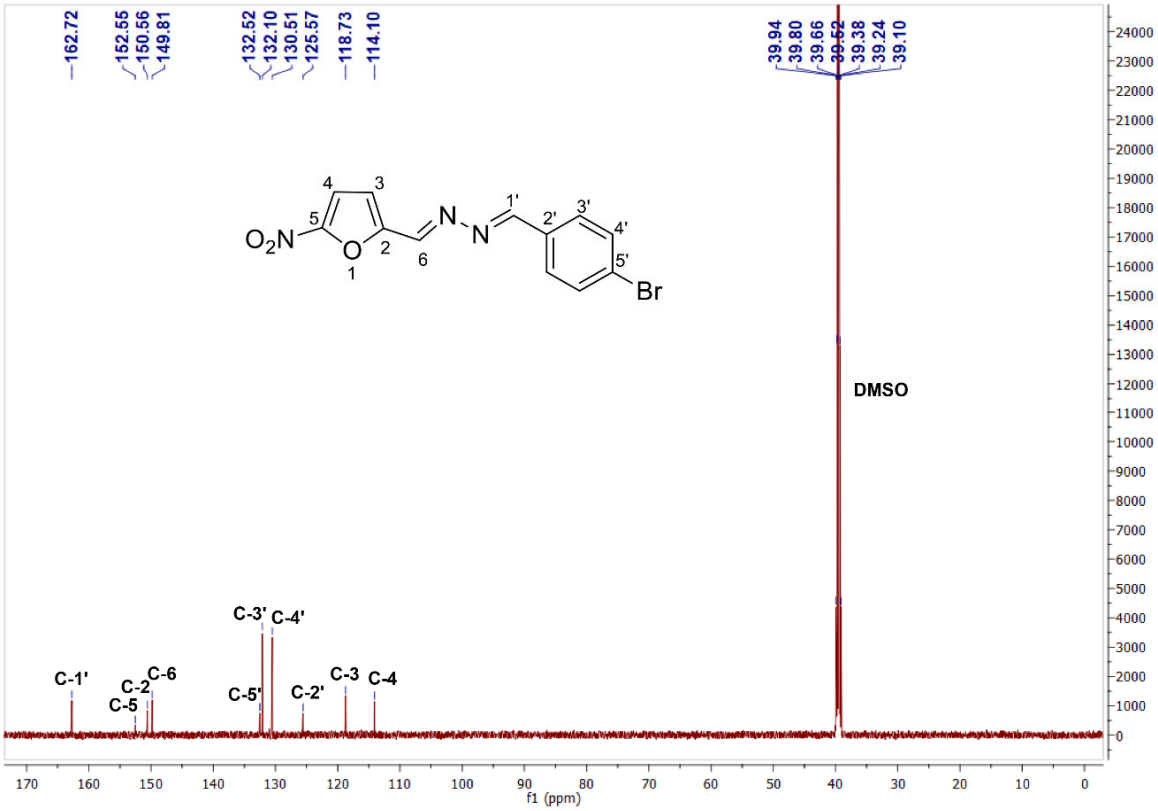
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
278.0334	1	C 12 H 9 Cl N 3 O 3	100.00	278.0327	-0.7	-2.5	12.6	9.5	even	ok

(*1E,2E*)-1-(4-Bromobenzylidene)-2-[(5-nitrofuran-2-yl)methylene]hydrazine (4a)

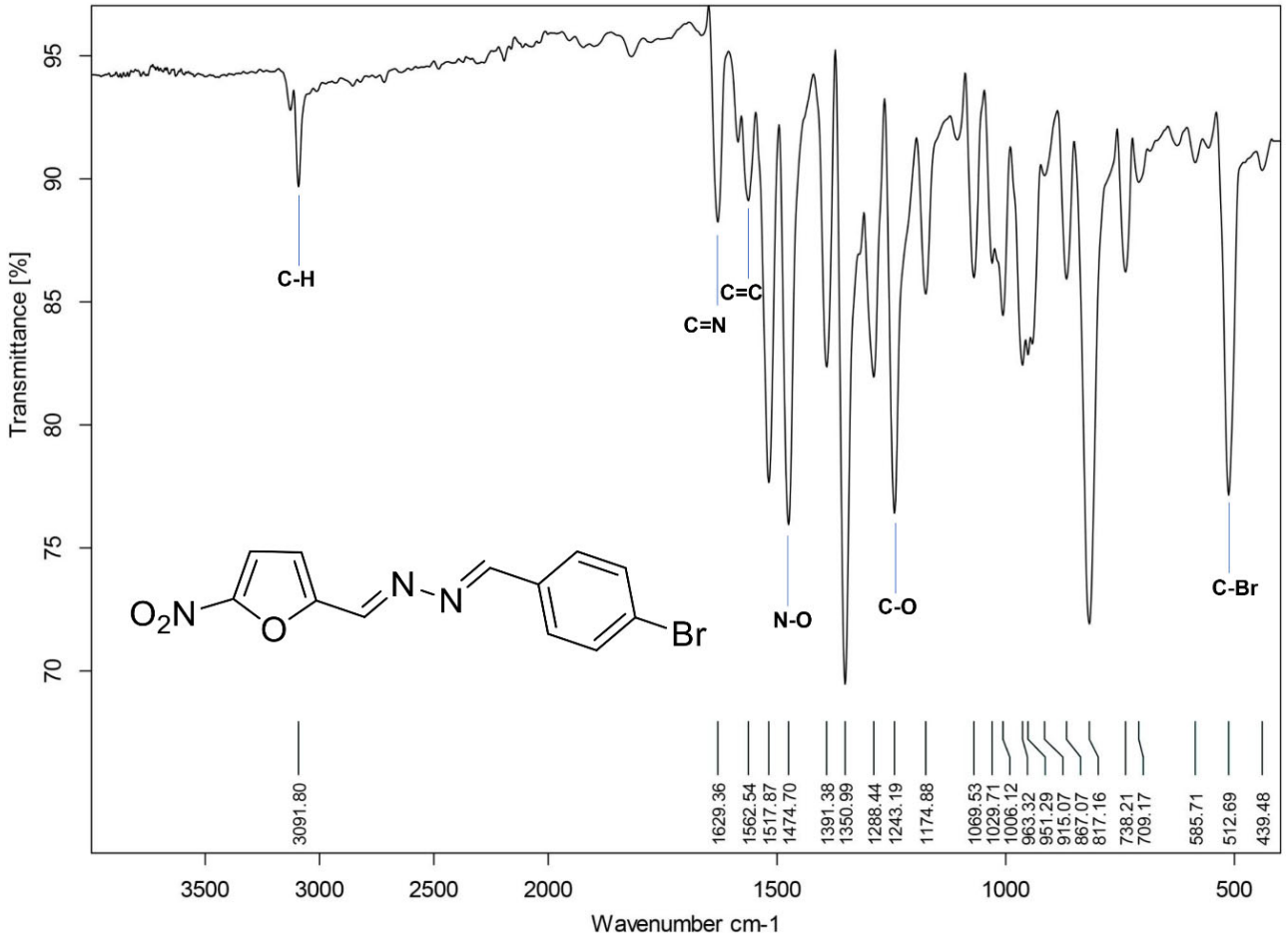
^{1}H NMR in DMSO



^{13}C NMR in DMSO



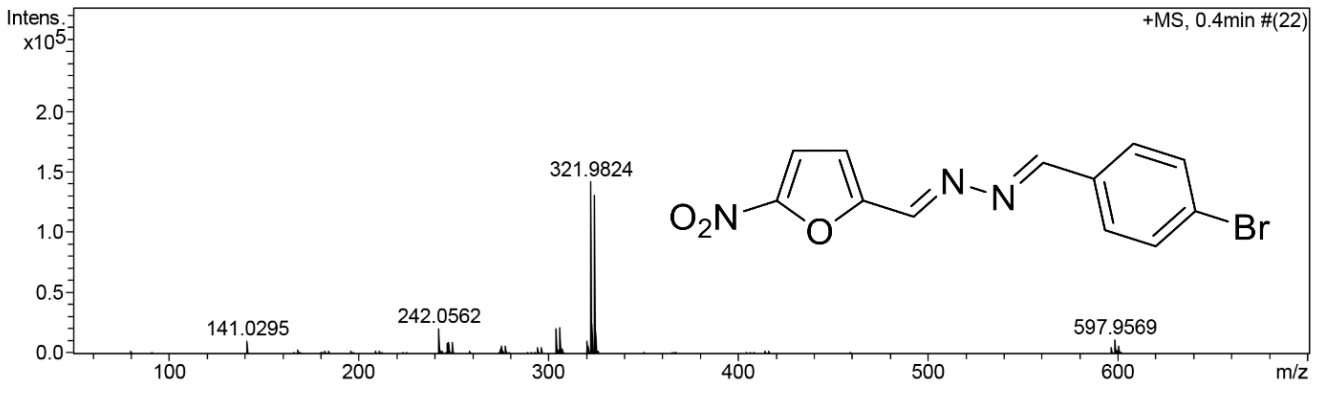
IR Spectrum



HRMS

Acquisition Parameter

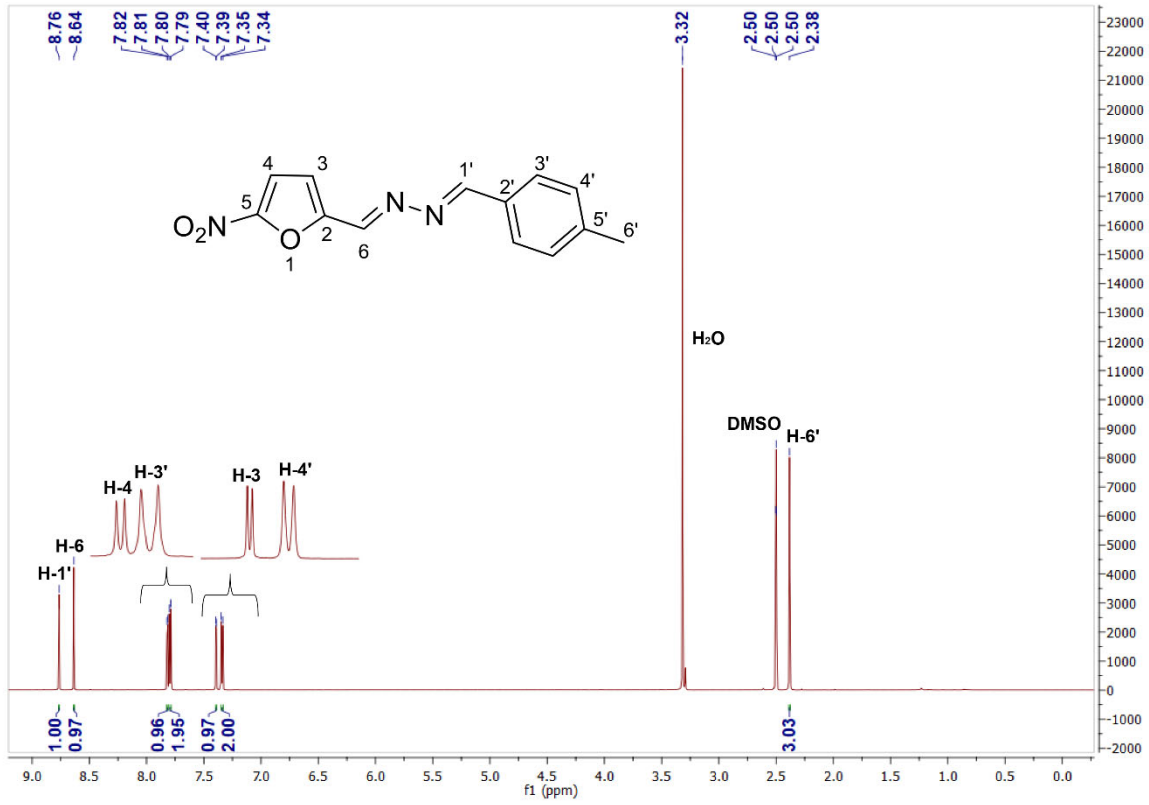
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



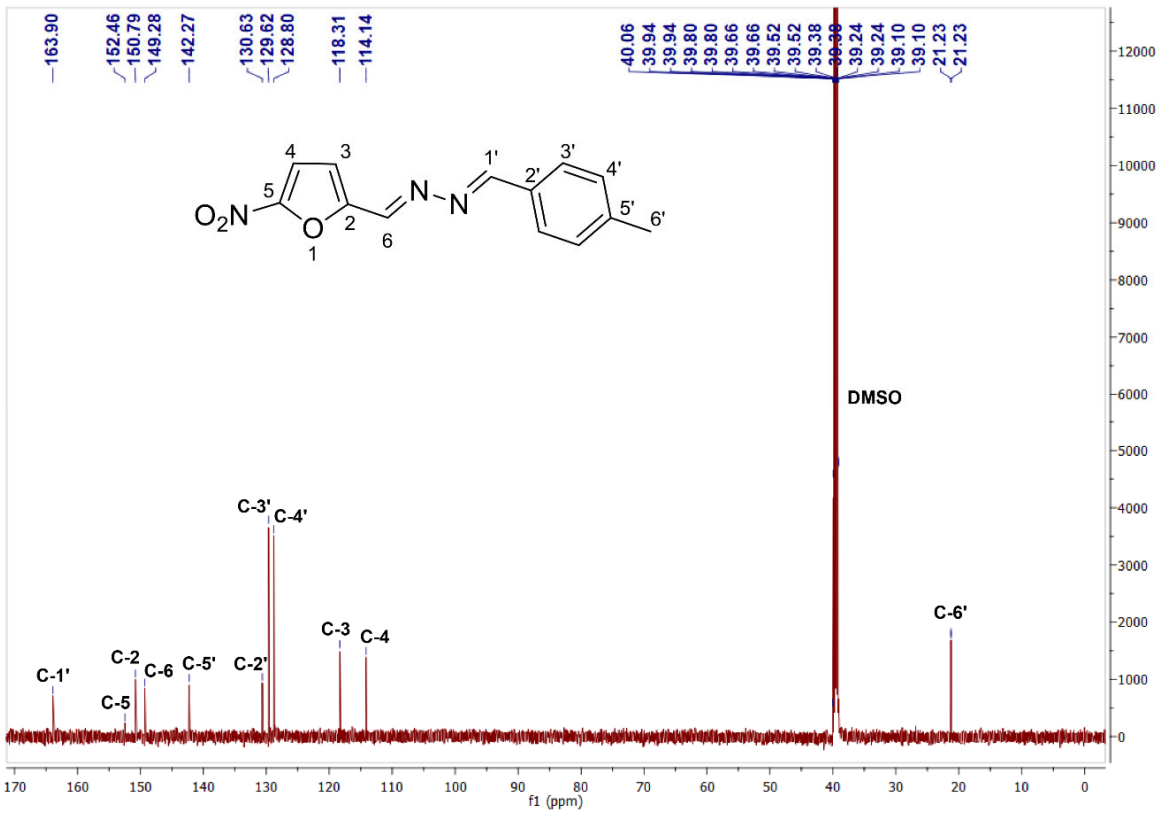
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
321.9824	1	C 12 H 9 Br N 3 O 3	100.00	321.9822	-0.3	-0.8	32.5	9.5	even	ok

(*1E,2E*)-1-(4-Methylbenzylidene)-2-[(5-nitrofuran-2-yl)methylene]hydrazine (5a)

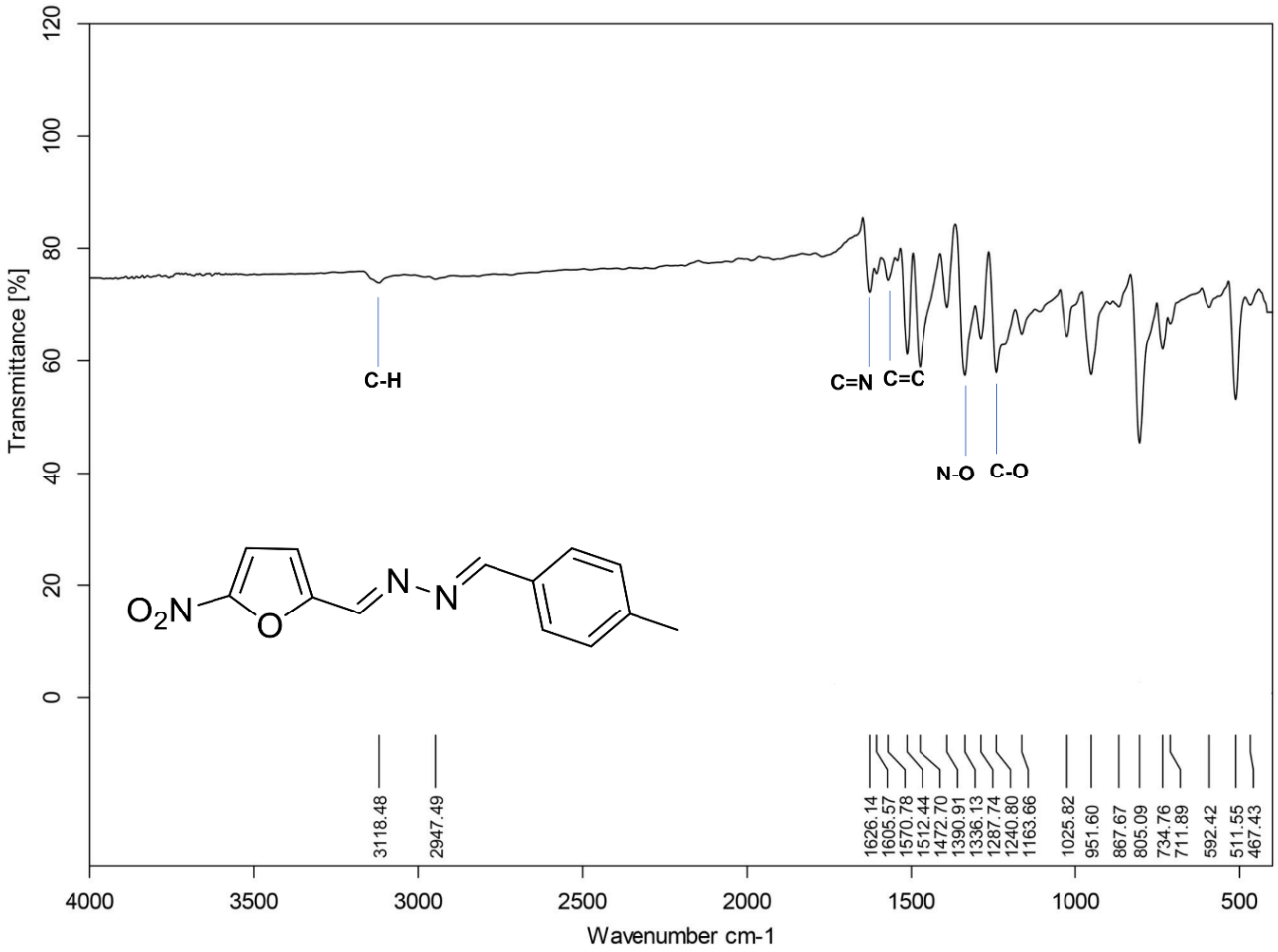
^{1}H NMR in DMSO



^{13}C NMR in DMSO



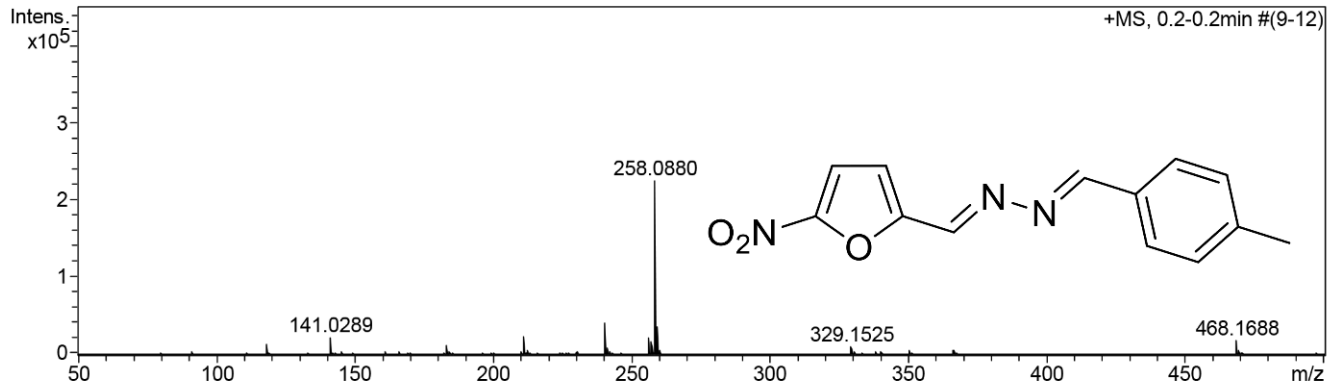
IR Spectrum



HRMS

Acquisition Parameter

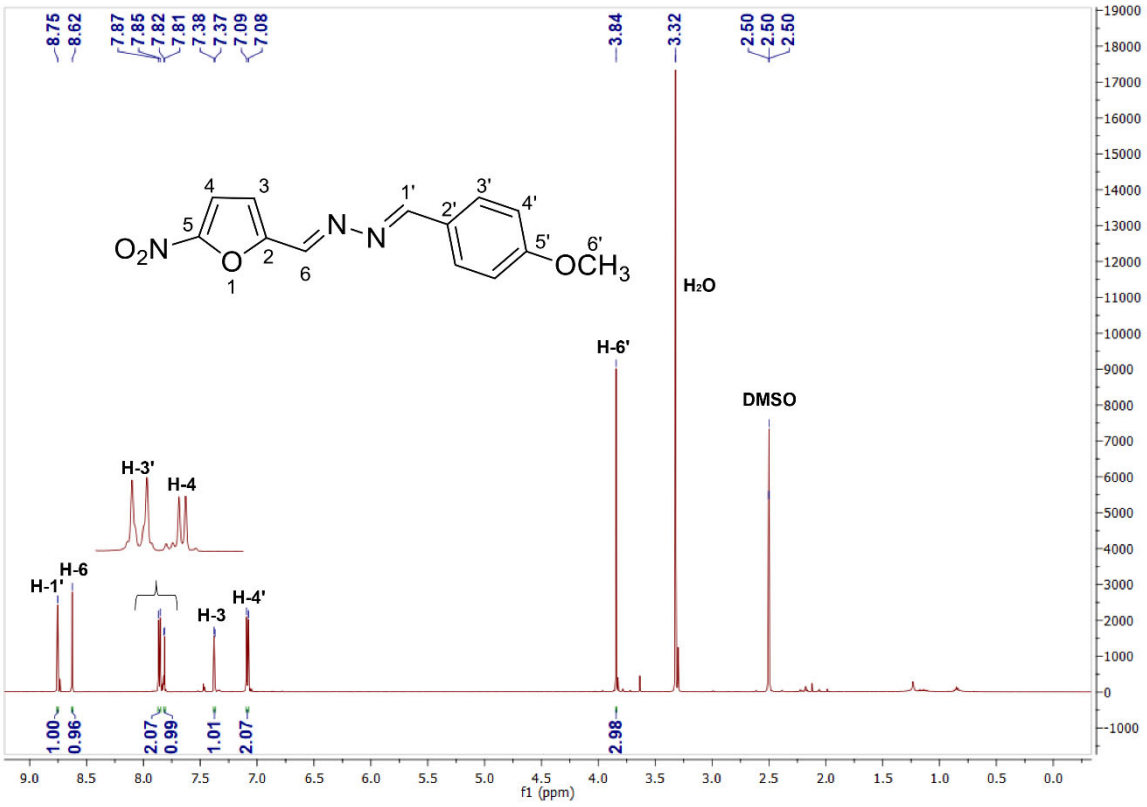
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



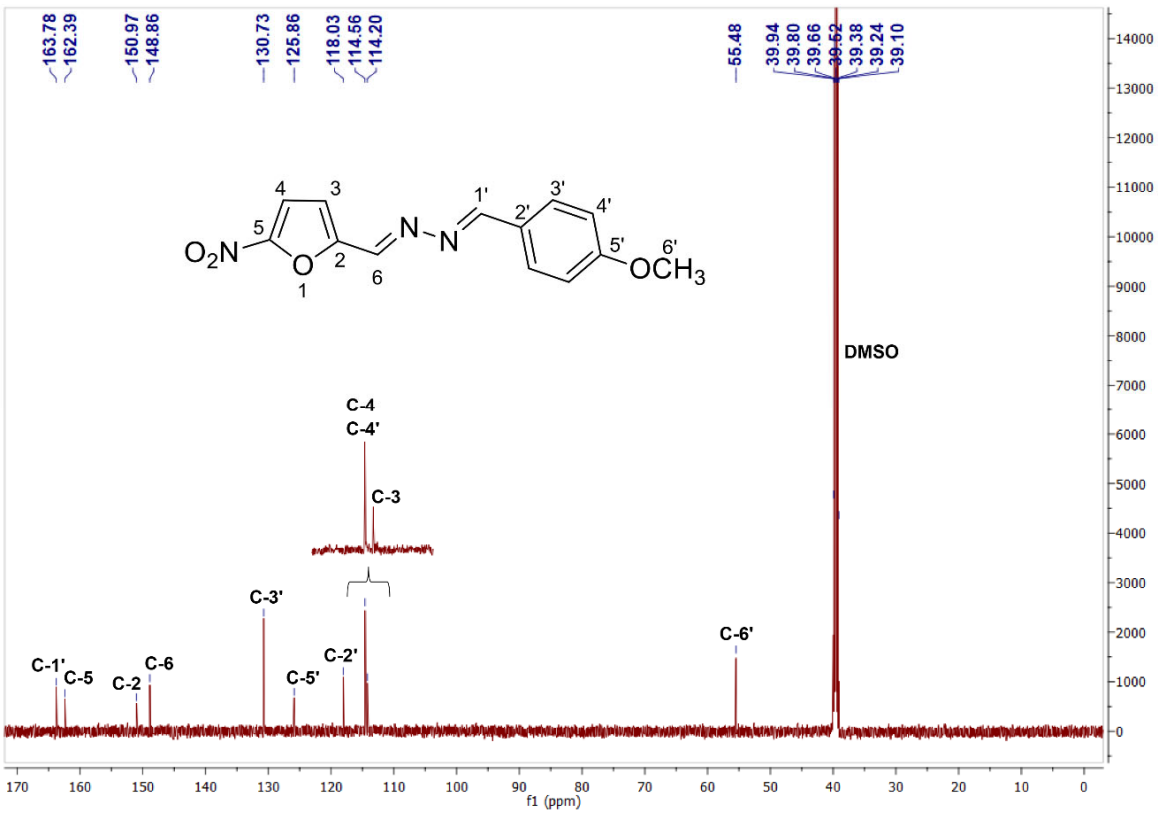
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
258.0880	1	C 13 H 12 N 3 O 3	100.00	258.0873	-0.6	-2.5	4.4	9.5	even	ok

(*1E,2E*)-1-(4-Methoxybenzylidene)-2-[(5-nitrofuran-2-yl)methylene]hydrazine (6a)

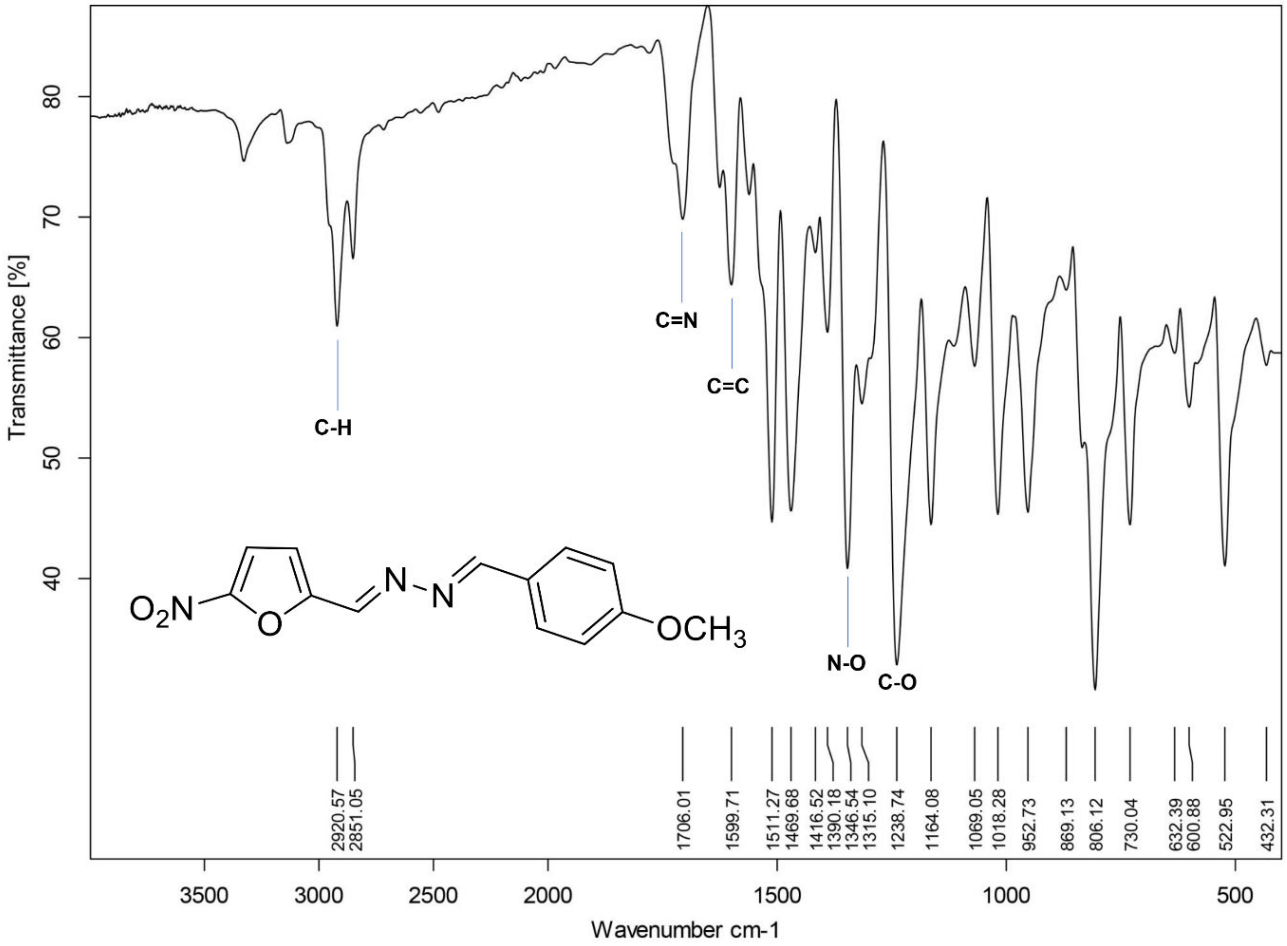
^{1}H NMR in DMSO



^{13}C NMR in DMSO



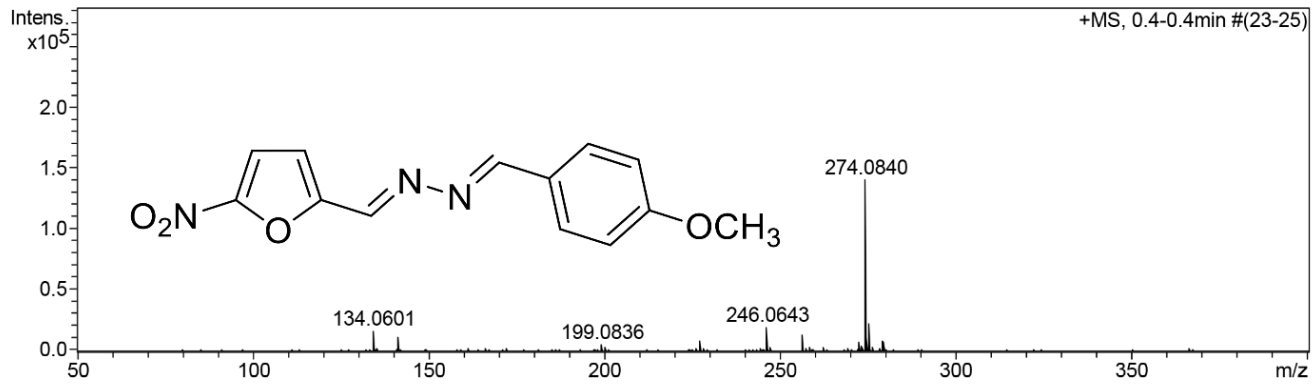
IR Spectrum



HRMS

Acquisition Parameter

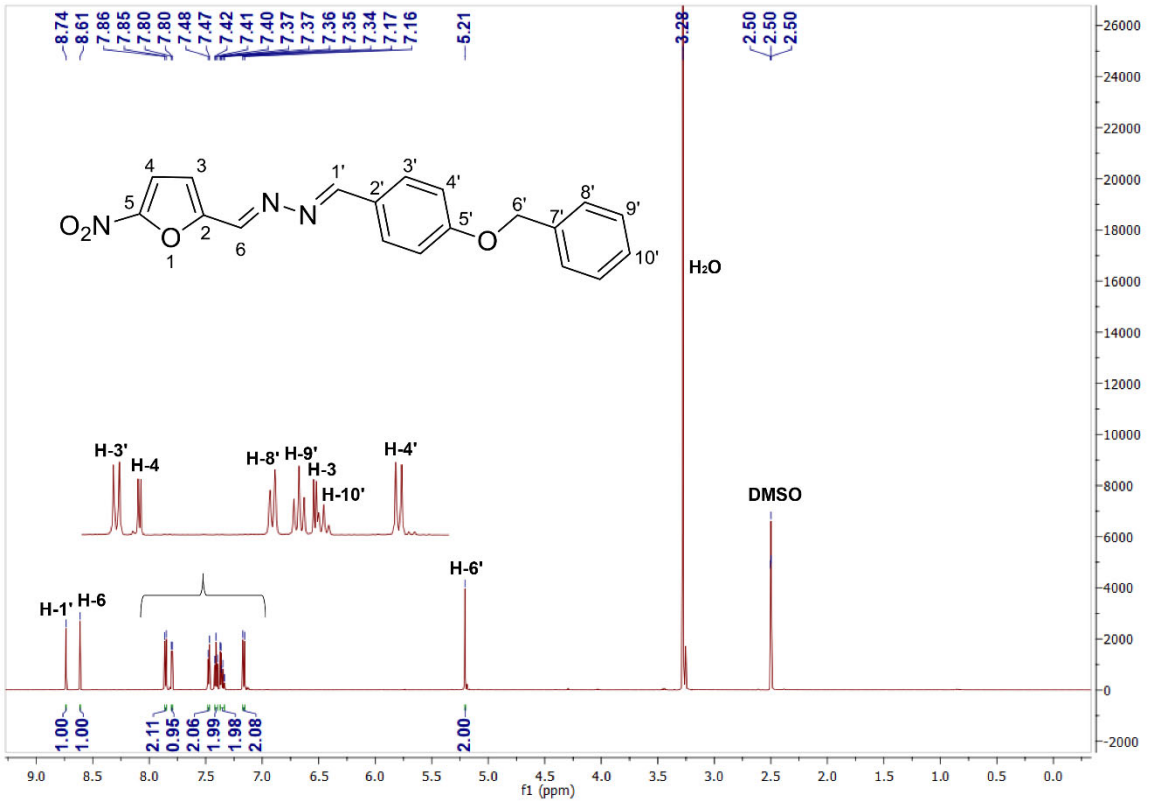
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



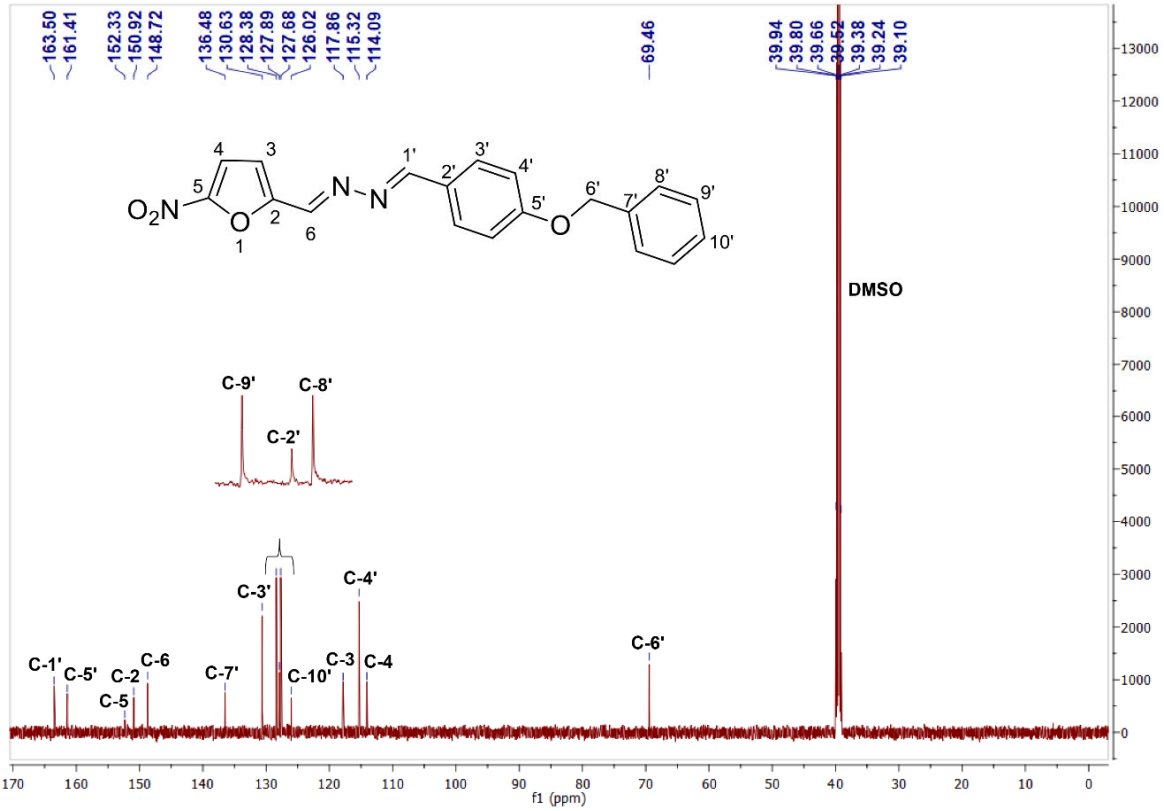
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
274.0840	1	C 13 H 12 N 3 O 4	100.00	274.0822	-1.8	-6.5	2.0	9.5	even	ok

(*1E,2E*)-1-[4-(Benzyloxy)benzylidene]-2-[(5-nitrofuran-2-yl)methylene]hydrazine (7a)

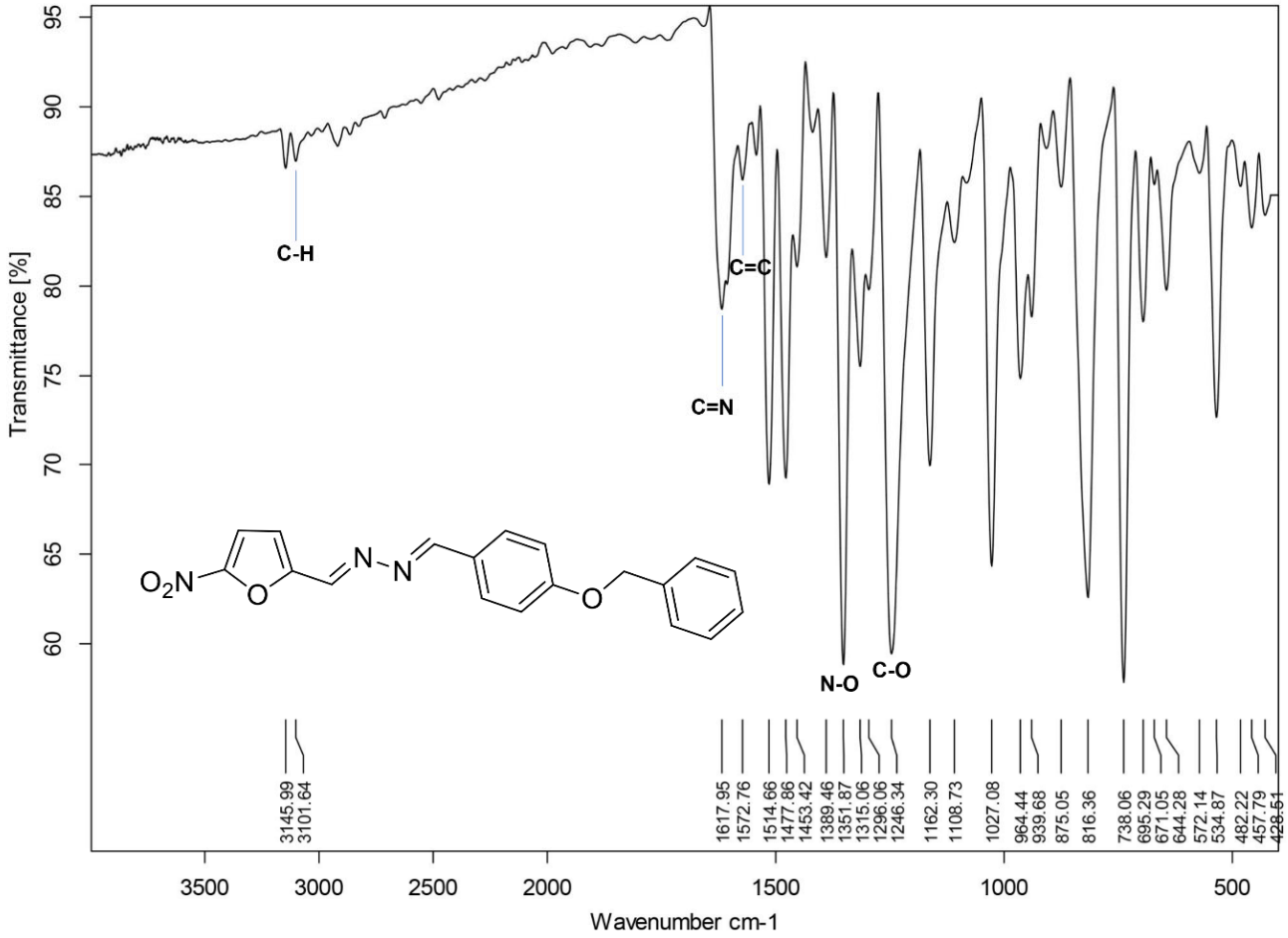
^{1}H NMR in DMSO



^{13}C NMR in DMSO



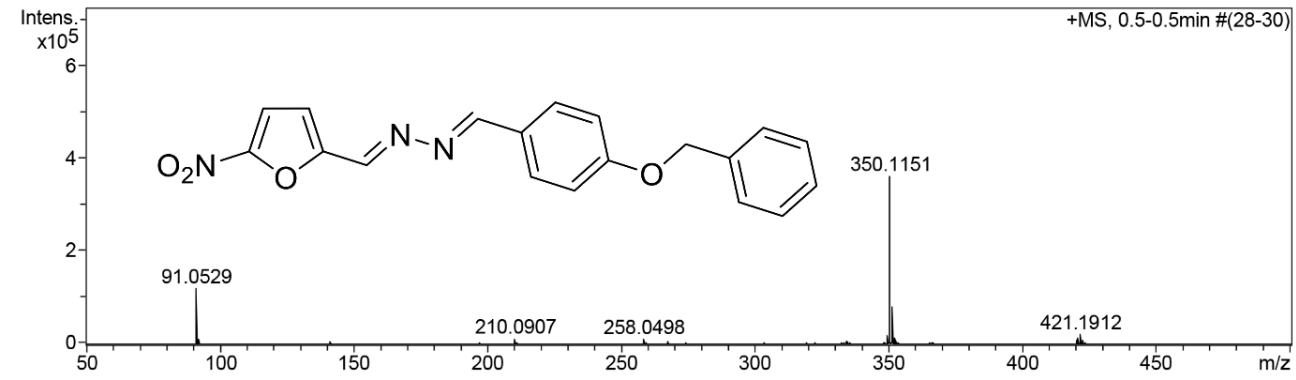
IR Spectrum



HRMS

Acquisition Parameter

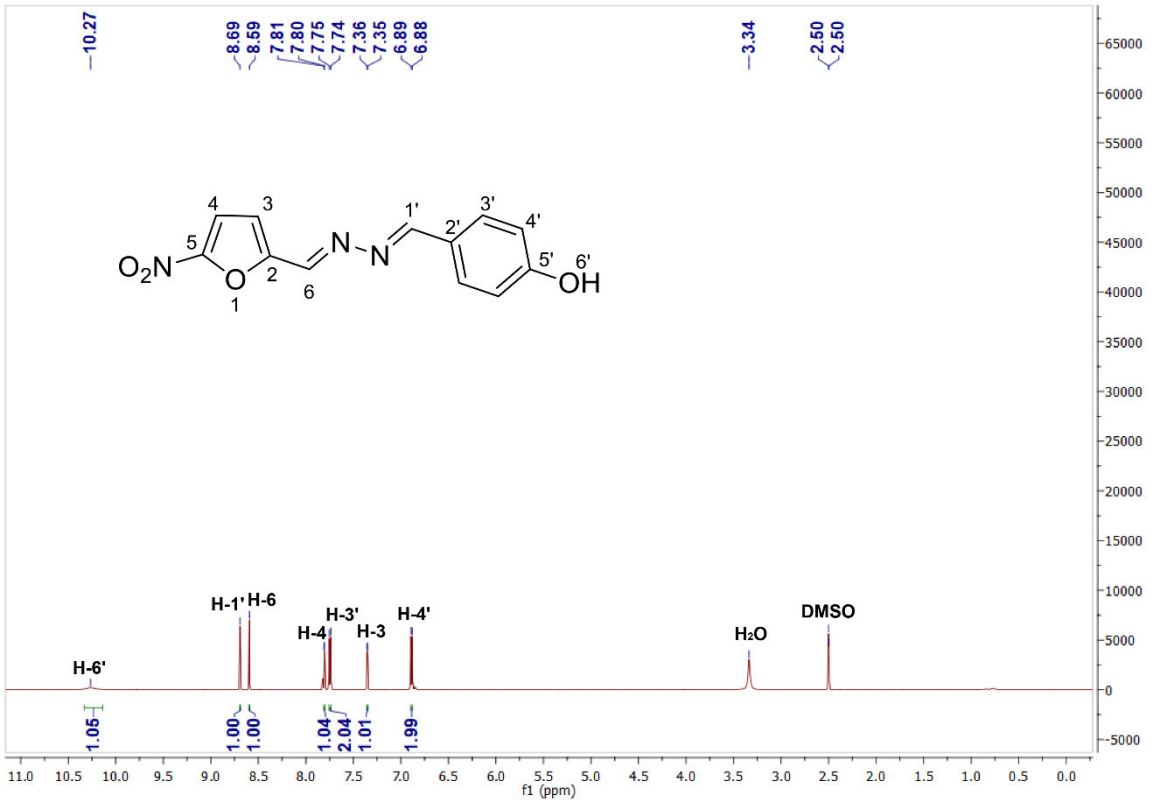
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



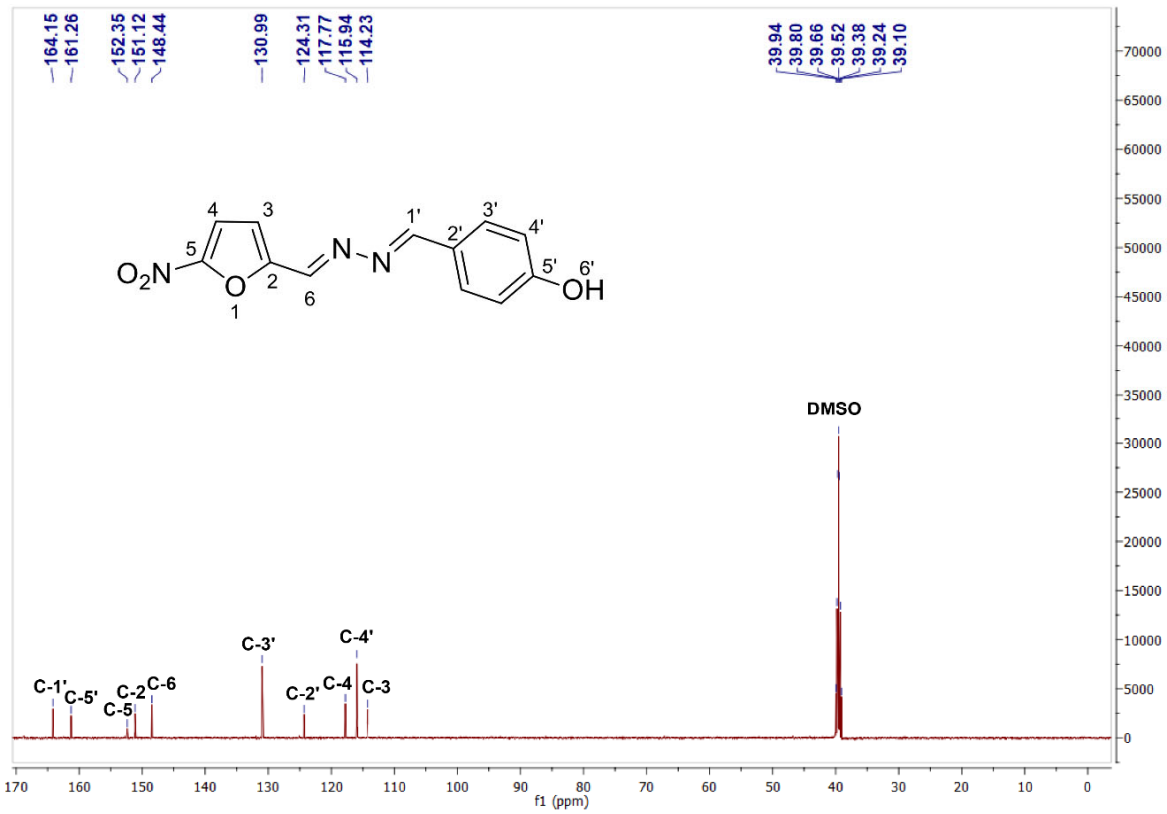
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
91.0529	1	C 7 H 7	100.00	91.0542	1.4	15.1	1.9	4.5	even	ok
350.1151	1	C 19 H 16 N 3 O 4	100.00	350.1135	-1.6	-4.5	2.3	13.5	even	ok

4-{(*E*)-{(*E*)-[(5-Nitrofuran-2-yl)methylene]hydrazono}methyl}phenol (8a)

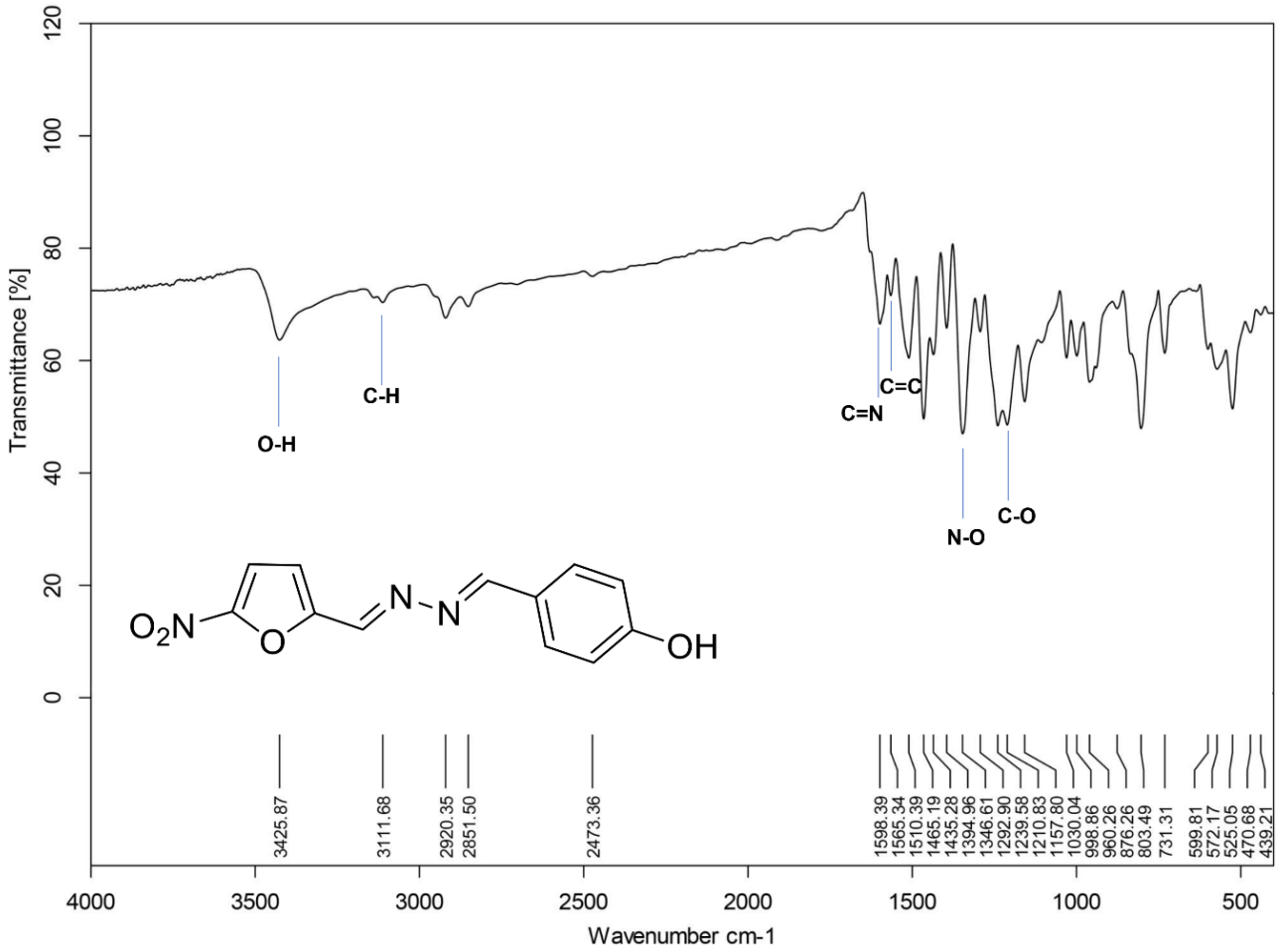
^{1}H NMR in DMSO



^{13}C NMR in DMSO



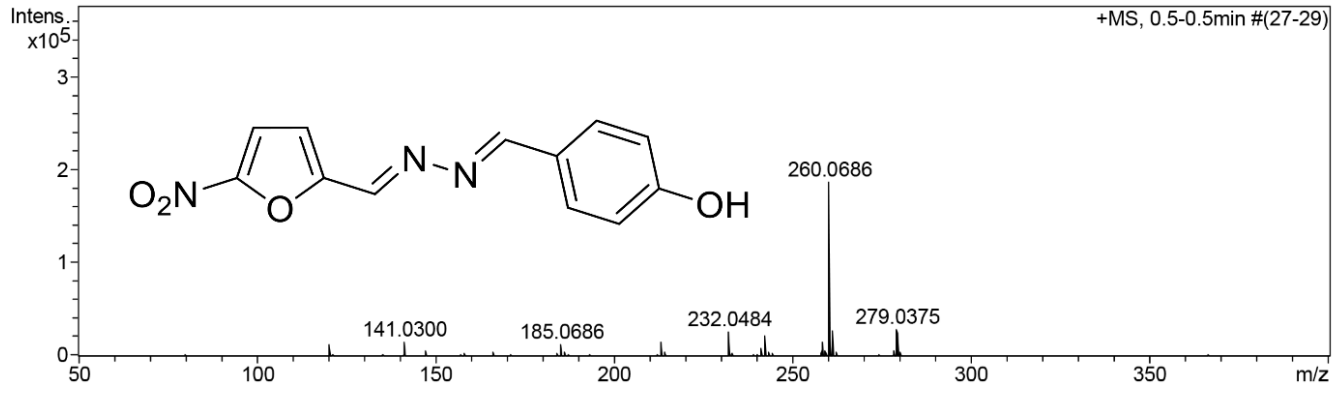
IR Spectrum



HRMS

Acquisition Parameter

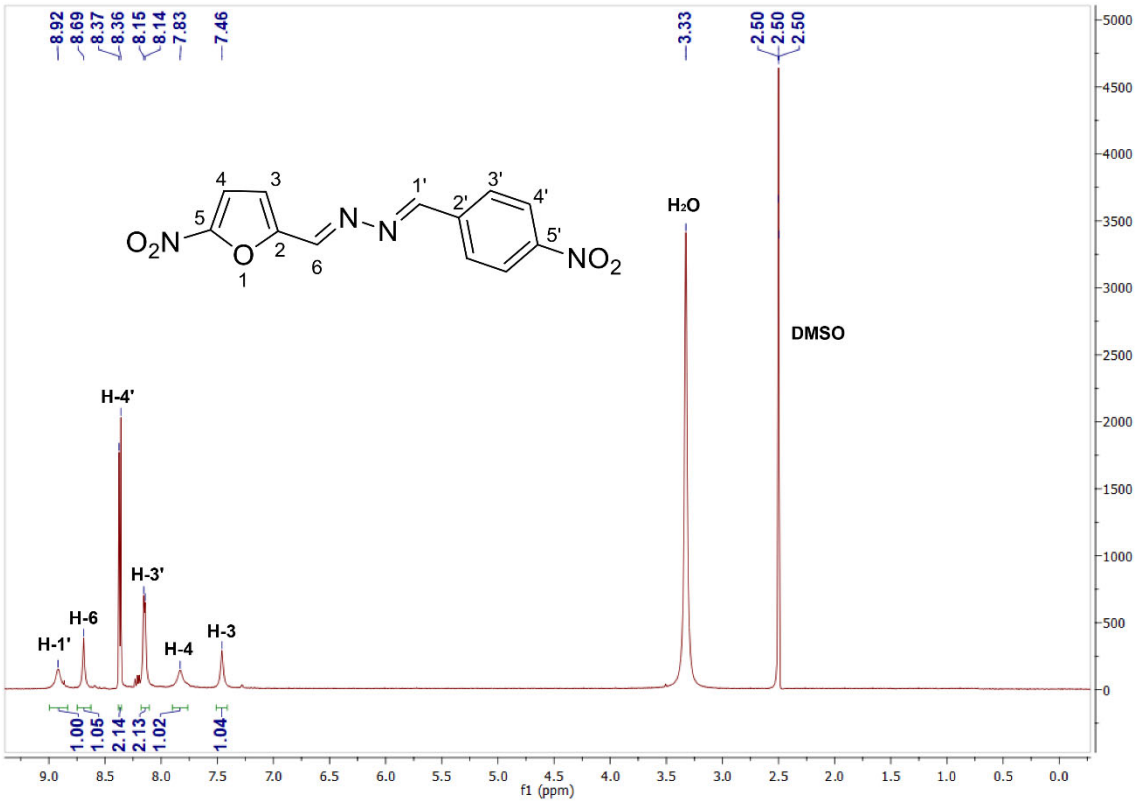
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



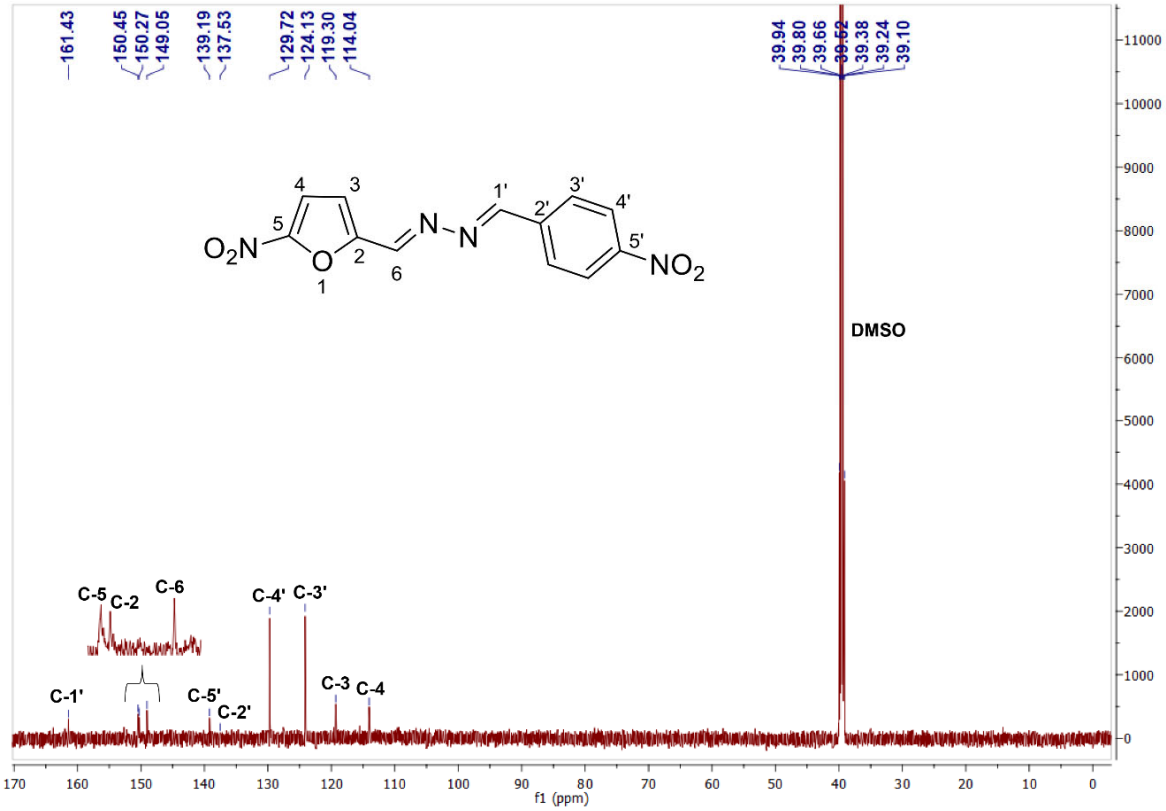
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
260.0686	1	C 12 H 10 N 3 O 4	100.00	260.0666	-2.0	-7.6	6.3	9.5	even	ok

(*1E,2E*)-1-(4-Nitrobenzylidene)-2-[(5-nitrofuran-2-yl)methylene]hydrazine (9a)

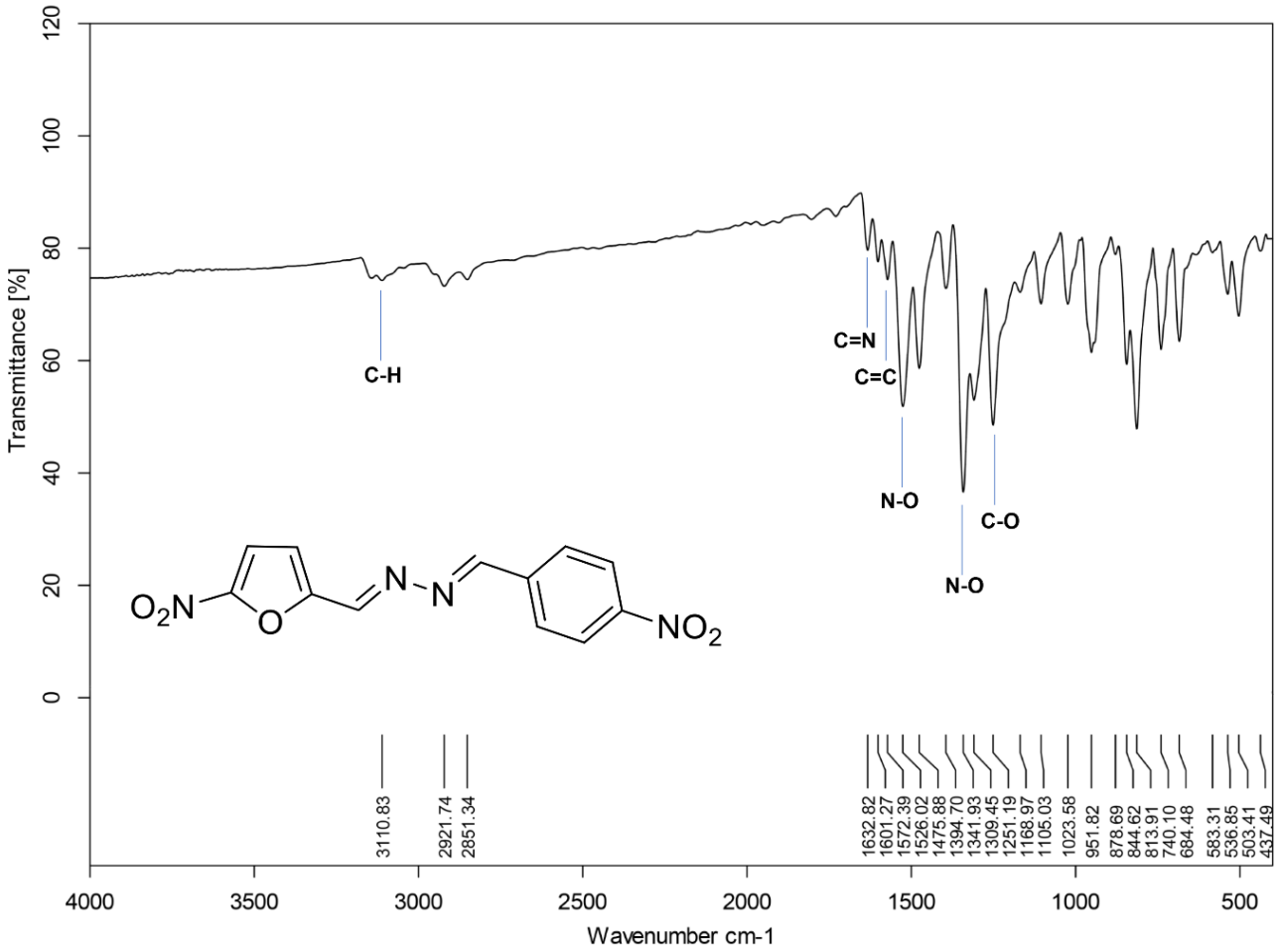
^{1}H NMR in DMSO



^{13}C NMR in DMSO



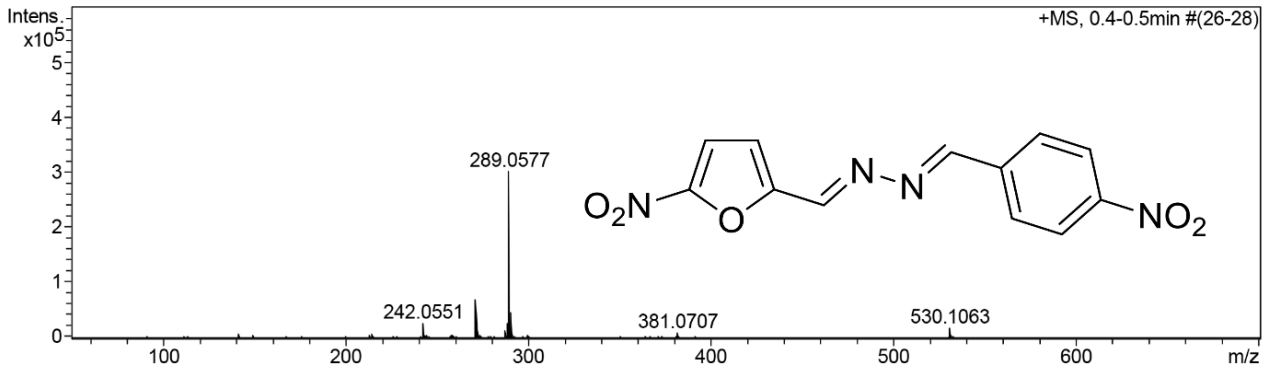
IR Spectrum



HRMS

Acquisition Parameter

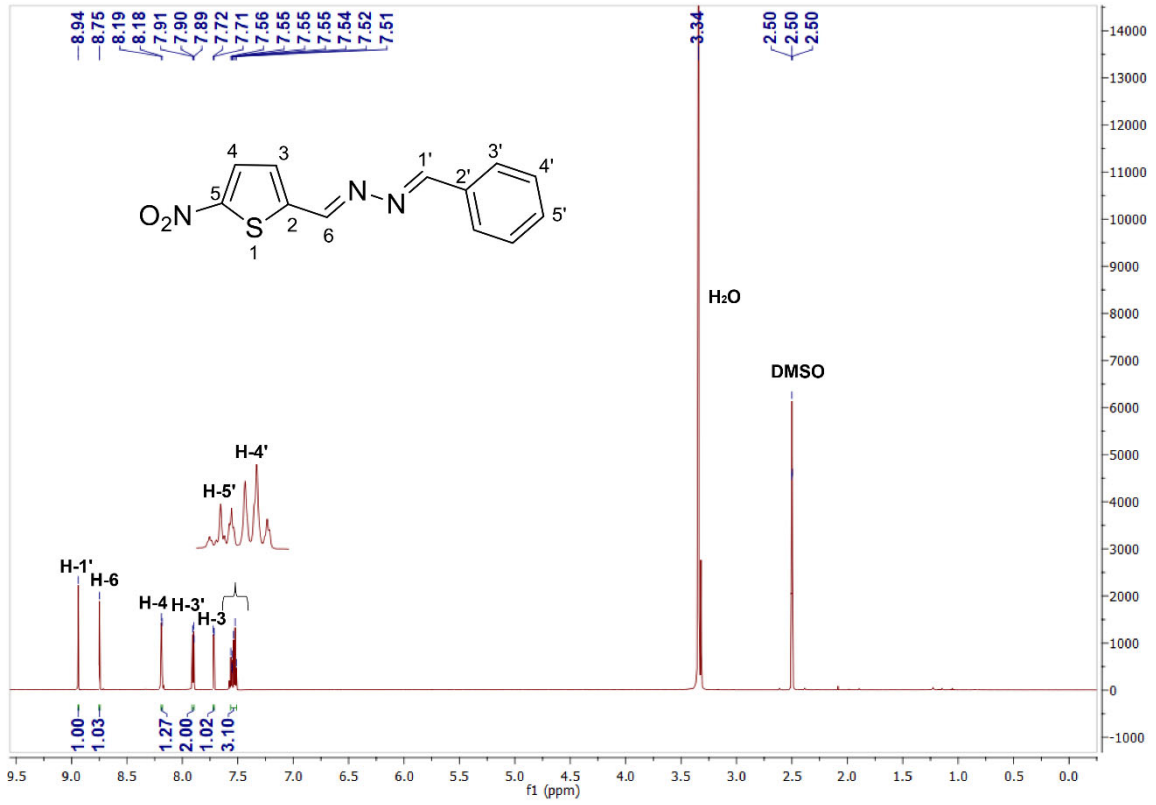
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



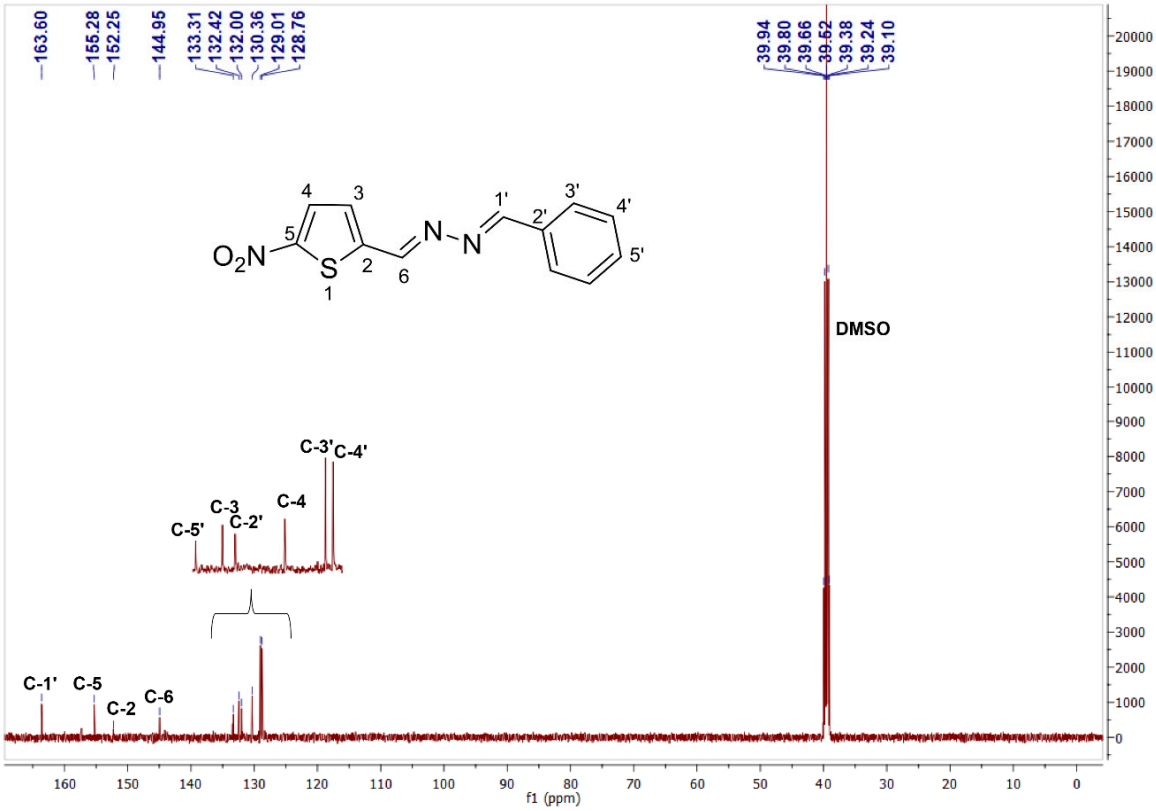
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
271.0462	1	C 12 H 7 N 4 O 4	100.00	271.0462	0.0	0.1	32.4	11.5	even	ok
289.0577	1	C 12 H 9 N 4 O 5	100.00	289.0567	-0.9	-3.2	2.1	10.5	even	ok

(*1E,2E*)-1-Benzylidene-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (1b)

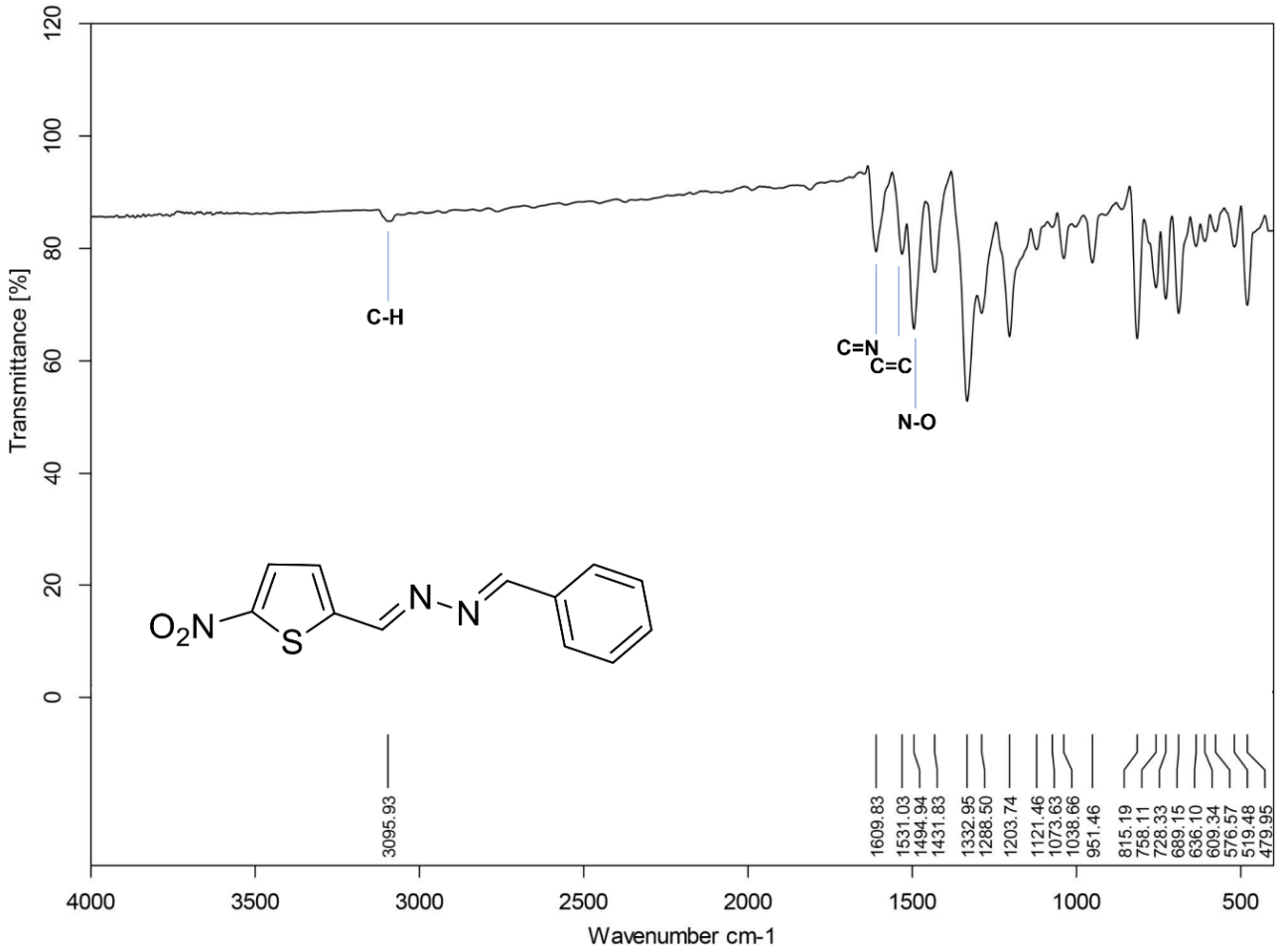
^{1}H NMR in DMSO



^{13}C NMR in DMSO



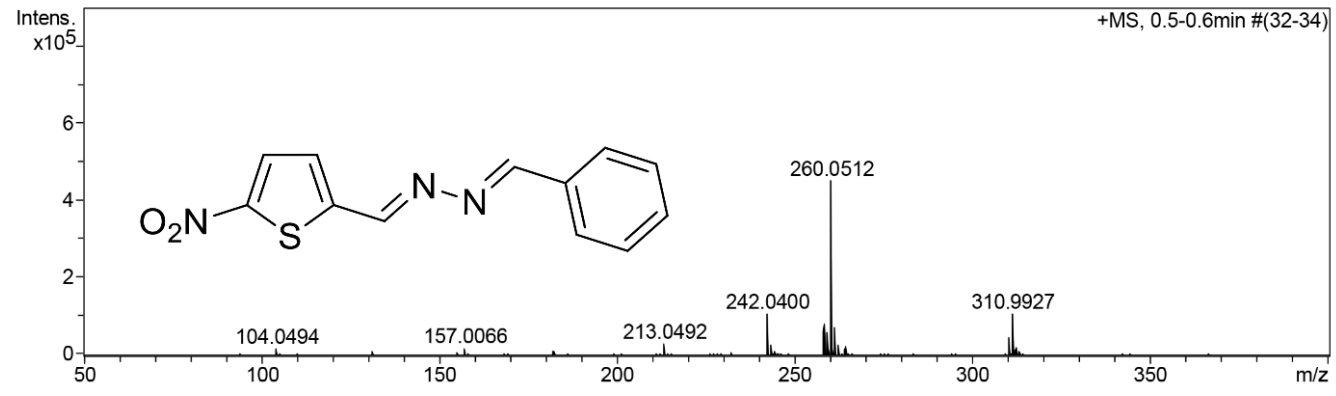
IR Spectrum



HRMS

Acquisition Parameter

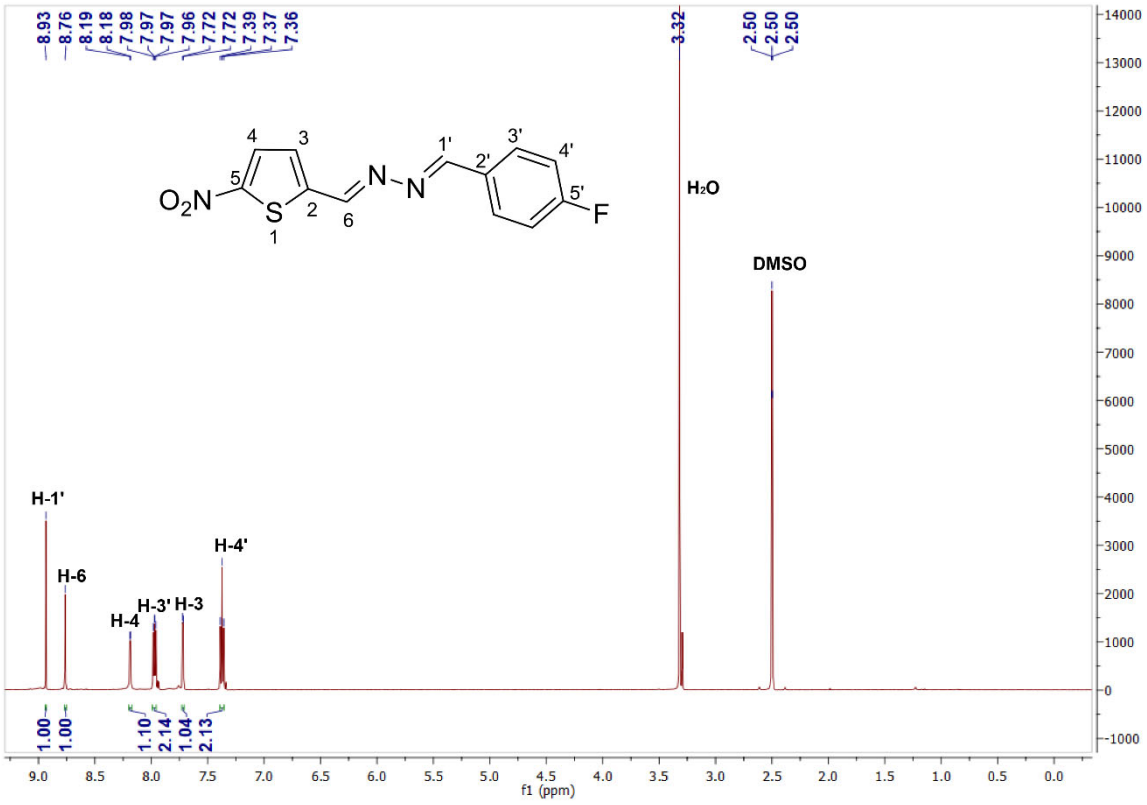
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



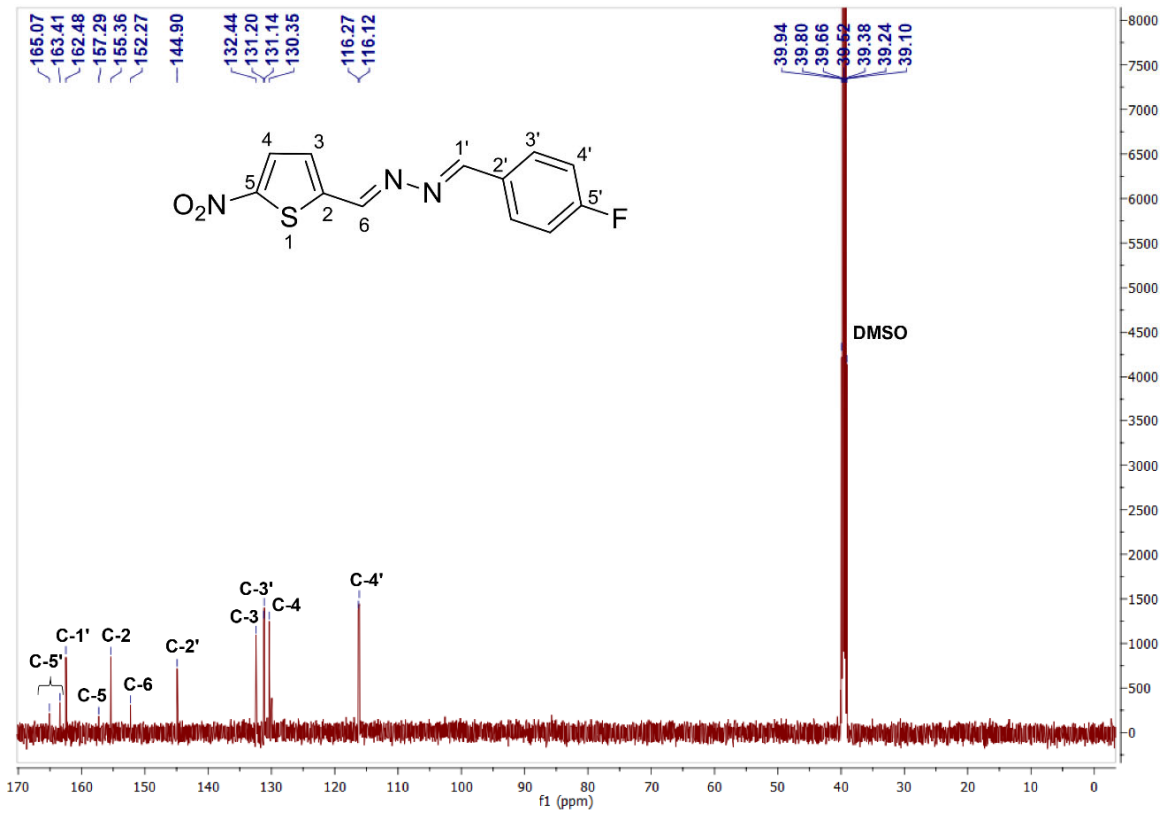
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
260.0512	1	C 12 H 10 N 3 O 2 S	100.00	260.0488	-2.4	-9.3	3.1	9.5	even	ok

(*1E,2E*)-1-(4-Fluorobenzylidene)-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (2b)

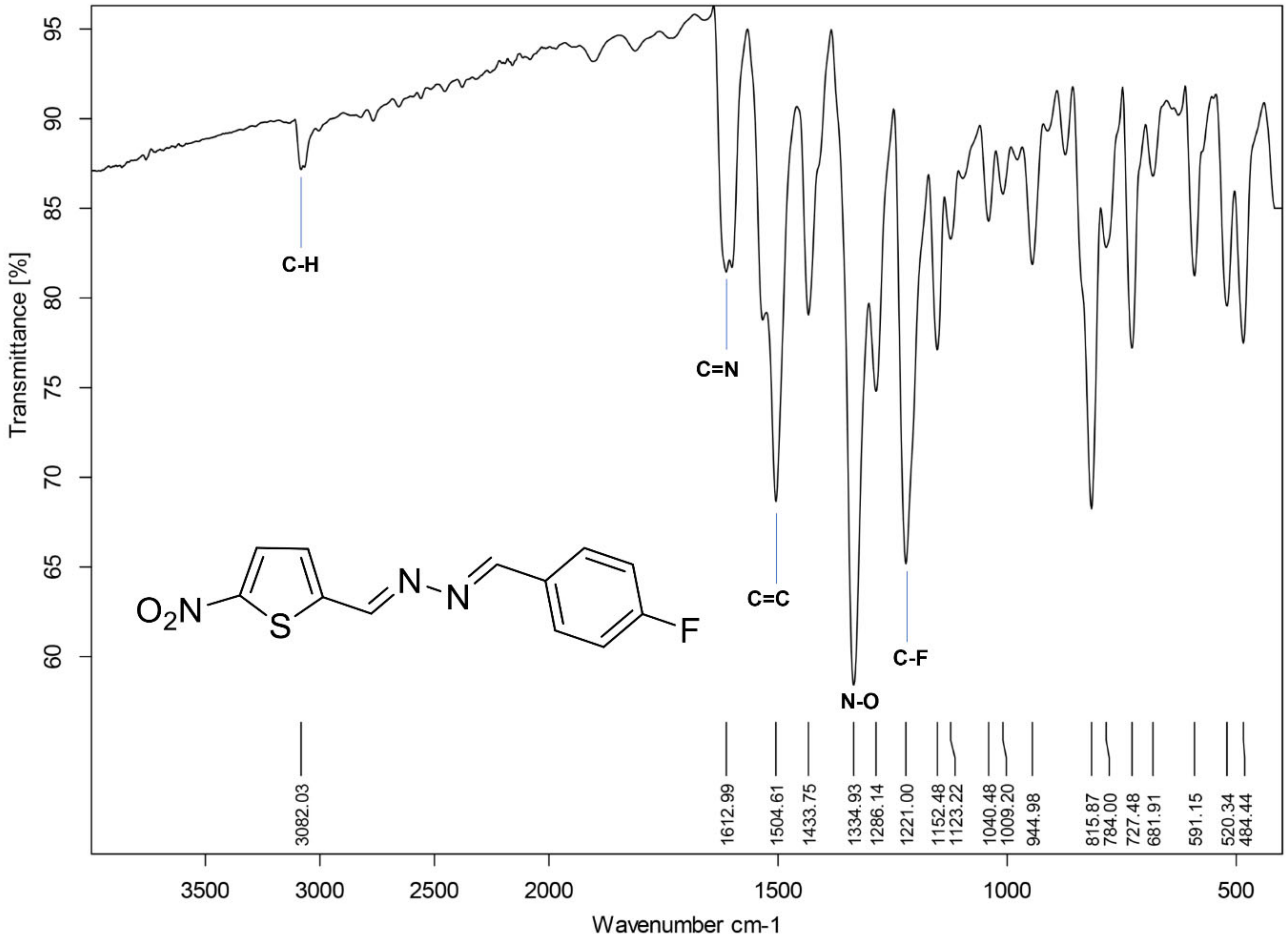
^{1}H NMR in DMSO



^{13}C NMR in DMSO



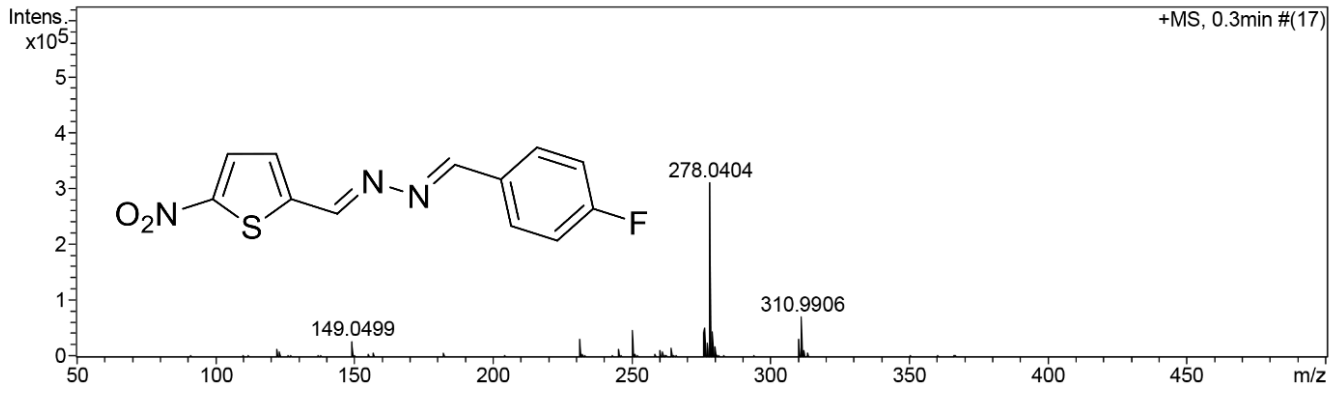
IR Spectrum



HRMS

Acquisition Parameter

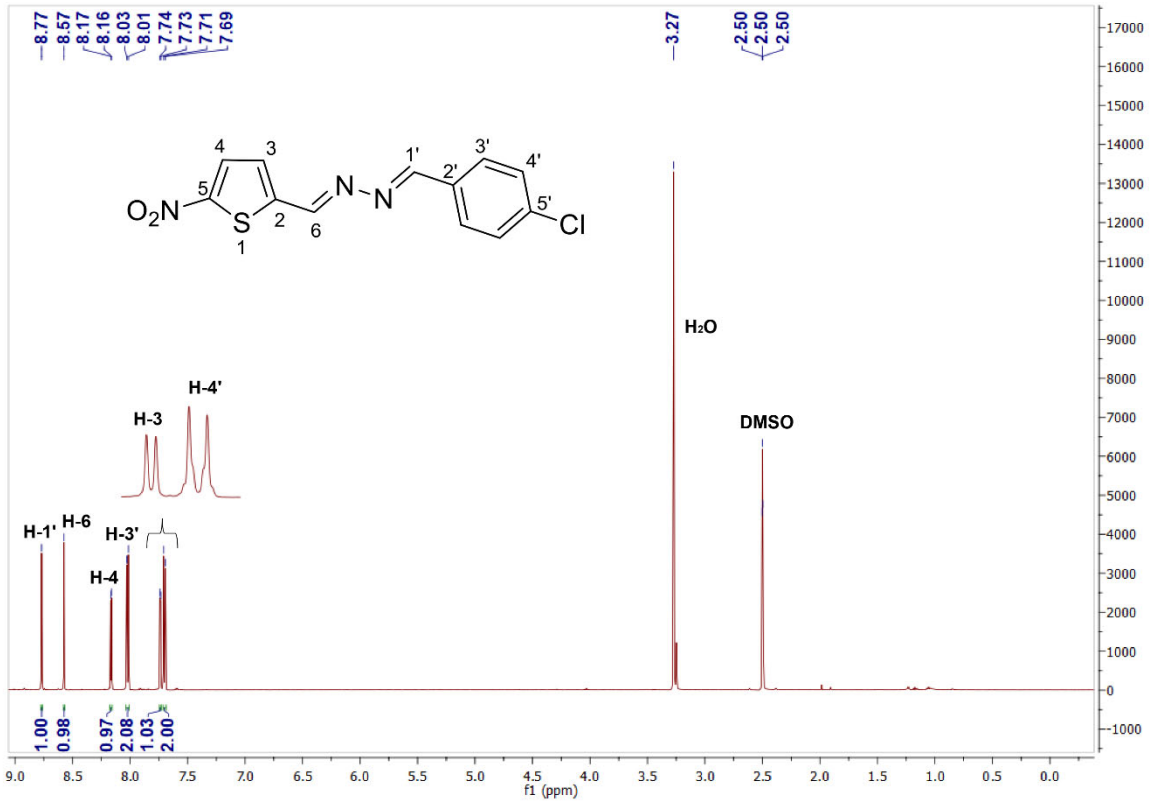
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



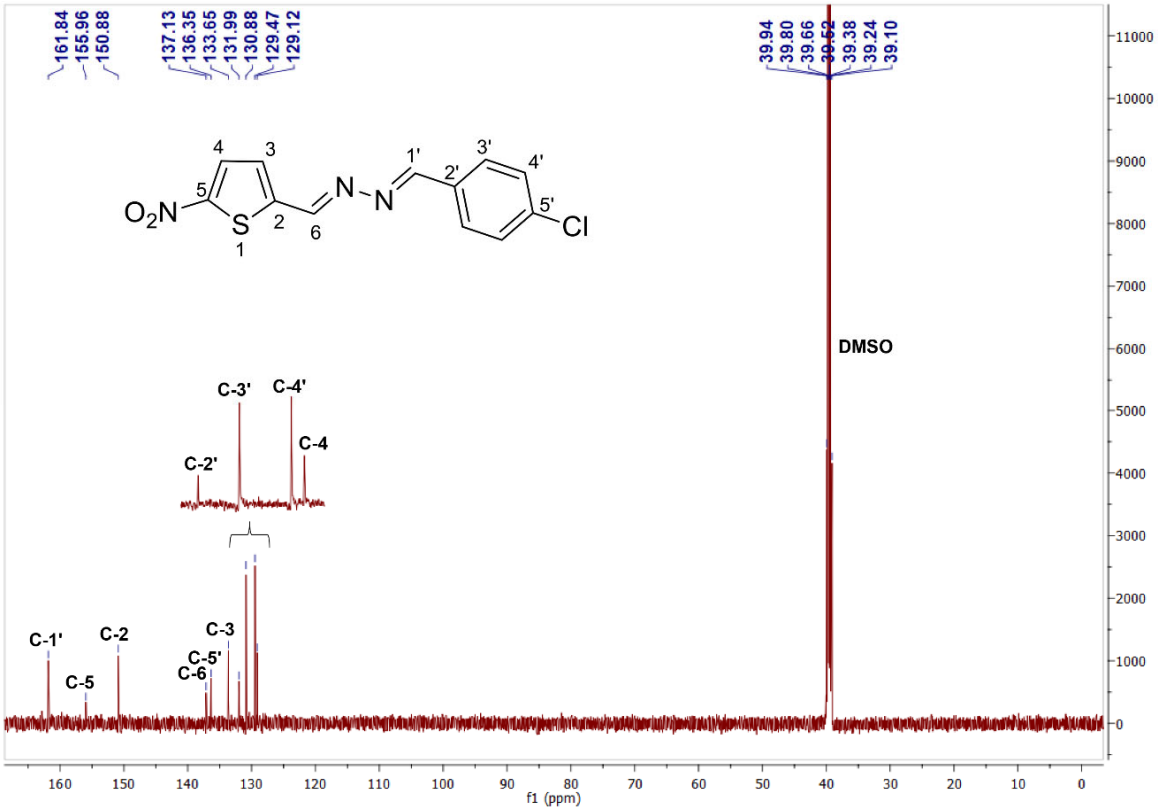
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
278.0404	1	C 12 H 9 F N 3 O 2 S	100.00	278.0394	-1.0	-3.5	0.9	9.5	even	ok

(*1E,2E*)-1-(4-Chlorobenzylidene)-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (3b)

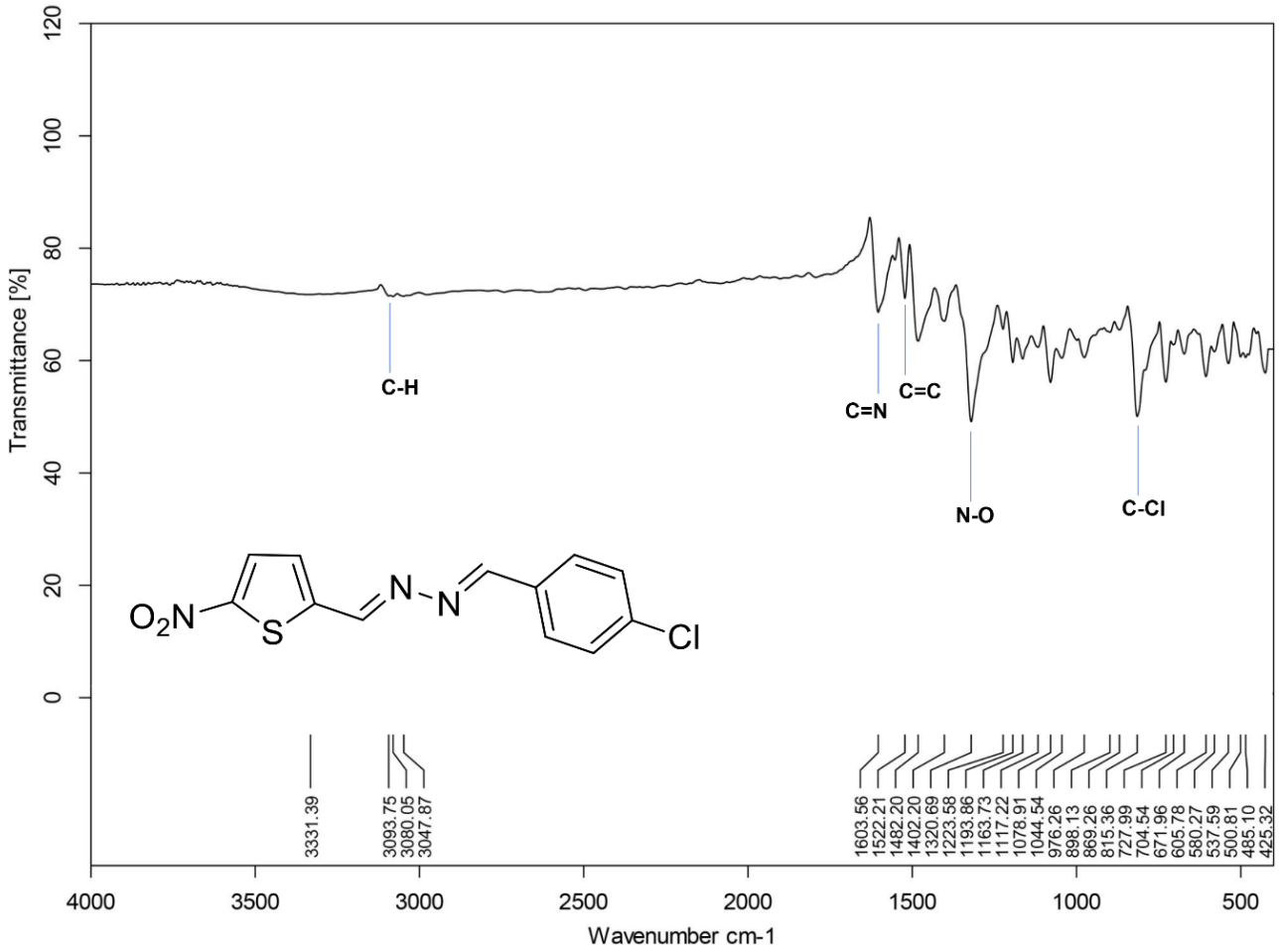
^{1}H NMR in DMSO



^{13}C NMR in DMSO



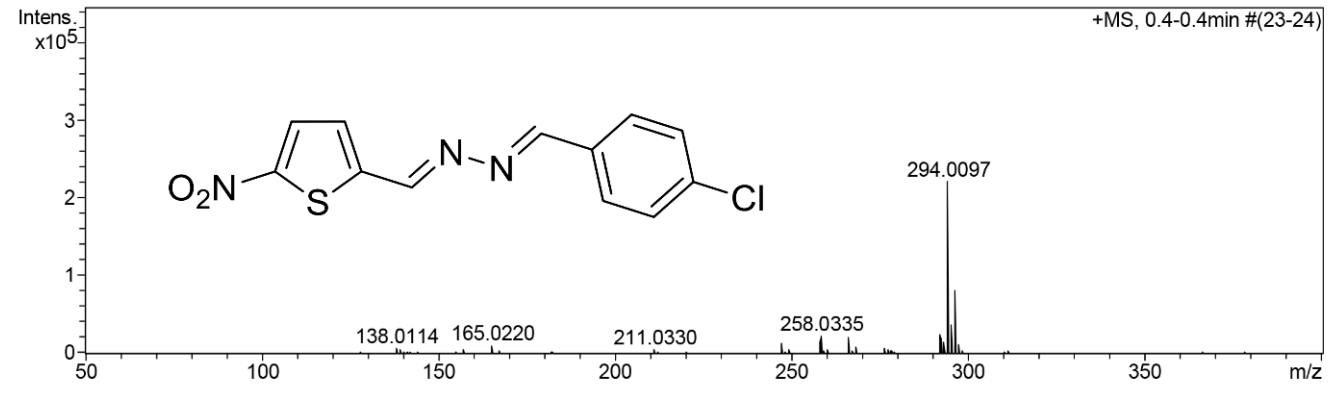
IR Spectrum



HRMS

Acquisition Parameter

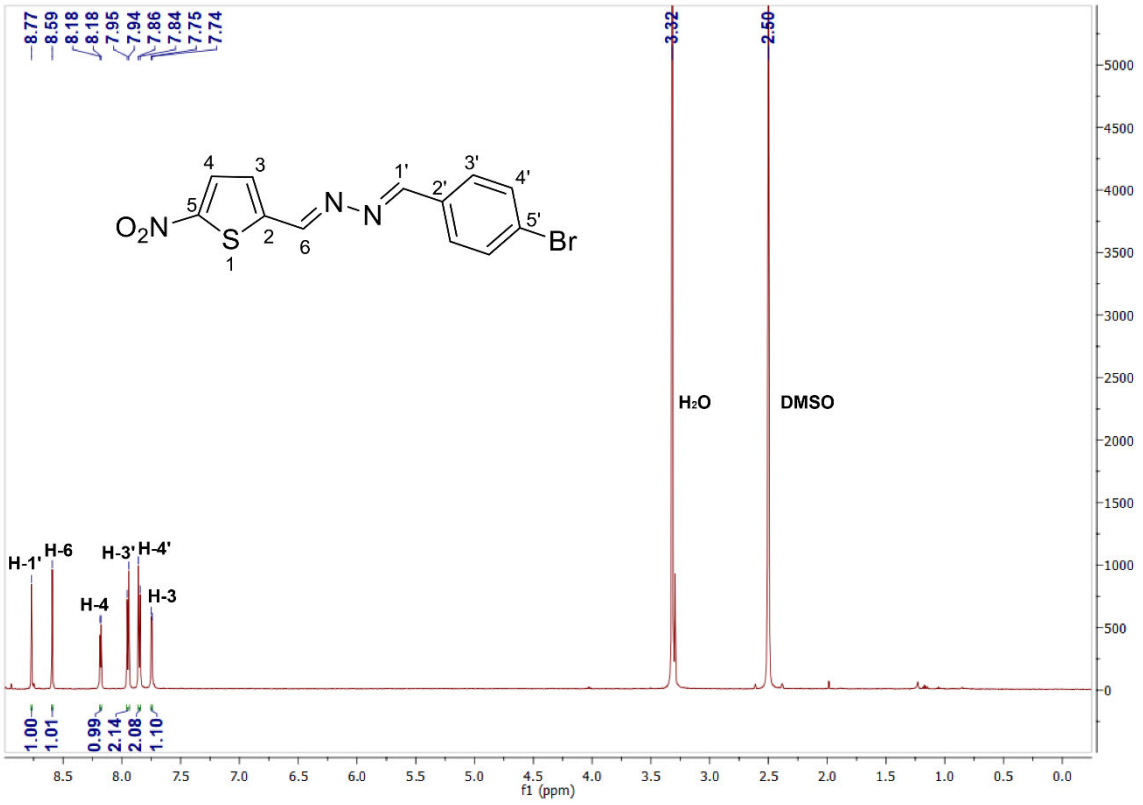
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



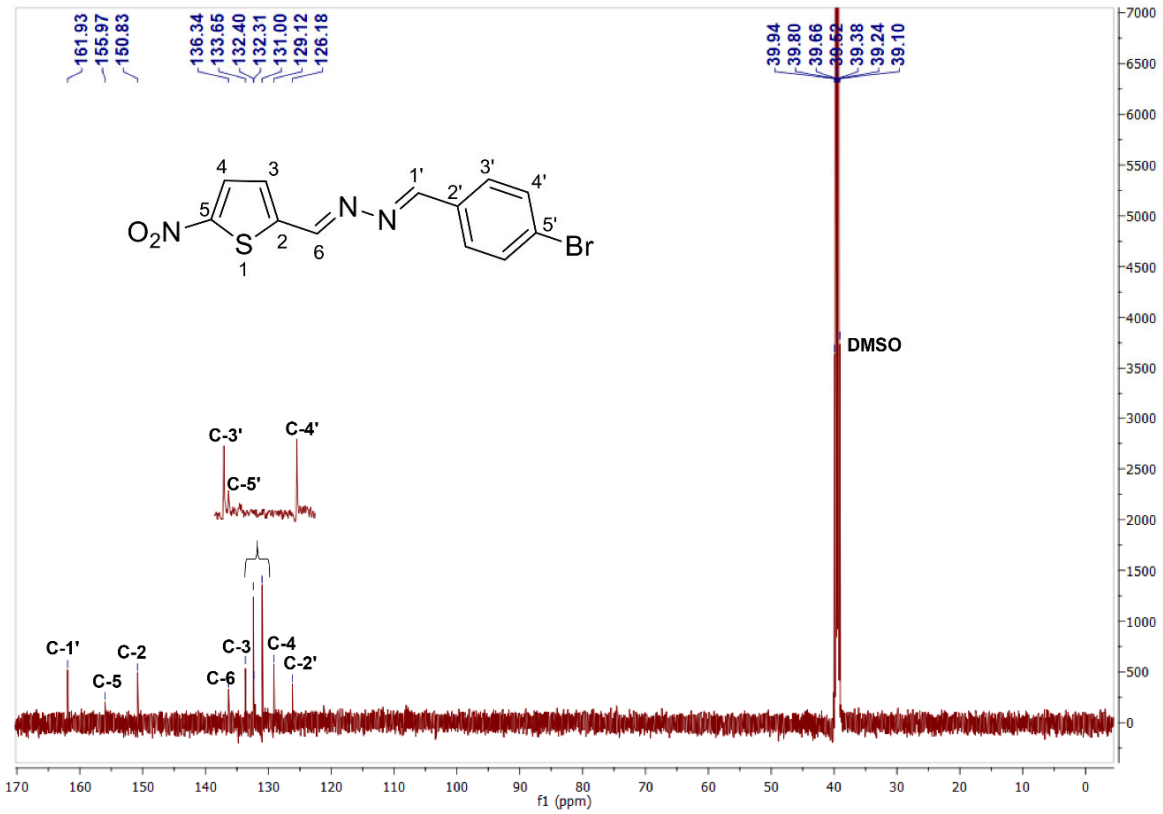
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
294.0097	1	C 12 H 9 Cl N 3 O 2 S	100.00	294.0099	0.2	0.6	8.0	9.5	even	ok

(*1E,2E*)-1-(4-Bromobenzylidene)-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (4b)

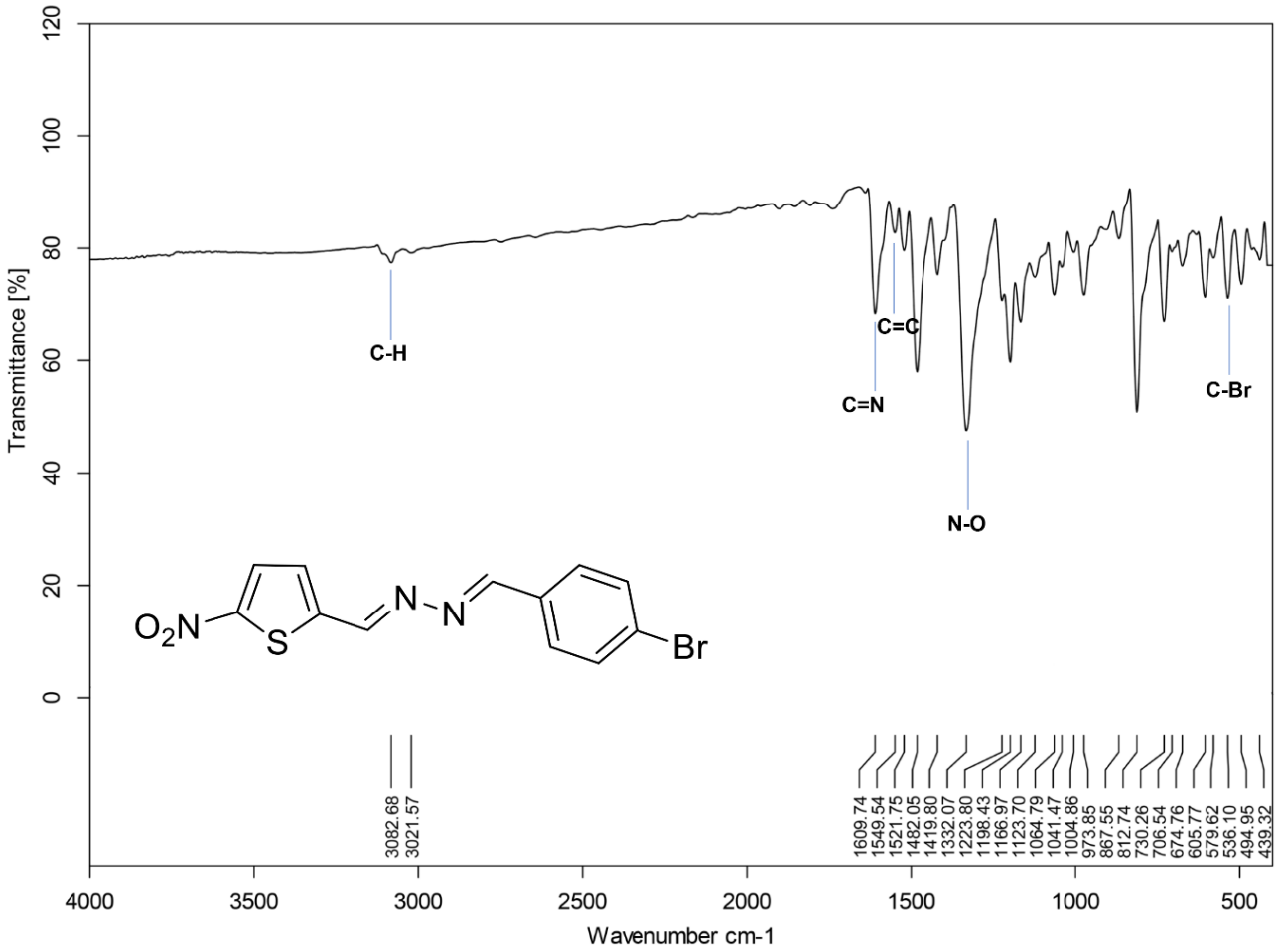
^{1}H NMR in DMSO



^{13}C NMR in DMSO



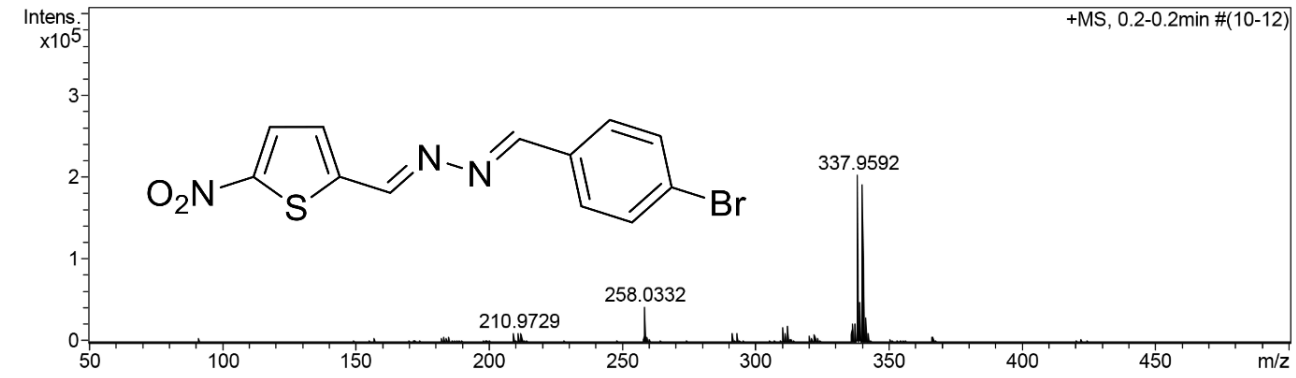
IR Spectrum



HRMS

Acquisition Parameter

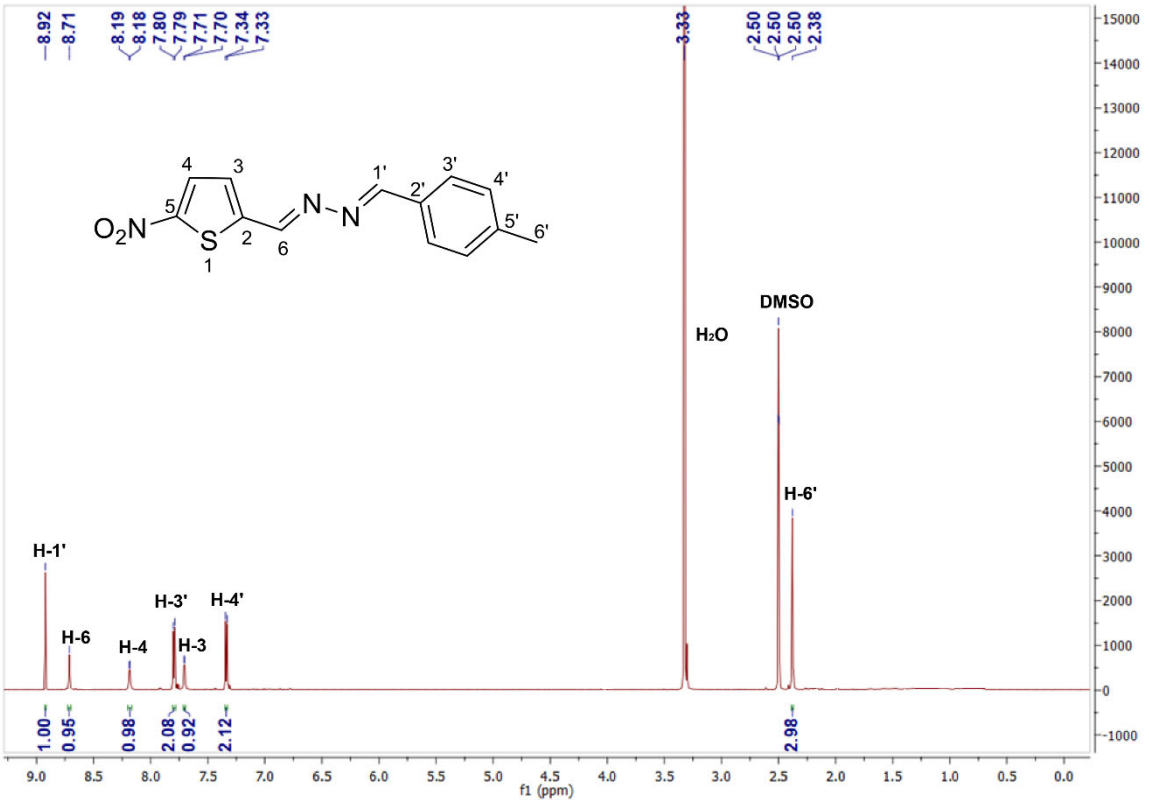
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



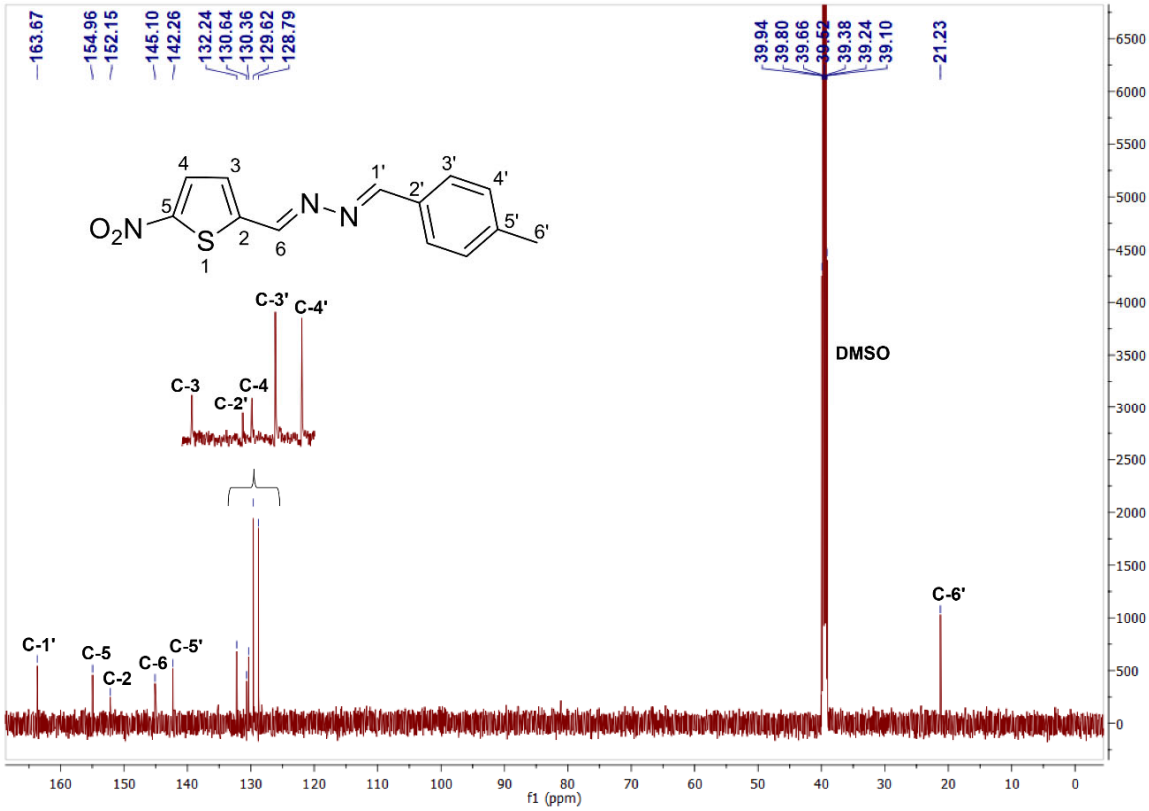
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
337.9592	1	C 14 H 11 Br O 3 S	51.79	337.9607	1.5	4.3	44.4	9.0	odd	ok
	2	C 12 H 9 Br N 3 O 2 S	100.00	337.9593	0.1	0.4	46.5	9.5	even	ok

(*1E,2E*)-1-(4-Methylbenzylidene)-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (5b)

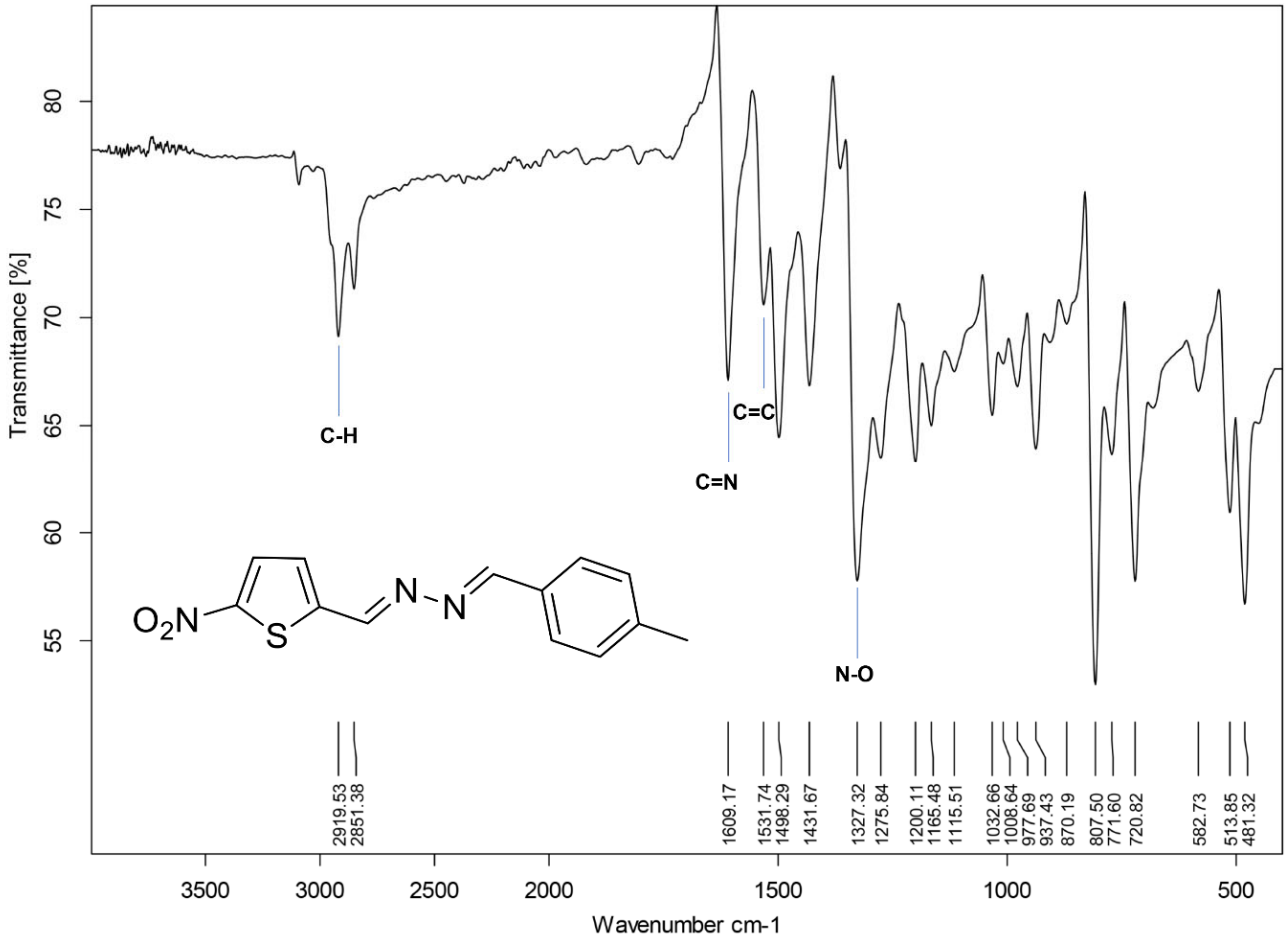
^{1}H NMR in DMSO



^{13}C NMR in DMSO



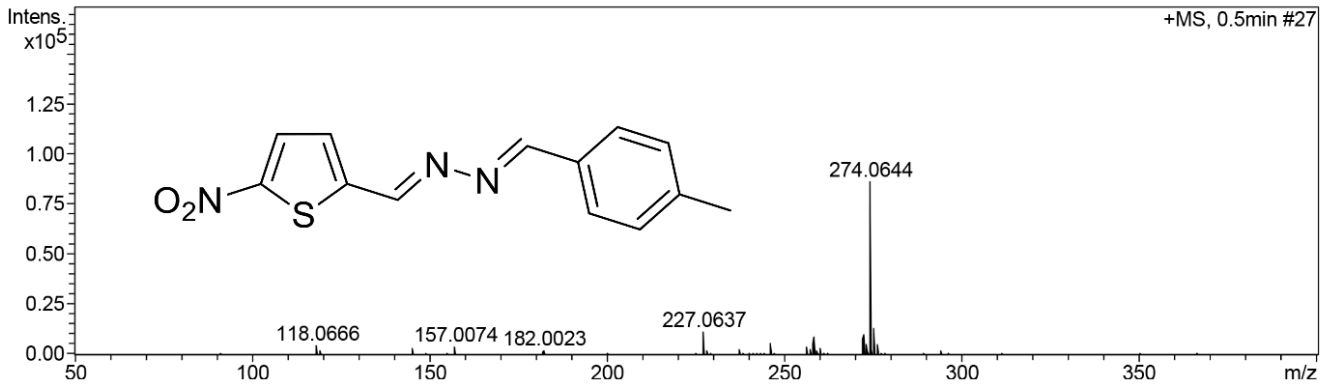
IR Spectrum



HRMS

Acquisition Parameter

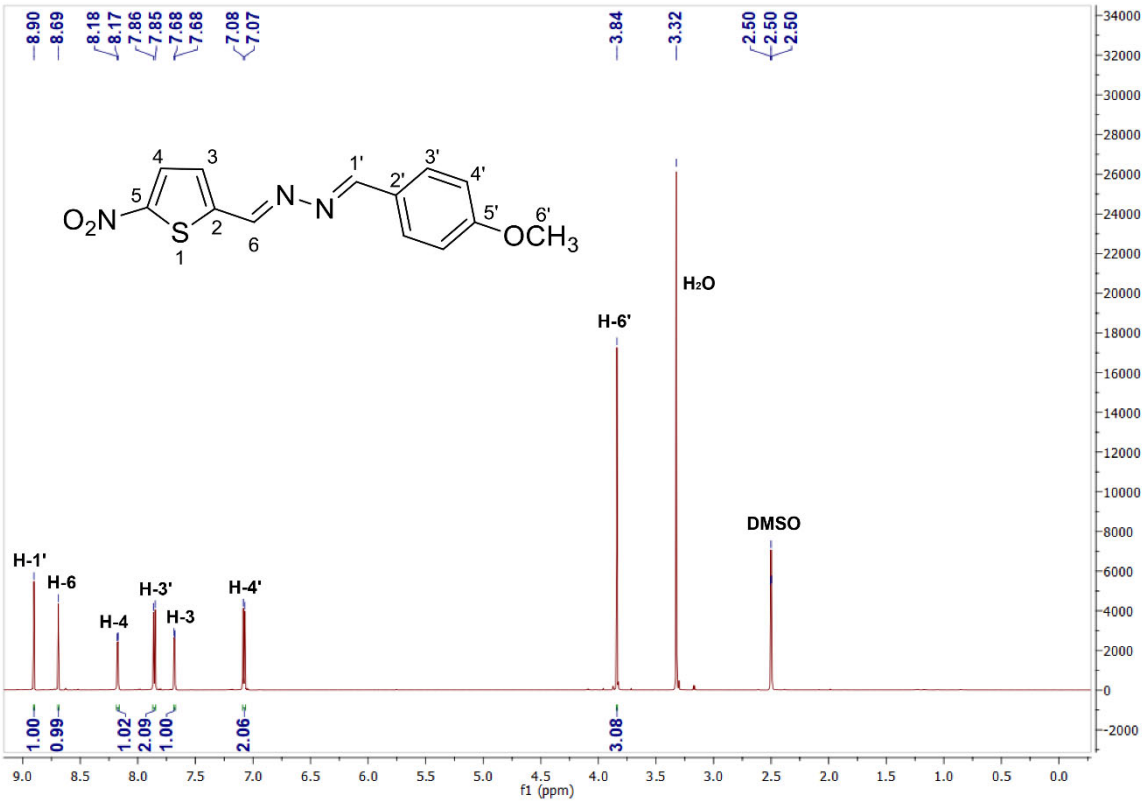
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



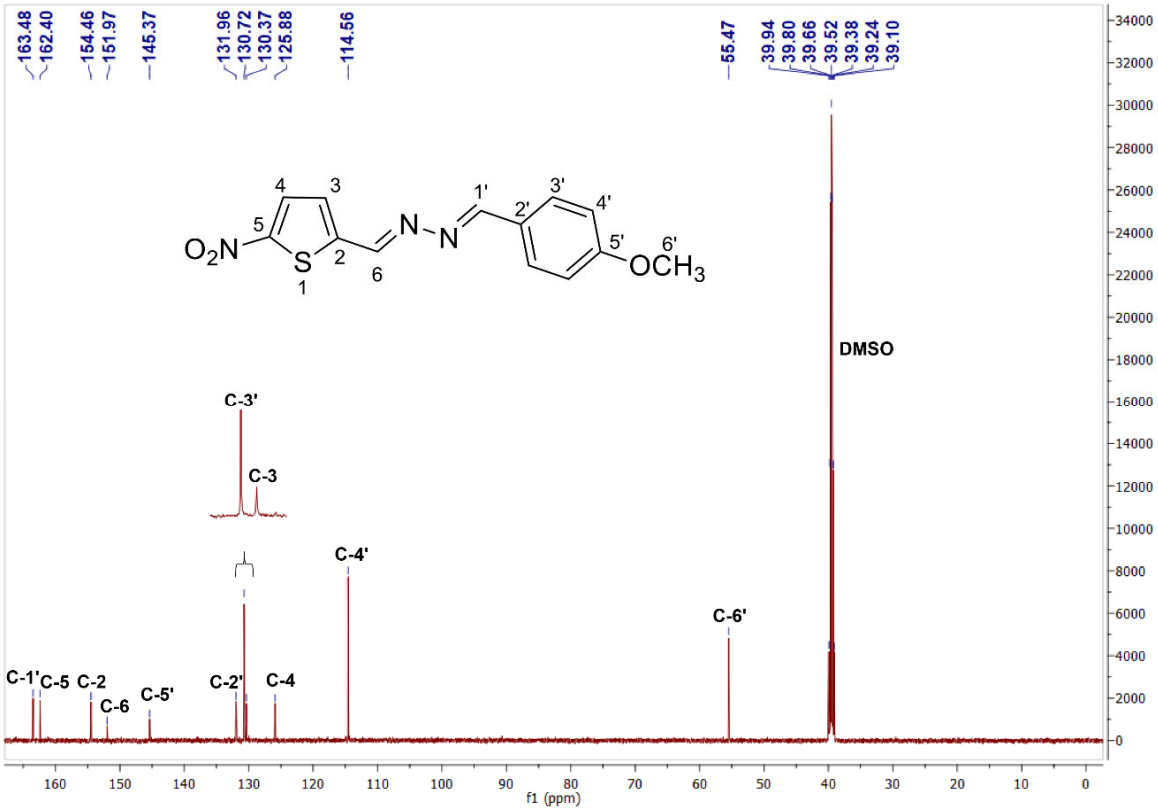
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
274.0644	1	C 13 H 12 N 3 O 2 S	100.00	274.0645	0.1	0.3	2.0	9.5	even	ok

(*1E,2E*)-1-(4-Methoxybenzylidene)-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (6b)

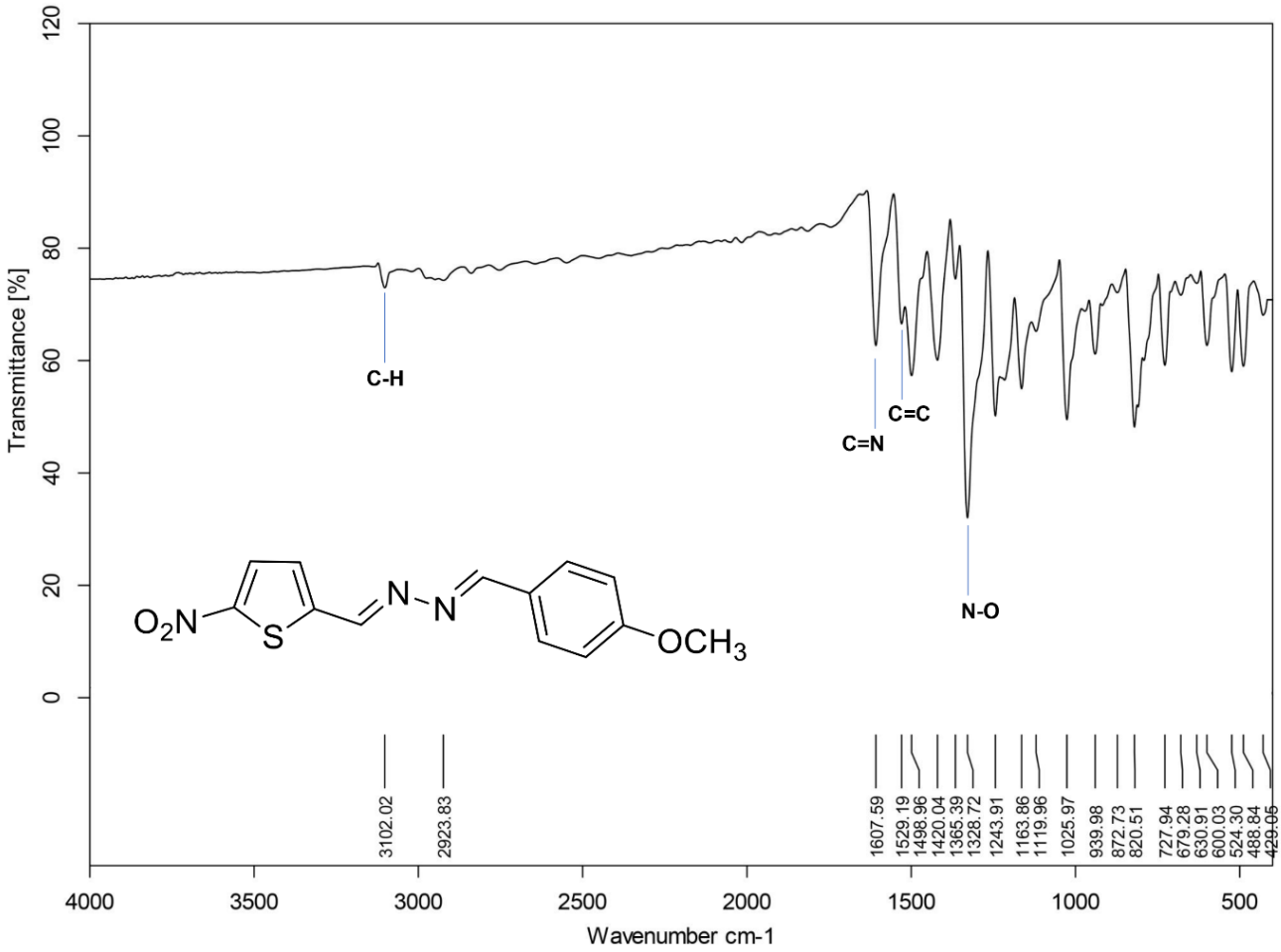
^{1}H NMR in DMSO



^{13}C NMR in DMSO



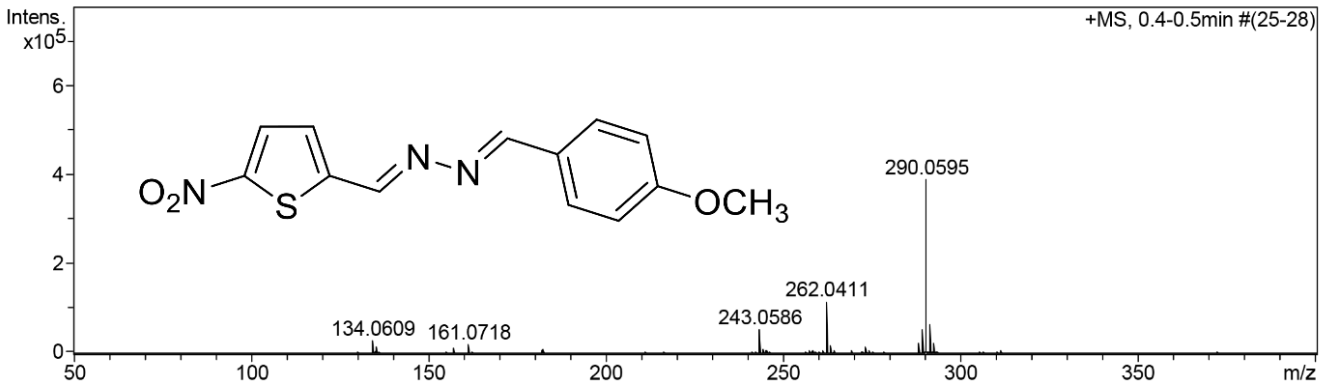
IR Spectrum



HRMS

Acquisition Parameter

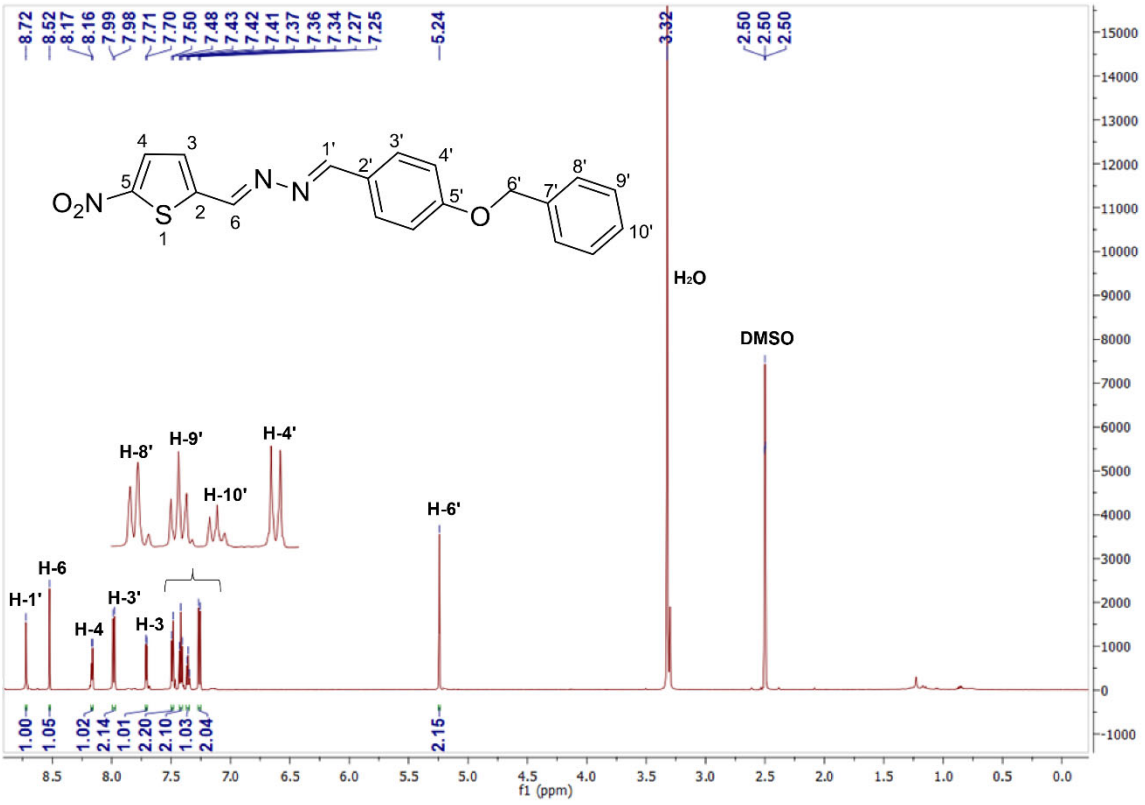
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



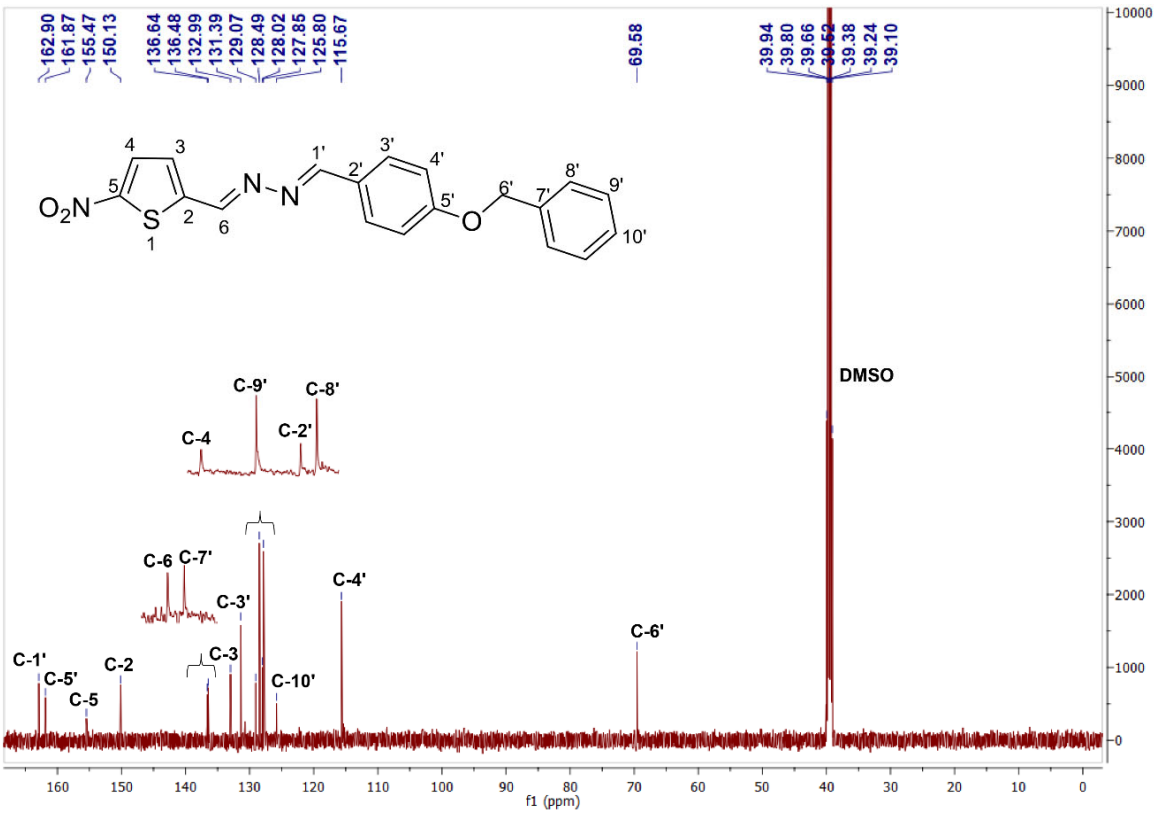
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
290.0595	1	C 13 H 12 N 3 O 3 S	100.00	290.0594	-0.1	-0.3	2.6	9.5	even	ok

(*1E,2E*)-1-[4-(Benzyloxy)benzylidene]-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (7b)

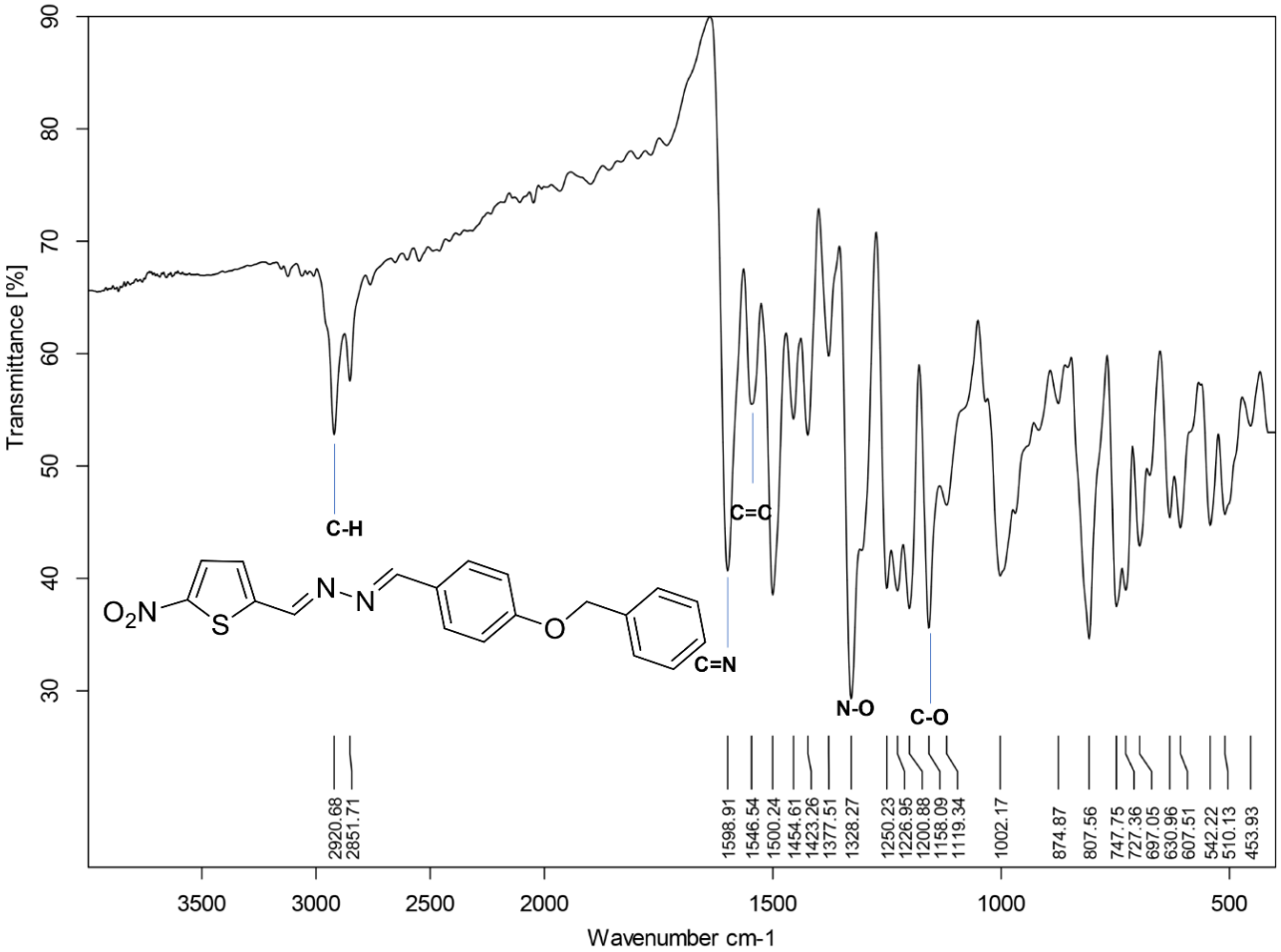
^{1}H NMR in DMSO



^{13}C NMR in DMSO



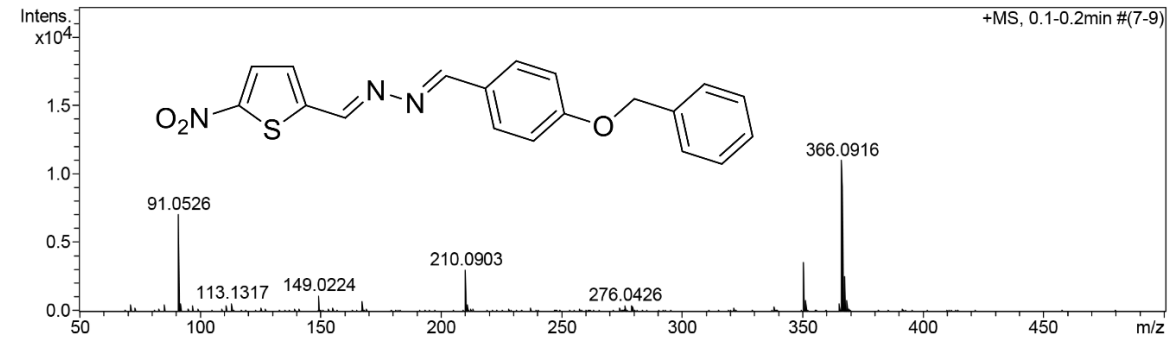
IR Spectrum



HRMS

Acquisition Parameter

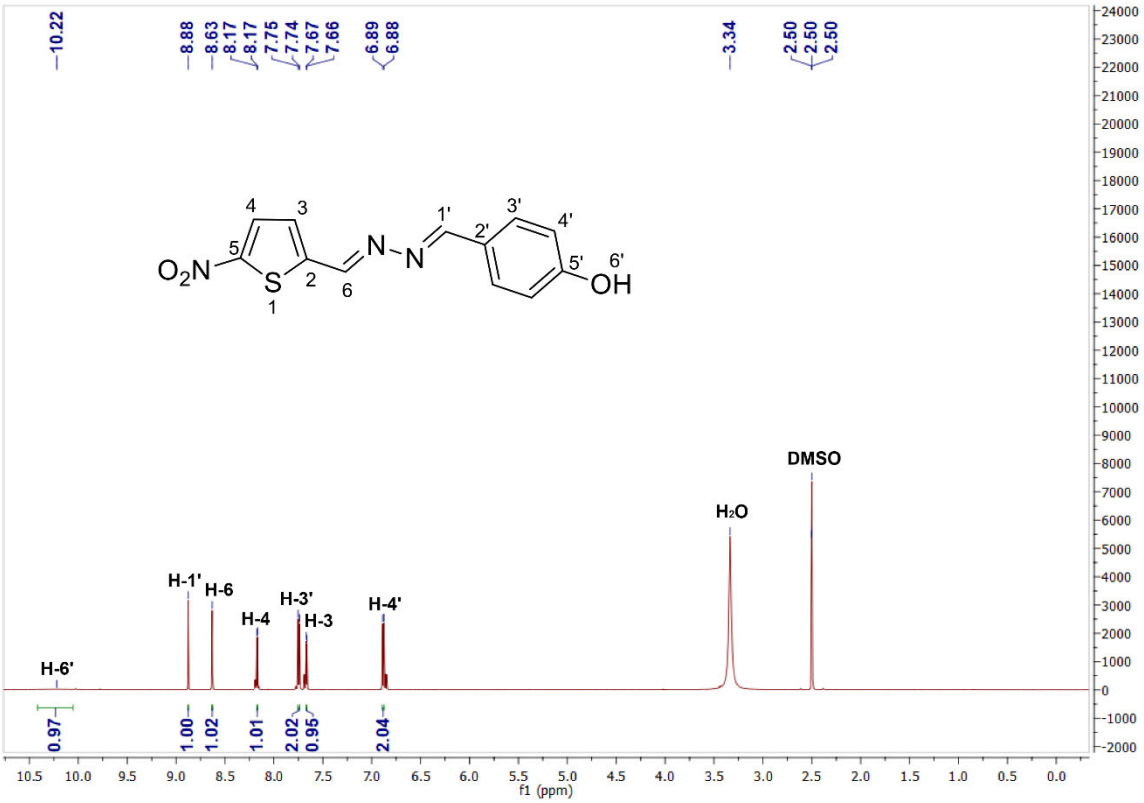
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



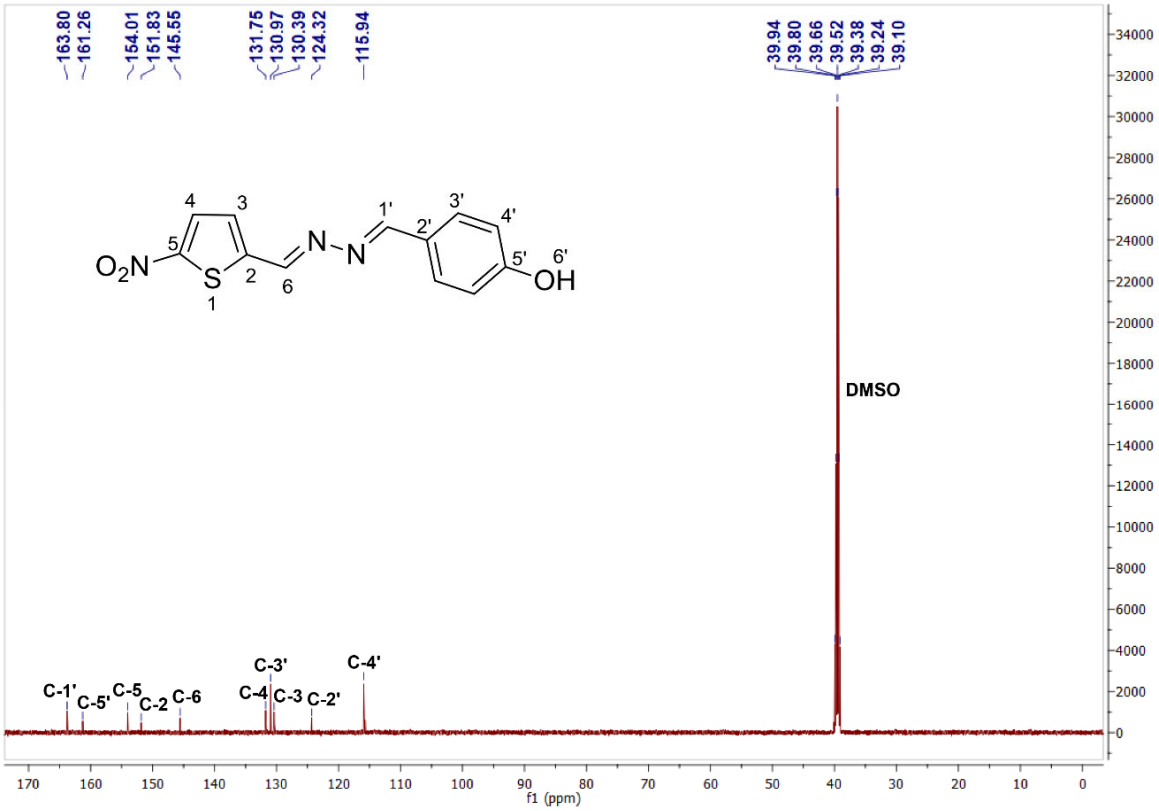
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
91.0526	1	C 7 H 7	100.00	91.0542	1.7	18.2	5.0	4.5	even	ok
210.0903	1	C 14 H 12 N O	100.00	210.0913	1.0	4.8	14.1	9.5	even	ok
	2	C 11 H 14 O 4	41.62	210.0887	-1.7	-7.9	34.4	5.0	odd	ok
350.1128	1	C 19 H 16 N 3 O 4	100.00	350.1135	0.7	2.1	17.7	13.5	even	ok
366.0916	1	C 19 H 16 N 3 O 3 S	100.00	366.0907	-0.9	-2.4	7.2	13.5	even	ok

4-(*E*)-(*E*)-[(5-Nitrothiophen-2-yl)methylene]hydrazonomethylphenol (8b)

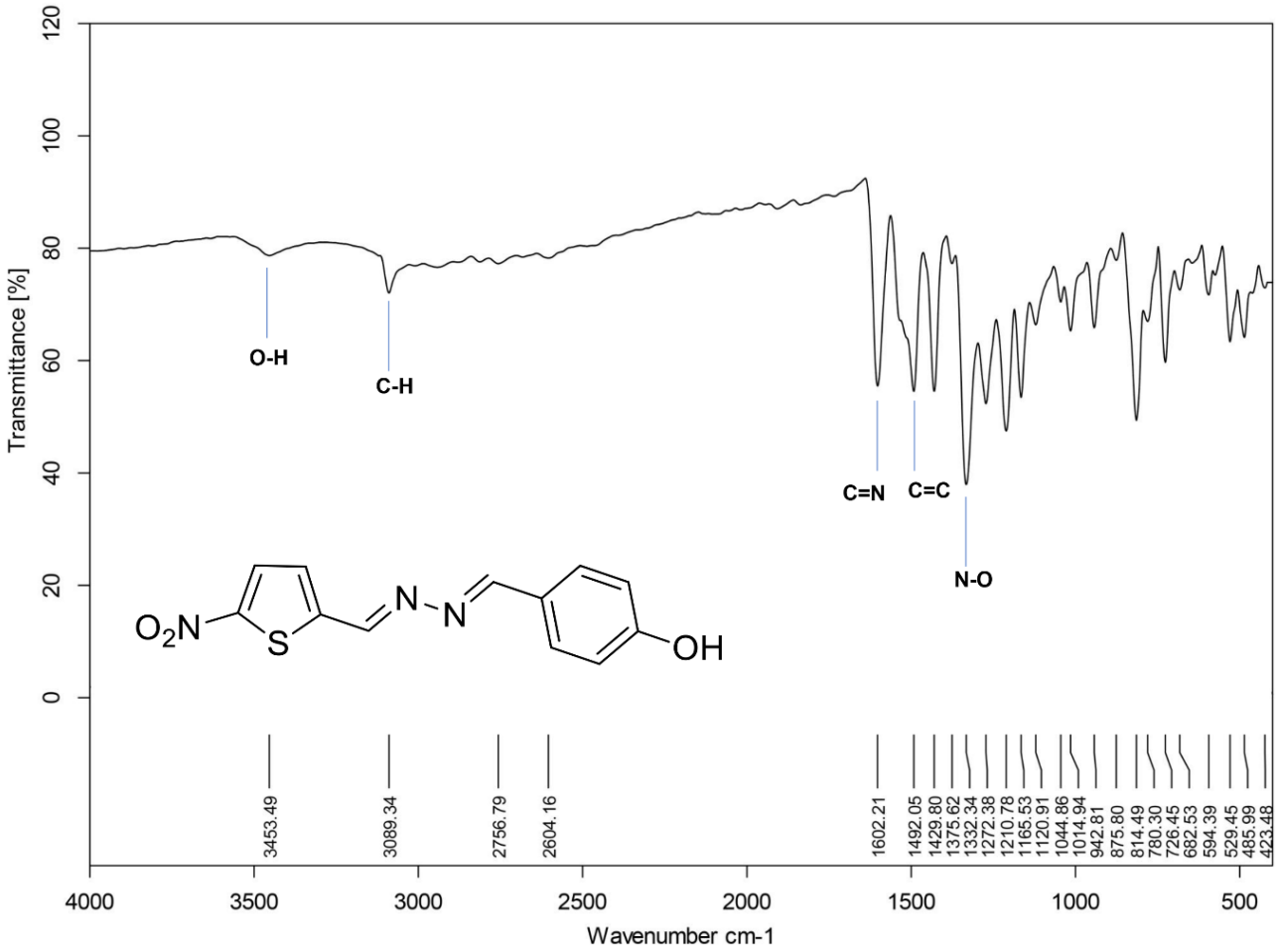
^{1}H NMR in DMSO



^{13}C NMR in DMSO



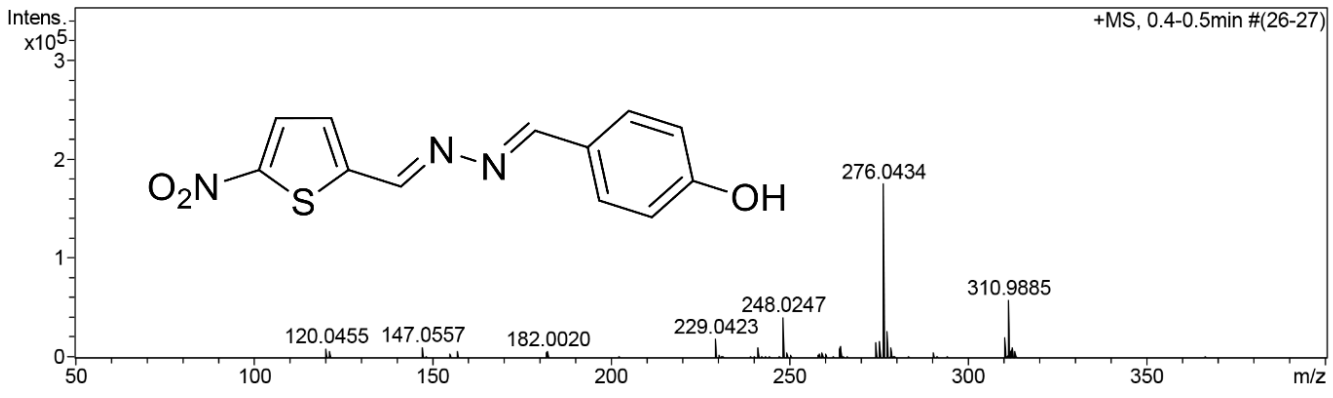
IR Spectrum



HRMS

Acquisition Parameter

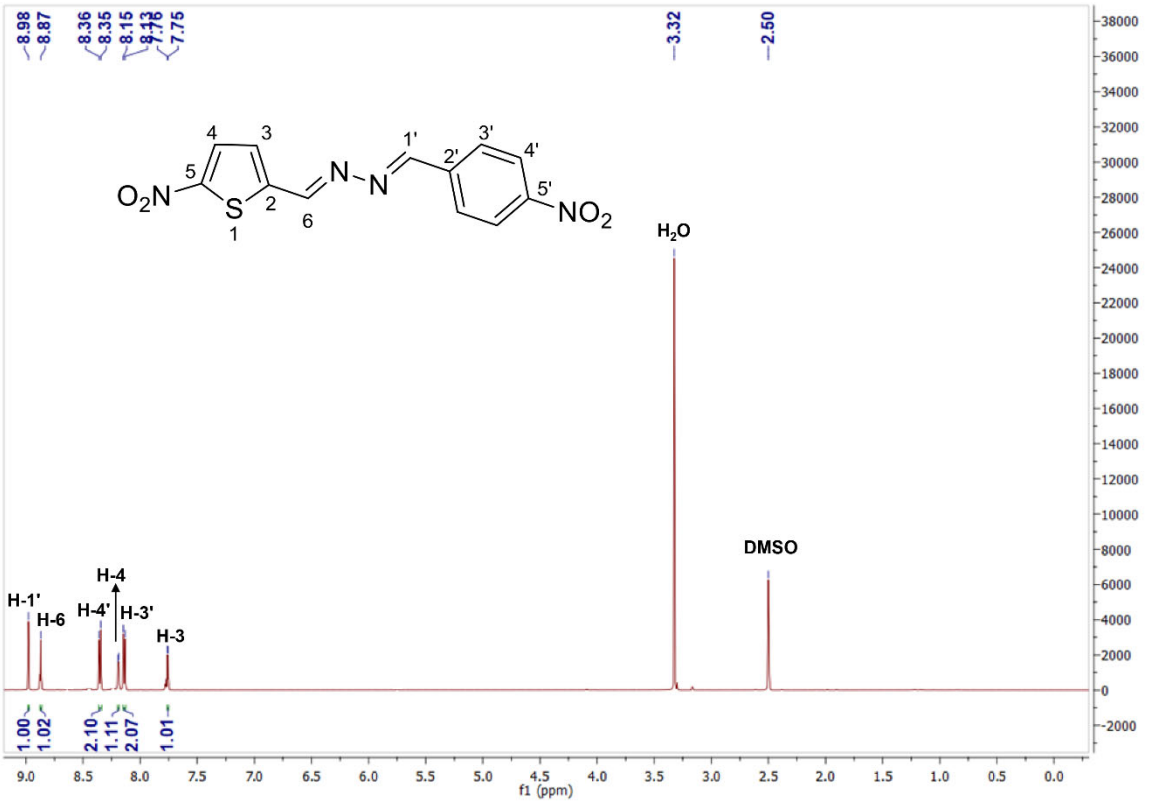
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



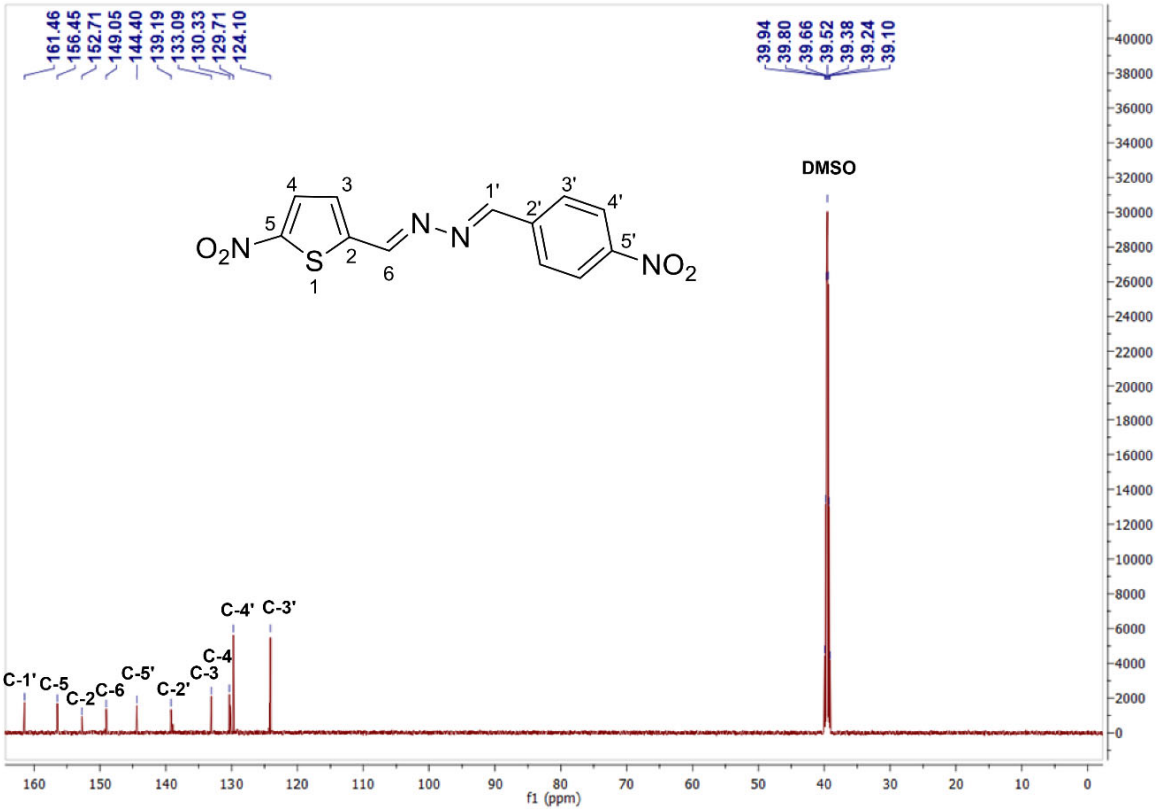
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
276.0434	1	C 12 H 10 N 3 O 3 S	100.00	276.0437	0.3	1.2	0.8	9.5	even	ok

(*1E,2E*)-1-(4-Nitrobenzylidene)-2-[(5-nitrothiophen-2-yl)methylene]hydrazine (9b)

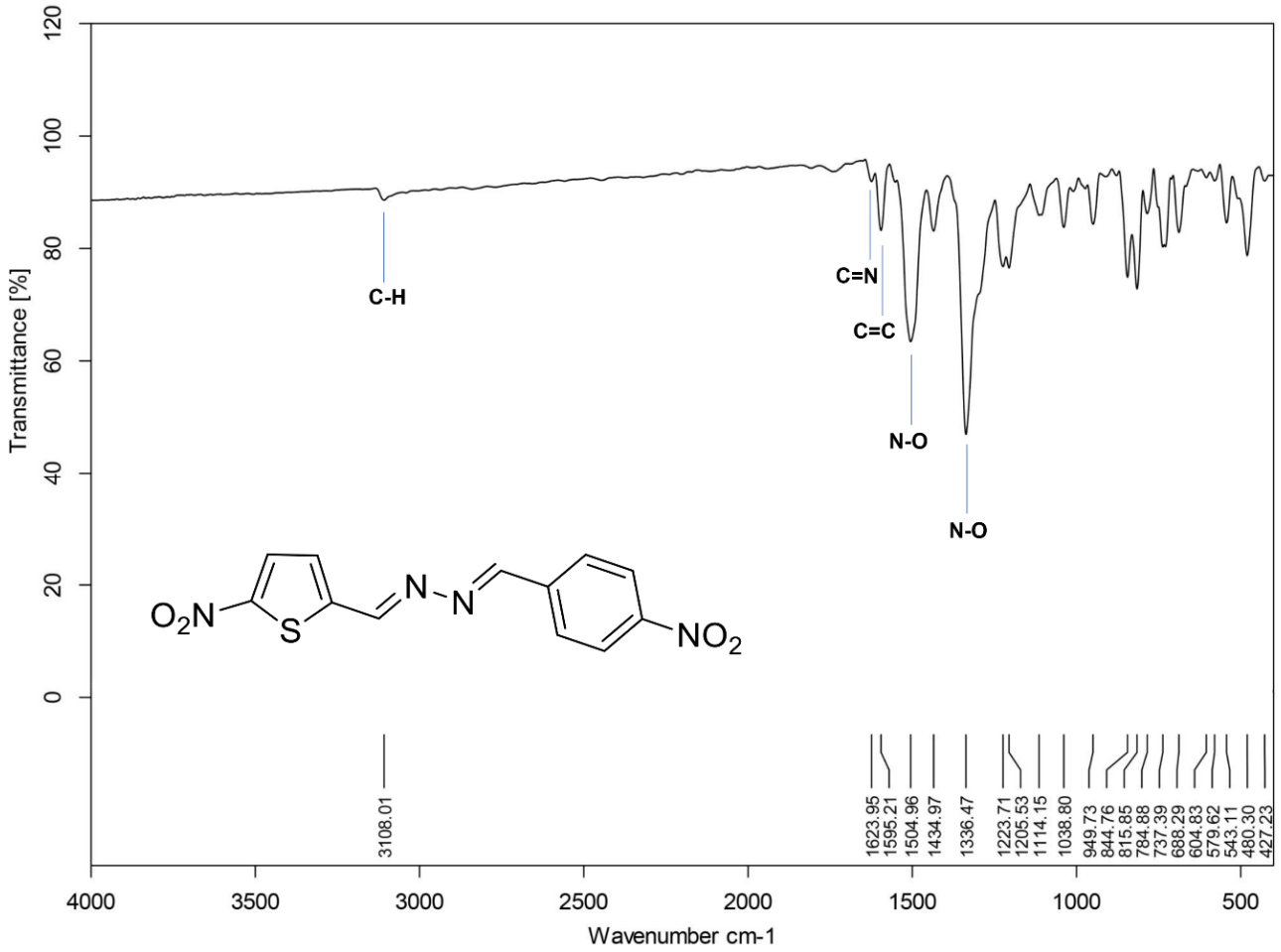
^{1}H NMR in DMSO



^{13}C NMR in DMSO



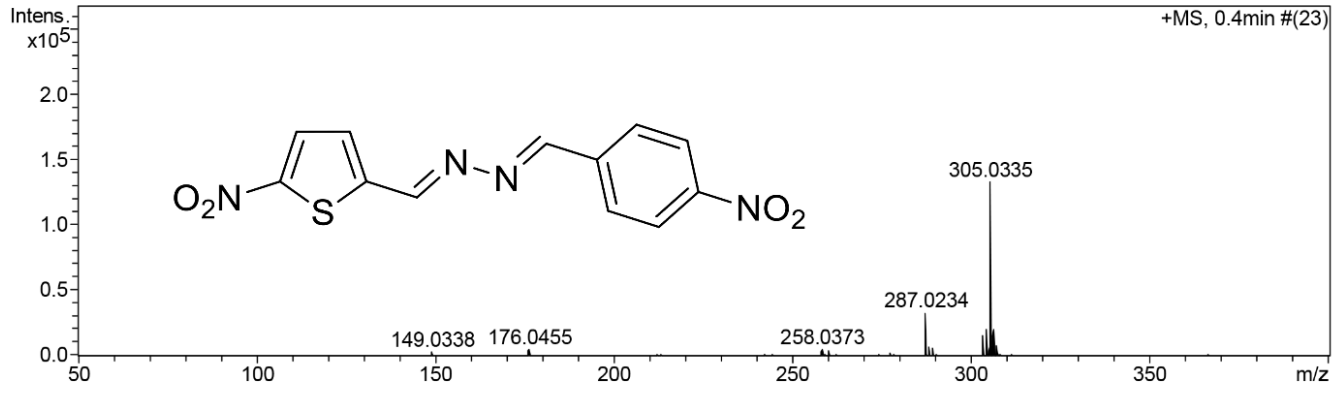
IR Spectrum



HRMS

Acquisition Parameter

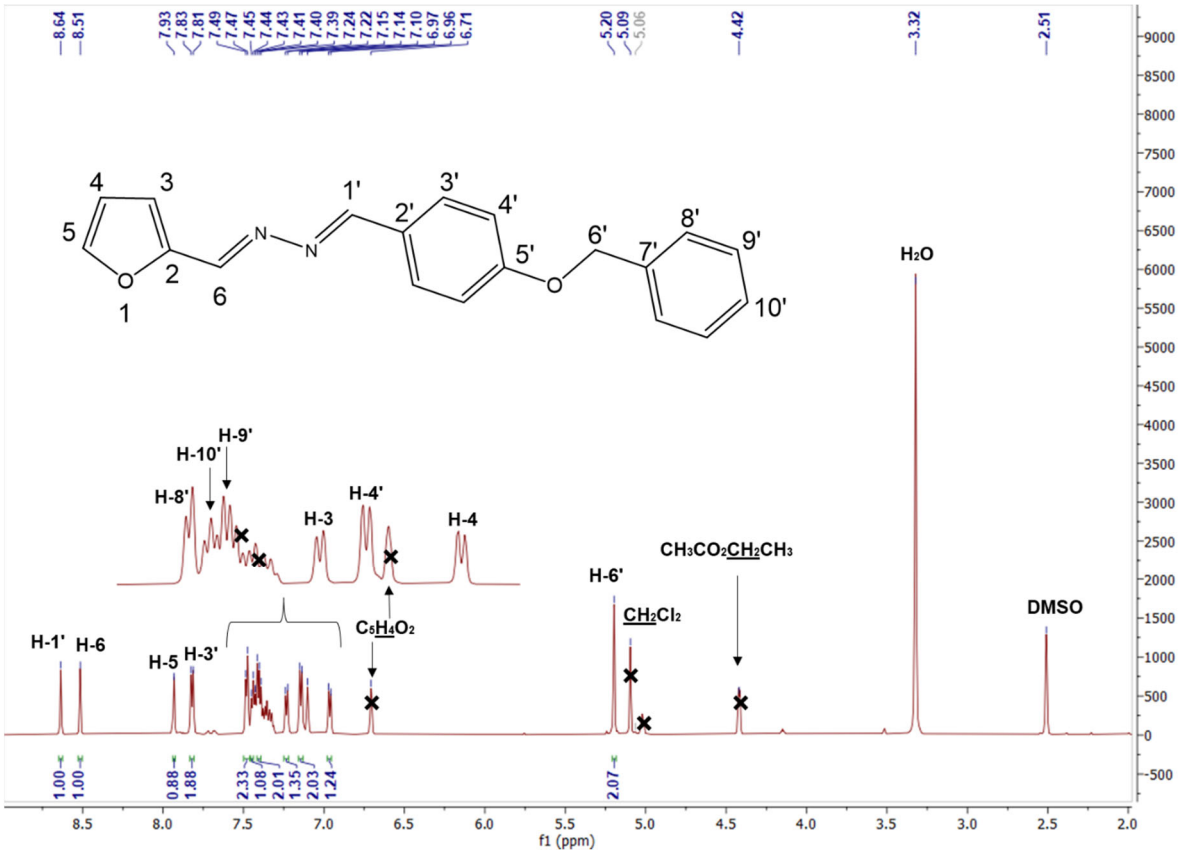
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1600 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



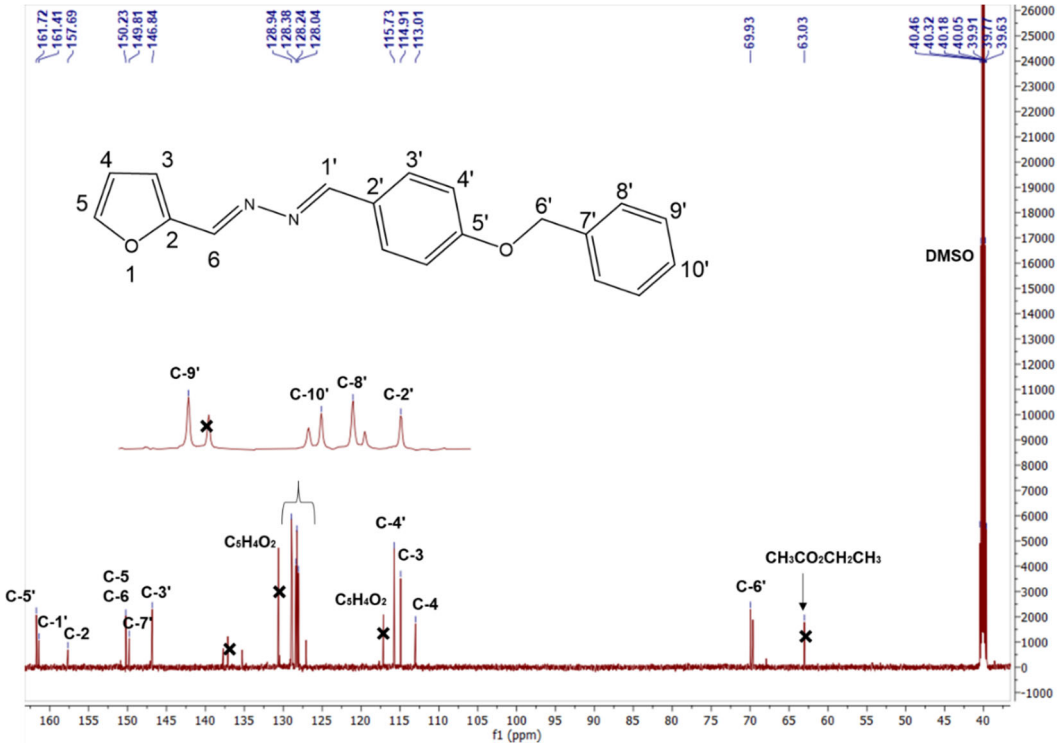
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
305.0335	1	C 12 H 9 N 4 O 4 S	100.00	305.0339	0.4	1.4	2.3	10.5	even	ok

(*E*)-1-[(*E*)-4-(Benzyloxy)benzylidene]-2-(furan-2-ylmethylene)hydrazine (11)

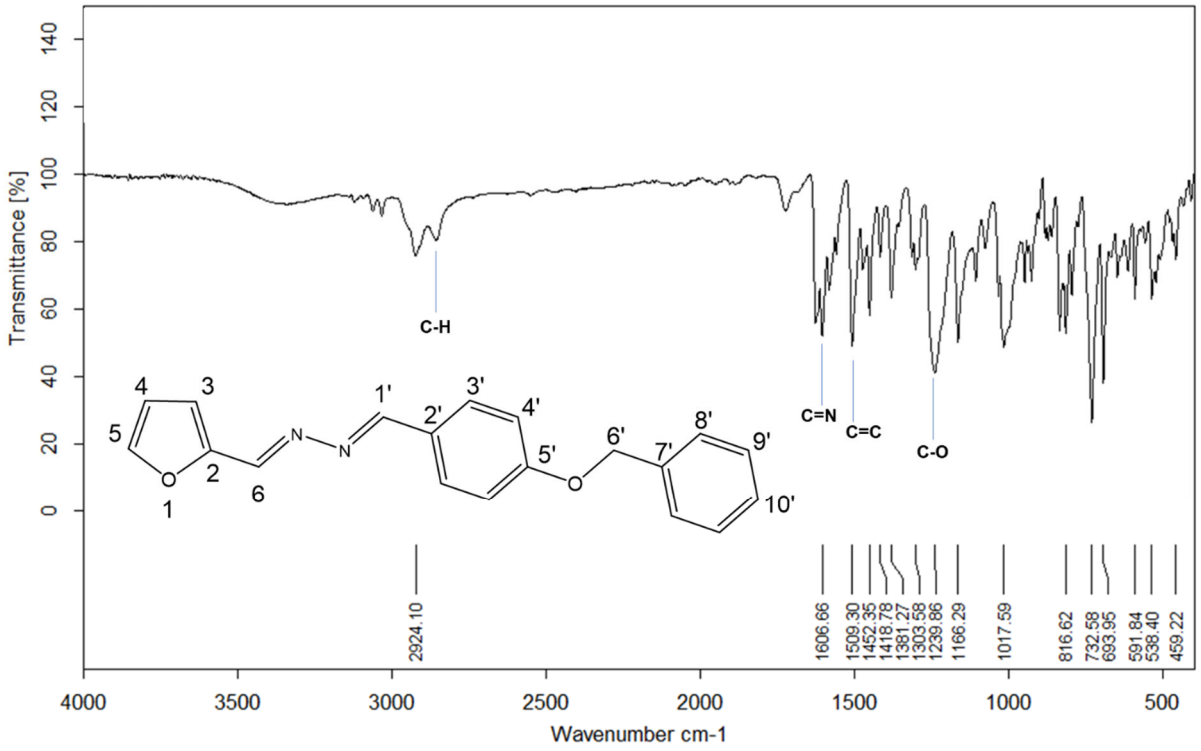
^{1}H NMR in DMSO



^{13}C NMR



IR


Transmittance [%]
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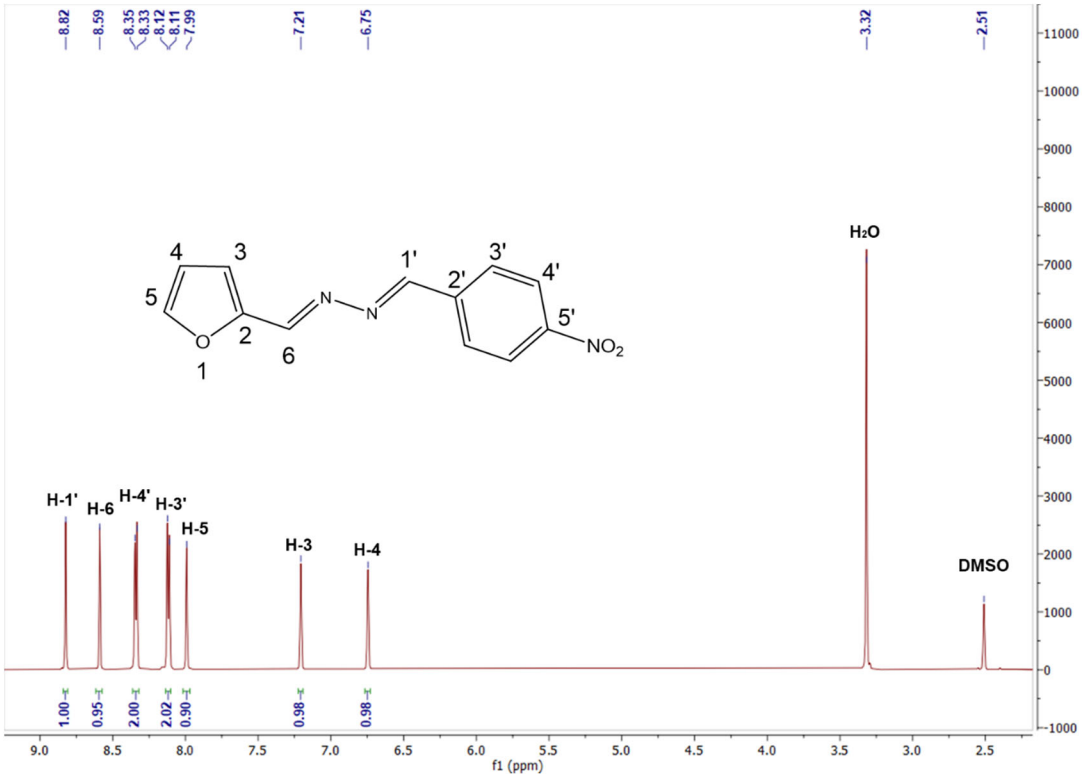
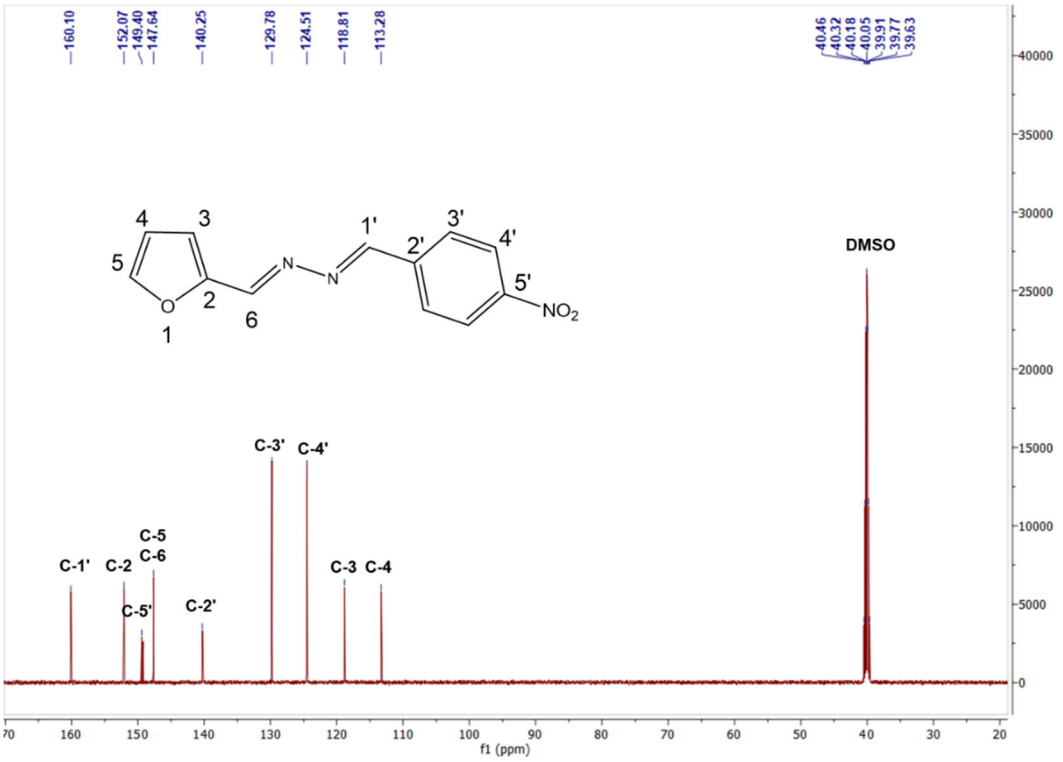
20
40
60
80
100
120
140
C-H
C=N
C=C
C-O
2924.10
1606.66
1509.30
1452.35
1418.78
1381.27
1303.58
1239.86
1166.29
1017.59
816.62
732.58
693.95
591.84
538.40
459.22
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

(*E*)-1-(Furan-2-ylmethylene)-2-[(*E*)-4-nitrobenzylidene]hydrazine (12)

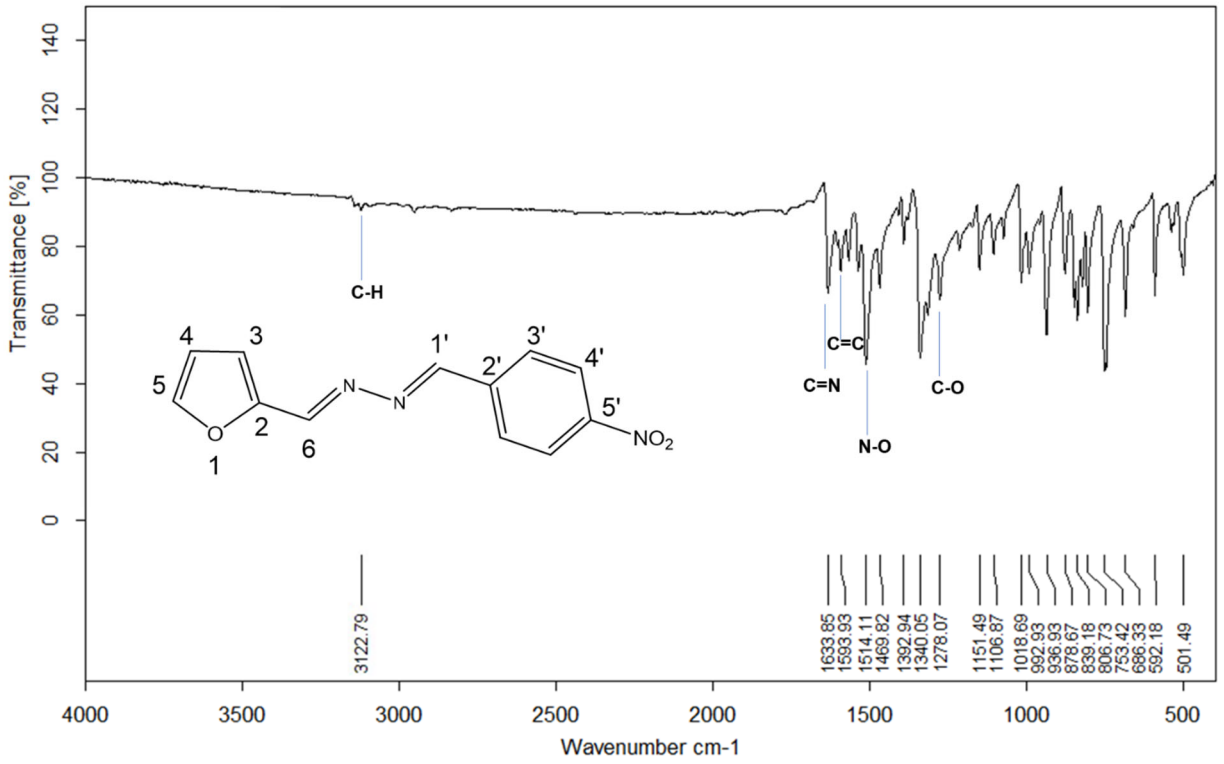
^{1}H NMR in DMSO



^{13}C NMR in DMSO



IR Spectrum


Transmittance [%]
0
20
40
60
80
100
120
140
C-H
C=C
C=N
C-O
N-O
4
3
5
2
1
6
N
N
1'
2'
3'
4'
5'
NO2
3122.79
1633.85
1593.93
1514.11
1469.82
1392.94
1340.05
1278.07
1151.49
1106.87
1018.69
992.93
936.93
878.67
839.18
806.73
753.42
686.33
592.18
501.49
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

APPENDIX B: *IN SILICO* PROPERTIES AND DATA

The physiochemical properties and subsequent absorption, distribution, metabolism, and excretion (collectively referred to as ADME) as well as drug-likeness and medicinal chemistry friendliness of test compounds were computed via the SwissADME web tool (*http://www.swissadme.ch*) [1]. The results are summarised below.

***

Table A1. Physiochemical properties of nitrofuranylazines

Compd.	Physiochemical properties								Lipophilicity	Water solubility	
	Chemical formula	Molecular weight	Fraction Csp3	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar refractivity	TPSA (Å^2)[a]	LogP$_{o/w}$ (MLogP)[b]	LogS (ESOL)[d]	LogS (Ali)[e]
NFA	$C_5H_3NO_4$	141.08	0	2	4	0	32.92	76.03	-0.93	-1.59	-2.2
1a	$C_{12}H_9N_3O_3$	243.22	0	4	5	0	69.16	83.68	1.83	-2.77	-3.34
2a	$C_{12}H_8FN_3O_3$	261.21	0	4	6	0	69.12	83.68	1.42	-2.92	-3.45
3a	$C_{12}H_8ClN_3O_3$	277.66	0	4	5	0	74.17	83.68	1.56	-3.35	-3.99
4a	$C_{12}H_8BrN_3O_3$	322.11	0	4	5	0	76.86	83.68	1.69	-3.67	-4.06
5a	$C_{13}H_{11}N_3O_3$	257.24	0.08	4	5	0	74.13	83.68	1.29	-3.06	-3.72
6a	$C_{13}H_{11}N_3O_4$	273.24	0.08	5	6	0	75.65	92.91	0.76	-2.83	-3.51
7a	$C_{19}H_{15}N_3O_4$	349.34	0.05	7	6	0	100.14	92.91	2.84	-4.75	-5.99
8a	$C_{12}H_9N_3O_4$	259.22	0	4	6	1	71.18	103.91	0.49	-2.62	-3.39
9a	$C_{12}H_8N_4O_5$	288.22	0	5	7	0	77.98	129.5	0.88	-2.81	-4.12
NTA	$C_5H_3NO_3S$	157.15	0	2	3	0	38.53	91.13	-0.91	-2.04	-3.08
1b	$C_{12}H_9N_3O_2S$	259.28	0	4	4	0	74.77	98.78	1.83	-3.82	-5.23
2b	$C_{12}H_8FN_3O_2S$	277.27	0	4	5	0	74.73	98.78	1.43	-3.97	-5.33
3b	$C_{12}H_8ClN_3O_2S$	293.73	0	4	4	0	79.78	98.78	1.56	-4.41	-5.88
4b	$C_{12}H_8BrN_3O_2S$	338.18	0	4	4	0	82.47	98.78	1.69	-4.72	-5.94
5b	$C_{13}H_{11}N_3O_2S$	273.31	0.08	4	4	0	79.74	98.78	1.29	-4.12	-5.61
6b	$C_{13}H_{11}N_3O_3S$	289.31	0.08	5	5	0	81.26	108.01	0.75	-3.88	-5.39
7b	$C_{19}H_{15}N_3O_3S$	365.41	0.05	7	5	0	105.75	108.01	2.83	-5.24	-6.95
8b	$C_{12}H_9N_3O_3S$	275.28	0	4	5	1	76.79	119.01	0.48	-3.68	-5.29
9b	$C_{12}H_8N_4O_4S$	304.28	0	5	6	0	83.59	144.6	0.84	-3.86	-6.01
NFX	$C_{12}H_9N_3O_5$	275.22	0	5	6	2	70.72	120.65	0.37	-2.95	-4.27
FZD	$C_8H_7N_3O_5$	225.16	0.25	3	6	0	56.97	100.86	-0.31	-1.24	-1.62
NFZ	$C_6H_6N_4O_4$	198.14	0	4	5	2	47.09	126.44	-1	-1.21	-2.45
NFT	$C_8H_6N_4O_5$	238.16	0.12	3	6	1	62.8	120.73	-0.76	-1.04	-1.6

[a]Topological Polar Surface Area (TPSA, Å): Calculated from [2]; [b]MLogP$_{o/w}$: Topological method implemented from [3-5]; [c]LogS scale: insoluble < -10 < poorly < -6 < moderately < -4 < soluble < -2 < very < 0 < highly; [d]LogS (ESOL): Topological method implemented from [6]; [e]LogS (Ali): Topological method implemented from [7]

Table A2. Pharmacokinetic properties of nitrofuranylazines

Compd.	Pharmacokinetic properties							
	GI absorption	BBB permeation	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
NFA	High	No	No	Yes	Yes	No	No	No
1a	High	No	No	Yes	Yes	Yes	No	No
2a	High	No	No	No	No	No	No	No
3a	High	No	No	No	No	No	No	No
4a	High	No	No	No	No	No	No	No
5a	High	No	No	Yes	Yes	Yes	No	No
6a	High	No	No	Yes	Yes	No	No	No
7a	High	No	No	Yes	Yes	Yes	No	No
8a	High	No	No	Yes	Yes	Yes	No	No
9a	High	No	No	Yes	Yes	Yes	No	No
NTA	High	No	No	Yes	Yes	Yes	No	No
1b	High	No	No	No	Yes	Yes	No	No
2b	High	No	No	Yes	Yes	Yes	No	No
3b	Low	No	No	Yes	Yes	Yes	No	No
4b	High	No	No	No	No	No	No	No
5b	High	No	No	No	No	No	No	No
6b	High	No	No	No	No	No	No	No
7b	High	No	No	Yes	Yes	No	No	No
8b	High	No	No	No	No	No	No	No
9b	High	No	No	Yes	Yes	Yes	No	No
NFX	High	No	No	No	No	No	No	No
FZD	High	No	No	No	No	No	No	No
NFZ	High	No	No	No	No	No	No	No
NFT	High	No	No	Yes	Yes	Yes	No	No

Table A3. Drug-likeness and medicinal chemistry friendliness of nitrofuranylazines

Compd.	Drug-likeness						Medicinal chemistry friendliness		
	Num. violations						Num. violations		
	Lipinski[a]	Ghose[b]	Veber[c]	Egan[d]	Muegge[e]	Bioavailability score[f]	PAINS[g]	Brenk[h]	Lead-likeness[i]
NFA	0	3 (MW<160, MR<40, #atoms<20)	0	0	1 (MW<200)	0.55	0	2 (aldehyde, nitro group)	1 (MW<250)
1a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	1 (MW<250)
2a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
3a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
4a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
5a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
6a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
7a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	1 (XLOGP3>3.5)
8a	0	0	0	0	0	0.55	1 (azine phenol)	2 azine, nitro group)	0
9a	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
NTA	0	3 (MW<160, MR<40, #atoms<20)	0	0	1 (MW<200)	0.55	0	2 (aldehyde, nitro group)	1 (MW<250)
1b	0	0	0	0	0	0.55	0	2 (azine, nitro group)	0
2b	0	0	0	0	0	0.55	0	2 (azine, nitro group)	1 (XLOGP3>3.5)
3b	0	0	0	0	0	0.55	0	2 (azine, nitro group)	1 (XLOGP3>3.5)
4b	0	0	0	0	0	0.55	0	2 azine, nitro group)	1 (XLOGP3>3.5)
5b	0	0	0	0	0	0.55	0	2 (azine, nitro group)	1 (XLOGP3>3.5)
6b	0	0	0	0	0	0.55	0	2 azine, nitro group)	0
7b	0	0	0	0	0	0.55	0	2 (azine, nitro group)	2 (MW>350, XLOGP3>3.5)
8b	0	0	0	0	0	0.55	1 (hydrazone phenol)	2 (azine, nitro group)	0
9b	0	0	1 (TPSA>140)	1 (TPSA>131.6)	0	0.55	0	2 (azine, nitro group)	0
NFX	0	0	0	0	0	0.55	0	2	0

								(imine, nitro group)	
FZD	0	0	0	0	0	0.55	0	2 (imine, nitro group)	1 (MW<250)
NFZ	0	0	0	0	1	0.55	0	2 (imine, nitro group)	1 (MW<250)
NFT	0	0	0	0	0	0.55	0	3 (hydantoin, imine, nitro group))	1 (MW<250)

[a]Lipinski: MW < 500, MLOGP < 4.15, N or O < 10, NH or OH < 5 [8]; [b]Ghose: 160 < MW < 480, -04 < WLOGP < 5.6, 40 < MR < 130, 20 < atoms < 70 [9]; [c]Veber: Num. rotatable bonds < 10, TPSA < 140 [10]; [d]Egan: WLOGP < 5.88, TPSA < 131.6 [11]; [e]Muegge: 200 < MW < 600, -2 < XLOGP < 5, TPSA < 150, num. rings < 7, num. carbon > 4, num. heteroatoms > 1, num. rotatable bonds < 15, num. H-bond acceptors < 10, num. H-bond donors < 5 [12]; [f]The probability that a compound will have > 10% bioavailability in rat or measurable Caco-2 permeability [13]; [g]Pan ssay interference structures (PAINS) [14]; [h]Brenk: structural alert [15]; [i]Lead-likeness: 250 < MW < 350, XLOGP3 < 3.5, num. rotatable bonds < 7 [16]. One violation of a rule is allowed.

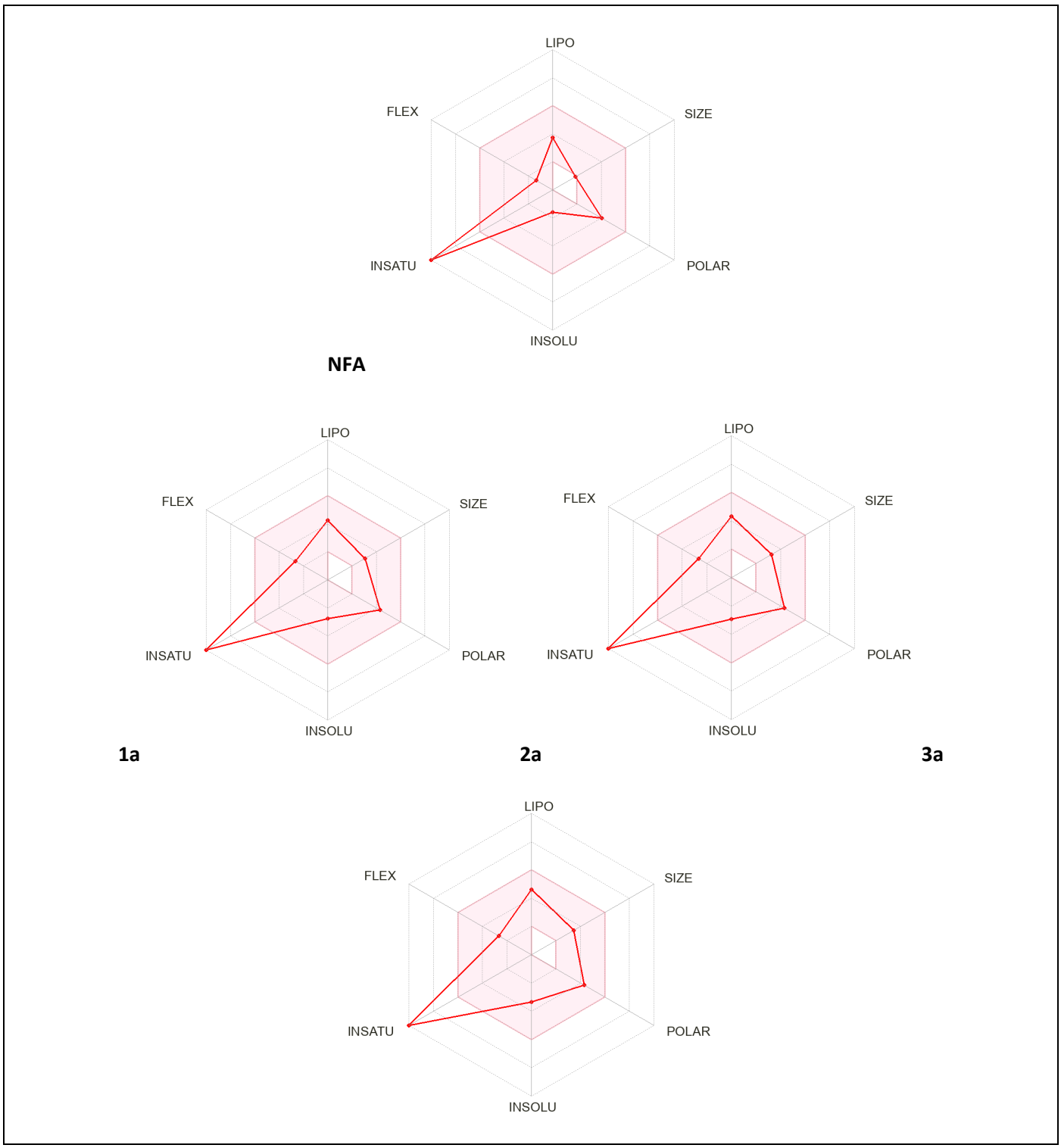


Figure B1. Bioavailability radar for nitrofuranylazines. The pink area represents the optimal range for lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU) and flexibility (FLEX) and the red lines represent these parameters of the compound. Any deviation represents a suboptimal physicochemical property for oral bioavailability.

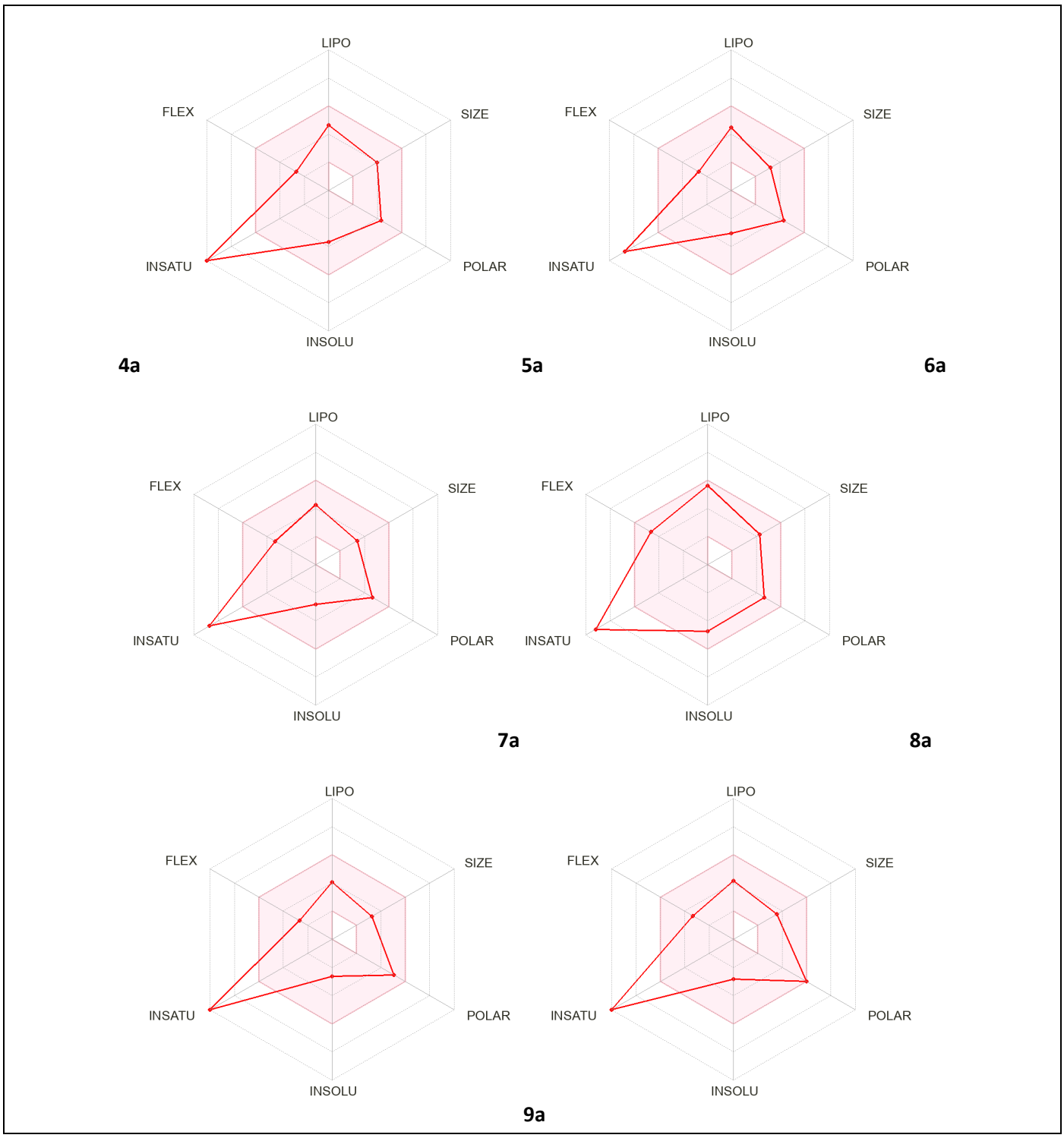


Figure B1 (continued). Bioavailability radar for nitrofuranylazines. The pink area represents the optimal range for lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU) and flexibility (FLEX) and the red lines represent these parameters of the compound. Any deviation represents a suboptimal physicochemical property for oral bioavailability.

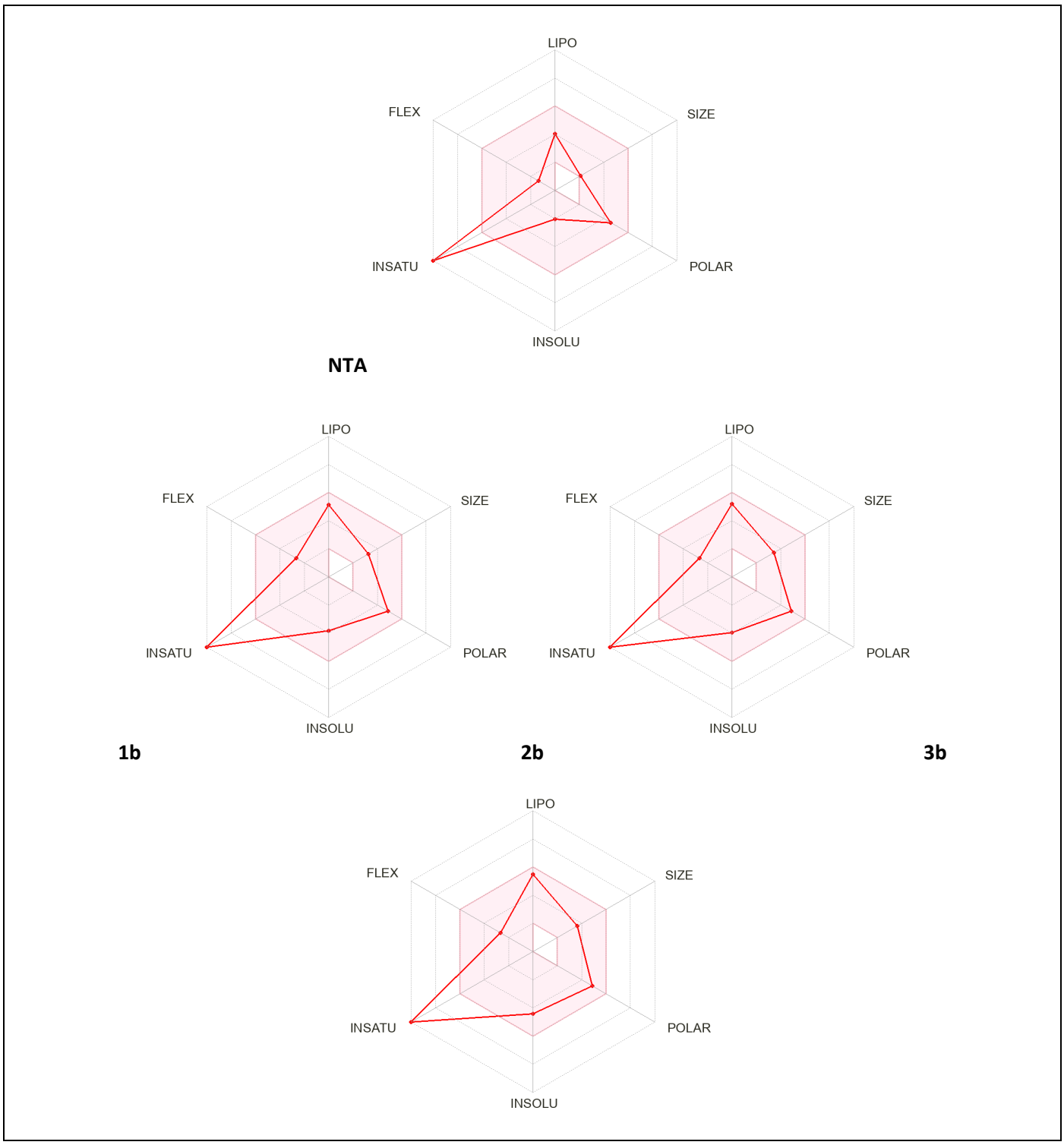


Figure B1 (continued). Bioavailability radar for nitrofuranylazines. The pink area represents the optimal range for lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU) and flexibility (FLEX) and the red lines represent these parameters of the compound. Any deviation represents a suboptimal physicochemical property for oral bioavailability.

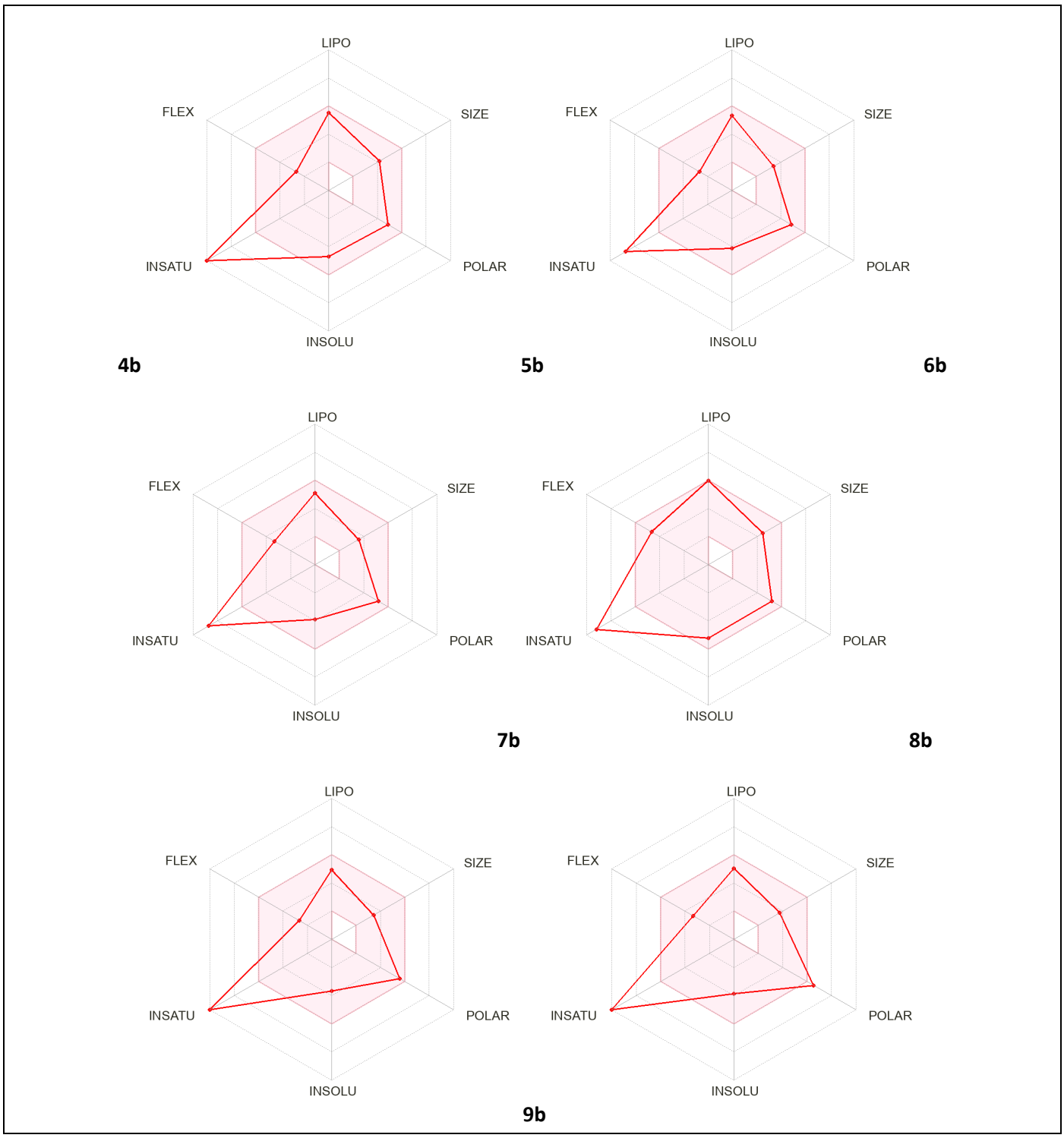


Figure B1 (continued). Bioavailability radar for nitrofuranylazines. The pink area represents the optimal range for lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU) and flexibility (FLEX) and the red lines represent these parameters of the compound. Any deviation represents a suboptimal physicochemical property for oral bioavailability.

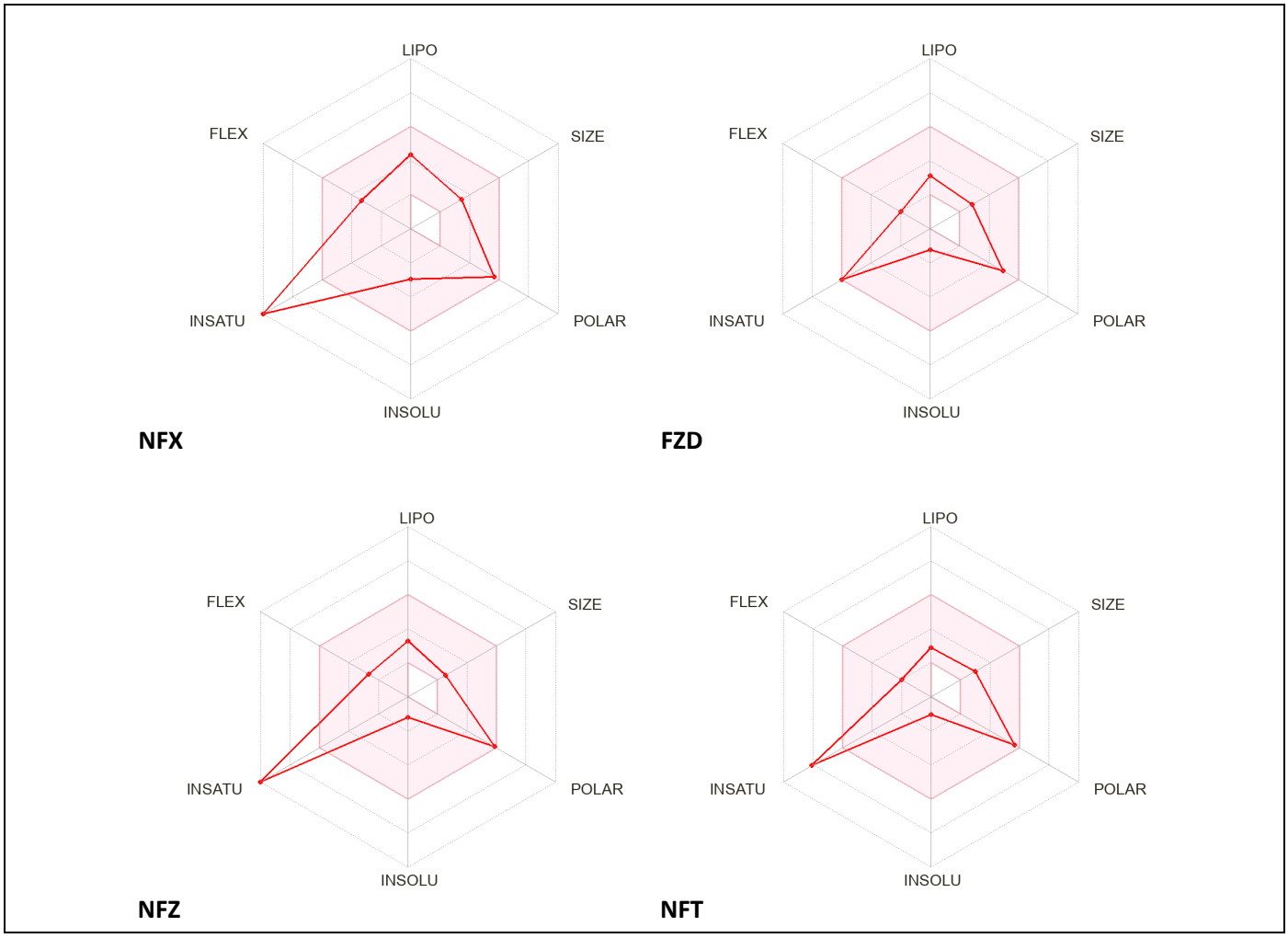


Figure B1 (continued). Bioavailability radar for nitrofuranylazines. The pink area represents the optimal range for lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU) and flexibility (FLEX) and the red lines represent these parameters of the compound. Any deviation represents a suboptimal physicochemical property for oral bioavailability.

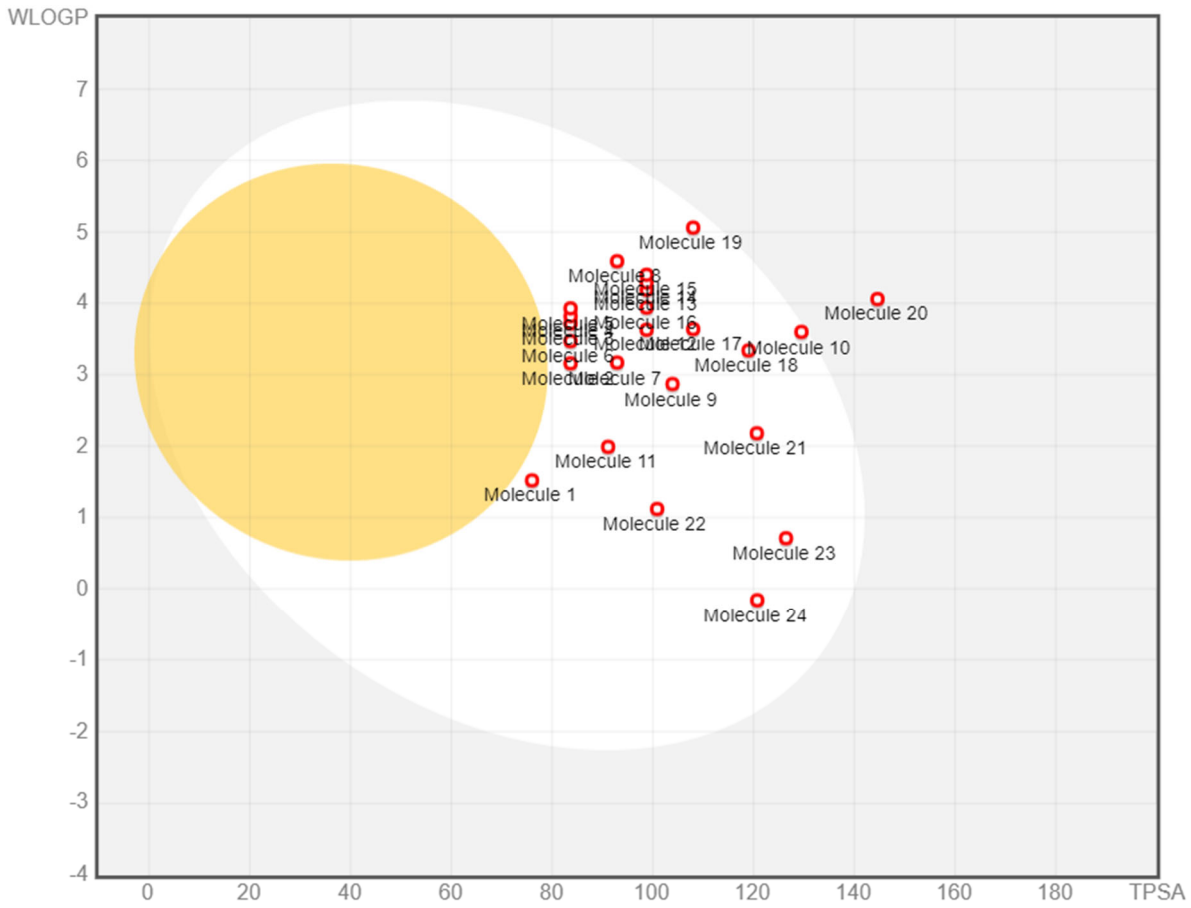


Figure B2. Nitrofuranylazines which are not substrates for Pgp (PGP-) are represented by the red circles. The white region is for high probability of passive absorption by the gastrointestinal tract (HIA), and the yellow region (yolk) is for high probability of brain penetration (BBB). White and yellow (yolk) regions are not mutually exclusive.

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