

Supporting Information

Malononitrile-activated Synthesis and anti-cholinesterase activity of Styrylquinoxalin-2(1*H*)-ones

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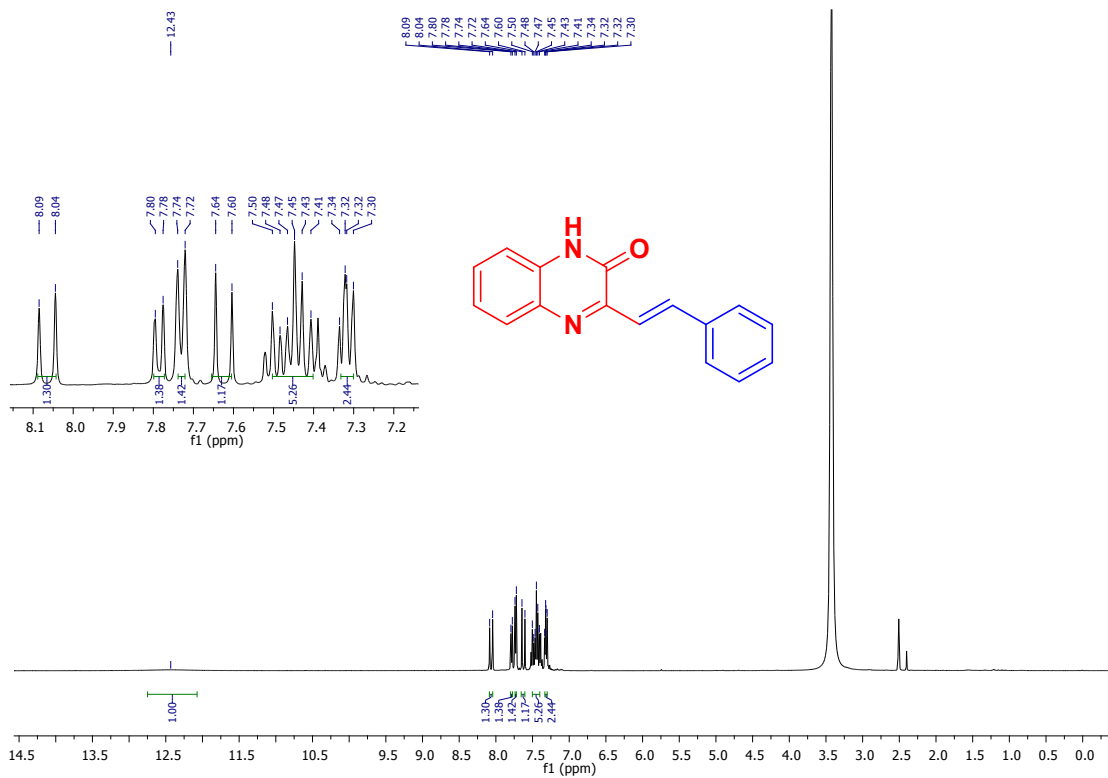
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Ghaziabad-201002, India

* Corresponding Author

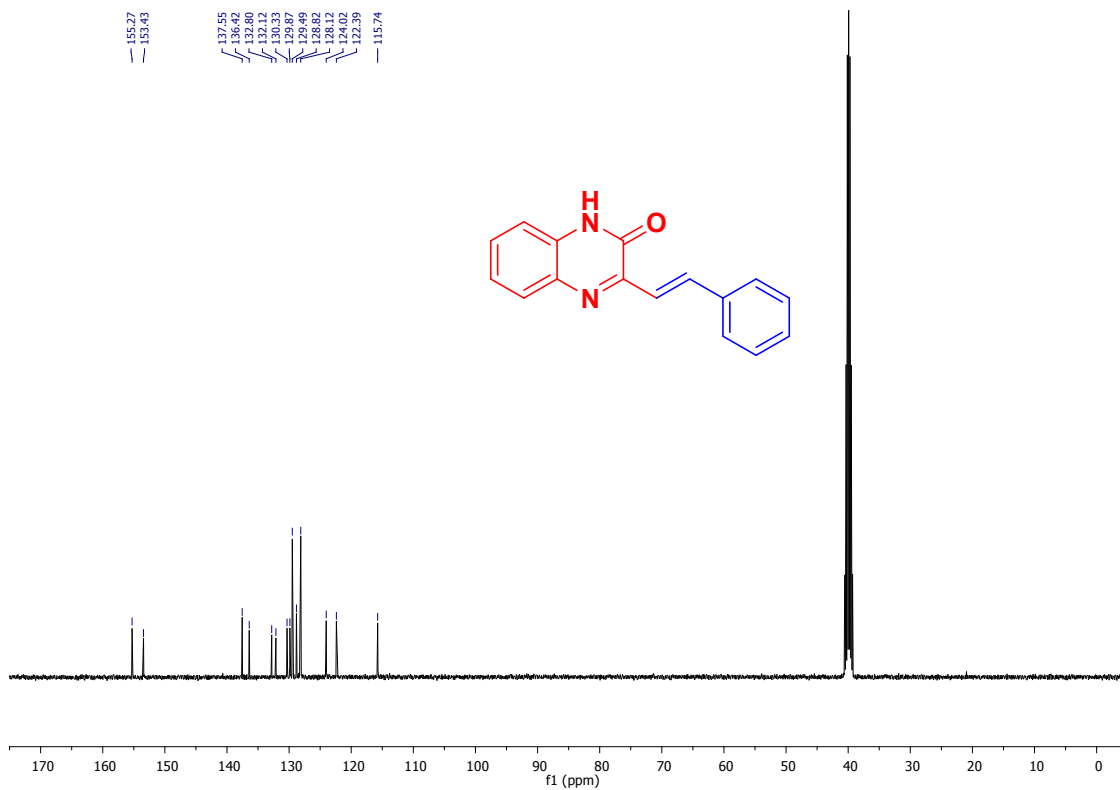
[†] Sheena Mahajan and Nancy Slathia have equally contributed as a first author.

4a. (E)-3-styrylquinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

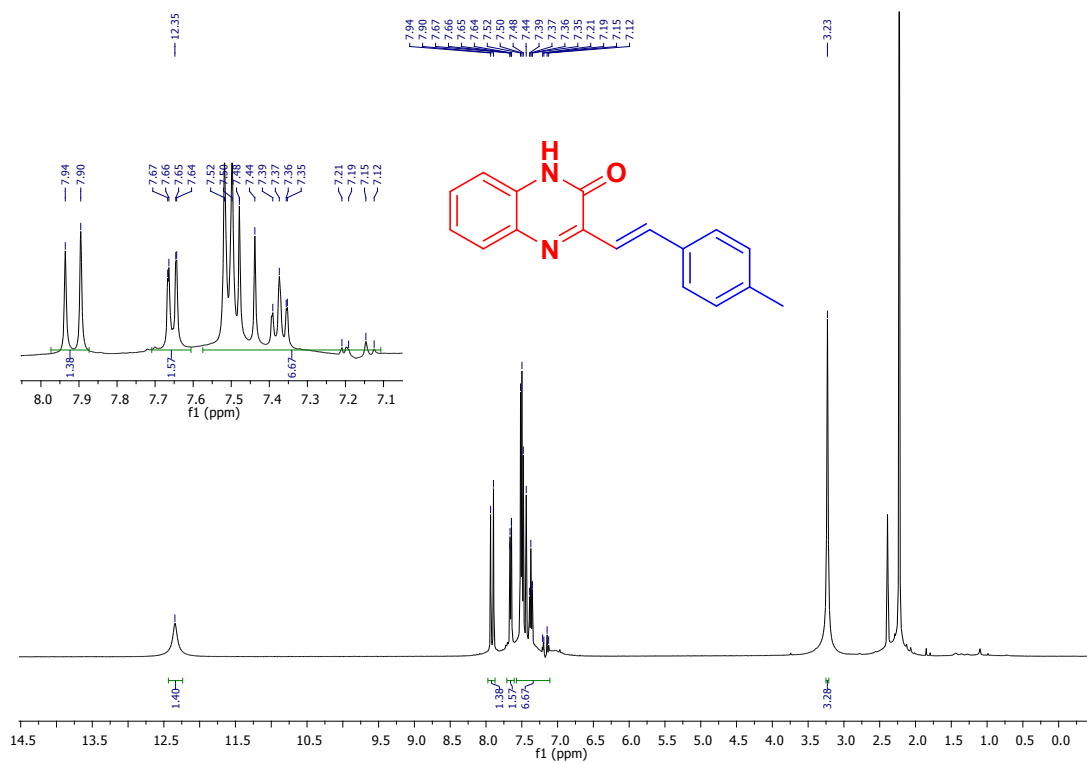


¹³C NMR spectrum (101 MHz, DMSO-d₆)

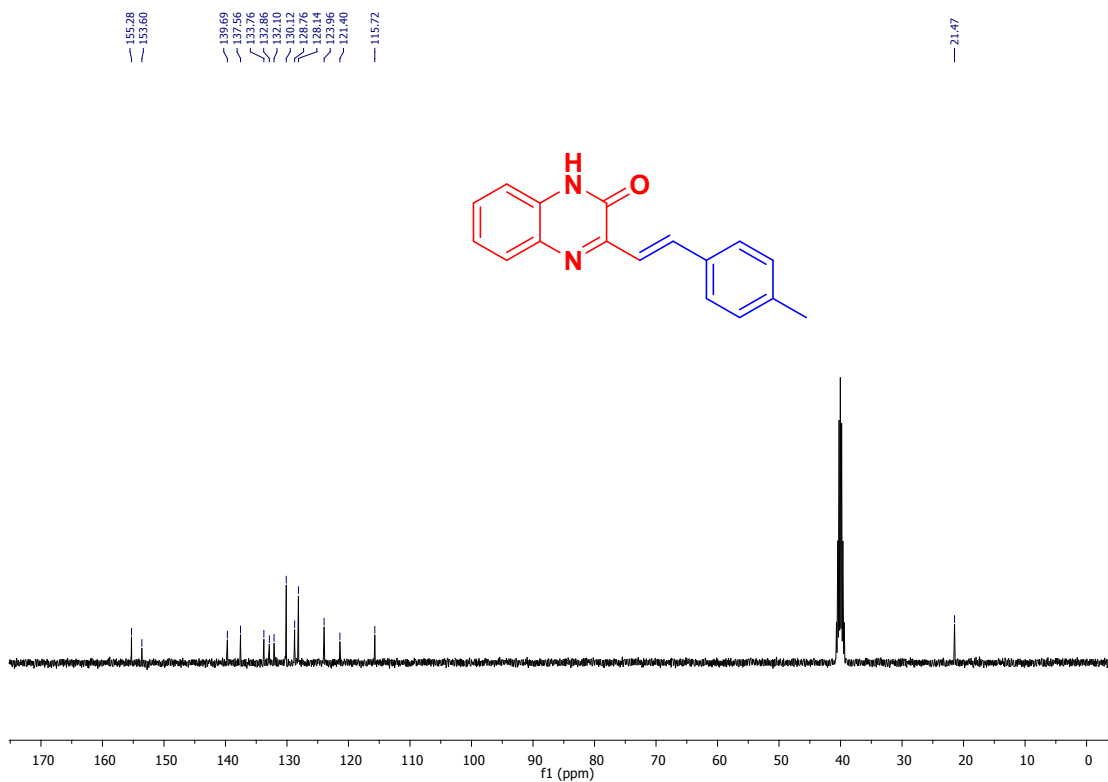


4b. (E)-3-(4-methylstyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

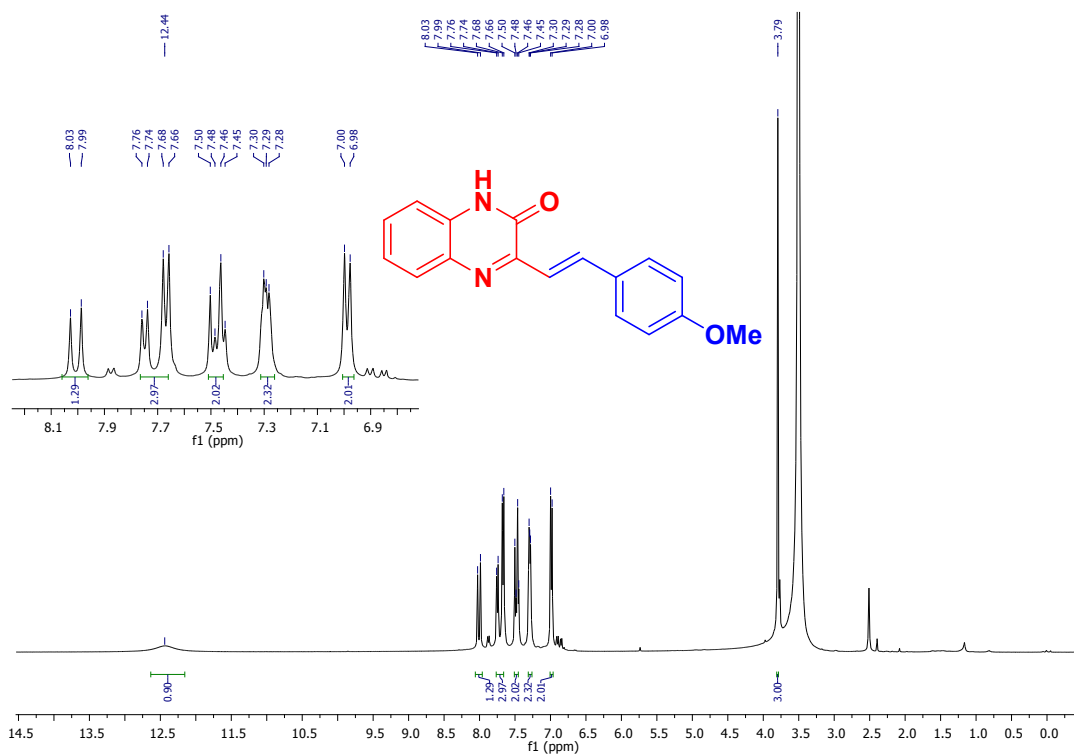


¹³C NMR spectrum (101 MHz, DMSO-d₆)

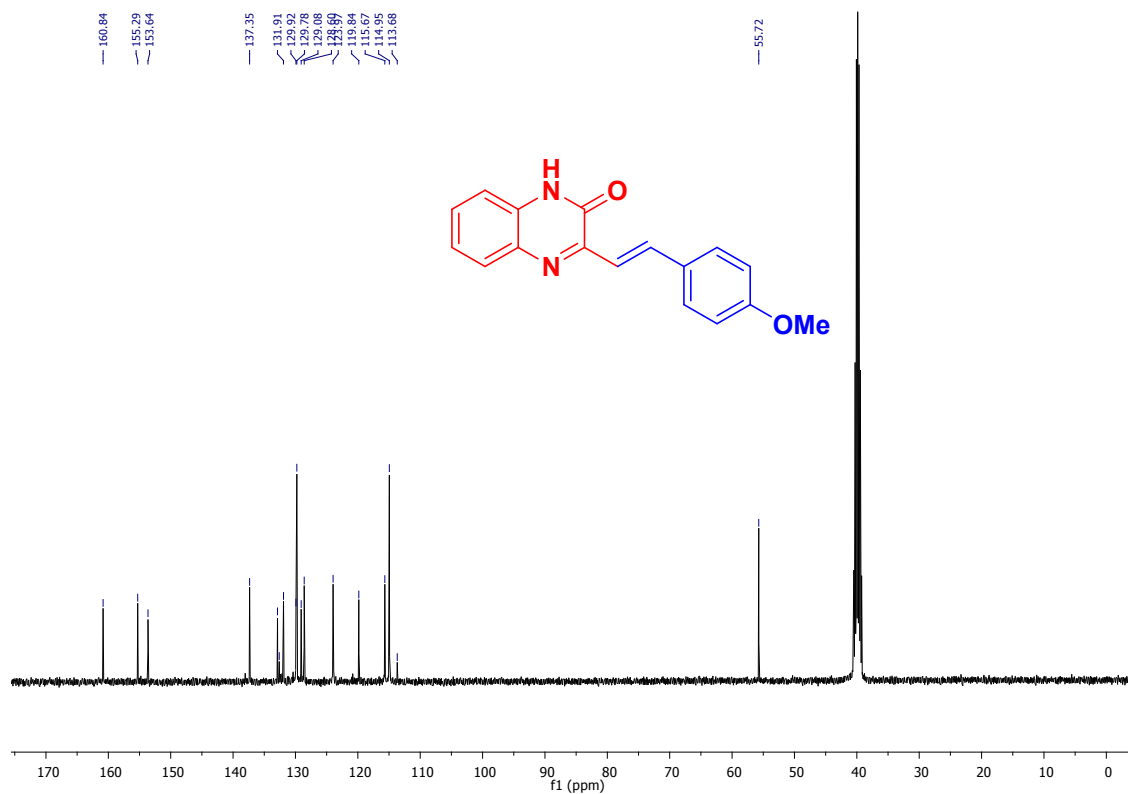


4c. (E)-3-(4-methoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

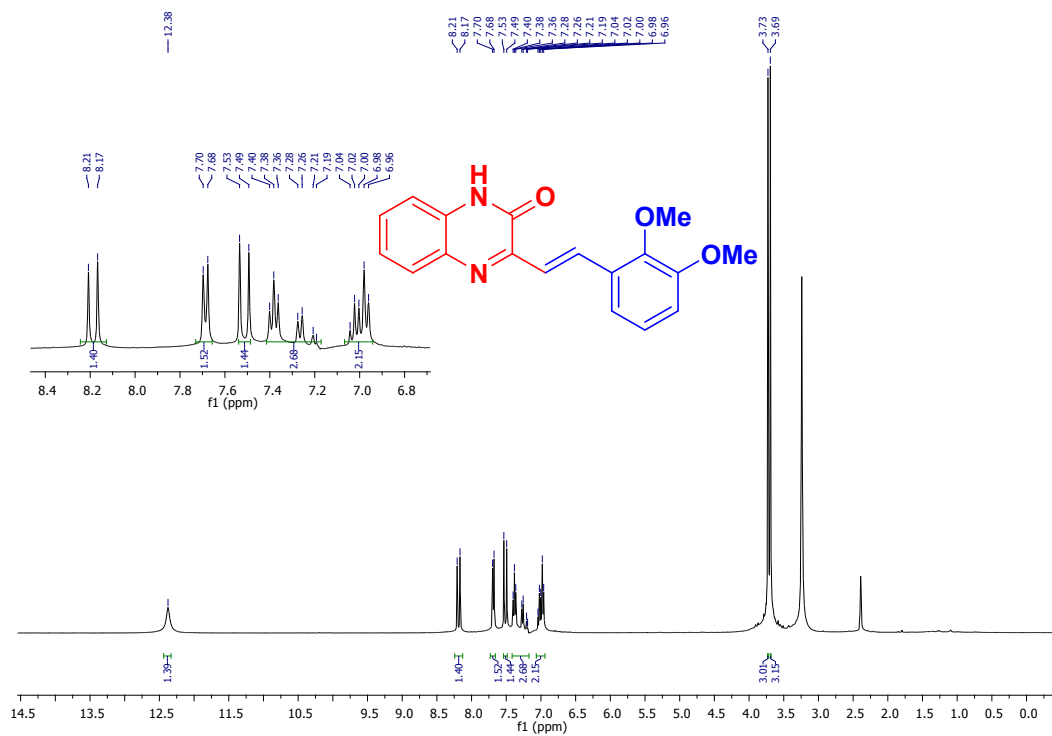


¹³C NMR spectrum (101 MHz, DMSO-d₆)

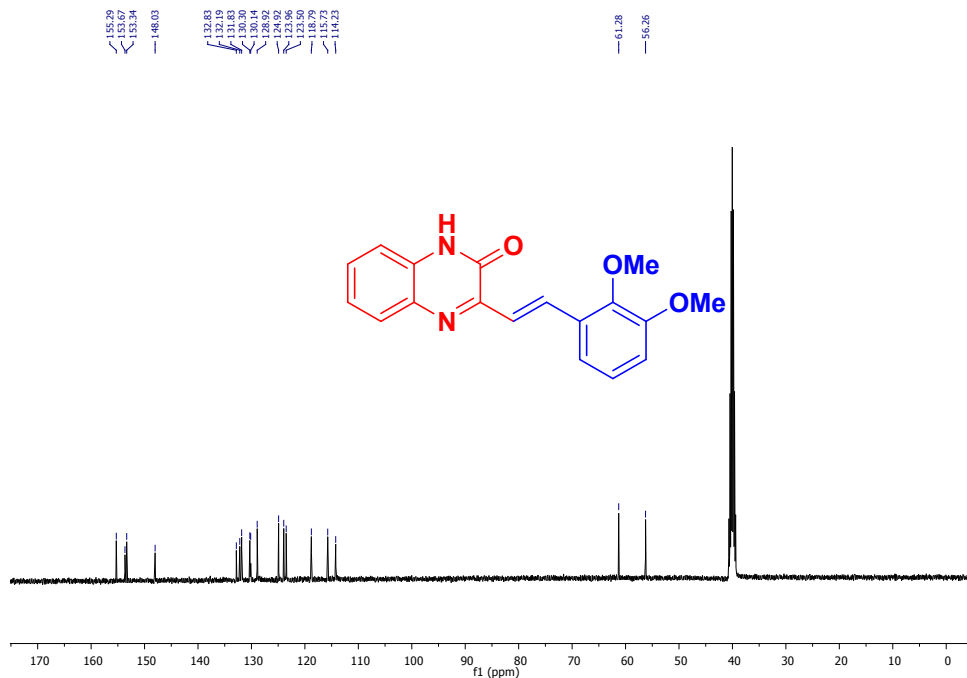


4d. (E)-3-(2,3-dimethoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

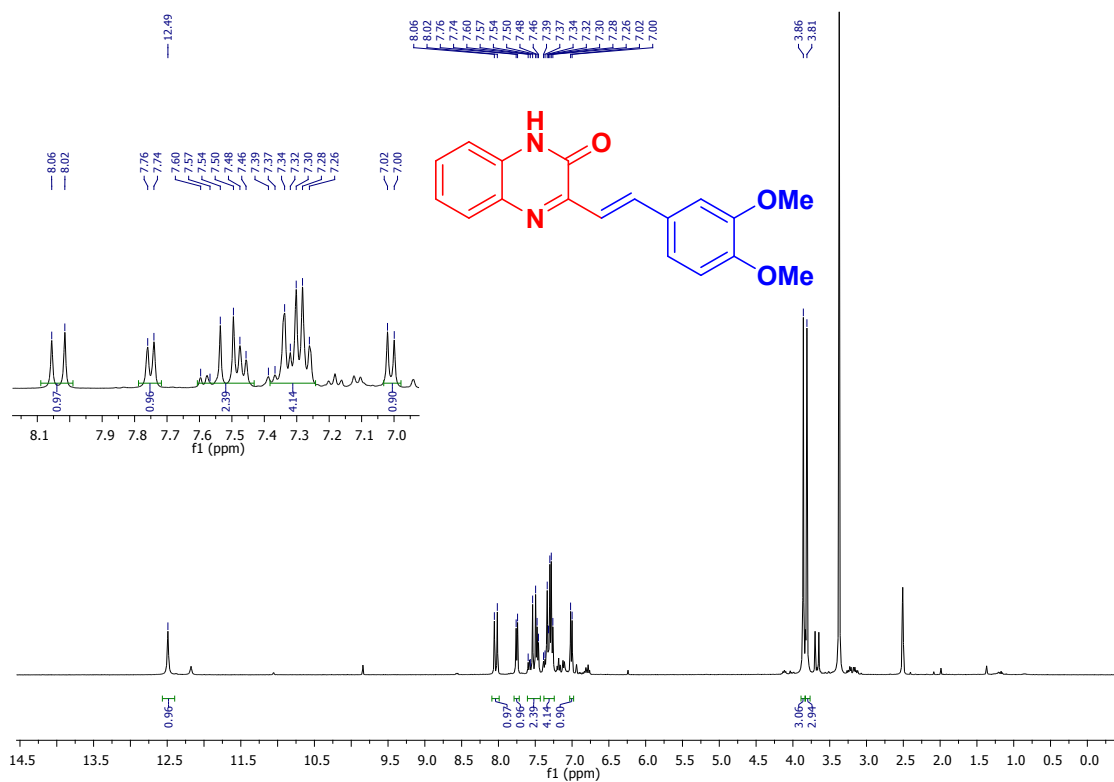


¹³C NMR spectrum (101 MHz, DMSO-d₆)

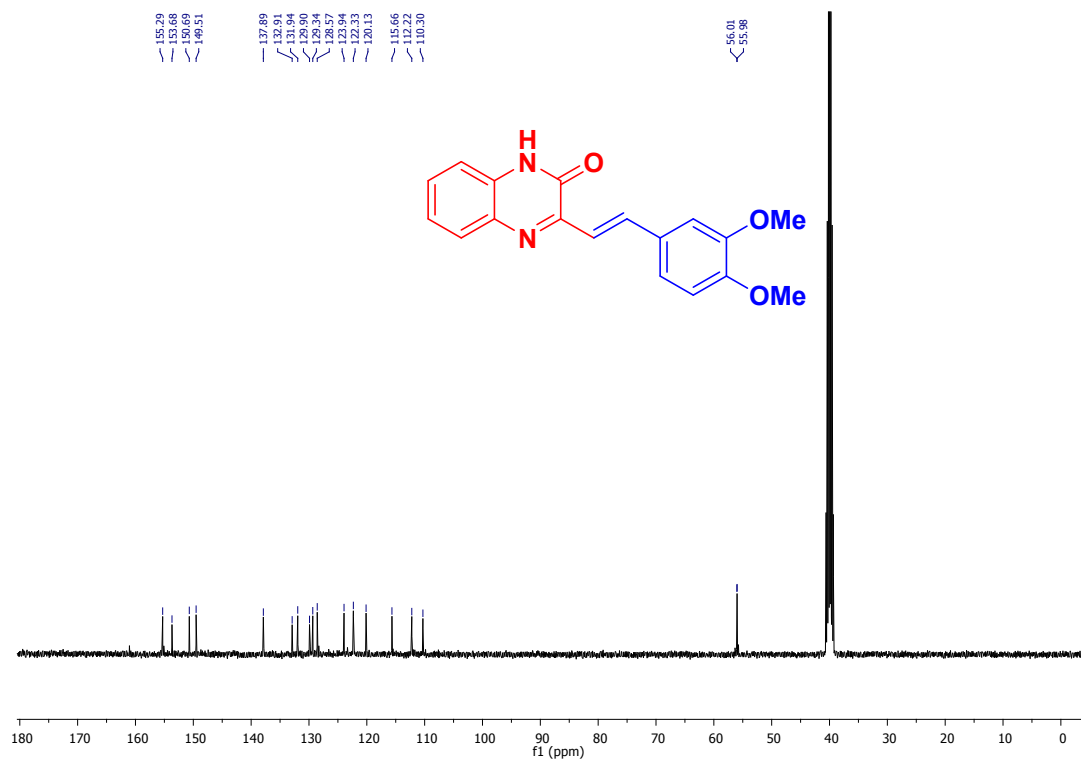


4e. (E)-3-(3,4-dimethoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

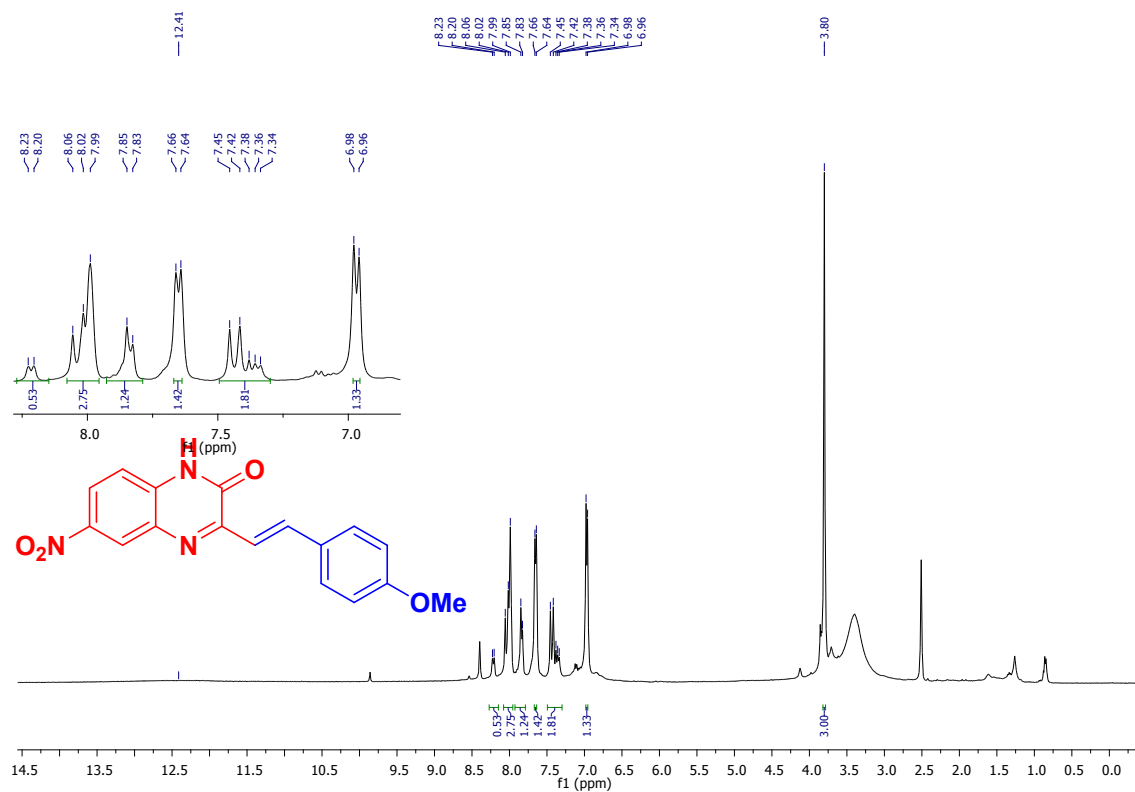


¹³C NMR spectrum (101 MHz, DMSO-d₆)

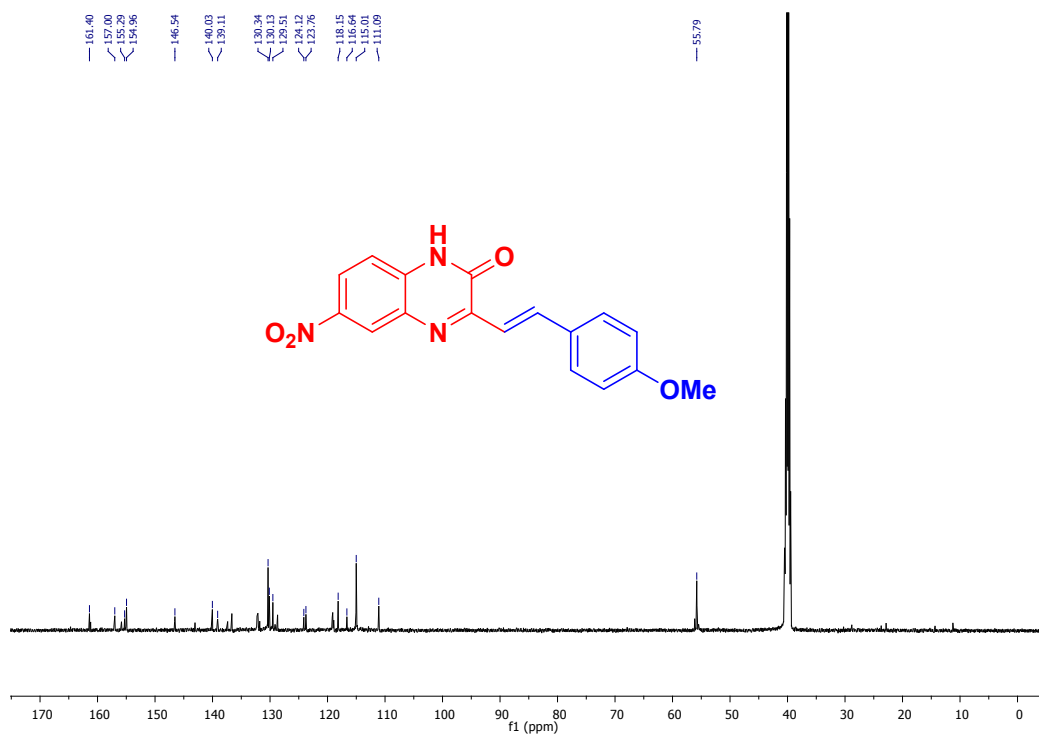


4f. (E)-3-(4-methoxystyryl)-6-nitroquinoxalin-2(1H)-one

^1H NMR spectrum (400 MHz, DMSO- d_6)



^{13}C NMR spectrum (101 MHz, DMSO- d_6)



HPLC Analysis of 4f

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

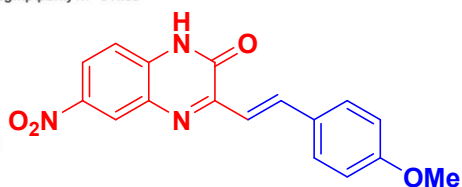
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:39:19 1 / 1

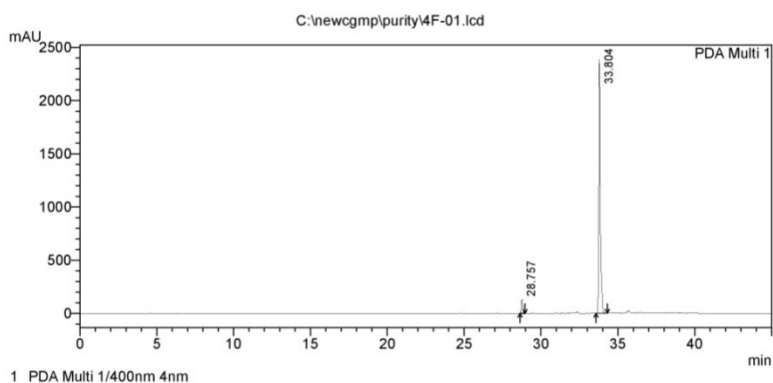
==== Shimadzu LCsolution Analysis Report ====

Acquired by : Admin
Sample Name : 4F-01
Sample ID : 4F-01
Tray# : 1
Vial # : 21
Injection Volume : 5 µL
Data File Name : 4F-01.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 25-11-2019 19:33:40
Data Processed : 25-11-2019 20:18:43

C:\newcgmp\purity\4F-01.lcd



<Chromatogram>



1 PDA Multi 1/400nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	28.757	790676	132179	4.868	5.245
2	33.804	15450172	2387966	95.132	94.755
Total		16240848	2520144	100.000	100.000

C:\newcgmp\purity\4F-01.lcd

HRMS Analysis of **4f**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

The source was operated in both positive and negative mode at an ion spray voltage of 3KV

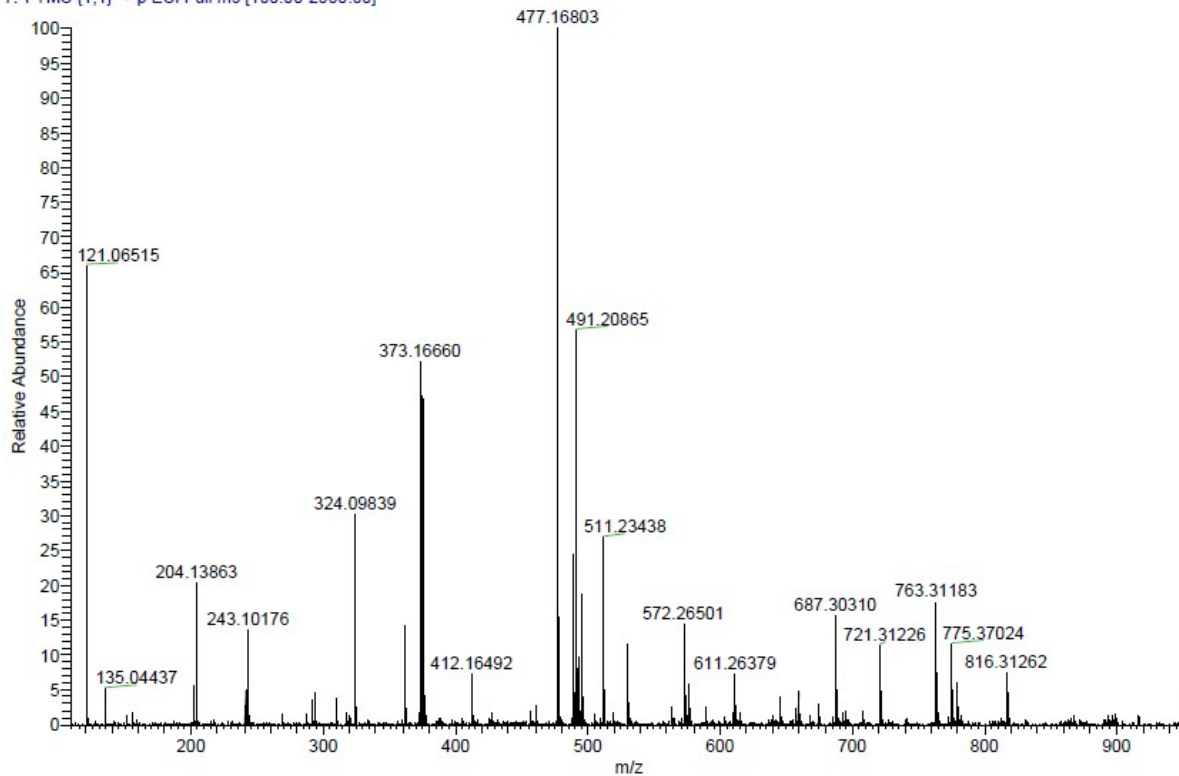
Oven temperature was set to 30°C

X:\Data\2018\July2019-Dec2019\4F

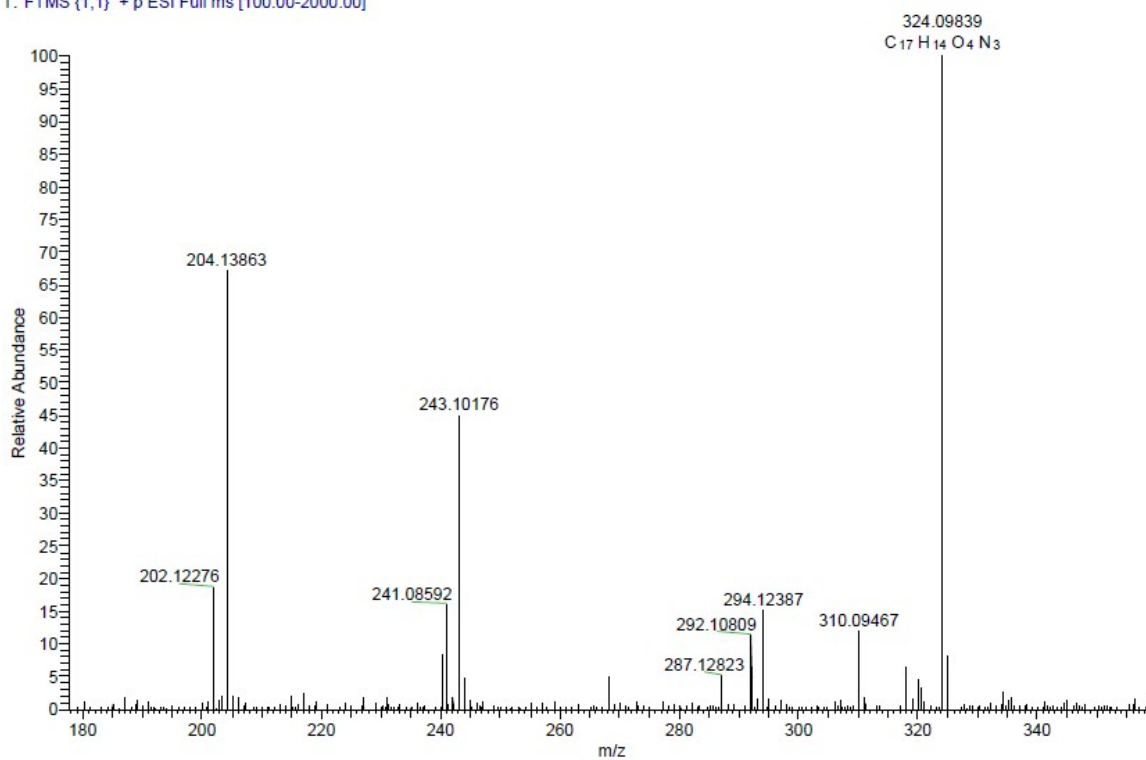
20-12-2019 16:00:50

4F #71 RT: 1.41 AV: 1 NL: 7.04E4

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

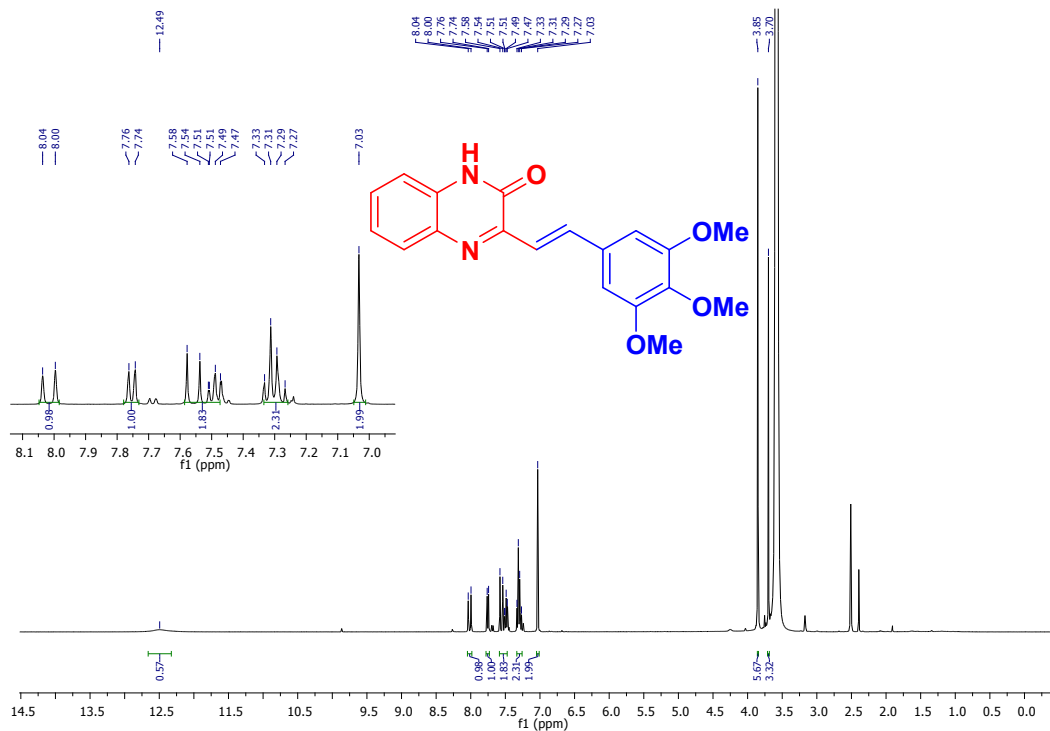


4F #71 RT: 1.41 AV: 1 NL: 2.13E4
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]

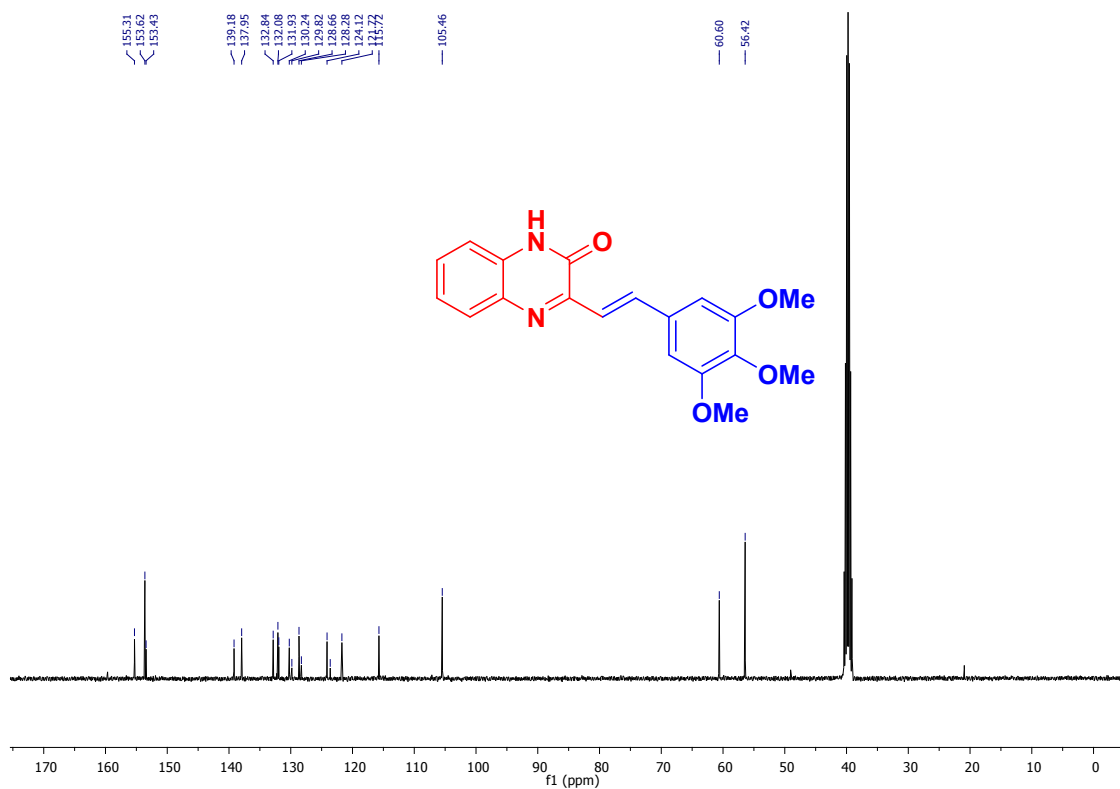


4g. (E)-3-(3,4,5-trimethoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4g

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

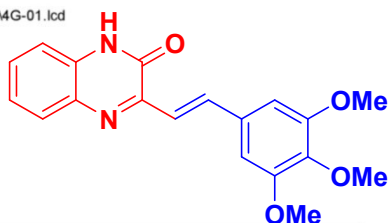
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

29-11-2019 11:16:33 1 / 1

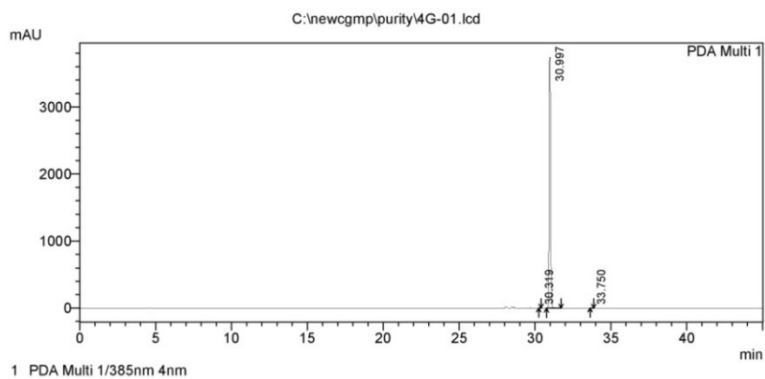
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
Sample Name : 4G
Sample ID : 4G-01
Tray# : 1
Vial # : 22
Injection Volume : 5 µL
Data File Name : 4G-01.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 28-11-2019 20:13:52
Data Processed : 29-11-2019 11:15:57

C:\newcgmp\purity\4G-01.lcd



<Chromatogram>



PeakTable					
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2	30.997	26328342	3744751	99.714	99.643
3	33.750	44270	7042	0.168	0.187
Total		26403888	3758153	100.000	100.000

C:\newcgmp\purity\4G-01.lcd

HRMS Analysis of **4g**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

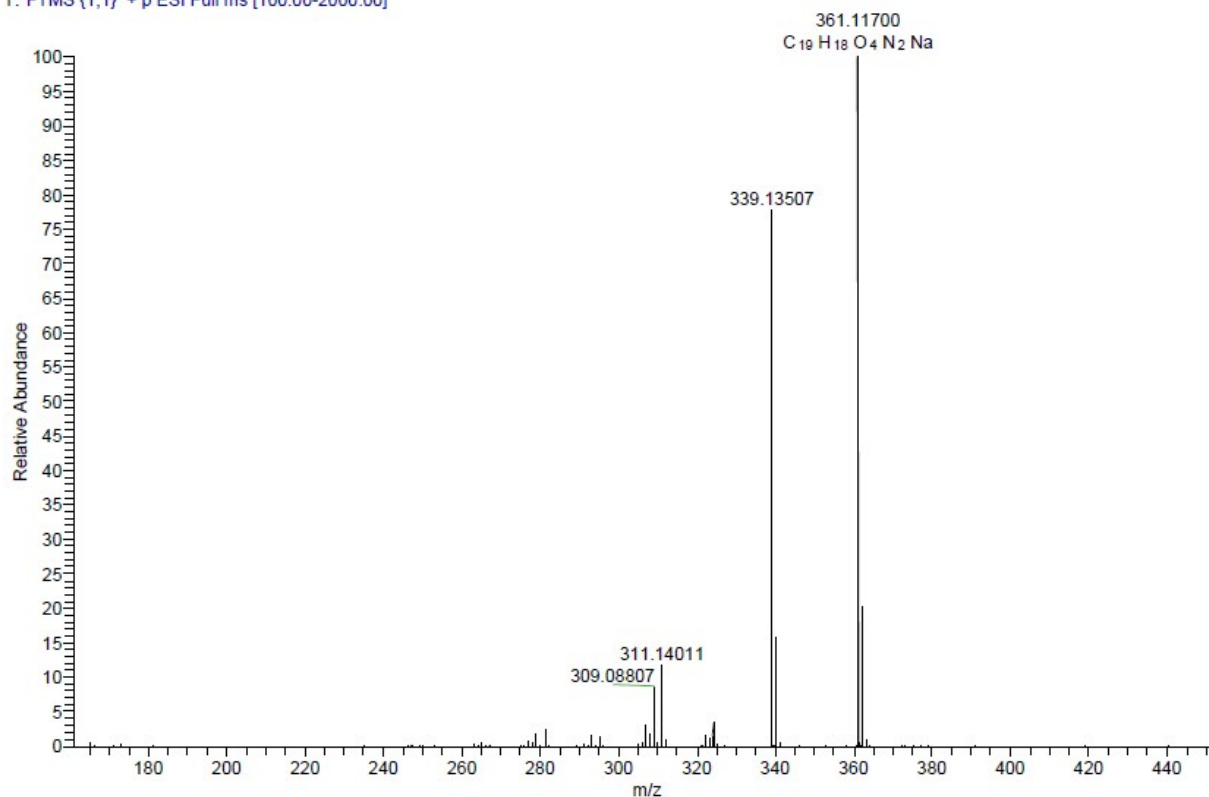
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

X:\Data\2018\July2019-DeC2019\4g

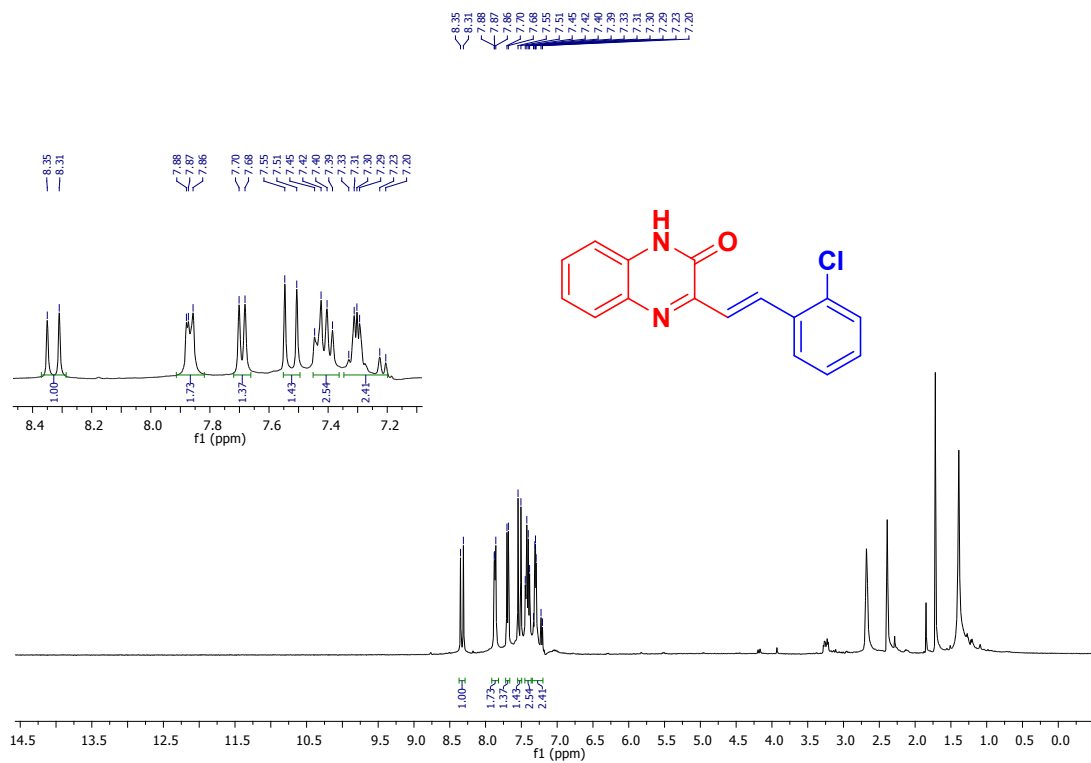
20-12-2019 16:06:28

4g #55 RT: 1.13 AV: 1 NL: 9.15E6
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

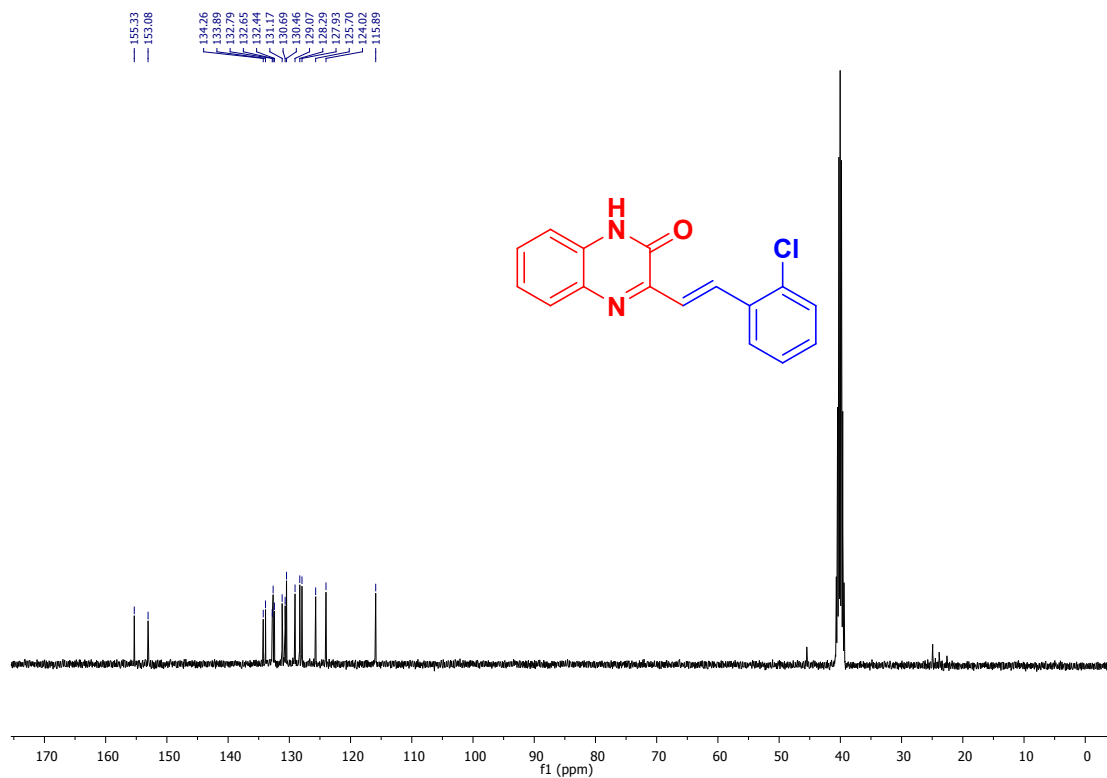


4h. (E)-3-(2-chlorostyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

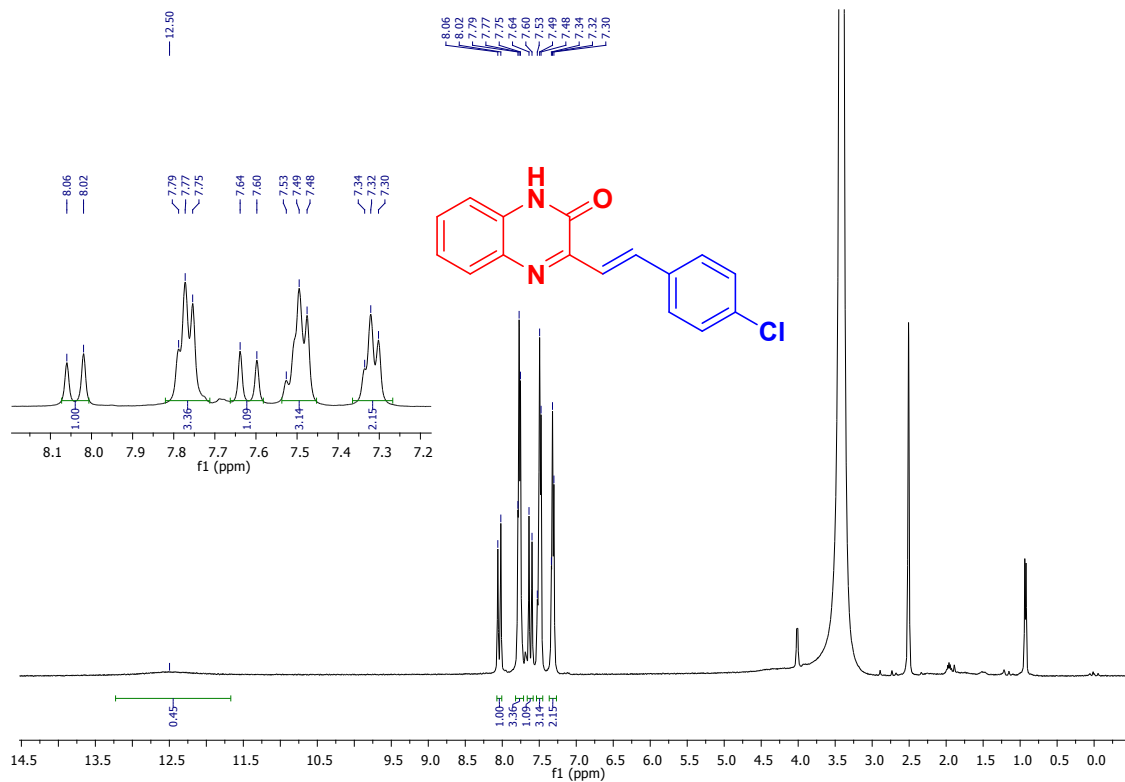


¹³C NMR spectrum (101 MHz, DMSO-d₆)

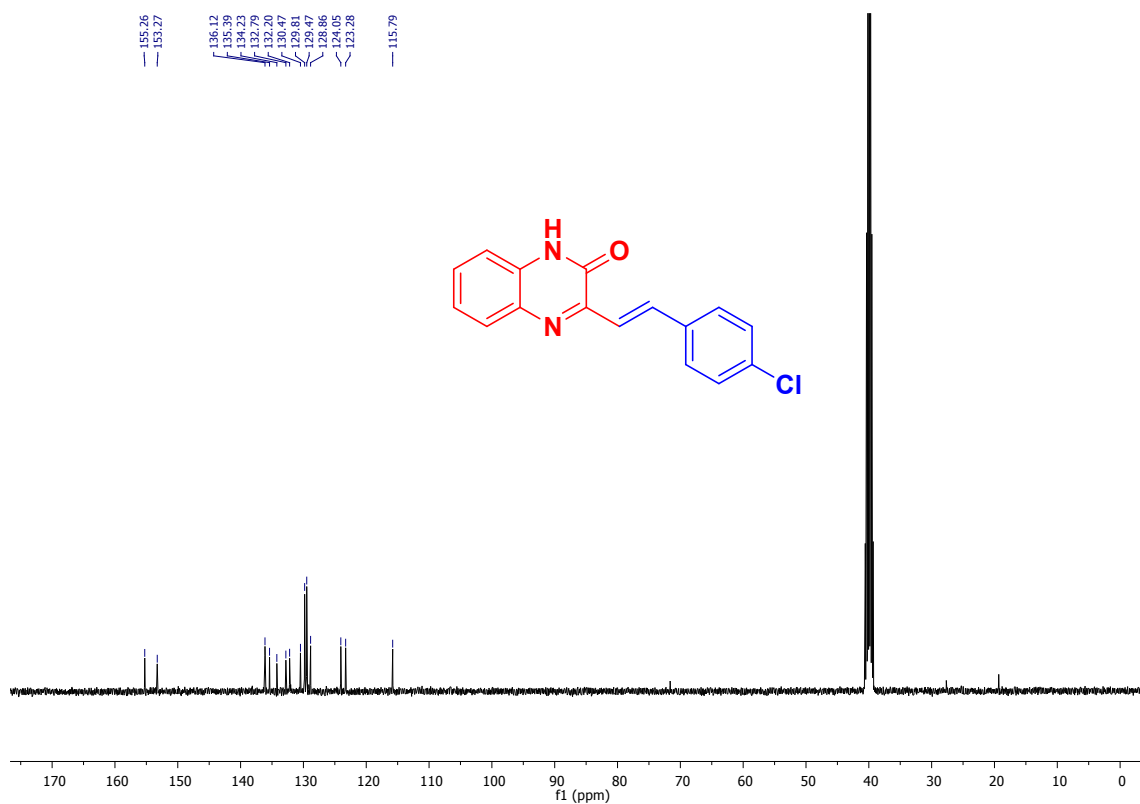


4i. (E)-3-(4-chlorostyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

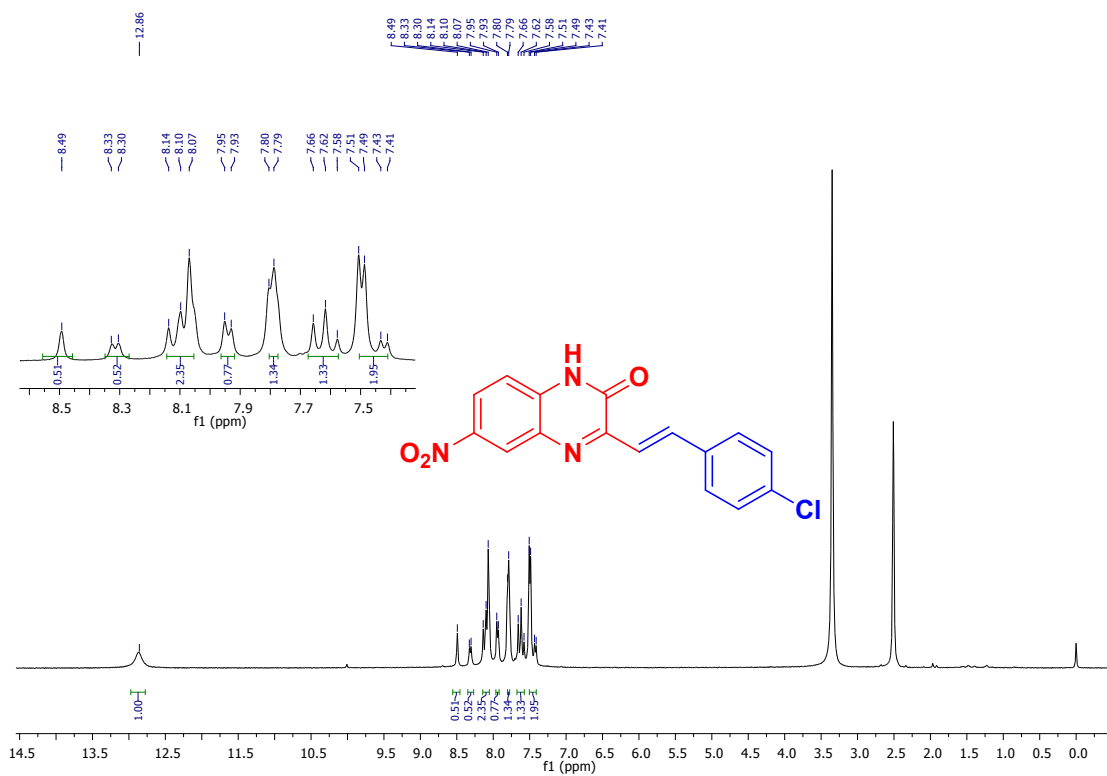


¹³C NMR spectrum (101 MHz, DMSO-d₆)

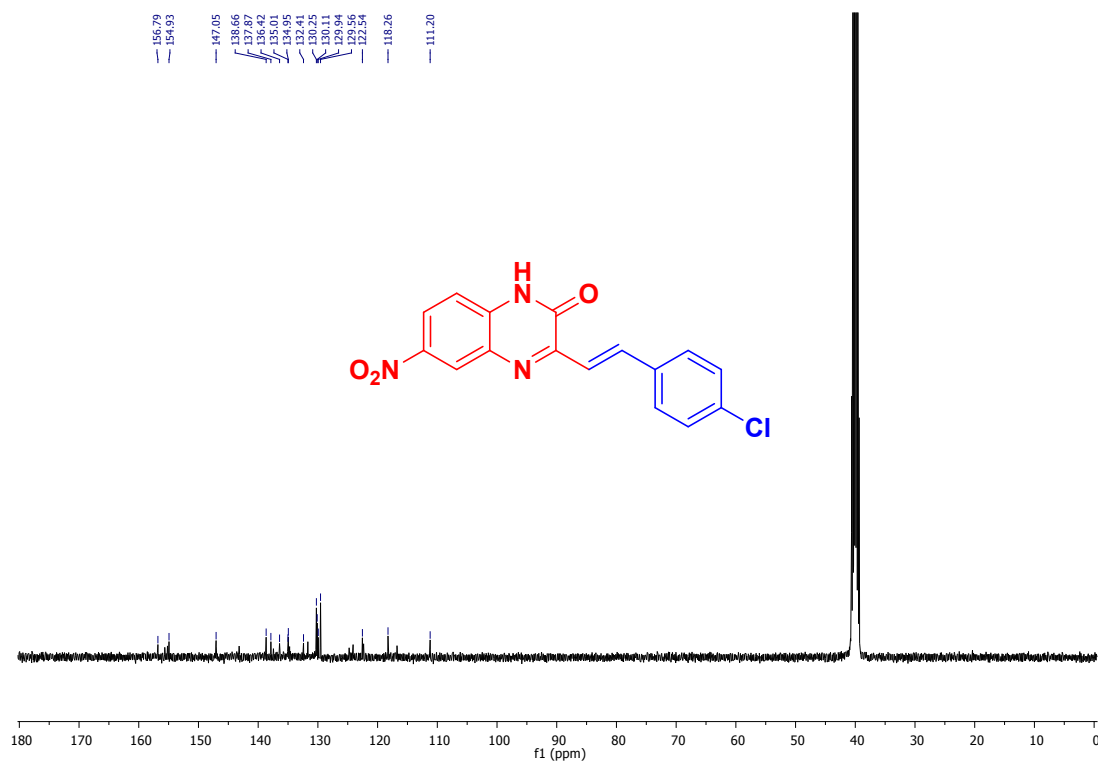


4j. (E)-3-(4-chlorostyryl)-6-nitroquinoxalin-2(1H)-one

^1H NMR spectrum (400 MHz, DMSO- d_6)



^{13}C NMR spectrum (101 MHz, DMSO- d_6)



HPLC Analysis of 4j

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

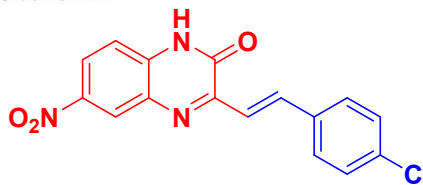
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:34:03 1 / 1

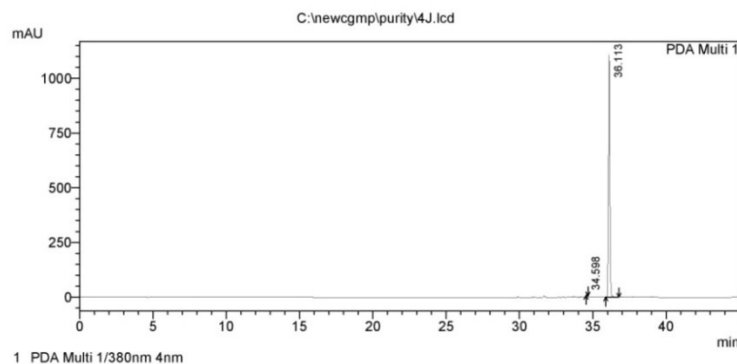
==== Shimadzu LCsolution Analysis Report ====

Acquired by : Admin
Sample Name : 4J
Sample ID : 4J
Tray# : 1
Vial # : 23
Injection Volume : 5 µL
Data File Name : 4J.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 25-11-2019 21:04:42
Data Processed : 25-11-2019 21:49:44

C:\newcgmp\purity\4J.lcd



<Chromatogram>



PDA Ch1 380nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	34.598	48569	11705	0.649	1.046
2	36.113	7436145	1106880	99.351	98.954
Total		7484715	1118585	100.000	100.000

C:\newcgmp\purity\4J.lcd

HRMS Analysis of 4j

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150µl /minute

The source was operated in both positive and negative mode at an ion spray voltage of 3KV

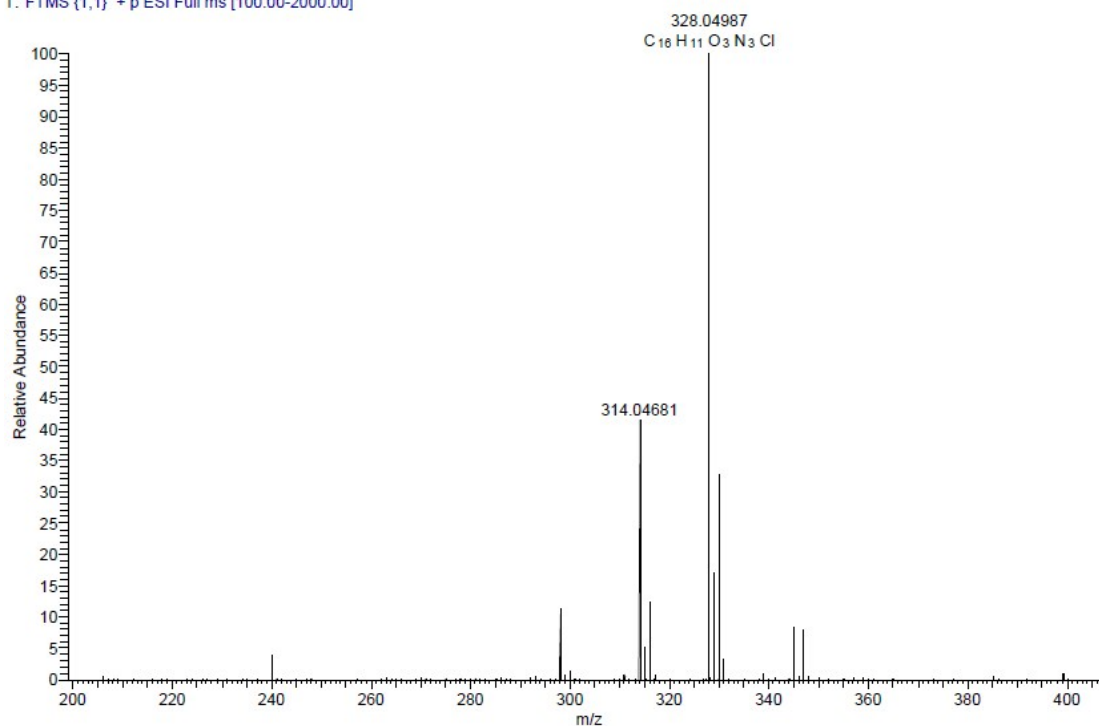
Oven temperature was set to 30°C

X:\Data\2018\July2019-DeC2019\4j

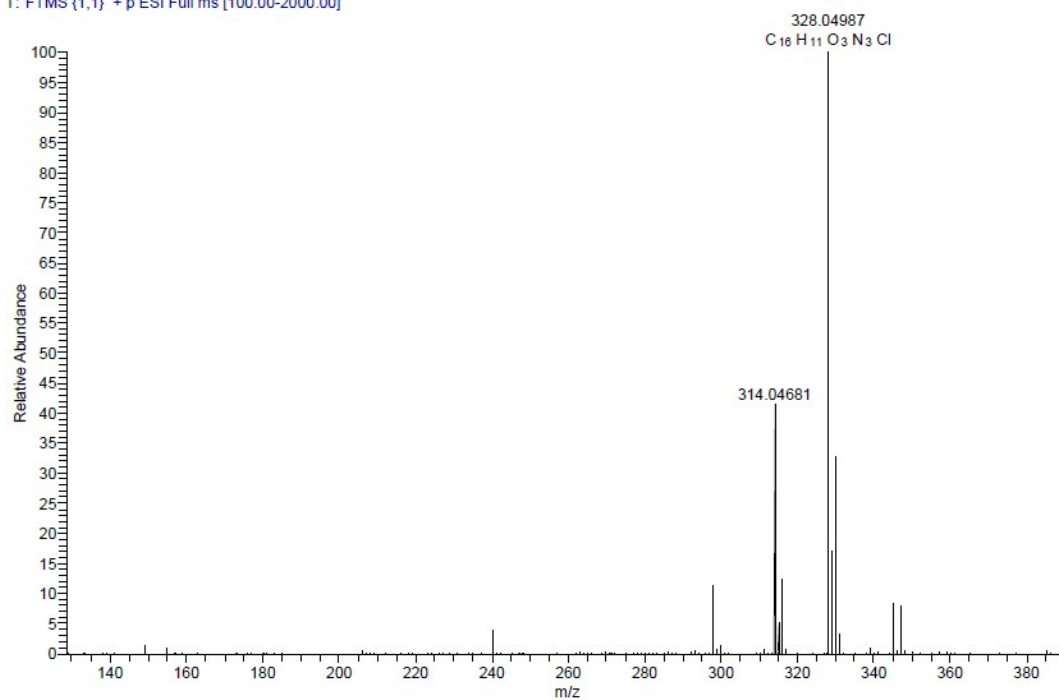
20-12-2019 16:12:10

4j #58 RT: 1.25 AV: 1 NL: 2.52E5

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

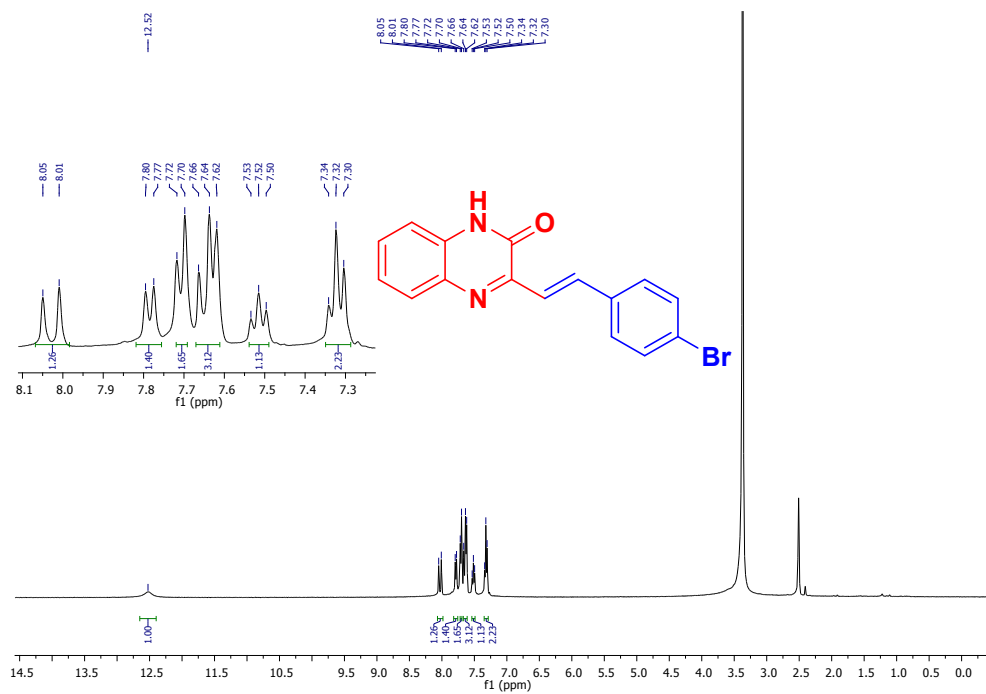


4j #58 RT: 1.25 AV: 1 NL: 2.52E5
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]

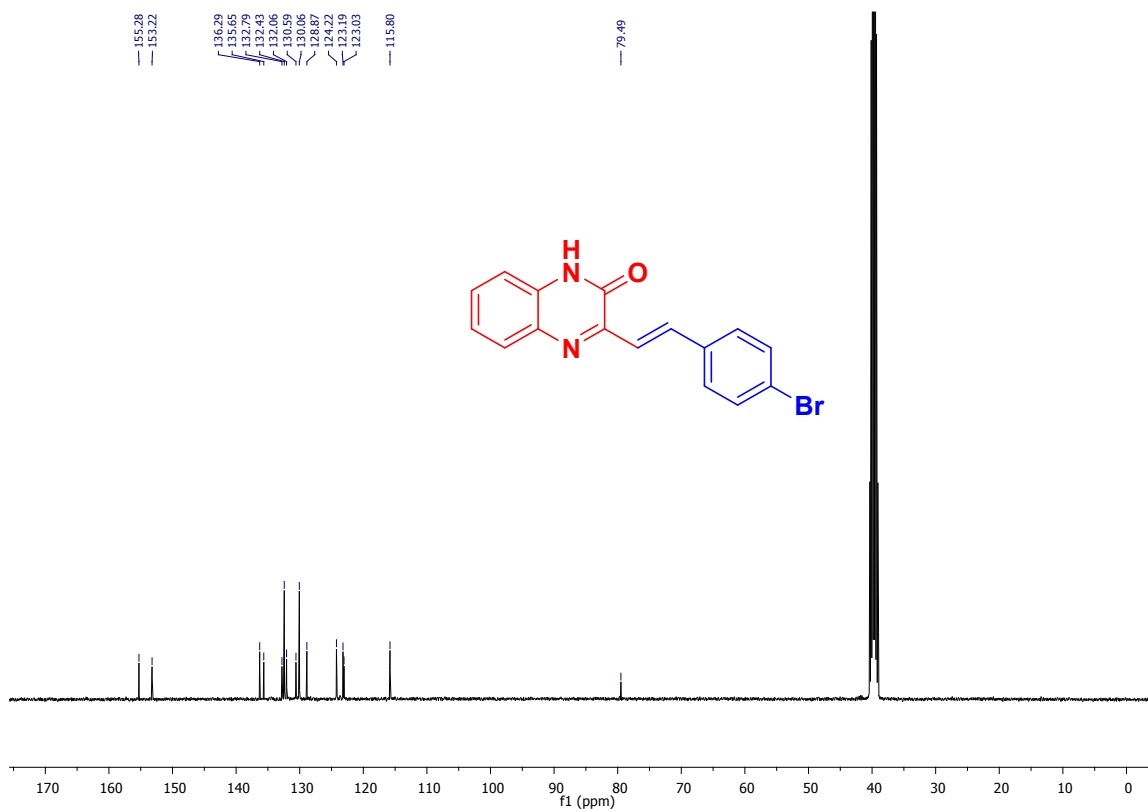


4k. (E)-3-(4-bromostyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

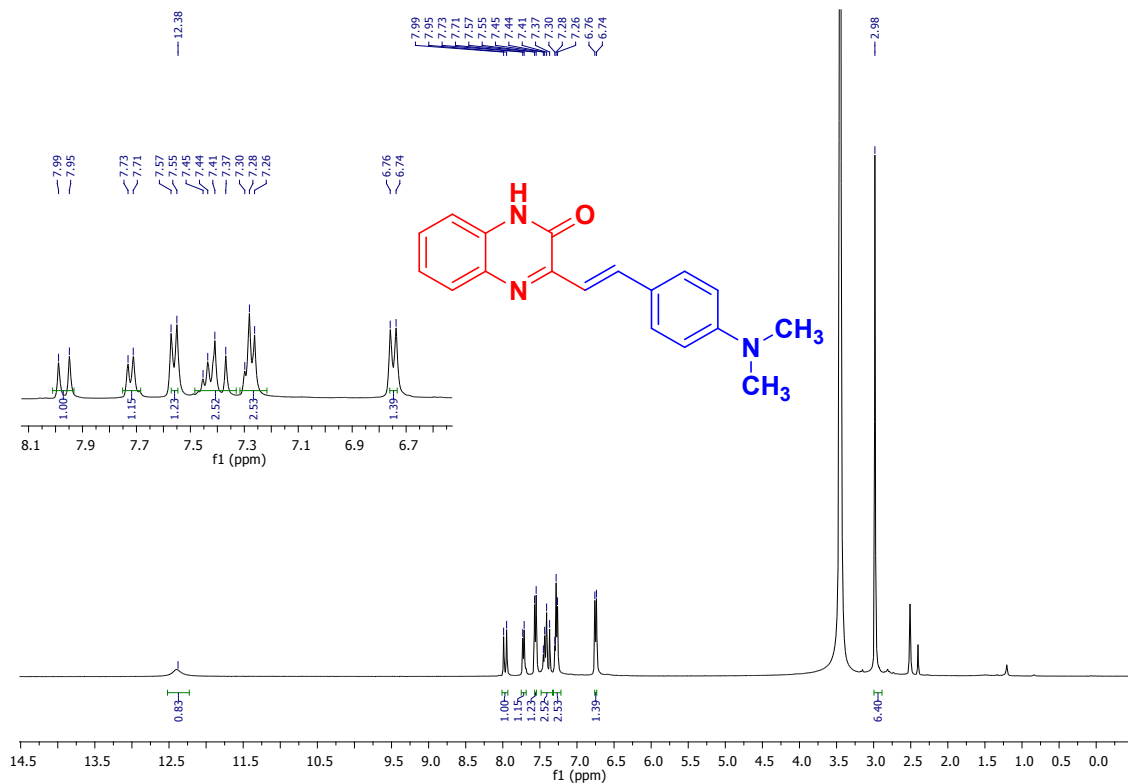


¹³C NMR spectrum (101 MHz, DMSO-d₆)

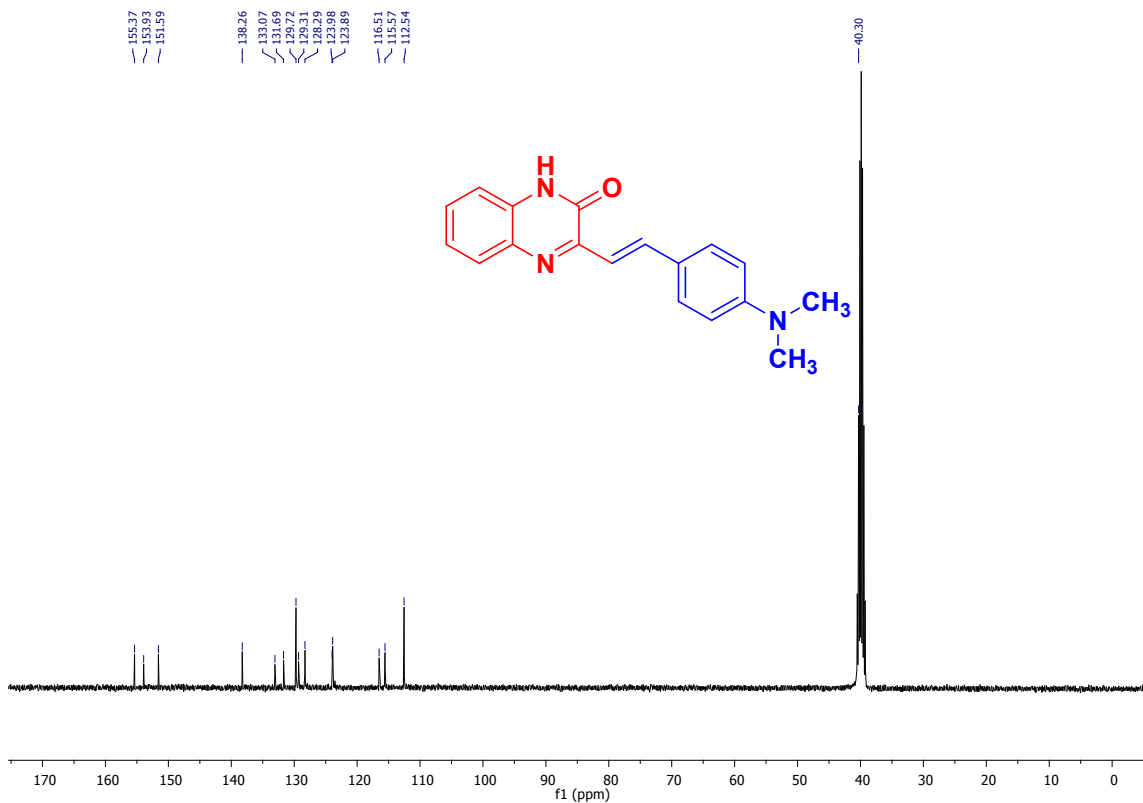


4l. (E)-3-(4-(dimethylamino)styryl)quinoxalin-2(1H)-one

^1H NMR spectrum (400 MHz, DMSO- d_6)

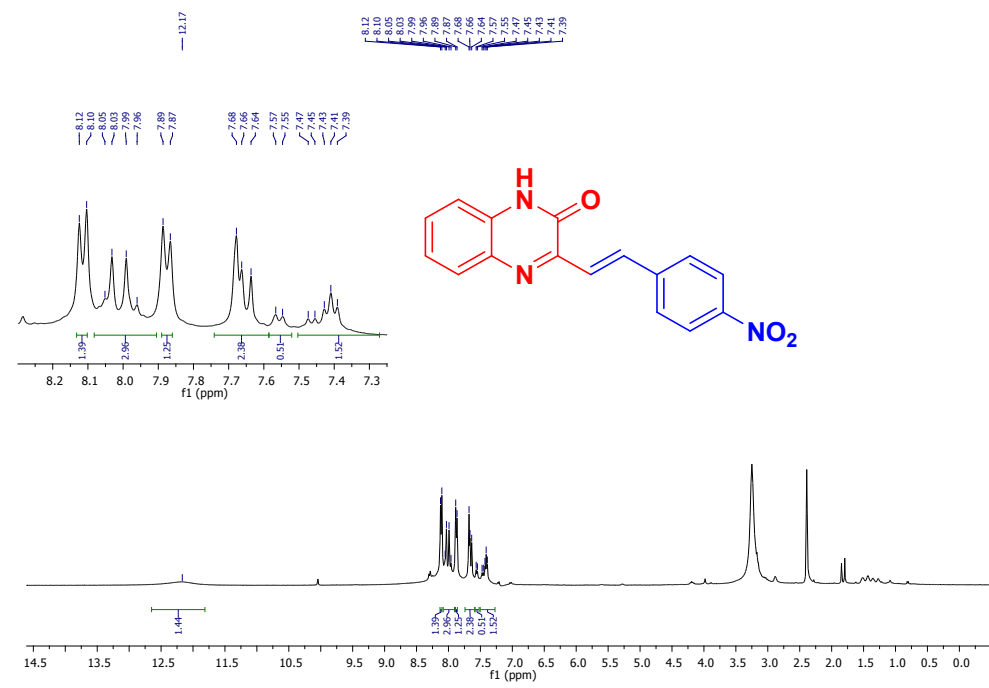


^{13}C NMR spectrum (101 MHz, DMSO- d_6)

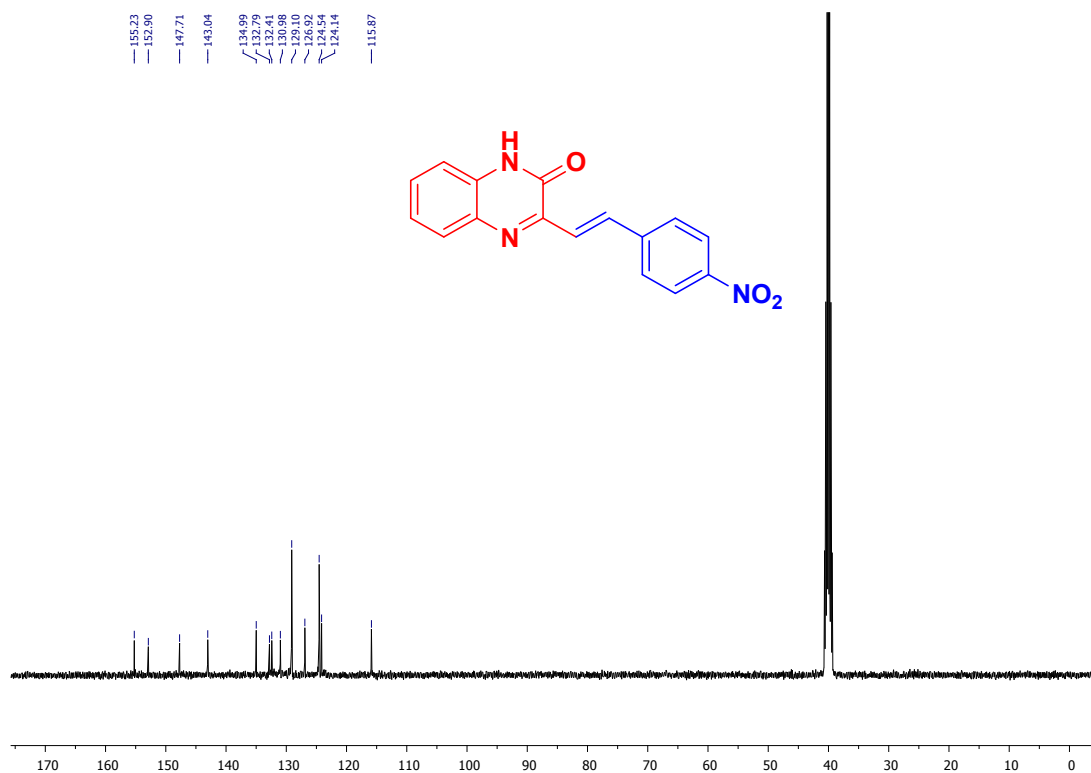


4m. (E)-3-(4-nitrostyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

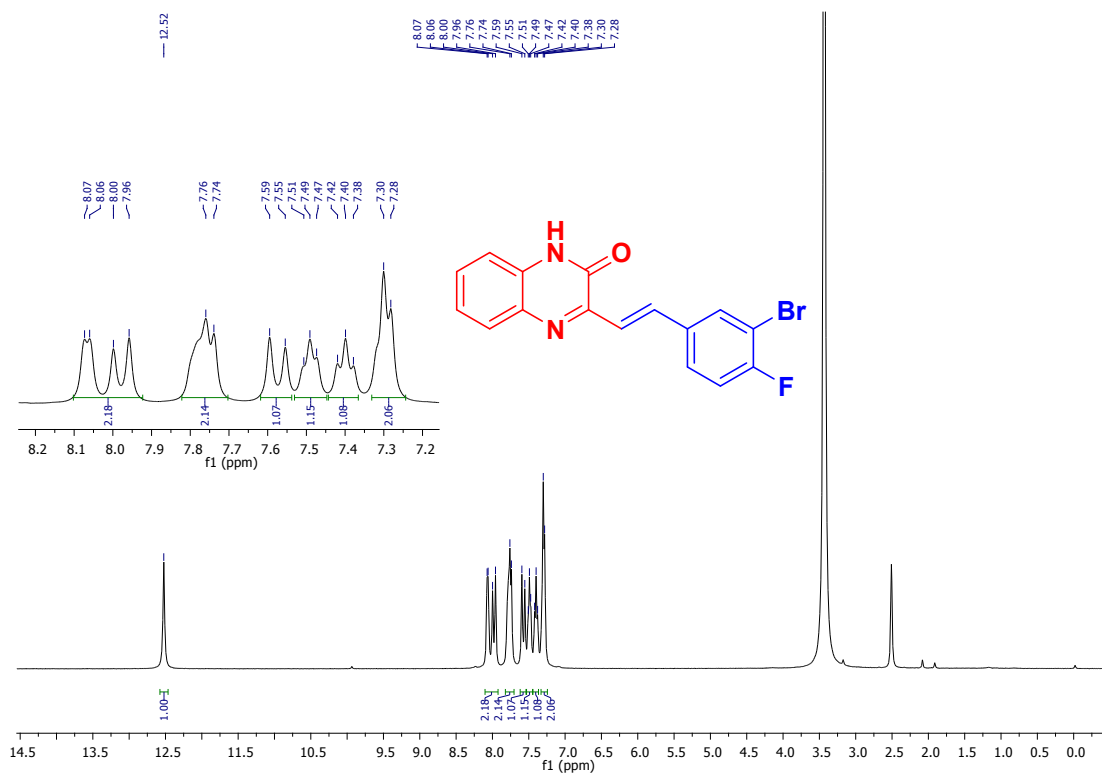


¹³C NMR spectrum (101 MHz, DMSO-d₆)

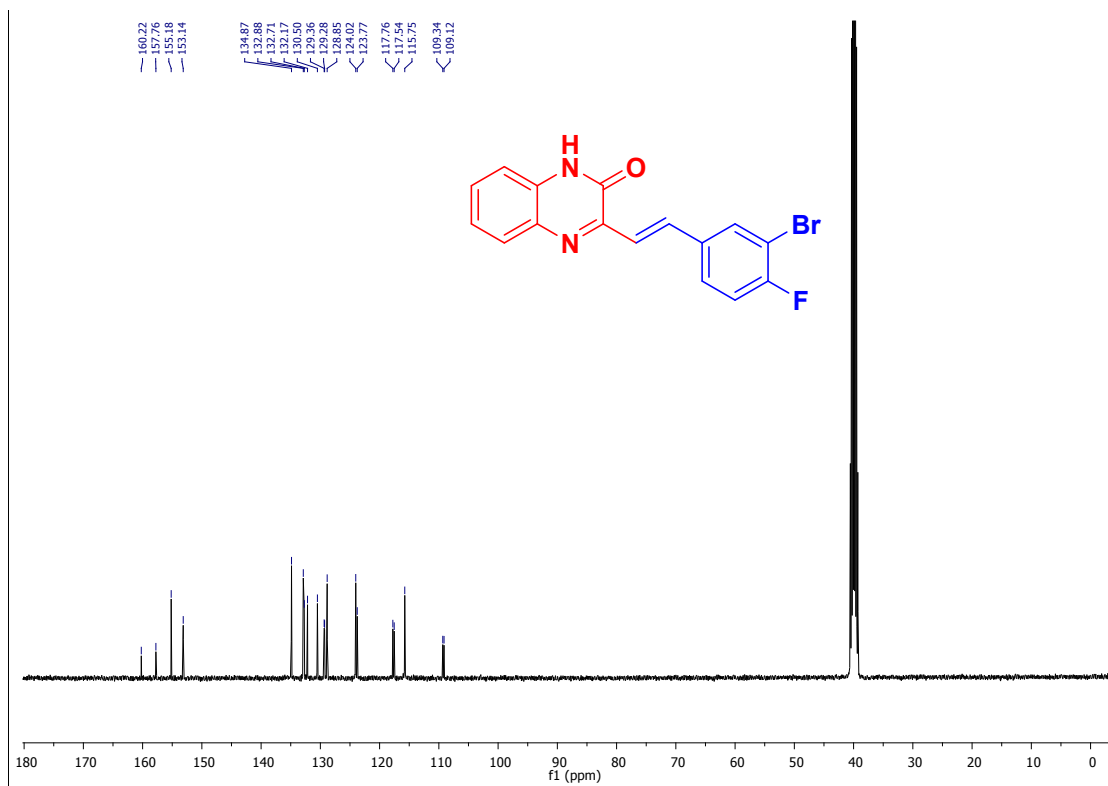


4n. (E)-3-(3-bromo-4-fluorostyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4n

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

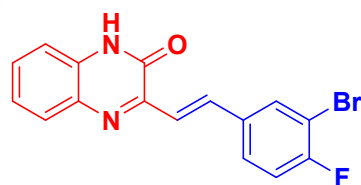
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:32:22 1 / 1

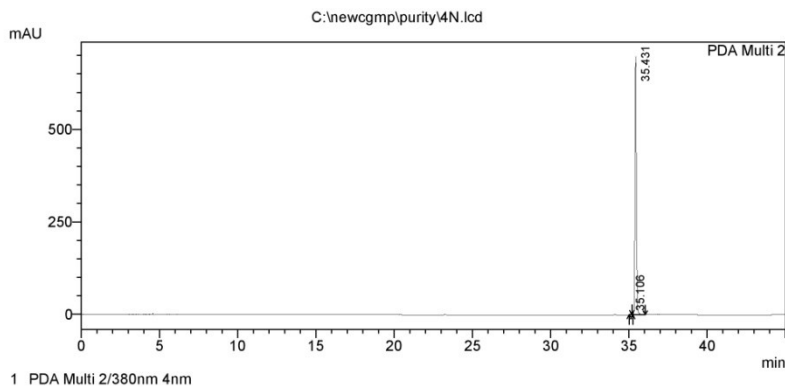
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
Sample Name : 4N
Sample ID : 4N
Tray# : 1
Vial # : 24
Injection Volume : 5 uL
Data File Name : 4N.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 25-11-2019 21:50:10
Data Processed : 25-11-2019 22:35:12

C:\newcgmp\purity\4N.lcd



<Chromatogram>



PeakTable					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	35.106	11021	2103	0.242	0.300
2	35.431	4551543	698094	99.758	99.700
Total		4562564	700198	100.000	100.000

C:\newcgmp\purity\4N.lcd

HRMS Analysis of **4n**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

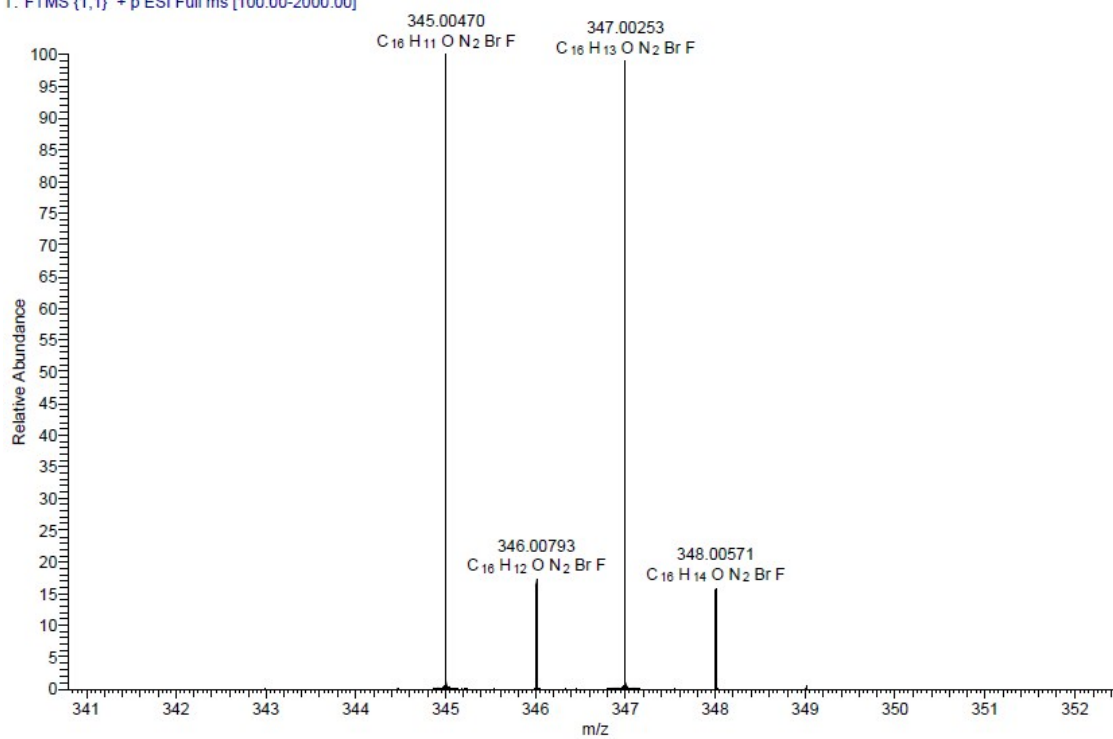
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C



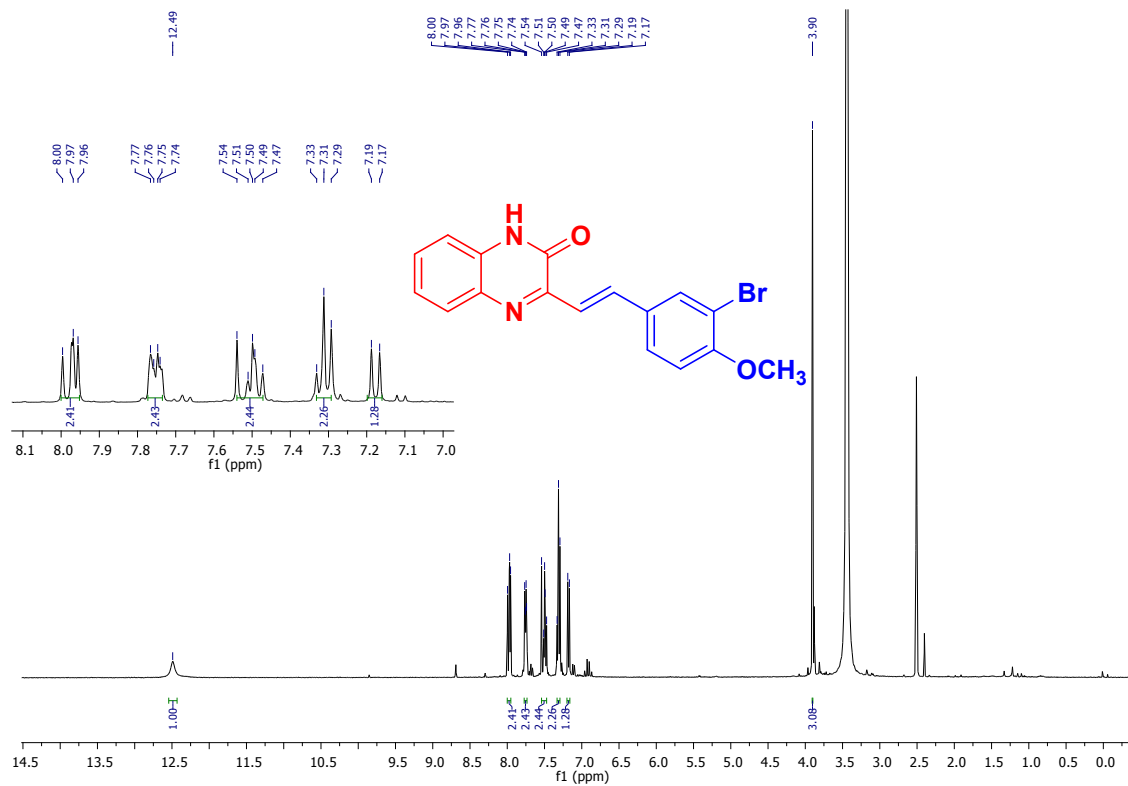
4n #61 RT: 1.28 AV: 1 NL: 4.29E6

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

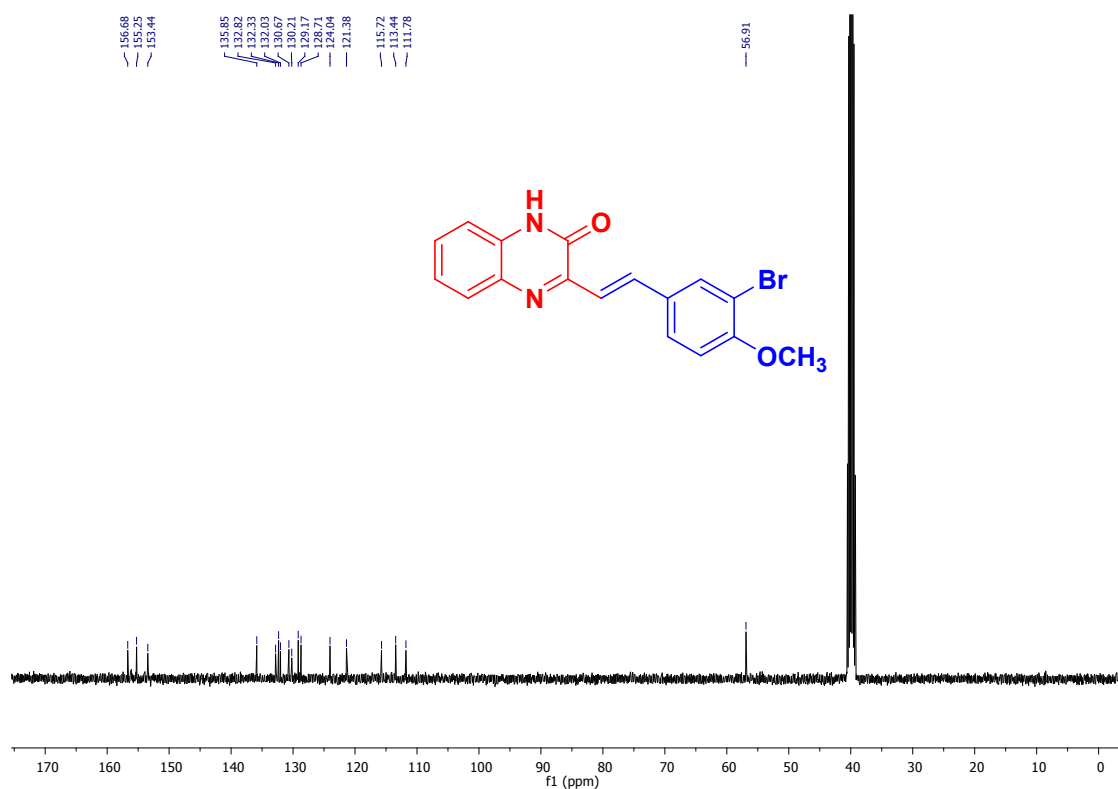


4o. (E)-3-(3-bromo-4-methoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4o

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

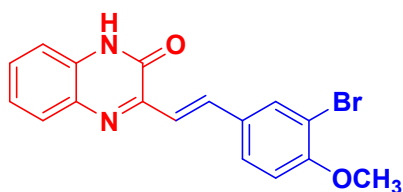
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:30:30 1 / 1

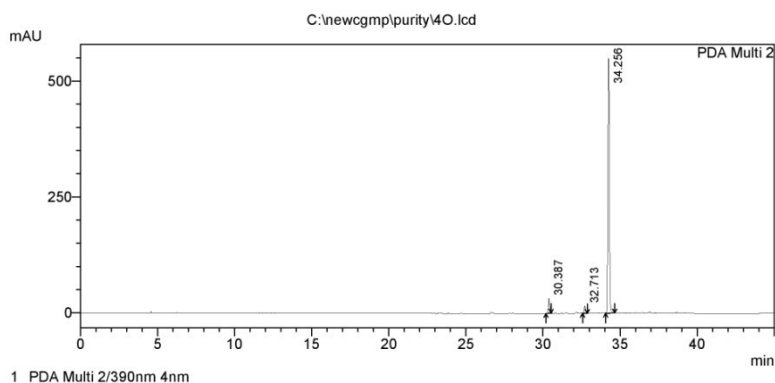
==== Shimadzu LCsolution Analysis Report ====

Acquired by : Admin
Sample Name : 4O
Sample ID : 4O
Tray# : 1
Vial # : 25
Injection Volume : 5 uL
Data File Name : 4O.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 25-11-2019 22:35:40
Data Processed : 25-11-2019 23:20:43

C:\newcgmp\purity\4O.lcd



<Chromatogram>



1 PDA Multi 2/390nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.387	185753	32094	4.854	5.375
2	32.713	99969	15756	2.612	2.639
3	34.256	3541079	549207	92.534	91.986
Total		3826802	597057	100.000	100.000

C:\newcgmp\purity\4O.lcd

HRMS Analysis of **4o**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

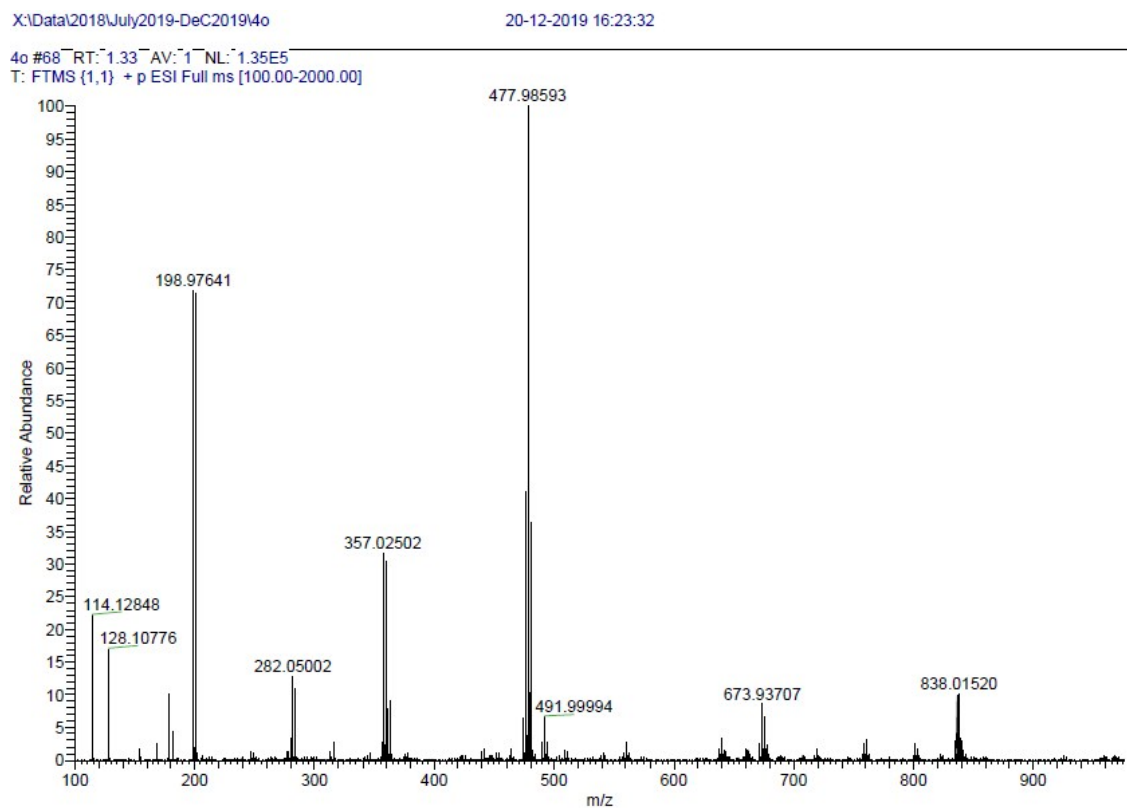
Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

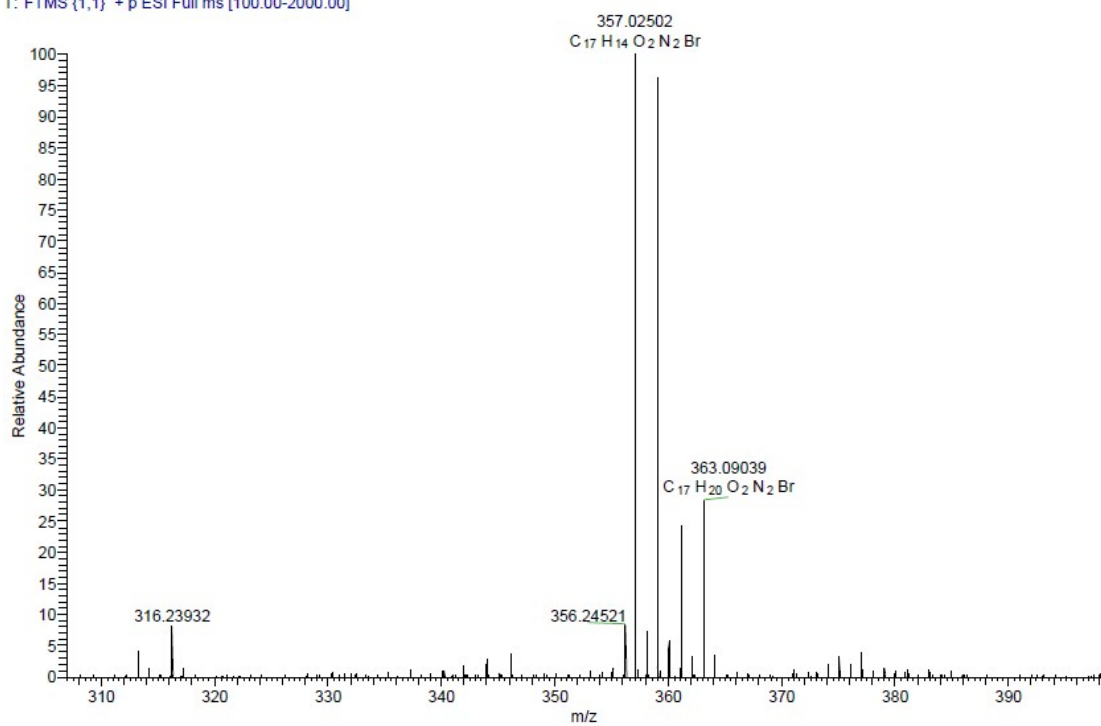
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C



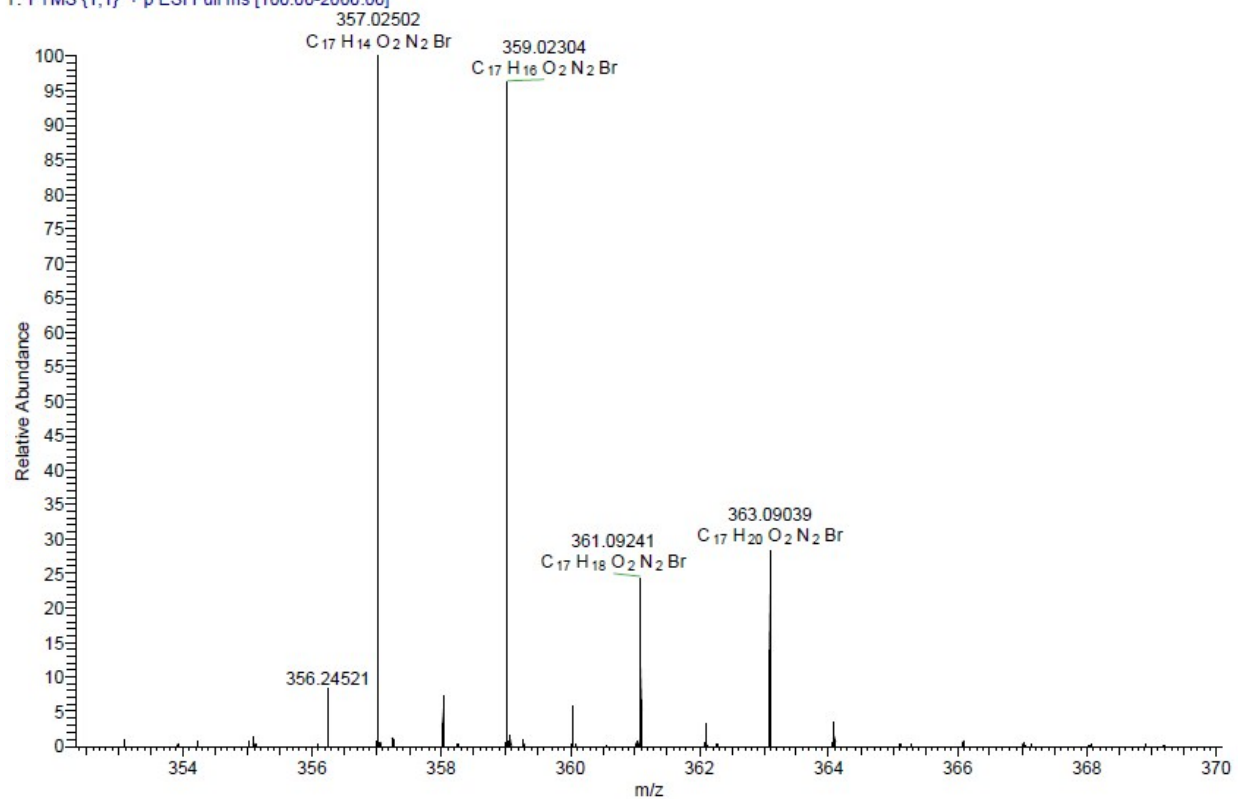
4o #68 RT: 1.33 AV: 1 NL: 4.25E4

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]



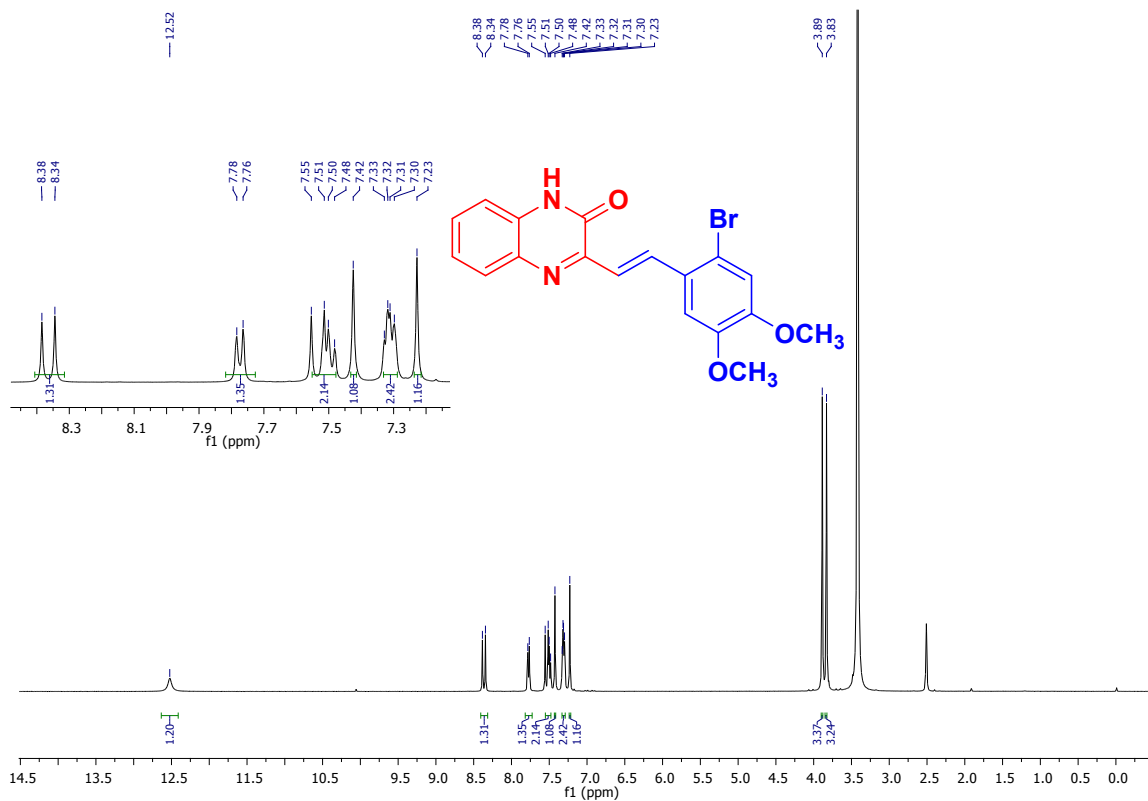
4o #68 RT: 1.33 AV: 1 NL: 4.25E4

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

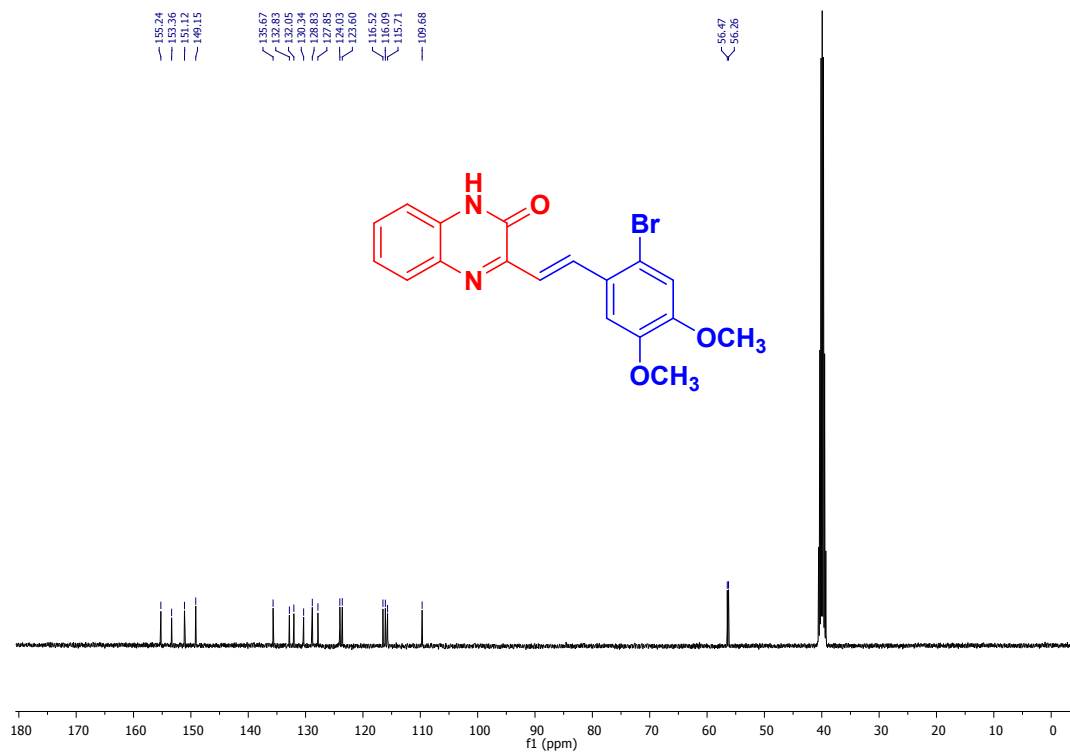


4p. *(E)*-3-(2-bromo-4,5-dimethoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4p

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

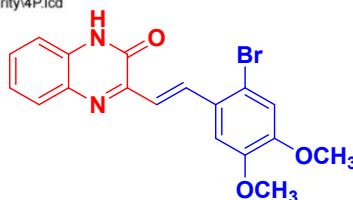
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

28-11-2019 17:13:23 1 / 1

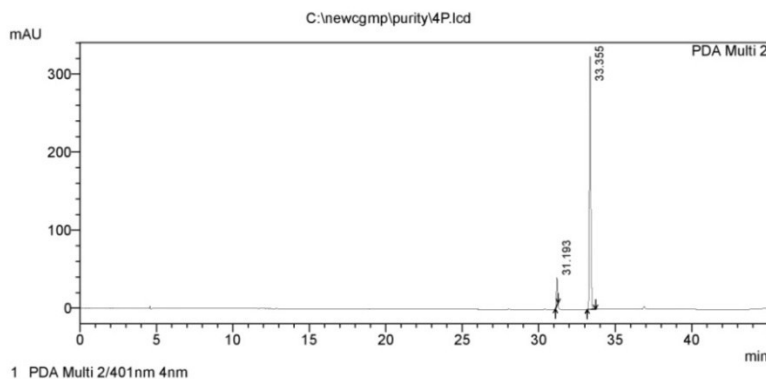
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
Sample Name : 4P
Sample ID : 4P
Tray# : 1
Vial # : 26
Injection Volume : 5 µL
Data File Name : 4P.Lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 25-11-2019 23:21:11
Data Processed : 26-11-2019 10:27:39

C:\newcgm\purity\4P.Lcd



<Chromatogram>



PeakTable					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	31.193	188291	35723	8.470	9.936
2	33.355	2034752	323807	91.530	90.064
Total		2223043	359530	100.000	100.000

C:\newcgm\purity\4P.Lcd

HRMS Analysis of **4p**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

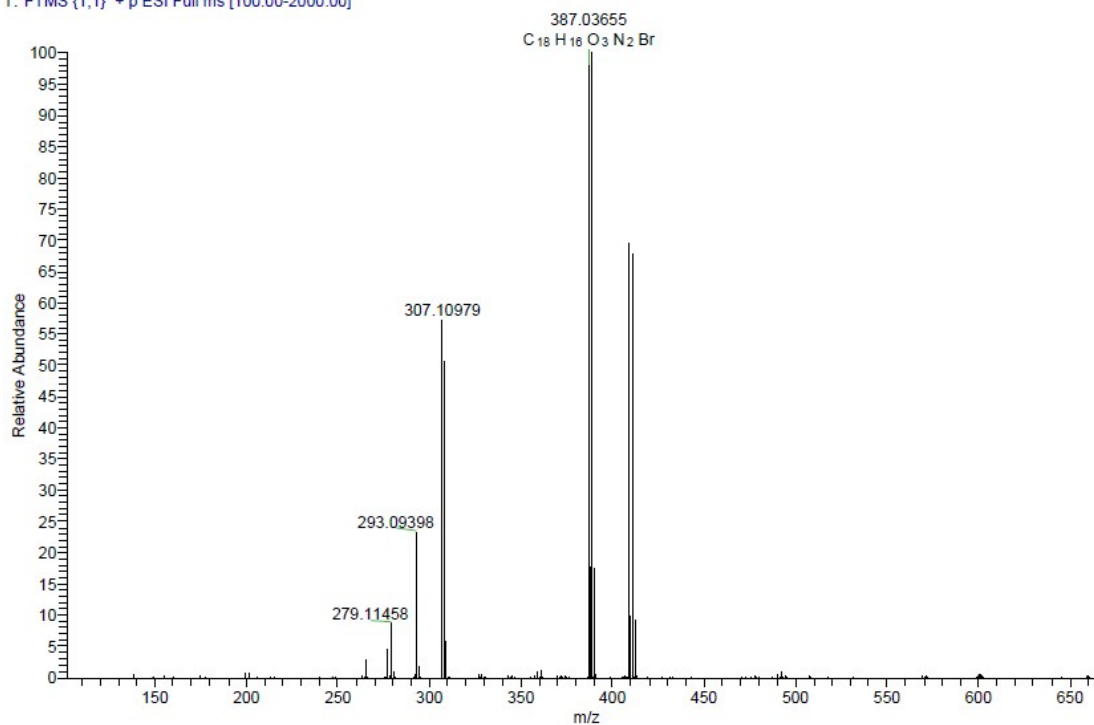
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

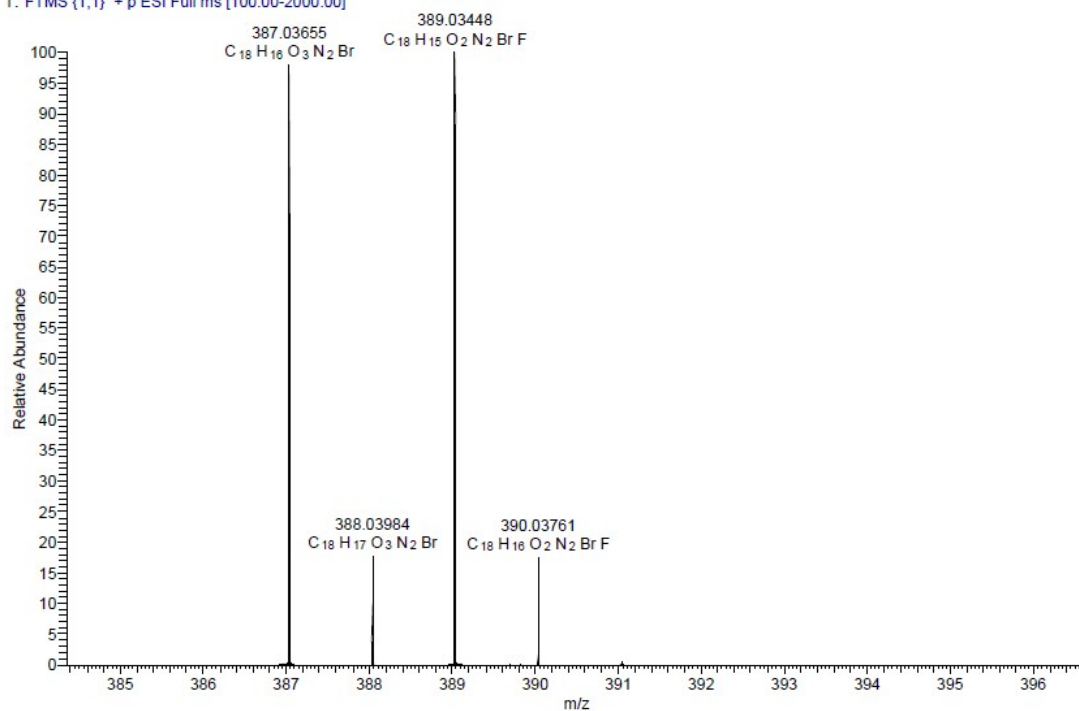
X:\Data\2018\July2019-DeC2019\4p

20-12-2019 16:29:14

4p #53-62 RT: 1.13-1.27 AV: 10 NL: 1.35E6
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

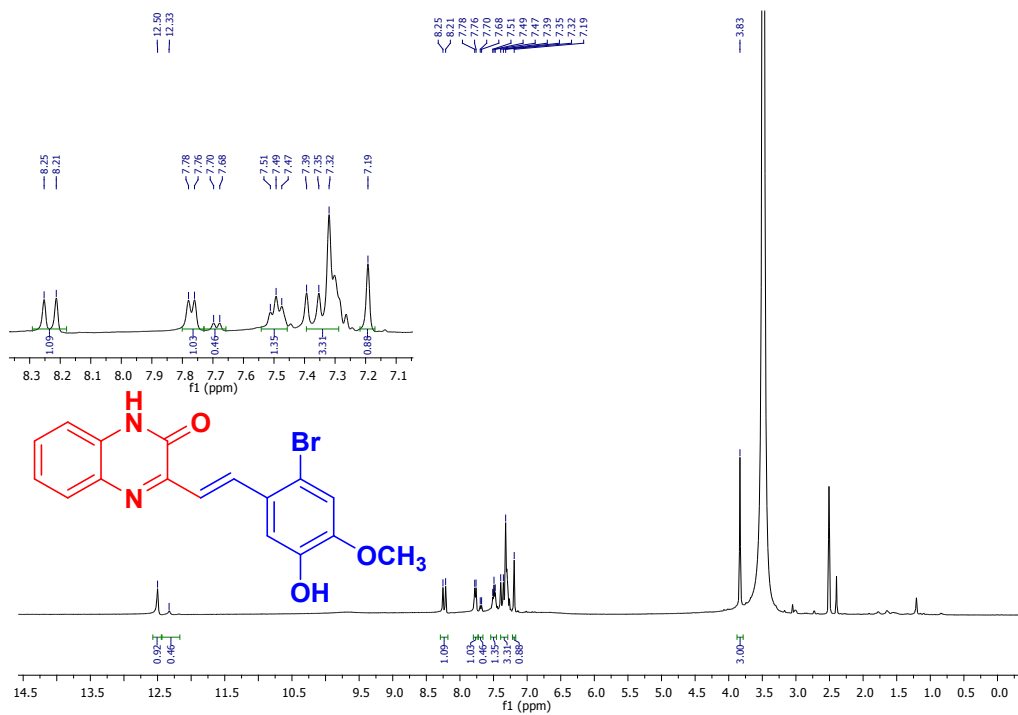


4p #53-62 RT: 1.13-1.27 AV: 10⁻¹ NL: 1.35E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]

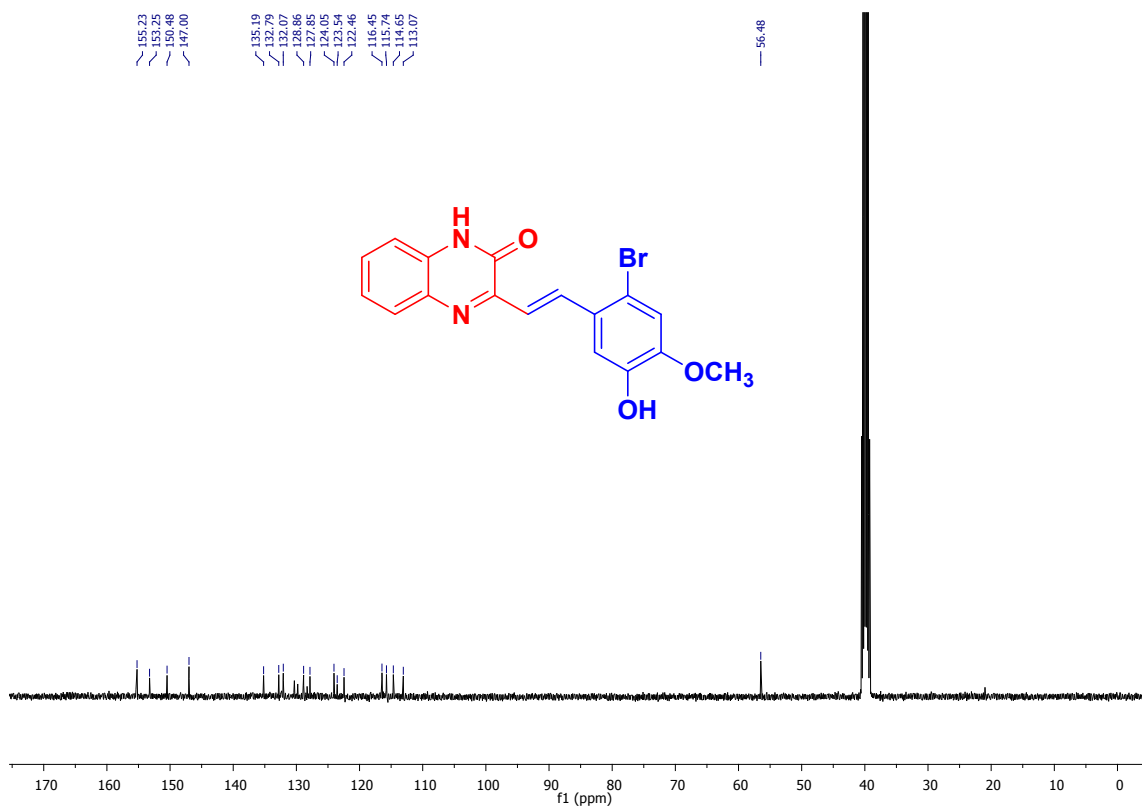


4q. (E)-3-(2-bromo-5-hydroxy-4-methoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4q

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

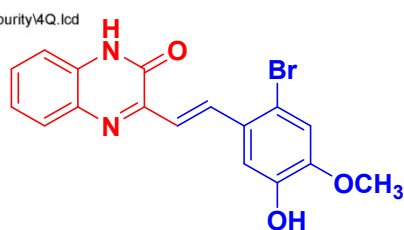
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:24:16 1 / 1

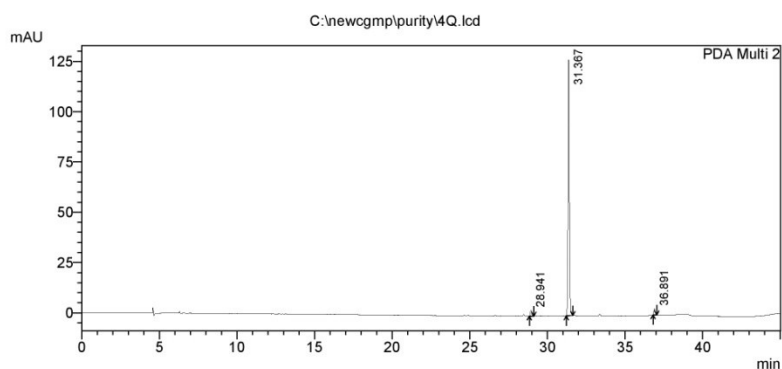
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
 Sample Name : 4Q
 Sample ID : 4Q
 Tray# : 1
 Vial # : 27
 Injection Volume : 5 uL
 Data File Name : 4Q.lcd
 Method File Name : purity.lcm
 Batch File Name : nancy compound purity.lcb
 Report File Name : Default.lcr
 Data Acquired : 26-11-2019 00:06:41
 Data Processed : 26-11-2019 00:51:43

C:\newcgmp\purity\4Q.lcd



<Chromatogram>



1 PDA Multi 2/401nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	28.941	17003	2924	2.133	2.196
2	31.367	760286	127178	95.396	95.492
3	36.891	19687	3079	2.470	2.312
Total		796976	133182	100.000	100.000

C:\newcgmp\purity\4Q.lcd

HRMS Analysis of **4q**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

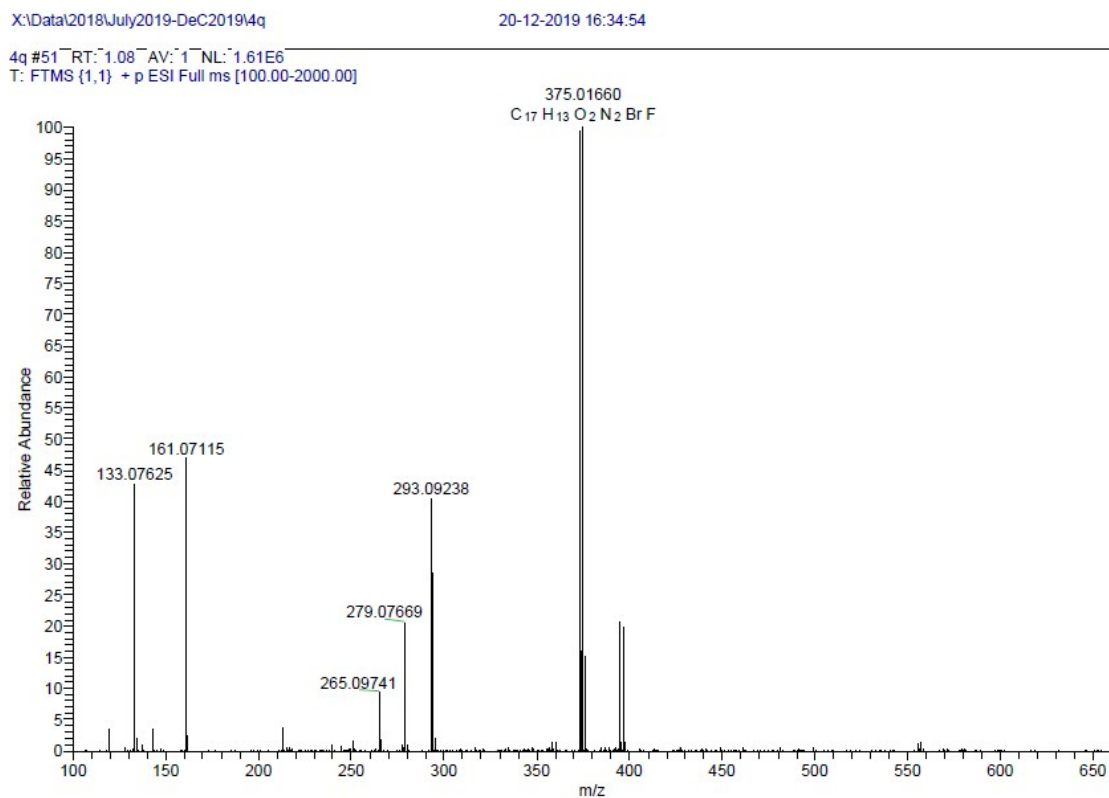
Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150µl /minute

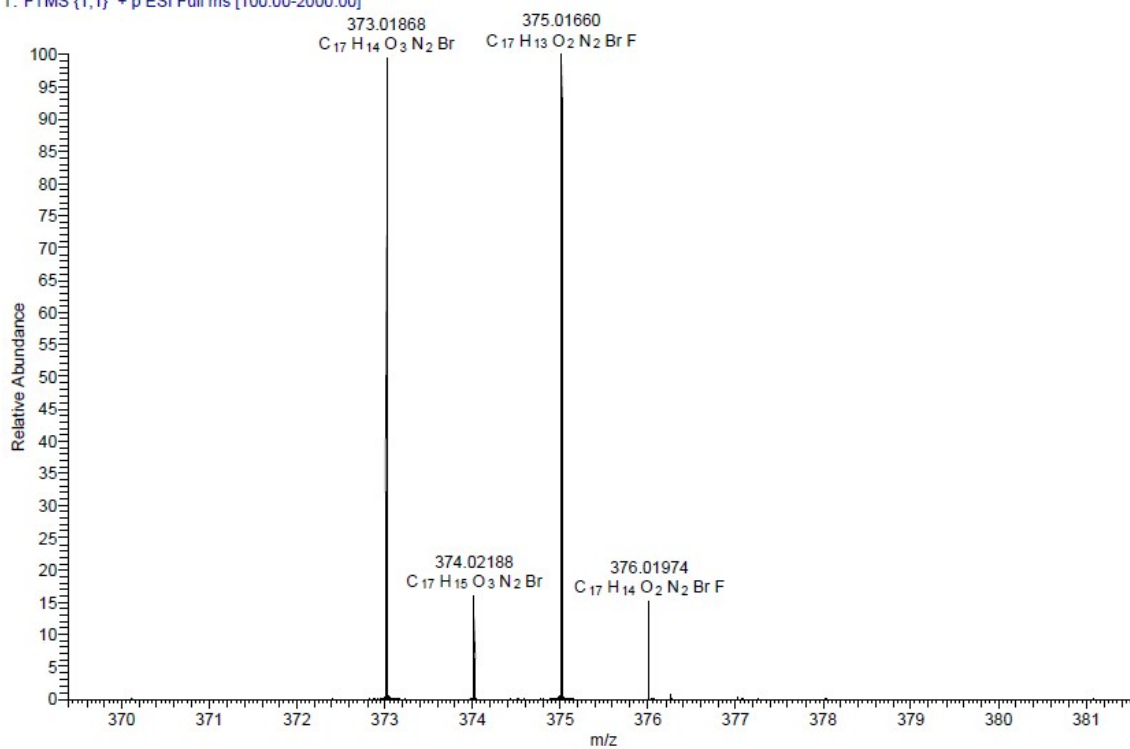
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C



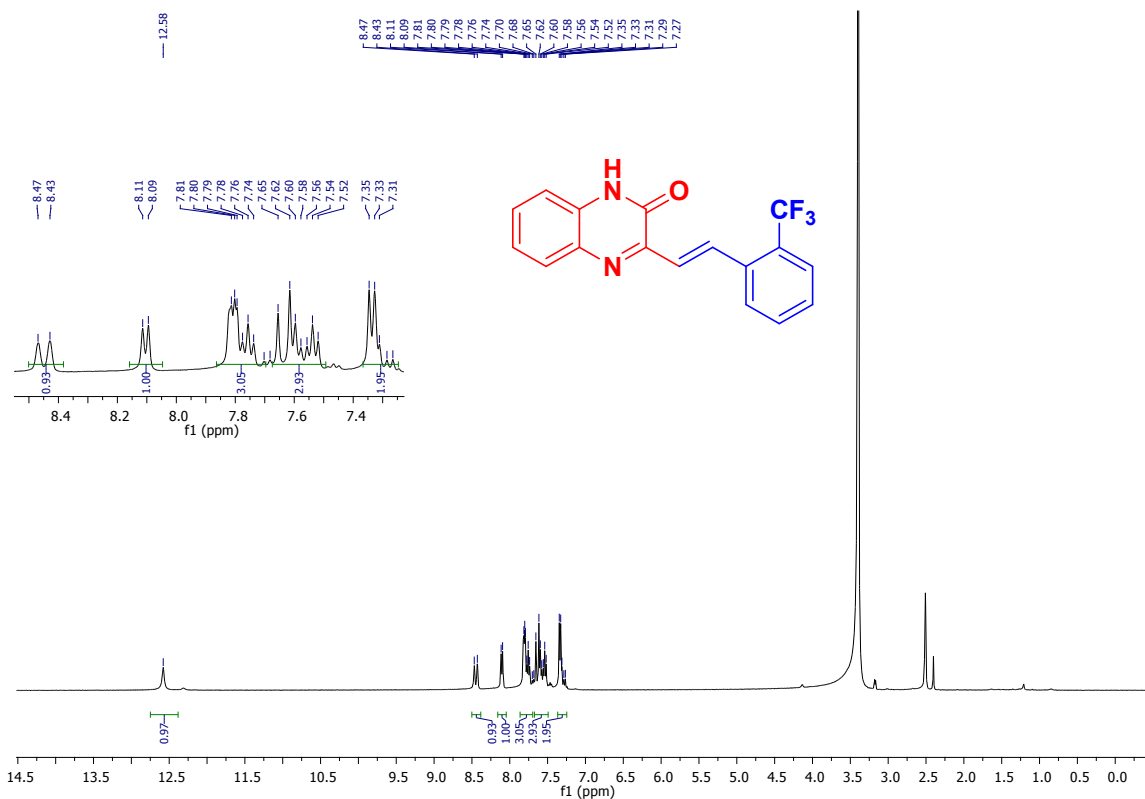
4q #51 RT: 1.08 AV: 1 NL: 1.61E6

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

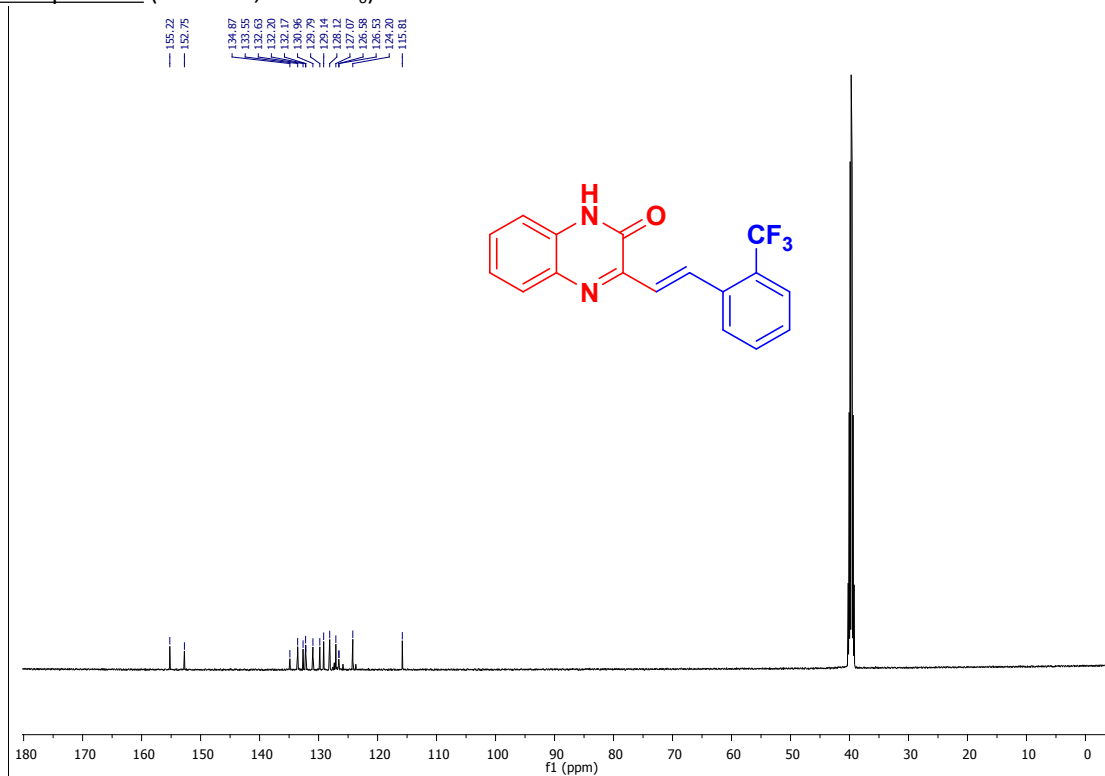


4r. (E)-3-(2-(trifluoromethyl)styryl)quinoxalin-2(1H)-one

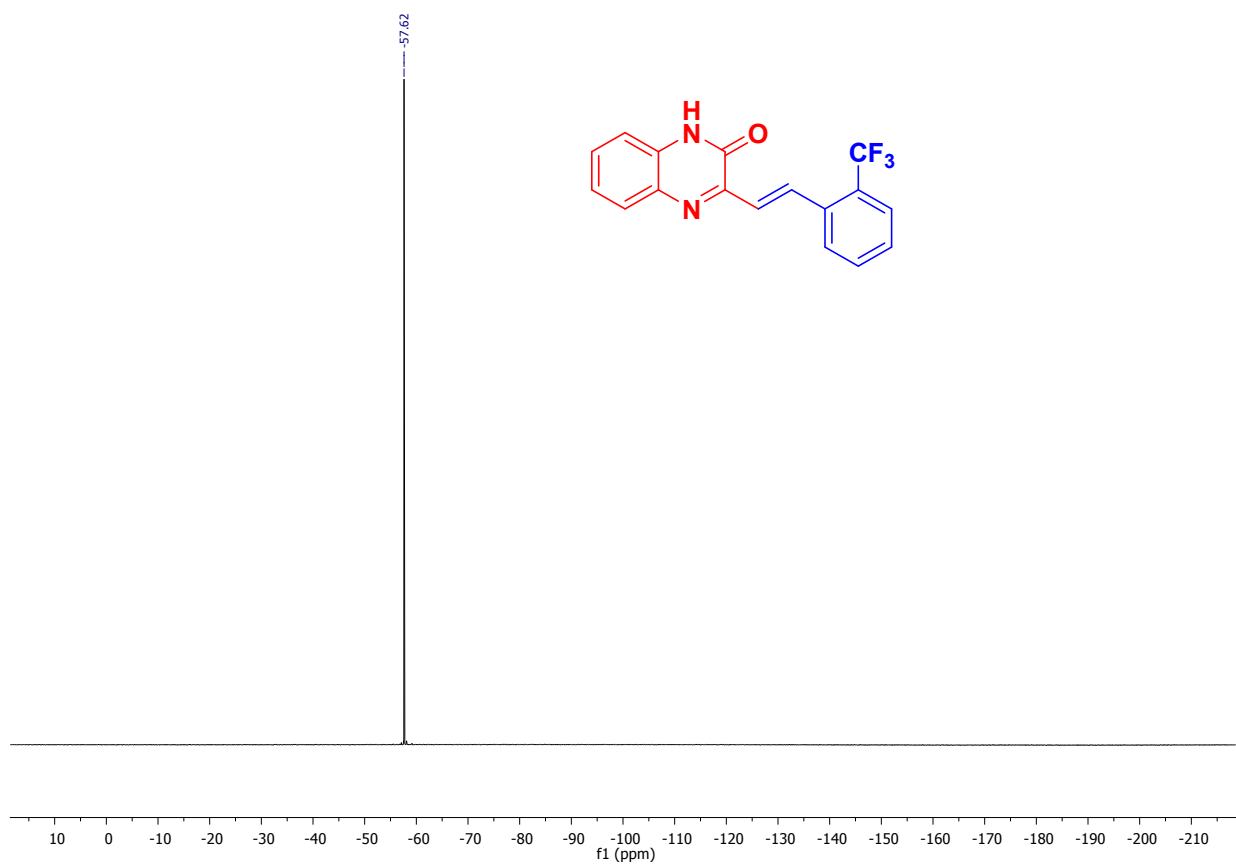
¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



¹⁹F NMR spectrum (376 MHz, DMSO-d₆)



HPLC Analysis of 4r

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 μ , 250 $\times$ 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 μ L. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

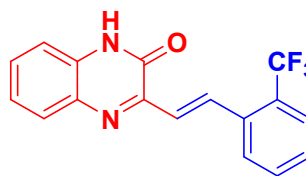
Table 2 : Parameters used in HPLC purity check.

Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

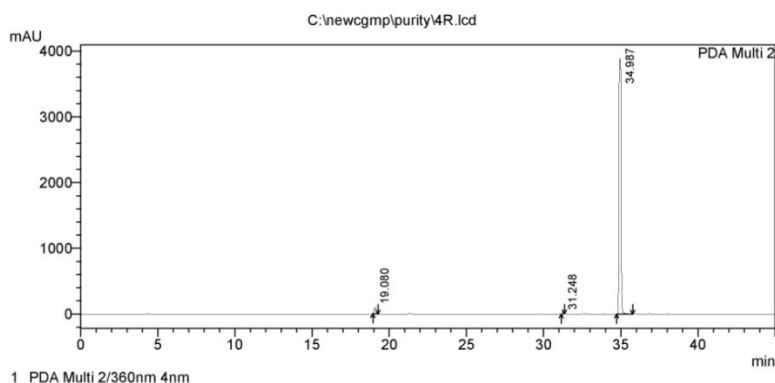
26-11-2019 10:19:41 1 / 1

==== Shimadzu LCsolution Analysis Report ====

Acquired by : Admin
Sample Name : 4R
Sample ID : 4R
Tray# : 1
Vial # : 28
Injection Volume : 10 μ L
Data File Name : 4R.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 26-11-2019 00:52:14
Data Processed : 26-11-2019 01:37:16



<Chromatogram>



1 PDA Multi 2/360nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	19.080	841793	103453	2.309	2.584
2	31.248	102596	19554	0.281	0.488
3	34.987	35516422	3880803	97.410	96.928
Total		36460811	4003809	100.000	100.000

C:\newcgmp\purity\4R.lcd

HRMS Analysis of **4r**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

The source was operated in both positive and negative mode at an ion spray voltage of 3KV

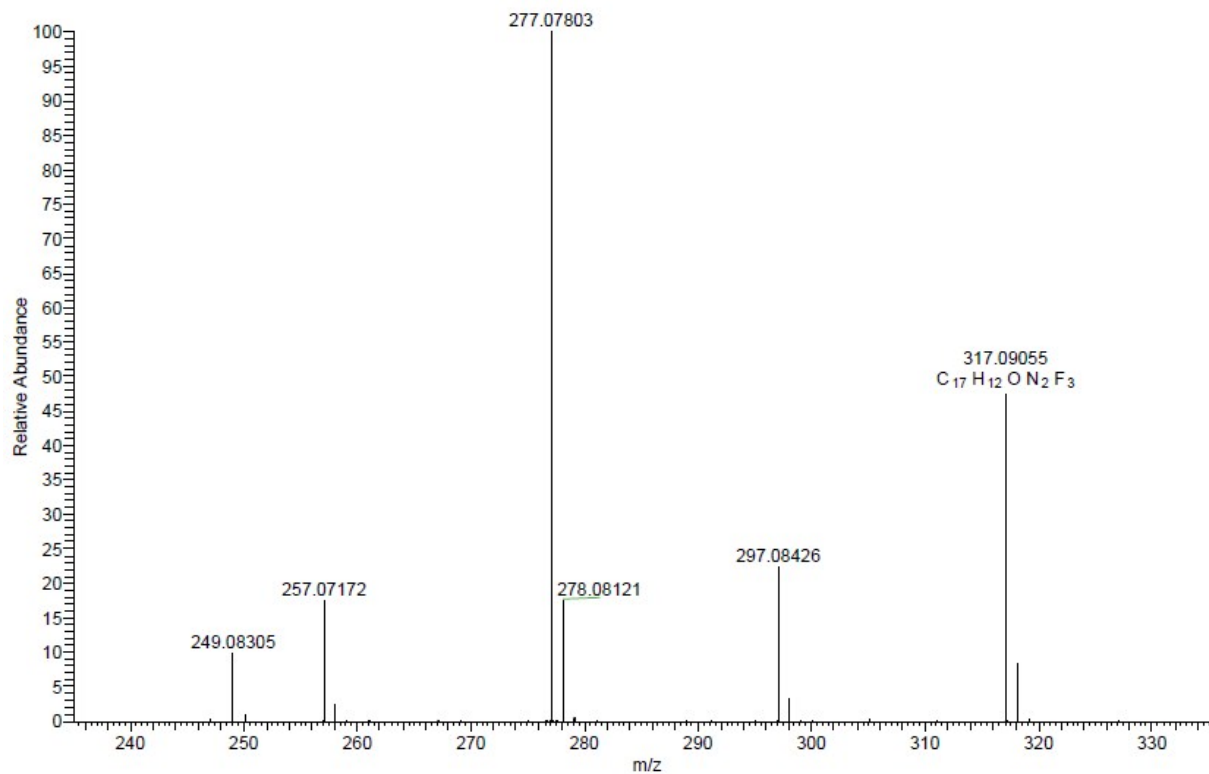
Oven temperature was set to 30°C

X:\Data\2018\July2019-DeC2019\4r

20-12-2019 16:40:34

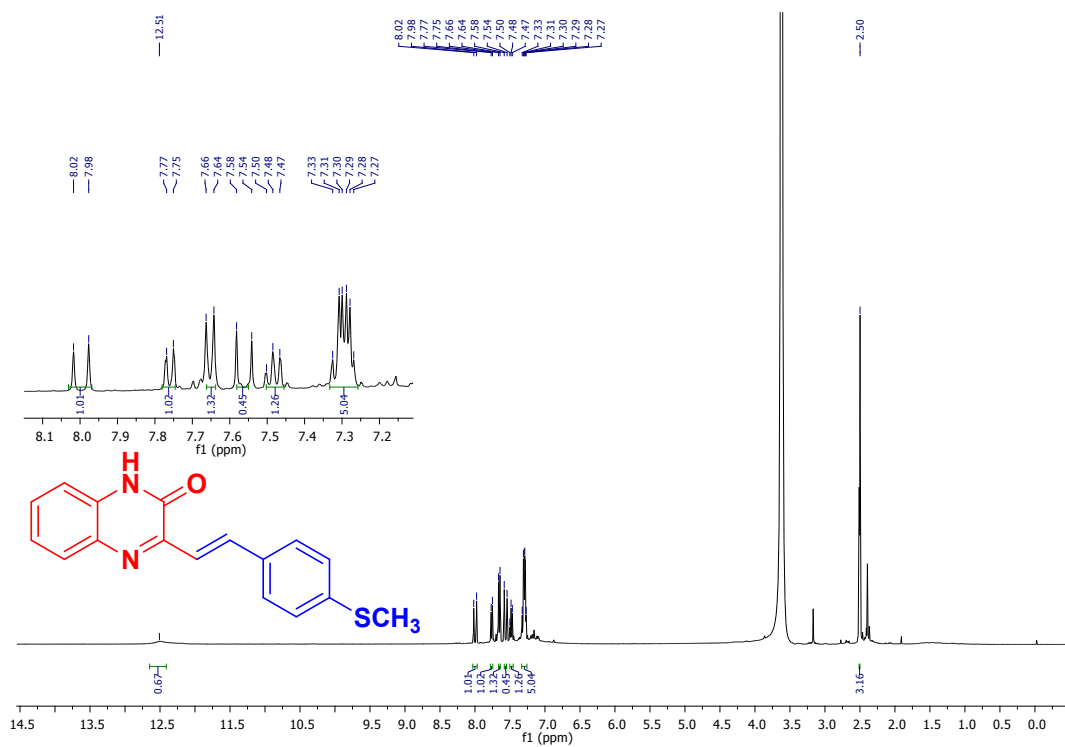
4r #58 RT: 1.19 AV: 1 NL: 1.44E7

T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

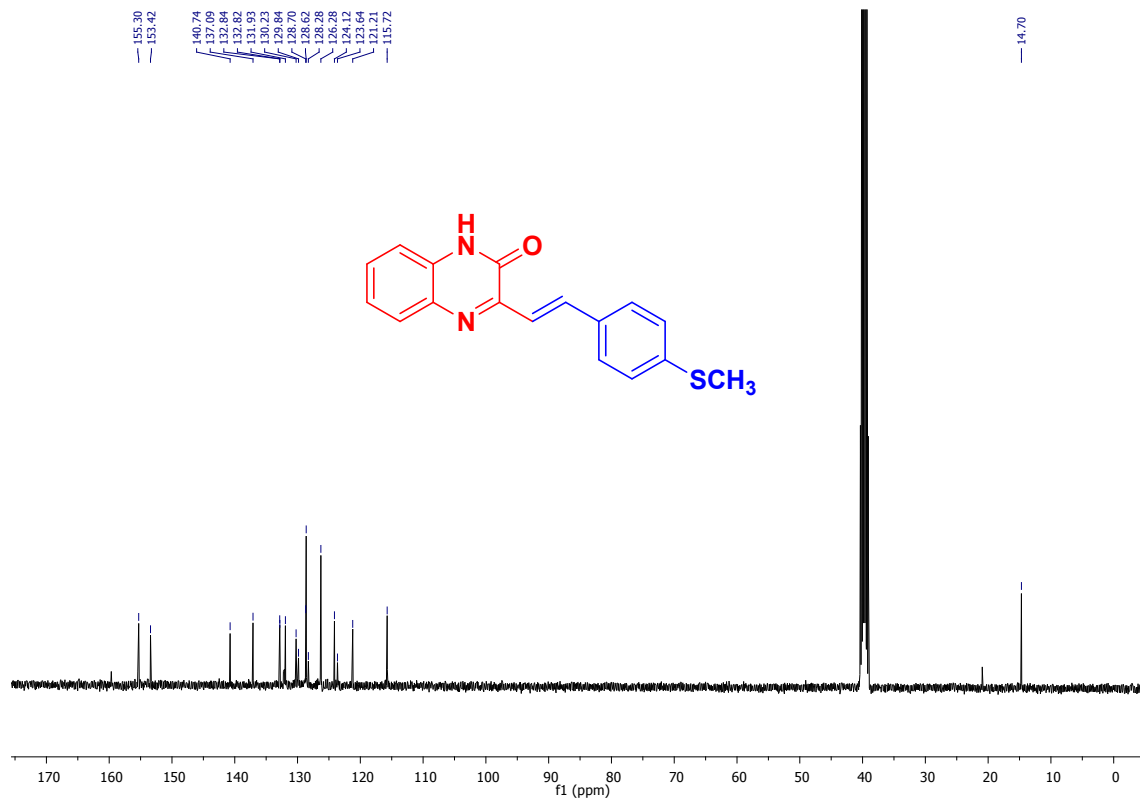


4s. (E)-3-(4-(methylthio)styryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4s

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

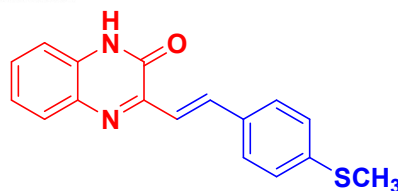
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

04-02-2020 16:26:19 1 / 1

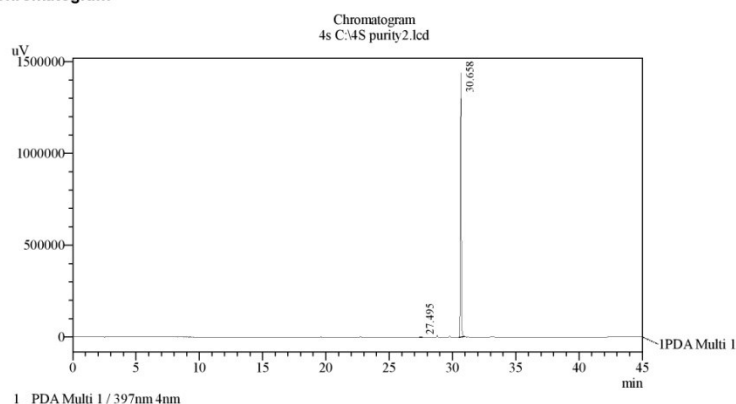
==== Shimadzu LCsolution Analysis Report ====

Acquired by : Admin
Sample Name : 4s
Sample ID : 4s
Tray# : 1
Vial # : 40
Injection Volume : 10 uL
Data File Name : 4s_purity2.lcd
Method File Name : purity.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 04-02-2020 15:33:28
Data Processed : 04-02-2020 16:18:31

C:\4S_purity2.lcd



<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	27.495	2712	651	0.039	0.045
2	30.658	7015045	1437867	99.961	99.955
Total		7017757	1438518	100.000	100.000

C:\4S_purity2.lcd

HRMS Analysis of **4s**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

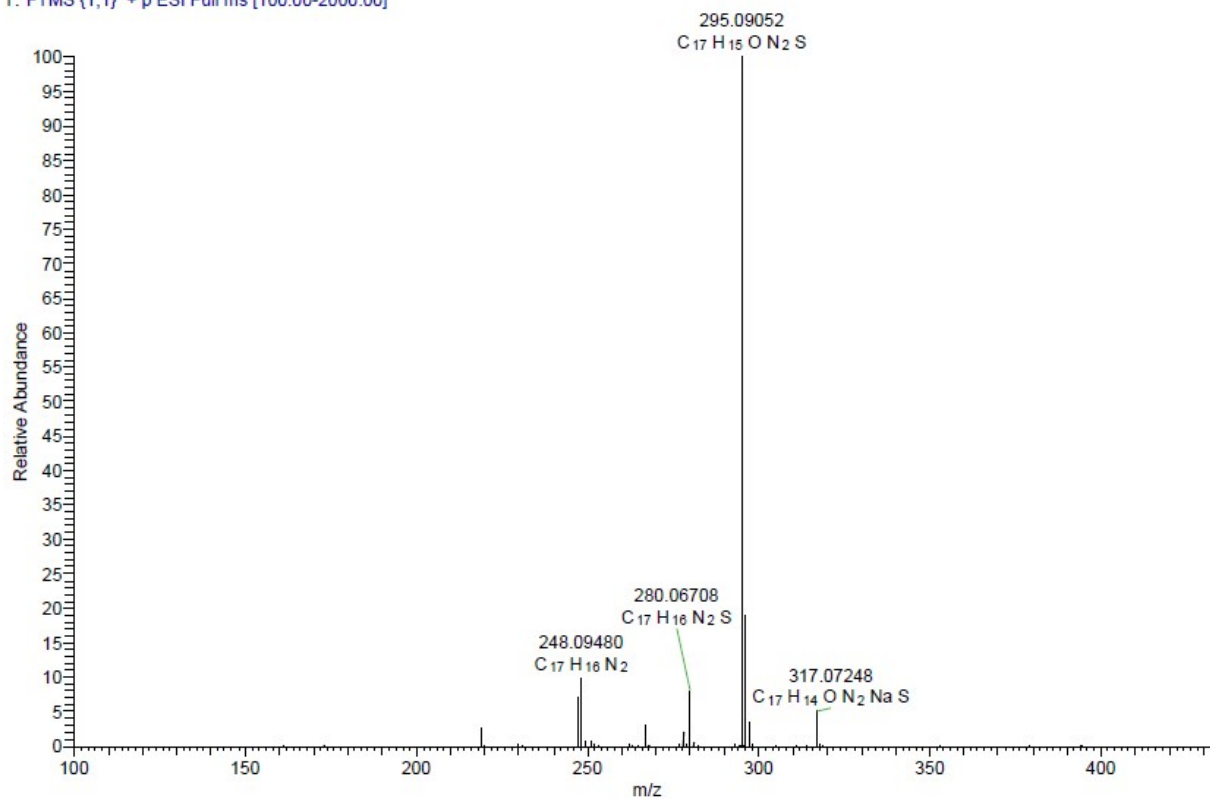
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

X:\Data\2018\July2019-DeC2019\4s

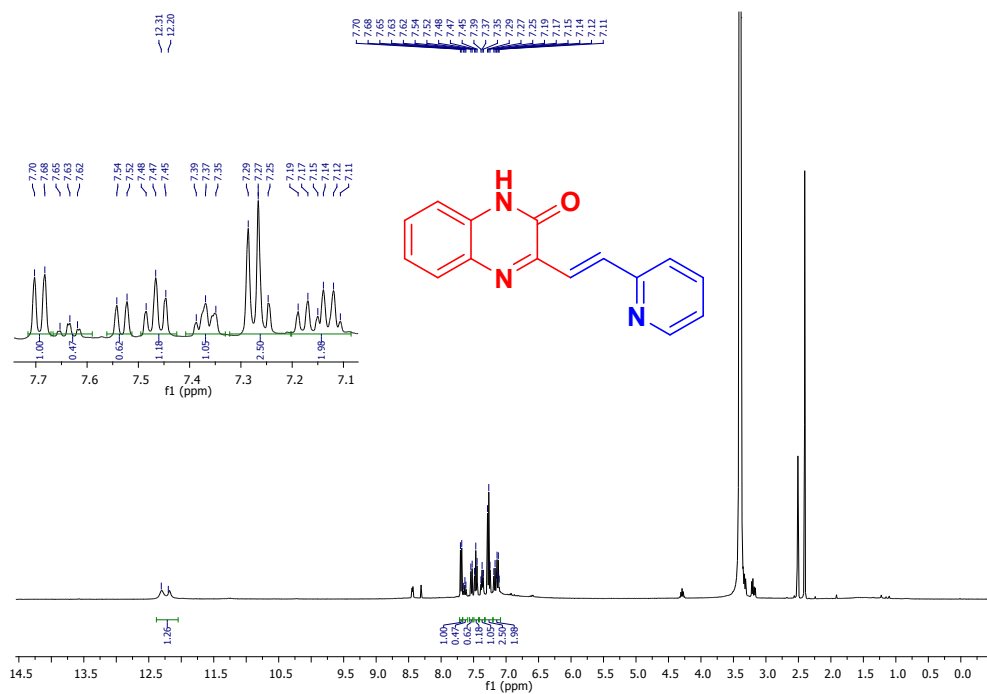
20-12-2019 16:46:16

4s #56 RT: 1.16 AV: 1 NL: 5.57E6
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

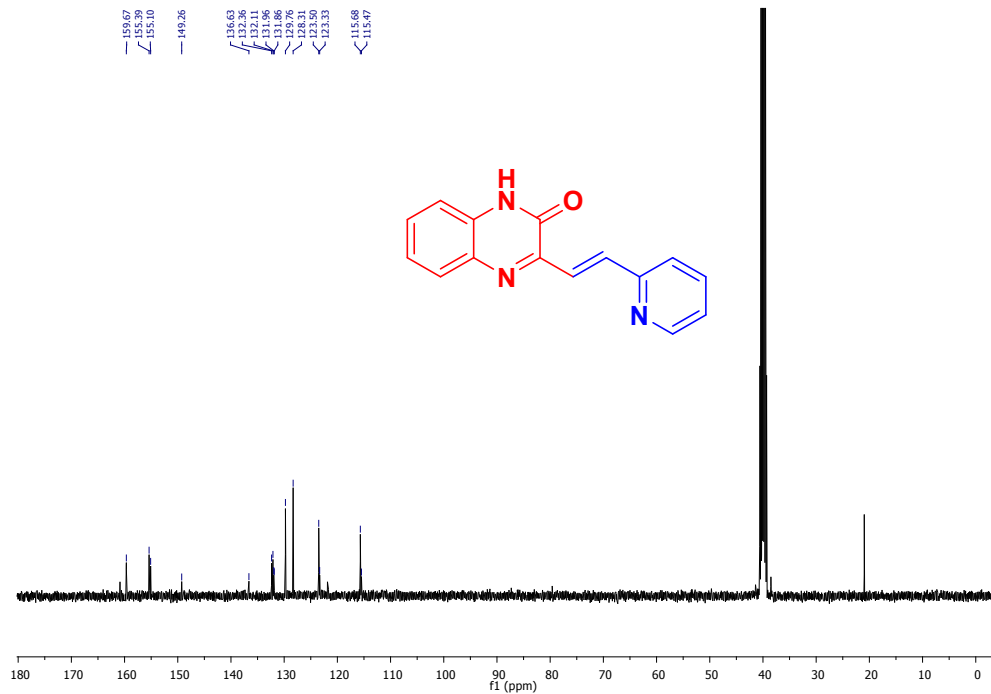


4t. (E)-3-(2-(pyridin-2-yl)vinyl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

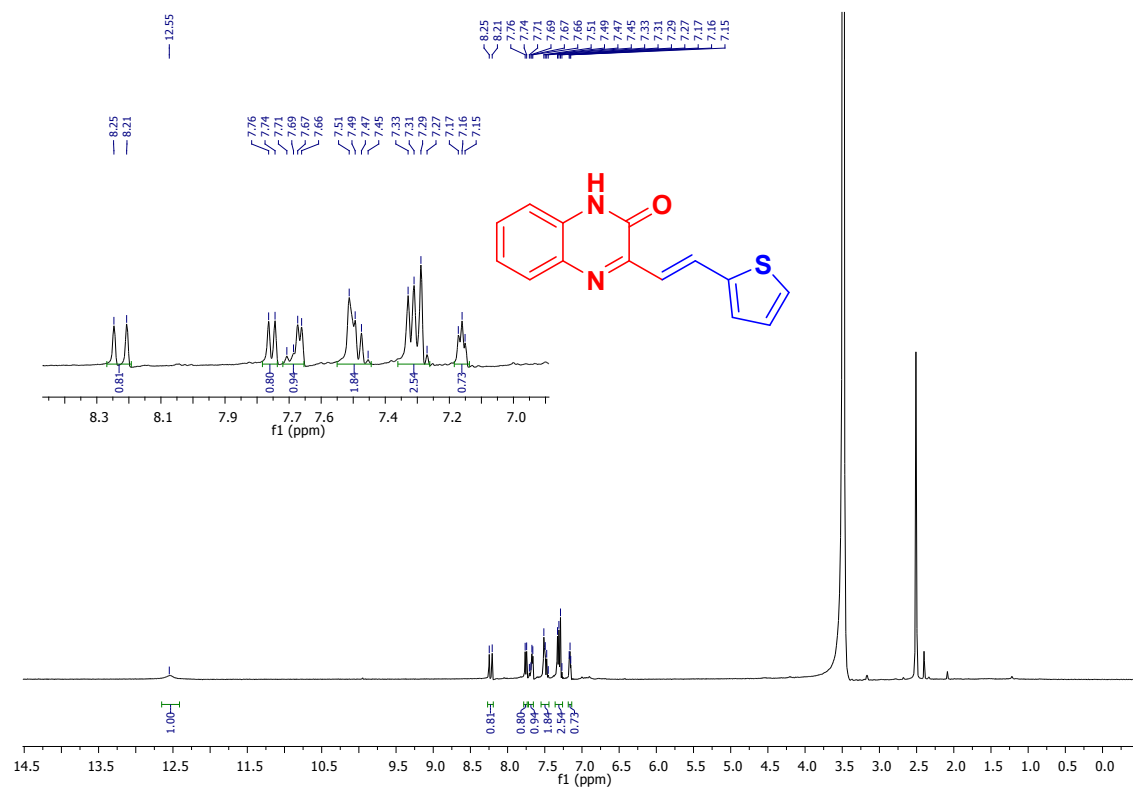


¹³C NMR spectrum (101 MHz, DMSO-d₆)

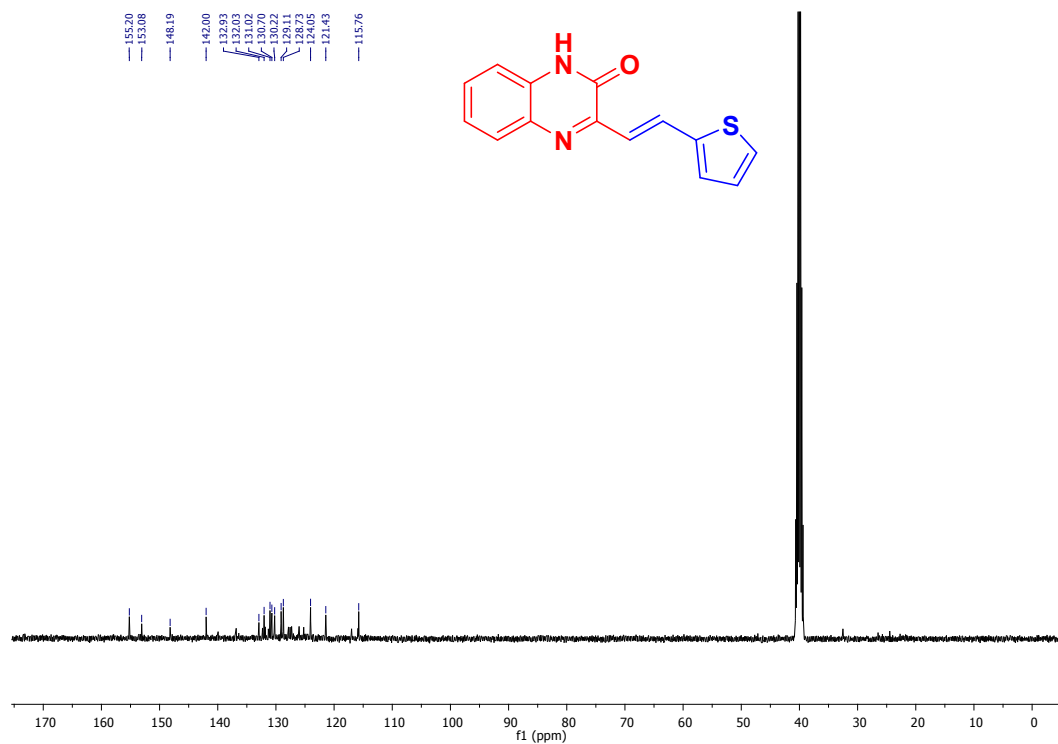


4u. (E)-3-(2-(thiophen-2-yl)vinyl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

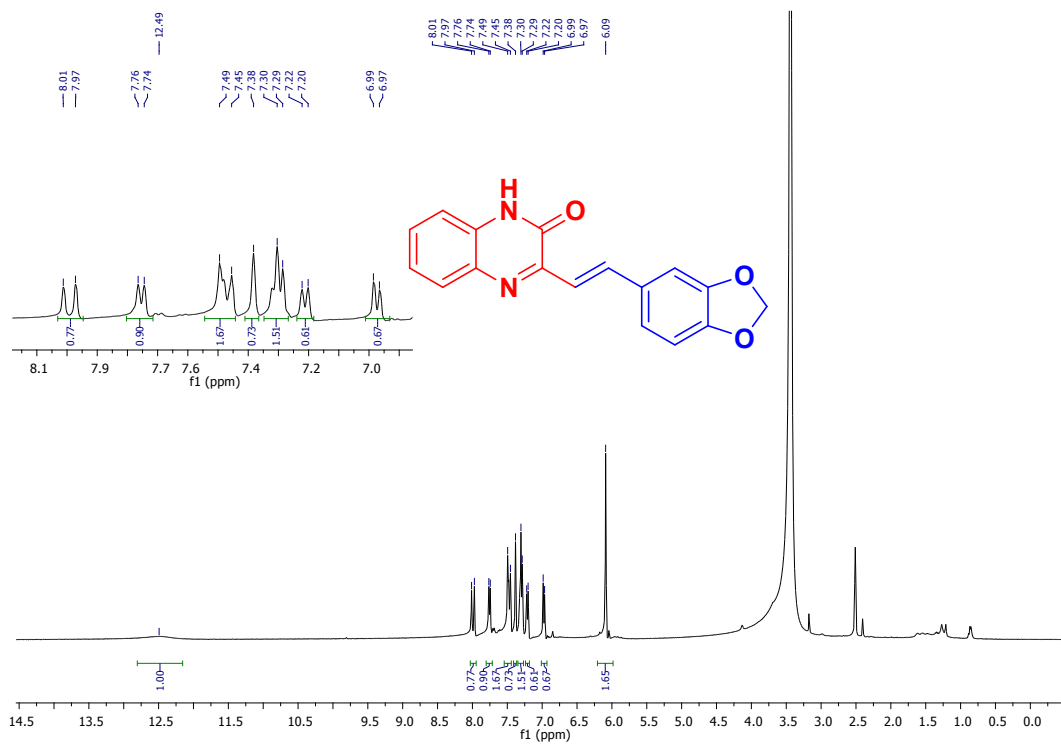


¹³C NMR spectrum (101 MHz, DMSO-d₆)

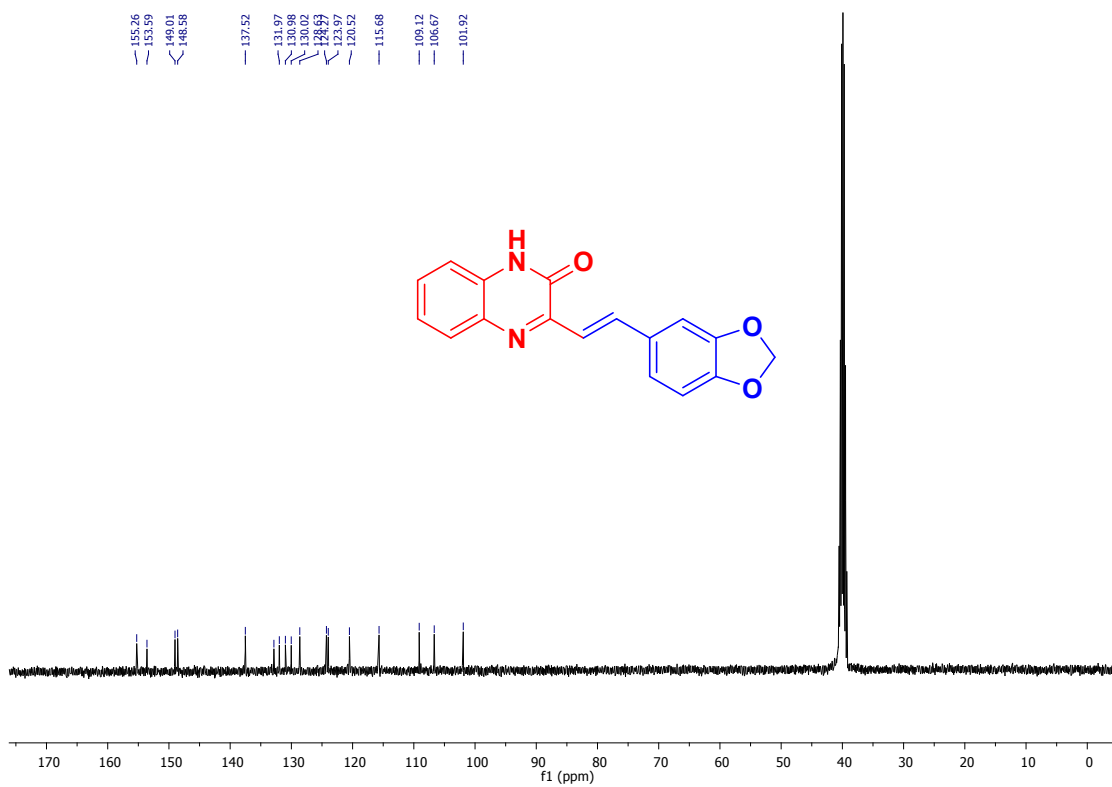


4v. (E)-3-(2-(benzo[d][1,3]dioxol-5-yl)vinyl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4v

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 μ , 250 $\times$ 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 μ L. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

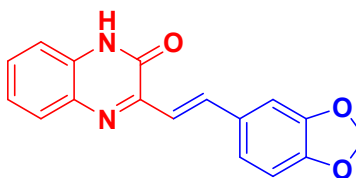
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:11:25 1 / 1

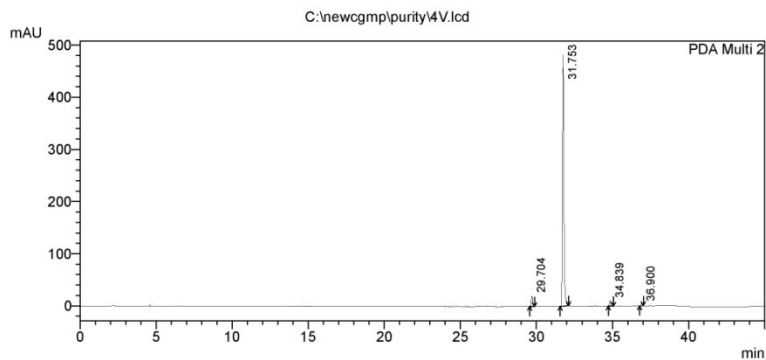
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
Sample Name : 4V
Sample ID : 4V
Tray# : 1
Vial # : 30
Injection Volume : 5 μ L
Data File Name : 4V.lcd
Method File Name : purity.lcm
Batch File Name : nancy compound purity.lcb
Report File Name : Default.lcr
Data Acquired : 26-11-2019 02:23:15
Data Processed : 26-11-2019 10:06:52

C:\newcgmp\purity4V.lcd



<Chromatogram>



1 PDA Multi 2/400nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	29.704	128838	20492	3.636	3.972
2	31.753	3322481	481271	93.777	93.294
3	34.839	70407	10686	1.987	2.071
4	36.900	21235	3416	0.599	0.662
Total		3542961	515865	100.000	100.000

C:\newcgmp\purity4V.lcd

HRMS Analysis of **4v**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

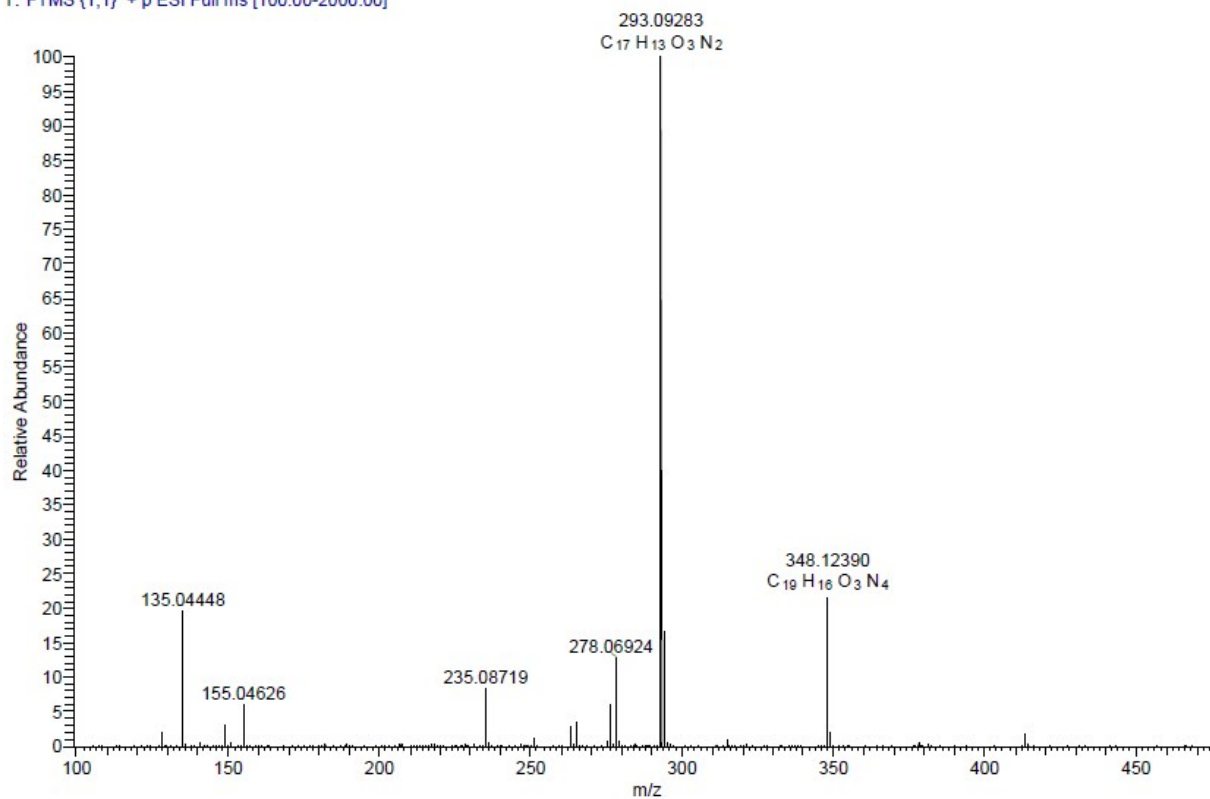
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

X:\Data\2018\July2019-DeC2019\4v

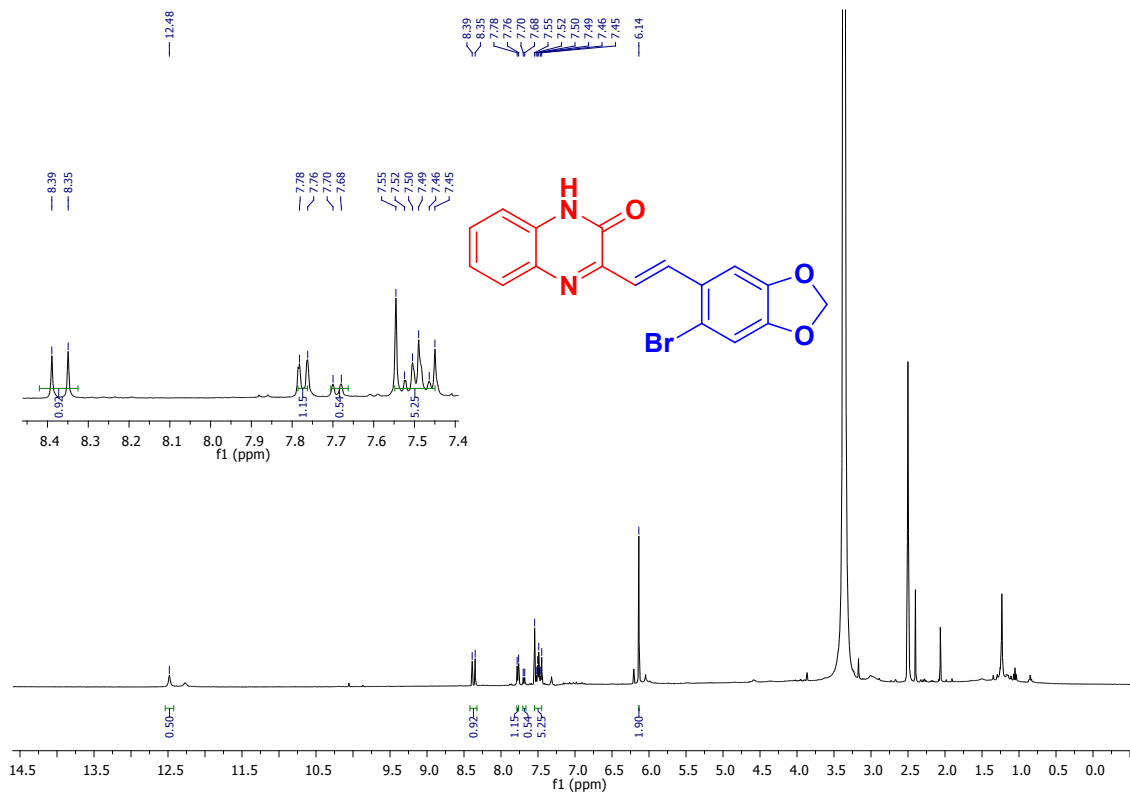
20-12-2019 16:51:57

4v #88 RT: 1.79 AV: 1 NL: 1.37E5
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

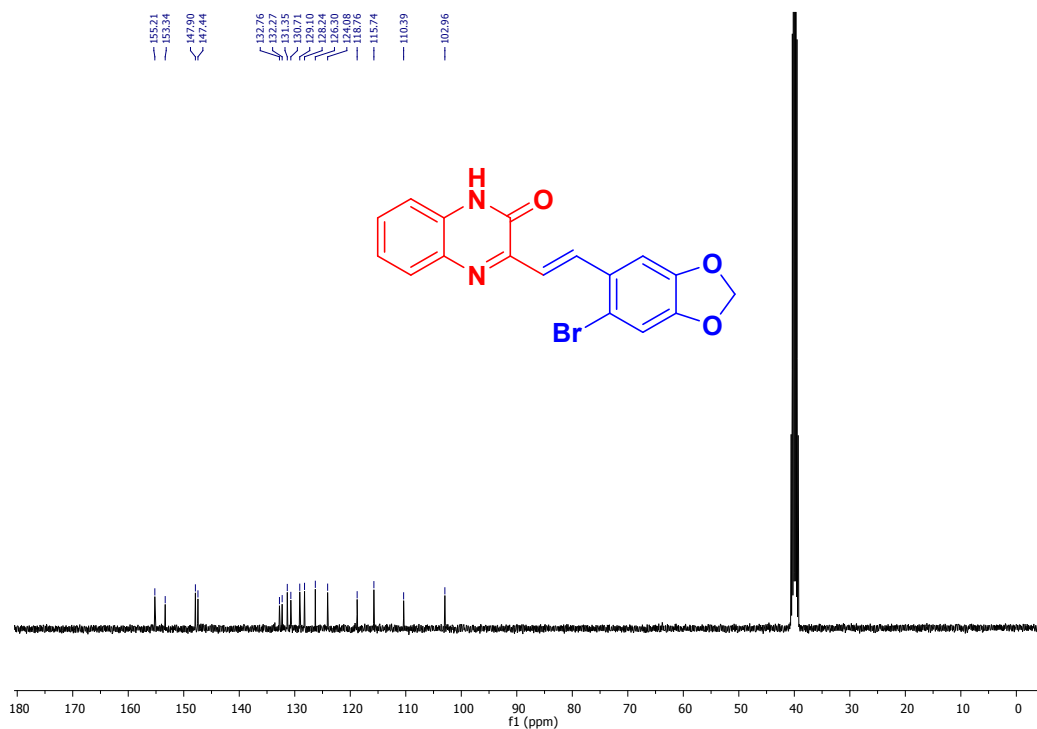


4w. (E)-3-(2-(4-bromobenzo[d][1,3]dioxol-5-yl)vinyl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4w

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

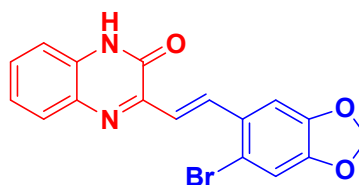
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

26-11-2019 10:03:25 1 / 1

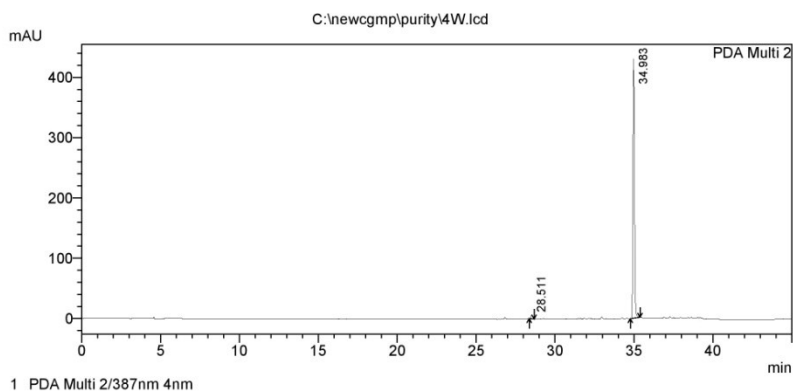
==== Shimadzu LCsolution Analysis Report ====

Acquired by : Admin
 Sample Name : 4W
 Sample ID : 4W
 Tray# : 1
 Vial # : 31
 Injection Volume : 5 µL
 Data File Name : 4W.lcd
 Method File Name : purity.lcm
 Batch File Name : nancy compound purity.lcb
 Report File Name : Default.lcr
 Data Acquired : 26-11-2019 03:08:47
 Data Processed : 26-11-2019 10:02:45

C:\newcgmpp\purity\4W.lcd



<Chromatogram>



1 PDA Multi 2/387nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	28.511	45655	6005	1.615	1.376
2	34.983	2781664	430275	98.385	98.624
Total		2827319	436280	100.000	100.000

C:\newcgmpp\purity\4W.lcd

HRMS Analysis of **4w**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

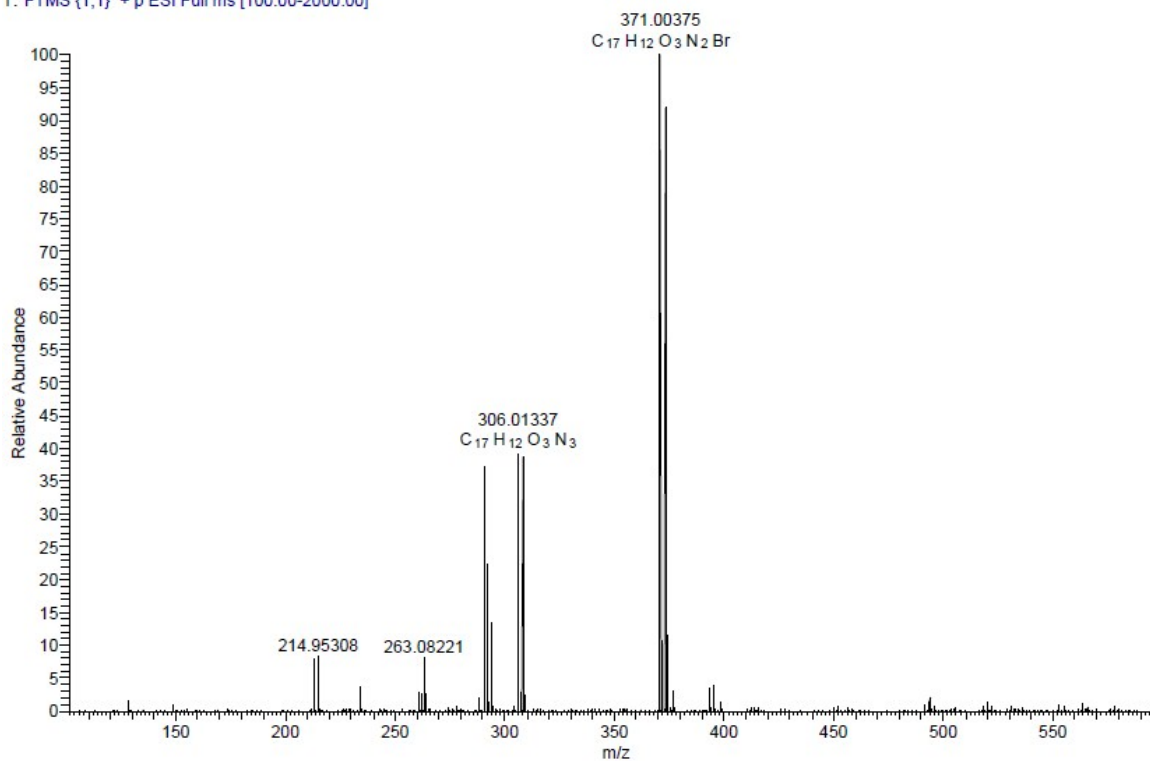
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

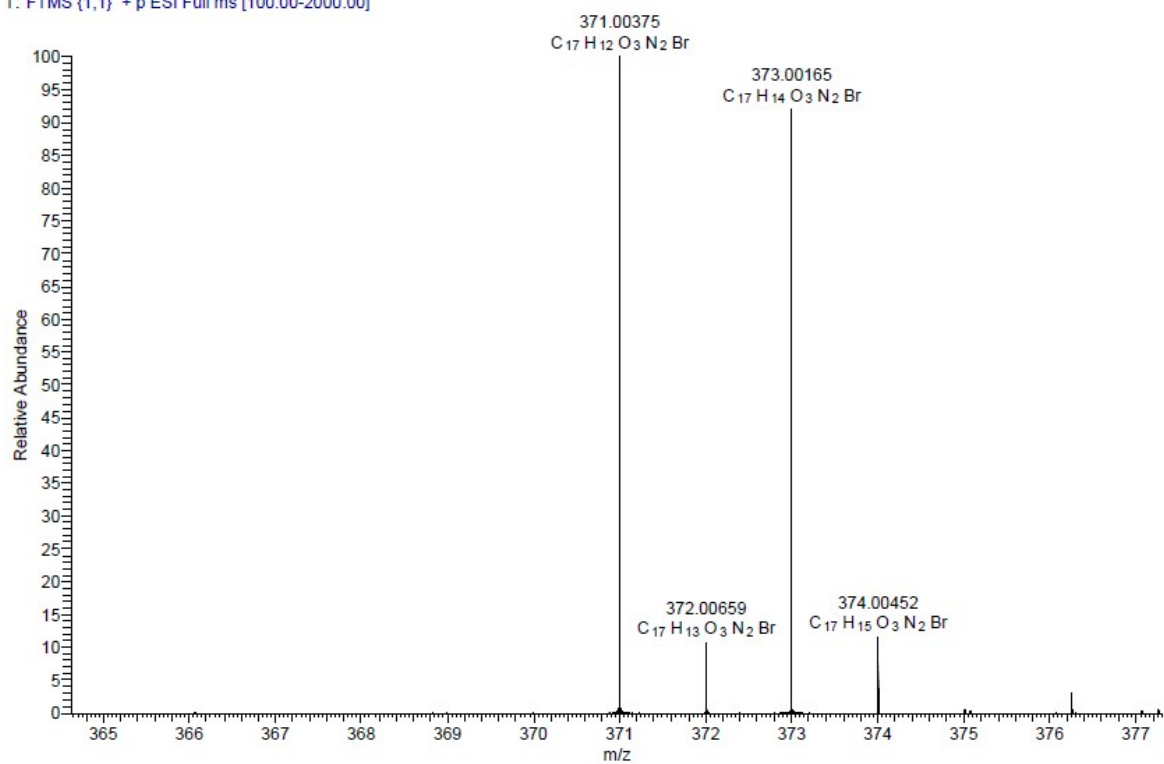
X:\Data\2018\July2019-DeC2019\4w

20-12-2019 16:57:37

4w #56 RT: 1.15 AV: 1 NL: 4.37E5
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

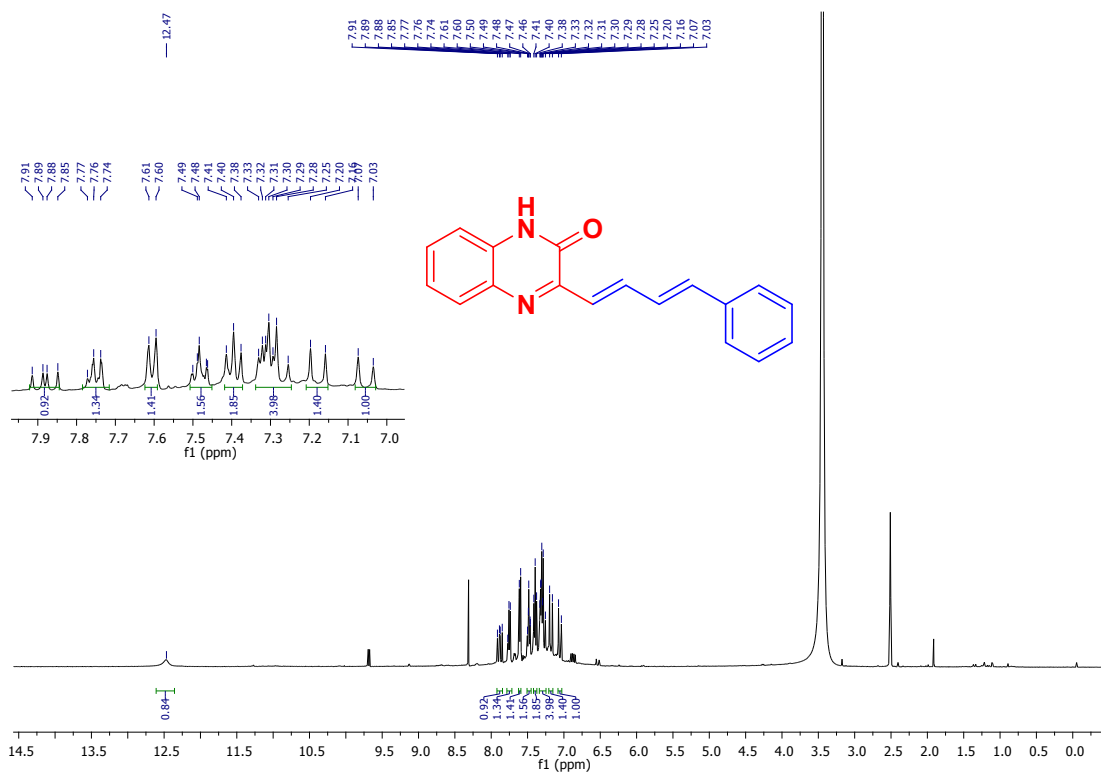


4w #56 RT: 1.15 AV: 1 NL: 4.37E5
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]

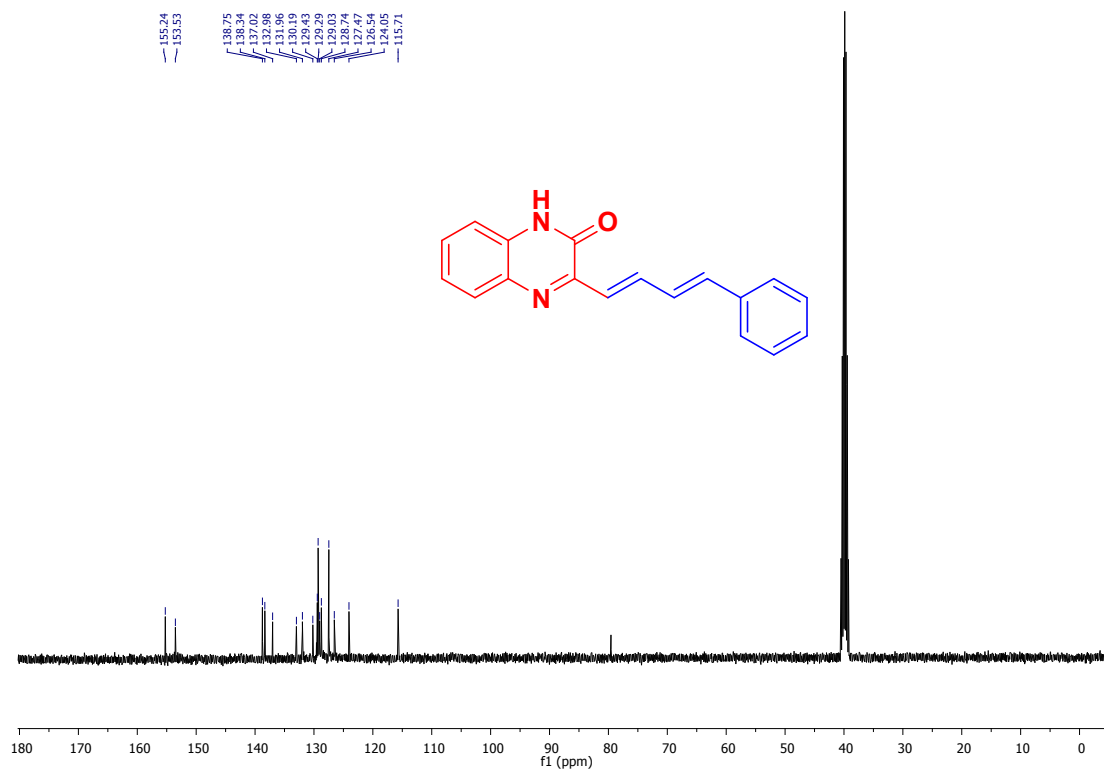


4x. 3-((1E,3E)-4-phenylbuta-1,3-dien-1-yl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

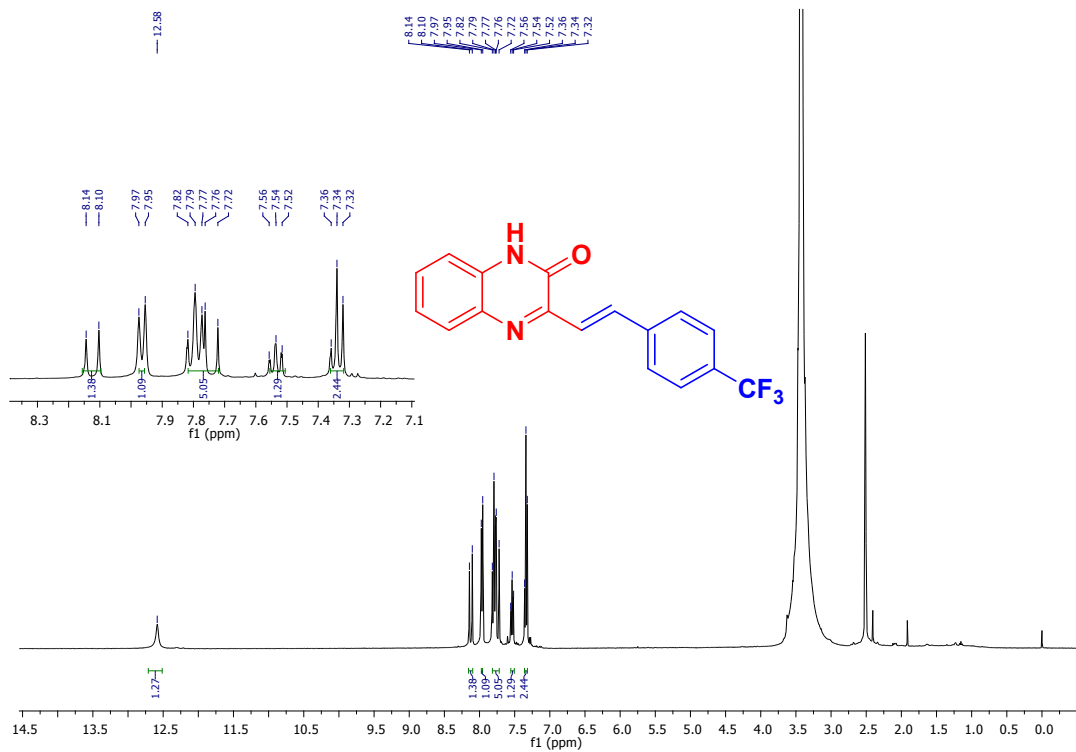


¹³C NMR spectrum (101 MHz, DMSO-d₆)

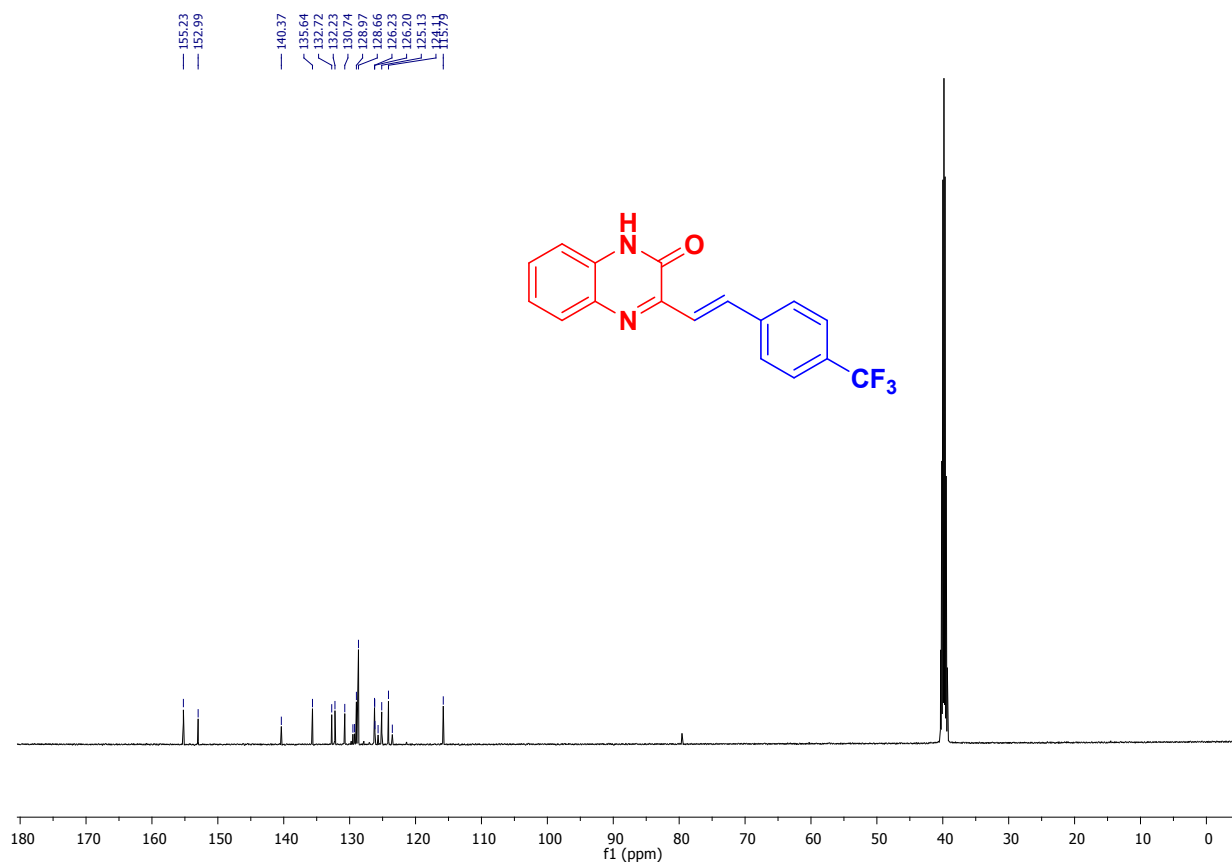


4y. (E)-3-(4-(trifluoromethyl)styryl)quinoxalin-2(1H)-one

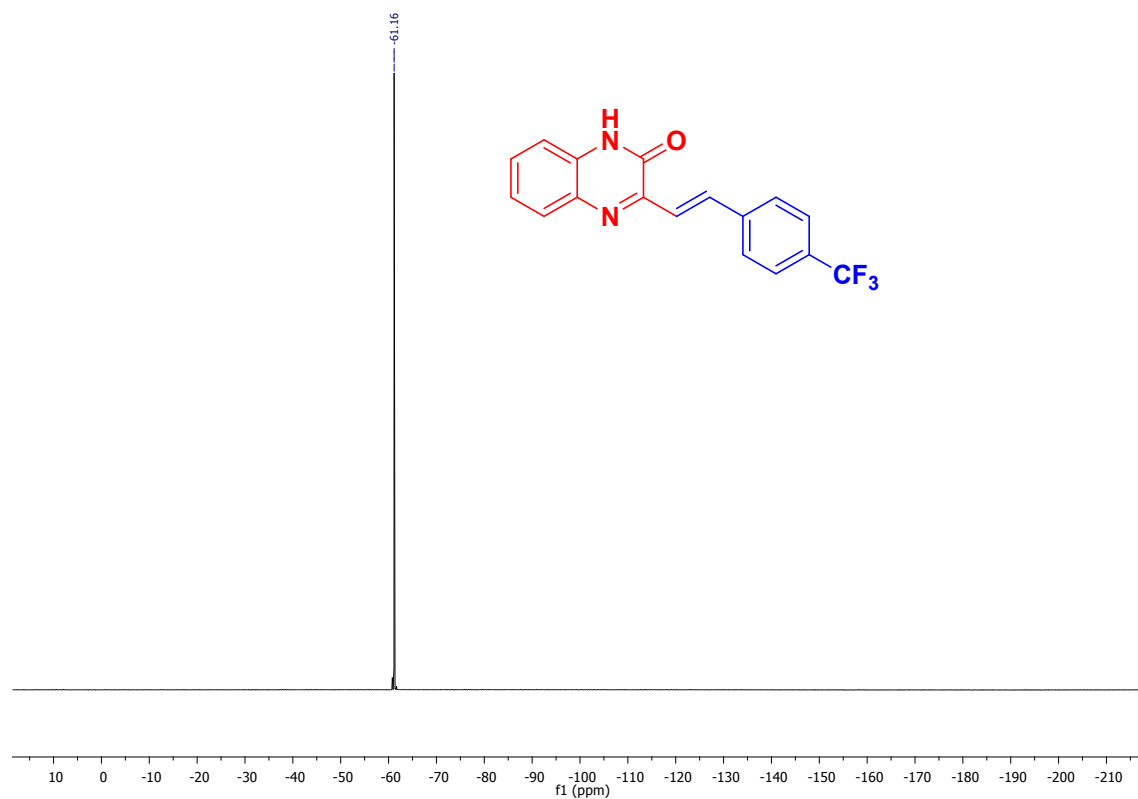
¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)

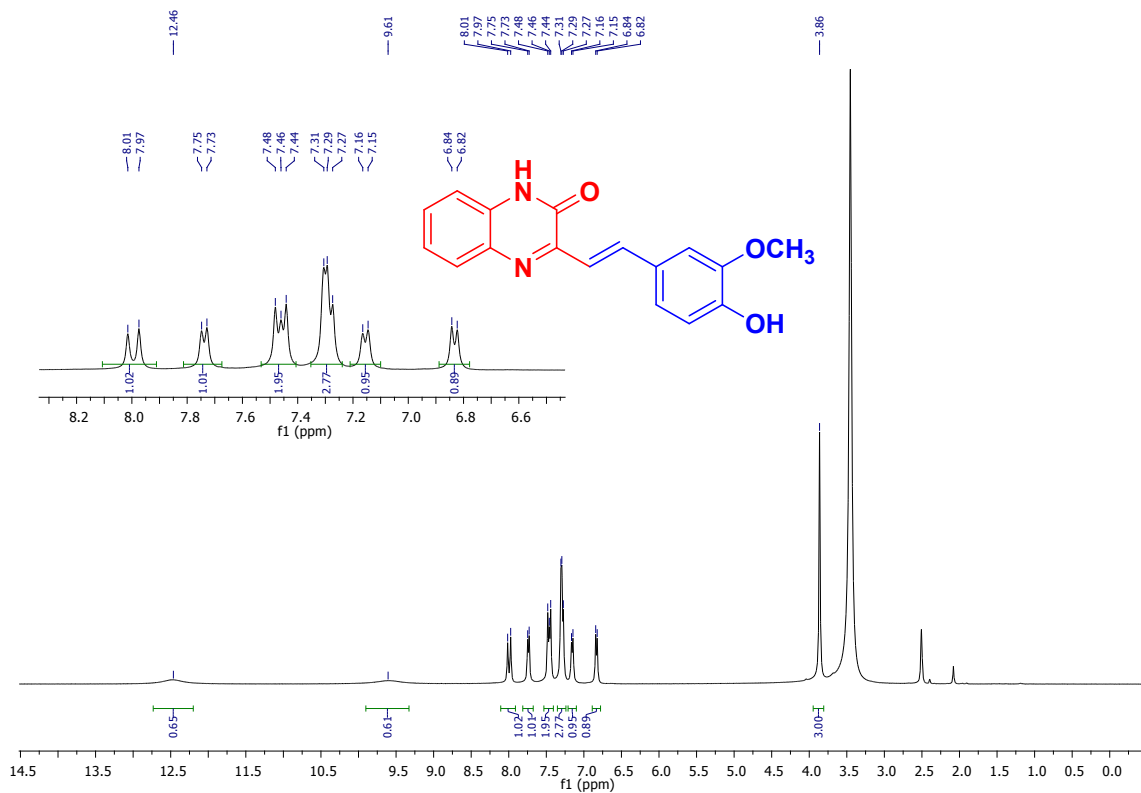


¹⁹F NMR spectrum (376 MHz, DMSO-d₆)

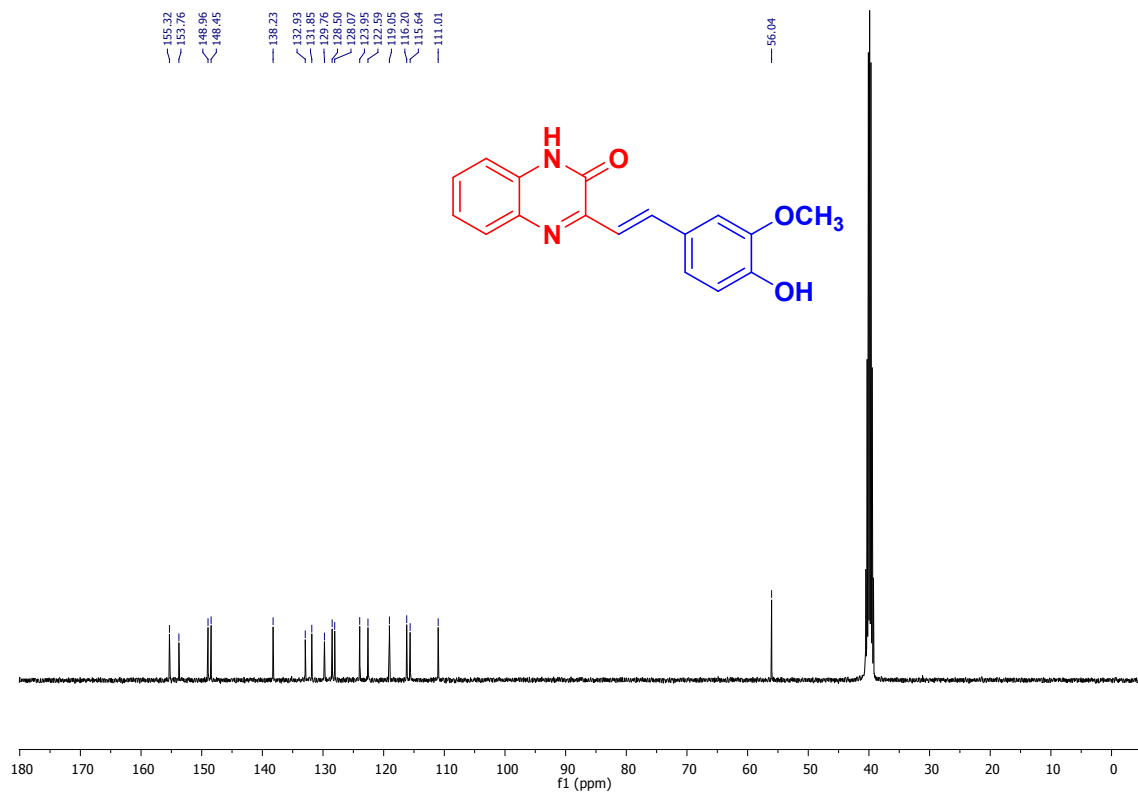


4z. (E)-3-(4-hydroxy-3-methoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4z

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

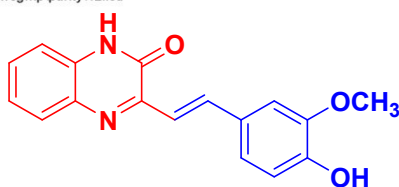
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

17-03-2020 14:42:09 1 / 1

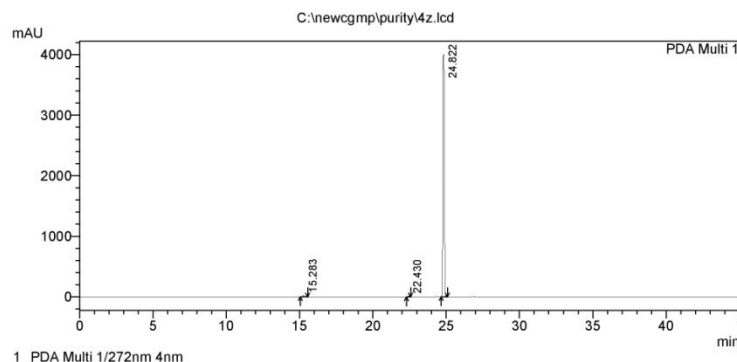
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
Sample Name : 4z
Sample ID : 4z
Tray# : 1
Vial # : 67
Injection Volume : 5 µL
Data File Name : 4z.lcd
Method File Name : purity.lcm
Batch File Name : 17032020.lcb
Report File Name : Default.lcr
Data Acquired : 17-03-2020 12:24:19
Data Processed : 17-03-2020 13:09:21

C:\newcgmp\purity\4z.lcd



<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.283	196459	24630	0.707	0.610
2	22.430	73917	10169	0.266	0.252
3	24.822	27531482	3999780	99.027	99.137
Total		27801858	4034579	100.000	100.000

C:\newcgmp\purity\4z.lcd

HRMS Analysis of **4z**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

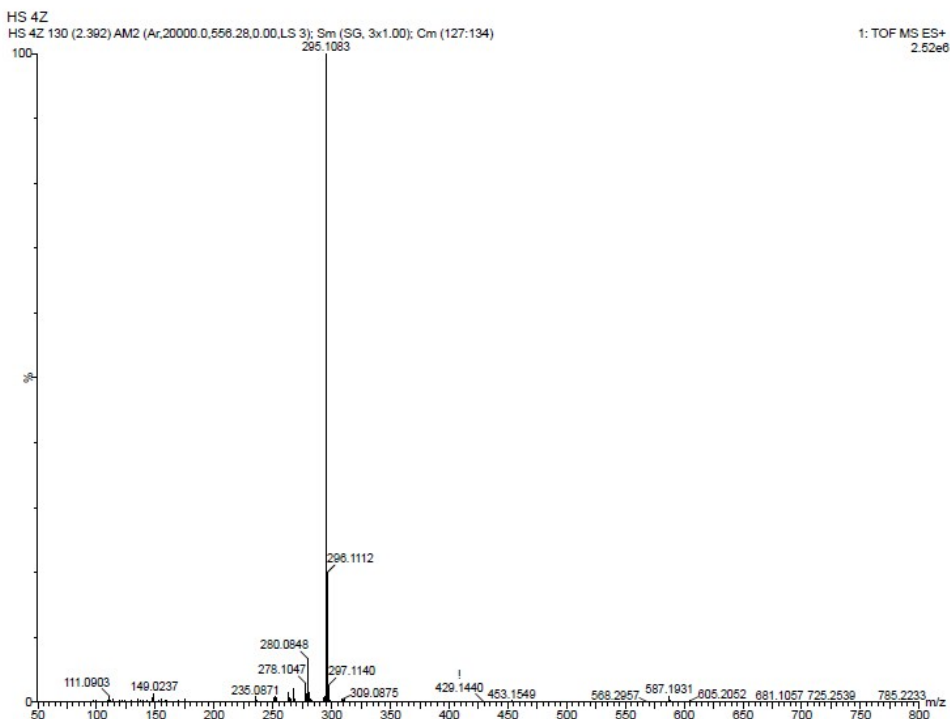
Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150µl /minute

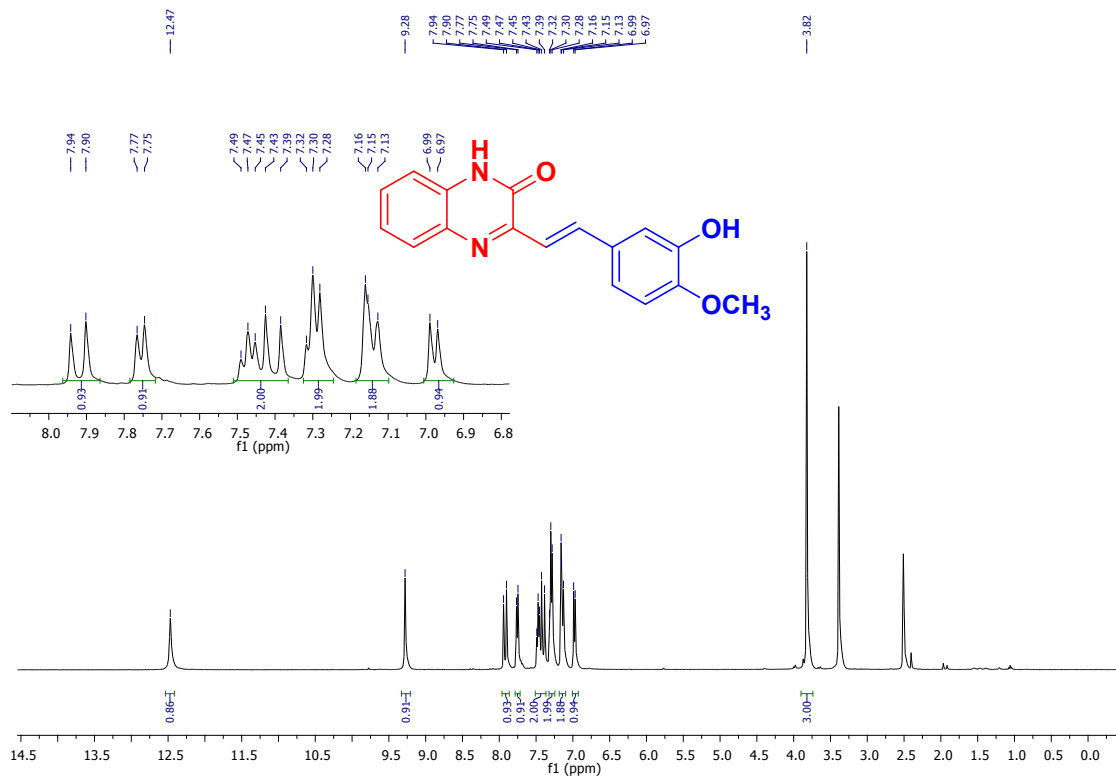
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

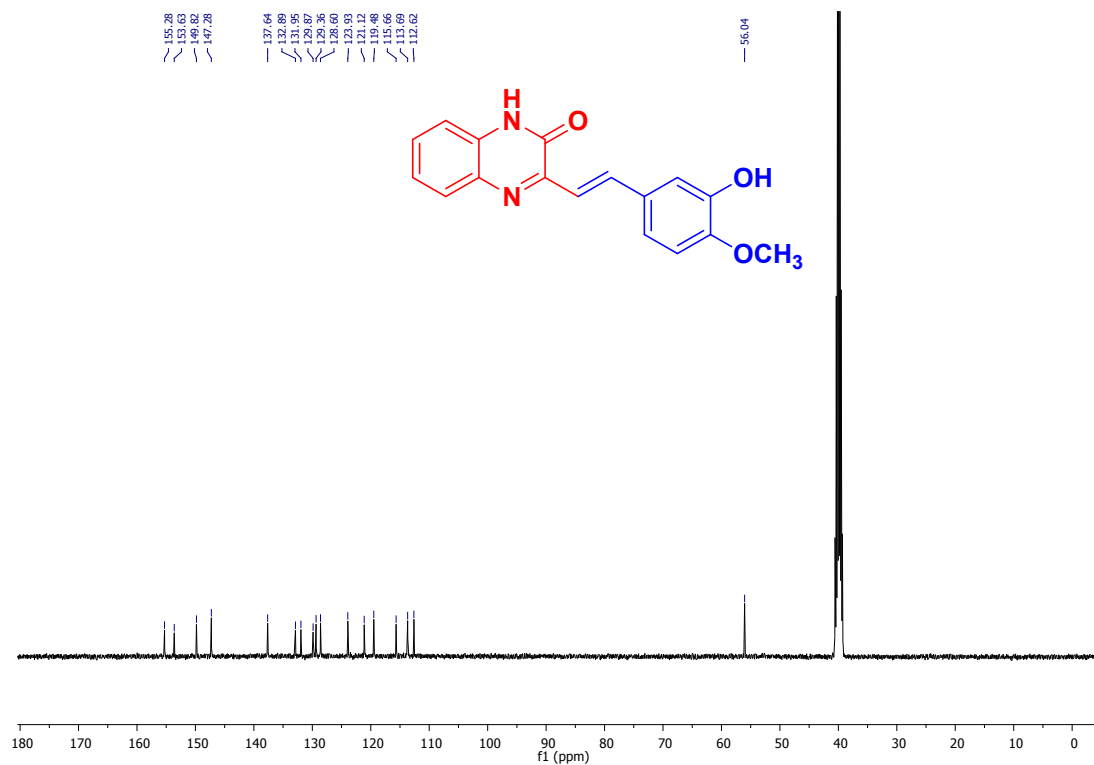


4aa. (E)-3-(3-hydroxy-4-methoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

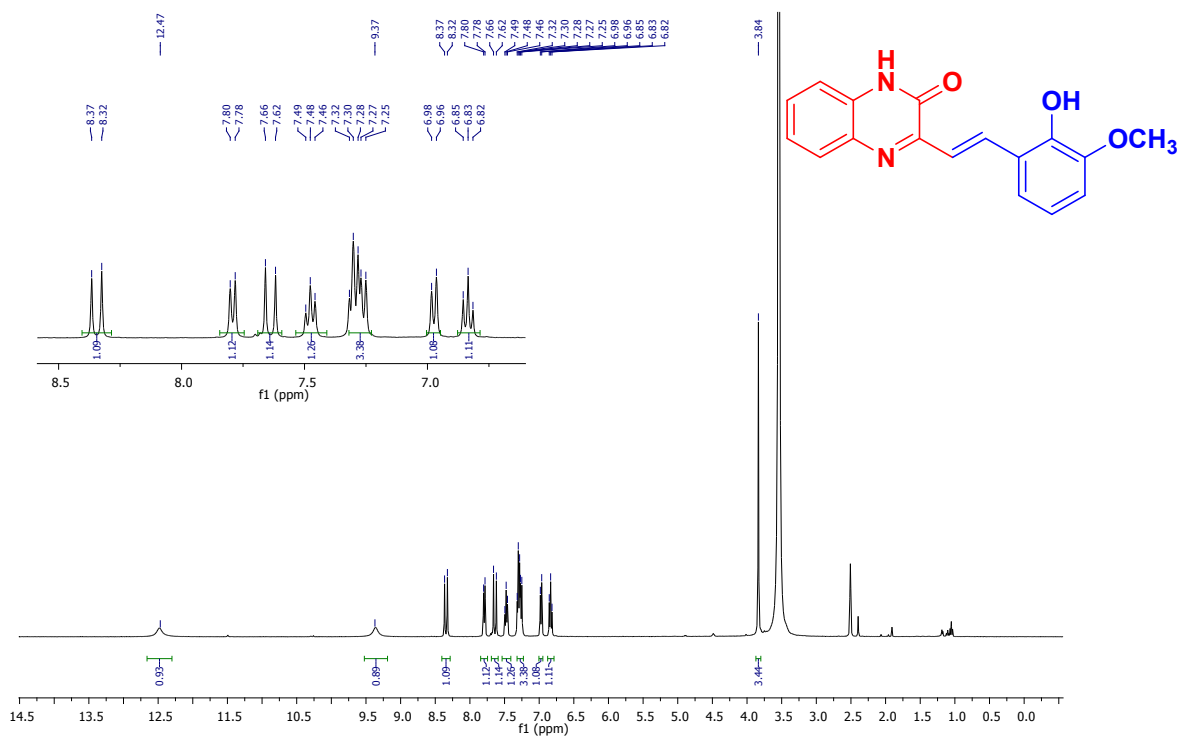


¹³C NMR spectrum (101 MHz, DMSO-d₆)

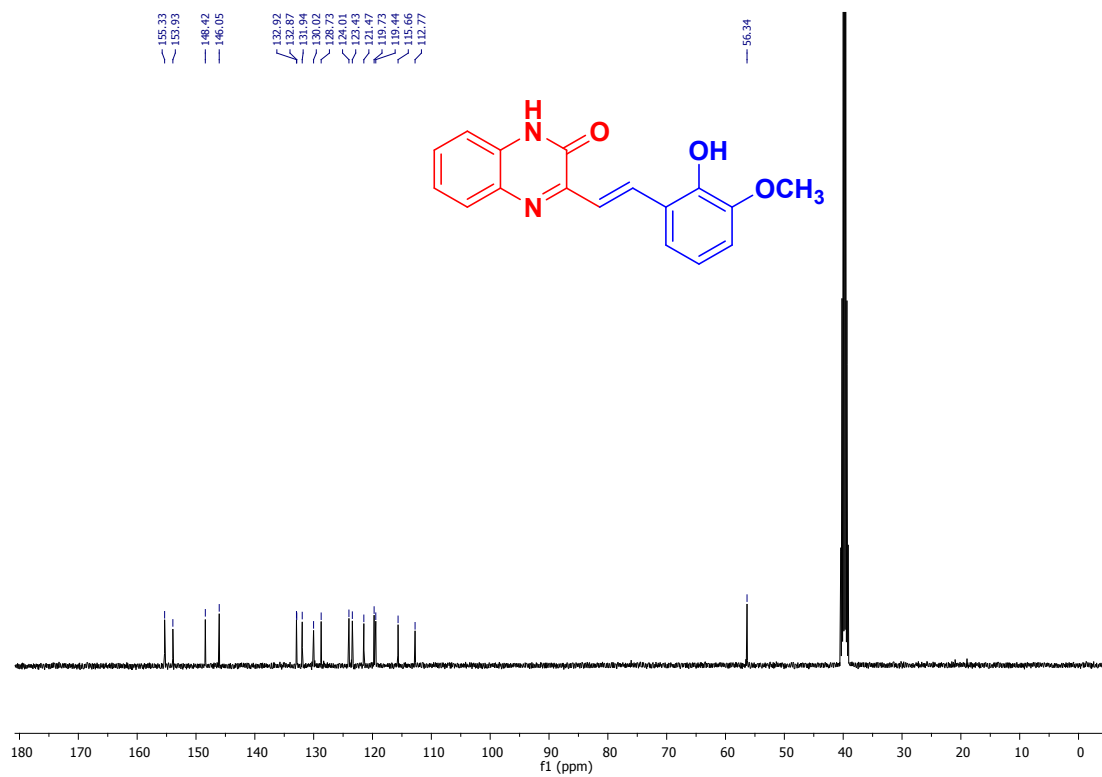


4ab. *(E)*-3-(2-hydroxy-3-methoxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4ab

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 μ , 250 $\times$ 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 μ L. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

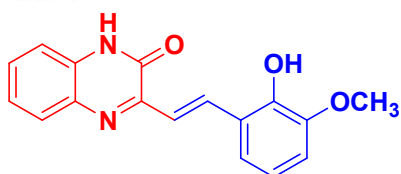
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

17-03-2020 14:33:52 1 / 1

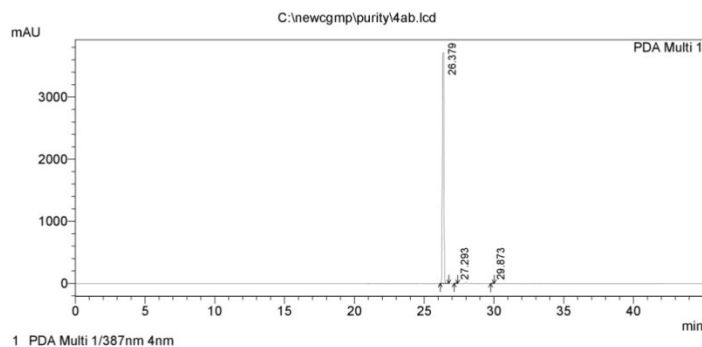
==== Shimadzu Lcsolution Analysis Report ====

Acquired by : Admin
Sample Name : 4ab
Sample ID : 4ab
Tray# : 1
Vial # : 65
Injection Volume : 5 μ L
Data File Name : 4ab.lcd
Method File Name : purity.lcm
Batch File Name : 17032020.lcb
Report File Name : Default.lcr
Data Acquired : 17-03-2020 10:53:22
Data Processed : 17-03-2020 11:38:25

C:\newcgmp\purity\4ab.lcd



<Chromatogram>



1 PDA Multi 1/387nm 4nm

PeakTable					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	26.379	29234547	3715062	99.275	99.264
2	27.293	120863	15285	0.410	0.408
3	29.873	92743	12260	0.315	0.328
Total		29448154	3742607	100.000	100.000

C:\newcgmp\purity\4ab.lcd

HRMS Analysis of **4ab**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

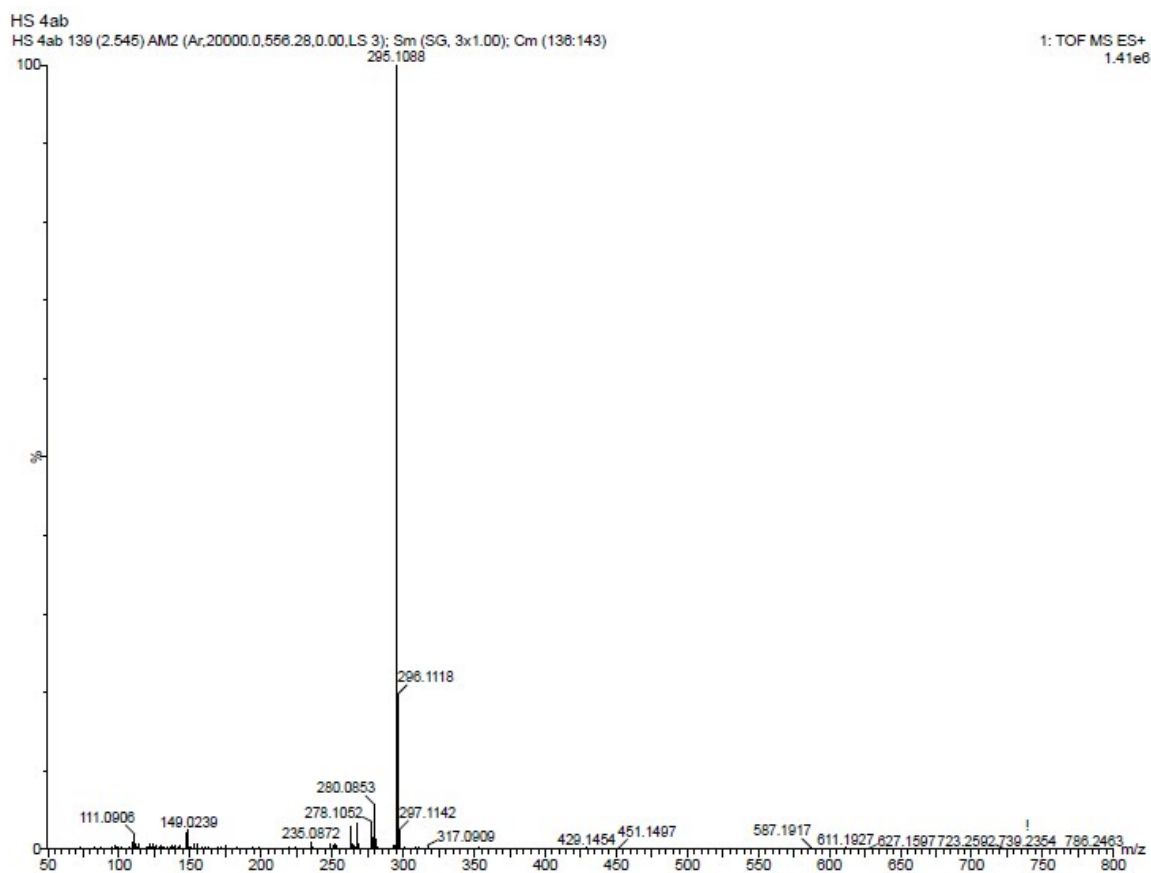
Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150 μ l /minute

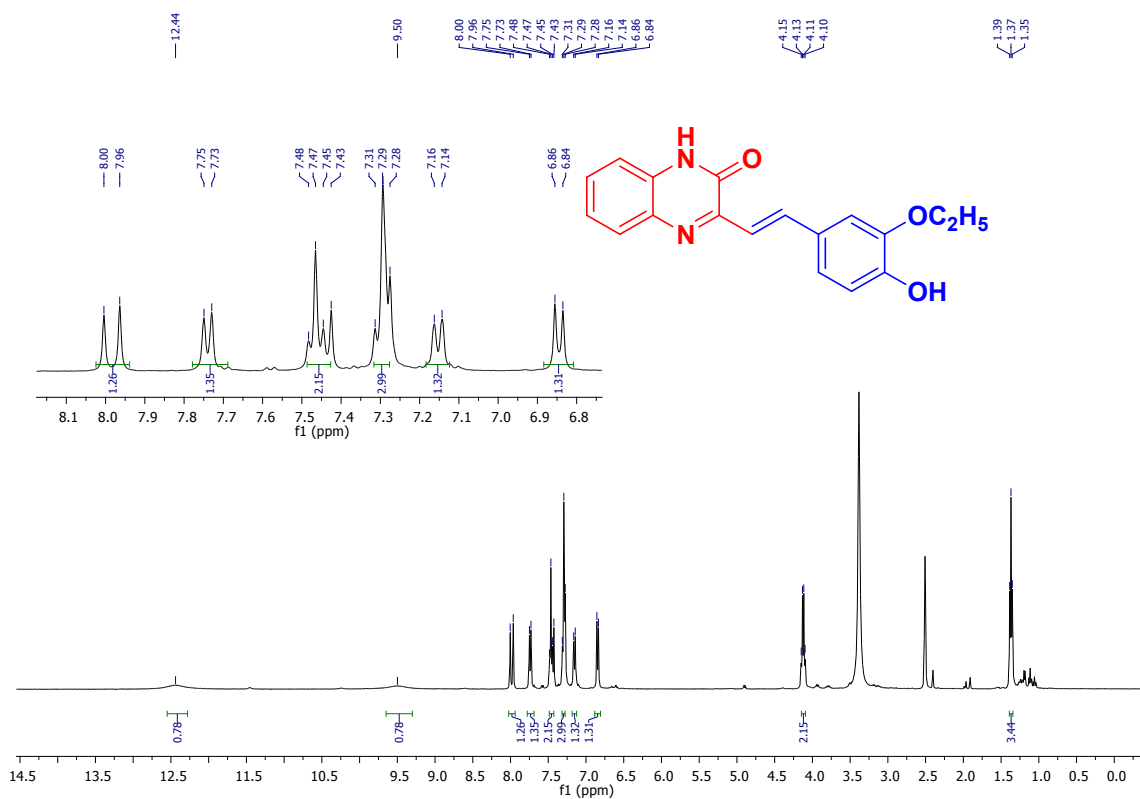
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

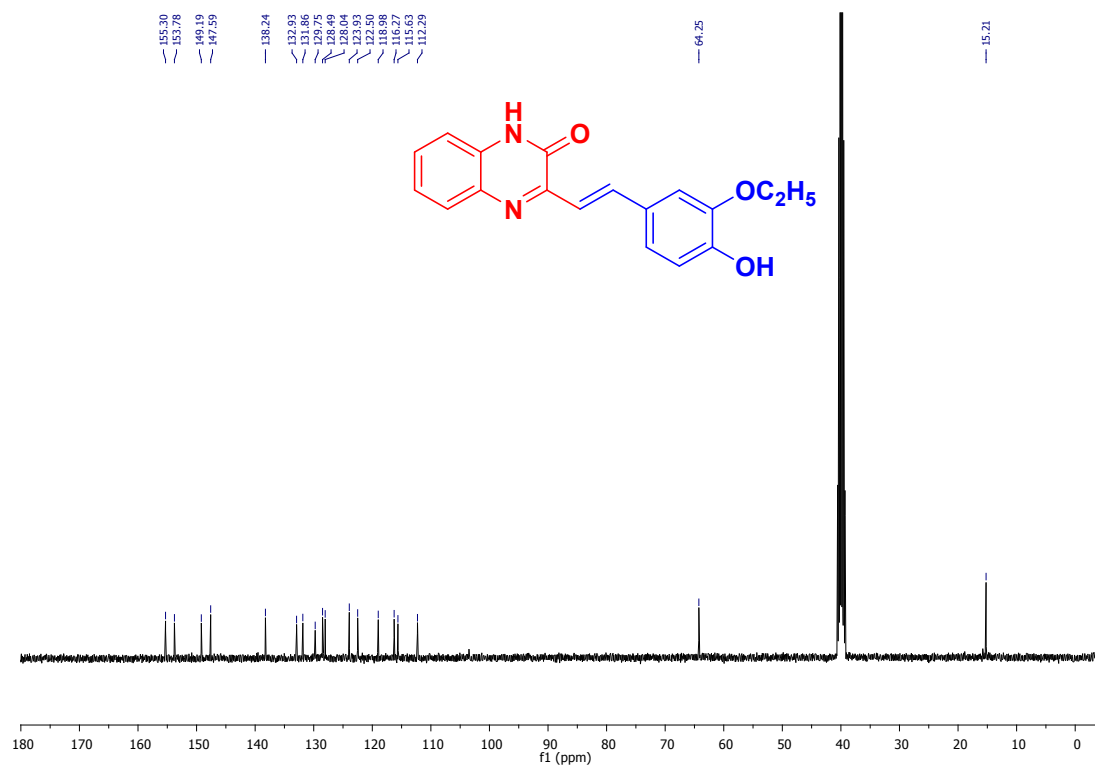


4ac. (E)-3-(3-ethoxy-4-hydroxystyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)



HPLC Analysis of 4ac

The HPLC purity was checked using Shimadzu HPLC system, consisting of purosphere C₁₈ (5 µ, 250 × 4.6 mm) column and a PDA detector. The flow rate was 0.6 mL/min with the injection volume of 10 µL. The total run time was 45 min with gradient elution using 0.1% v/v formic acid in water (A) and mobile phase of acetonitrile (B). The gradient (WRT % v/v of A and B) was as shown in the **Table 2** :

Table 2 : Parameters used in HPLC purity check.

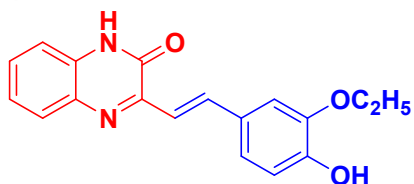
Time (in min.)	WRT % v/v of B	WRT % v/v of A
0	0	100
10	10	90
20	30	70
30	60	40
35	80	20
40	0	100
45	Stop	Stop

19-03-2020 15:13:27 1 / 1

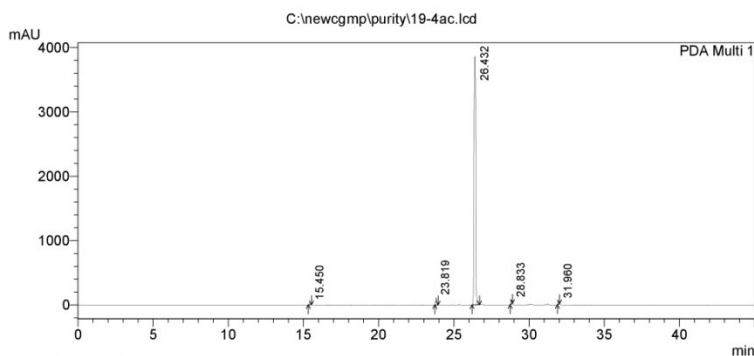
==== Shimadzu LcSolution Analysis Report ====

Acquired by : Admin
Sample Name : 19-4ac
Sample ID : 19-4ac
Tray# : 1
Vial # : 19
Injection Volume : 5 µL
Data File Name : 19-4ac.lcd
Method File Name : purity.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 19-03-2020 14:21:38
Data Processed : 19-03-2020 15:06:39

C:\newcgmp\purity\19-4ac.lcd



<Chromatogram>



1 PDA Multi 1/362nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.450	215756	30873	0.668	0.752
2	23.819	597720	109428	1.850	2.665
3	26.432	30994112	3858272	95.911	93.980
4	28.833	293771	61265	0.909	1.492
5	31.960	214089	45596	0.662	1.111
Total		32315449	4105434	100.000	100.000

C:\newcgmp\purity\19-4ac.lcd

HRMS Analysis of **4ac**

LC HRMS- THERMOSCIENTIFIC- EXACTIVE

C18 COLUMN- Hypersil

MOBILE PHASE- methanol and water (0.1% formic acid)

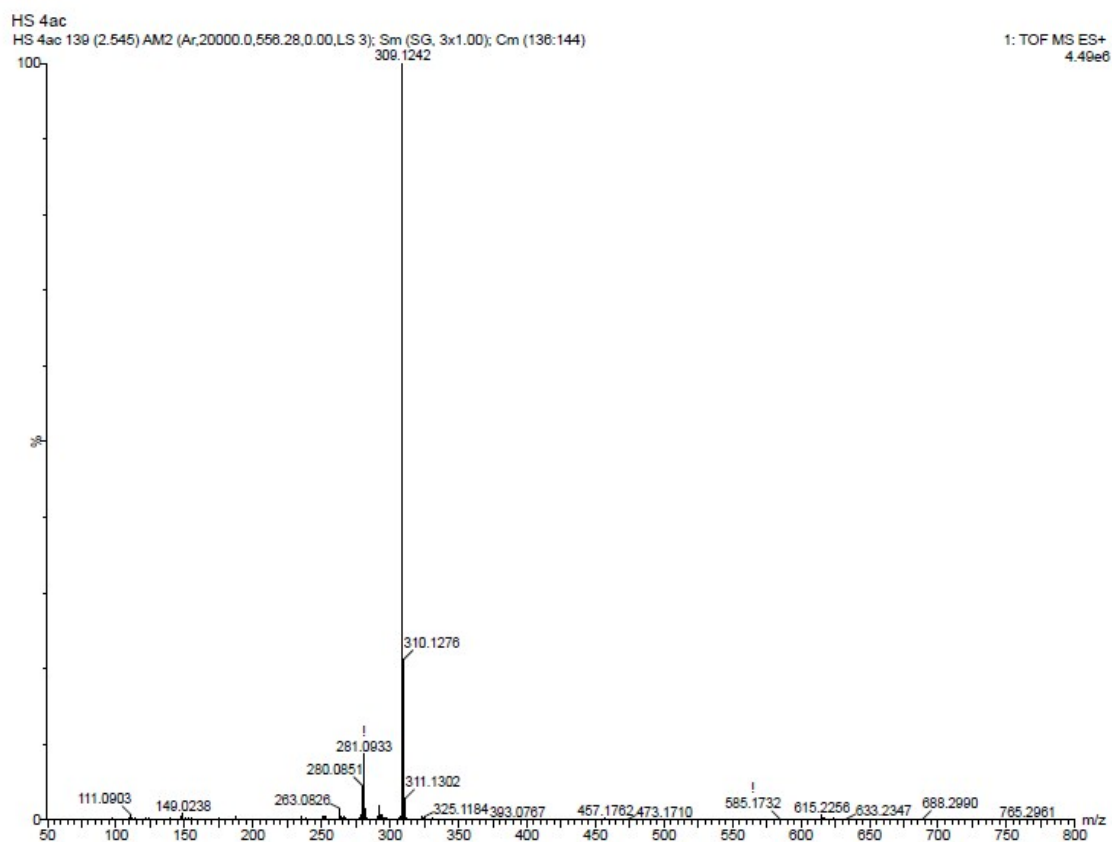
Gradient method : 97% methanol and 3% water for 5 minutes.

Injected amount : 2Microlitre

Flow rate of solvent 150µl /minute

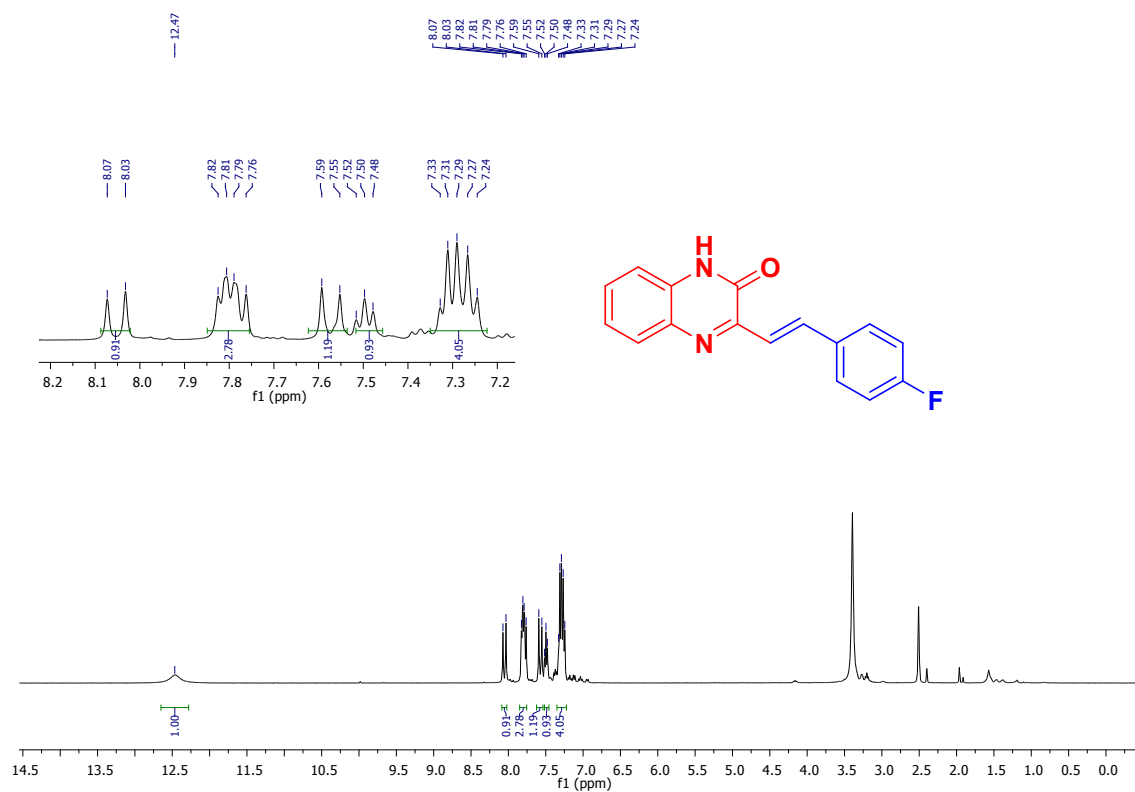
The source was operated in both positive and negative mode at an ion spray voltage of 3KV

Oven temperature was set to 30°C

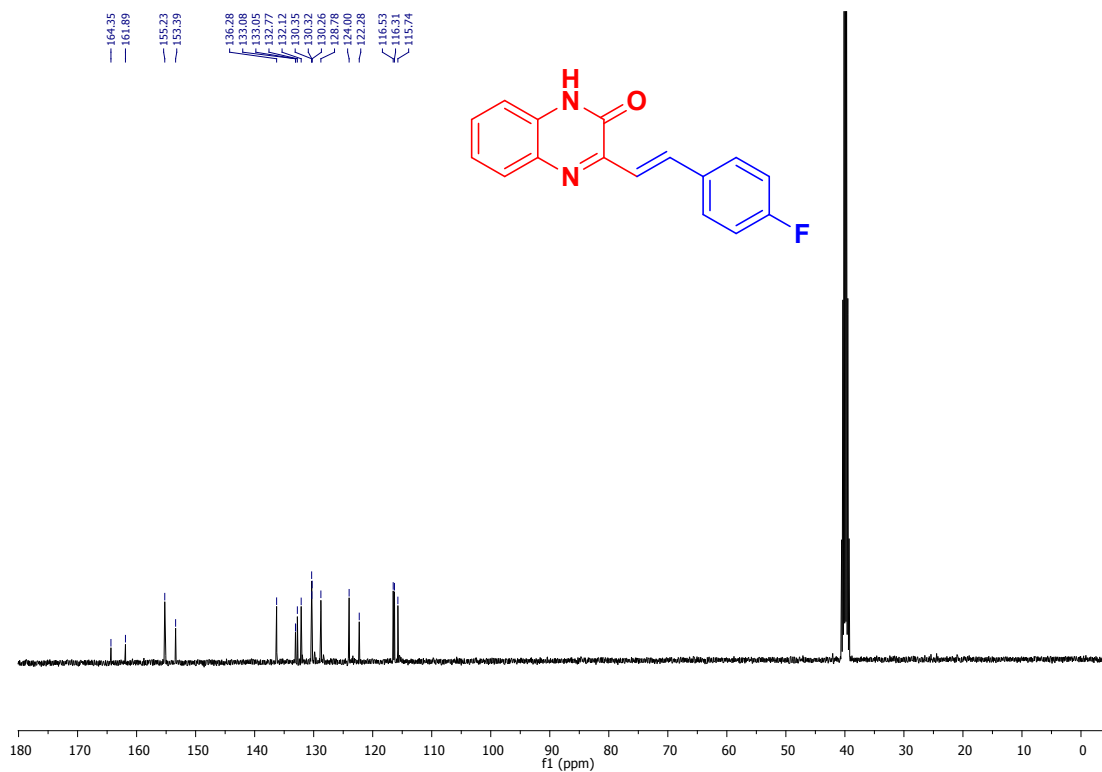


4ad. (E)-3-(4-fluorostyryl)quinoxalin-2(1H)-one

¹H NMR spectrum (400 MHz, DMSO-d₆)

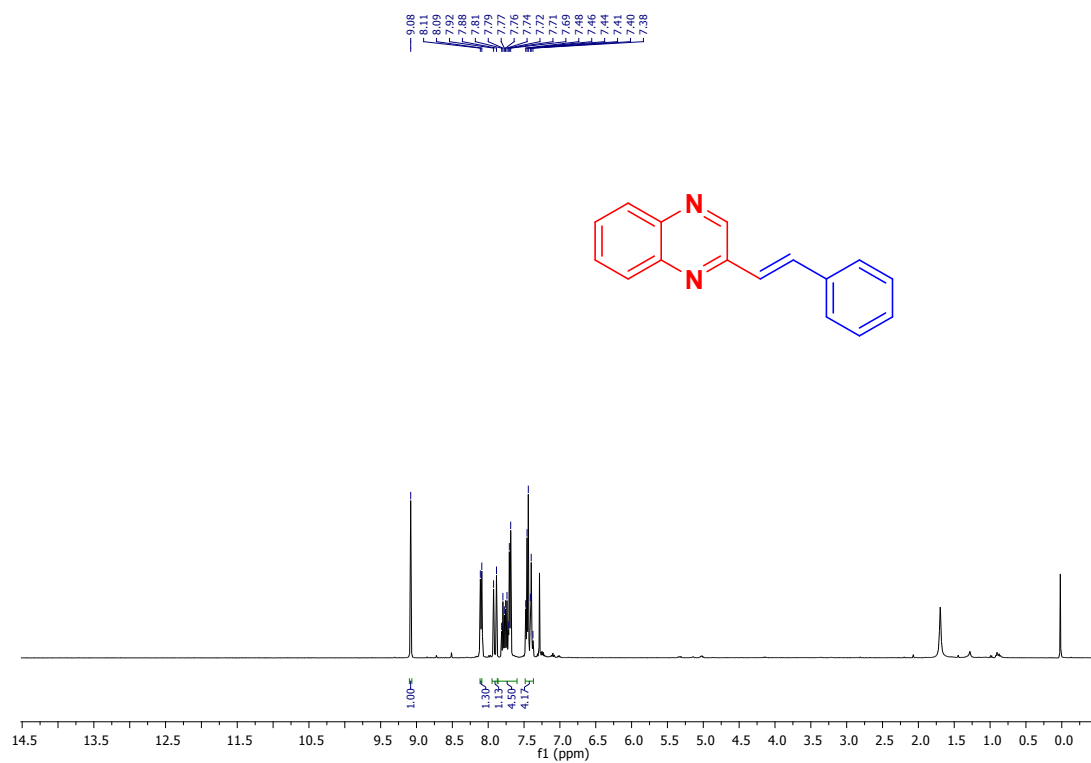


¹³C NMR spectrum (101 MHz, DMSO-d₆)

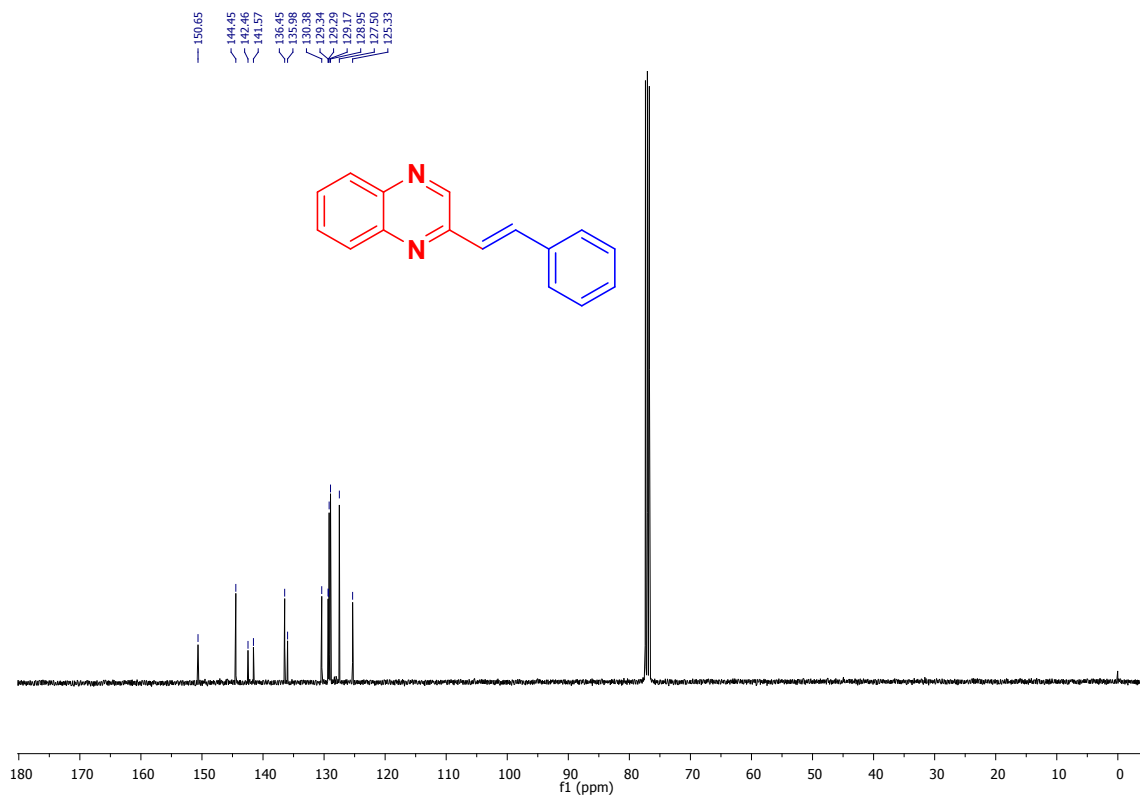


4ae. (*E*)-2-styrylquinoxaline

¹H NMR spectrum (400 MHz, CDCl₃)

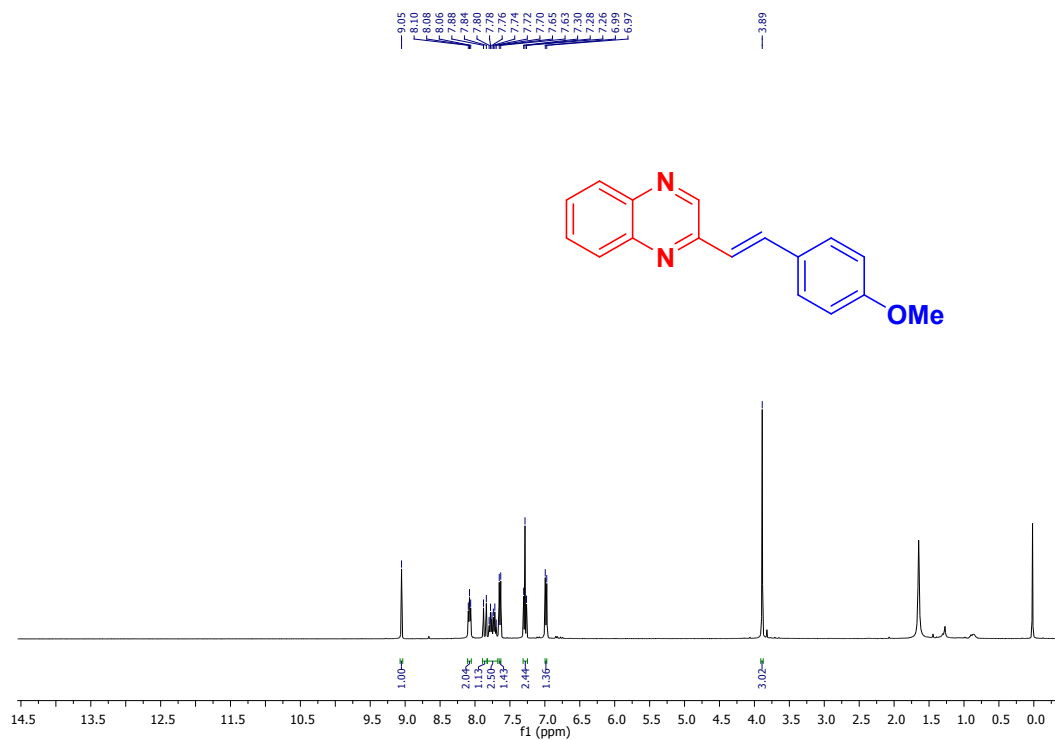


¹³C NMR spectrum (101 MHz, CDCl₃)

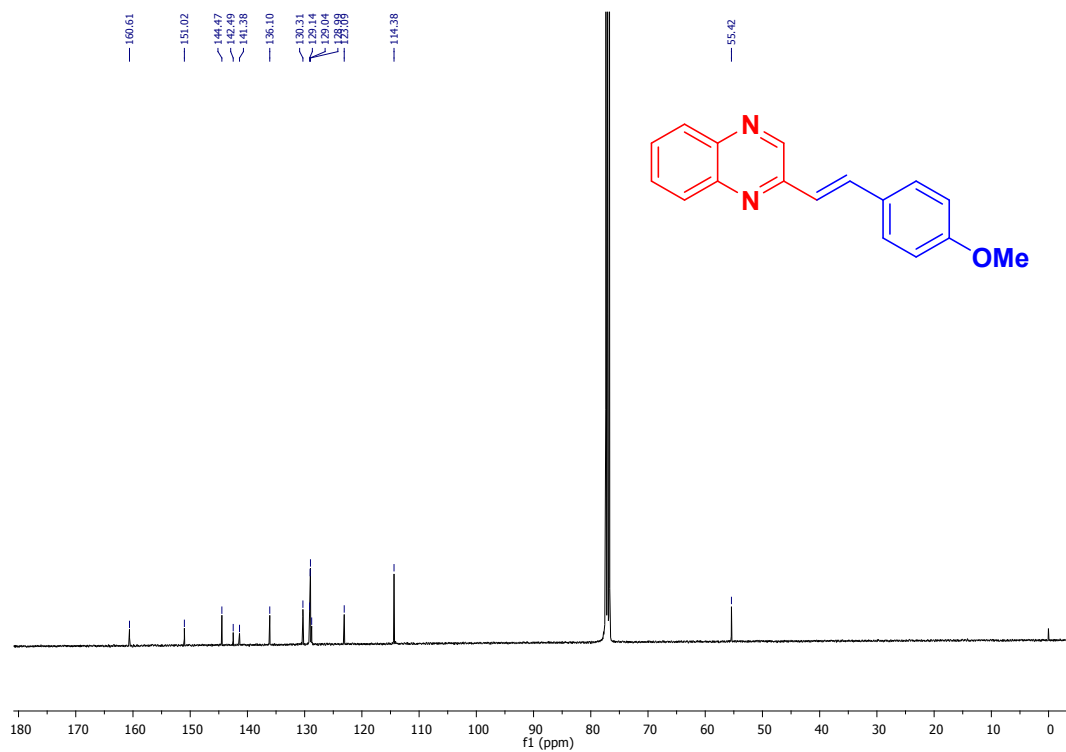


4af. *(E)*-2-(4-methoxystyryl)quinoxaline

^1H NMR spectrum (400 MHz, CDCl_3)

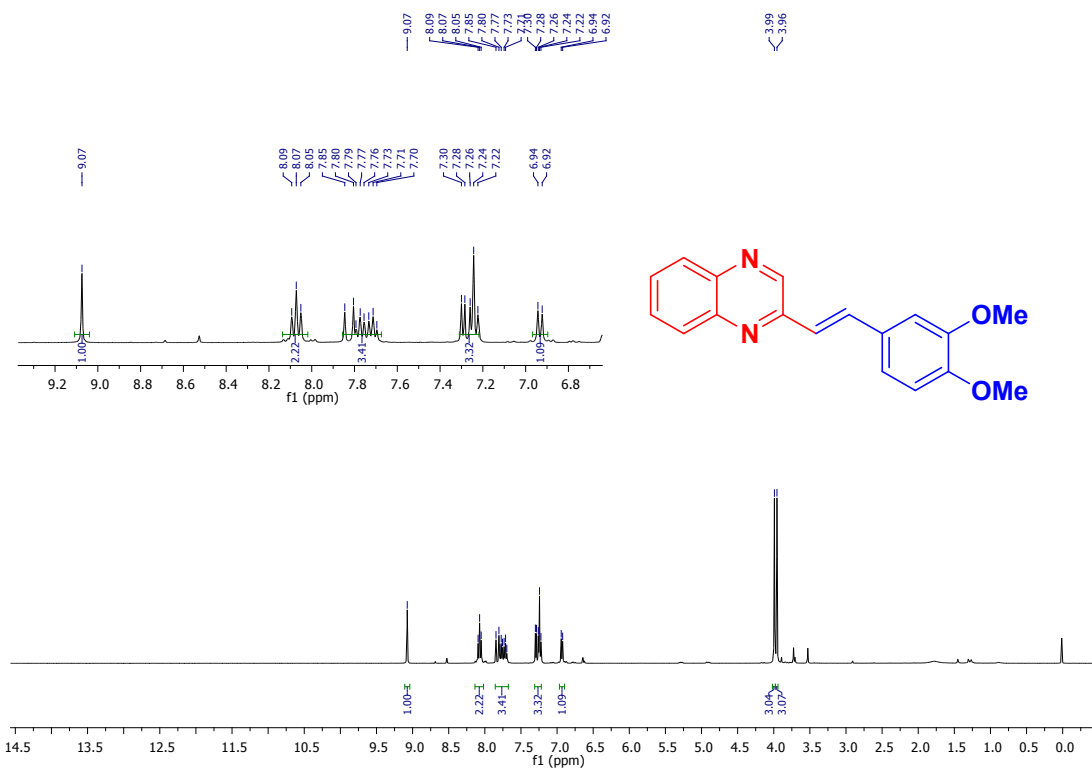


^{13}C NMR spectrum (101 MHz, CDCl_3)

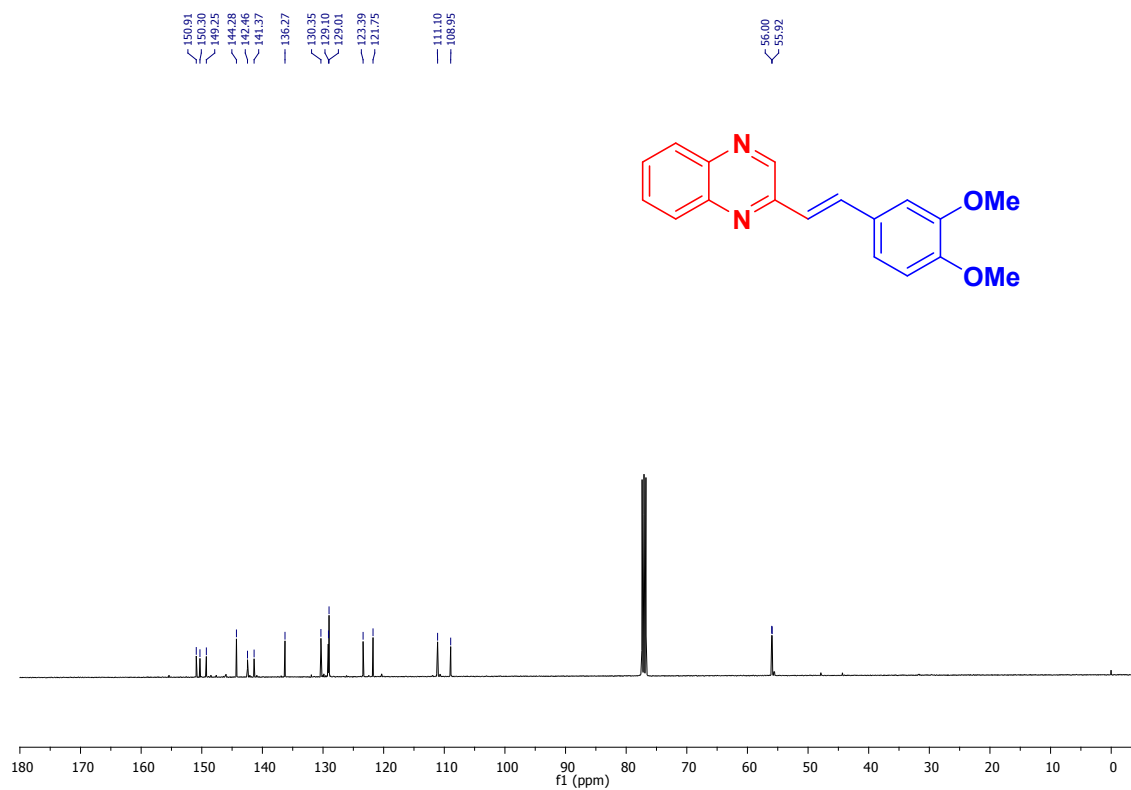


4ag. (E)-2-(3,4-dimethoxystyryl)quinoxaline

¹H NMR spectrum (400 MHz, CDCl₃)

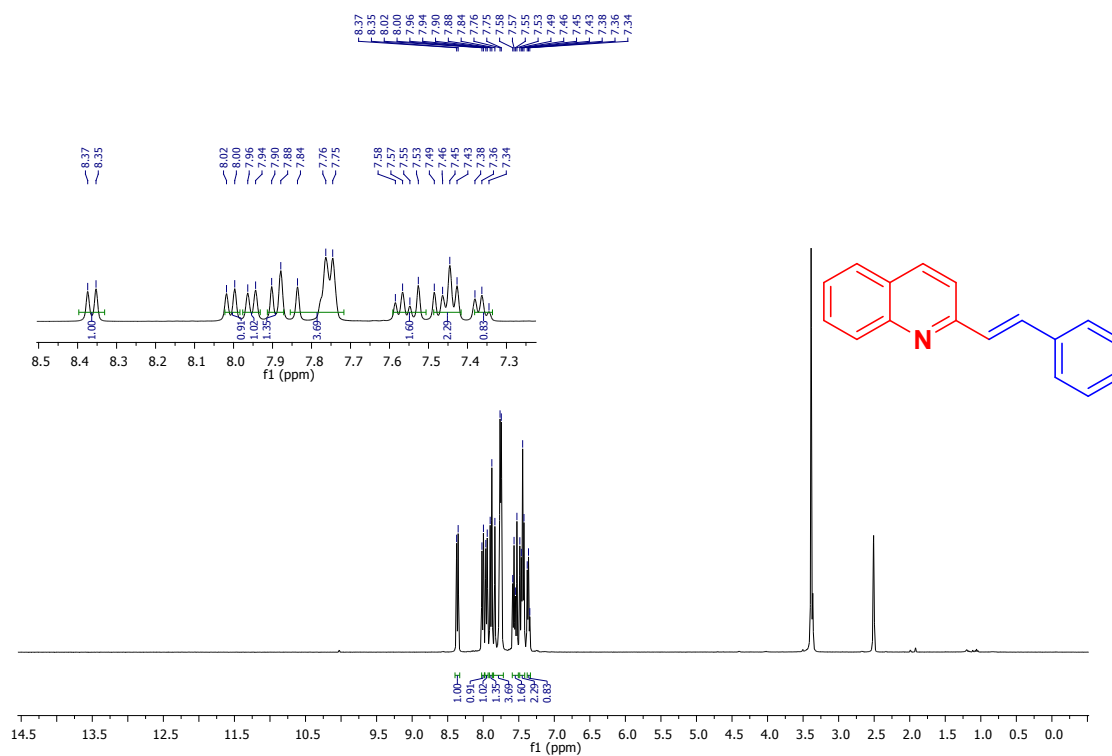


¹³C NMR spectrum (101 MHz, CDCl₃)

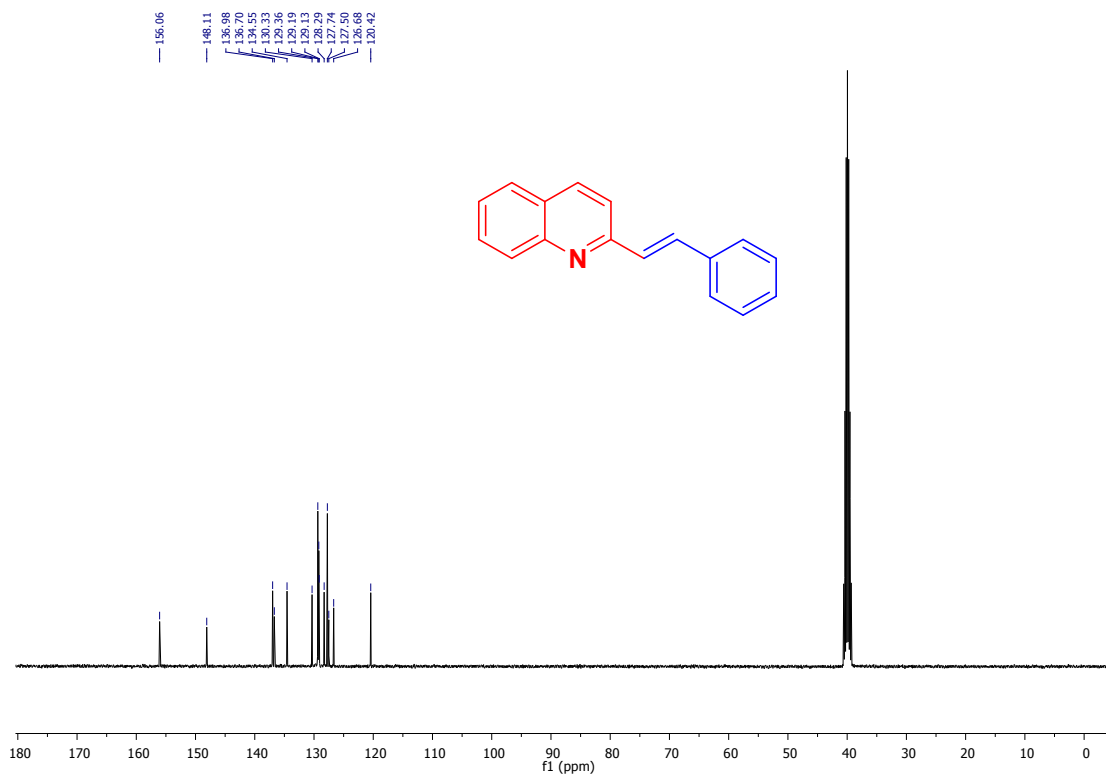


4ah. (*E*)-2-styrylquinoline

¹H NMR spectrum (400 MHz, DMSO-d₆)

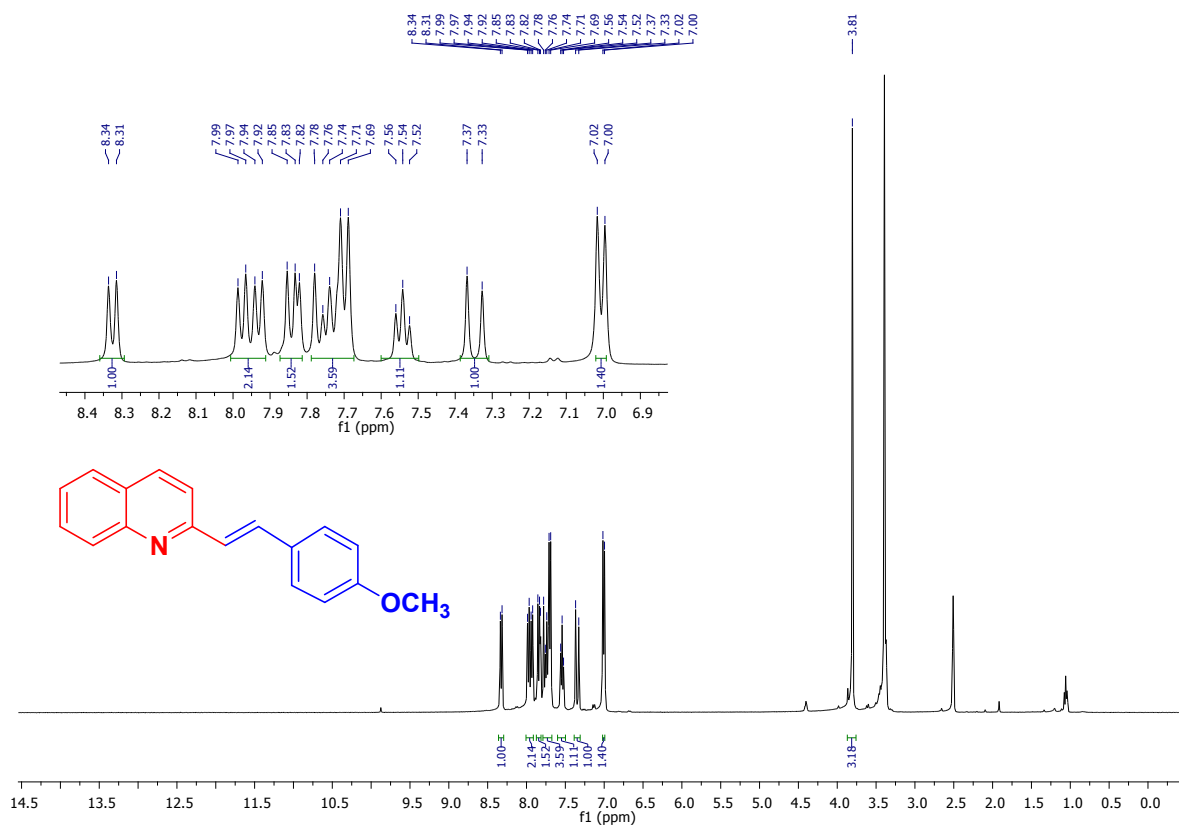


¹³C NMR spectrum (101 MHz, DMSO-d₆)

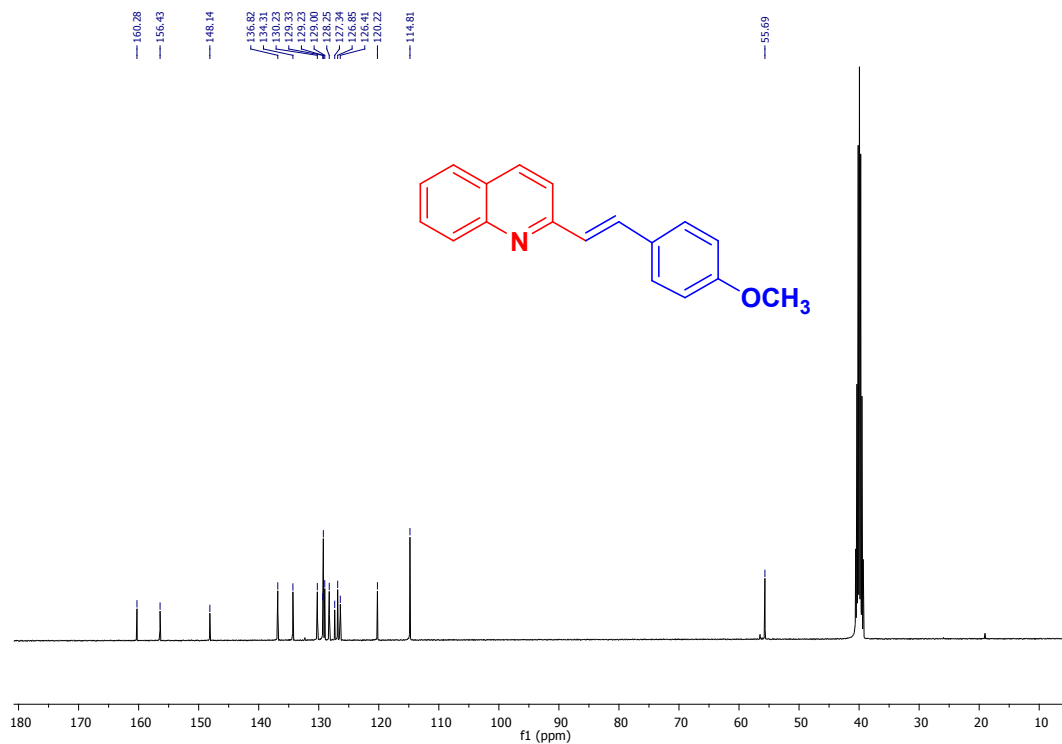


4ai. (E)-2-(4-methoxystyryl)quinoline

¹H NMR spectrum (400 MHz, DMSO-d₆)

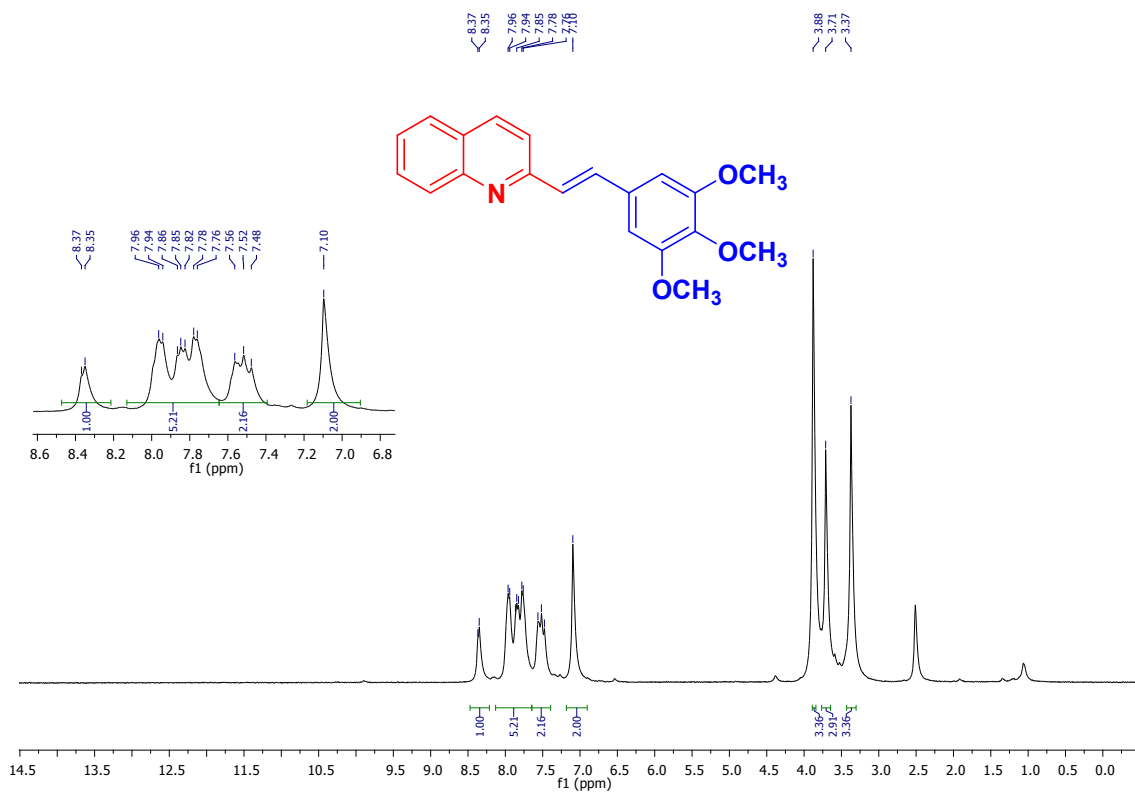


¹³C NMR spectrum (101 MHz, DMSO-d₆)

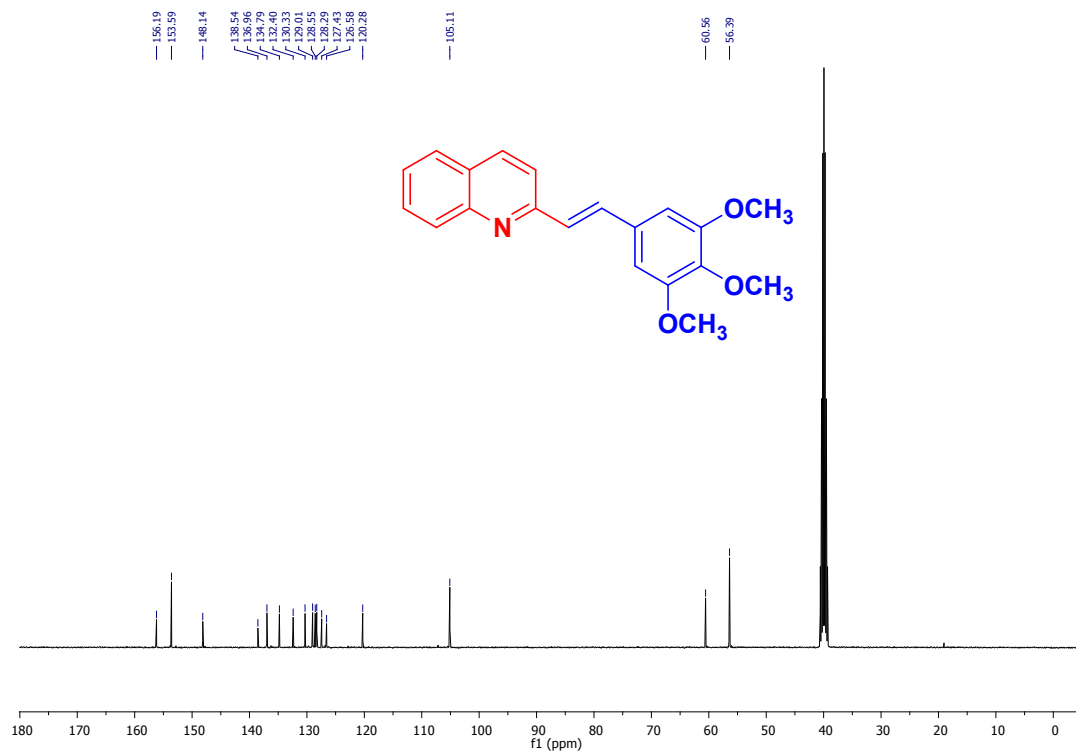


4aj. (E)-2-(3,4,5-trimethoxystyryl)quinoline

¹H NMR spectrum (400 MHz, DMSO-d₆)

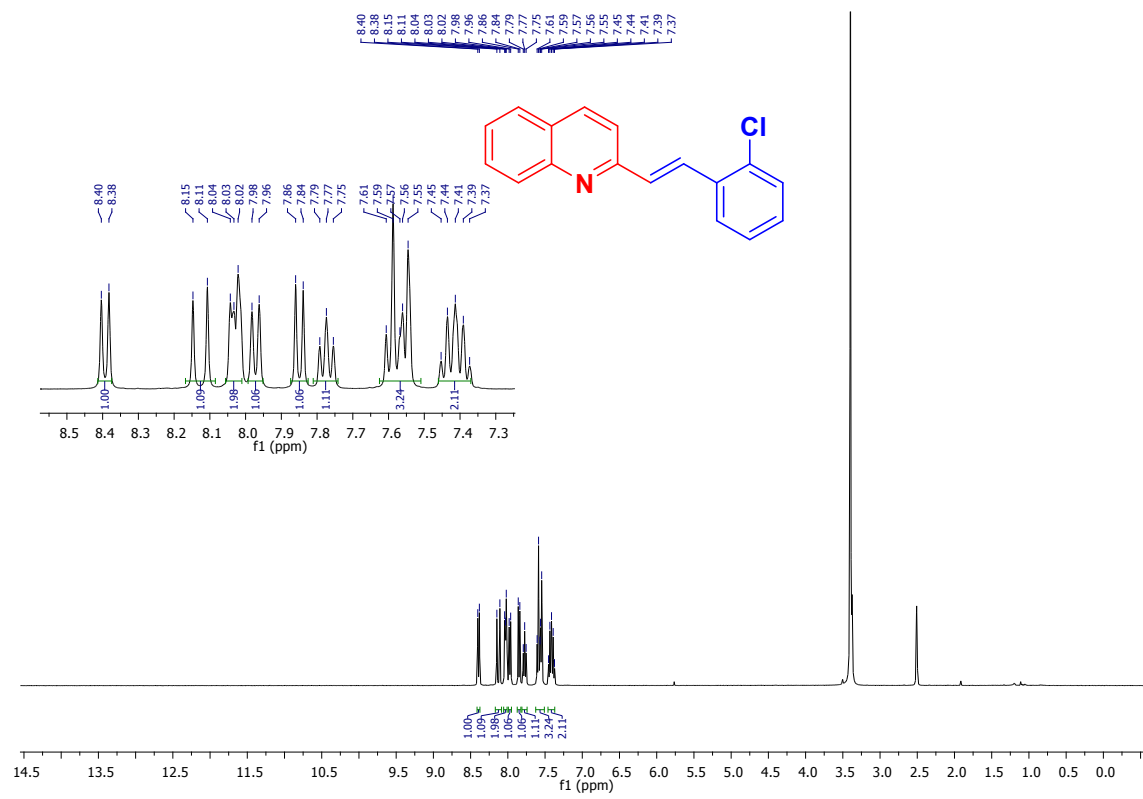


¹³C NMR spectrum (101 MHz, DMSO-d₆)

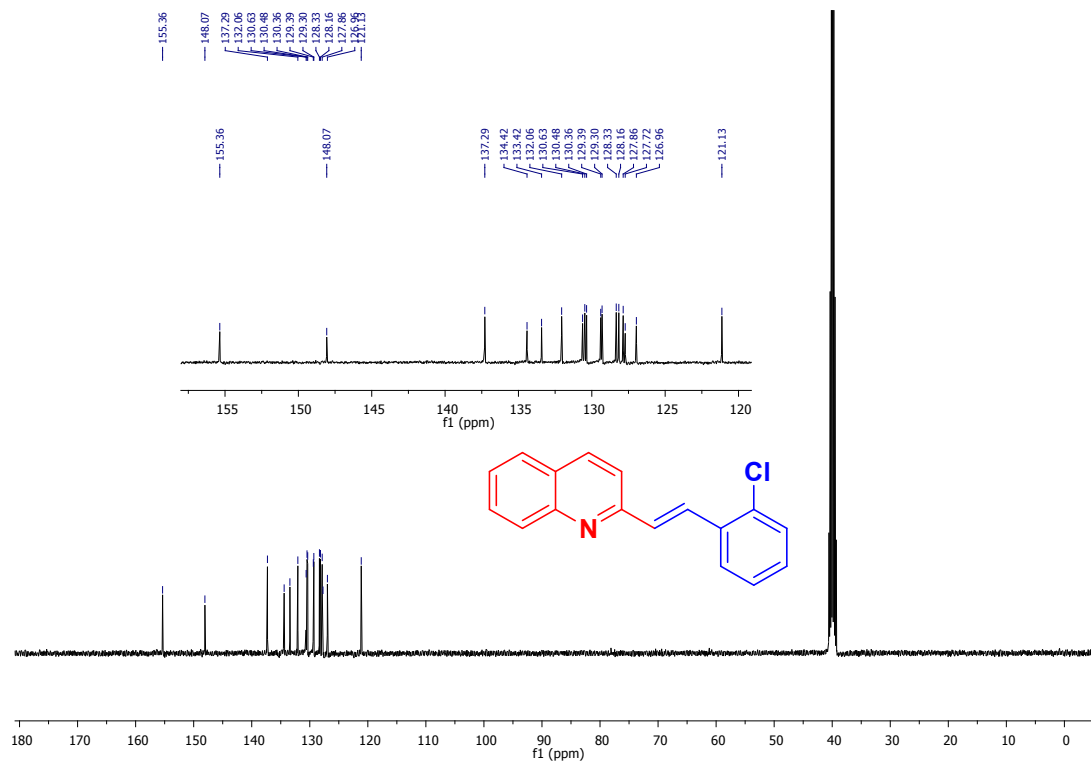


4ak. (E)-2-(2-chlorostyryl)quinoline

¹H NMR spectrum (400 MHz, DMSO-d₆)

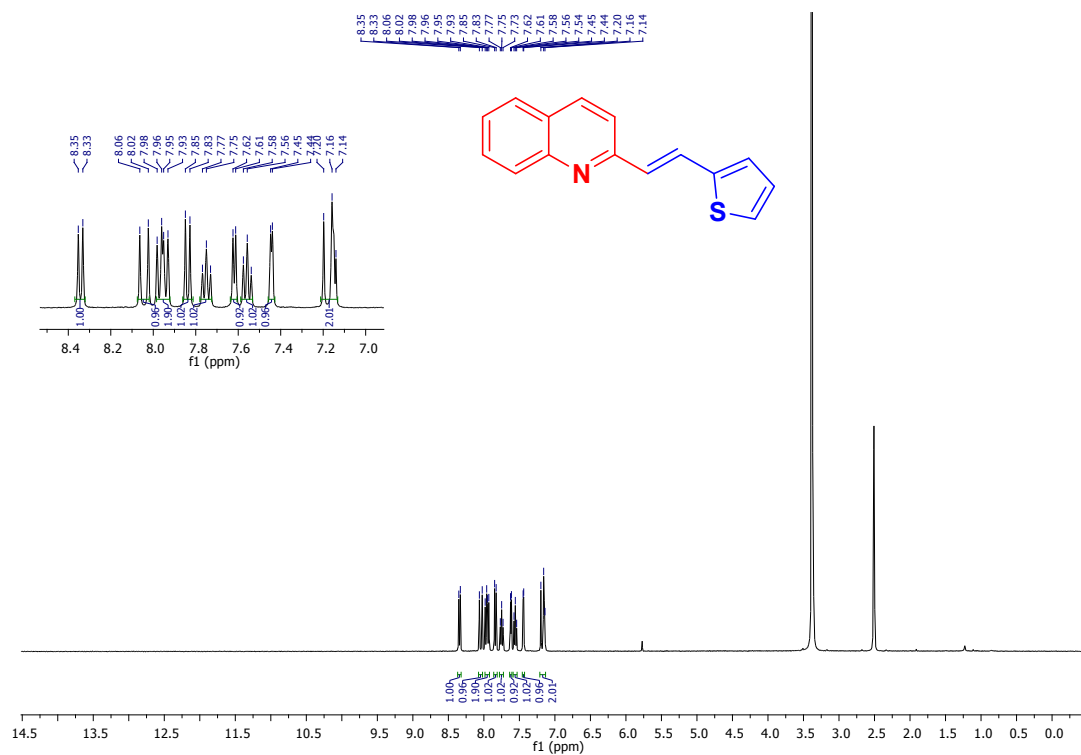


¹³C NMR spectrum (101 MHz, DMSO-d₆)



4al. (E)-2-(2-(thiophen-2-yl)vinyl)quinoline

¹H NMR spectrum (400 MHz, DMSO-d₆)



¹³C NMR spectrum (101 MHz, DMSO-d₆)

