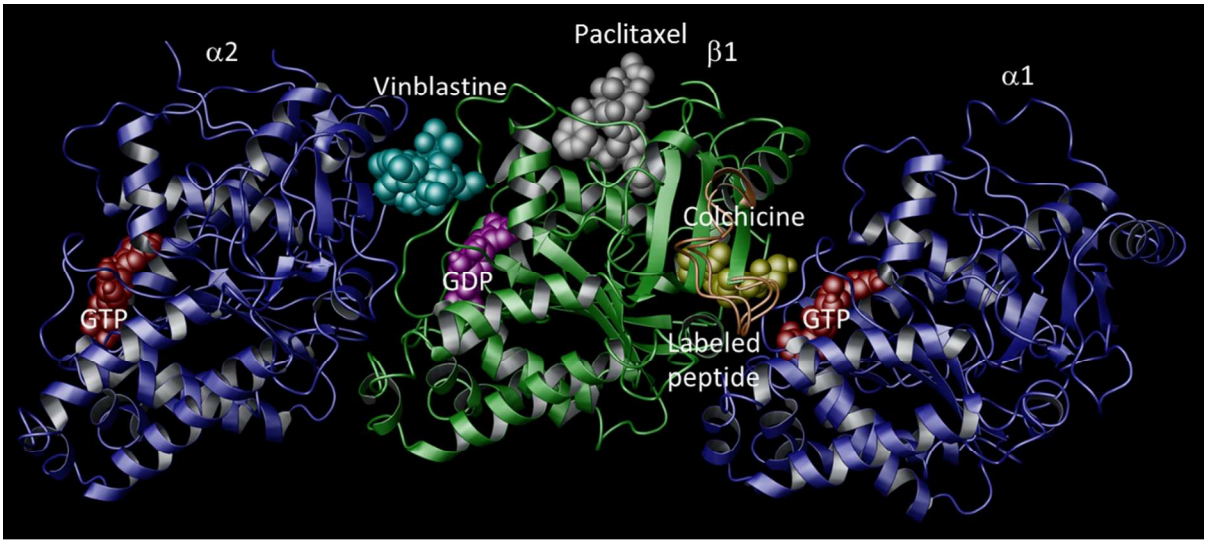
Supporting Information for

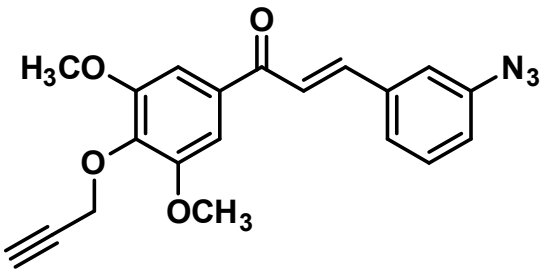
An indole-chalcone inhibits multidrug-resistant cancer cell growth by targeting microtubules

Materials

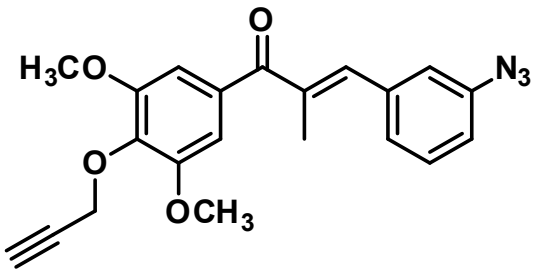
All purchased reagents and solvents were used without further purification. Silica gel chromatography was performed on Whatman silica gel 60 Å (230–400 mesh). Nuclear magnetic resonance (NMR) spectra (^{1}H and ^{13}C) were recorded on a spectrometer (Bruker Ascend 400) and calibrated using the deuterated solvent residual as an internal reference. High-resolution mass spectrometry (HRMS) was performed using a Q-TOF micro mass spectrometer. Purities of the compounds were analyzed by HPLC (Agilent 1100) using an ODS-A column (YMC Pack; 10 × 250 mm, 5 μm) with methanol:H_2O (100:0 to 80:20 over 20 min and 80:20 thereafter) as the mobile phase with a flow rate of 2 mL/min. The separation was monitored at wavelengths of 254 and 365 nm. The purities of all final compounds were higher than 95%.

Human A549, A549/T, A549/DDP, HCT-116, HCT-116/L, HL60, HL60/DOX, K562, K562/HHT300, CCRF-CEM, and CCRF-CEM/VLB100 cells were authenticated via DNA analysis by Genetica DNA Laboratories (Cincinnati, OH, USA) or by the University of Arizona Genomics Core. Cells were cultured following our standard protocols and tested monthly for *Mycoplasma* contamination. De-identified mobilized peripheral blood (MPB) was obtained after informed consent according to protocols approved by the University of Minnesota Institutional Review Board.





Negative Probe (1)
GI_{50} = 28600 ± 2200 nM



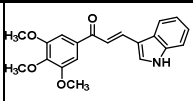
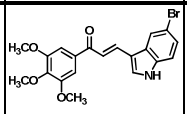
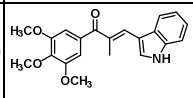
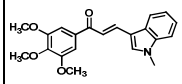
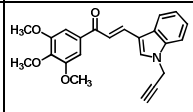
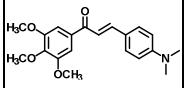
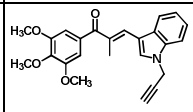
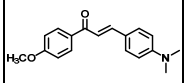
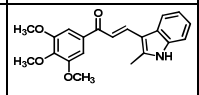
Positive Probe (2)
GI_{50} = 380 ± 10 nM

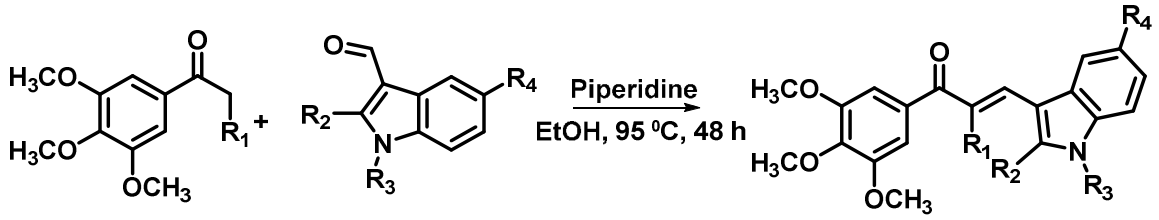
Figure S1. Structural assembly of α- and β-tubulin and its known binding sites for paclitaxel (white), vinblastine (cyan), colchicine (yellow), GDP (red) and GTP (purple) and positive chalcone probe labeled peptide (orange). The structures of probes and their cytotoxicity (GI_{50}) towards A549 human non-small lung adenocarcinoma cell line were provided.

Indole-chalcones and the structure-activity relationship

Based on our previous SAR results [1] that an α-methyl substitution significantly increased chalcone's cytotoxicity and compound **3** was previously reported as a highly cytotoxic agent [2], **FC77** and a few analogs were synthesized with their cytotoxicity determined against A549 human non-small lung adenocarcinoma cancer cell line [1, 3-6]. A clear SAR was observed (Table S1). The introduction of an α-methyl substituent significantly increased the cytotoxic potency (two probe with a 75-fold difference [1, 6], **3** and **FC77** with a 14-fold difference, and **5** and **6** with a 30-fold difference). The chalcones with an indole moiety (**3** – **9**) had potent cytotoxicity against A549 cancer cells with the GI_{50} values in the low nanomolar to low micromolar range. The free 1-amino group of indole is critical to the cytotoxicity as the compounds with 1-amino substitutions showed 40-, 2- and 19-fold loss in potency comparing **3** and **5**, **3** and **9**, and **FC77** and **6**, respectively. A 5-bromo substitution was highly unfavorable to the cytotoxicity (**3** and **8** with a 178-fold difference). A 2-methyl substitution also led to significant loss of cytotoxicity (**5** and **9** with a 15-fold difference). These chalcones with the addition of **10** and **11** were docked into the colchicine-binding site (**10** and **11** were selected in order to cover a wider range of cytotoxicity because they are much less cytotoxic, particularly **11**).

Table S1. The structures and the 48-hour cytotoxicity of the indole chalcones towards A549 human non-small lung adenocarcinoma cell line.

Entry	Structure	A549 GI_{50} (nM)	Entry	Structure	A549 GI_{50} (nM)
3		38±3	**8**		6630±570
4 (FC77)		2.7± 0.4	**9**		77±8
5		1510±210	**10** [1]		920± 180
6		50±5	**11** [1]		>60000
7		560±32			



Scheme S1. Synthesis of indole-chalcones.

(*E*)-3-(1*H*-indol-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3**) [7]

Recrystallization yield: 10%. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.71 (1H, br, NH), 8.13 (1H, d, J = 15.6 Hz, =CH), 8.00 (1H, m, Ar-H), 7.63 (1H, s, Ar-H), 7.55 (1H, d, J = 15.2 Hz, =CH), 7.46 (1H, m, Ar-H), 7.31 (4H, m, Ar-H), 3.96 (9H, s, 3OCH_3). ^{13}C NMR (100MHz, $CDCl_3$): δ 189.86, 153.10, 138.78, 137.23, 134.44, 130.08, 123.57, 120.54, 117.82, 114.52, 111.99, 105.96, 60.97, 56.40. HRMS (ESI^+) m/z Calculated for $C_{20}H_{19}NO_4$ 338.1387; Observed 338.1385 ($M+H^+$). HPLC Purity: 97.8%, R_t = 35.70 min, UV 254 nm.

Intermediate 1: 1-(prop-2-yn-1-yl)-1*H*-indole-3-carbaldehyde [8]

Recrystallization yield: 38.2%. ^{1}H NMR (400MHz, $CDCl_3$): δ 10.03 (1H, s, CHO), 8.34 (1H, dd, J_1 = 1.6 Hz, J_2 = 8.3 Hz, Ar-H), 7.90 (1H, s, Ar-H), 7.32-7.44 (3H, m, Ar-H), 4.94 (2H, s, CH_2), 2.55 (1H, s, alkynyl-H). ^{13}C NMR (100MHz, $CDCl_3$): δ 184.58, 137.46, 136.80, 125.54, 124.28, 123.30, 123.30, 122.34, 118.70, 109.81, 75.81, 75.57, 36.65.

(*E*)-3-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**5**)

Recrystallization yield: 28.4%. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.09 (1H, d, *J* = 15.6 Hz, =CH), 8.01 (1H, d, *J* = 7.8 Hz, Ar-H), 7.67 (1H, s, Ar-H), 7.52 (2H, m, =CH), 7.30-7.39 (4H, m, Ar-H), 4.93 (2H, s, CH_2), 3.97 (6H, s, $2OCH_3$), 3.94 (3H, s, OCH_3), 2.51 (1H, s, alkynyl-H). ^{13}C NMR (100MHz, $CDCl_3$): *δ* 189.66, 153.10, 142.00, 138.22, 137.18, 134.43, 132.38, 123.48, 121.98, 121.68, 120.82, 117.75, 113.77, 110.23, 105.95, 74.85, 60.97, 56.41, 36.30. HRMS (ESI^+) m/z Calculated for $C_{23}H_{21}NO_4$ 376.1543; Observed 376.1542 ($M+H^+$). HPLC Purity: 97.7%, R_t = 14.33 min, UV 254 nm.

(*E*)-3-(1*H*-indol-3-yl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**6**)

Recrystallization yield: 4.3%. ^{1}H NMR (400MHz, $CDCl_3$): *δ* 7.66 (2H, s, =CH, Ar-H), 7.58 (1H, d, *J* = 7.3 Hz, Ar-H), 7.45 (1H, d, *J* = 7.6 Hz, Ar-H), 7.36 (1H, m, Ar-H), 7.16-7.23 (1H, m, Ar-H), 7.03 (2H, s, Ar-H), 4.98 (2H, s, CH_2), 3.95 (6H, s, $2OCH_3$), 3.90 (3H, s, OCH_3), 2.51 (1H, s, alkynyl-H), 2.34 (3H, s, CH_3). ^{13}C NMR (100MHz, $CDCl_3$): *δ* 197.84, 152.78, 140.75, 135.54, 134.51, 134.02, 131.65, 128.77, 128.73, 123.32, 121.25, 118.77, 112.72, 109.80, 106.93, 74.59, 60.98, 56.26, 36.39, 15.46. HRMS (ESI^+) m/z Calculated for $C_{24}H_{23}NO_4$ 390.1700; Observed 390.1697 ($M+H^+$). HPLC Purity: 95.50%, R_t = 9.35 min, UV 365 nm.

(*E*)-3-(2-methyl-1*H*-indol-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**7**)

Recrystallization yield: 7.2%. ^{1}H NMR (400MHz, $CDCl_3$): *δ* 8.37 (1H, br, NH), 8.17 (1H, d, *J* = 15.4 Hz, =CH), 7.95 (d, *J* = 7.3 Hz, Ar-H), 7.54 (1H, d, *J* = 15.4 Hz, =CH), 7.37 (1H, m, Ar-H), 7.33 (2H, s, Ar-H), 7.29 (1H, m, Ar-H), 3.97 (6H, s, $2OCH_3$), 3.95 (3H, s, OCH_3), 2.64 (3H, s, CH_3). ^{13}C NMR (100MHz, DMSO-d_6): *δ* 188.31, 153.23, 144.42, 141.56, 138.12, 136.53, 134.76, 126.29, 122.50, 121.64, 120.55, 114.75, 111.92,

109.60, 105.97, 60.59, 56.51, 12.41. HRMS (ESI^+) m/z Calculated for $C_{21}H_{21}NO_4$ 352.1543; Observed 352.1543 ($M+H^+$). HPLC Purity: 98.5%, R_t = 17.91 min, UV 254 nm.

(*E*)-3-(5-bromo-1*H*-indol-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**8**)

Recrystallization yield: 15.6%. ^1H NMR (400MHz, $CDCl_3$): δ 8.69 (1H, br, NH), 8.13 (1H, s, Ar-H), 8.04 (1H, d, J = 15.6 Hz, =CH), 7.62-7.63 (1H, s, Ar-H), 7.51 (1H, d, J = 15.6 Hz, =CH), 7.41 (1H, d, J = 8.6 Hz, Ar-H), 7.33 (3H, m, Ar-H), 3.97 (6H, s, $2OCH_3$), 3.95 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.73, 153.32, 142.33, 137.83, 135.93, 134.31, 130.51, 126.64, 123.55, 118.72, 115.30, 114.27, 113.46, 103.10, 61.15, 56.53. HRMS (ESI^+) m/z Calculated for $C_{20}H_{18}BrNO_4$ 416.0492; Observed 416.0492 ($M+H^+$). HPLC Purity: 96.4%, R_t = 37.92 min, UV 254 nm.

(*E*)-3-(1-methyl-1*H*-indol-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**9**) [7]

Recrystallization yield: 54.1%. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (1H, d, J = 15.5 Hz, =CH), 7.96 (1H, d, J = 7.3 Hz, Ar-H), 7.29 – 7.55 (5H, m, =CH, Ar-H), 7.22 (2H, s, Ar-H), 3.93 (6H, s, $2OCH_3$), 3.91 (3H, s, OCH_3), 3.83 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.81, 153.19, 141.99, 138.75, 138.39, 134.72, 134.62, 126.28, 123.32, 121.72, 120.76, 116.95, 113.09, 110.32, 105.99, 77.58, 77.16, 76.74, 61.10, 56.51, 33.44. HRMS (ESI^+) m/z Calculated for $C_{21}H_{21}NO_4$ 352.1543; Observed 352.1543 ($M+H^+$). HPLC Purity: 95.1%, R_t = 9.23 min, UV 254 nm.

Docking and molecular modeling

The positive probe was docked into the colchicine binding site of tubulin (PDB entry: 1SA0) using Schrodinger Glide package with no constraint (Coordinate, X: 40.9, Y: 52.51, Z: -9.03). As shown in Fig. S1, the azide group of the positive probe is within 2.2Å and 2.5Å to the backbone oxygen and the amide sidechain atoms of N349 of the labeled peptide. Using the same method, eleven chalcone compounds (compounds **1-11**) with a wide range of cytotoxicity were docked into the colchicine binding site of tubulin (PDB entry: 1SA0). Their best binding modes were obtained and imported into the atom-based QSAR panel [9]. The model was generated by Schrodinger Phase package using default parameters. The GI_{50} and predicted activity values were presented in units of –log [concentration, μM]. As shown in Fig. S3, the predicted activity and the GI_{50} showed a nice correlation with a R^2 value of 0.70.

A

#	Compound 2 /ctrl ratio		
	Round 1	Round 2	Round 3
Y310-R318	44	51	105
F242-R251	36	38	104
L253- R262	20	38	179
I381-R390	47	37	162
I47-K58	109	50	87
I163-K174	24	92	179
E325-K336	44	42	63
A63-R77	25	44	130
A283-K297	58	59	82
N337-K350	6	8	16
G104-R121	27	35	64
L217-R241	39	58	42
S78-K103	22	34	39
F20-R46	41	36	95
Average (99% confidence)	39 ± 20	44 ± 15	96 ± 41

B

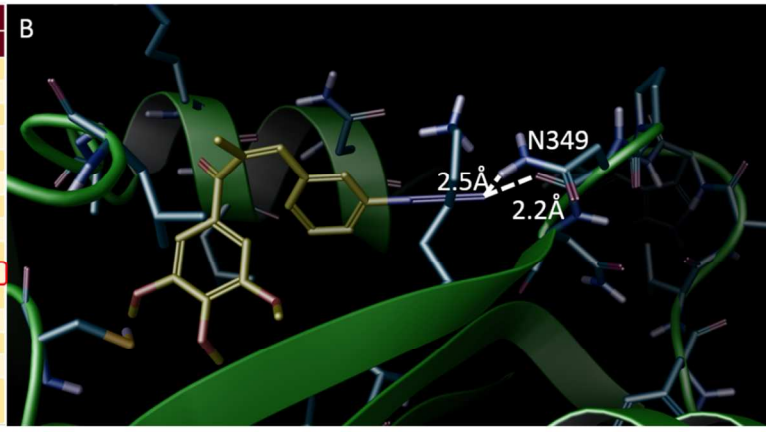
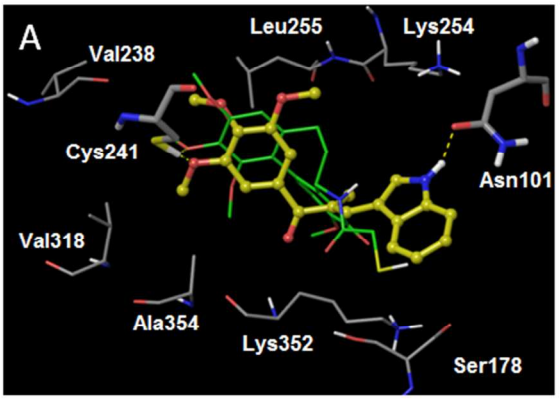
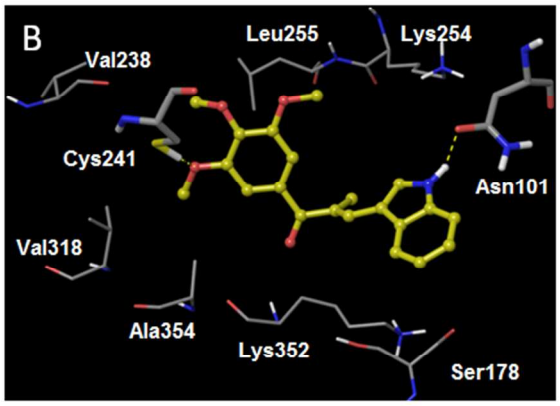
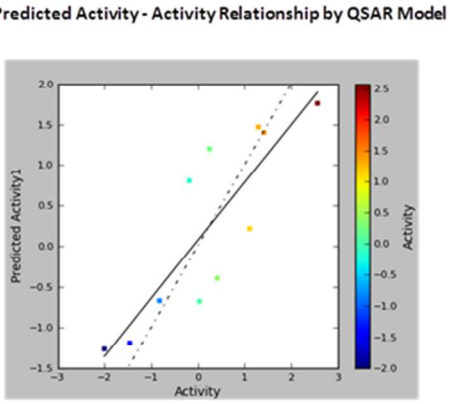


Figure S2. (A) Proteomic study suggested the colchicine binding site as the target for the positive chalcone probe [1, 6]. (B) Molecular modelling study of this probe showed that the azide group is within 2.2Å and 2.5Å to the backbone oxygen and the amide sidechain atoms of N349 of the labeled peptide.





C



Ligand name	Activity	Predicted Activity	Prediction Error
1	-1.456	-1.1877	0.268296
2	0.420	-0.37855	-0.817855
3	1.420	1.40119	-0.0188068
4	2.569	1.76136	-0.807242
5	-0.179	0.805015	0.984015
6	1.301	1.46966	0.168664
7	0.252	1.20141	0.949407
8	-0.820	-0.670958	0.148542
9	1.114	0.216125	-0.897875
10	0.036	-0.679375	-0.715375
11	-2.000	-1.26177	0.738231

Figure S3. Docking studies of **FC77** with tubulin: (A) Superposition of the docked conformation of **FC77** and the original ligand of 1SA0 (colchicine); (B) Predicted binding mode of **FC77** and tubulin. **FC77** was presented in yellow carbons, the original ligand of 1SA0 was in green carbons, the tubulin was in gray carbons with representative amino acids labeled. The nitrogen and oxygen were presented in blue and red, respectively. The hydrogen bonds were labeled in yellow dash line. (C) An atom-based QSAR model was generated by Schrodinger Phase package. The GI_{50} (activity in the table) and predicted activity values were presented in units of –log [concentration, μM]. The activity value of compound **11** with a GI_{50}>60 μM was presented as -2.0.

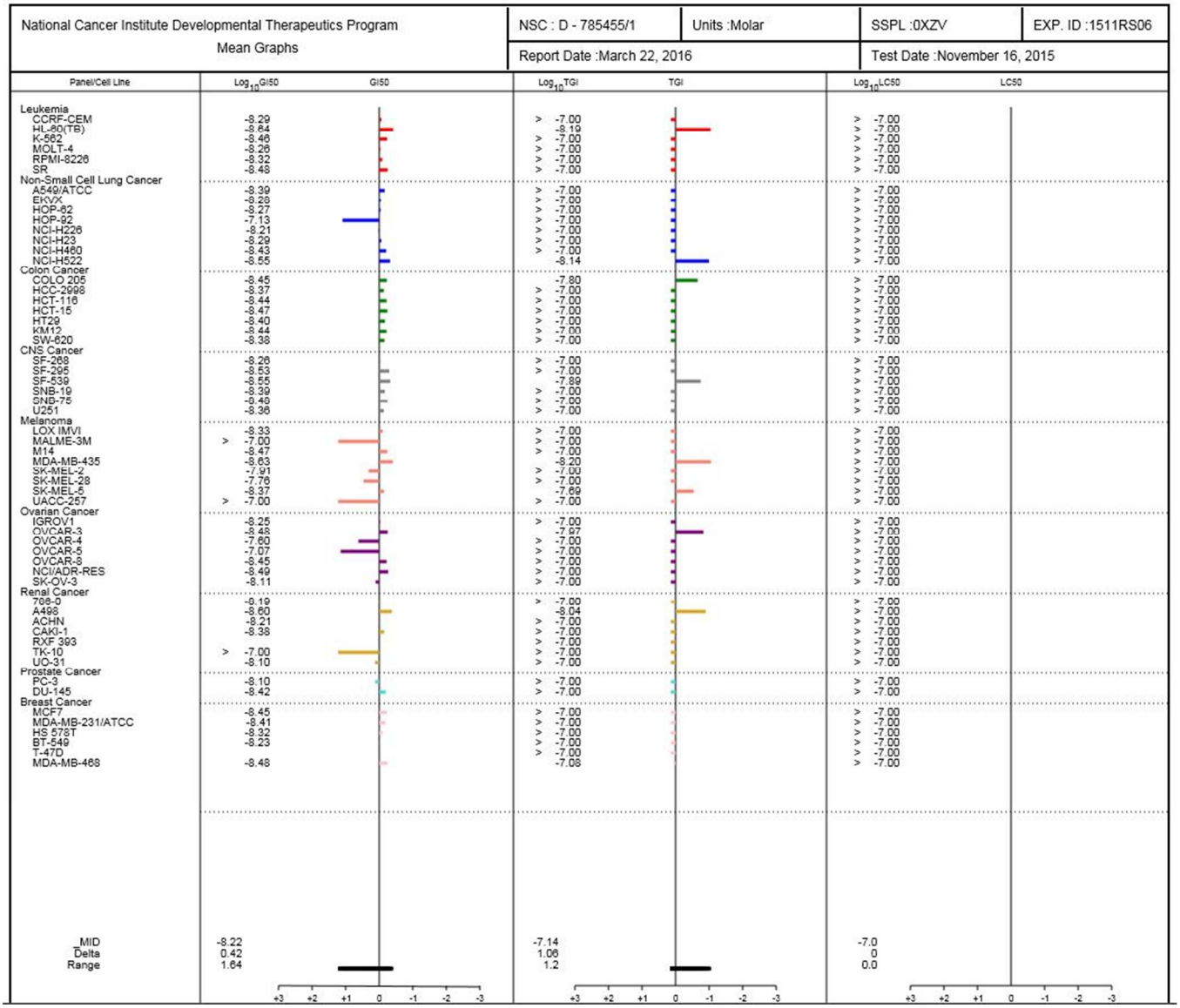


Figure S4. Original NCI-60 data. The Seed NSC of **FC77** is D-785455/1.

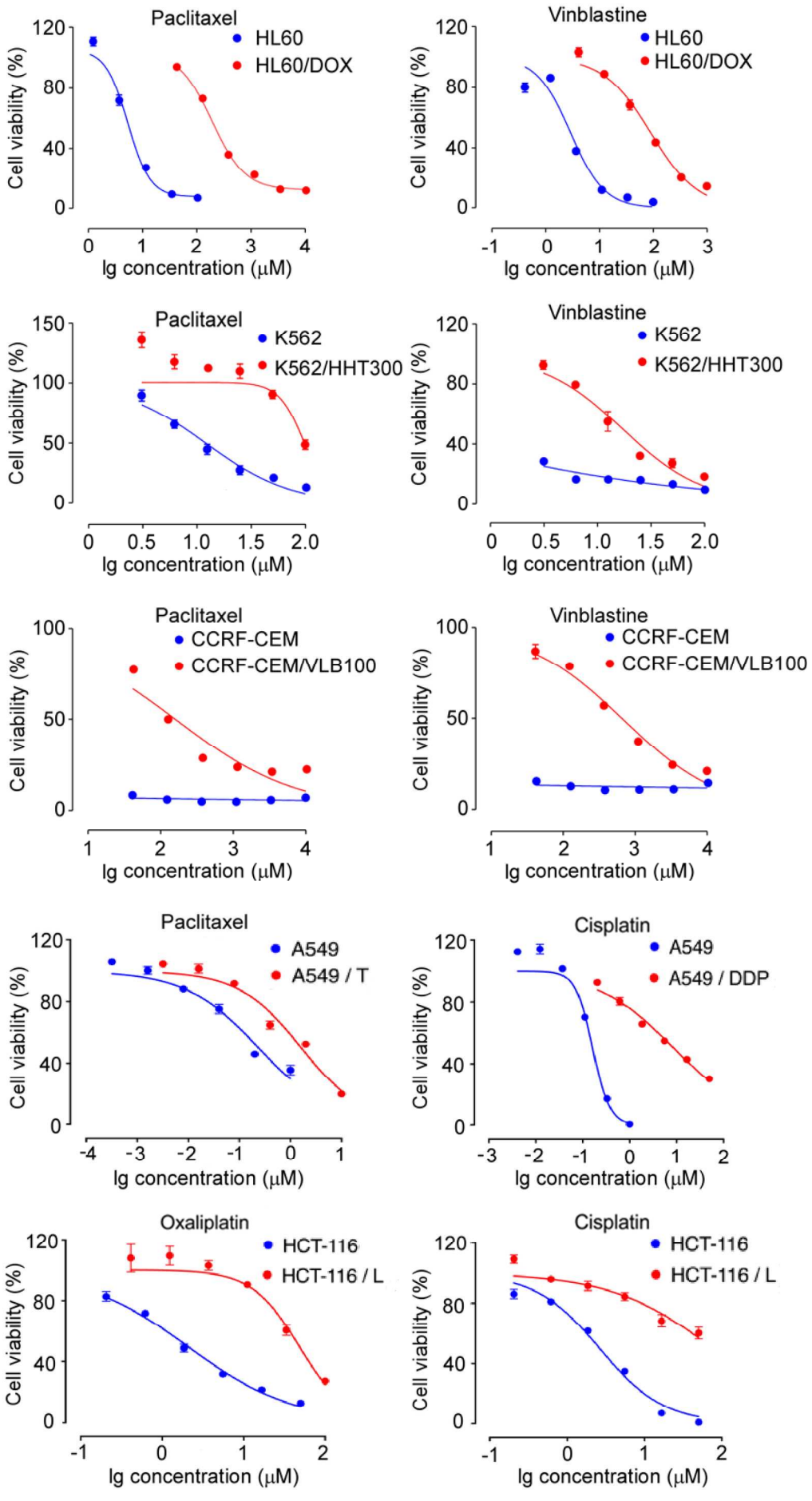

Paclitaxel
HL60
HL60/DOX
Cell viability (%)
lg concentration (μM)
Vinblastine
HL60
HL60/DOX
Cell viability (%)
lg concentration (μM)
Paclitaxel
K562
K562/HHT300
Cell viability (%)
lg concentration (μM)
Vinblastine
K562
K562/HHT300
Cell viability (%)
lg concentration (μM)
Paclitaxel
CCRF-CEM
CCRF-CEM/VLB100
Cell viability (%)
lg concentration (μM)
Vinblastine
CCRF-CEM
CCRF-CEM/VLB100
Cell viability (%)
lg concentration (μM)
Paclitaxel
A549
A549 / T
Cell viability (%)
lg concentration (μM)
Cisplatin
A549
A549 / DDP
Cell viability (%)
lg concentration (μM)
Oxaliplatin
HCT-116
HCT-116 / L
Cell viability (%)
lg concentration (μM)
Cisplatin
HCT-116
HCT-116 / L
Cell viability (%)
lg concentration (μM)

Figure S5. Characterization of the multi-drug resistant phenotype of parental and drug-resistant cancer cells.

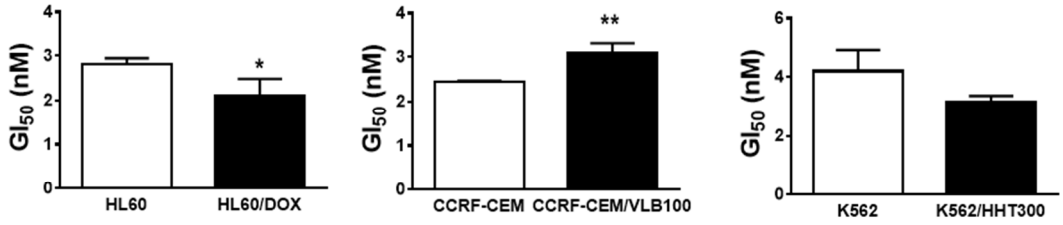


Figure S6. Characterization of the cytotoxicity of combretastatin A-4 towards the three multi-drug resistant leukemia cell lines in comparison to their parental cancer cell lines. *$p < 0.05$. **$p < 0.01$.

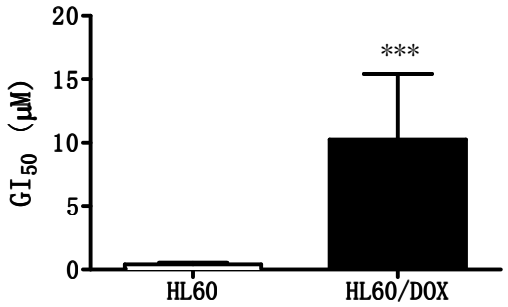
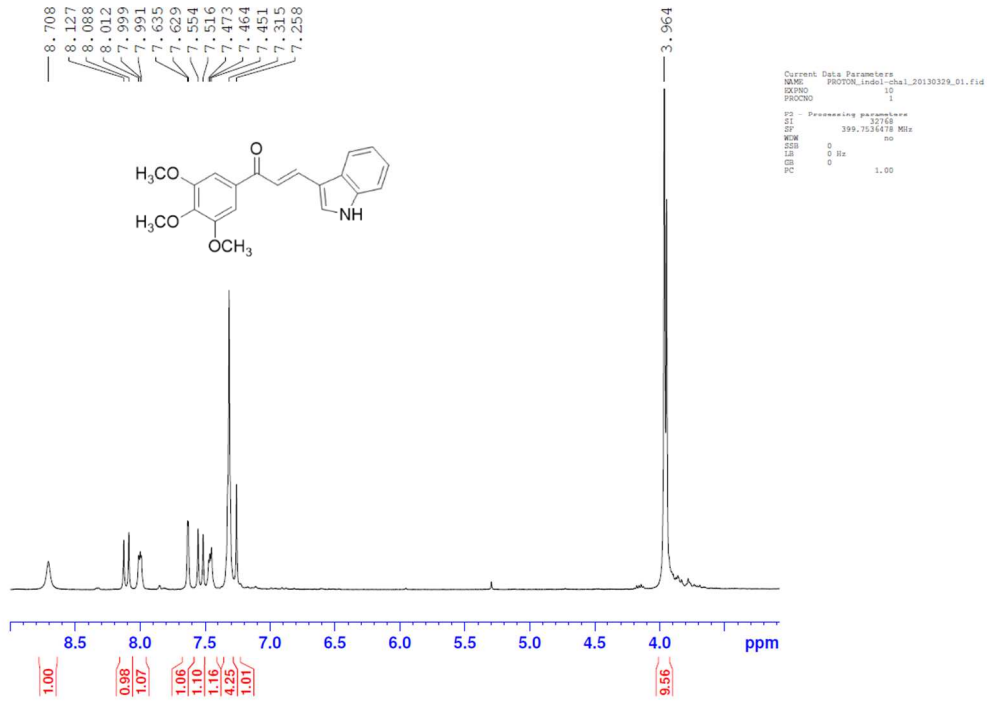
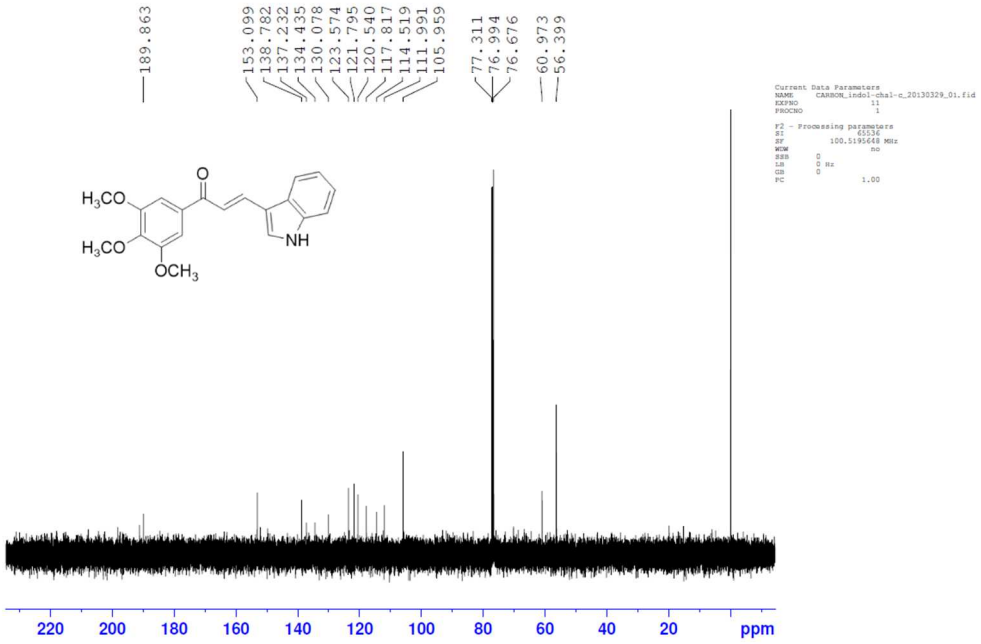
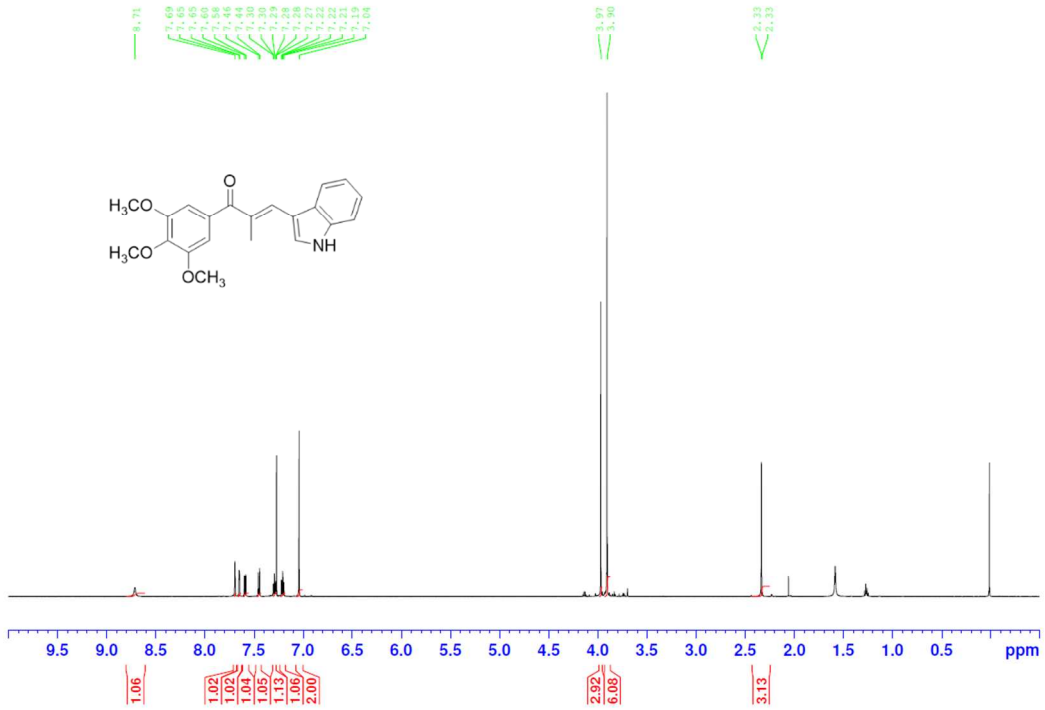


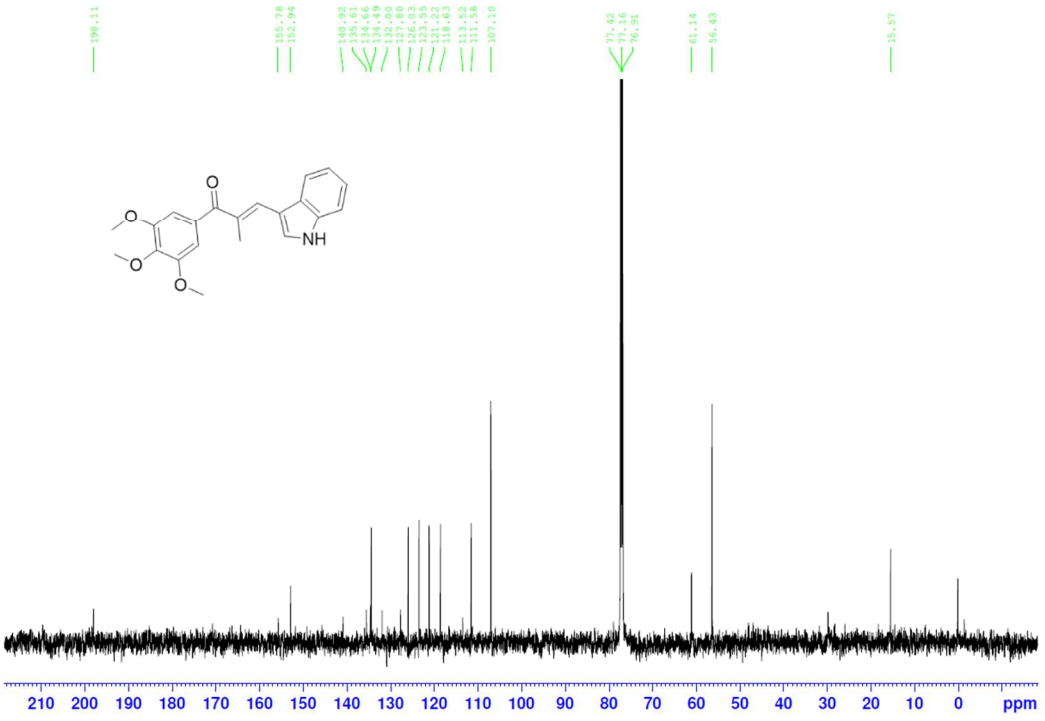
Figure S7. Characterization of the cytotoxicity of colchicine towards the HL60 and HL60/DOX. ***$p < 0.001$.

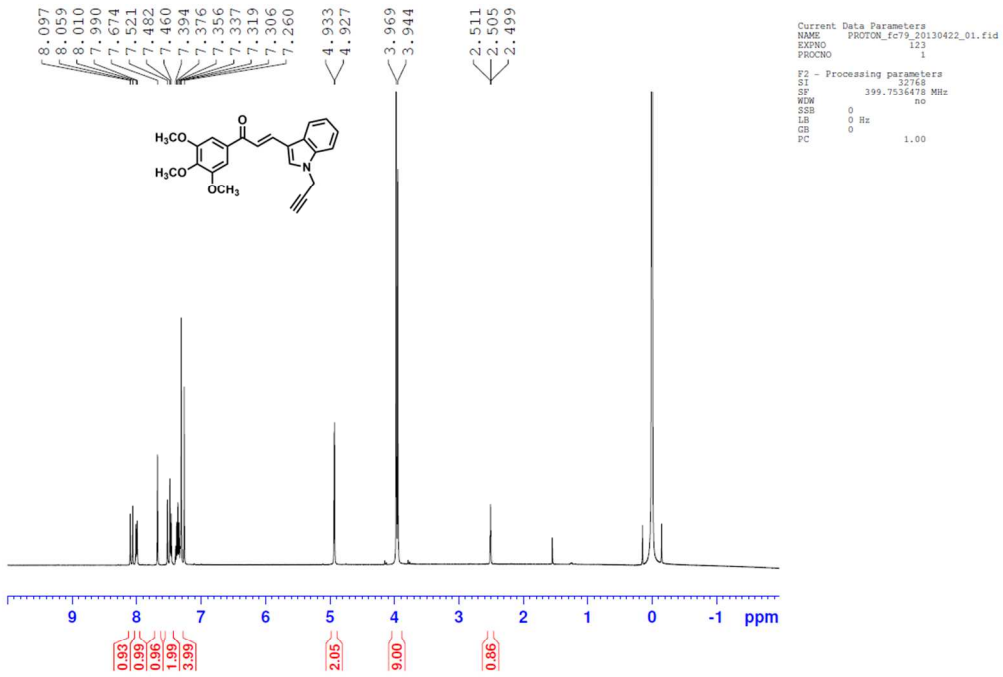
^{1}H-NMR and ^{13}C-NMR

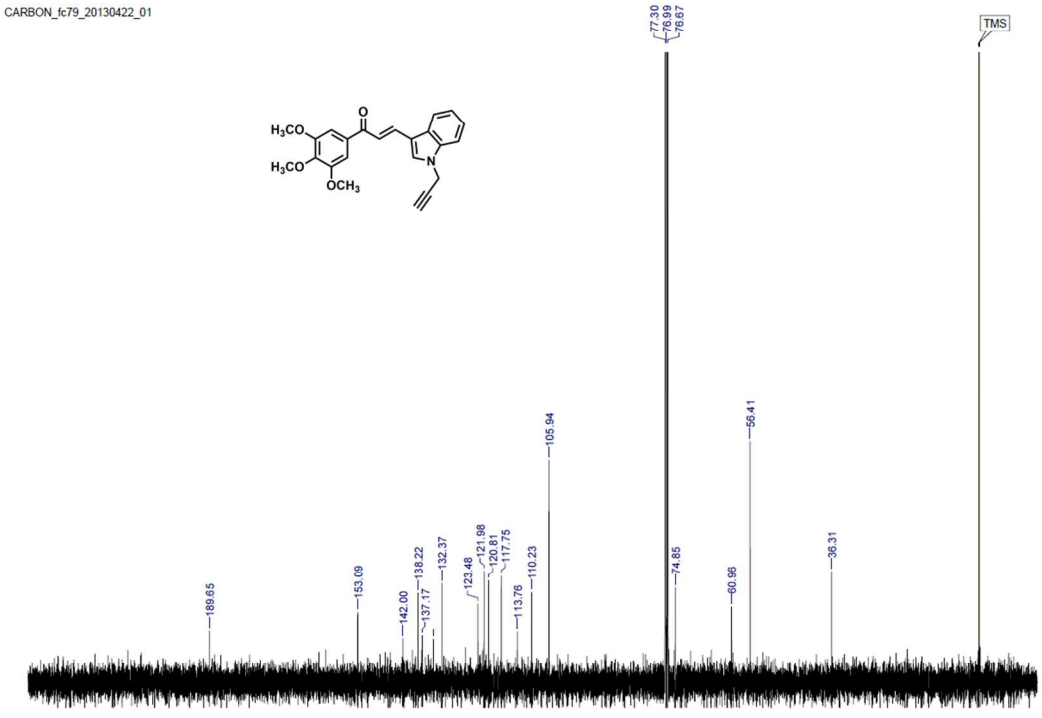


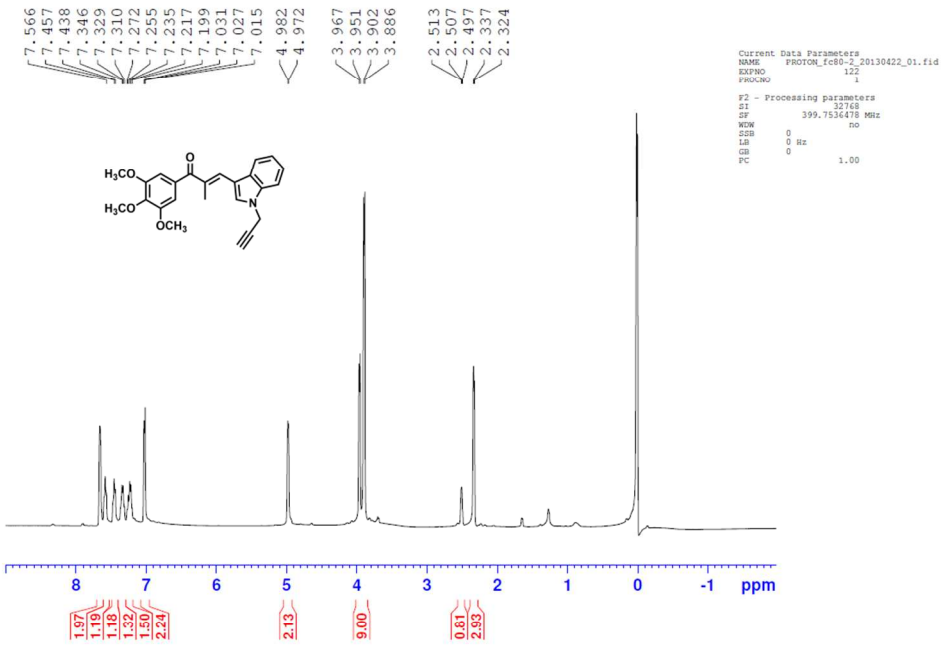


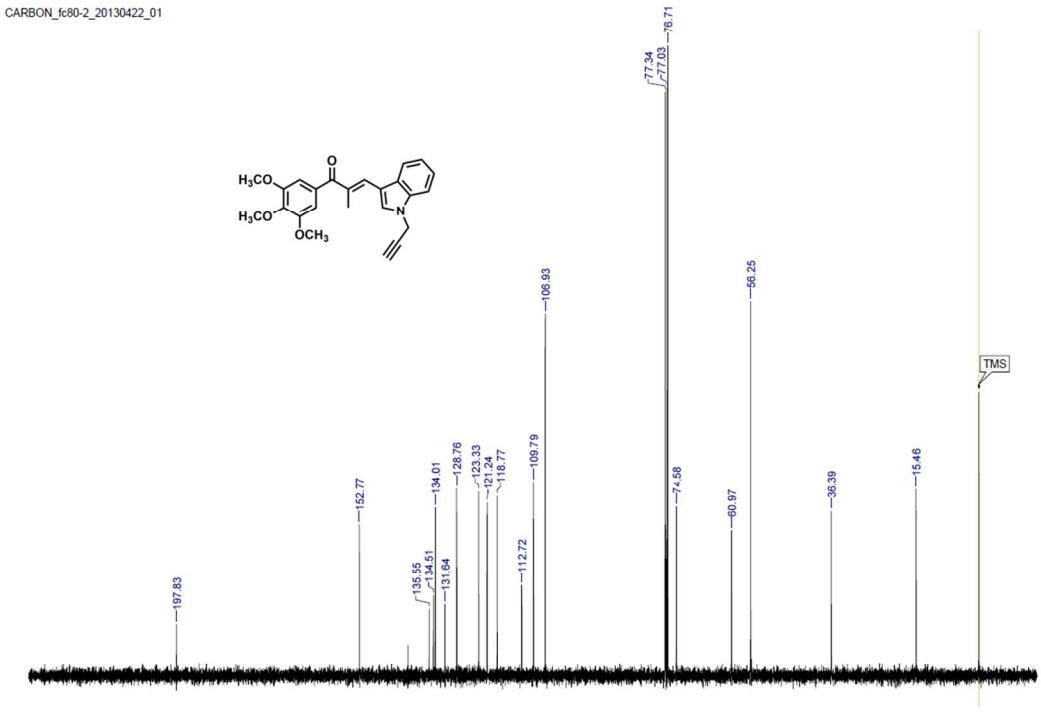

9.71
7.69
7.65
7.60
7.58
7.46
7.44
7.30
7.29
7.28
7.27
7.22
7.21
7.19
7.04
3.97
3.90
2.33
2.33
O
H3CO
H3CO
OCH3
NH
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.06
1.02
1.02
1.04
1.05
1.13
1.06
2.00
2.92
6.08
3.13

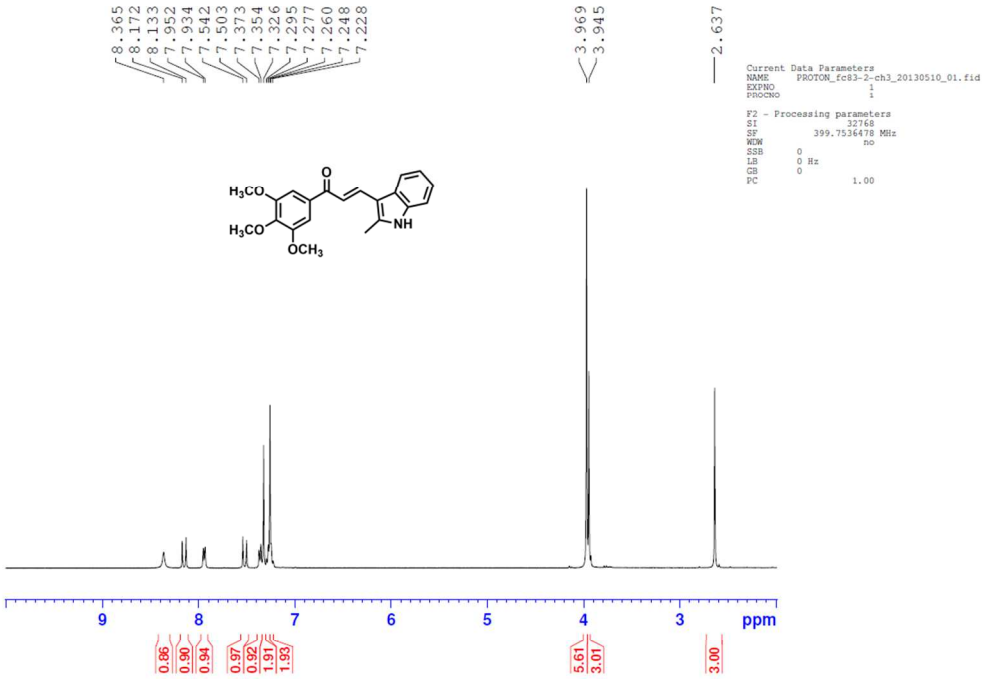

198.11
155.78
152.94
140.92
135.61
134.46
134.49
132.00
127.80
126.03
123.25
121.22
118.63
113.52
111.58
107.10
77.42
77.16
76.91
61.14
56.43
15.57
O
O
O
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

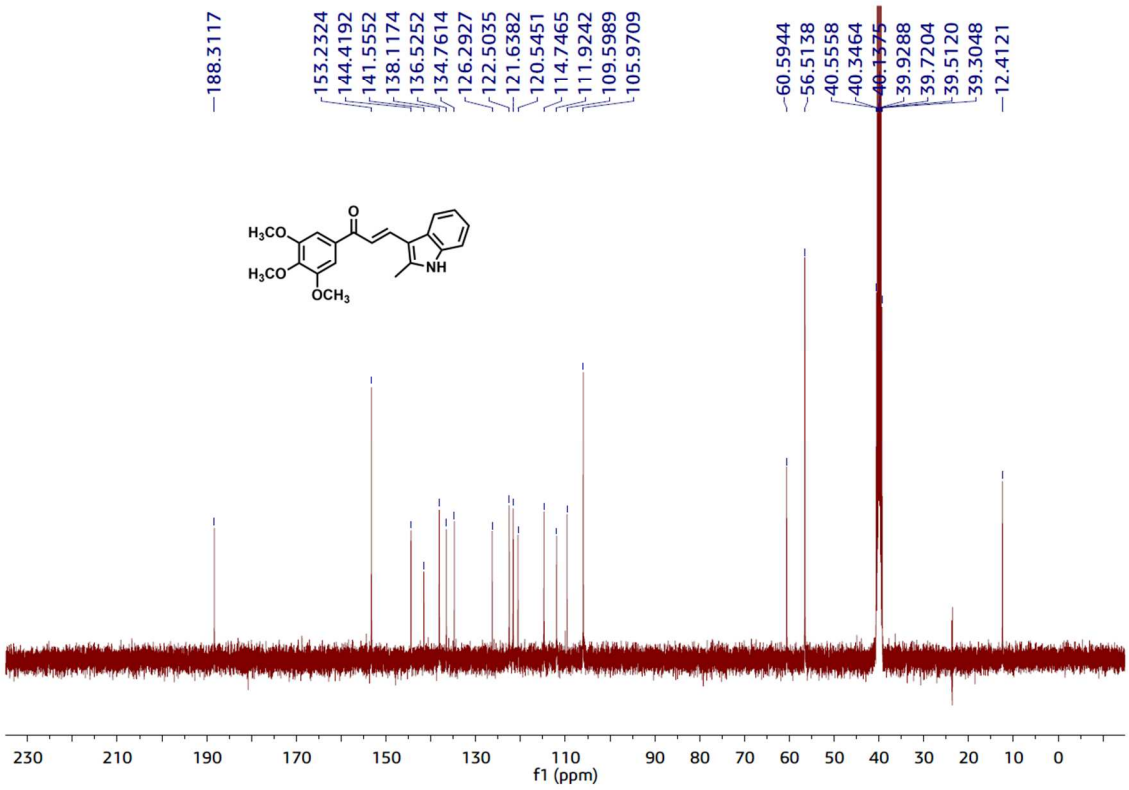

8.097
8.059
8.010
7.990
7.674
7.521
7.482
7.460
7.394
7.376
7.356
7.337
7.319
7.306
7.260
4.933
4.927
3.969
3.944
2.511
2.505
2.499
H3CO
H3CO
OCH3
O
N
Current Data Parameters
NAME PROTON_fc79_20130422_01.fid
EXPNO 123
PROCNO 1
F2 - Processing parameters
SI 32768
SF 399.7536478 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
9 8 7 6 5 4 3 2 1 0 -1 ppm
0.93
0.99
0.96
1.99
3.99
2.05
9.00
0.86

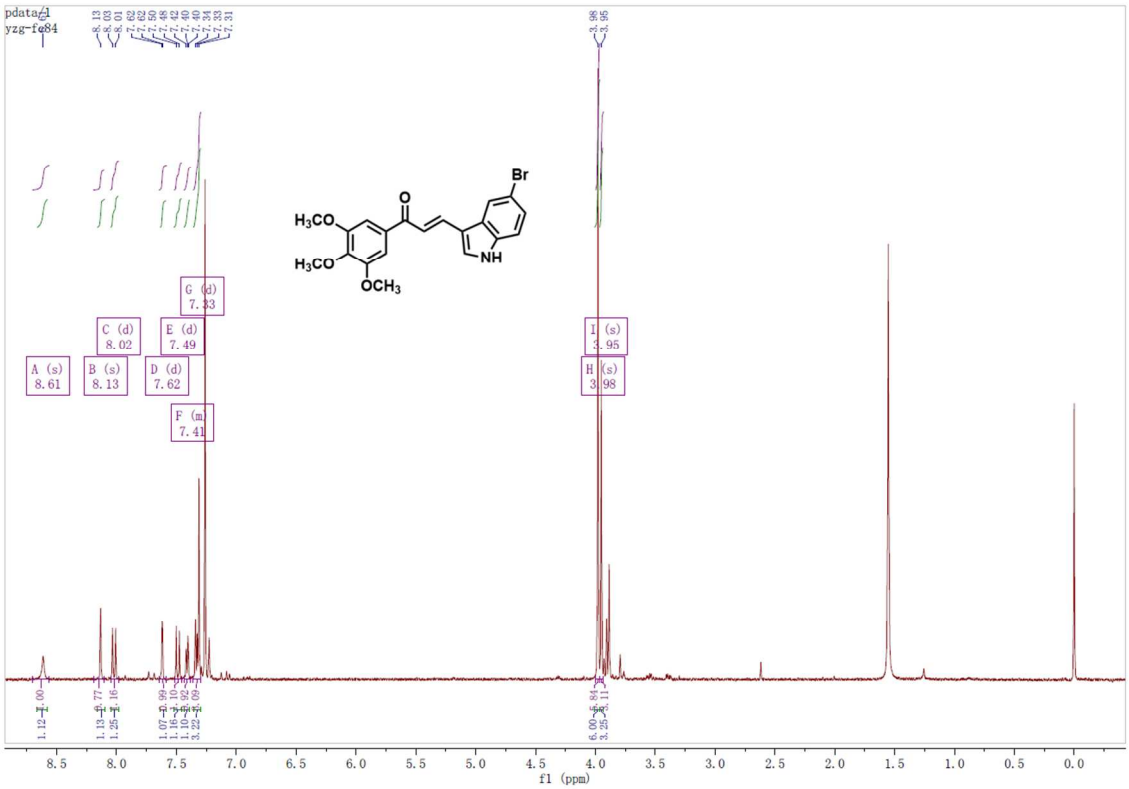

CARBON_fc79_20130422_01
H3CO
H3CO
OCH3
O
N
77.30
76.99
76.67
TMS
105.94
56.41
189.65
153.09
142.00
138.22
137.17
132.37
123.48
121.98
120.81
117.75
113.76
110.23
74.85
60.96
36.31

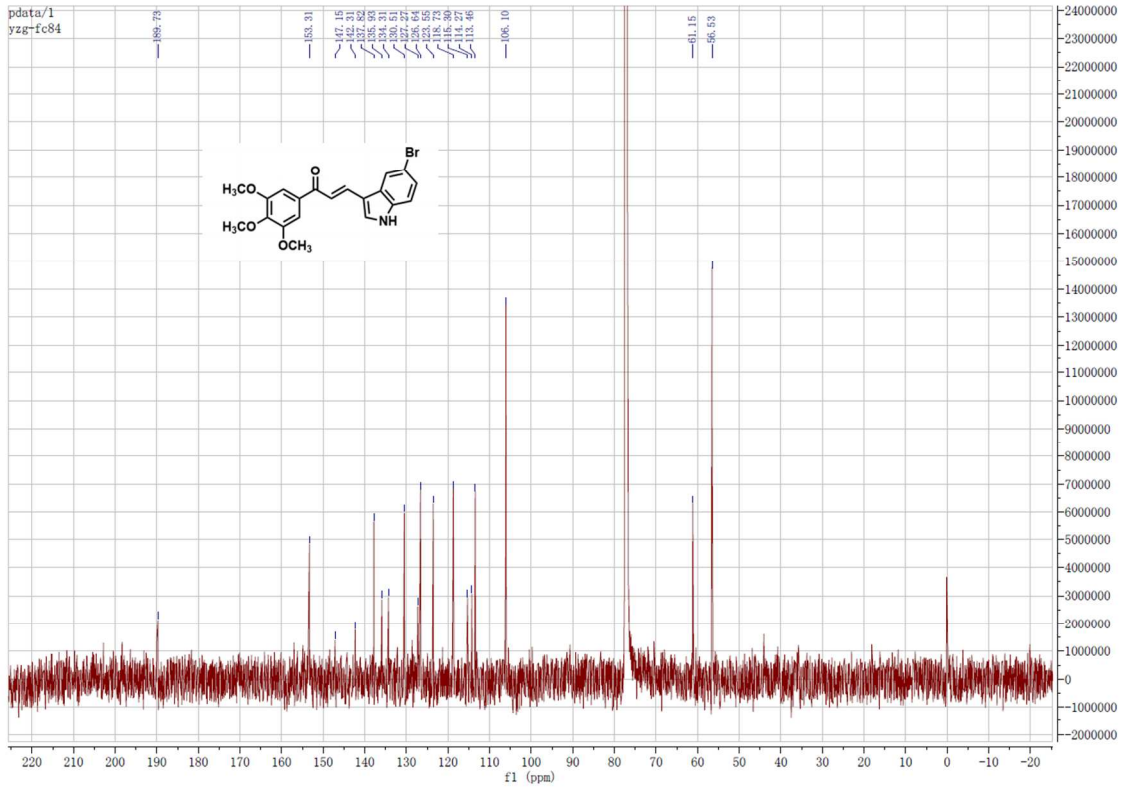

7.566
7.457
7.438
7.346
7.329
7.310
7.272
7.255
7.235
7.217
7.199
7.031
7.027
7.015
4.982
4.972
3.967
3.951
3.902
3.886
2.513
2.507
2.497
2.337
2.324
Current Data Parameters
NAME PROTON_fc80-2_20130422_01.fid
EXPNO 122
PROCNO 1
F2 - Processing parameters
SI 32768
SF 399.7536478 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
H3CO
H3CO
OCH3
O
N
8
7
6
5
4
3
2
1
0
-1
ppm
1.97
1.19
1.18
1.32
1.50
2.24
2.13
9.00
0.81
2.93

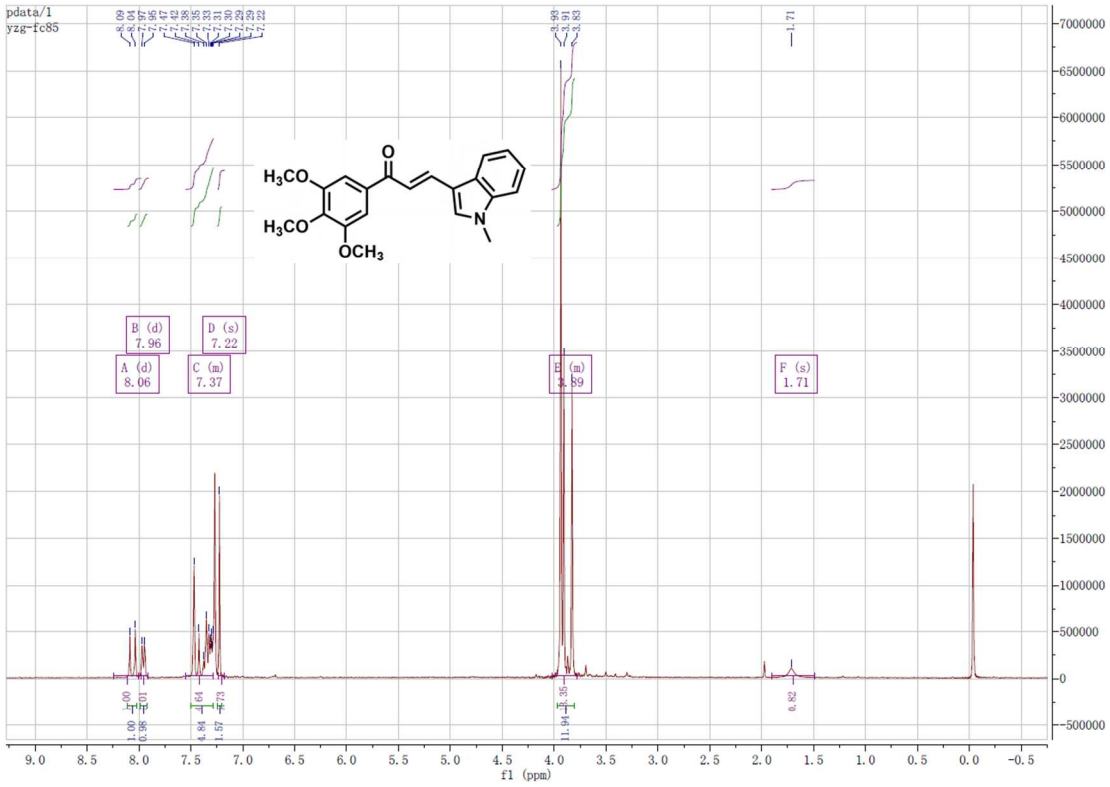

CARBON_fc80-2_20130422_01
H3CO
H3CO
OCH3
O
N
78.71
77.34
77.03
106.93
56.25
TMS
152.77
134.01
128.76
123.33
121.24
118.77
109.79
135.55
134.51
131.64
112.72
74.58
60.97
36.39
15.46
197.83

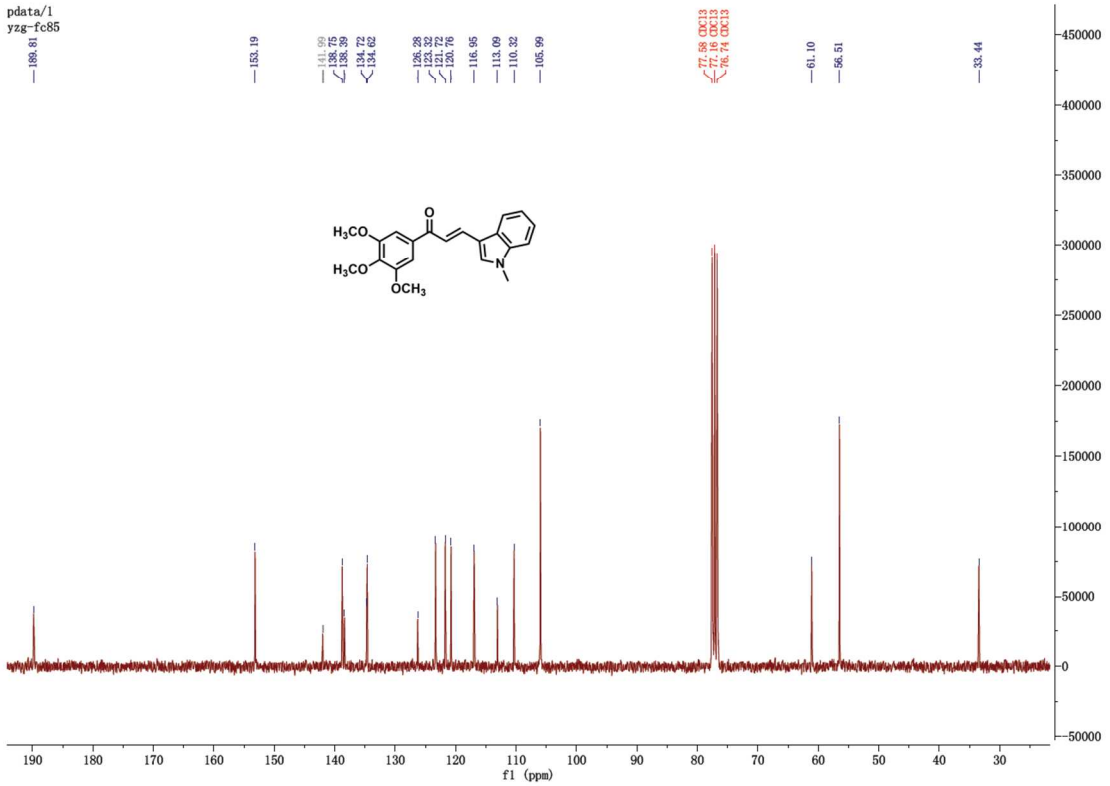

8.365
8.172
8.133
7.952
7.934
7.542
7.503
7.373
7.354
7.326
7.295
7.277
7.260
7.248
7.228
3.969
3.945
2.637
Current Data Parameters
NAME PROTON_fc83-2-ch3_20130510_01.fid
EXPNO 1
PROCNO 1
F2 - Processing parameters
SI 32768
SF 399.7536478 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
H3CO
H3CO
OCH3
O
NH
9
8
7
6
5
4
3
ppm
0.86
0.90
0.94
0.97
0.92
1.91
1.93
5.61
3.01
3.00


188.3117
153.2324
144.4192
141.5552
138.1174
136.5252
134.7614
126.2927
122.5035
121.6382
120.5451
114.7465
111.9242
109.5989
105.9709
60.5944
56.5138
40.5558
40.3464
40.1375
39.9288
39.7204
39.5120
39.3048
12.4121
H3CO
H3CO
OCH3
O
NH
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


pdata/1
yzg-fc84
A (s)
8.61
B (s)
8.13
C (d)
8.02
D (d)
7.62
E (d)
7.49
F (m)
7.41
G (d)
7.33
H (s)
3.98
I (s)
3.95
f1 (ppm)


pdata/1
yzg-fc84
f1 (ppm)


pdata/1
yzg-fc85
B (d)
7.96
D (s)
7.22
A (d)
8.06
C (m)
7.37
E (m)
3.89
F (s)
1.71
f1 (ppm)

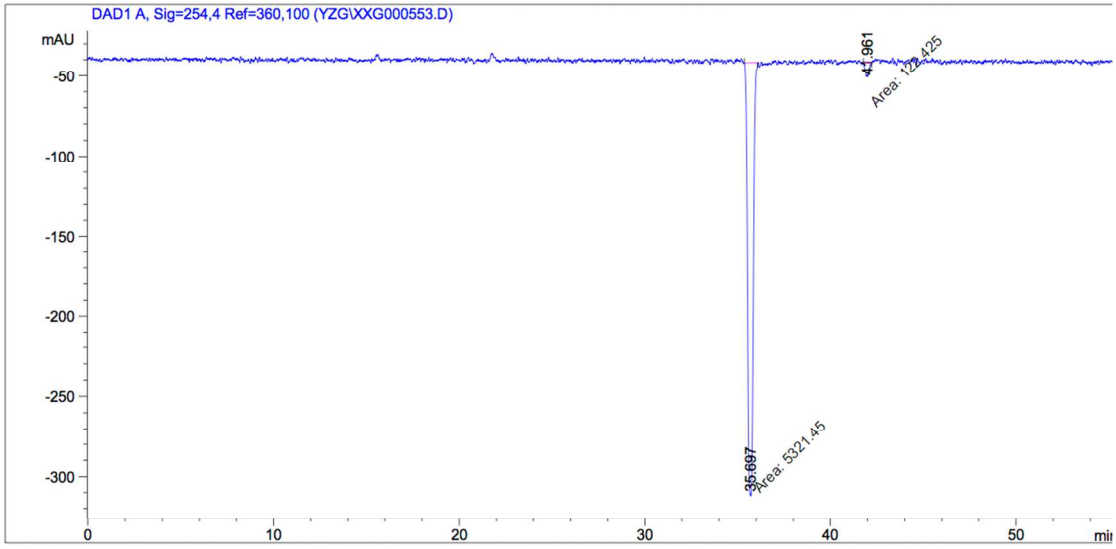

pdata/1
yzg-fc85
189.81
153.19
141.99
138.75
138.39
134.72
134.62
126.28
123.32
121.72
120.76
116.95
113.09
110.32
105.99
77.58 CDCl3
77.16 CDCl3
76.74 CDCl3
61.10
56.51
33.44
f1 (ppm)

HPLC purity

Compound 3

Data File D:\JACK\YZG\XXG000553.D
Sample Name: Fc58

```
==========================================================================
Acq. Operator   : Yzg
                                                    Location : Vial 1
Injection Date  : 11-Mar-17, 14:28:35
Acq. Method     : YIZHE SUN.M
Analysis Method : D:\JACK\YIZHE SUN\YIZHE SUN.M
Last changed    : 3/13/2017 1:26:44 PM by ZYB
                  (modified after loading)
```

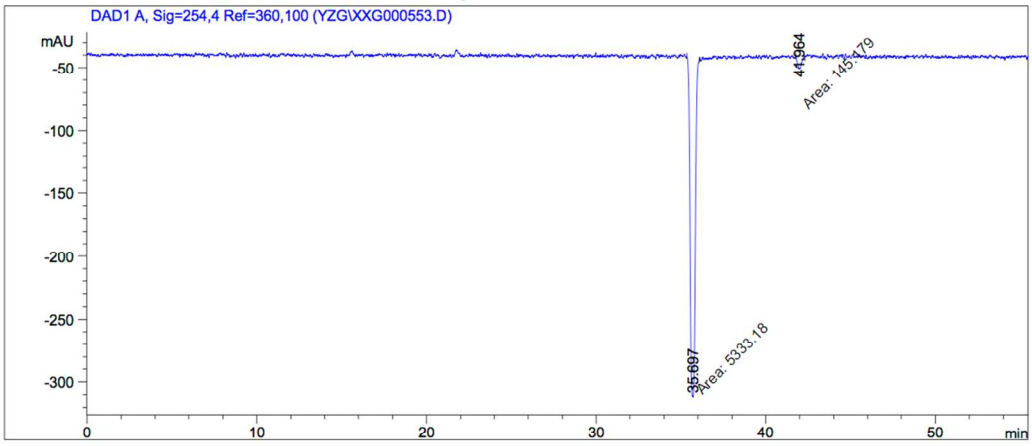



```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  35.697 PP N  0.3284 5321.45215  270.08963  97.7512
   2  41.961 MP N  0.2300  122.42458    8.87006   2.2488

Totals :                  5443.87673  278.95969


==========================================================================
                        *** End of Report ***
```

Compound 4

Data File D:\JACK\YZG\XXG000553.D
Sample Name: Fc77

```
==========================================================================
Acq. Operator   : Yzg
                                                    Location : Vial 1
Injection Date  : 11-Mar-17, 14:28:35
Acq. Method     : YIZHE SUN.M
Analysis Method : D:\JACK\YIZHE SUN\YIZHE SUN.M
Last changed    : 3/15/2017 8:27:57 PM by wzl
                  (modified after loading)
```

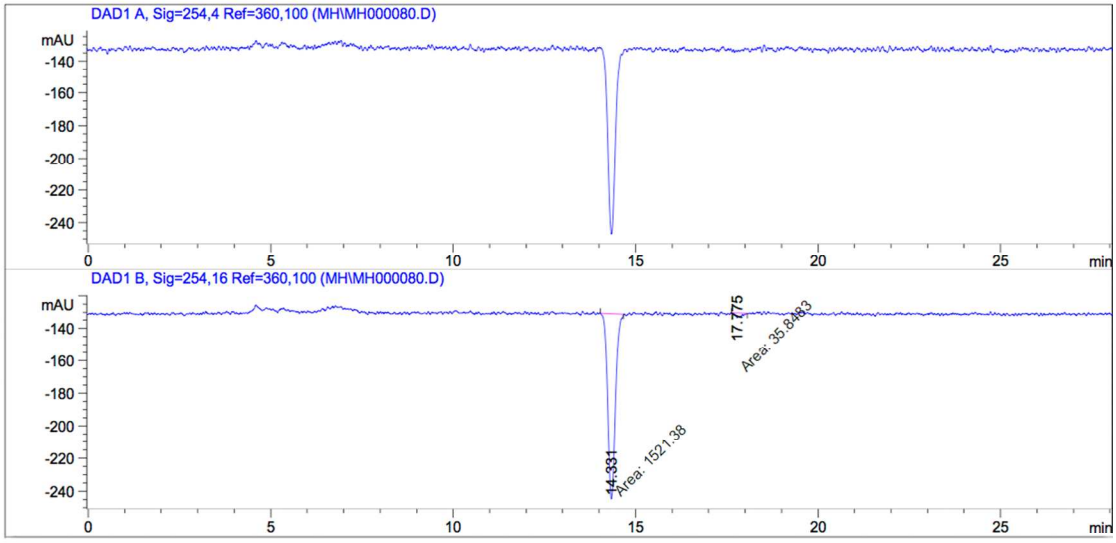



```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  35.697 MP N  0.3288 5333.17773  270.36365  97.3500
   2  41.964 PM N  0.2561  145.17870    9.44740   2.6500

Totals :                  5478.35643  279.81105


==========================================================================
                          *** End of Report ***
```

Compound 5

Data File D:\JACK\MH\MH000080.D
Sample Name: Fc 79

```
==========================================================================
Acq. Operator    : MH
                                                    Location : Vial 1
Injection Date   : 25-May-17, 16:43:42
Method           : D:\JACK\YIZHE SUN\YIZHE SUN.M
Sample Info      : 28ACN
```

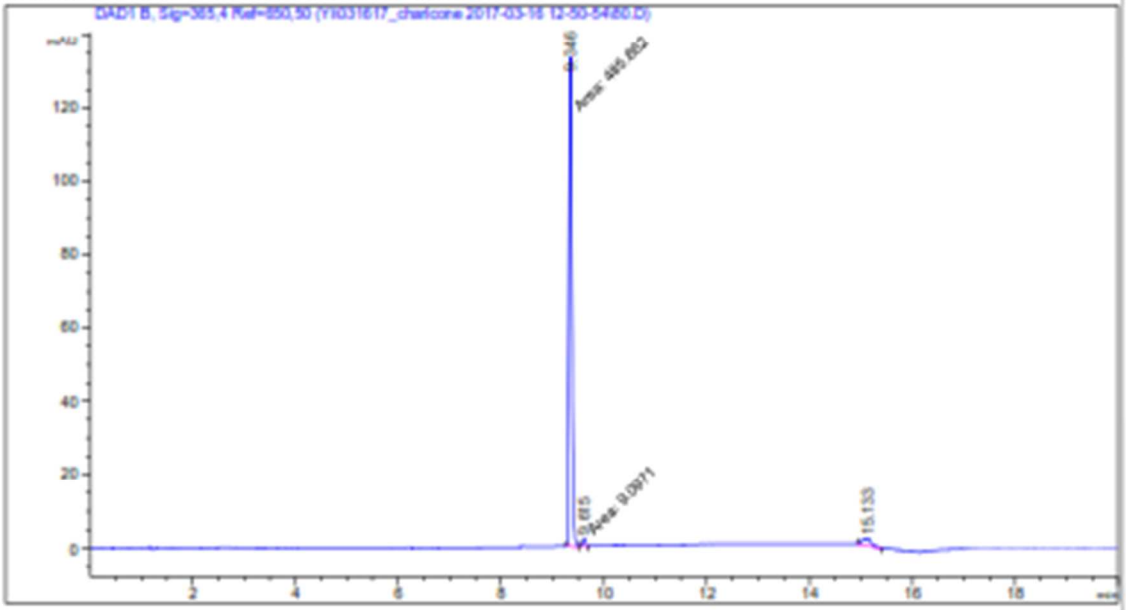



```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  14.331 PP N  0.2231 1521.38330  113.63955  97.6979
   2  17.775 MM N  0.2116   35.84830    2.82298   2.3021

Totals :                  1557.23161  116.46253

==========================================================================
                          *** End of Report ***
```

Compound 6

Data File C:\Chem32\1\Data\Yi\031617_charlcone 2017-03-16 12-50-54\80.D
Sample Name: 80

```
================================================================================
Acq. Operator    : SYSTEM                          Seq. Line :   4
Acq. Instrument : LC_1100                          Location :   3
Injection Date  : 3/16/2017 1:57:52 PM                 Inj :   1
                                                  Inj Volume : 10.000 µl
Method          : C:\Chem32\1\Data\Yi\031617_charlcone 2017-03-16 12-50-54\031617_chalcone.M
                  (Sequence Method)
Last changed    : 3/16/2017 12:34:01 PM by SYSTEM
Additional Info : Peak(s) manually integrated
```

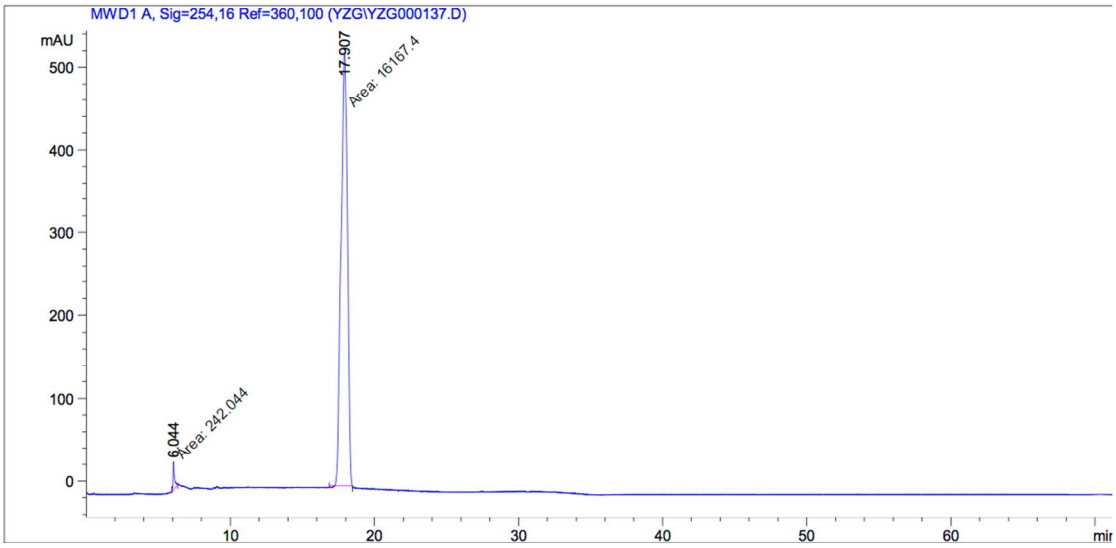



```
================================================================================
                Area Percent Report with Performance
================================================================================

Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=365,4 Ref=650,50


RetTime    k'     Area       Height   Symm.  Width    Plates Resol Select
 [min]          [mAU*s]     [mAU]           [min]           ution ivity
-------|------|----------|----------|-----|-------|-------|-----|------
  9.346    -    485.86179  133.84888  0.82  0.0573  147600    -      -
  9.615    -      9.09710    2.24821  0.65  0.0593  145766  2.71   1.03
 15.133    -     24.31011    2.04329  1.47  0.2067   29702 24.38   1.57


================================================================================
                          *** End of Report ***
```

Compound 7

Data File D:\JACK\YZG\YZG000137.D
Sample Name: Fc83

```
==========================================================================
Acq. Operator   : YZG
Acq. Instrument : LC 1200                        Location : Vial 1
Injection Date  : 3/30/2017 10:40:12 AM
Acq. Method     : D:\JACK\YIZHESUN.M
Last changed    : 3/30/2017 10:39:23 AM by YZG
                  (modified after loading)
Analysis Method : D:\JACK\YIZHE SUN\YIZHE SUN.M
Sample Info     : 25%
```

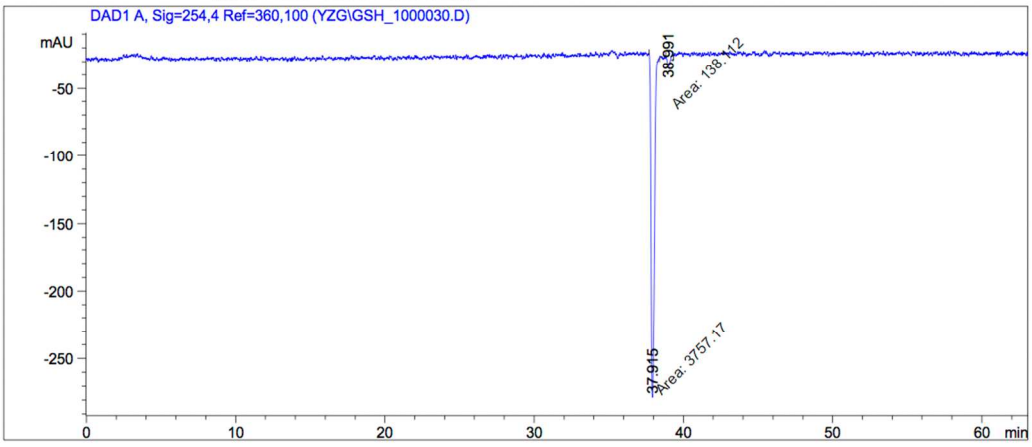



```
==========================================================================================
                              Fraction Information
==========================================================================================
Fraction collection off
==========================================================================================
No Fractions found.
==========================================================================================
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: MWD1 A, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.044 MM    0.1161  242.04439   34.73186   1.4750
   2  17.907 MM    0.5147 1.61674e4   523.52673  98.5250

Totals :                  1.64094e4   558.25859
==========================================================================
                          *** End of Report ***
```

Compound 8

Data File D:\JACK\YZG\GSH_1000030.D
Sample Name: FC84

```
==========================================================================
Acq. Operator   : YZG
                                                    Location : Vial 1
Injection Date  : 11-Mar-17, 14:28:35
Acq. Method     : YIZHE SUN.M
Analysis Method : D:\JACK\YIZHE SUN\YIZHE SUN.M
Last changed    : 3/15/2017 8:27:57 PM by wzl
                  (modified after loading)
```

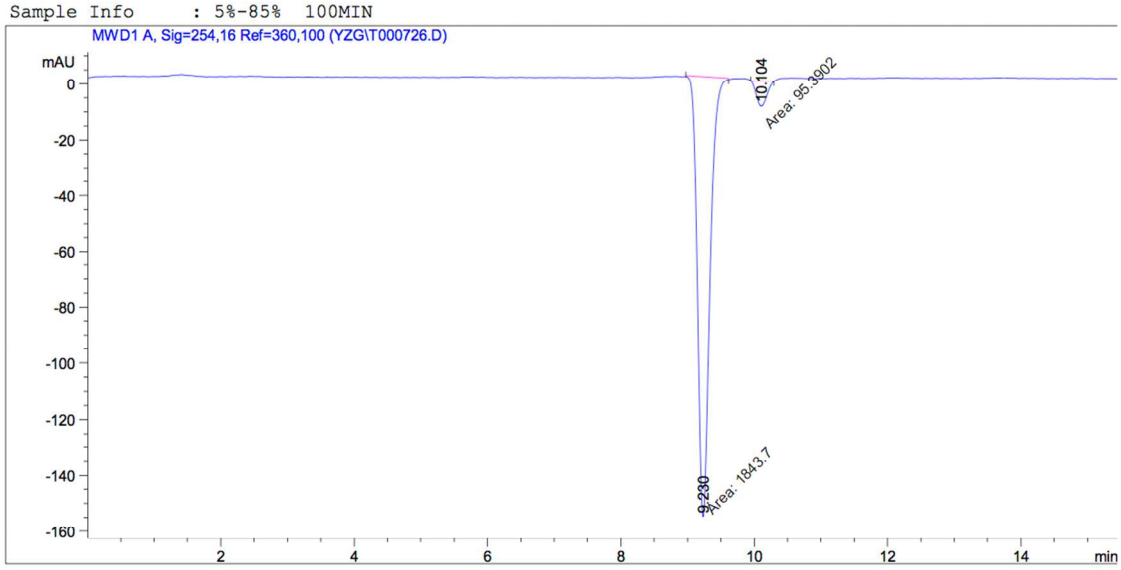



```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  37.915 PP N  0.2475 3757.17114  252.97966  96.4544
   2  38.991 PM N  0.2627  138.11179    8.76226   3.5456

Totals :                  3895.28293  261.74192


==========================================================================
                        *** End of Report ***
```

Compound 9

Data File D:\JACK\YZG\T000726.D
Sample Name: FC85-100%

```
==========================================================================
Acq. Operator   : YZG
Acq. Instrument : LC 1200                       Location : Vial 1
Injection Date  : 5/16/2017 7:46:20 AM
Acq. Method     : D:\JACK\YIZHESUN.M
Last changed    : 5/16/2017 7:35:12 AM by YZG
                  (modified after loading)
Analysis Method : D:\JACK\YIZHE SUN\YIZHE SUN.M
Sample Info     : 5%-85%  100MIN
```




```
==========================================================================================
                              Fraction Information
==========================================================================================
Fraction collection off
==========================================================================================
No Fractions found.
==========================================================================================
==========================================================================
                           Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Signal 1: MWD1 A, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   9.230 MM N  0.1953 1843.70142  157.36546  95.0807
   2  10.104 PM N  0.1774   95.39015    8.96282   4.9193

Totals :                  1939.09157  166.32829

==========================================================================
                           *** End of Report ***
```

References:

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3. He, W.; Wang, Q.; Srinivasan, B.; Xu, J.; Padilla, M. T.; Li, Z.; Wang, X.; Liu, Y.; Gou, X.; Shen, H. M.; Xing, C.; Lin, Y. A JNK-mediated autophagy pathway that triggers c-IAP degradation and necroptosis for anticancer chemotherapy. *Oncogene* **2014,** *33*, (23), 3004-13.
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5. Zhang, Y.; Srinivasan, B.; Xing, C.; Lu, J. A new chalcone derivative (E)-3-(4-methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one suppresses prostate cancer involving p53-mediated cell cycle arrests and apoptosis. *Anticancer research* **2012,** *32*, (9), 3689-98.
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9. Gupta, M. K.; Misra, K. Atom-based 3D-QSAR, molecular docking and molecular dynamics simulation assessment of inhibitors for thyroid hormone receptor alpha and beta. *Journal of molecular modeling* **2014,** *20*, (6), 2286.