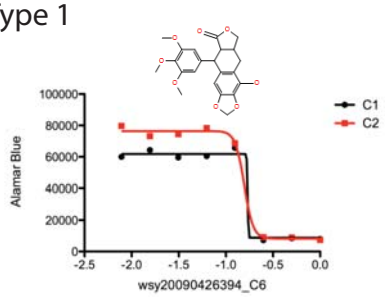
Introduction to Supporting Information Fig.S1

There are two types of dose response curve in the Fig.S1

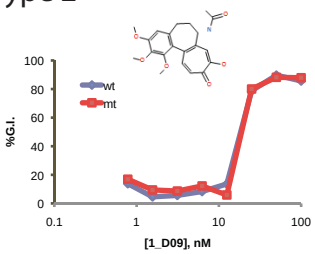
Type1 shows the fluorescence intensity of alamar blue, a viability dye, from compound treated cell culture. Two breast cancer cell lines (MCF10A-derived) were used to create this cell viability curve. X-axis represents compound concentration in nM. The highest concentration is 100nM and each data point represents mean of duplicate data. Cells were treated with compound in 384-well assay plate for 24 hours.

Type 1



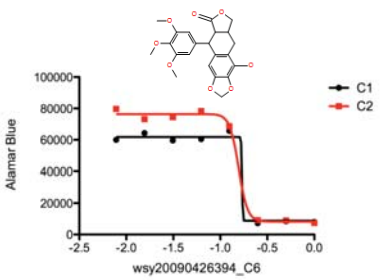
Type2 measures normalized cell viability in two immortalized fibroblast cell lines (BJ-derived). Cells were treated with each compound in 384-well assay plate for 24 hours. The highest concentration of the compound is 100nM and each data point represents mean of duplicate data. Alamar blue dye was used to determine cell viability.

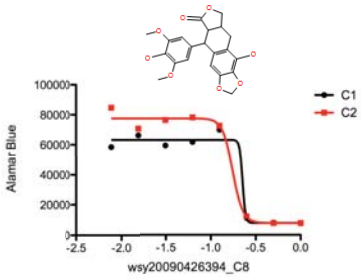
Type 2

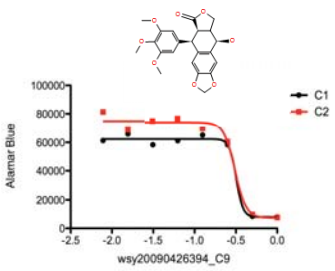


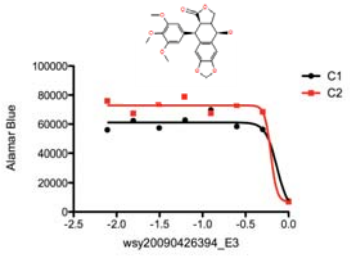
In every dose-response curve, we included the structure and the name of the compound. If the name of the compound is unknown, we indicated PubChem compound ID as a reference.

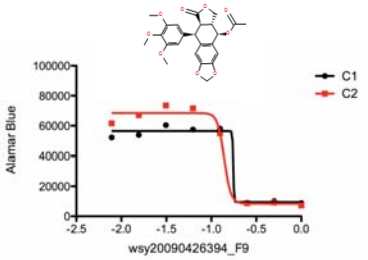
Group 1: Microtubule inhibitor
Subgroup a. Podopyllotoxin and its analogs

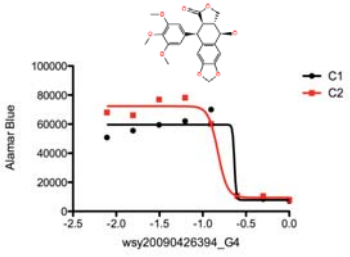




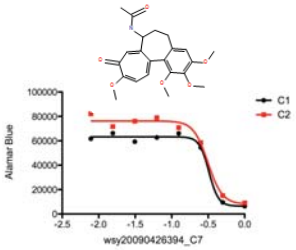


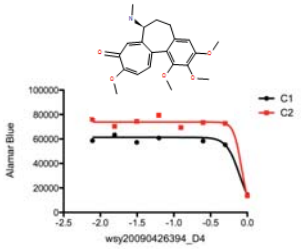


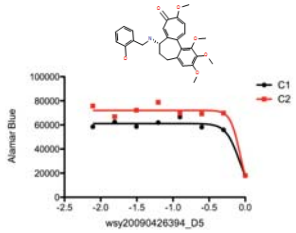


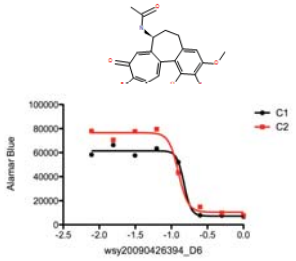


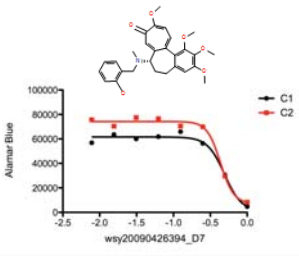
Group 1: Microtubule inhibitor
Subgroup b. Colchichine and its analogs

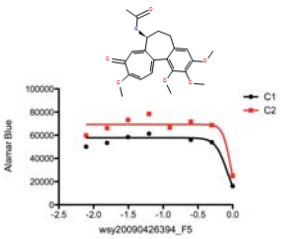


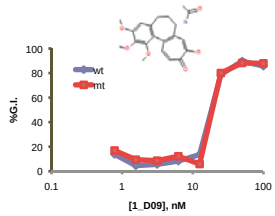




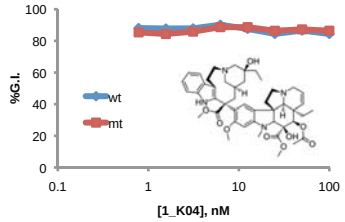




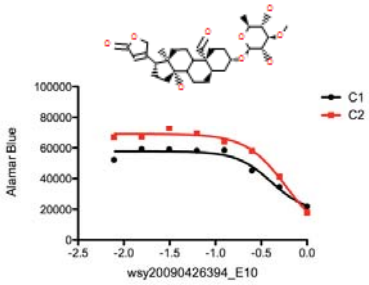


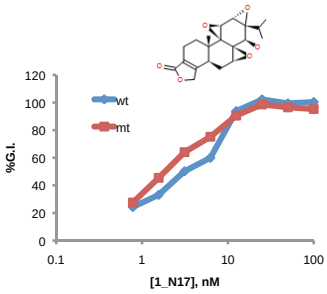


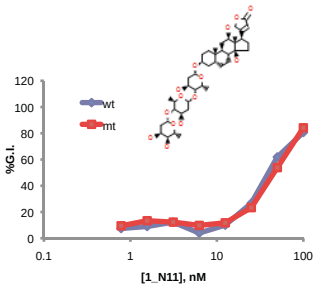
Group 1: Microtubule inhibitor
Subgroup c. Paclitaxel

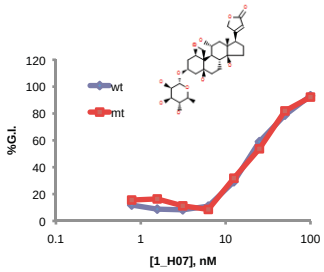


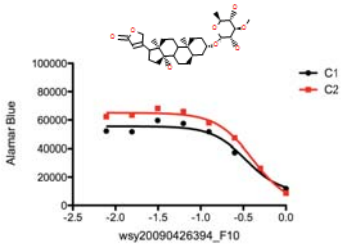
Group 1: Microtubule inhibitor
Subgroup d. Estradiol-derivatives

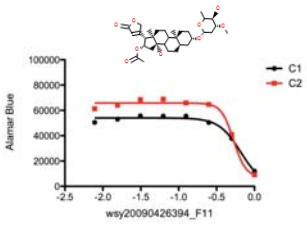




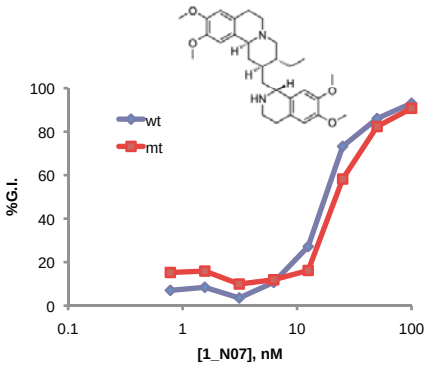




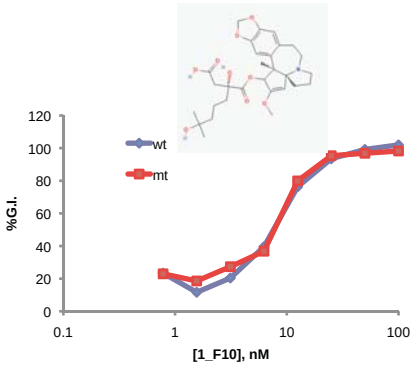




Group 2: Translation inhibitor

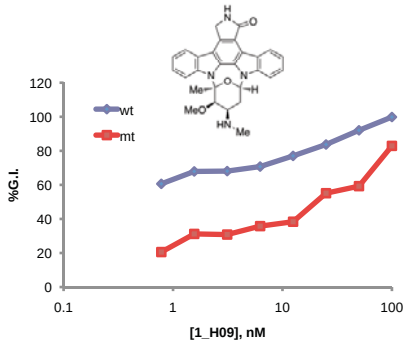


Emitine



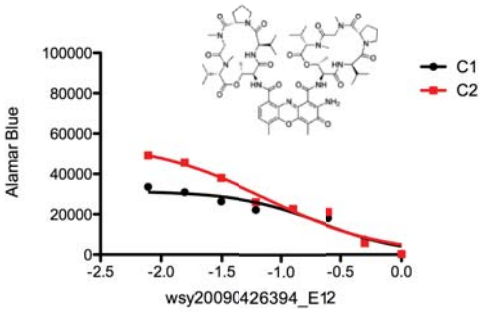
Homoharringtonine

Group 3: Pan-kinase inhibitor



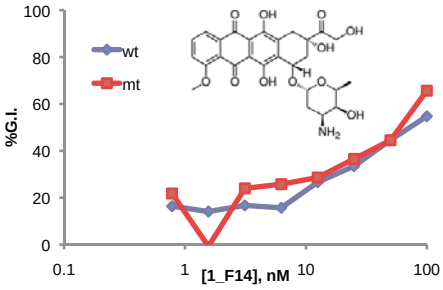
Staurosporine

Group 4: Transcription inhibitor



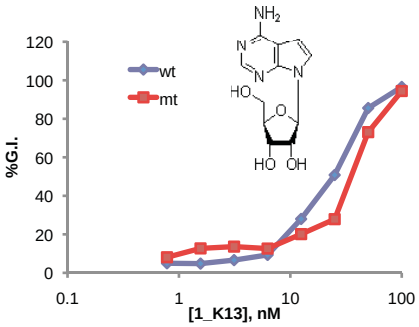
Actinomycin D

Group 5: Topo II inhibitor



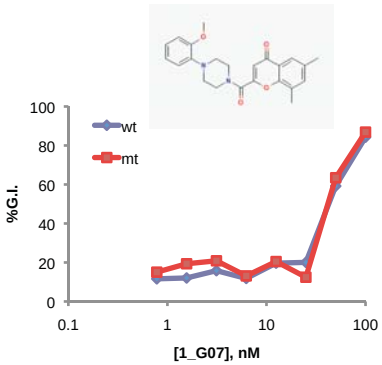
Doxorubicin

Group 6: Nucleoside analog

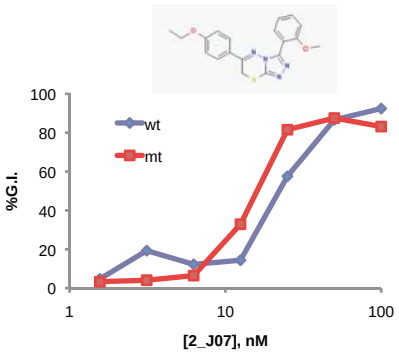


Tubercidin

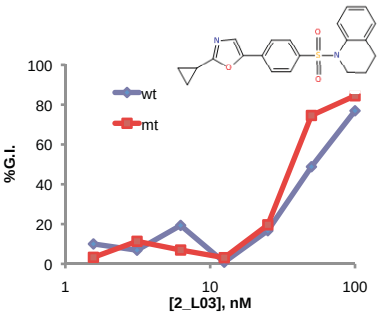
Unknown Groups:



CID 4970947
cmpd 1



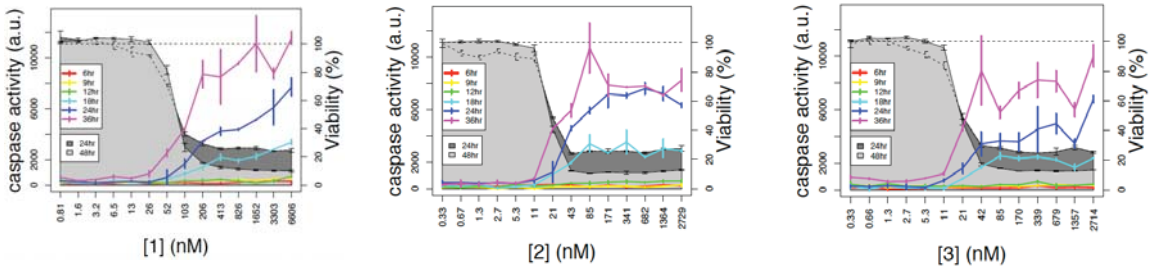
CID 663143
cmpd 2



CID 20959105
cmpd 3

Supporting information Figure S2.

A



B

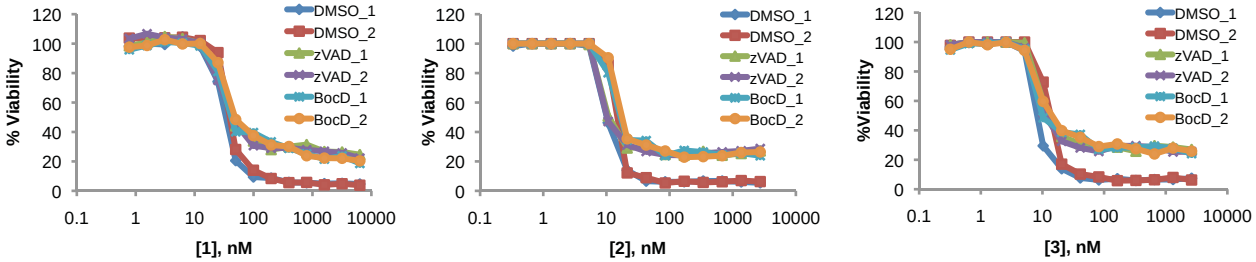
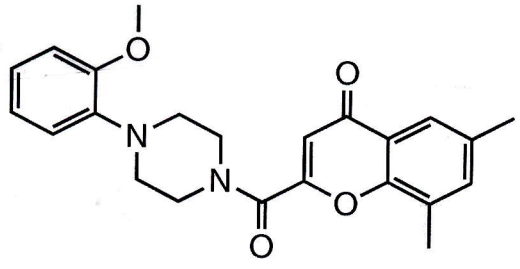


Figure S2. Microtubule inhibition by the identified small molecules activates cellular caspases. However, the activation of caspases partially accounts for cell death induced by microtubule inhibitors as blocking of caspase activation could not rescue cell death completely. (A) Time- and dose-dependent activation of cellular caspases upon compound treatment. (B) HT1080 cells were treated with the indicated compound in the presence or absence of two caspase inhibitors, Boc-D-fmk and zVAD-fmk. The concentration of caspase inhibitors was 50uM and the incubation time was for 24 hours. The cell death was only partially rescued by the caspase inhibitor treatment.

SI Fig.S3 page 1

[Elemental Composition]
Data : Oct1405
Date : 07-Oct-2011 13:51
Sample: Compd1
Note : NBA
Inlet : Direct
Ion Mode : FAB+
RT : 1.28 min
Scan#: (12,16)
Elements : C 28/3, H 100/8, O 4/0, N 2/0
Mass Tolerance : 1000ppm, 1mmu if m/z < 1, 3mmu if m/z > 3
Unsaturation (U.S.) : -0.5 - 100.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
393.1809	100.0	-1.3 / -0.5	12.5	C 23 H 25 O 4 N 2



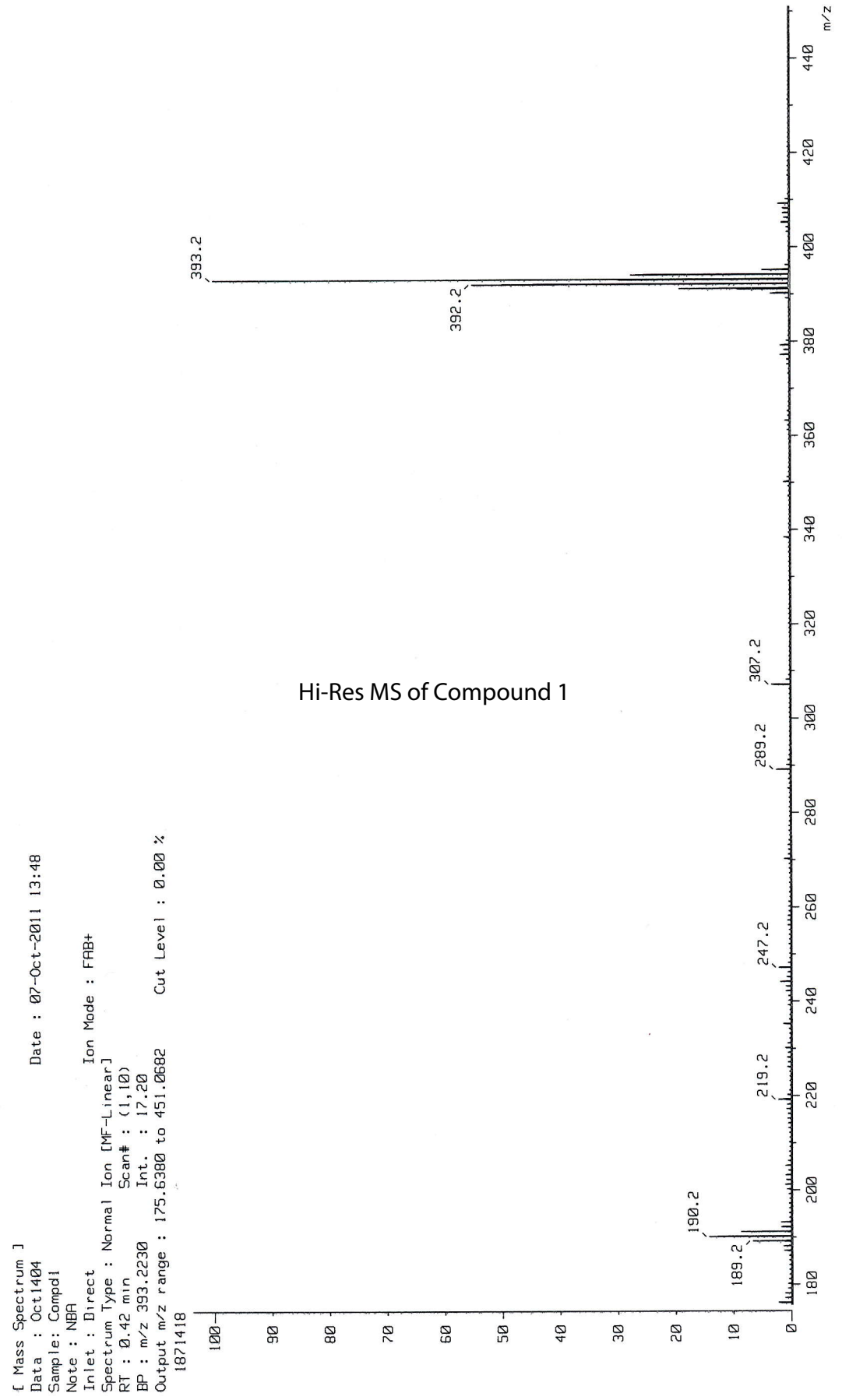
Hi-Res MS of Compound 1

SI Fig.S3 page 2

[Theoretical Ion Distribution]
Molecular Formula : C23 H25 O4 N2
(m/z 393.1814, MW 393.4625, U.S. 12.5)
Base Peak : 393.1814, Averaged MW : 393.4639(a), 393.4647(w)

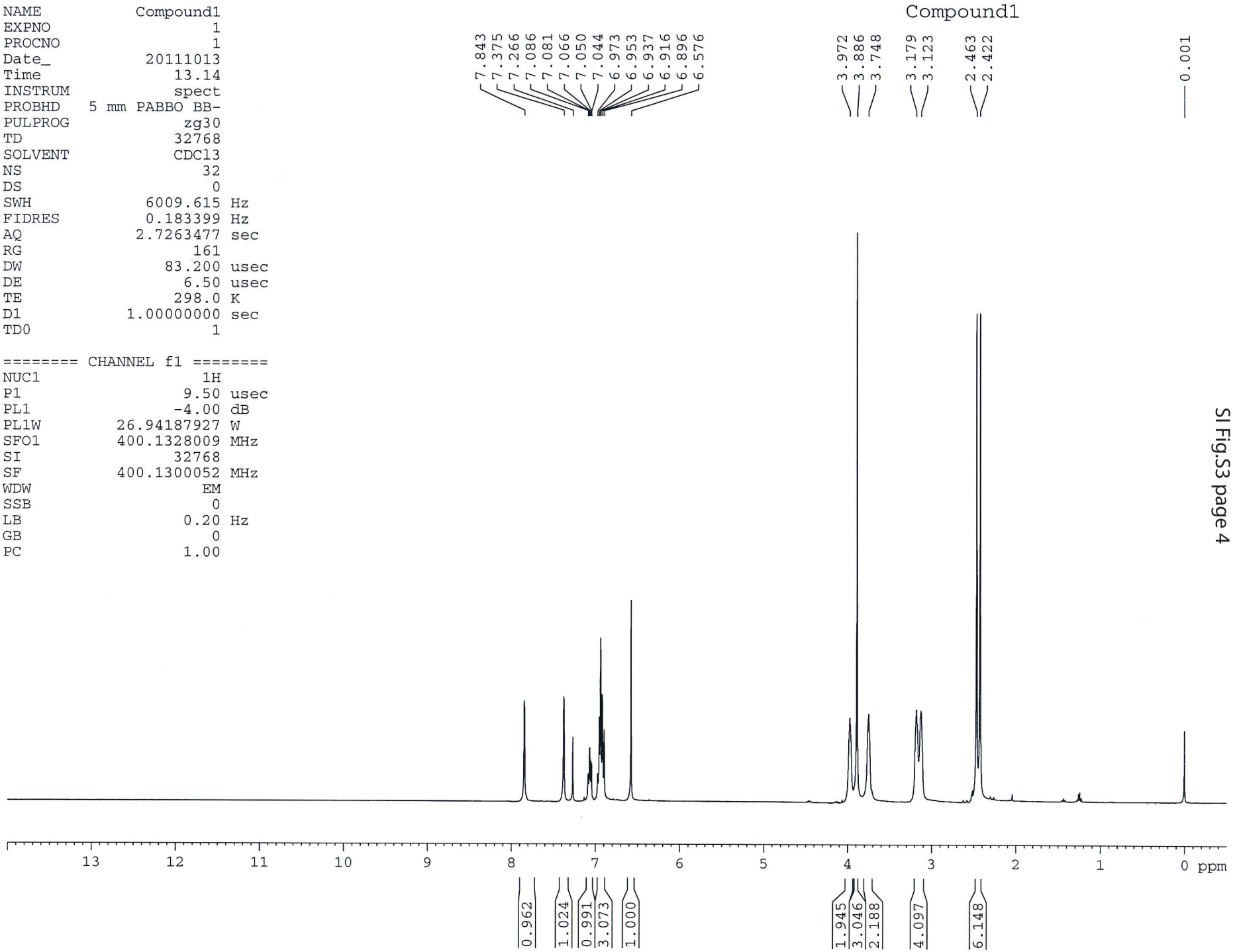
m/z	INT.	
393.1814	100.0000	**********
394.1847	26.8435	**********
395.1875	4.2611	**
396.1902	0.4998	
397.1928	0.0469	
398.1954	0.0037	
399.1980	0.0002	

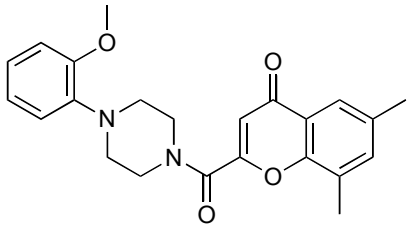
Hi-Res MS of Compound 1



Hi-Res MS of Compound 1

NAME	Compound1
EXPNO	1
PROCNO	1
Date_	20111013
Time	13.14
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	32
DS	0
SWH	6009.615 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	161
DW	83.200 usec
DE	6.50 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.50 usec
PL1	-4.00 dB
PL1W	26.94187927 W
SFO1	400.1328009 MHz
SI	32768
SF	400.1300052 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00





Compound1

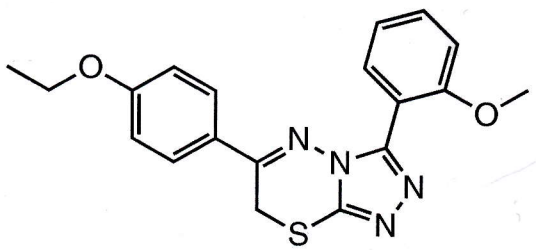
^{1}H NMR ($CDCl_3$, 400MHz, ppm) δ 7.84(s, 1H), 7.37(s, 1H), 7.06(t, J = 8.0Hz, 1H), 6.97-6.89(m, 3H), 6.57(s, 1H), 3.97(b, 2H), 3.88(s, 3H), 3.74(b, 2H), 3.18(b, 2H), 3.12(b, 2H), 2.46(s, 3H), 2.42(s, 3H).

SI Fig.S3 page 6

[Elemental Composition]
Data : Oct1401 Date : 07-Oct-2011 13:13
Sample: Compd2
Note : NBA
Inlet : Direct Ion Mode : FAB+
RT : 0.95 min Scan#: (7,13)
Elements : C 25/3, H 100/8, O 2/0, N 4/0, S 1/0
Mass Tolerance : 1000ppm, 1mmu if m/z < 1, 3mmu if m/z > 3
Unsaturation (U.S.) : -0.5 - 100.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
367.1227	100.0	-0.4 / -0.2	13.5	C 19 H 19 O 2 N 4 S

thenticity of c analysis



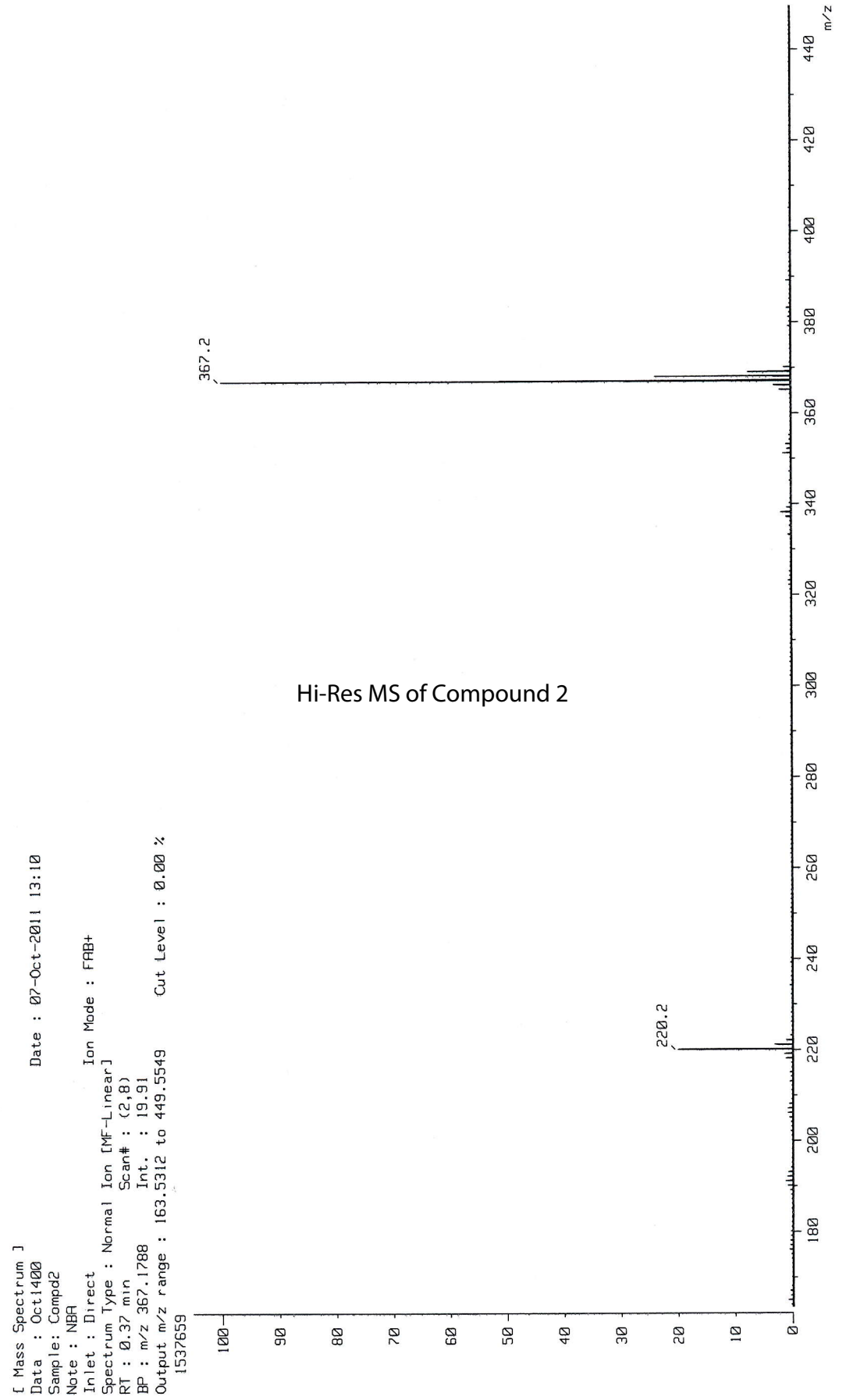
lysis (Check)), CI( ), MALD

Hi-Res MS of Compound 2

[Theoretical Ion Distribution]
Molecular Formula : C19 H19 O2 N4 S
(m/z 367.1229, MW 367.4515, U.S. 13.5)
Base Peak : 367.1229, Averaged MW : 367.4511(a), 367.4523(w)

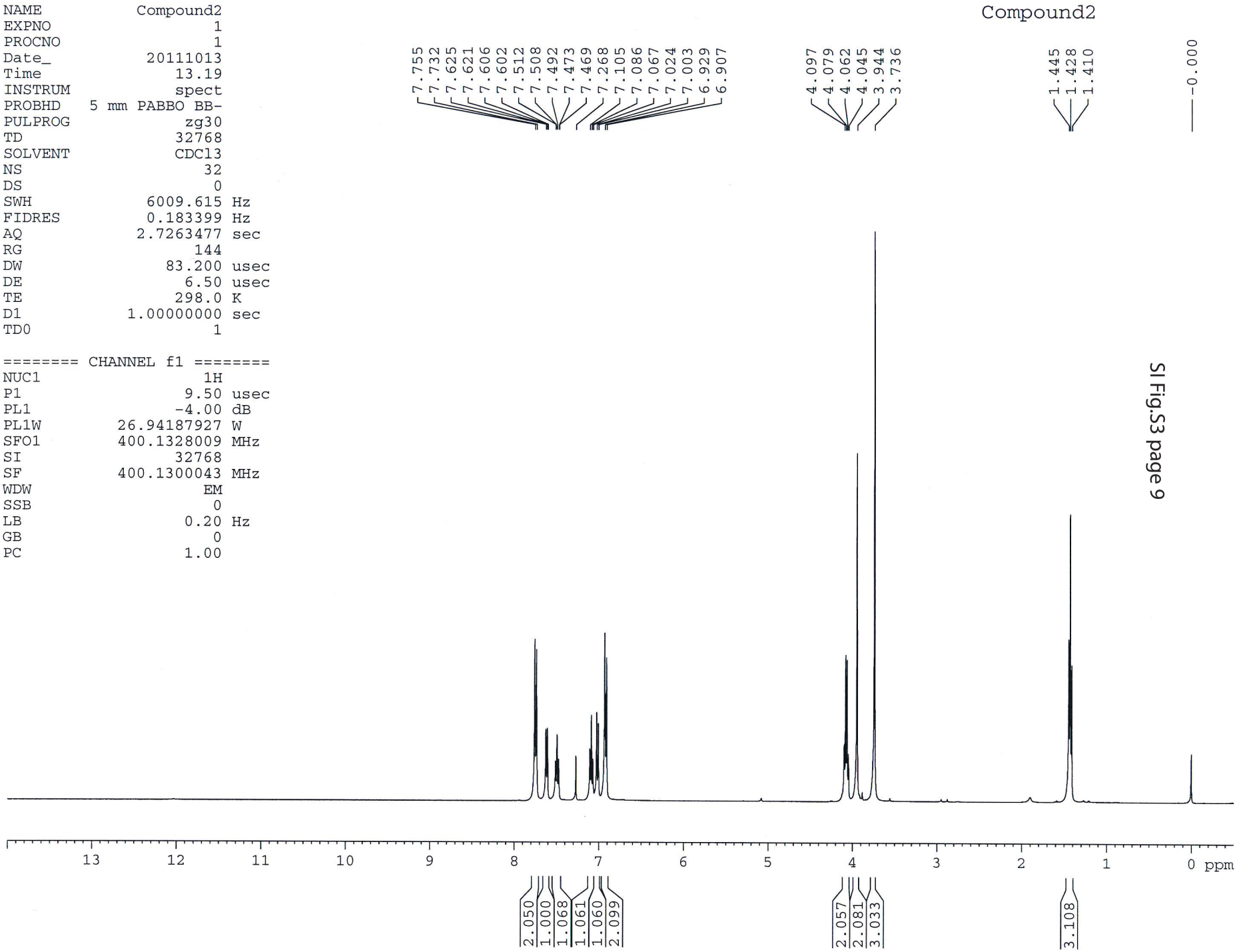
m/z	INT.	
367.1229	100.0000	**********
368.1257	23.7524	**********
369.1227	7.5291	****
370.1237	1.3074	*
371.1247	0.1715	
372.1260	0.0179	
373.1275	0.0015	
374.1293	0.0001	

Hi-Res MS of Compound 2

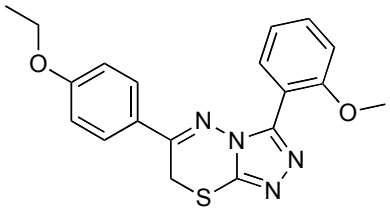


Hi-Res MS of Compound 2

NAME	Compound2
EXPNO	1
PROCNO	1
Date_	20111013
Time	13.19
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	32
DS	0
SWH	6009.615 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	144
DW	83.200 usec
DE	6.50 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.50 usec
PL1	-4.00 dB
PL1W	26.94187927 W
SFO1	400.1328009 MHz
SI	32768
SF	400.1300043 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00



SI Fig.S3 page 9

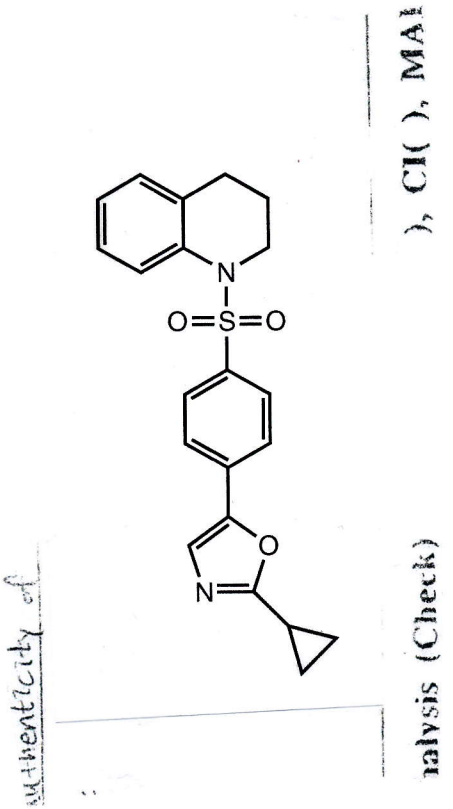


Compound2

^{1}H NMR ($CDCl_3$, 400MHz, ppm) δ 7.60(d, J = 9.2Hz, 2H), 7.51(d, J = 7.6Hz, 1H), 7.48(t, J = 7.6Hz, 1H), 7.09(t, J = 7.6Hz, 1H), 7.01(d, J = 8.4Hz, 1H), 6.92(d, J = 8.8Hz, 2H), 4.07(q, J = 868Hz, 2H), 3.94(s, 2H), 3.74(s, 3H), 1.42(t, J = 6.8Hz, 3H).

[Elemental Composition]
Data : Oct1403 Date : 07-Oct-2011 13:39
Sample: Compd3
Note : NBA
Inlet : Direct Ion Mode : FAB+
RT : 0.74 min Scan#: (4,13)
Elements : C 28/3, H 100/8, O 3/0, N 2/0, S 1/0
Mass Tolerance : 1000ppm, 1mmu if m/z < 1, 2mmu if m/z > 2
Unsaturation (U.S.) : -0.5 - 100.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
381.1266	100.0	-1.7 / -0.6	13.5	C 21 H 21 O 3 N 2 S

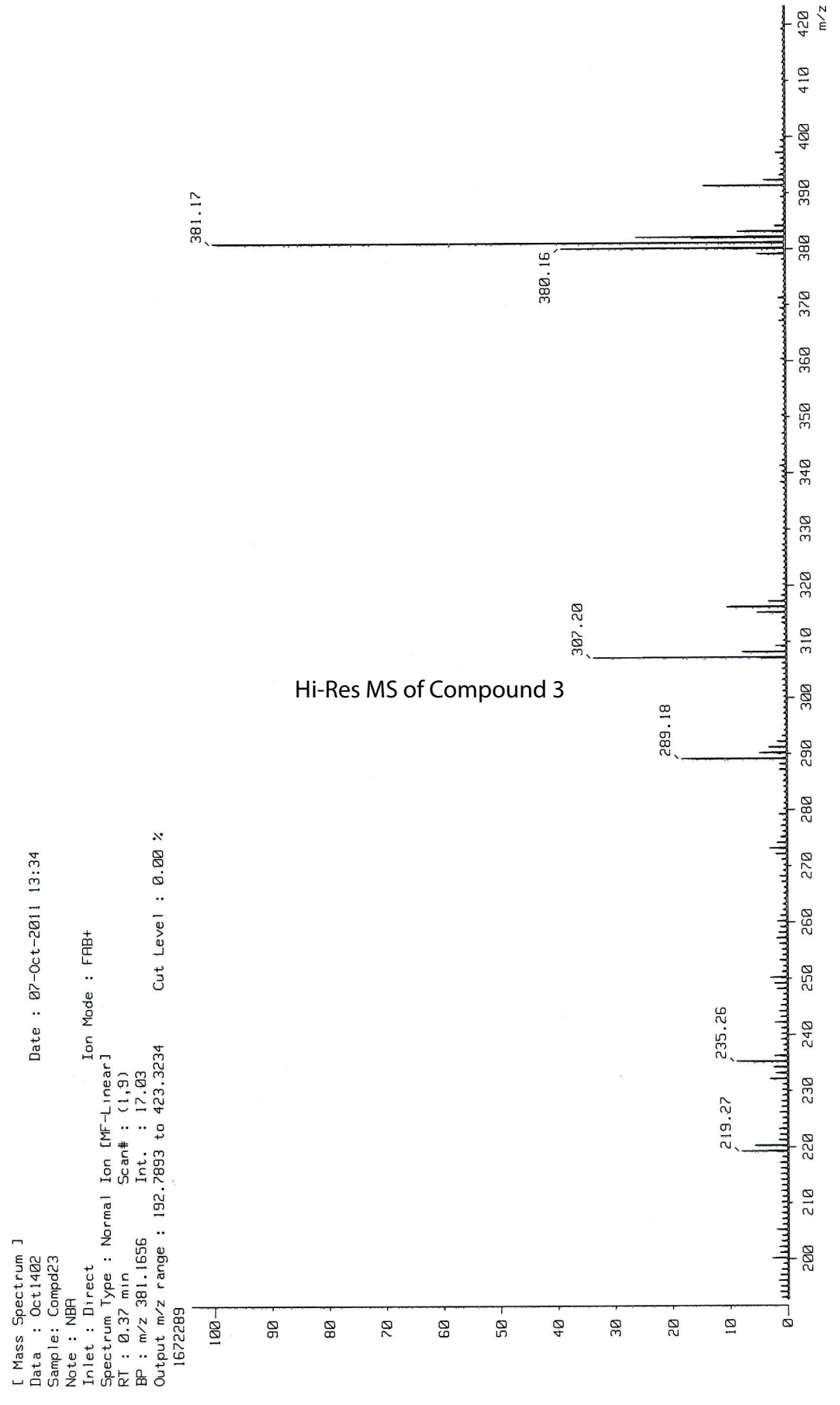


Hi-Res MS of Compound 3

[Theoretical Ion Distribution]
Molecular Formula : C21 H21 O3 N2 S
(m/z 381.1273, MW 381.4753, U.S. 13.5)
Base Peak : 381.1273, Averaged MW : 381.4750(a), 381.4762(w)

m/z	INT.	
381.1273	100.0000	**********
382.1304	25.3102	**********
383.1276	8.1007	*****
384.1288	1.4756	*
385.1299	0.2078	
386.1313	0.0235	
387.1330	0.0022	
388.1347	0.0002	

Hi-Res MS of Compound 3



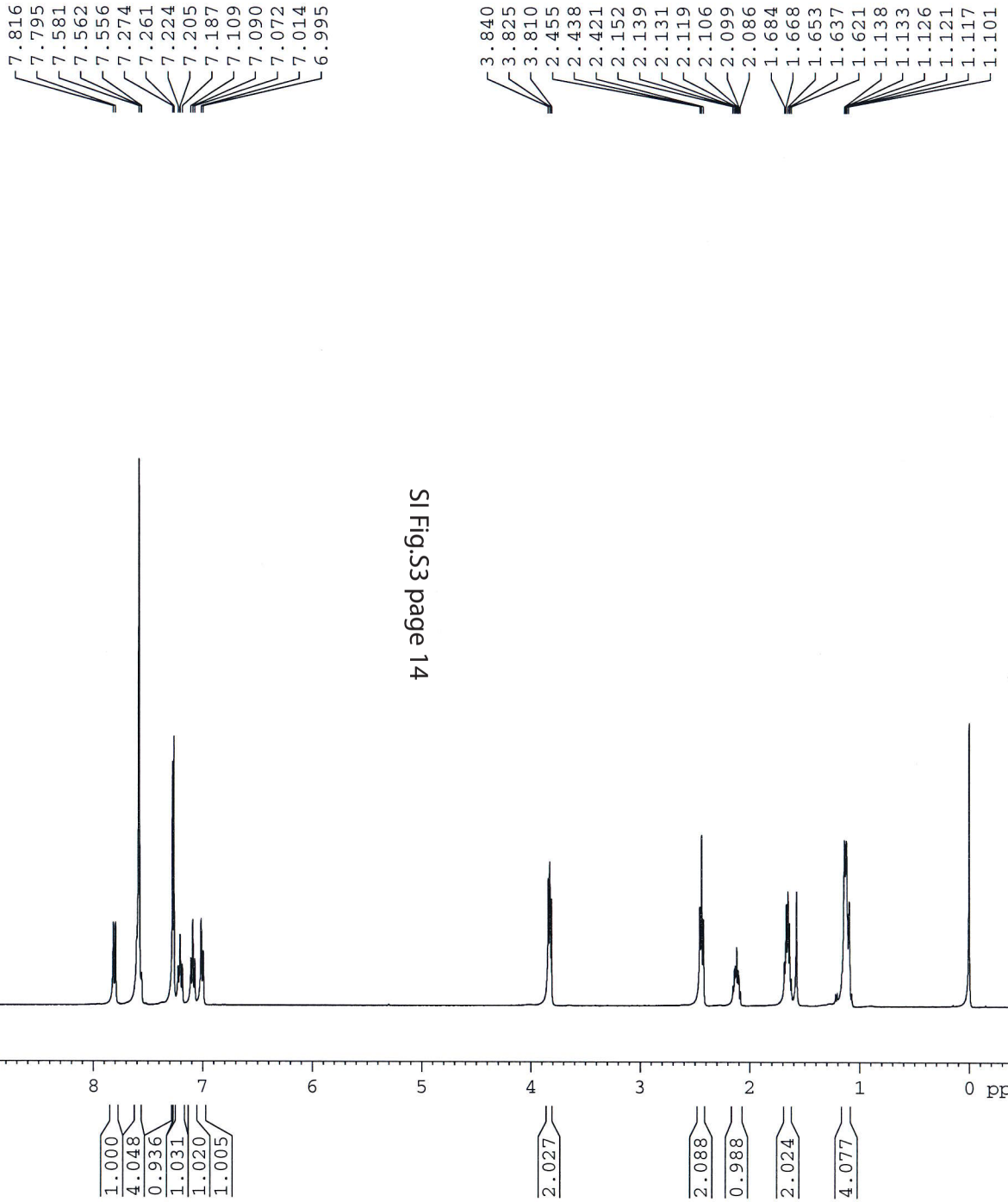
Hi-Res MS of Compound 3

NAME	Compound 3
EXPNO	1
PROCNO	1
Date_	20111012
Time	18.30
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	126
DS	0
SWH	6009.615 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	456
DW	83.200 usec
DE	6.50 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

NUC1	1H
P1	9.50 usec
PL1	-4.00 dB
PL1W	26.94187927 W
SFO1	400.1328009 MHz
SI	32768
SF	400.1300073 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00

Compound 3



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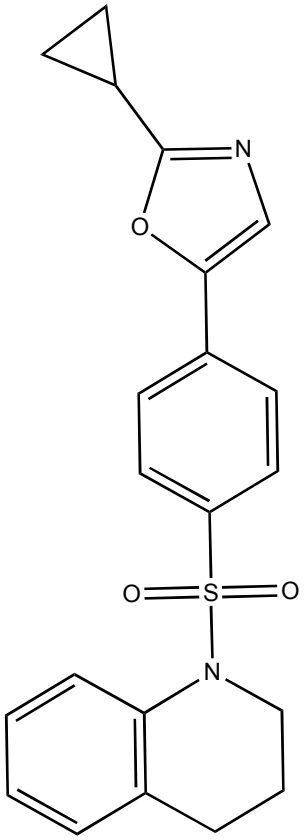


Compound 3 !

!

^{1}H NMR ($CDCl_3$, 400MHz, ppm) δ 7.80(d, J = 7.6Hz, 1H), 7.58-7.55(m, 4H), 7.27(s, 1H), 7.20(t, J = 7.6Hz, 1H), 7.09(t, J = 7.6Hz, 1H), 7.00(d, J = 7.6Hz, 1H), 3.82(t, J = 6.0Hz, 2H), 2.43(t, J = 6.8Hz, 2H), 2.11(m, 1H), 1.65(q, J = 6.4Hz, 2H), 1.13-1.09(m, 4H)

Supplementary information Table S1. List of cell death modulators use in Fig.3.

Abbreviation	Chemical or Genetic Modulator	Mechanism	Concentration used
DFOM	Deferoxamine	Chelates iron	100uM
Co2+	Cobalt (II)	Blocks calcium channels	656uM
ALLN	Calpain Inhibitor I	Inhibitor of calpain I and II, cathepsins B,L	7.5uM
Lmim	L-mimosine	Inhibits G1-S cell cycle transition	200uM
$p53^{KD}$	Knock down of Tumor protein 53	Initiates apoptosis in response to DNA damage	-
CHX	Cycloheximide	Protein synthesis inhibitor	1.5uM
Dig	Digoxin	Na+/K+ ATPase inhibitor	0.13uM
zVAD	Cbz-val-ala-asp(OMe)-fluormethylketone	Broad spectrum caspase inhibitor	50uM
aTOC	a-tocopherol	Antioxidant	100uM
U0126	1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene	Mek 1/2 inhibitor	10uM
3MA	3-methyladenine	Inhibitor of autophagosome formation	5mM
ATA	Aurintricarboxylic Acid	Nuclease inhibitor	38uM
NAD+	Nicotinamide adenine dinucleotide	Activates sirtuins, prevents energetic depletion	2mM
ActD	Actinomycin D	RNA synthesis inhibitor	0.016uM
BocD	t-butoxycarbonyl-asp-fluormethylketone	Broad spectrum caspase inhibitor	50uM
$Bcl2^{OE}$	Overexpression of B-cell leukemia/lymphoma 2	Prevents mitochondrial outer membrane permeabilization	-
Nec1	Necrostatin-1	Inhibitor of necroptosis	19.5uM
$Survivin^{OE}$	Overexpression of Survivin	Inhibits caspases	
BHA	Butylated hydroxyanisole	Antioxidant	50uM
BHT	Butylated hydroxytoluene	Antioxidant	50uM
DPQ	3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone	Inhibitor of PARP1	10uM
CspA	Cyclosporin A	Binds cyclophilin	5uM

Cell lines

HT-1080 cells were grown in DEME supplemented with 10% fetal bovine serum and non-essential amino acids. BJeLR cells were maintained in 4:1 mixture of DMEM to M199 supplemented with 15% heat-inactivated fetal bovine serum. MCF10A cells were maintained in the culture media described previously[1]. Penicillin and streptomycin were used as antibiotics in all media. Cells were incubated in a tissue culture incubator at 37°C in a humidified incubator containing 5% CO2.

Dose curve generation (Figure 1)

Compound plates were prepared by diluting compound stock solution (in DMSO) to the cell growth media and by making 2-fold dilution series on the 384-well microplates as 10x concentrated solution. Assay plates were prepared by seeding 1000 cells per well in 36 µl of growth media in black, clearbottom, 384-well plates. Cells were treated with compound by transferring 4uL solution from the compound plates to the assay plates. One day later, alamar blue (Invitrogen, Carlsbad, CA, USA, catalog number DAL1100) was added to the assay plates in order to determine cell viability. All liquid handling was carried out using a Biomek FX AP384 module (Beckman Coulter, Fullerton, CA, USA). Percent growth inhibition (%GI) was calculated from the following formula using alamar blue readout:

$$\%GI = 100 \times (1 - (X - N)/(P - N))$$

where X is values from cells with compound treatment, N is the values from media, and P is the values from cells and DMSO. All experiments were performed in triplicate.

Immunohistochemistry (Figure 2A)

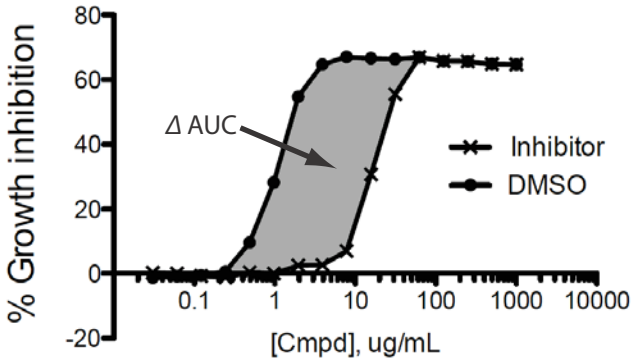
Cells were grown on coverslips to ~50% confluency and treated with indicated amount of MTIs for 4 hours. Cells were fixed with 3.7% formaldehyde solution in PBS for 15-30min followed by washing with PBS 5 times. Cell membrane was permeabilized with 0.2% Triton-X in PBS for 10min and rinsed once with TBS (10mM Tris [pH 7.5], 150mM NaCl). The permeabilized sample was blocked with 10% goat serum in TTBS (0.1% Tween-20 in TBS) for 30-60min and washed once with TTBS. The microtubule network was probed with anti-tubulin antibody (Santa Cruz cat# sc-32293) in 1% goat serum in TTBS for 30-60min at room temperature followed by washing in TTBS for 10min. Alexa Fluor anti-mouse antibody (Invitrogen, cat#A-11005) was used as the secondary antibody to visualize the microtubule network using the 60x lens of an epifluorescence microscope.

Modulatory profiling (Figure 3)

HT-1080 cells were trypsinized, counted, and combined with modulators or with vehicle and seeded into 384-well plates at 1000 cells/well. Various MTIs were dissolved in DMSO and arrayed in 14-point dilution series in a 384-well polypropylene plate (Greiner, cat. #781280) and stored at −80°C. The plate was diluted 1:25 into cell culture media in polypropylene plates, then 1:10 into the assay plates approximately one hour after cells were seeded. After 48 hours, a 50%

Alamar blue solution was added to a final concentration of 10% Alamar blue. After 16 hours of incubation, the fluorescence intensity was determined using a Victor 3 plate reader (Perkin Elmer) with a 535 nm excitation filter and a 590 nm emission filter. All assays were done in at least triplicate.

To generate a heat map shown in Figure 3, we created dose response curve of MTIs with or without the modulator and calculated differences in AUC (area under the curve).



$$\text{Normalized AUC} = \frac{\Delta\ \text{AUC}}{\text{AUC}_{\text{DMSO}}} = \frac{\text{AUC}_{\text{DMSO}} - \text{AUC}_{\text{Inh}}}{\text{AUC}_{\text{DMSO}}}$$

For example, the graph shown above contains two dose-response curves from two conditions; one from lethal compound with DMSO and the other from lethal compound with a cell death inhibitor. AUC (Area Under the Curve) of each curve was calculated and Δ AUC was determined by subtracting AUC_{Inh} from AUC_{DMSO}. The normalized AUC was determined by dividing Δ AUC by AUC_{DMSO} and was used to create the heatmap shown in Fig. 3.

1. Debnath, J.; Muthuswamy, S. K.; Brugge, J. S., Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods* **2003,** *30* (3), 256-68.