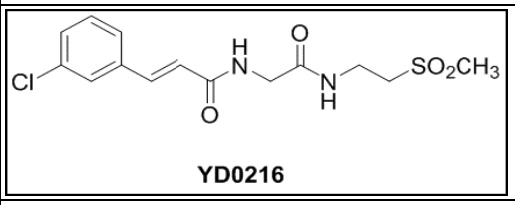
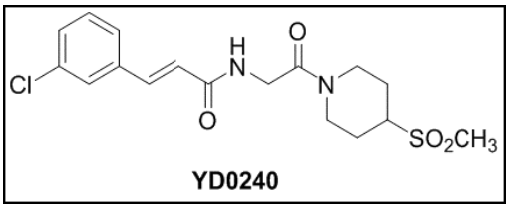
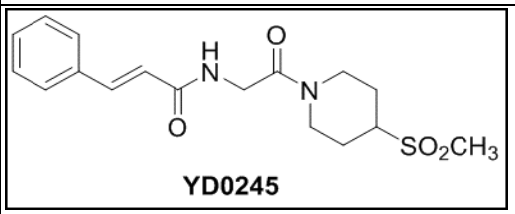
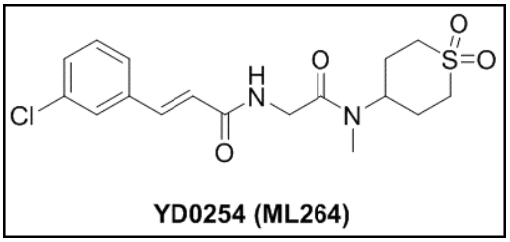
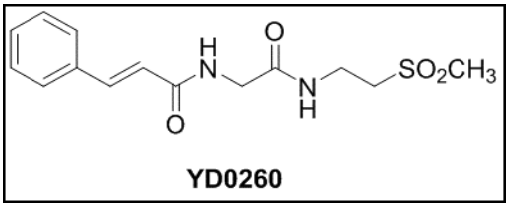
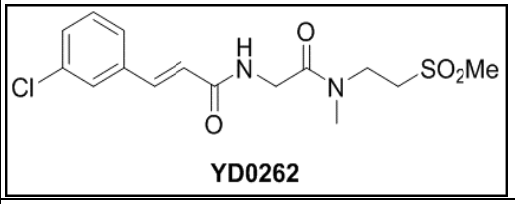
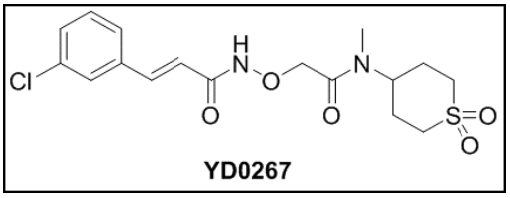
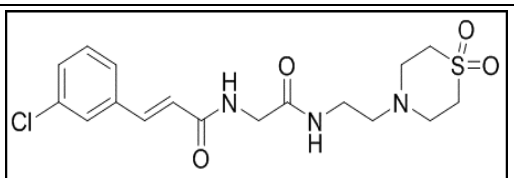
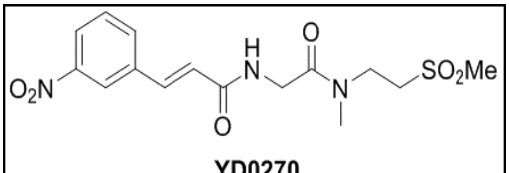
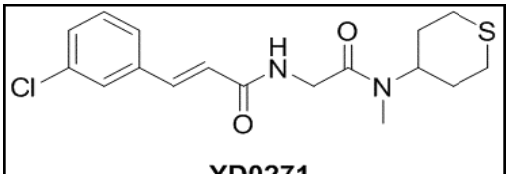
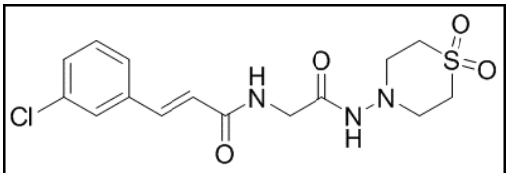
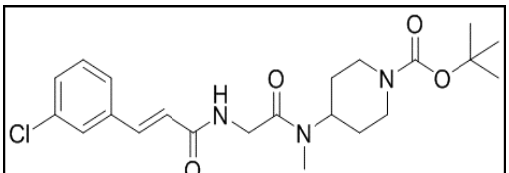
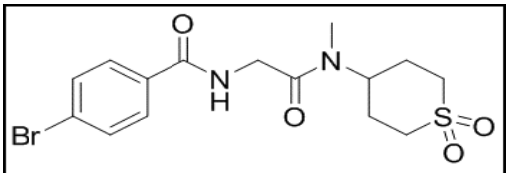
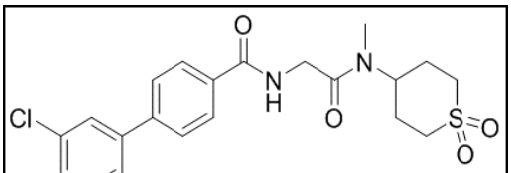
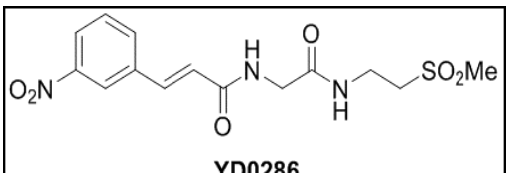
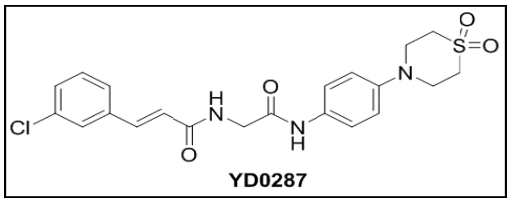
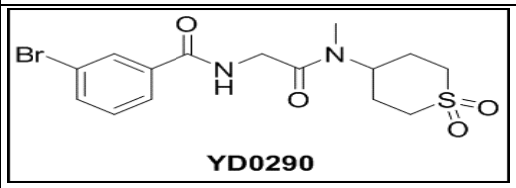
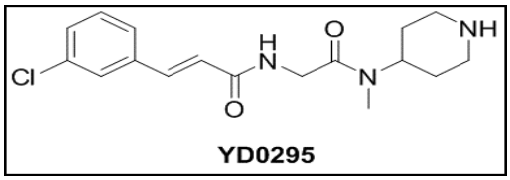
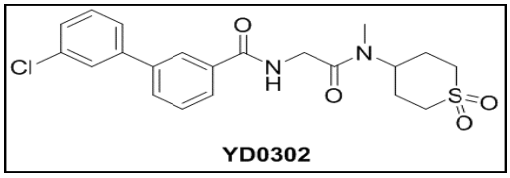
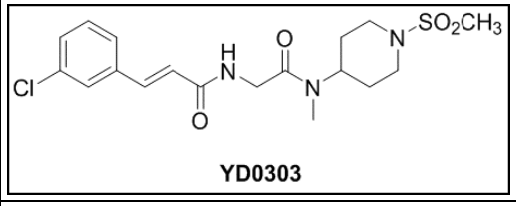
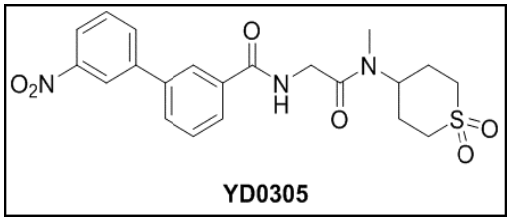
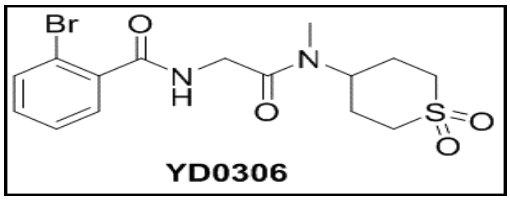
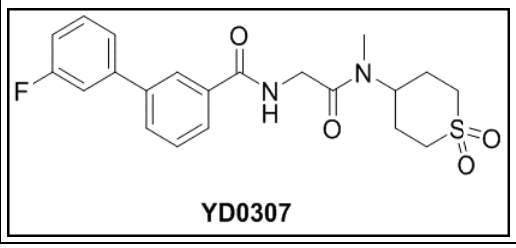
Supplementary Tables

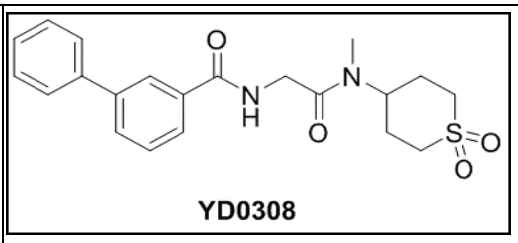
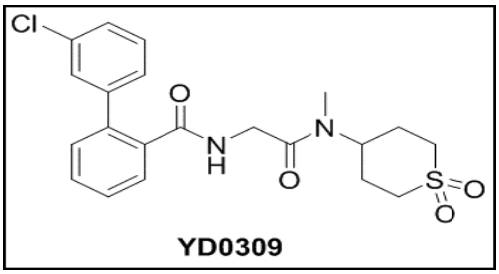
Supplementary Table 1

The structures of 25 compounds

No.	Compound ID	Molecular weight	Structures
1	**YD-2-16**	344.8138	 YD0216
2	**YD-2-40**	384.8776	 YD0240
3	**YD-2-45**	350.4326	 YD0245
4	**YD-2-54**	384.8776	 YD0254 (ML264)
5	**YD-2-60**	310.3687	 YD0260
6	**YD-2-62**	358.8404	 YD0262
7	**YD-2-67**	400.877	 YD0267

8	**YD-2-69**	399.8923	 YD0269
9	**YD-2-70**	369.3929	 YD0270
10	**YD-2-71**	352.8788	 YD0271
11	**YD-2-72**	371.8391	 YD0272
12	**YD-2-77**	435.9443	 YD0277
13	**YD-2-83**	403.2914	 YD0283
14	**YD-2-85**	434.9363	 YD0285
15	**YD-2-86**	355.3663	 YD0286

16	**YD-2-87**	447.9351	 YD0287
17	**YD-2-90**	403.2914	 YD0290
18	**YD-2-95**	335.8285	 YD0295
19	**YD-3-2**	434.9363	 YD0302
20	**YD-3-3**	413.9189	 YD0303
21	**YD-3-5**	445.4888	 YD0305
22	**YD-3-6**	403.2914	 YD0306
23	**YD-3-7**	418.4817	 YD0307

24	**YD-3-8**	400.4913	 YD0308
25	**YD-3-9**	434.9363	 YD0309

Supplementary Table 2

The antibodies used for Western Blot

Name	MW (KDa)	Source	Company	Dilution	Product Number
PARP	116/89	Rabbit	Cell signaling	1:1000	9532S
Caspase3	32/21/14	Rabbit	Cell signaling	1:1000	9662S
cleave-Caspase 3	19/17	Rabbit	Cell signaling	1:1000	9661S
Caspase8	57/43/18	Mouse	Cell signaling	1:1000	9746S
Caspase9	47/37/35/17	Rabbit	Cell signaling	1:1000	9502S
Caspase7	35/20	Rabbit	Cell Signaling	1:1000	9494S
CyclinD1	36	Rabbit	Cell signaling	1:1000	2978S
p21	21	Rabbit	Cell signaling	1:1000	2947S
p27	27	Mouse	BD Biosciences	1:1000	610241
Bcl2	26	Rabbit	Cell signaling	1:1000	2870S
Bclxl	30	Rabbit	Cell signaling	1:1000	2764S
DR4	55	Rabbit	Cell Signaling	1:1000	42533
DR5	55/40	Rabbit	Sigma	1:1000	D3938
BID	22/15	Rabbit	Cell signaling	1:1000	9942
Bak	25	Rabbit	Cell signaling	1:1000	9942
Bax	20	Rabbit	Cell Signaling	1:1000	9942
AKT	60	Rabbit	Cell signaling	1:1000	4685S
p-AKT(Ser473)	60	Rabbit	Cell Signaling	1:1000	9018P
GAPDH	37	Rabbit	Santa Cruz Biotechnology	1:1000	Sc-25778
Bip	78	Rabbit	Cell signaling	1:1000	3177S
IRE1α	130	Rabbit	Cell signaling	1:1000	3294S
JNK	46,54	Rabbit	Cell signaling	1:1000	9252S
p-JNK	46,54	Rabbit	Cell signaling	1:1000	9251S
c-Jun	43,48	Rabbit	Cell signaling	1:1000	9165S
p-c-Jun	48	Rabbit	Cell signaling	1:1000	9164S
PREK	130	Rabbit	Cell signaling	1:1000	5683S
CHOP	19	Mouse	Cell signaling	1:1000	2895S
ATF6	75	Mouse	abcam	1:1000	ab37149
KLF5	55	Rabbit	JTD	1:1000	/
Anti-Rabbit IgG	/	Goat	eBio-science	1:5000	/
Anti-Mouse IgG	/	Goat	eBio-science	1:5000	/

Supplementary Table 3

The IC_{50} values of ML264 in cell lines

	Cell lines	**IC50（μM）**
ERα + Breast Cancer	MCF 7	11
	T-47D	>12
TNBC	SUM 149PT	>12
	MDA-MB-231	12
	MDA-MB-453	12
	MDA-MB-468	>12
	Hs 578T	>12
	HCC 1806	>12
	HCC 1937	>12
Normal Cells	MCF 10A	7
	HC 11	NA
	NIH3T3	NA
Colorectal Cancer	HCT 116	7

Supplementary Materials and Methods

Plasmid transfection

One day before transfection, MDA-MB-231 cells were plated in growth medium without antibiotics in 30-70% confluence. Plasmid DNA were first diluted in the appropriate amount of Opti-MEM® I Medium without serum. Then Lipofectamine™ 2000 were diluted in the appropriate amount of Opti-MEM® I Medium without serum. Following that, DNA was mixed with Lipofectamine™ 2000 gently and incubated for 20 minutes at room temperature to allow complex formation. Finally, the DNA -Lipofectamine™ 2000 complexes were added to the cells and mixed gently by rocking the plate back and forth. The cells were incubated at 37°C in a CO_2 incubator for 48 hours.

Supplementary Figure Legends

Figure S1. YD277 induced cell death can be partially inhibited by classical caspase inhibitor Z-VAD-fmk

A. MDA-MB-231 cells were seeded in a 6-well plate. Eighteen hours later , the cells were pre-treated with Z-VAD-fmk (MCE, New Jersey, USA, Cat#HY-16658, 100 μM) for 1 hour and treated with YD277 for 36 hours. Caspase-7 and PARP cleavage were examined by Western blotting.

B. MDA-MB-231 cells were seeded in 48 well plates. Eighteen hours later , the cells were pre-treated with Z-VAD-fmk for 1 hour and treated with YD277 for 36 hours. The SRB assay was used to measure cell viability. Data is shown as mean ± S.D.（n=3）. **p < 0.01.

Figure S2. YD277 does not chenge the expression levels of DR4/5, Bak, Bax, and Bid proteins

MDA-MB-231 and MDA-MB-468 cells were treated with YD277 (1, 3, 10 μM), ML264 (10 μM), or DMSO for 36 h, and the expression levels of apoptosis-related proteins were detected by WB. β-actin normalized quantitative data were shown below their panel.

Figure S3. Over-expression of Bcl2/Bclxl partially rescued YD277-induced cell death

A. The pBabe-Bcl2 (β isoform) plasmid was used to generate retroviruses and to obtain stable Bcl2 over-expression MDA-MB-231 cell populations. Following that, the cells were transfected with pBabe-Bclxl plasmid for 36 hours and treated with YD277 (3 μM) for another 36 hours. DMSO used as the negative control. The protein expression levels of Bclxl, Bcl2, and PARP were examined by WB. β-actin normalized quantitative data were shown below their panel.

B. The SRB assay was used to detect the cell viability. Data was shown as mean ± S.D.（n=3）. *p < 0.05，**p < 0.01.

Figure S4. YD277 inhibits the activation of AKT

MDA-MB-231 cells were treated with YD277 (3 μM) or DMSO for 36 h. MDA-MB-468 cells were treated with YD277 (3 μM) for 48 hours. The expression levels of p-AKT and total AKT proteins were detected by WB. AKT normalized quantitative data for p-AKT was shown below the panel.

Figure S5. YD277 induces IRE1α in MDA-MB-231 cells at the transcriptional level

A. YD277 increased *IRE1α* mRNA levels in a dose dependent manner. SYBR Select Master Mix (Life technologies) was used for quantitative RT-PCR. MDA-MB-231 cells were treated with YD277 (1, 3, 10 μM) for 24 hours. Total RNA was extracted for RT-qPCR. The expression levels of *IRE1α* was normalized to *GAPDH.* Human IRE1α forward primer sequence is 5′-TGTCAGAGATCTCCTCCGAG -3′ and its reverse primer sequence is 5′-TCTCTCGTGGCTGCACAGCTCCA -3′. Human GAPDH forward primer sequence is 5′- GAAAGCCTGCCGGTGACTAA -3′ and its reverse primer sequence is 5′- GCCCAATACGACCAAATCAGAGA -3′. *, p<0.05, student's t-test.

B. YD277 increased *IRE1α* mRNA levels in a time dependent manner. YD277 (1 μM) was used to treat MDA-MB-231 cells for 12, 24, 36, and 48 hours. Total RNA was extracted for RT-qPCR. The expression levels of *IRE1α* was normalized to *GAPDH.* *P < 0.05, **P < 0.01, Student's t-test.

C. YD277 increased *IRE1α* mRNA levels in an actinomycin D dependent manner. YD277(1 μM) was used to treat MDA-MB-231 cells for 12 or 24 hours. Actinomycin D(1 μg/ml)was added with YD277 at the same time as

indicated. Total RNA was extracted for RT-qPCR. The expression levels of *IRE1α* was normalized to *GAPDH.* **P < 0.01, Student's t-test.

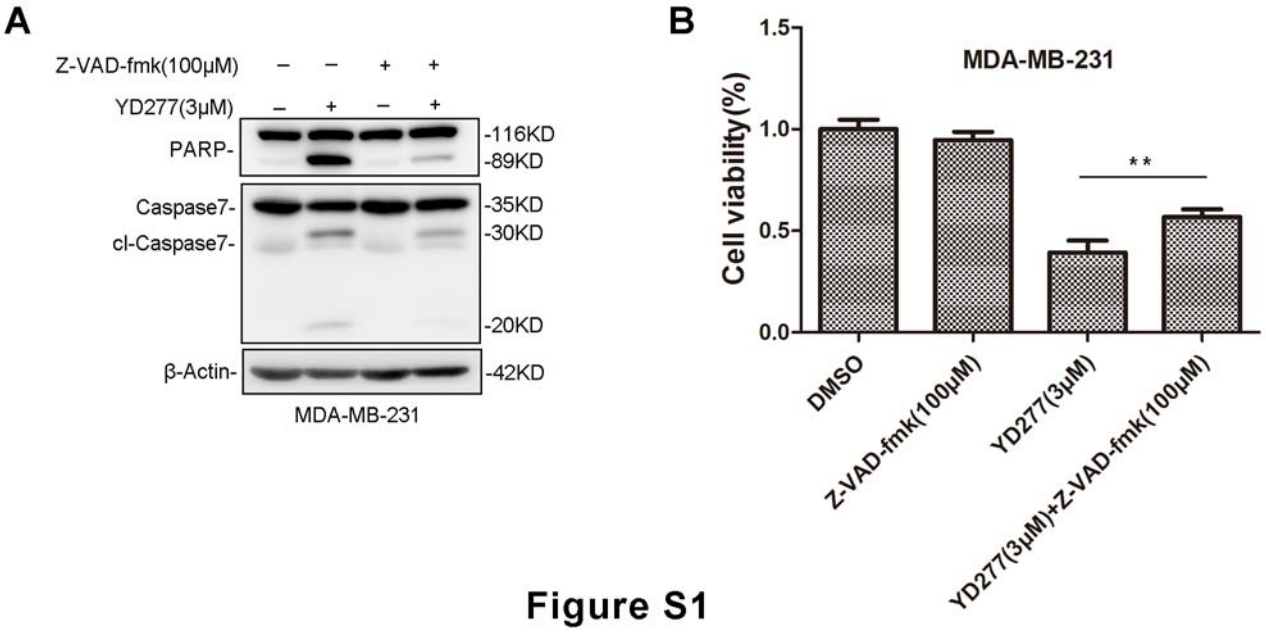

A
Z-VAD-fmk(100μM) – – + +
YD277(3μM) – + – +
PARP-
-116KD
-89KD
Caspase7-
cl-Caspase7-
-35KD
-30KD
-20KD
β-Actin-
-42KD
MDA-MB-231
B
MDA-MB-231
Cell viability(%)
1.5
1.0
0.5
0.0
**
DMSO
Z-VAD-fmk(100μM)
YD277(3μM)
YD277(3μM)+Z-VAD-fmk(100μM)

Figure S1

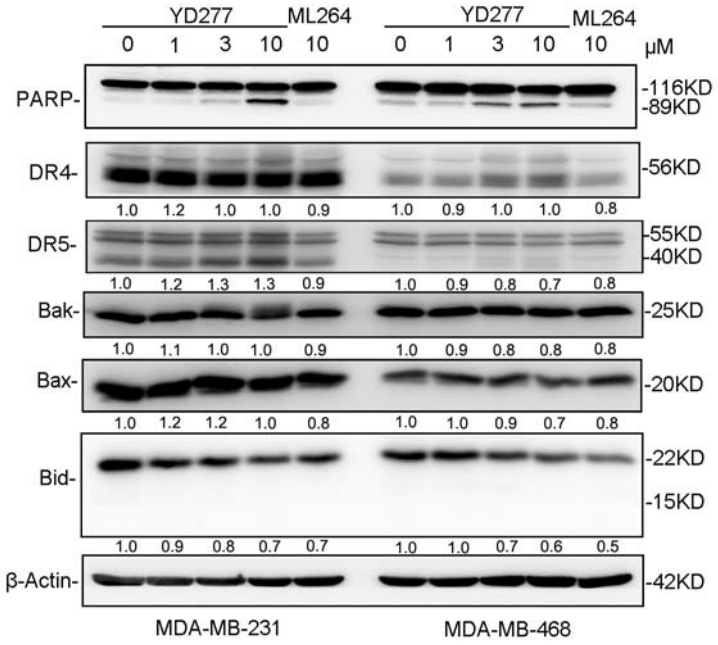

YD277
ML264
YD277
ML264
0 1 3 10 10 0 1 3 10 10 μM
PARP-
-116KD
-89KD
DR4-
-56KD
1.0 1.2 1.0 1.0 0.9 1.0 0.9 1.0 1.0 0.8
DR5-
-55KD
-40KD
1.0 1.2 1.3 1.3 0.9 1.0 0.9 0.8 0.7 0.8
Bak-
-25KD
1.0 1.1 1.0 1.0 0.9 1.0 0.9 0.8 0.8 0.8
Bax-
-20KD
1.0 1.2 1.2 1.0 0.8 1.0 1.0 0.9 0.7 0.8
Bid-
-22KD
-15KD
1.0 0.9 0.8 0.7 0.7 1.0 1.0 0.7 0.6 0.5
β-Actin-
-42KD
MDA-MB-231
MDA-MB-468

Figure S2

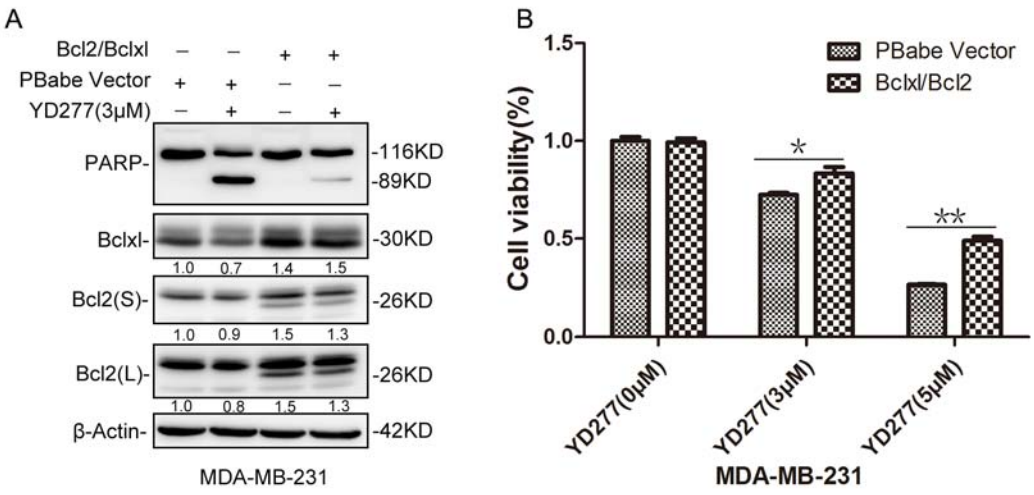

A
Bcl2/Bclxl – – + +
PBabe Vector + + – –
YD277(3μM) – + – +
PARP-
-116KD
-89KD
Bclxl-
-30KD
1.0 0.7 1.4 1.5
Bcl2(S)-
-26KD
1.0 0.9 1.5 1.3
Bcl2(L)-
-26KD
1.0 0.8 1.5 1.3
β-Actin-
-42KD
MDA-MB-231
B
PBabe Vector
Bclxl/Bcl2
Cell viability(%)
1.5
1.0
0.5
0.0
*
**
YD277(0μM)
YD277(3μM)
YD277(5μM)
MDA-MB-231

Figure S3

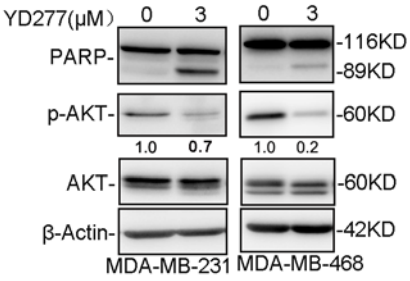

YD277(μM)
0
3
0
3
PARP-
-116KD
-89KD
p-AKT-
-60KD
1.0
0.7
1.0
0.2
AKT-
-60KD
β-Actin-
-42KD
MDA-MB-231
MDA-MB-468

Figure S4

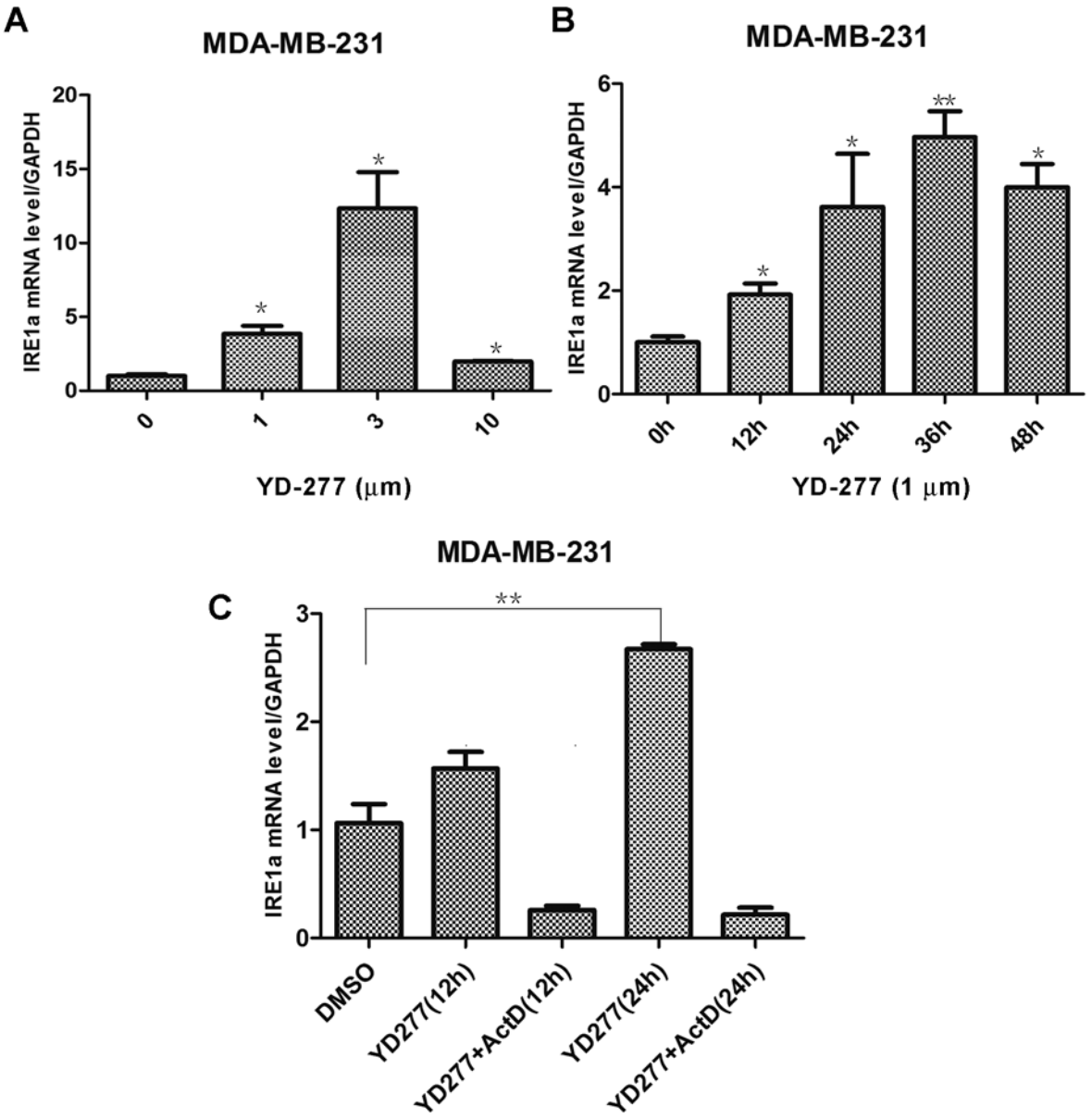

A
MDA-MB-231
IRE1a mRNA level/GAPDH
20
15
10
5
0
*
*
*
0
1
3
10
YD-277 (μm)
B
MDA-MB-231
IRE1a mRNA level/GAPDH
6
4
2
0
*
*
**
*
0h
12h
24h
36h
48h
YD-277 (1 μm)
C
MDA-MB-231
**
IRE1a mRNA level/GAPDH
3
2
1
0
DMSO
YD277(12h)
YD277+ActD(12h)
YD277(24h)
YD277+ActD(24h)

Figure S5