Identification of a non -

Table S1. The ^{13}C and ^{1}H NMR data of AG-08 (0 +] / S S P b)Q & ' & O

H/C	/C (ppm)	/H (ppm), ***J*** (Hz)
1	34 t	1.57 m
2	25.4 t	1.91 m
3	90.6 d	4.2 dd (10.6, 5.9)
4	38.7 s	-
5	52.2 d	1.73 brs
6	126.2 d	5.61 m
7	129.3 d	5.60 m
8	43.7 d	2.82 brs
9	145 s	-
10	38.2 s	-
11	113.7 d	5.14 dt (5.1, 2.3)
12	37.9 t	1.95 (m), 2 (m)
13	44.6 s	-
14	43.6 s	-
15	44.1 t	2.06 (m), 1.55 (m)
16	73.3 d	4.7 ddd (7.8, 7.8, 6)
17	56.4 d	2.22 d (7.6)
18	18.7 q	0.65 s
19	20.1 q	0.99 s
20	87.1 s	-
21	27.9 q	1.24 s
22	34.6 t	2.56 q (10.5), 1.6 m
23	25.7 t	2 (m)
24	81.6 d	3.76 dd (7.3, 7.3)
25	72.1 s	-
26	27.9 q	1.30 s
27	26.7 q	1.15 s
28	27.7 q	0.79 s
29	16.5 q	0.86 s
30	18.9 q	0.96 s
¶	134.9 s	-
¶	127.8 d	7.8 d (8.1)
¶	129.8 d	7.32 d (8.4)
¶	144.5 s	-
¶	129.8 d	7.32 d (8.4)
¶	127.8 d	7.8 d (8.1)
¶	21.8 q	2.44 s

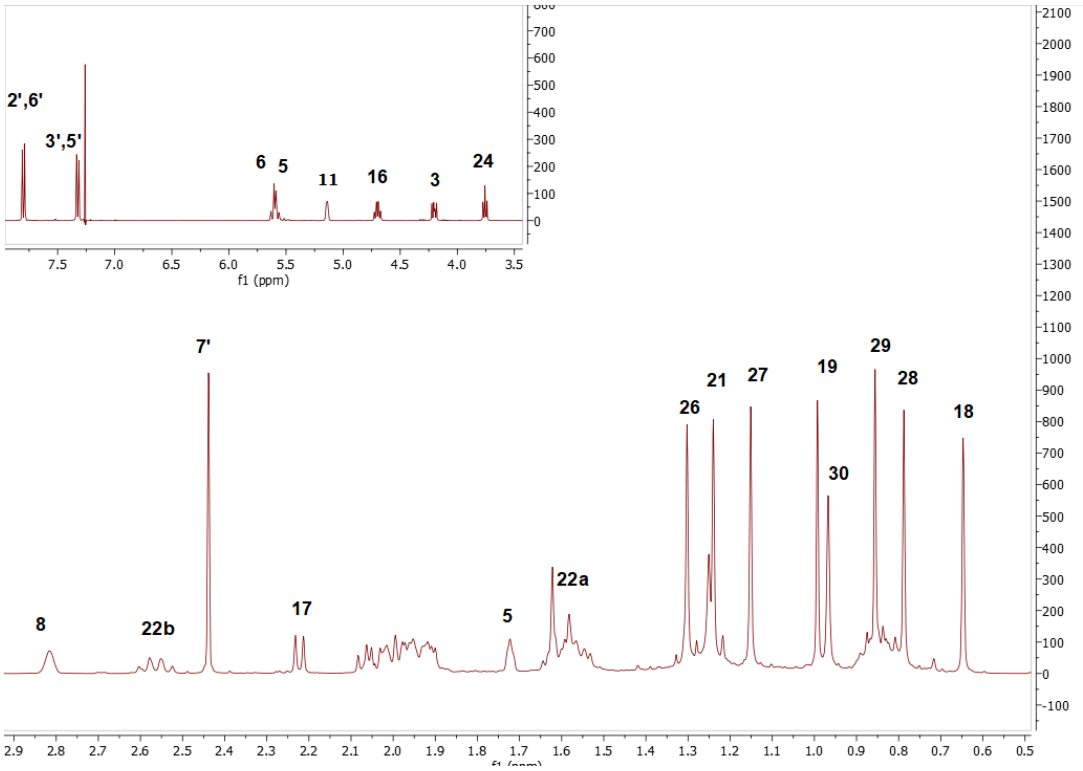


Figure S2. ^{1}H NMR Spectrum of AG-08 ($CDCl^{3}$, 400 MHz)

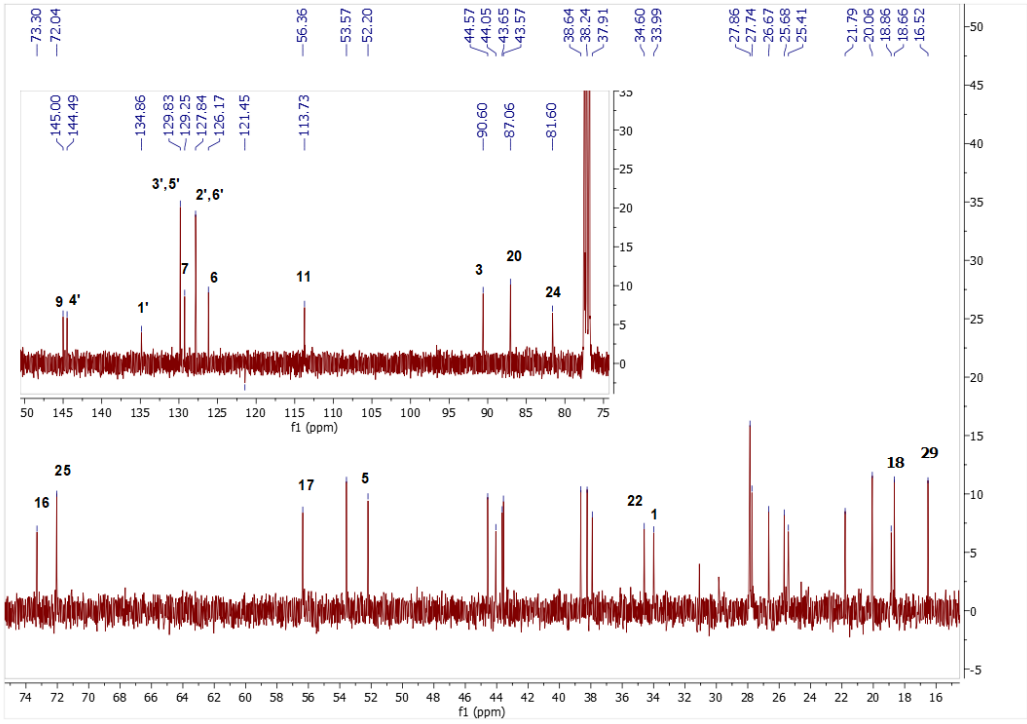


Figure S3. ^{13}C NMR spectrum of AG-08 ($CDCl^{3}$, 100 MHz)

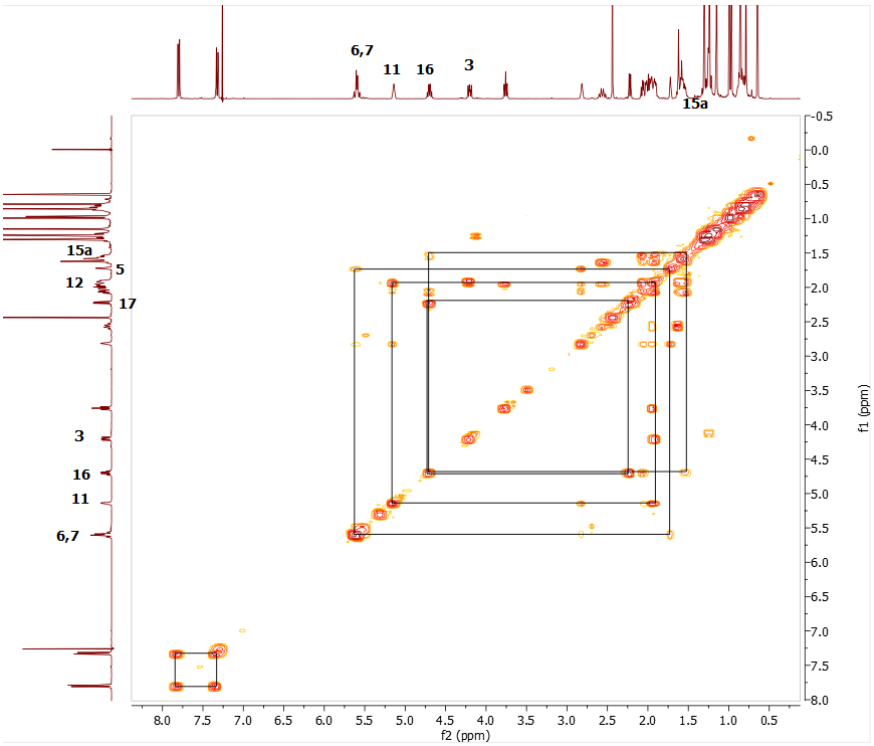


Figure S4. COSY Spectrum of AG-08

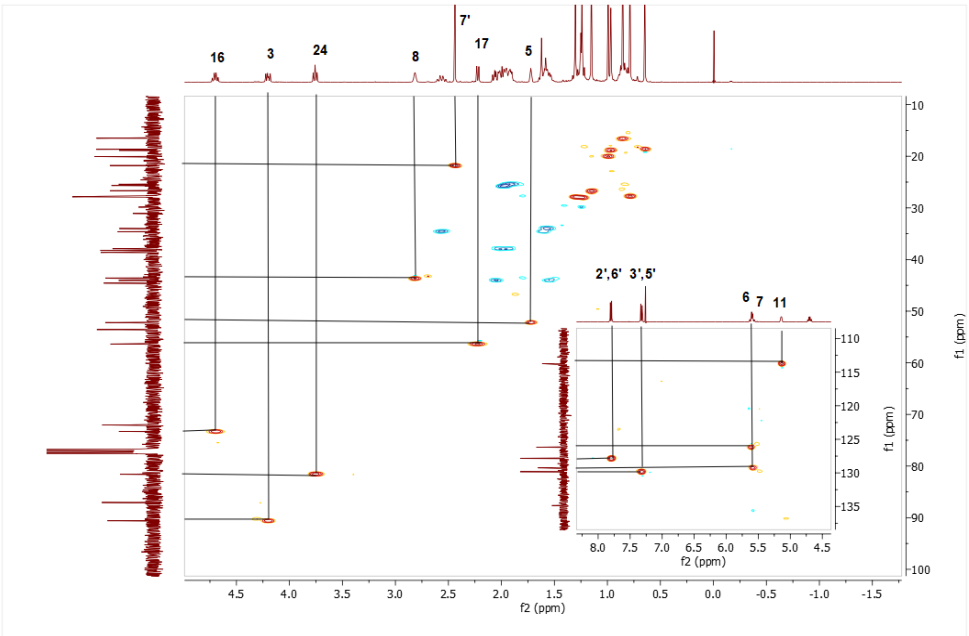


Figure S5. HSQC Spectrum of AG-08

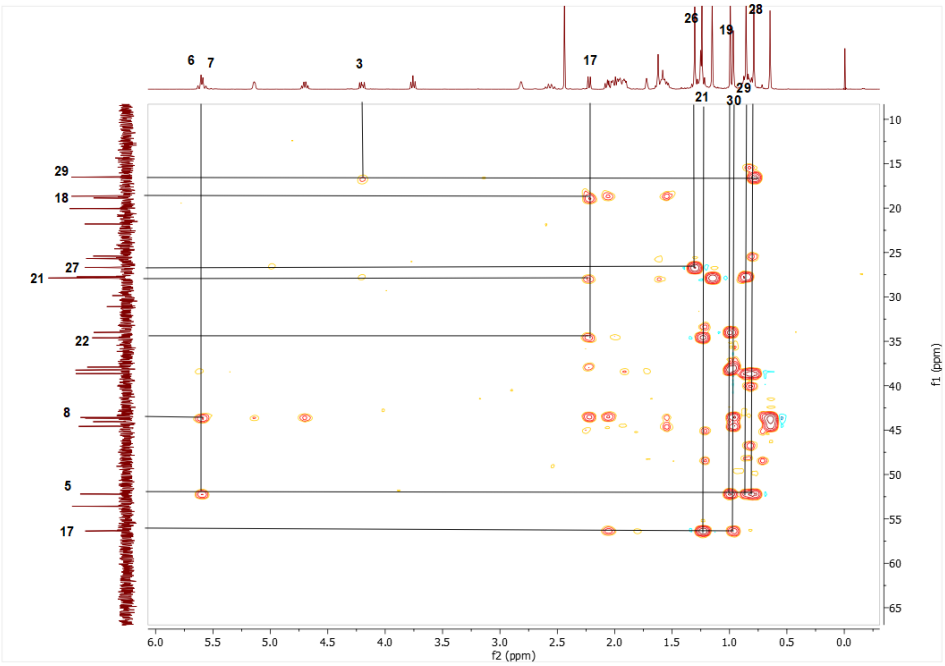


Figure S6. HMBC Spectrum of AG-08

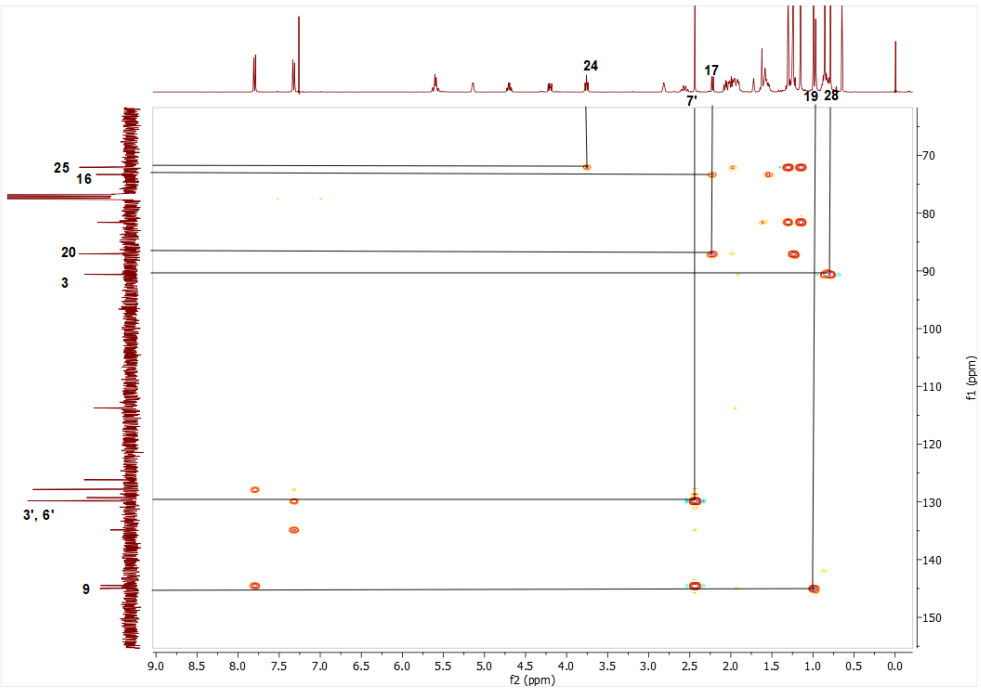


Figure S7. HMBC Spectrum of AG-08

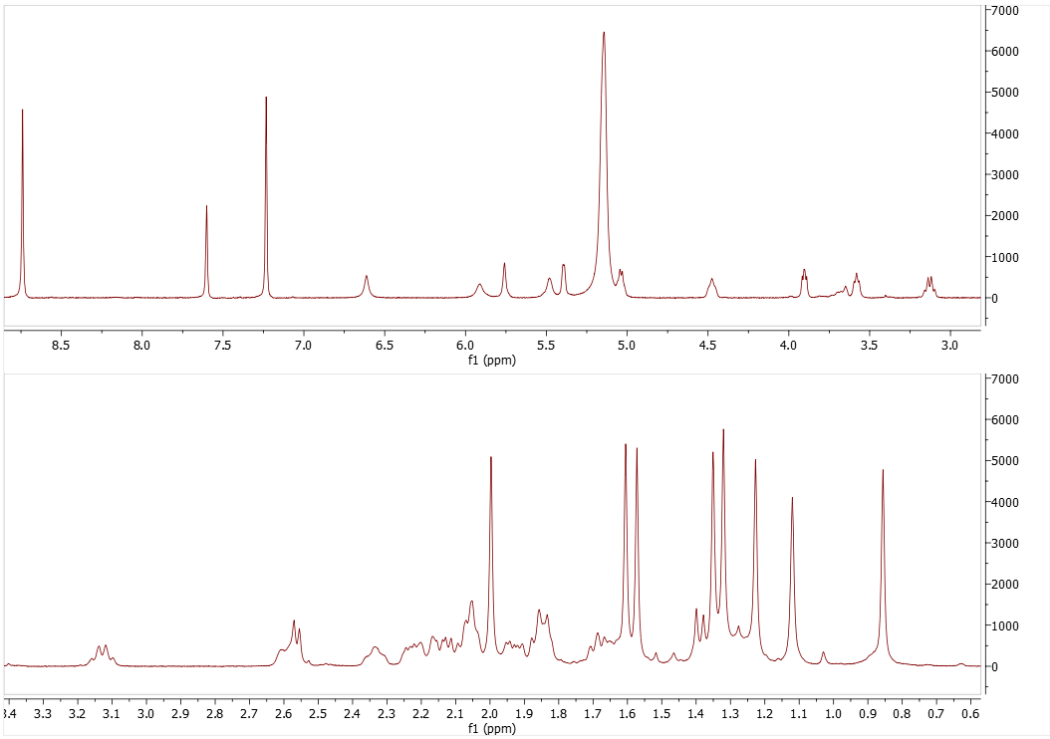


Figure S8. ^{1}H NMR Spectrum of AG (C_5D_5N, 500 MHz)

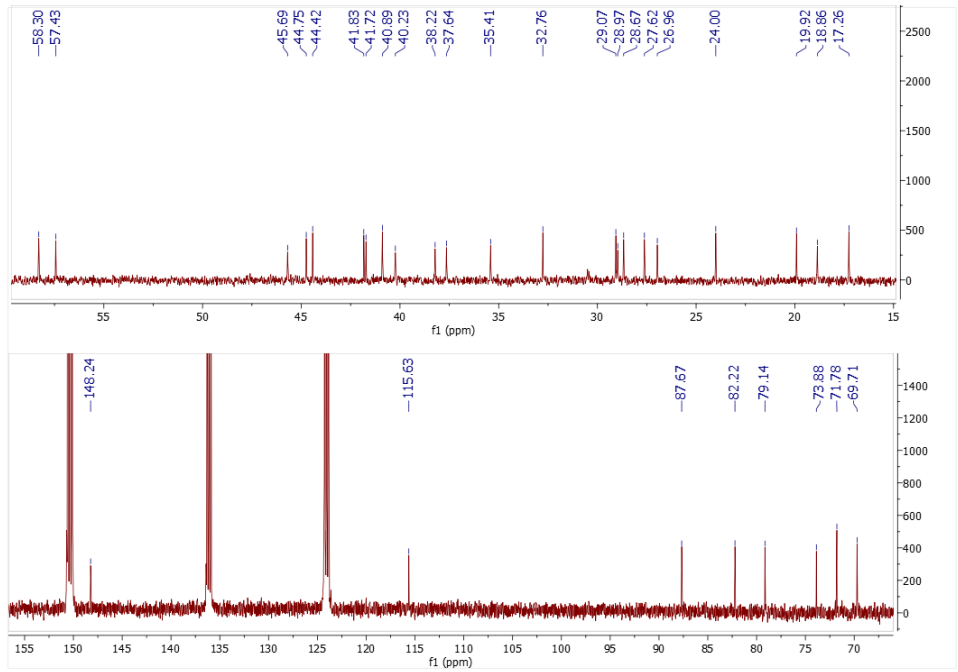


Figure S9. ^{13}C NMR spectrum of AG (C_5D_5N, 125 MHz)

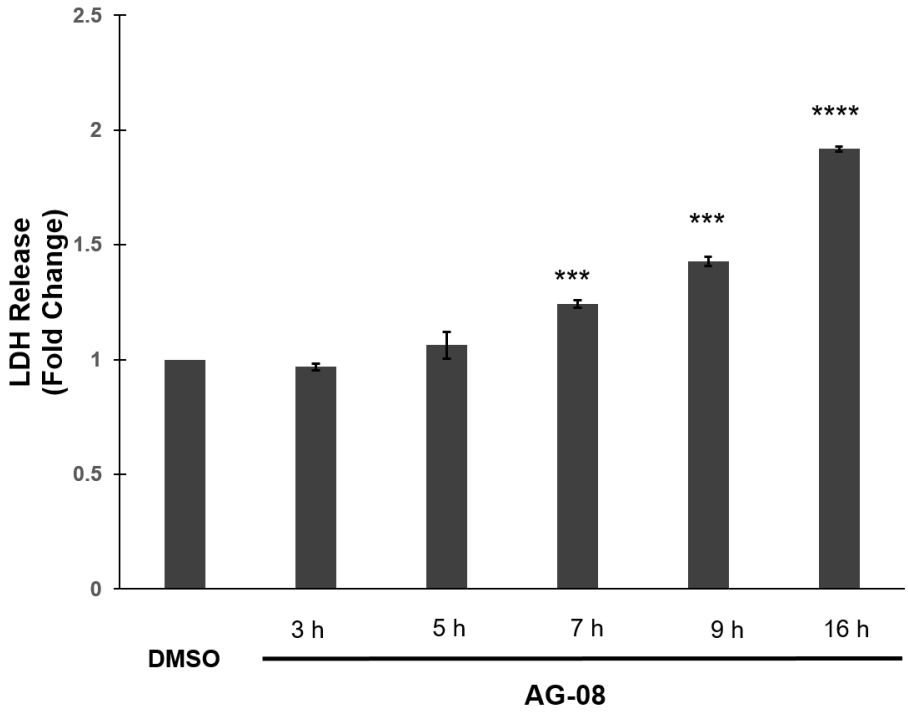


Figure S10. AG -08 invoke s necrotic cell death. LDH release was determined after W K H W U H D W P H Q W R AG-08 for 3, 5, 7, 9 and 16 h. The level of released LDH was calculated as fold change relative to vehicle. The reported values correspond W R P H D Q Y D O X H V " V W D Q G D U G G H Y L D W L R Q " V G R I W ****p<0.0001.

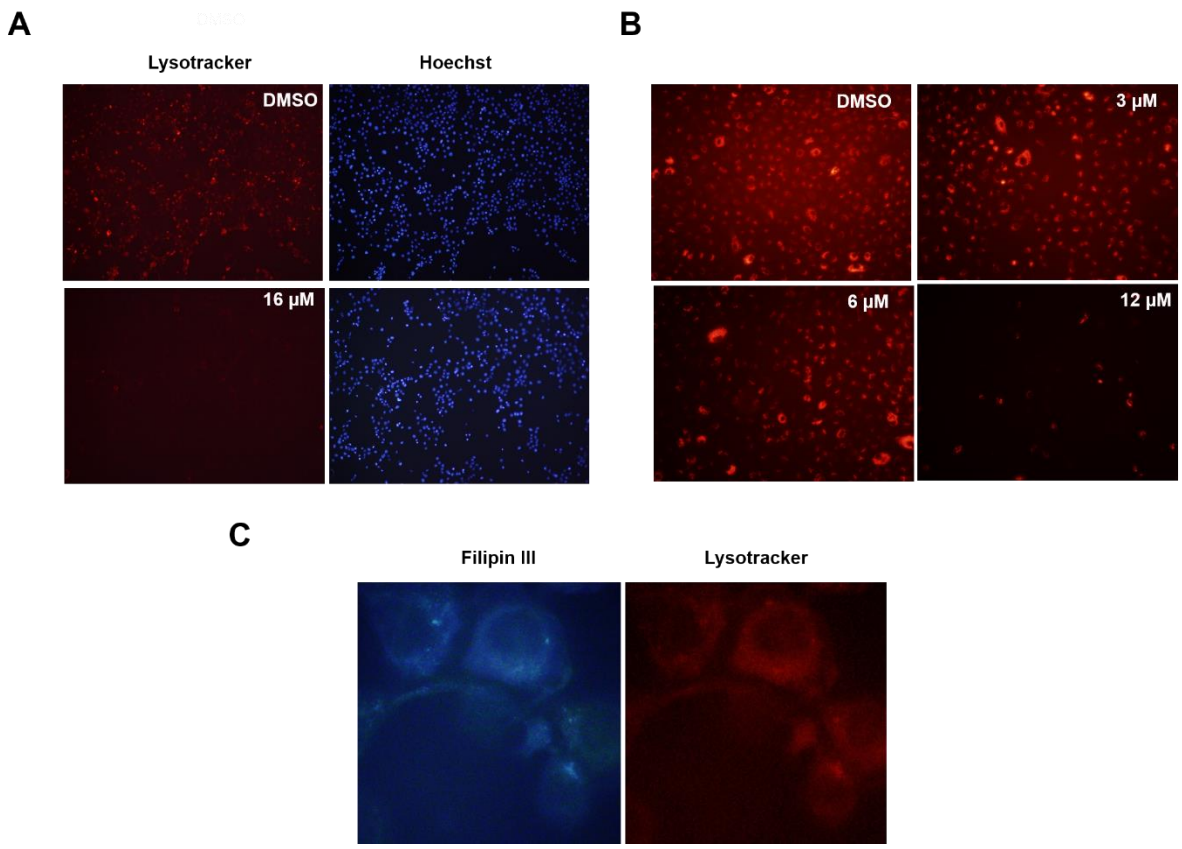


Figure S1 1. AG-08 induces lysosomal impairment. (A) HCC1937 cells were treated with 16 — 0 $ * R U Y H K L F O H I R U K 7 K H Q / - 9 9 R 7 U D F N H U Š 5 H C was used to stain acidic lysosome accompanied by Hoechst staining of nucleus. (B) A549 cells were incubated with 3, 6, 1 — 0 $ * R U Y H K L F O H I R U K / \ V R 7 U D F N H 99 was used to shown lysosomal acidity of cells. (C) After HCC1937 cells were treated with 16 — 0 $ * I R U K / \ V R 7 U D F N - 9 9 U Š 5 H C Filipin III was used to showing co-localization of lysosomes and cholesterol.

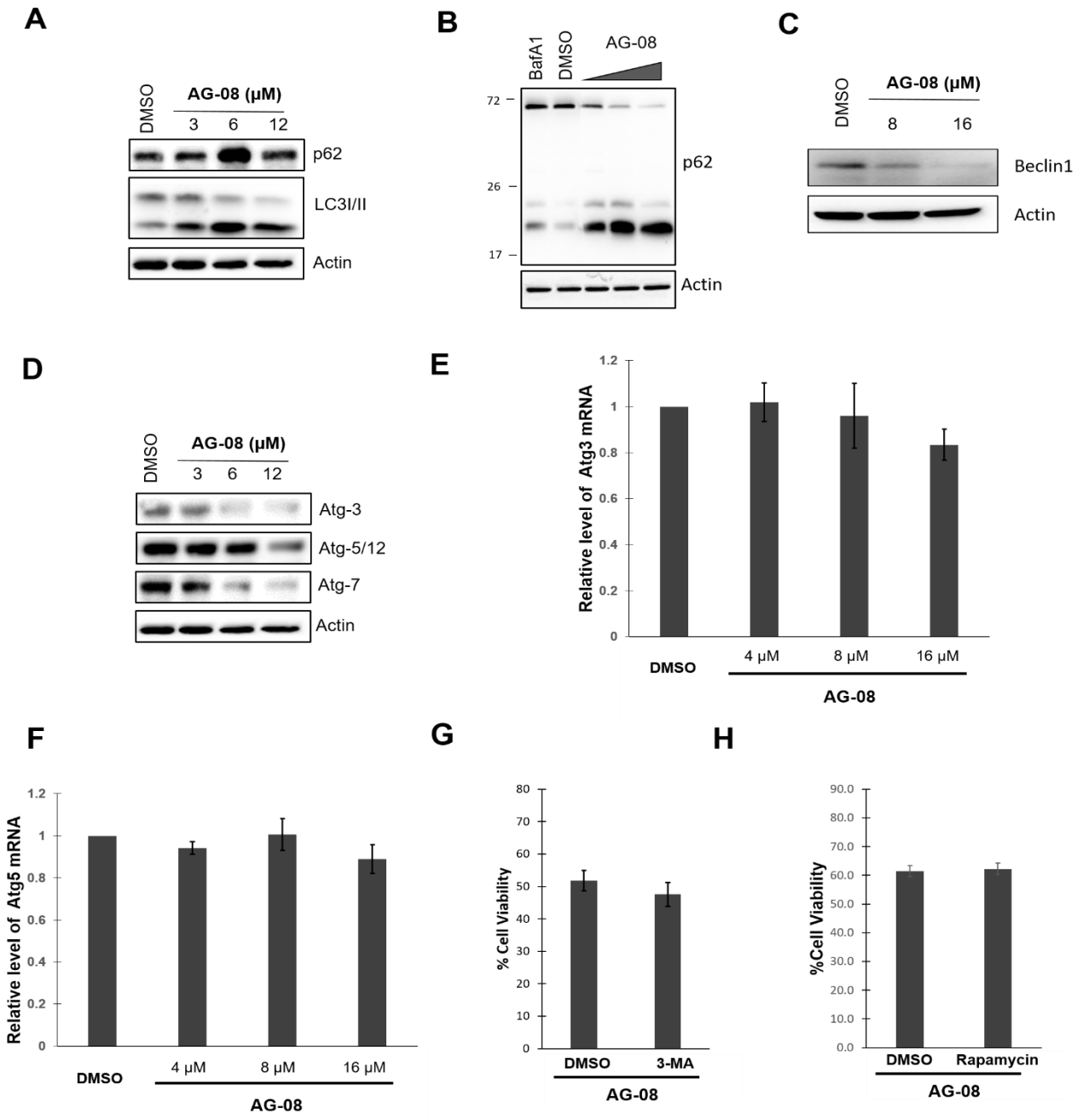


Figure S12. AG-08 inhibits autophagy. (A) AG-08 inhibits autophagic flux in A549 F H O O V $ F H O O V Z H U H W U H D W H G Z L W K D Q G R U H G L F L Q H I R U 2 4 h. Then protein levels of LC3-I/LC3-II and p62 were evaluated via IB. The experiments were repeated two times independently; with one representative result shown. (B) Following treatment

Z L W K D Q G -08 or vehicle for 16 h, p62 protein level of HCC1937 cells was investigated by IB. Experiments were repeated three times independently; with one representative result shown. (C) + & & F H O O V Z H U H W U H D W H G Z L W AG-08 or vehicle for 16 h and protein level of Beclin-1 was investigated via IB (D) After $ F H O O V Z H U H W U H D W H G Z L W -08 or vehicle for 24 h, protein level of Atg-3, Atg-5/12, and Atg-7 was determined via IB. (E-F) HCC1937 cells were treated with D Q G -08 or vehicle for 16 h and q-RTPCR was used to determine mRNA level of Atg-5 and Atg-3. (G-H) Following pre-treatment with 3 mM 3-MA and 500 nM 5 D S D P \ F L Q I R U K + & & F H O O V Z H U H W U H D W H G Z L W K -08 for 24 h, cell viability was calculated. Error bars represent s.e.

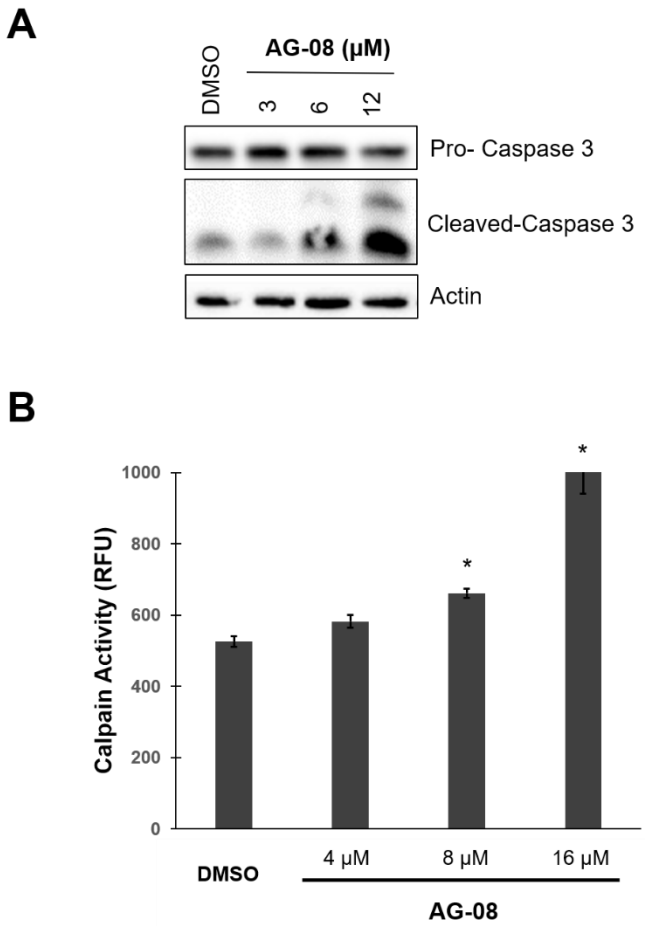
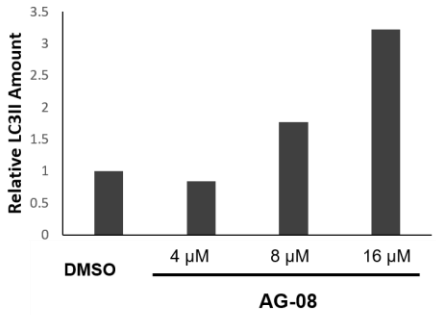
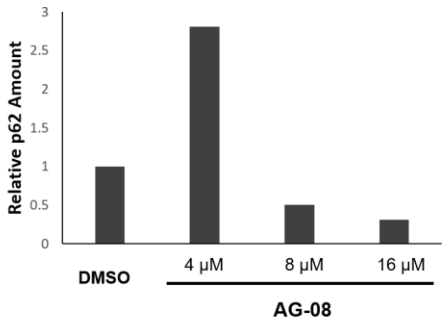


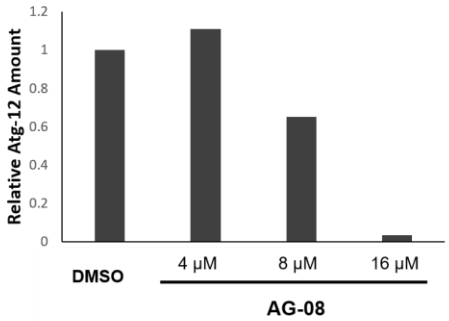
Figure S13. AG-08 induces the activity of proteases in A549 cell line. A549 cells were treated with AG-08 or vehicle for 24 h. (A) The expression of full length and cleaved caspase 3 were investigated via IB. (B) Calpain activity of cells was determined. Error bars are the standard deviations (n=3). p-values were calculated with respect to vehicle-treated cells (*$p < 0.05$).

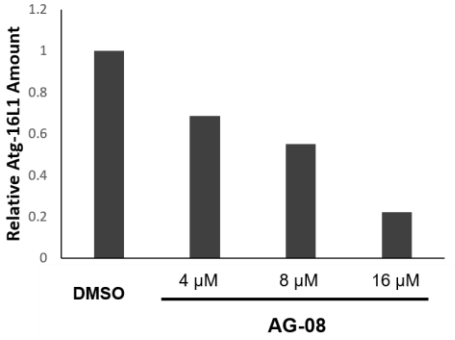
A

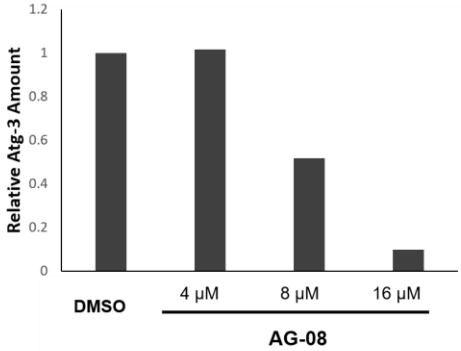

Relative LC3II Amount
3.5
3
2.5
2
1.5
1
0.5
0
DMSO
4 μM
8 μM
16 μM
AG-08

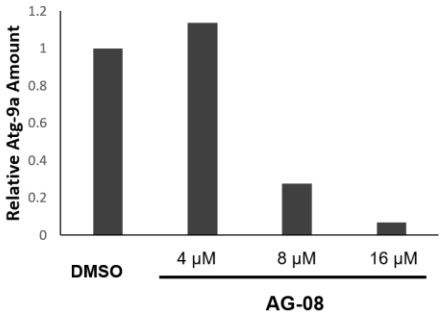

Relative p62 Amount
3
2.5
2
1.5
1
0.5
0
DMSO
4 μM
8 μM
16 μM
AG-08

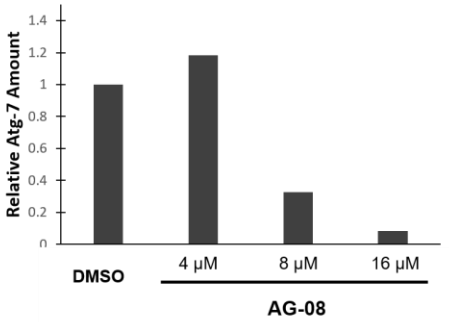
B

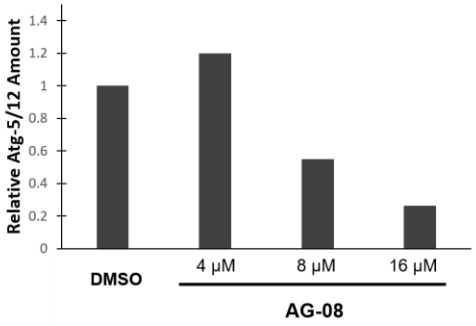

Relative Atg-12 Amount
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
4 μM
8 μM
16 μM
AG-08


Relative Atg-16L1 Amount
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
4 μM
8 μM
16 μM
AG-08

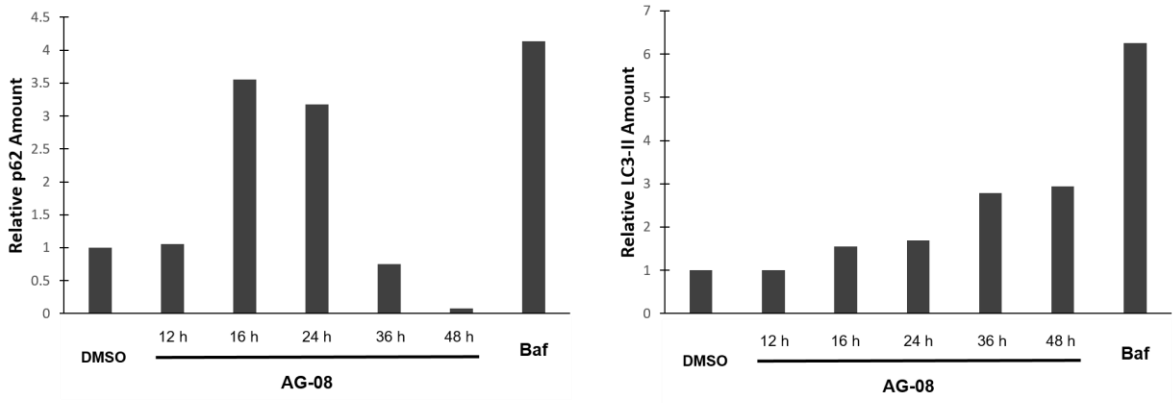

Relative Atg-3 Amount
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
4 μM
8 μM
16 μM
AG-08


Relative Atg-9a Amount
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
4 μM
8 μM
16 μM
AG-08


Relative Atg-7 Amount
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
4 μM
8 μM
16 μM
AG-08


Relative Atg-5/12 Amount
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
4 μM
8 μM
16 μM
AG-08

C



D

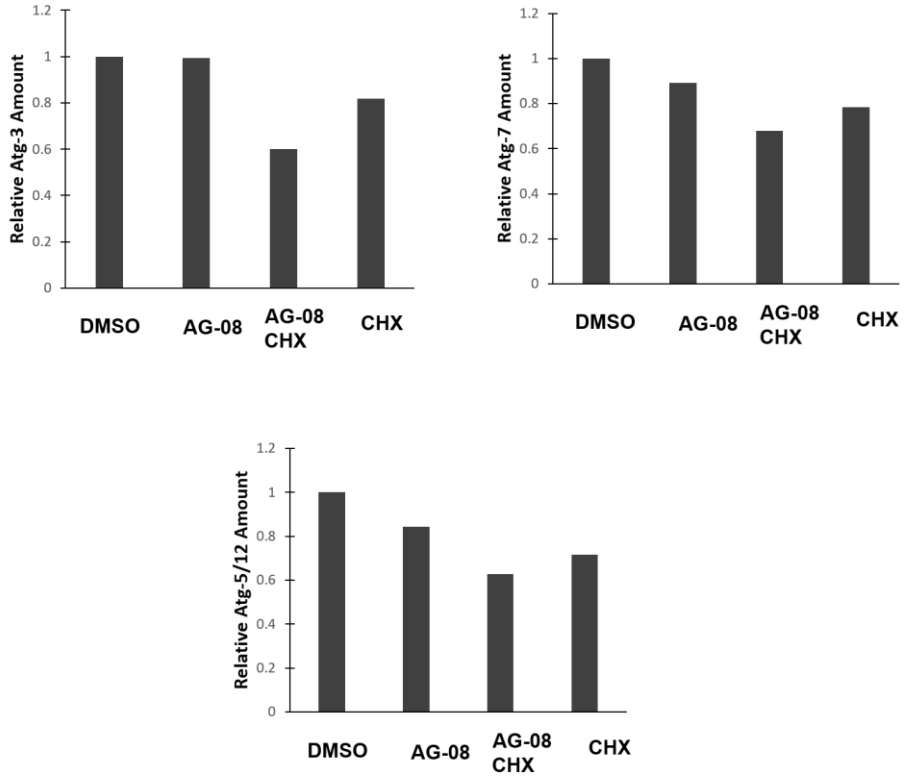


Figure S14. AG-08 inhibits autophagy. Western blot densitometric analysis of (A) Figure 4A, (B) Figure 4B and (C) Figure 4C, (D) Figure 4E.

Table S2. AG and CG are not cytotoxic. The experiment repeated three times. 30 and

— 0 D U H P D [L P X P V R O X E L O L W \ F R Q F H Q W U D W L R Q R I $ * D

Cell lines	AG IC_{50} **(μM)**	CG IC_{50} **(μM)**
HeLa	>30	>50
HCC1937	>30	>50
MRC5	>30	>50
MCF7	>30	>50
A549	>30	>50

Table S3. q-RT-PCR primer list

Gene	Forward Sequence	Reverse Sequence
Atg-5	CCCTCTTGGGGTACATGTCTGT	CTCGAAGCACATCTTTGTCATCTG
Atg-3	AACAGTGACCATTGAAAATCAC	CGTTAACAGCCATTTTGCCACTAA
p62	GTGCAACATGGGGCTTGAGAA	GTCAATTCCTCGTCACTGGAAAAC
CHOP	AGTCTAAGGCACTGAGCGTATC	CTTTCAGGTGTGGTGATGTATGAA
TBP1	GAGTTCTGGGATTGTACCGCA	CGTGGTTCGTGGCTCTCTT