Necroptosis-Inducing Rhenium(V) Oxo Complexes

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JACS *ASAP*

CANCER AND CISPLATIN

Estimated 8.2 million cancer related deaths in 2012

Increased from 2008 by 600,000

- Cisplatin has been used to treat a large number of cancers:
  - Approved: Bladder, ovarian, and testicular
  - Also used for: head and neck, mesothelioma, cervical, lung, esophageal, brain, and neuroblastomas
- Discovered in1968, due to broad spectrum of activity, became and still is a widely used cancer chemotherapeutic
  - Over 50% of cancer treatment regimens involve cisplatin and its derivatives

http://www.nlm.nih.gov/medlineplus/druginfo/meds/a684036.html
J. Med. Chem., **2011**, *54*, 3–25.

CISPLATIN

- Disadvantages (*common* side effects)
  - Kidney damage
  - Decreased blood levels of magnesium, potassium, and calcium
  - Nausea/vomiting
  - Low white blood cell, red blood cell, and platelet counts
  - Taste changes, including metallic taste of foods
  - Sensation of pins and needles or numbness in hands and/or feet caused by irritation of nerves
  - Swelling in hands, feet, or legs
  - Fetal changes if pregnant during treatment
- A number of other platinum based compounds have been pursued
  - Carboplatin, approved 1989 (improved safety)
  - Oxaliplatin, FDA approved 2002 (broader spectrum)
  - Satraplatin, not approved (orally bioavailable)
  - Picoplatin, phase III results unsatisfactory (active in some Cisplatin resistant cancers)

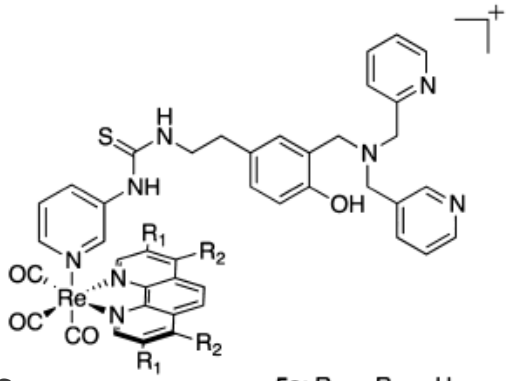
American Cancer Society, http://www.cancer.org/treatment/treatmentsandsideeffects/guidetocancerdrugs/cisplatin
Nat. Rev. Cancer, **2007**, *7*, 573-584.

PLATINUM ALTERNATIVES

- Due to high cross resistance of platinum drugs, other transition metal based compounds have been sought as a replacement
- Some classes of transition metal containing complexes:
  - Iridium, titanium, iron, ruthenium, osmium, gold, silver, molybdenum, gallium, rhenium
  - Various mechanisms of action from DNA binding, apoptosis induction, nucleobase binding, to induction of ROS production
- Several have begun phase I and phase II trials
- Difficulties:
  - Aqueous solubility
  - Hydrolytic stability
  - Toxicity

Anti-Cancer Agent. Me., **2014**, *14*, 1199-1212.

RHENIUM

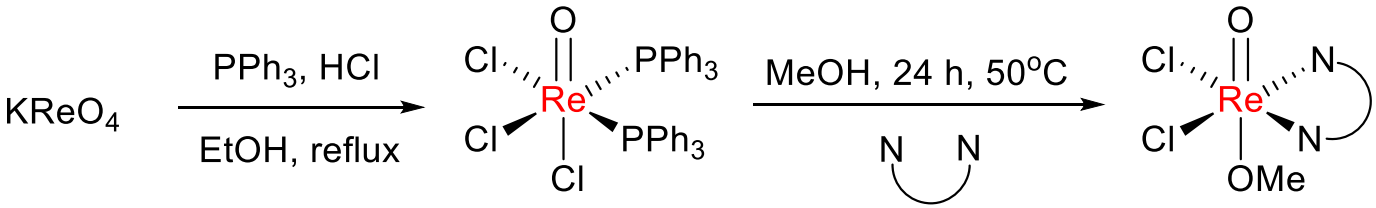


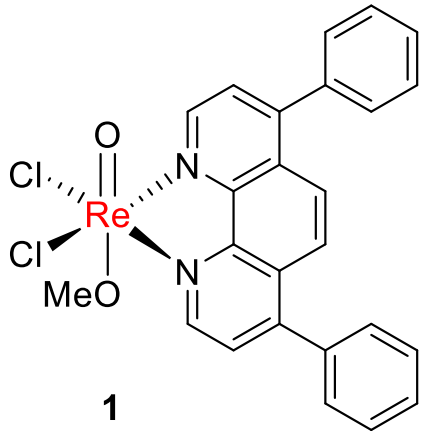
5a: $R_1 = R_2 = H$
5b: $R_1 = R_2 = Me$
5c: $R_1 = H$ $R_2 = Ph$

- Re agents have been used as in vitro and in vivo imaging agents
- Also ^{186}Re and ^{188}Re have been used in radiotherapy
  - However, its antiproliferative activities have not been studied as well
- Appealing for catalytic potential and lipophllicity
- Re(I) compounds have proven to be some of the most active Re antiproliferative compounds reported (acting via covalent interaction with DNA or protein side chains
  - IC_{50} as low as 700 nM
- In 2010, Mitsopoulou and co workers showed that several oxo Re(V) complexes were able to intercalate into DNA and upon irradiation cause DNA strand breaks suggesting potential use as cancer chemotherapeutic

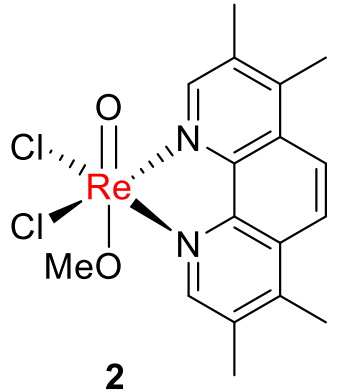
ACS Chem. Biol. **2014**, *9*, 2180−2193.
Bioinorg. Chem. Appl., **2010**, *2010,* 973742.

COMPOUNDS 1 AND 2





1



2

IN VITRO ACTIVITY

IC_{50} Values (nM) in Various Cancerous and Healthy Cell Lines after 72 h Exposure

Cell line	Cancer type	**1**	**2**	Cisplatin
A549	Lung carcinoma	207 ± 4	157 ± 15	3230 ± 467
HeLa	Cervical adenocarcinoma	445 ± 4	695 ± 21	4100 ± 113
U2OS	Bone osteosarcoma	274 ± 6	209 ± 31	4600 ± 600[a]
NTERA-2	Testis carcinoma	230 ± 28	255 ± 35	385 ± 49
A2780	Ovarian carcinoma	670 ± 40	150 ± 10	700 ± 200[a]
A2780CP70	Ovarian carcinoma	42 ± 15	56 ± 2	8415 ± 205
MRC-5	Lung fibroblast	1351 ± 228	709 ± 76	530 ± 600[a]

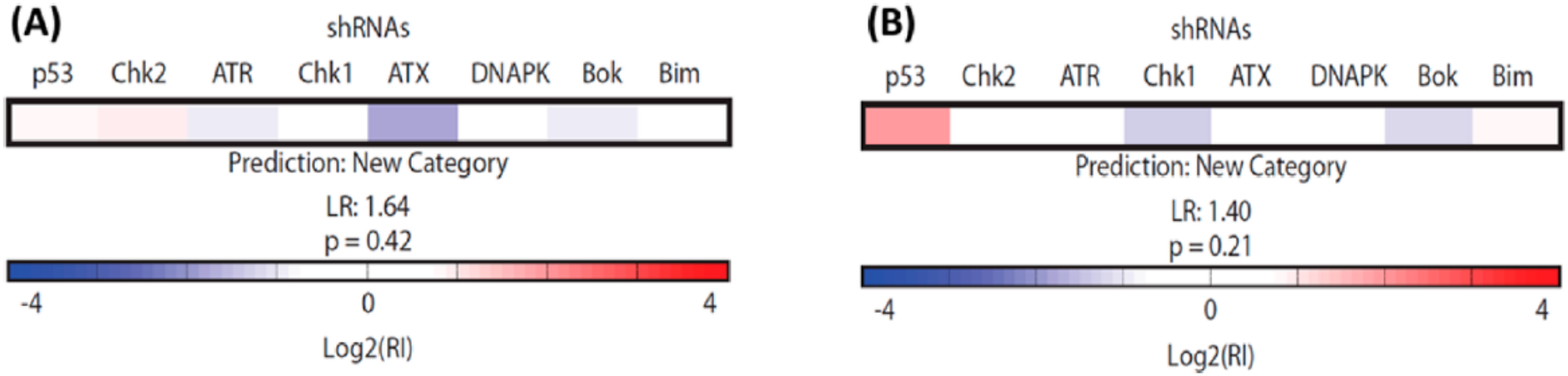
[a]Values from literature

ACTIVITY IN CISPLATIN RESISTANT CELL LINES

IC_{50} Values (nM) a Panel of Cisplatin-Resistant Cell Lines after 72 h Exposure

Cell line	Cancer type	**1**	**2**	Cisplatin
HT-29	Colorectal adenocarcinoma	85 ± 11	95 ± 20	29640 ± 1329
MDA-MB-231	Breast adenocarcinoma	475 ± 161	1735 ± 275	43600 ± 7071
MCF-7	Breast adenocarcinoma	285 ± 35	805 ± 21	9740 ± 537
PC-3	Prostate adenocarcinoma	270 ± 14	780 ± 10	10250 ± 919
DU 145	Prostate carcinoma	2840 ± 381	1370 ± 84	>100000

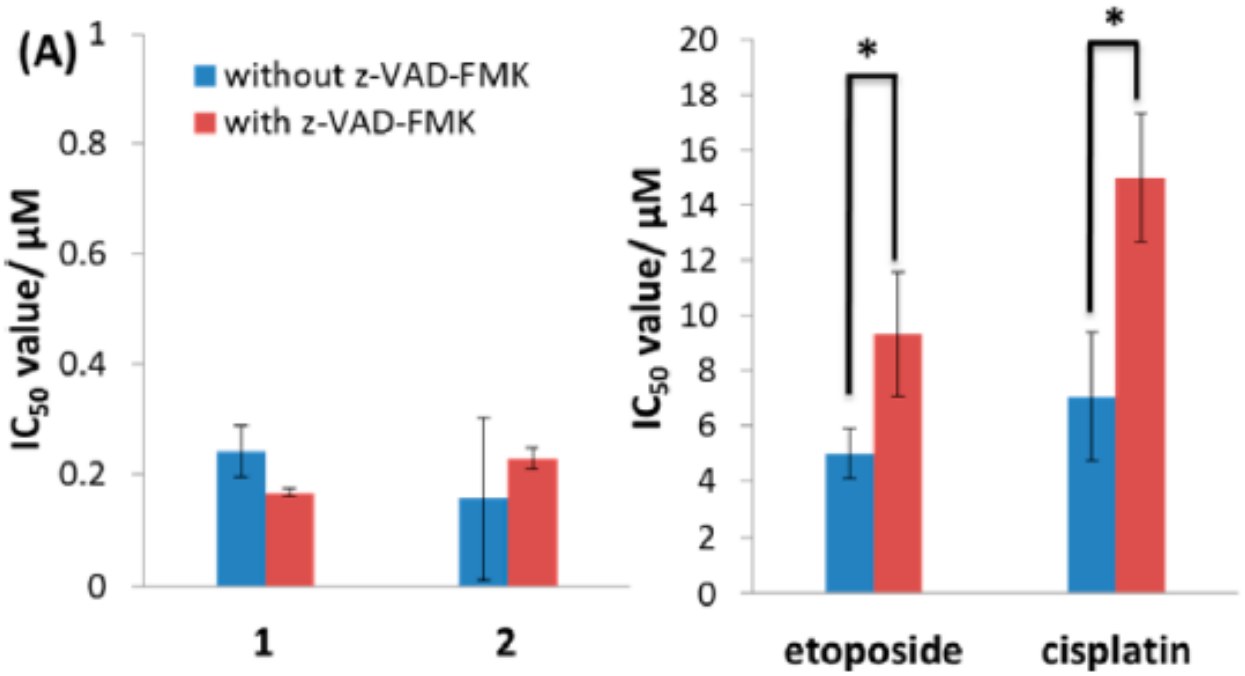
CLASSIFICATION OF **1** AND **2** MECHANISM OF ACTION



RNAi signature of Eμ-Myc$^{p19arf-/-}$ lymphoma cells treated with 1 μM (LD80-90) **1** (**A**) or **2** (**B**) after 72 h.

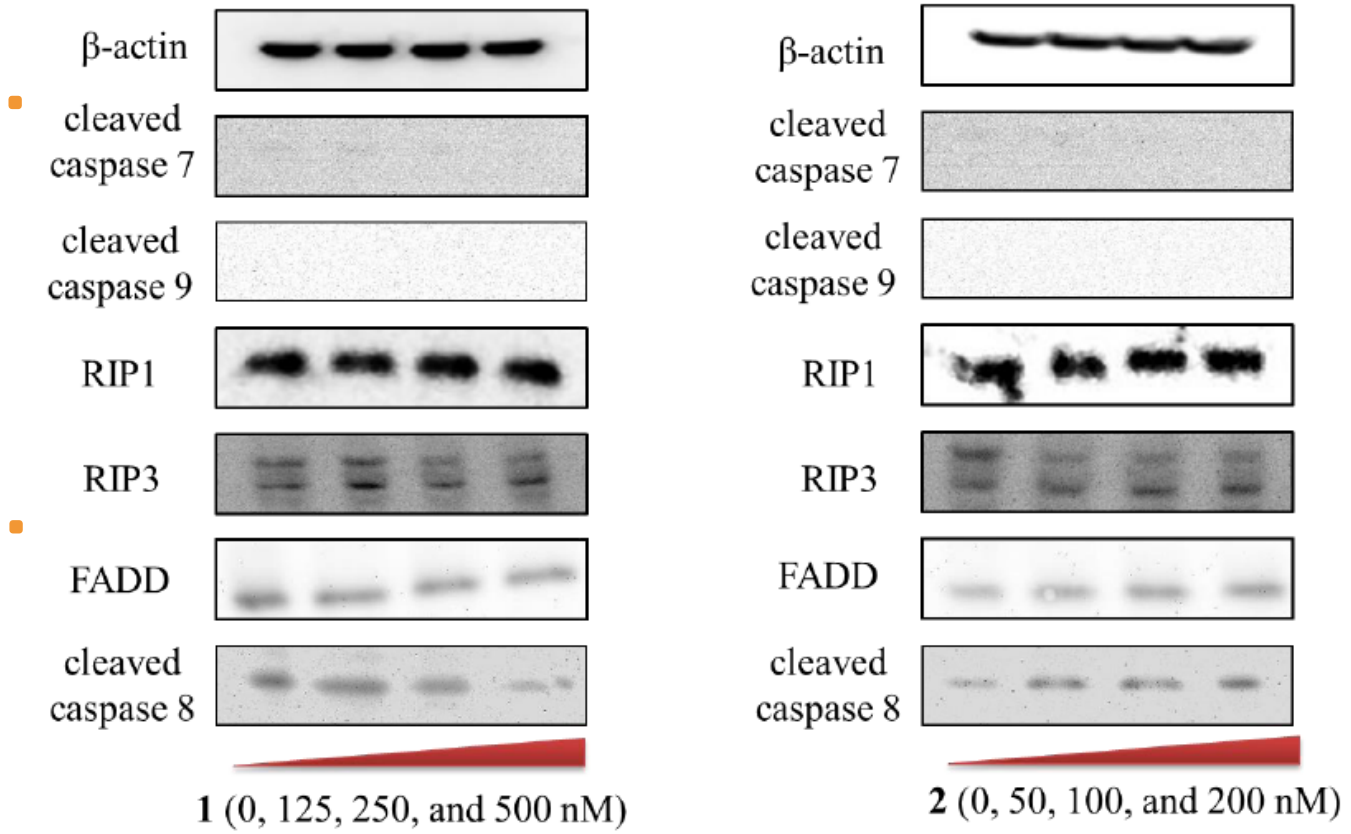
- Neither **1** nor **2** act via a mechanism of action similar to that of drugs in the reference set
- **1** and **2** are a novel class (mechanistically) of cancer drug compound

MOA: RELATED TO CASPASE-MEDIATED APOPTOSIS?



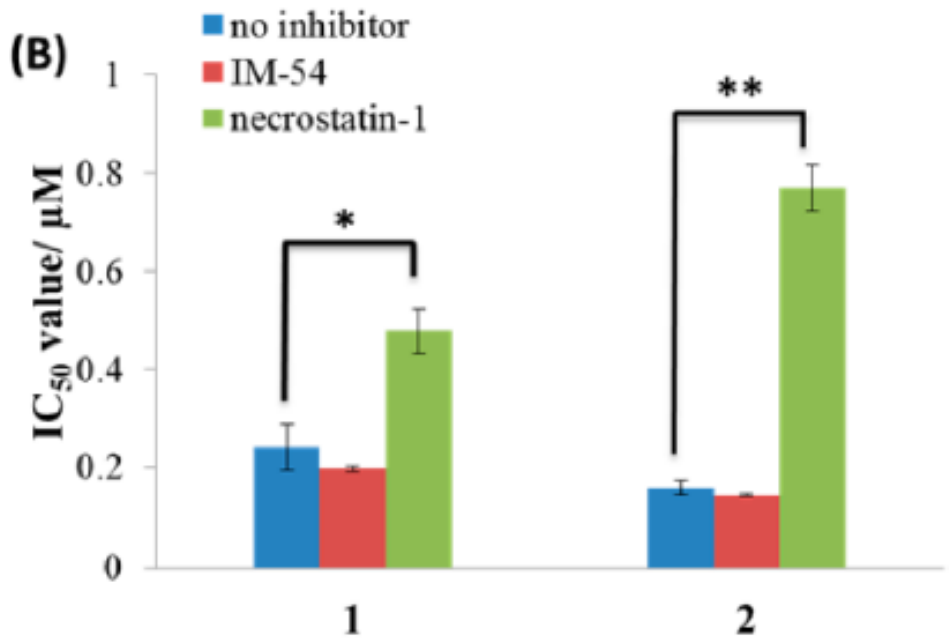
- **1** and **2** do not induce caspase-mediated apoptosis

MOA: RELATED TO APOPTOSIS?



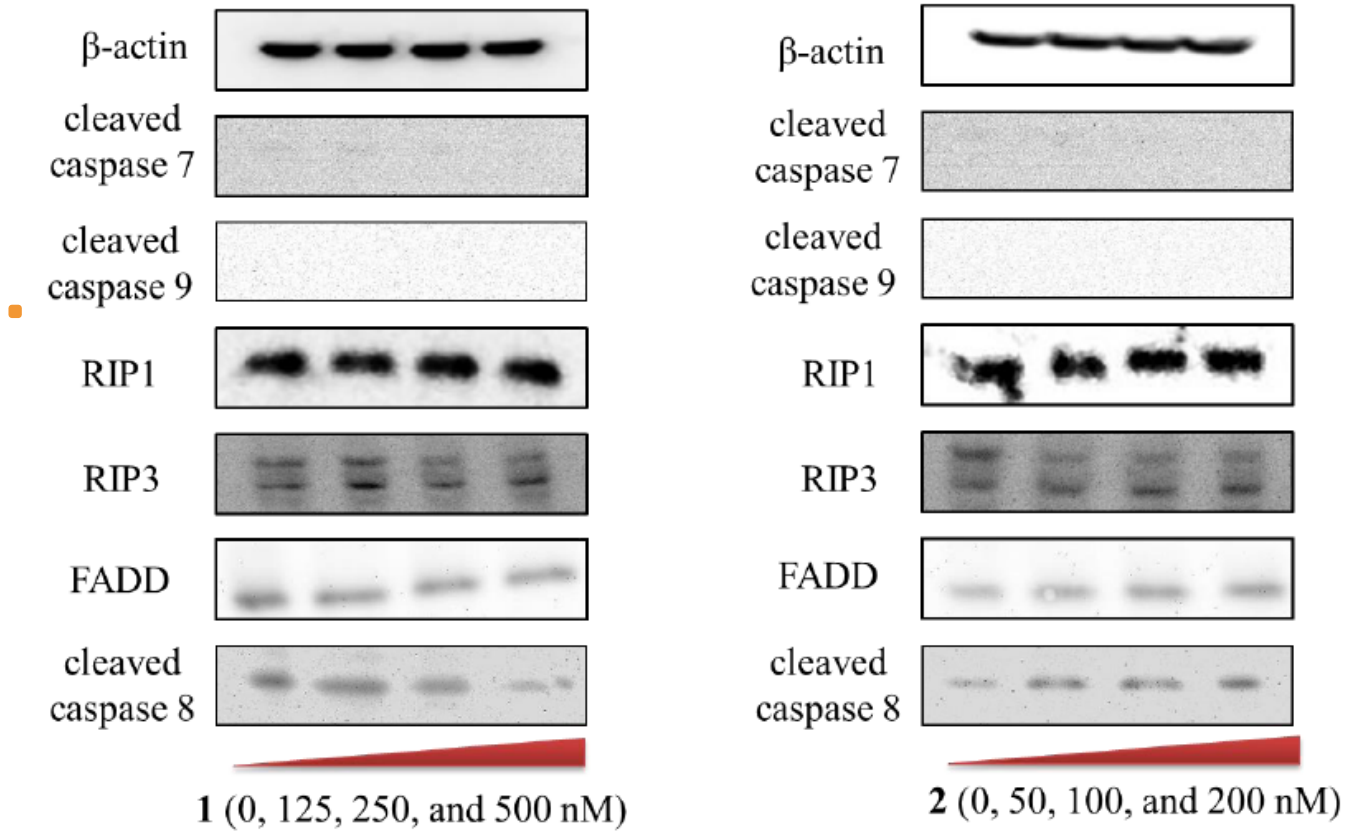
- **1** and **2** do not induce apoptosis via caspase cascade nor ripoptosome formation

MOA: RELATED TO NECROPTOSIS?



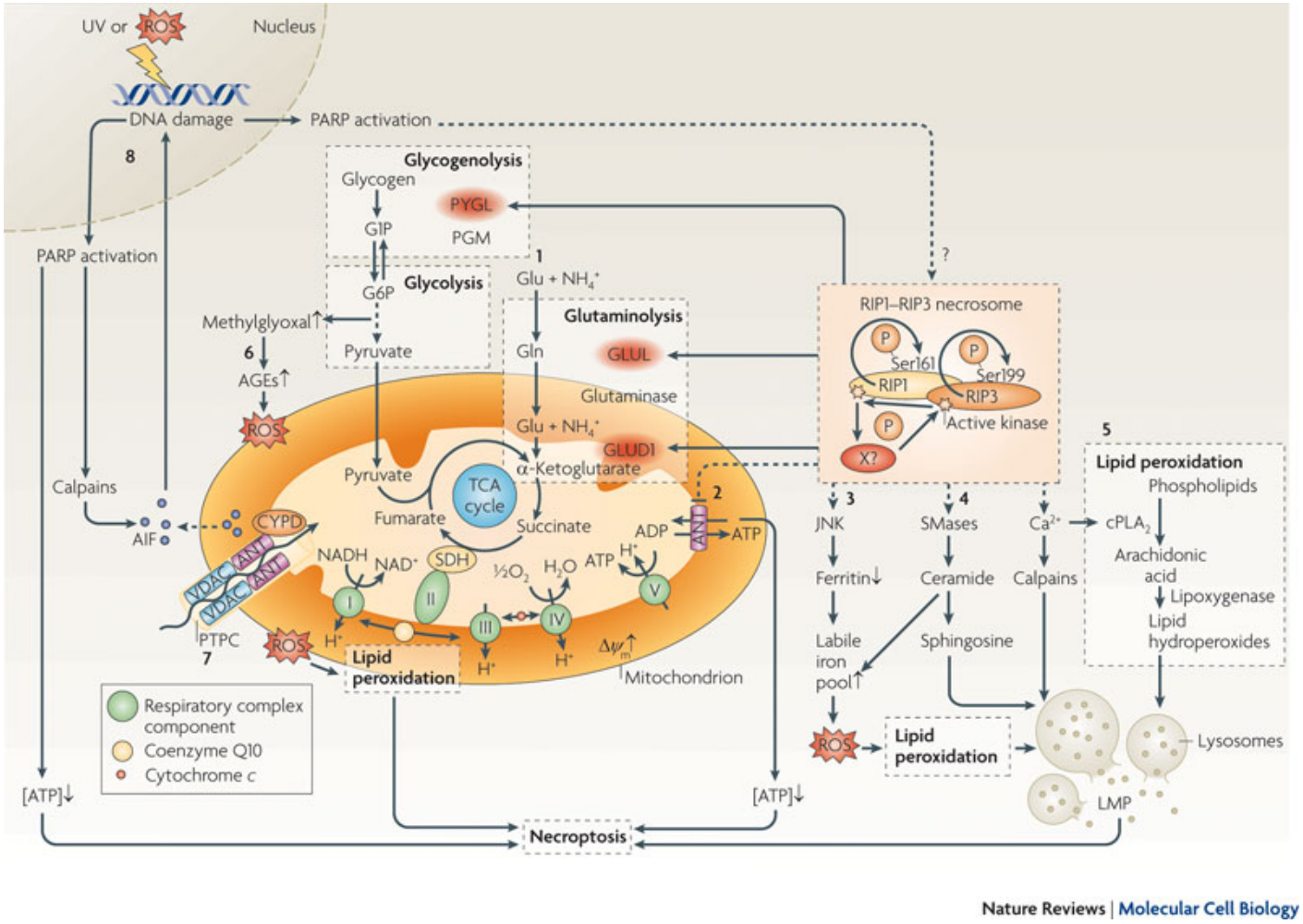
- Effect seen in various cell lines
- **1** and **2** induce cell death via necroptosis but not unregulated necrosis

MOA: RELATED TO NECROPTOSIS



- **1** and **2** act via the RIP1-RIP3 necrosome rather than upregulation of either individual protein

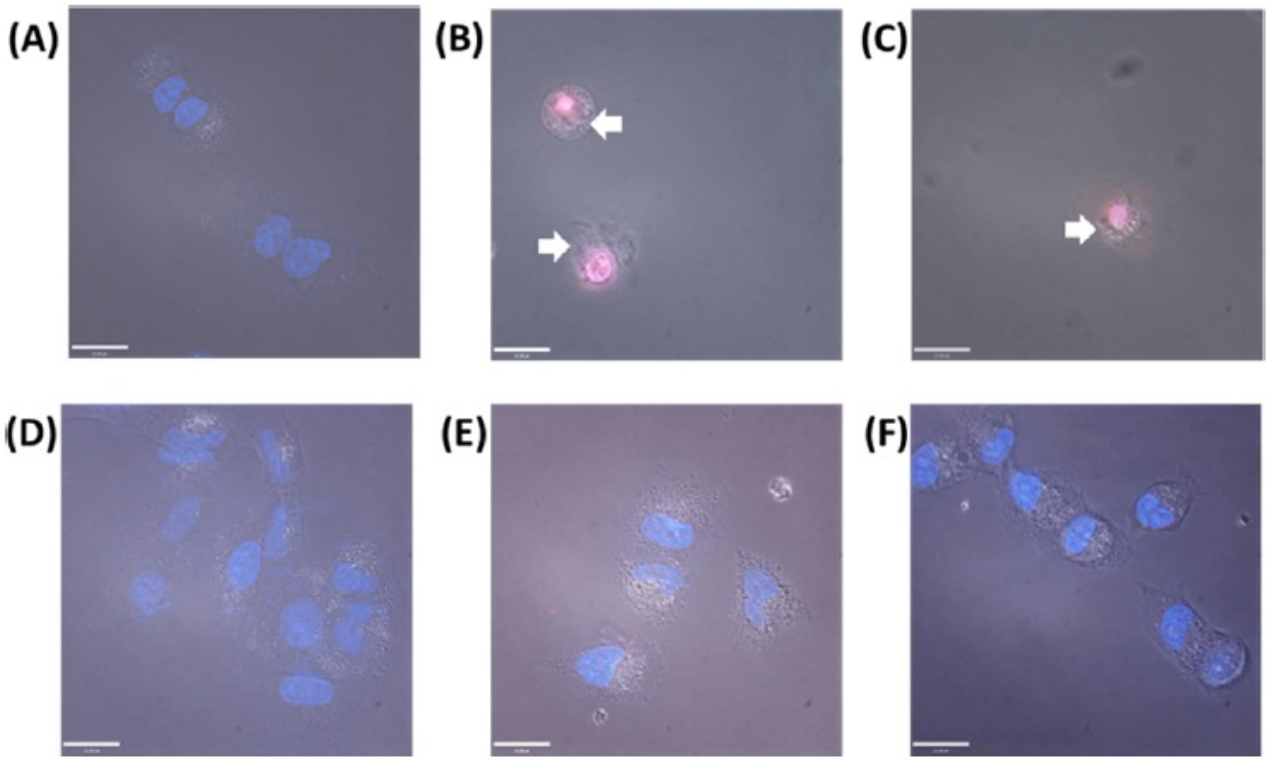
NECROPTOSIS



Nature Reviews | Molecular Cell Biology

Nat. Rev. Mol. Cell. Bio **2010**, *11*, 700-714.

HOW DO **1** AND **2** INDUCE NECROPTOSIS?



A. Untreated cells.

B. Treatment with **1**.

C. Treatment with **2**.

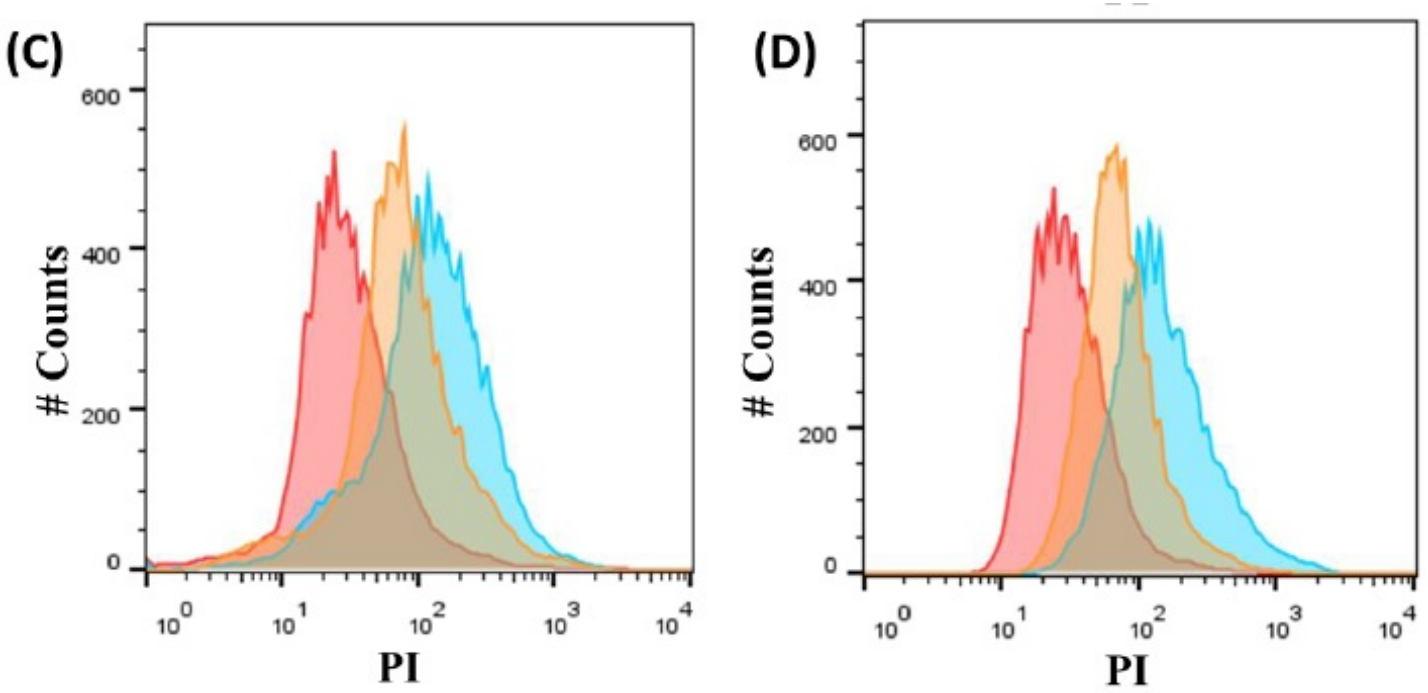
D. Treatment with **1** and necrostatin-1

E. Treatment with **2** and necrostatin-1

F. Stained with Hoechst 33258 and PI

- Necrostatin-1 blocks **1** and **2** induced necroptosis
- Necrosome formation is important in **1** and **2** mechanism of action

HOW DO **1** AND **2** INDUCE NECROPTOSIS?



Red = PI stained cells

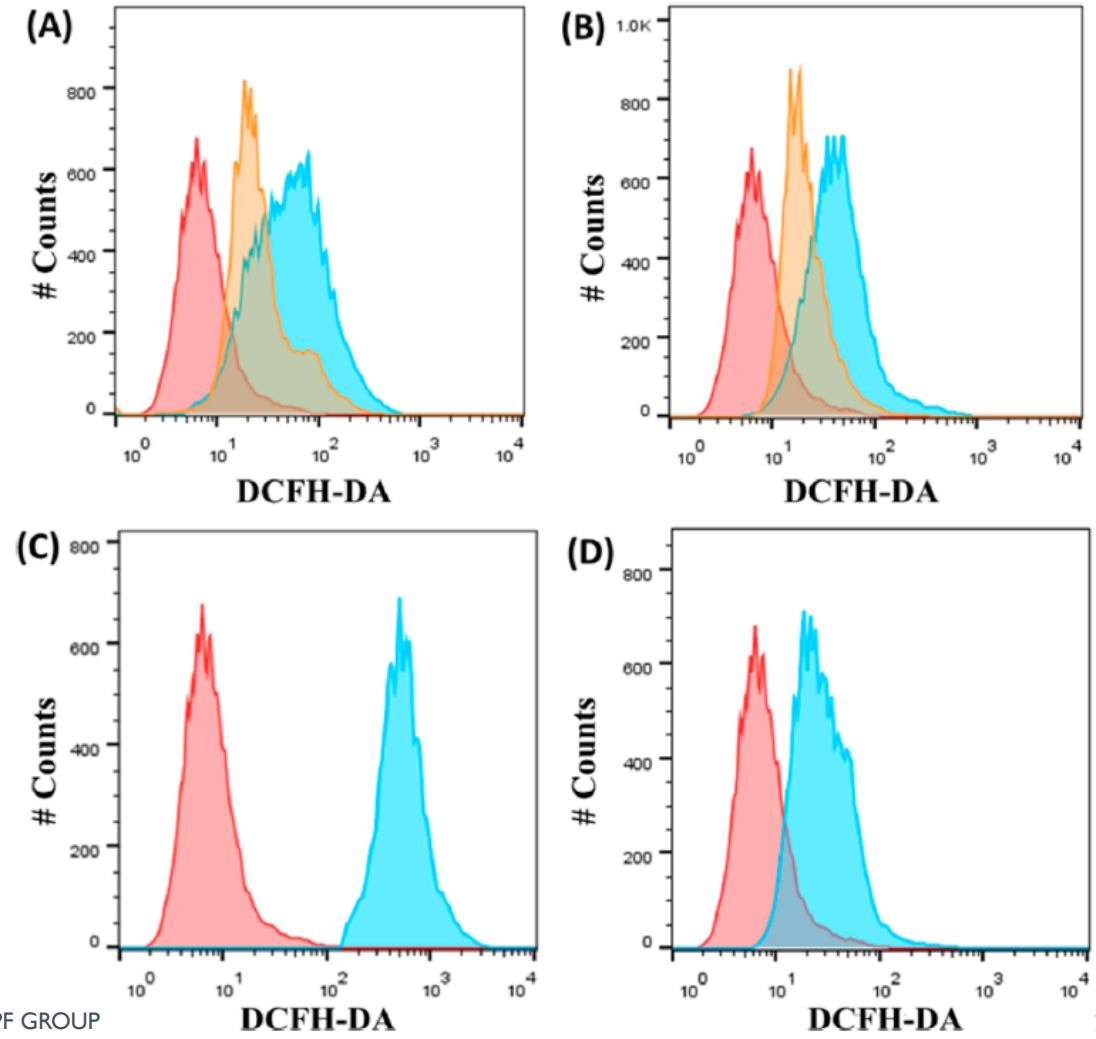
Blue = cells treated with **1** (A) or **2** (B)

Orange = cells pretreated with *N*-acetylcysteine (1 h) followed by **1** (A) or **2** (B)

Pretreatment with a ROS inhibitor (*N*-acetylcysteine)

- **1** and **2** cause increase in ROS in order to cause necroptic cell death

HOW DO **1** AND **2** INDUCE NECROPTOSIS?



Red = ROS levels in untreated cells

Blue = in cells treated with **1** (A) or **2** (B)

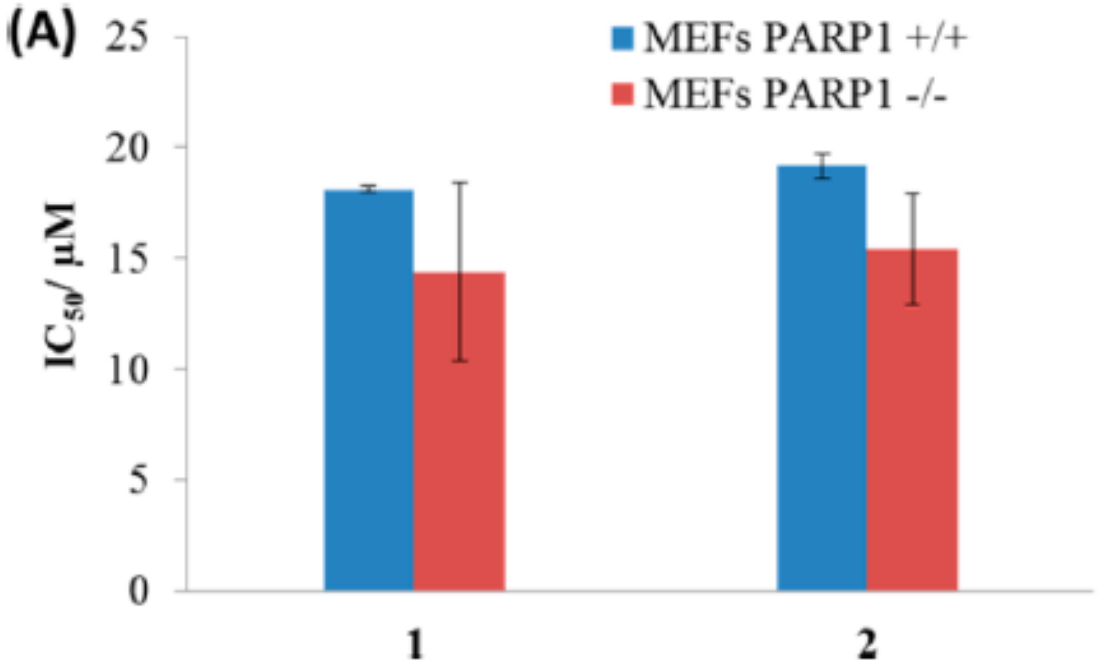
Orange = in cells treated with **1** and necrostatin-1

C. ROS levels after treatment with H_2O_2 (1 mM)

D. ROS levels after treatment with shikonin (necroptosis inducer)

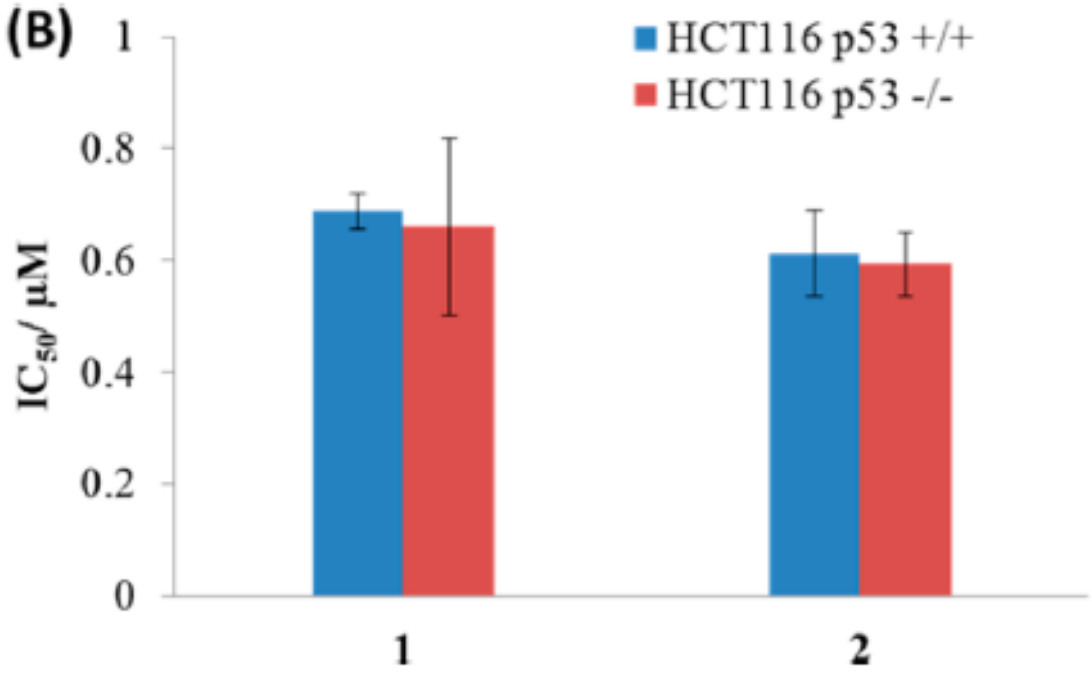
- **1** and **2** induce necroptosis in a necrosome-dependent manner via elevated ROS levels

HOW DO **1** AND **2** INDUCE NECROPTOSIS?



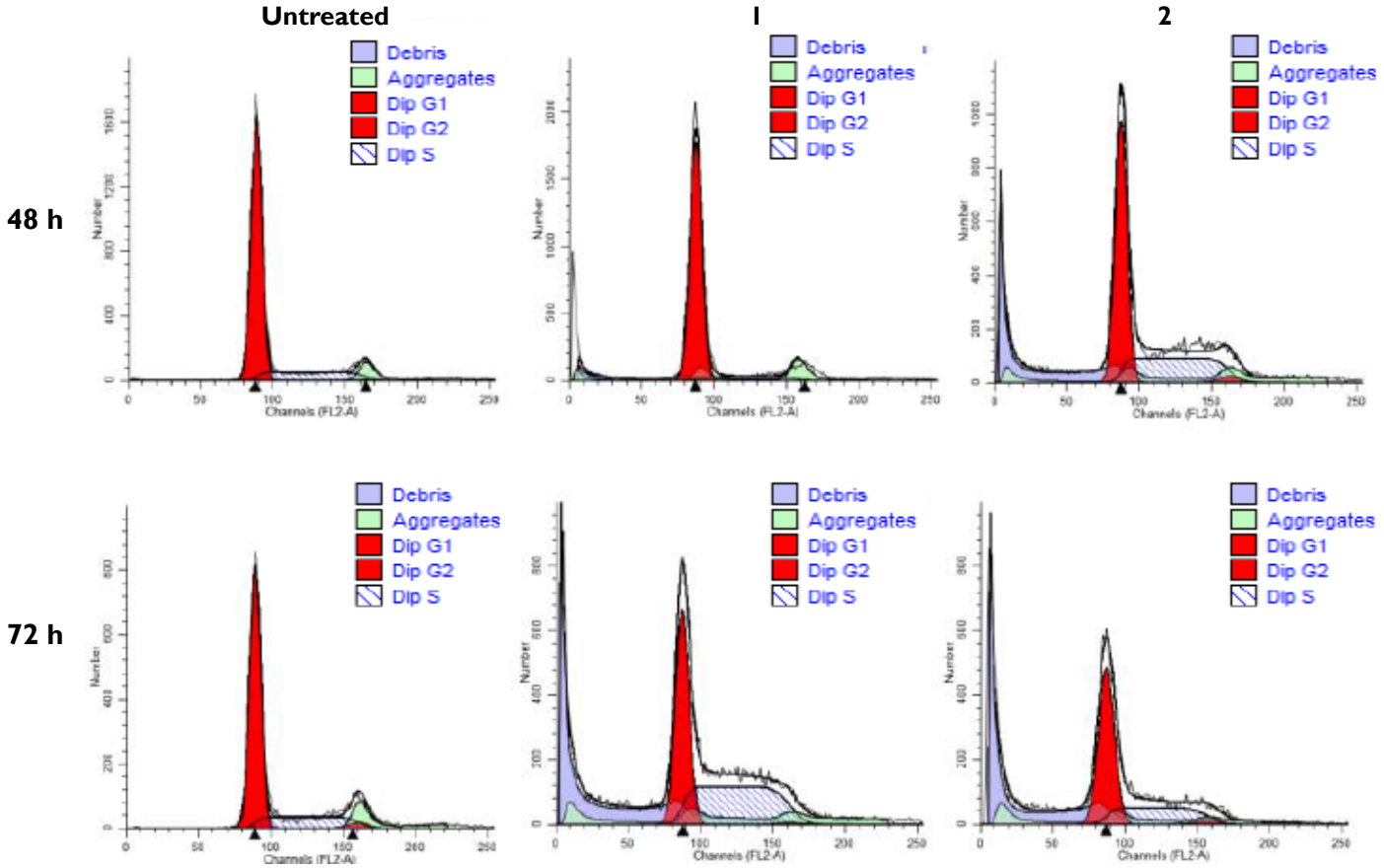
- Supported by immunoblotting which showed no increase in expression of markers of DNA damage
- **1** and **2** mechanism of action is independent of PARP-1

HOW DO **1** AND **2** INDUCE NECROPTOSIS?



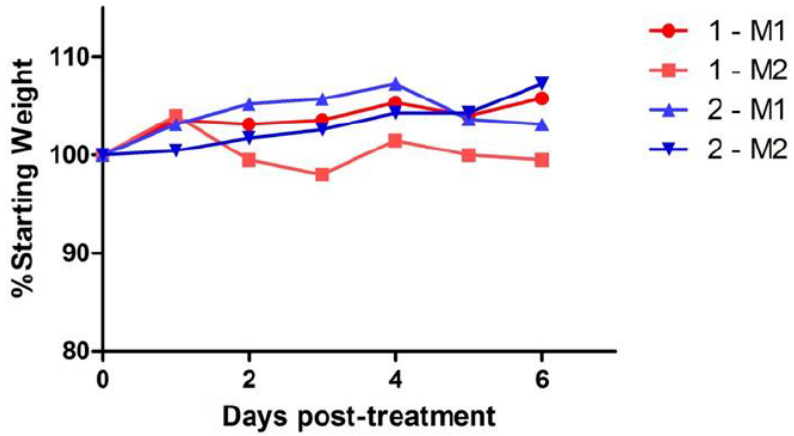
- Supported by RNAi data which show little to no correlation between **1** and **2** activity and p53 status
- **1** and **2** mechanism of action is independent of p53

EFFECTS OF 1 AND 2 ON THE CELL CYCLE



- Treated cells become stalled in G1 and then a large amount of cellular debris is seen indicating cell death (pattern characteristic of necrotopsis)

IN VIVO TOXICITY AND STABILITY



- Mice injected with up to 36 mg/kg (single dose, IP) and monitored 6 days post injection
- No significant acute toxicity
  - 30 mg/kg Cisplatin causes acute nephrotoxicity
- $t_{1/2}$ in whole human blood = 29.1 min
  - $t_{1/2}$ for cisplatin = 21.6 min

CONCLUSION / FUTURE WORK

- Compounds **1** and **2** selectively killed cancer cells (including cisplatin resistant cell lines) over normal cells
  - With greater potency over cisplatin (up to nearly 350x improved potency in some cases)
- **1** and **2** appear to induce cell death via a novel mechanism of action – necroptosis
  - Via necrosome activity, increased ROS generation, G1 cell cycle arrest, and cell membrane disruption
- Potentially very useful due to no cross resistance between apoptosis inducing agents and **1** and **2** for the treatment of chemoresistant cancers
- What is the cellular target of compounds **1** and **2**?
- In vivo activity?