SELECTED OPPORTUNITIES IN ONCOLOGY

TRIARYLPYRIDINE COMPOUNDS AND USE THEREOF FOR TREATING CANCER (BIO 20164)

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Product factsheet

In vitro PoC

- **Target:**
  - G-quadruplex DNA
- **Product:**
  - Tested: new bis-triazole 2,4,6-triarylpyridines
- **Application:**
  - Cancer
- **Rational:**
  - Lysosomal drug sequestration is widely considered to be an important mechanism of resistance to cancer chemotherapy. However, little is know regarding the possible lysosomal drug sequestration of G-quadruplex DNA ligands which have interesting antiproliferative activity
- **POC:**
  - The lead compound within this family, **20A**, accumulates within the lysosomes and promotes their enlargment
  - The new bis-triazole 2,4,6-triarylpyridines are able to induce cancer cells death either as a standalone or, synergistically, in combination with a lysosomotropic agent, such as chloroquine
  - The compounds are active against a variety of cancer cells such as HeLa (cervical cancer cell), A549 (lung carcinoma), and PDX-2 or PDX-3 (lung adenocarcinoma)
- **Patent and publication:**
  - *Triarylpyridine Compounds and Chloroquine Act in Concert to Trigger Lysosomal Membrane Permeabilization and Cell Death in Cancer Cells*. Jennifer Beauvarlet, Rabindra Nath Das, Karla Alvarez-Valadez, Isabelle Martins, Alexandra Muller, Elodie Darbo, Elodie Richard, Pierre Soubeyran, Guido Kroemer, Jean Guillon, Jean-Louis Mergny, Mojgan Djavaheri-Mergny. **Cancers (Basel).** 2020 Jun 18;12(6):1621.
  - EP20305567: TRIARYLPYRIDINE COMPOUNDS AND USE THEREOF FOR TREATING CANCER

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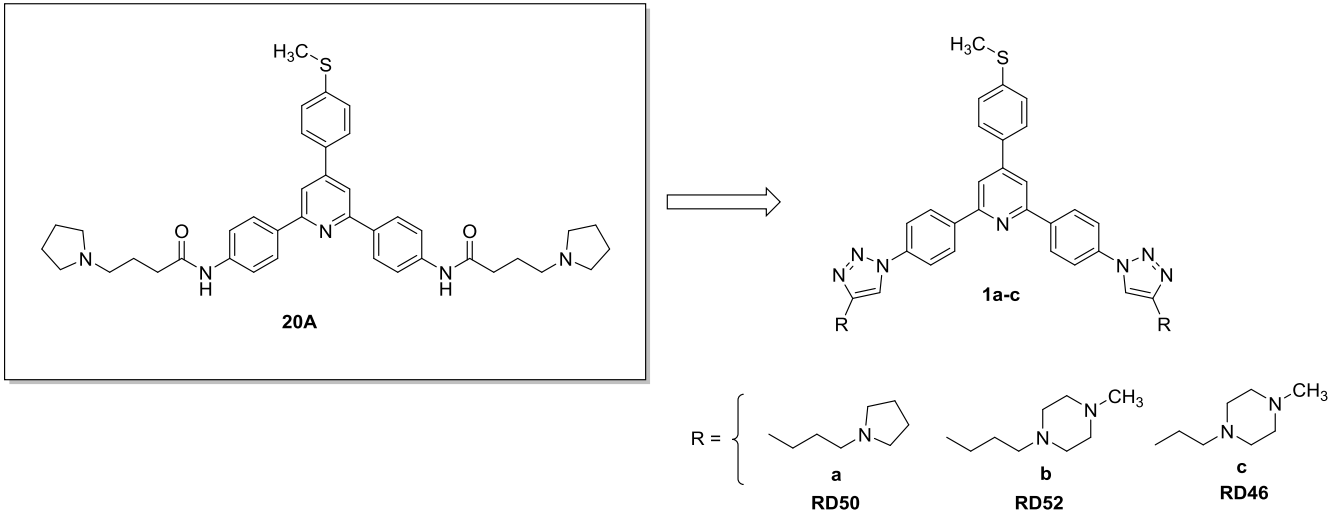
Proof of concept

Structure of new bis-triazole 2,4,6-triarylpyridines



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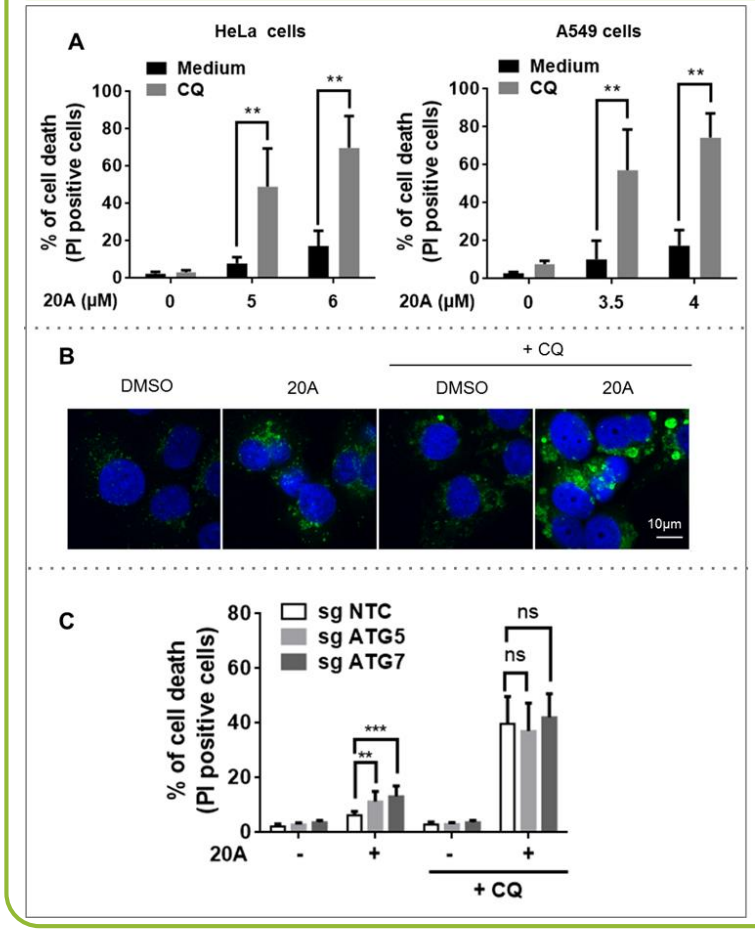
Proof of concept

20A and chloroquine act in concert to trigger enlarged lysosomes associated with a robust cell death



A) HeLa cells (left) and A549 cells (right) were treated with the indicated concentration of **20A** with or without 25 µM of chloroquine for 24h. Cell death was evaluated by scoring the percentage of propidium iodide (PI) -positive cells after flow cytometer analysis. The data represents the mean ± SD of 6 values obtained from three independent experiments each performed in duplicate. ** *p-value* < 0.01 using Mann-Whitney test.
B) Galectin3-mCherry expressing U2OS cells were treated or not with 3 µM **20A**, either the presence or absence of 25 µM chloroquine for 24h and then immunostained for LAMP1. Representative z-projection of merge confocal images of nuclei (blue signal) and LAMP1 (green signal), scale bar 10 µm.
C) Autophagy-proficient (NTC) and -deficient (ATG5 and ATG7 KO) HeLa cells were treated with 5 µM **20A** either in the presence (25 µM) or absence of chloroquine for 24h. Cell death was evaluated by scoring the percentage of PI-positive cells after flow cytometer analysis. The data represents the mean ± SD of 9 values obtained from three independent experiments each performed in triplicate. *P-value* ** < 0.01 and *** < 0.001, ns = non significant using Mann-Whitney test.

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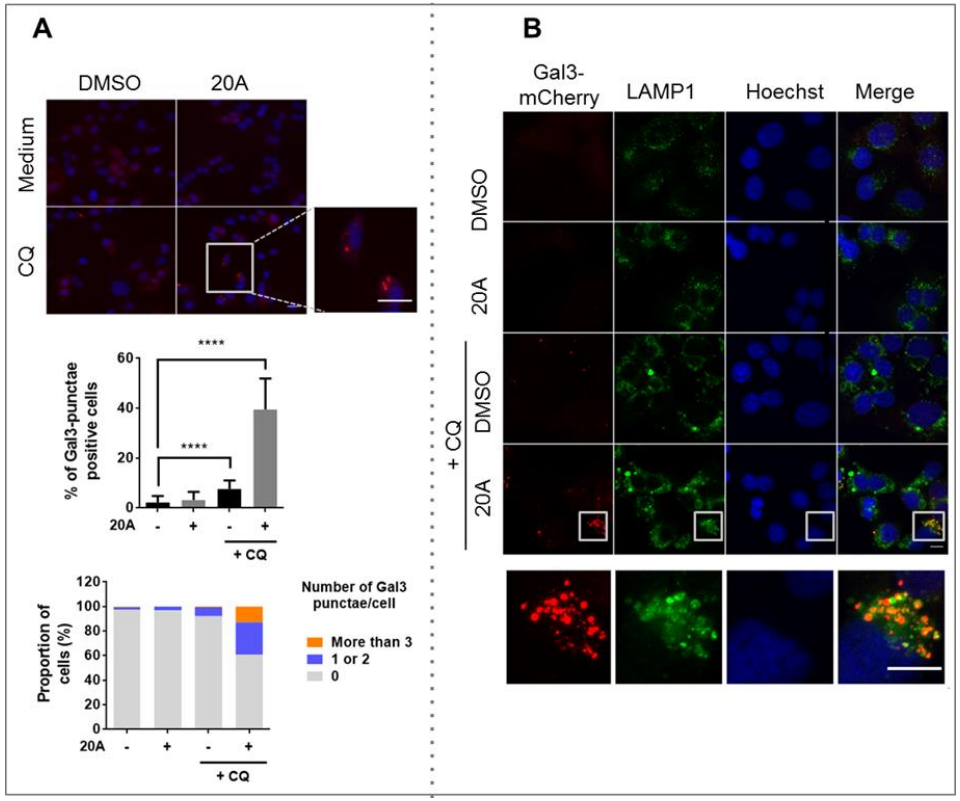
Proof of concept

20A and chloroquine act in concert to trigger LMP in cancer cells



A) Top, Galectin3-mcherry expressing U2OS cells were treated or not with 3 µM 20A in either the presence or absence of 25 µM chloroquine for 24h. Representative epifluorescence of merge images are presented with Galectin 3 (red signal) and Hoechst (blue signal), scale bar 25 µm. Middle, Percentage of cells displaying at least one Galectin 3 punctae are scored. Data are presented as mean ± SD of 15 values obtained from 5 randomly chosen fields in each of the three independent experiments. Bottom, The number of Galectin 3 punctae per cell was scored and results are expressed as percentage of cells with indicated Gal-3 puncta. Data are presented as the mean percentage of at least 1000 cells analyzed from 5 randomly chosen fields in each of the three independent experiments. **** p-value < 0.0001 using Mann-Whitney test

B) Galectin3-mcherry expressing U2OS cells were treated or not with 3µM 20A either in the presence or absence of 25µM chloroquine for 24h and then immunostained for LAMP1. Representative z-projection of confocal images of Galectin 3 (red signal), nuclei (blue signal) and LAMP1 (green signal), scale bar 10µm. LMP is assessed by colocalization of LAMP1 with Galectin 3 punctae, as observed on the magnified view from box area presented for 20A-treated cells.

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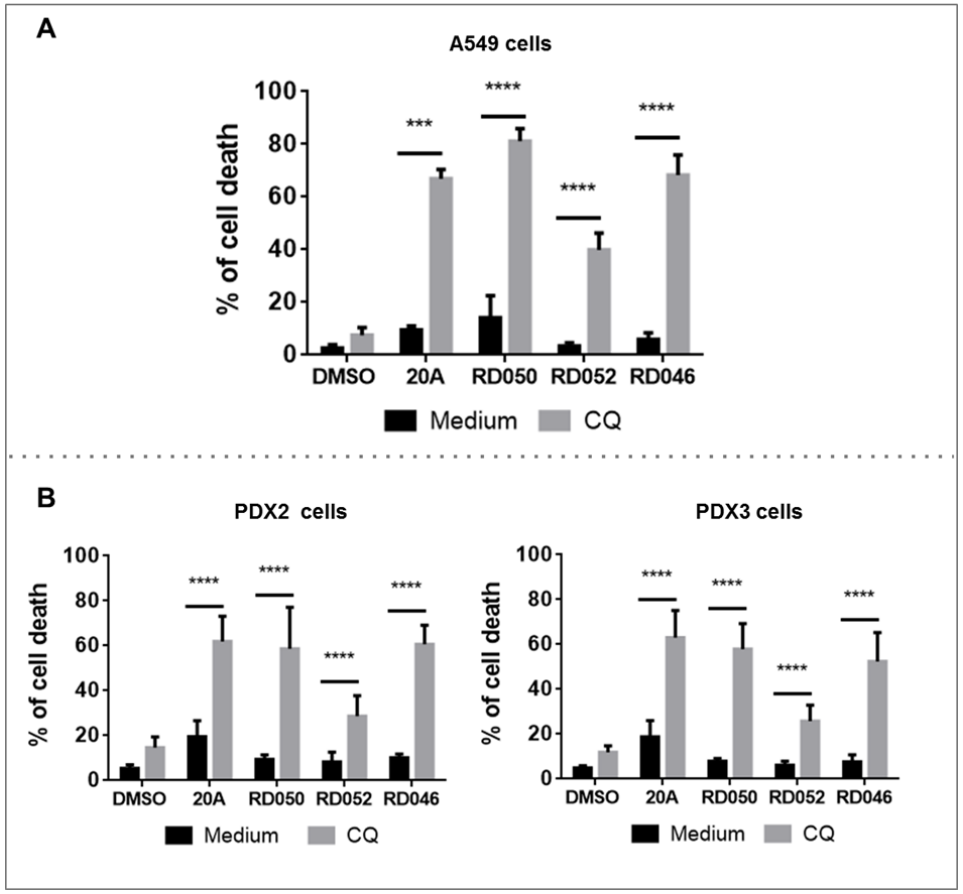
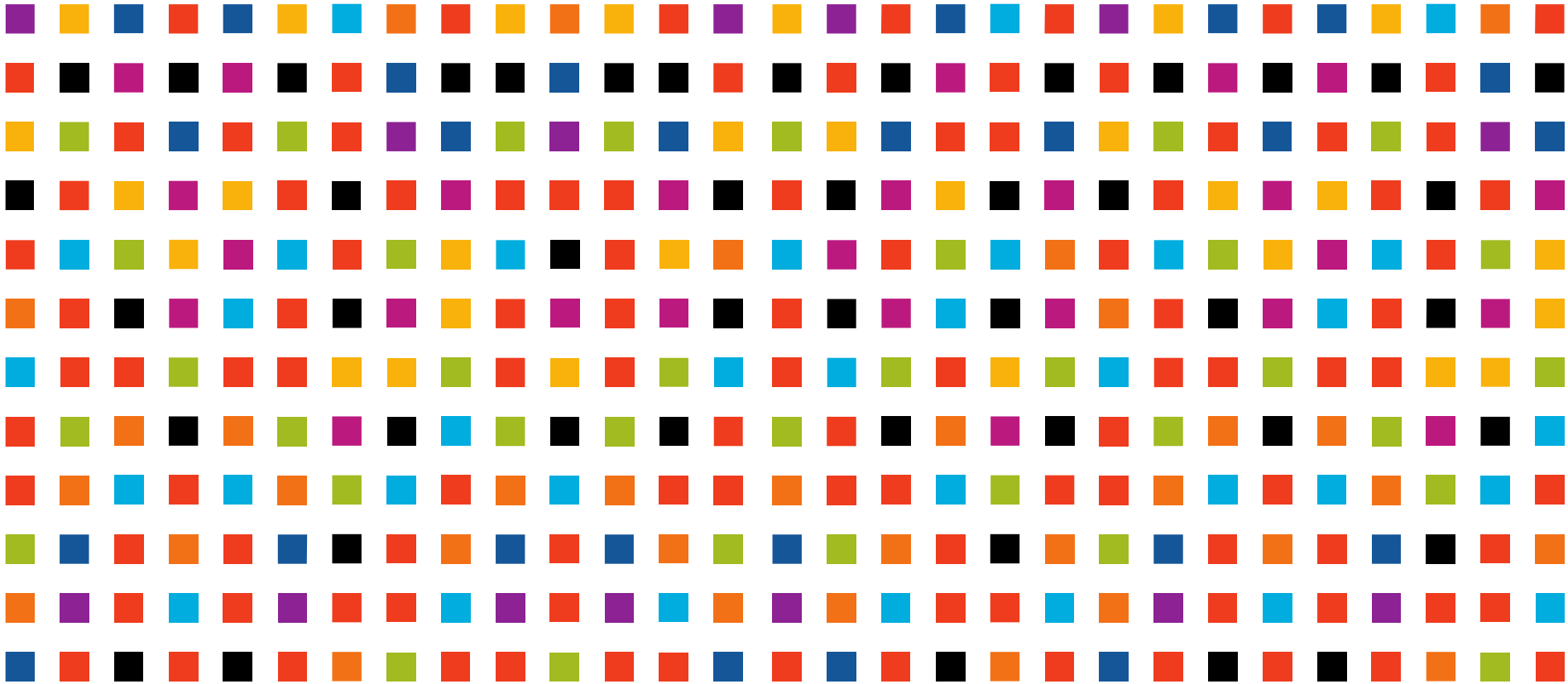
Combined treatment with chloroquine and 20A–derived compounds significantly activates cell death in both A549 lung cancer cells and patient –derived xenograft cell lines from lung cancer



A and B. A549 lung cancer cell lines (A) and two PDX from lung cancer PDX2 (left) and PDX3 (right) (B) were treated with either 3.5 µM 20A, 2.5 µM 1a (RD050), 1.5 µM 1b (RD052) or 2 µM 1c (RD046) in either the presence or absence of 25 µM chloroquine for 24h. Cell death was evaluated by scoring the percentage of PI-positive cells by flow cytometer analysis. The data represents the mean ± SD of 9 values obtained from three independent experiments each performed in triplicate. p-value *** < 0.001 and **** < 0.0001 using Mann-Whitney test.



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