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The potential of novel Benzo[*a*]phenoxazine derivatives for colorectal cancer treatment

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UNIVERSIDADE DO MINHO

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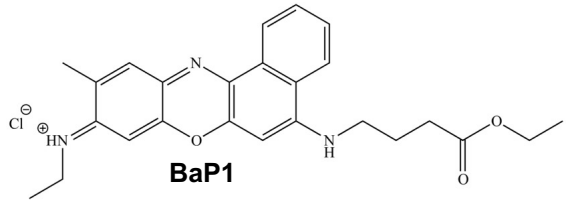
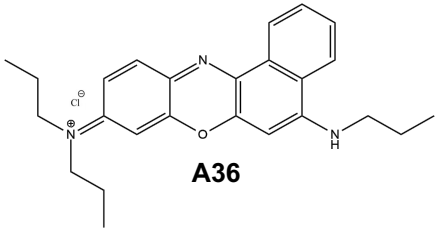
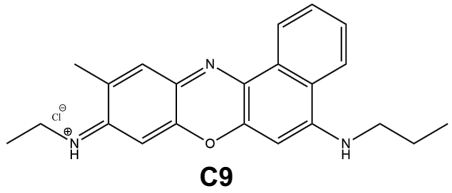
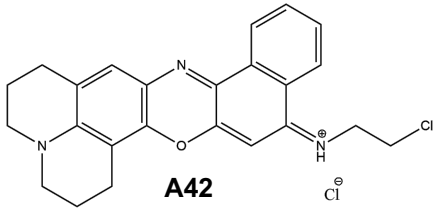








Anticancer activity

Abstract:

Cancer is expected to rank as the leading cause of death and the most important barrier to increase life expectancy in the 21st century. Colorectal cancer (CRC) has been ranked as one of the most incident cancer types and one of the most mortal.

Overall, the number of effective anti-cancer agents approved for use in humans is still very limited. Moreover, tumor resistance and secondary effects stemming from classical chemotherapy remain a major clinical problem, reinforcing the need for the development of novel drugs.

In the recent years, benzo[*a*]phenoxazines derivatives have shown to possess anticancer activity, which has created interest in exploring the potential of these compounds as anticancer drugs. We have recently synthetized and evaluated the biological activity of an array of new benzo[*a*]phenoxazines and demonstrated that they display a varied antiproliferative activity against *Saccharomyces cerevisiae*.

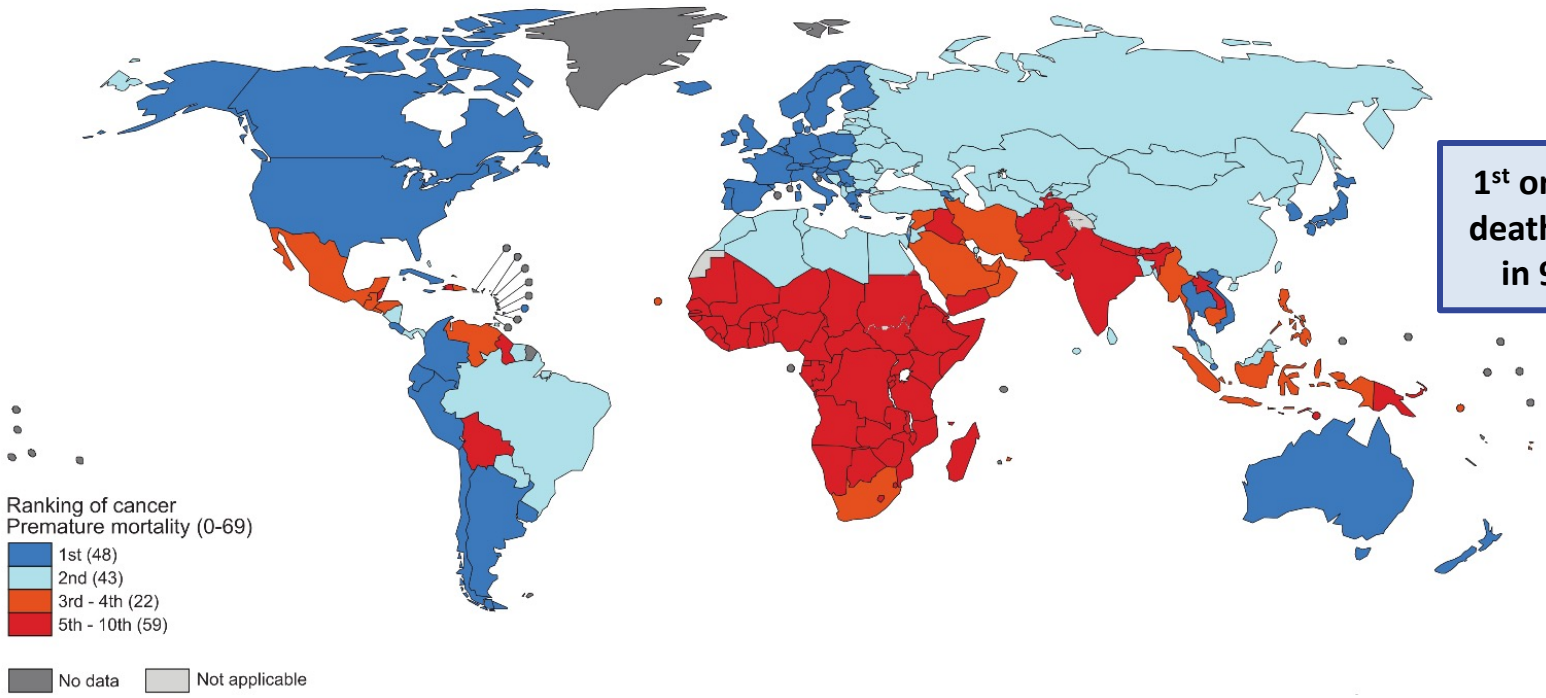
In the present study, we selected four of our most active compounds and evaluated their anticancer activity in Colorectal Cancer (CRC) cells. Our results showed that all compounds had a more toxic effect for CRC cell lines compared to non-tumor cell lines**.** We detected that the compounds accumulated on the lysosomes and induced lysosomal membrane permeabilization (LMP) that resulted cytosolic acidification and apoptotic cell death in CRC cells. These observations highlight compounds of this class as a promising candidates to be explored as new anticancer targeted agents for CRC treatment, using LMP as a novel cancer therapeutic approach.

Keywords: Colorectal Cancer, Anticancer Drugs, Benzo[*a*]phenoxazines, Nile Blue

State of art

Cancer Incidences

Leading cause of death and the most important barrier to increasing life expectancy in the 21st century



1st or 2nd leading cause of death before age 70 years in 91 of 172 countries

State of art

Anticancer Drugs

Cytotoxic agents

Antimetabolites — Structural analogues of pyrimidine or purine - disrupt nucleic acids synthesis;

DNA interactive agents — Interact directly with DNA (alkylating agents, cross-linking agents, intercalating agents, topoisomerase inhibitors and DNA-cleaving agents)

Antitubulin agents — Interfere with microtubule dynamics (taxanes and vinca alkaloids)

Act on both tumor cells and healthy cells

Hair loss;
Nausea;
Bone marrow suppression;

Gastrointestinal tract lesions;
Development of clinical resistance;

State of art

New era of anticancer Drugs

Targeted molecular therapies

- **Monoclonal antibodies**
- **Oral targeted tyrosine kinase inhibitors**

Substantial benefits for patients. Primary or secondary drug resistance and drug adverse effects still limit their use.

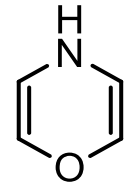
Extremely expensive

Monthly treatments costing $6,800–10,300
Durations of treatment >12 months

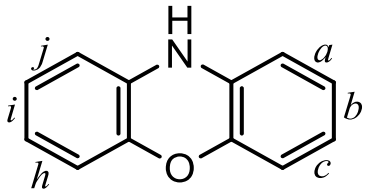
State of art

Benzo[*a*]phenoxazines

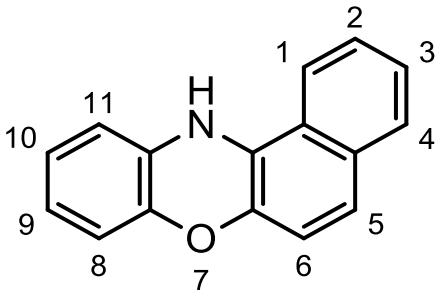
Structure and Nomenclature



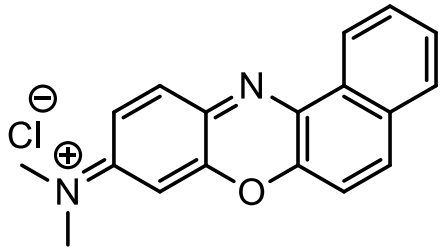
Oxazine



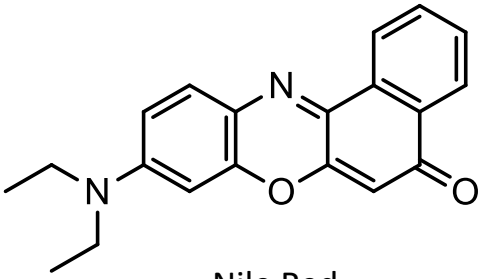
Phenoxazine



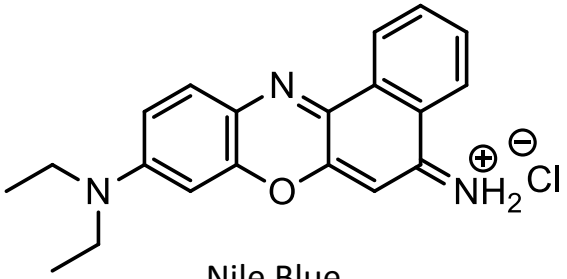
Benzo[*a*]phenoxazine



Meldola's Blue



Nile Red



Nile Blue

Known Benzo[*a*]phenoxazines

State of art

Benzo[*a*]phenoxazines

Applications

They have been mainly used as **Florescent Probes**

Common uses

- Labeling of lipids
- Labeling of nucleic acids
- Blotting experiments
- Gel electrophoresis
- Living cell assays

- **Nile Red**: Lipid droplets, membrane systems
- **Nile Blue**: peptides, DNA, tumor cells

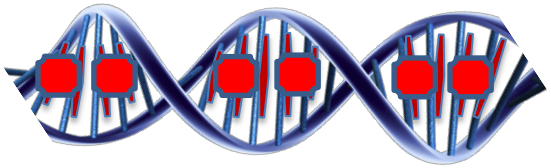
State of art

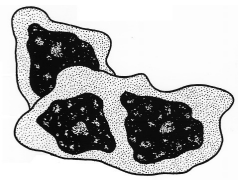
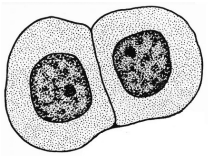
Benzo[*a*]phenoxazines

Applications → Possess **Antiproliferative Properties**

They exert their antiproliferative effect → Metabolic conversion → Oxidative Stress

Actin on DNA, through the intercalation of their planar structure between the DNA base pairs



Cancer cell	Normal cell
	

Higher accumulation
Higher toxicity

Aims

Objective

Explore the biological activity of new benzo[*a*]phenoxazine derivatives and assess their potential application as anti-cancer agents.

Uncover compounds with potential for therapeutic application

Results and Discussion

Evaluation of the potential anti-cancer activity of BaP1, C9, A36 and A42 in human cells

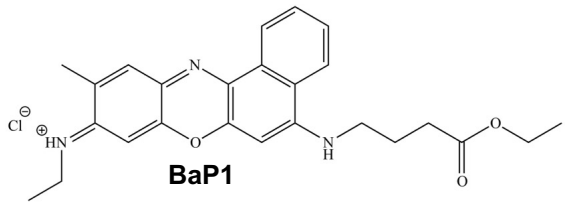
BaP1 C9, A36 and A42

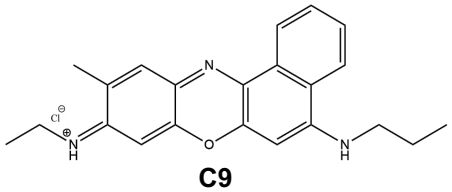
Normal derived cell line:
NCM460

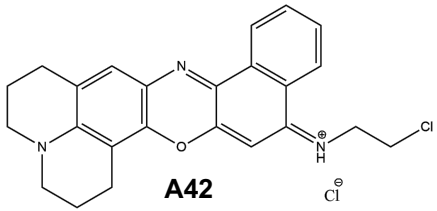
Effect

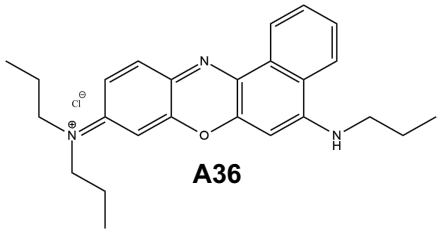
Colorectal cancer cells:
RKO, Sw480 and Hct116

IC50 - Sulforhodamine B



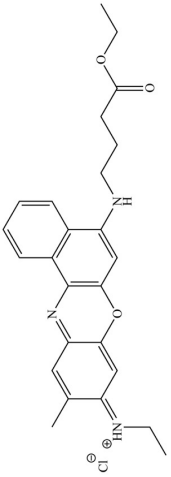






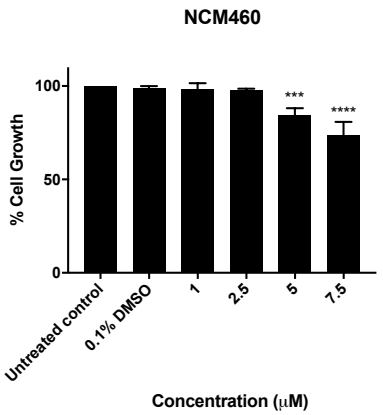
Results and Discussion

Effect on "normal" and cancer cells

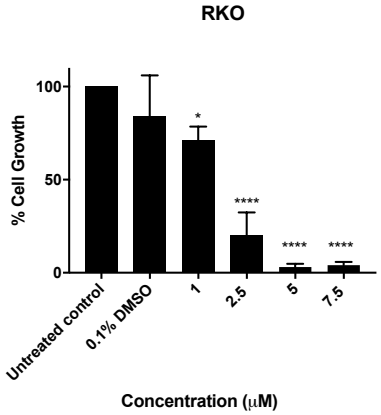


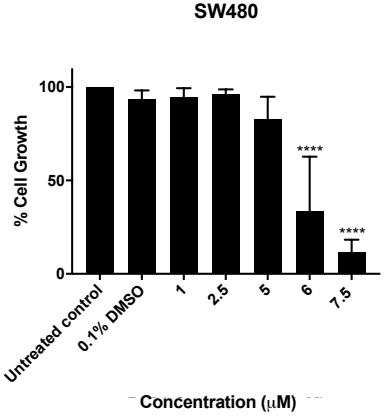
BaP1

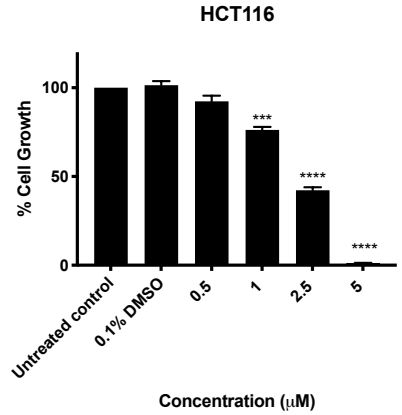
Normal derived cell line

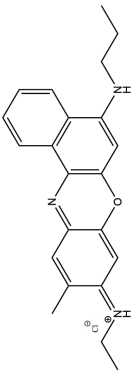


Cancer cell lines

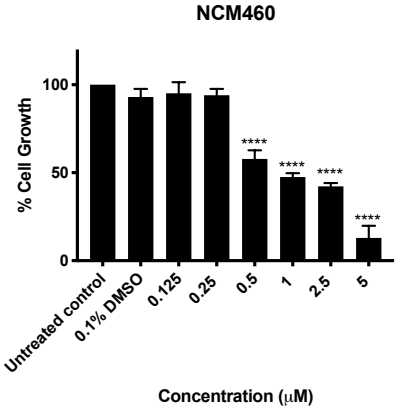


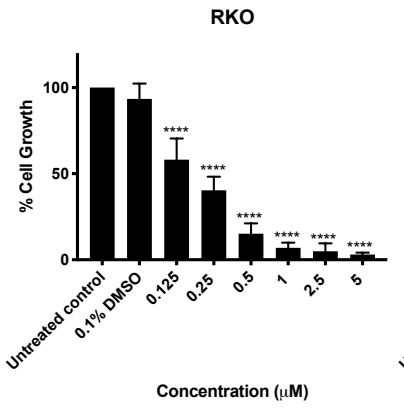


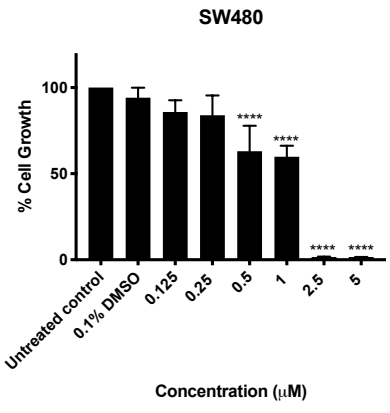


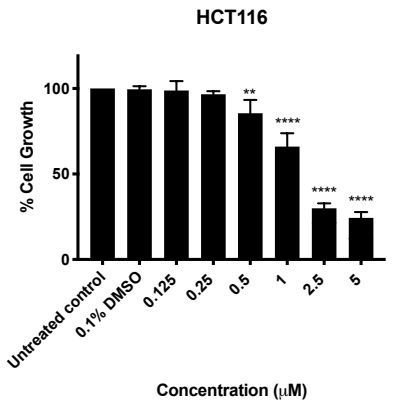


C9







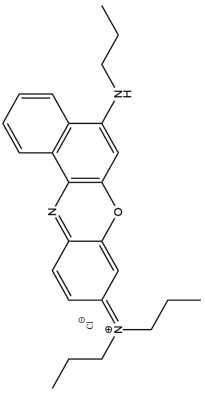


Results and Discussion

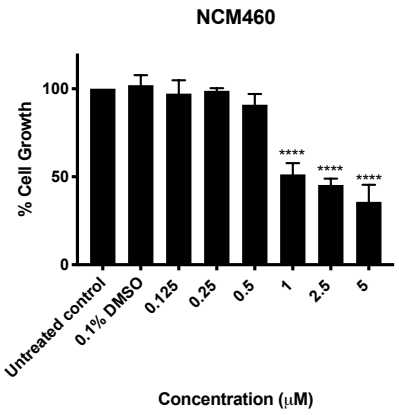
Effect on "normal" and cancer cells

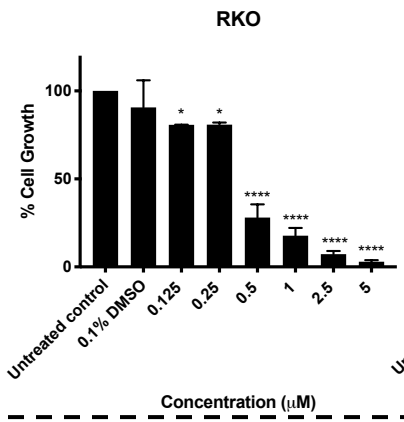
Normal derived cell line

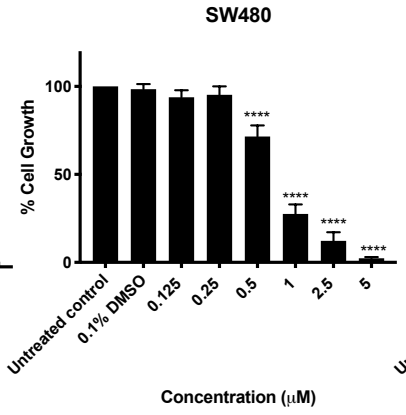
Cancer cell lines

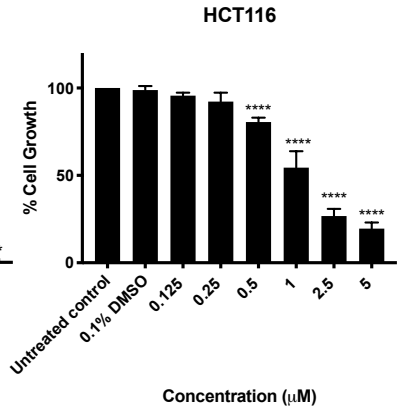


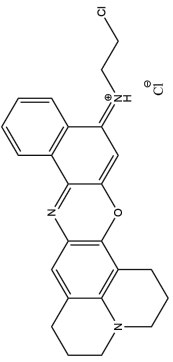
A36



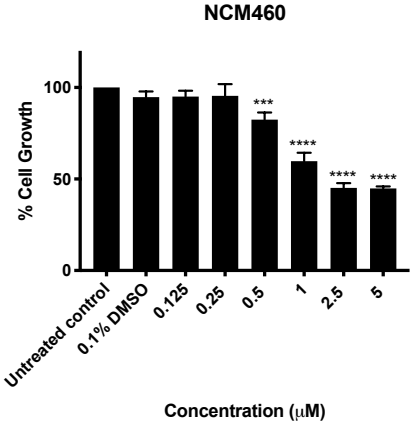


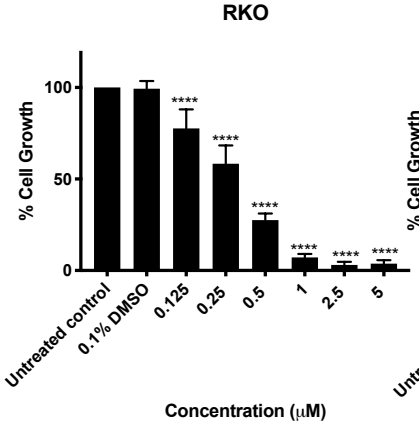


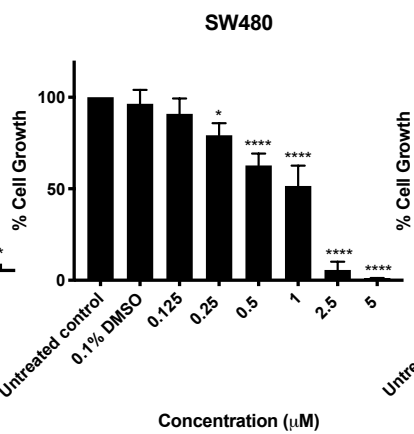


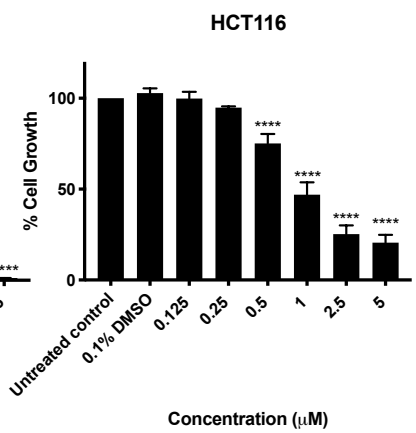


A42









Results and Discussion

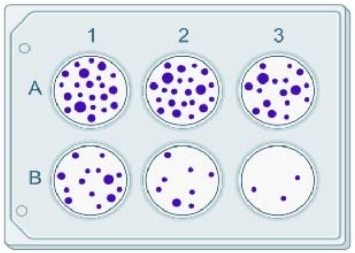
IC50 and Selectivity Index determination
BaP1, C9, A36, and A42

Cell Lines	BaP1 IC_{50} (μM)	C9 IC_{50} (μM)	A36 IC_{50} (μM)	A42 IC_{50} (μM)
NCM460	**12.80** ± 2.05	**1.12** ± 0.13	**2.06** ± 0.25	**2.59** ± 0.31
SW480	**5.60** ± 0.19	**0.78** ± 0.09	**0.72** ± 0.03	**0.78** ± 0.07
RKO	**1.40** ± 0.08	**0.17** ± 0.01	**0.37** ± 0.03	**0.29** ± 0.01
HCT116	**1.90** ± 0.09	**1.64** ± 0.09	**1.28** ± 0.06	**1.13** ± 0.07

Cell Lines	Selectivity Index (Colon)			
	BaP1	C9	A36	A42
SW480	2.26	1.44	2.86	3.32
RKO	9.14	6.59	5.56	8.93
HCT116	6.74	0.68	1.61	2.29

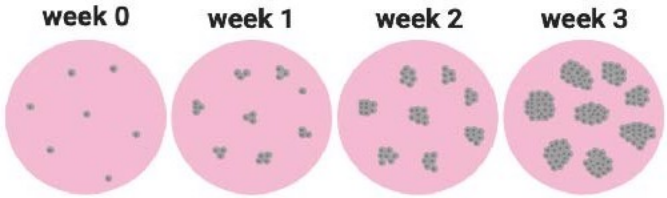
Results and Discussion

Colony formation assay



CFU

Evaluate cellular growth, and the **cytotoxic** or **genotoxic** effects of agents with potential clinical application

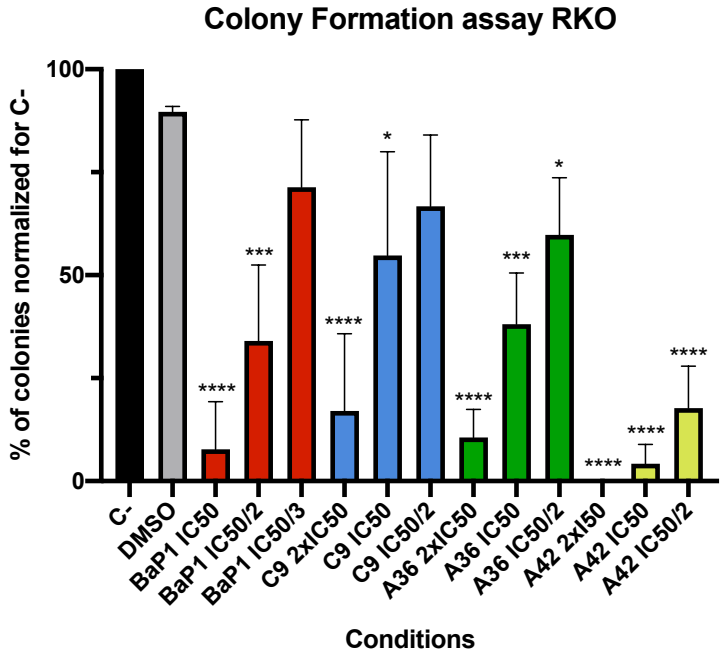


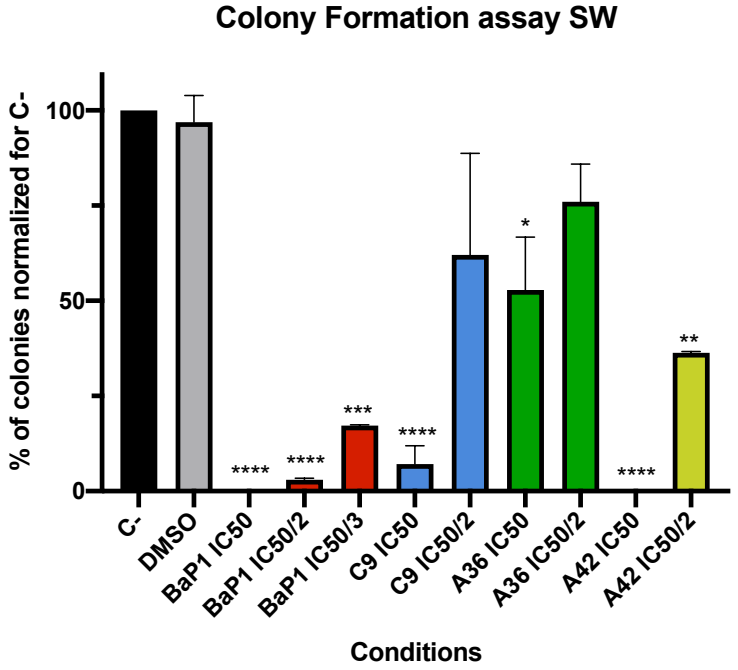
Ability of a single cell to grow into a **colony**

Are the compounds capable to reduce the formation of colonies ?

Results and Discussion

CFU for RKO and SW480



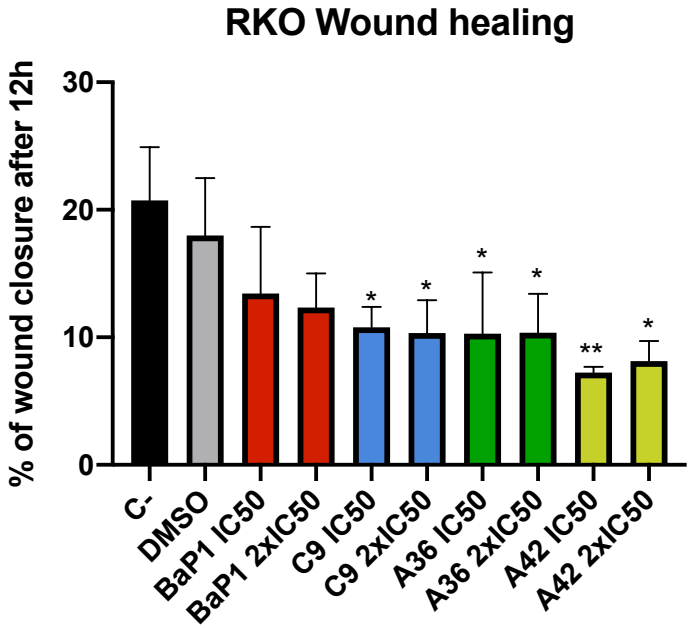


Results and Discussion

Wound healing assay

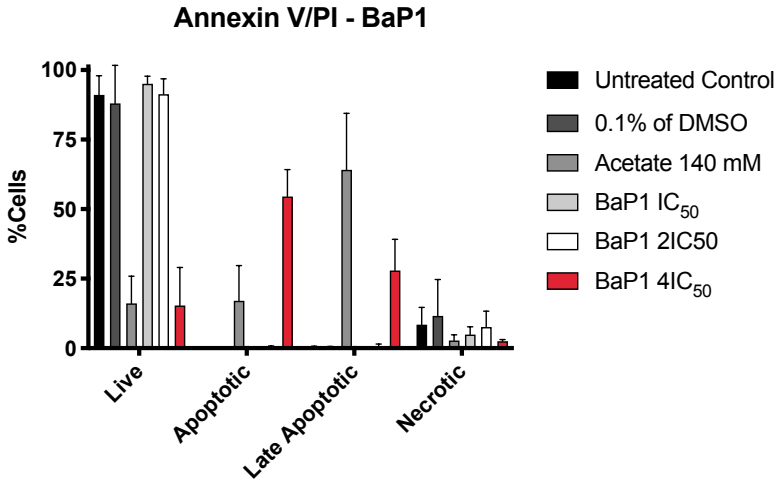
Evaluate the effect of a compound on cell migration

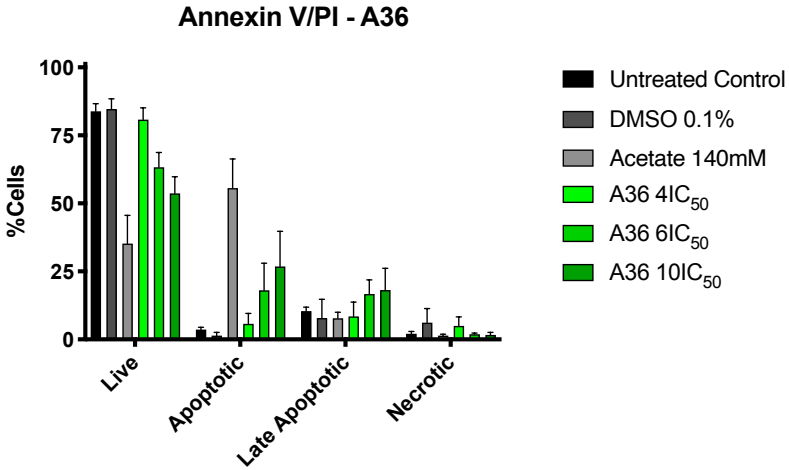
RKO

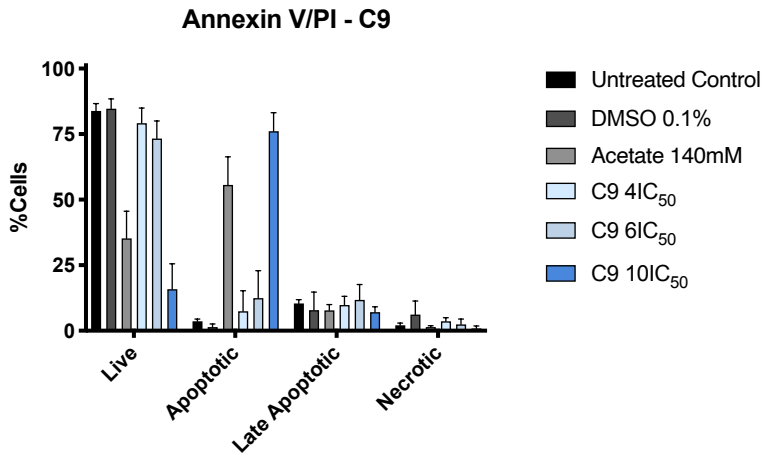


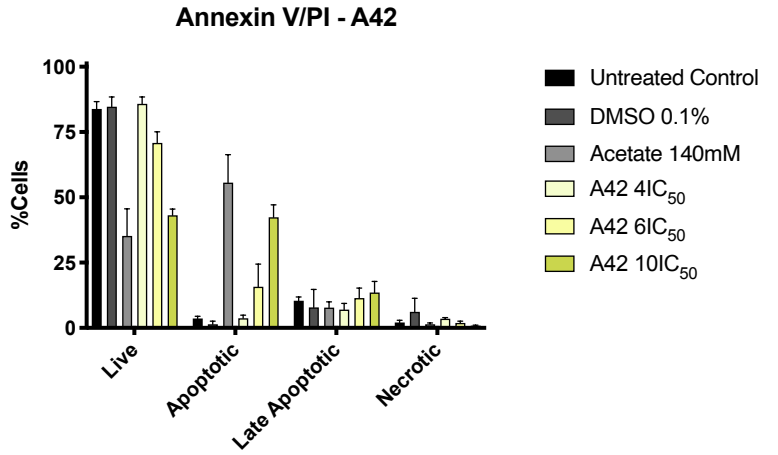
Results and Discussion

Annexin V/PI assay for C9, A36 and A42









Results and Discussion

BaP1 characterization

BaP1 High Selectivity and inhibitory effect on Colon Cancer Cell Lines

Cell Lines	Selectivity Index
SW480	2.26
RKO	9.14
HCT116	6.74

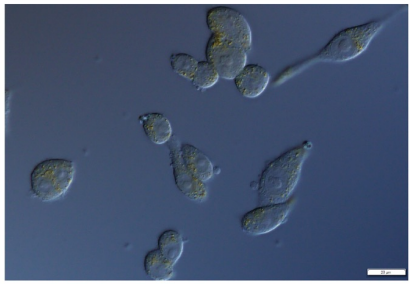
What are the mechanisms behind BaP1 toxicity?

Results and Discussion

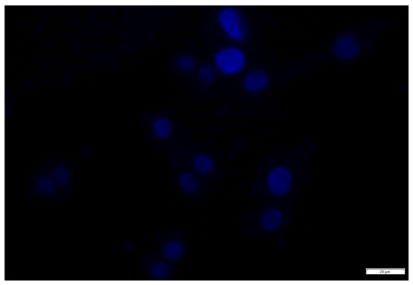
BaP1 lysosome accumulation

Acridine Orange co-stain

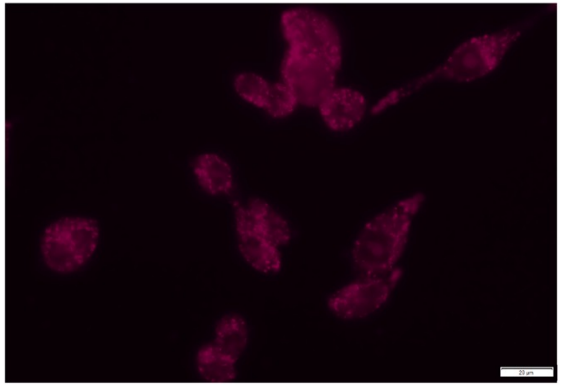
Dic



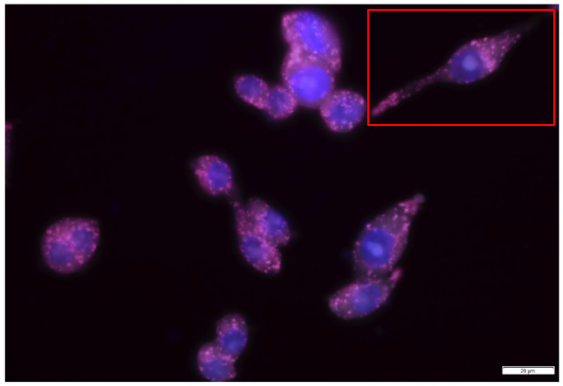
Blue (DAPI)

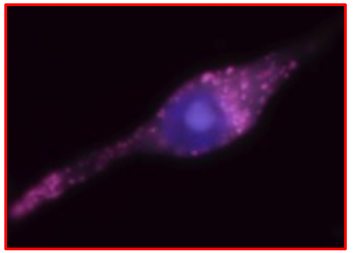


Far Red (BaP1)

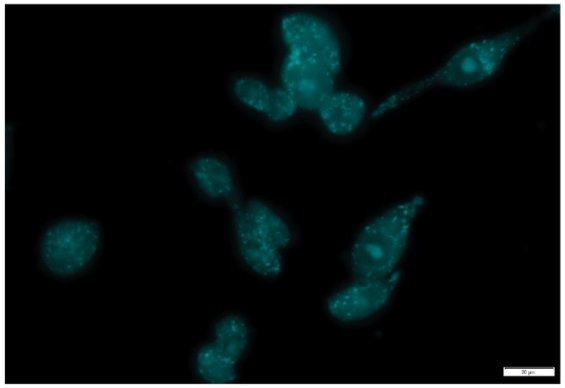


Fluorescence Overlay

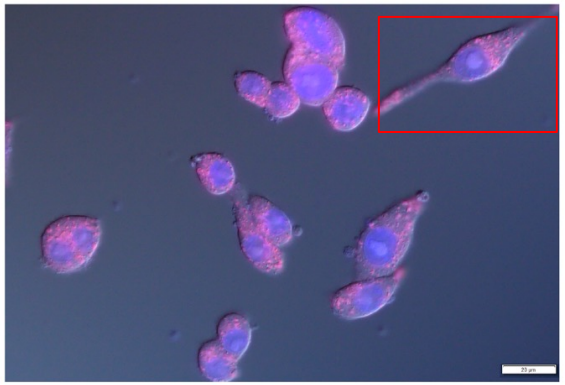




Cyane Blue (AO)



Total Overlay

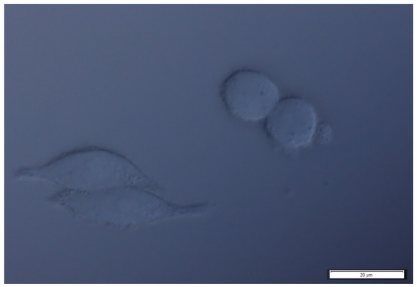


Results and Discussion

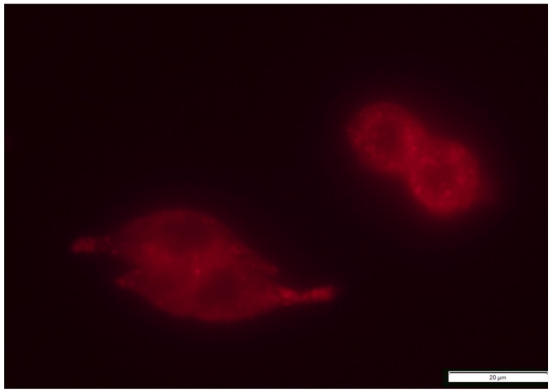
BaP1 lysosome accumulation

LysoTracker Green co-stain

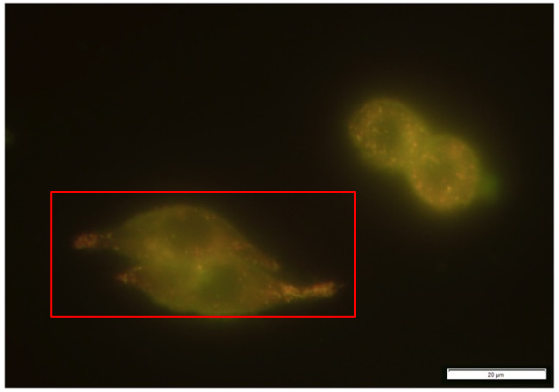
Dic

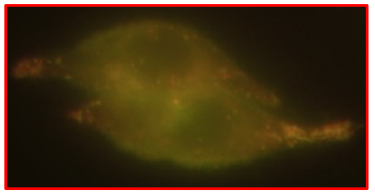


Far Red (BaP1)

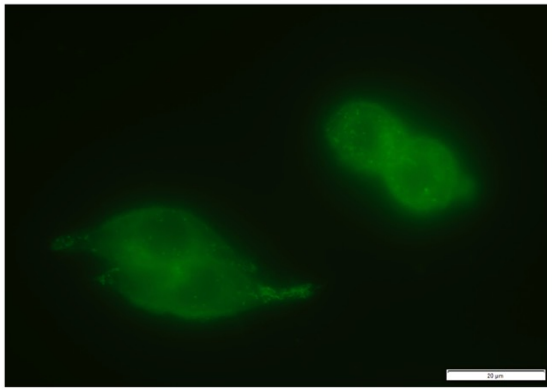


Fluorescence Overlay

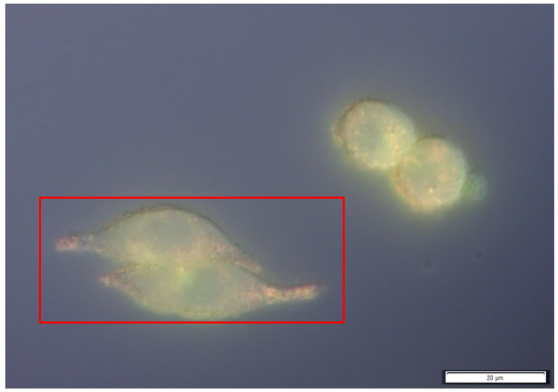


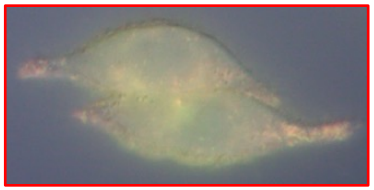


Green (LysoTracker Green)



Total Overlay





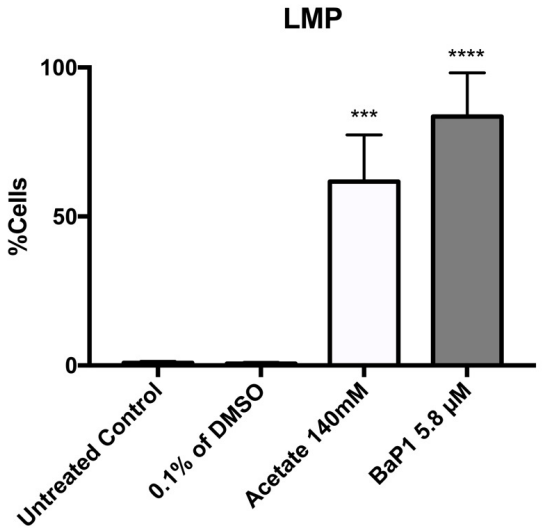
Results and Discussion

Implications of lysosome accumulation

BaP1 → **Acridine Orange**, **Lysotracker Green** → Accumulate and stain the lysosomes

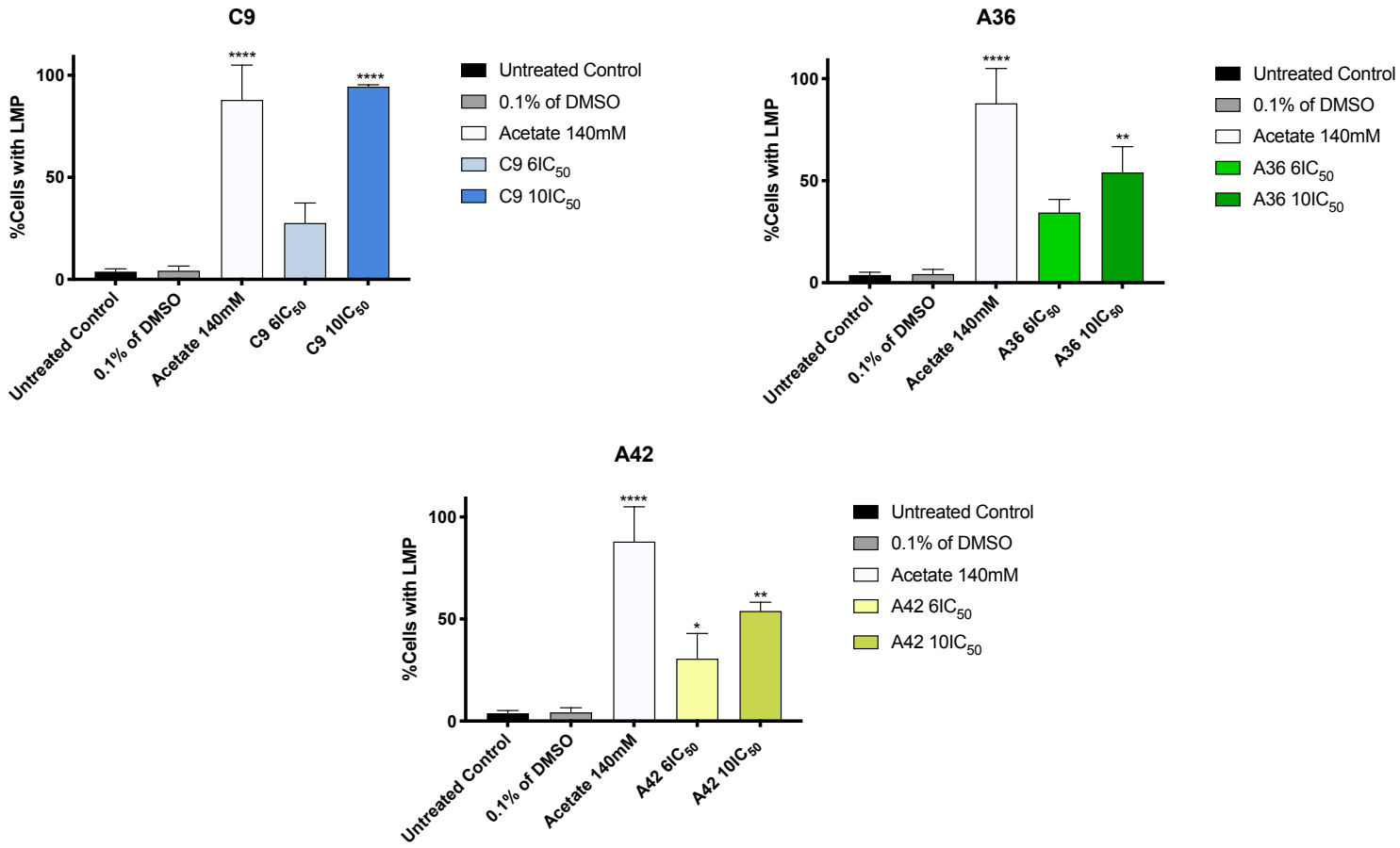
BaP1 induce lysosome membrane permeabilization (LMP) in RKO cell line?

LMP - measure as a decrease in AO red fluoresce



Results and Discussion

C9, A36 and A42 lysosome permeabilization

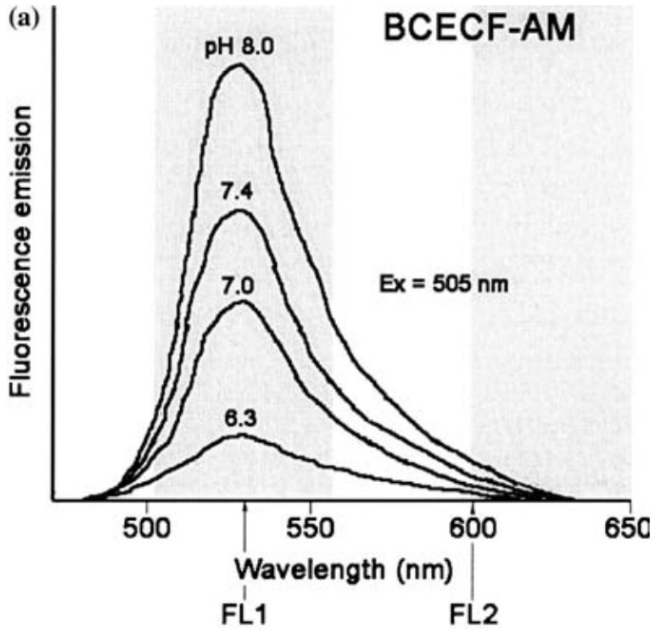


Results and Discussion

Does BaP1 induces cytosolic acidification?

BCECF-AM

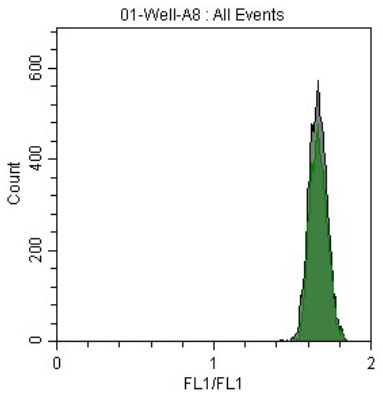
Determine the cytosolic pH decrease by the FL1/FL2 ratio



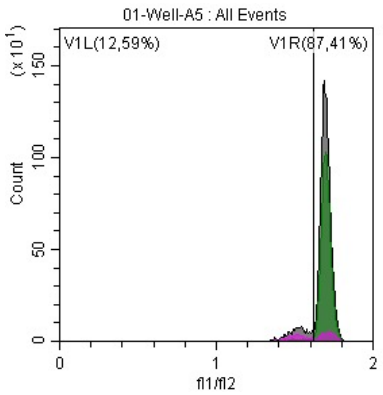
Results and Discussion

Does BaP1 induces cytosolic acidification?

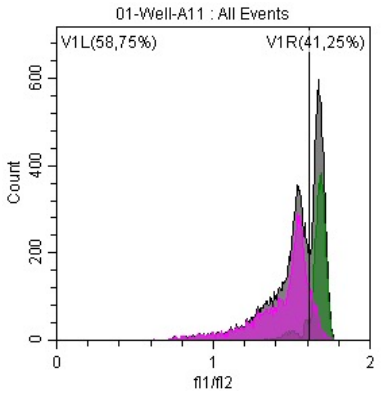
Untreated control



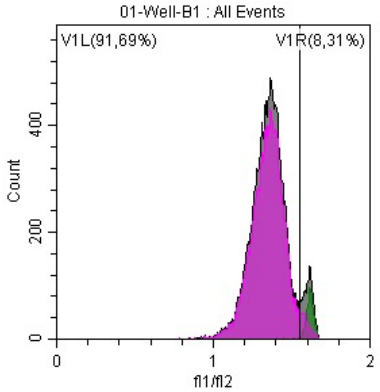
DMSO

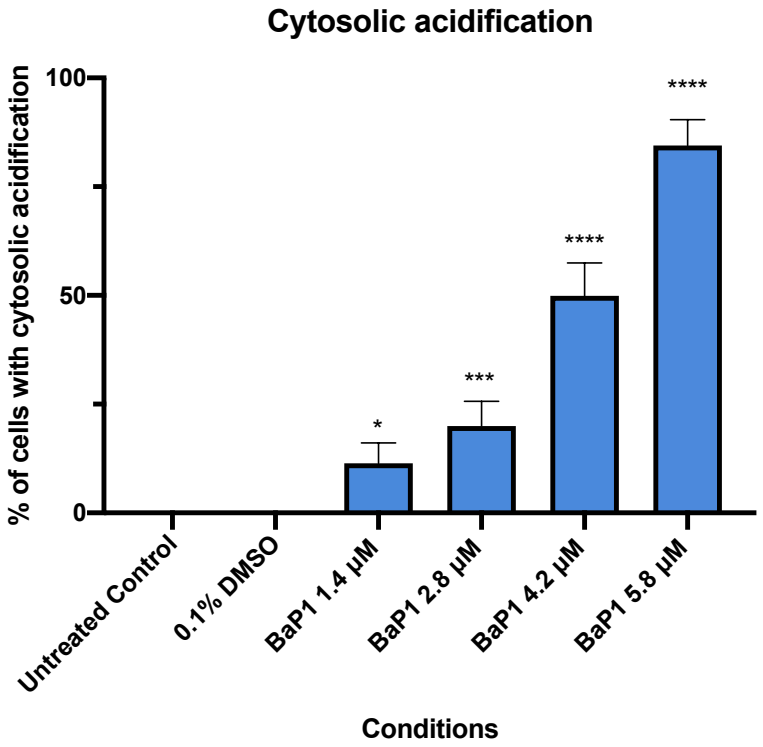


BaP1 4.2 µM



BaP1 5.8 µM





Conclusions

Final Remarks

- BaP1, C9, A36 and A42 reduce cell proliferation in CRC cells (RKO, SW480 and HCT116) having low effect in normal colon cells (NCM460);
- BaP1, C9, A36 and A42 reduce colony formation and wound closing;
- The compounds induce a regulated cell death process of an apoptotic nature in RKO cell line;
- BaP1 accumulates on RKO lysosomes;
- The compounds lysosome accumulation leads to lysosomal membrane permeabilization and cytosolic acidification;

Promising candidates to be exploited as new anticancer targeted agent, using LMP as a therapeutic approach in CRC

Thank you for your attention!

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