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Combination therapy assays with doxorubicin and cathepsin L inhibitors against the triple-negative breast cancer line MDA-MB-231

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

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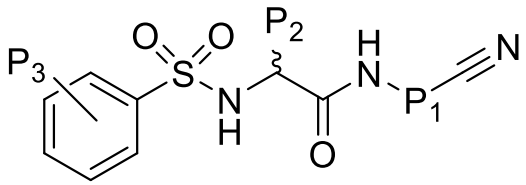
Talita Valdes, Isabela Marques, Andrei Leitão*

Medicinal & Biological Chemistry Group (NEQUIMED)
The São Carlos Institute of Chemistry (IQSC)
University of São Paulo (USP)

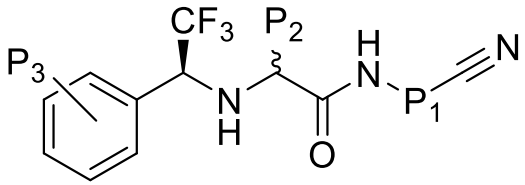
* Corresponding author: andleitao@iqsc.usp.br

Combination therapy assays with doxorubicin and cathepsin L inhibitors against the triple-negative breast cancer line MDA-MB-231

Graphical Abstract

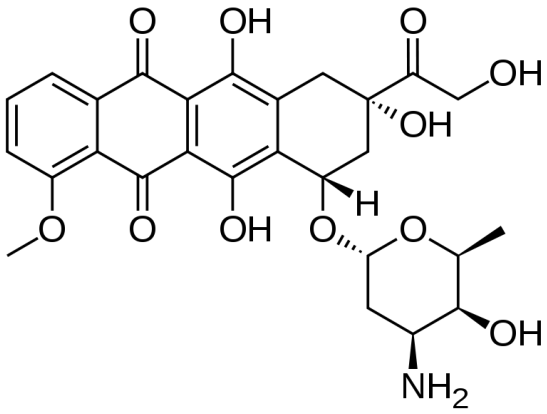


or



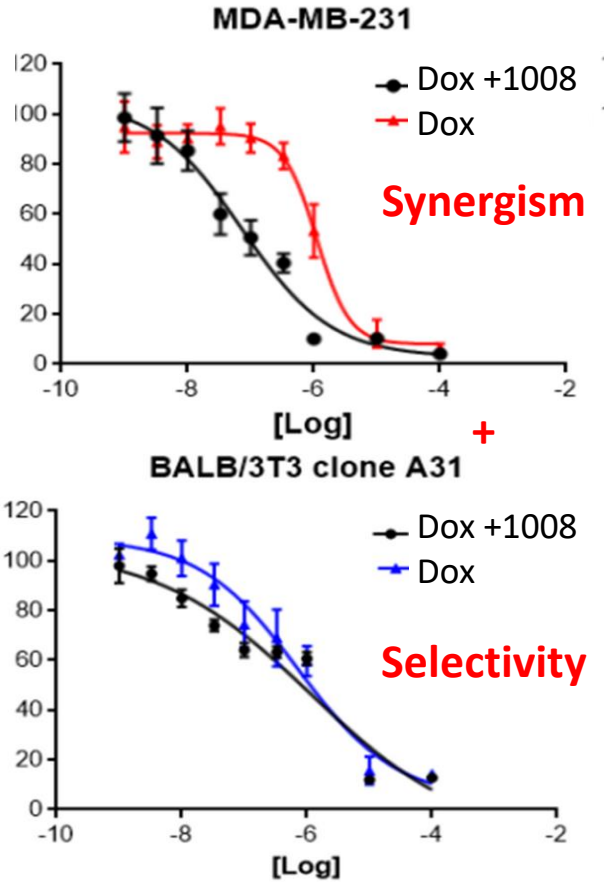
CatL inhibitors

+



Doxorubicin

=



Abstract:
Breast cancer is a worldwide health problem, being one of the most prevalent types of tumors in the female population. Despite the availability of many therapies, including doxorubicin, novel chemotherapeutic approaches are being studied for this disease, focusing on triple-negative breast cancer cells. Cathepsin L is a cysteine protease highly expressed in many tumors, where novel dipeptidyl nitrile inhibitors have been designed and studied over time in our research group. Here, an approach involving the combination therapy of twelve novel cathepsin L inhibitors and doxorubicin was assayed against the triple-negative human breast cancer cell line MDA-MB-231. The cells were cultivated using DMEM medium supplemented with 10% FBS. They were added to 96-plates at a concentration of $1.0x10^5$ cells/well (100 µL/well). After 24 h incubation, the medium was removed to add 10 micromolar cathepsin L inhibitors and a range of doxorubicin concentrations (0.10-1.0 nM). The system was incubated for 72 h, being subject to MTT assay. The Bliss test was used to evaluate the concentration-dependent assay of these chemicals, which led to synergism for many chemicals. The best combination led to almost 8-times higher potency improvement than doxorubicin alone. The SAR was described for the set of dipeptidyl nitriles. It is not yet known how these chemicals could act in combination, and this is the current subject of our efforts to exploit biological mechanisms.

Keywords: cell-based assays; combination therapy; drug discovery; *in vitro* study.

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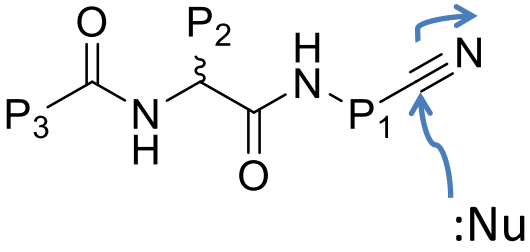
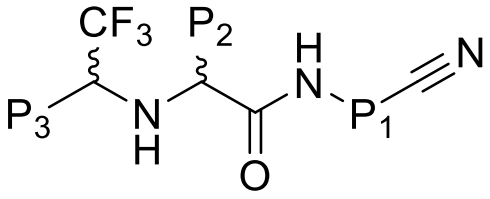
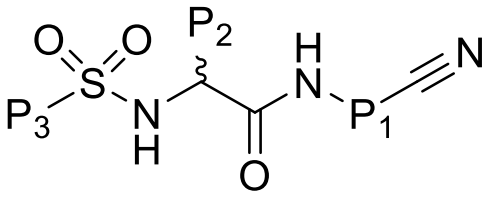
Introduction

Current knowledge

- Cysteine cathepsins are involved in many neoplastic processes *in vivo* (including different types of breast cancers), which make them therapeutic targets of interest for the design and development of novel therapeutic approaches based on dipeptidyl nitrile derivatives.

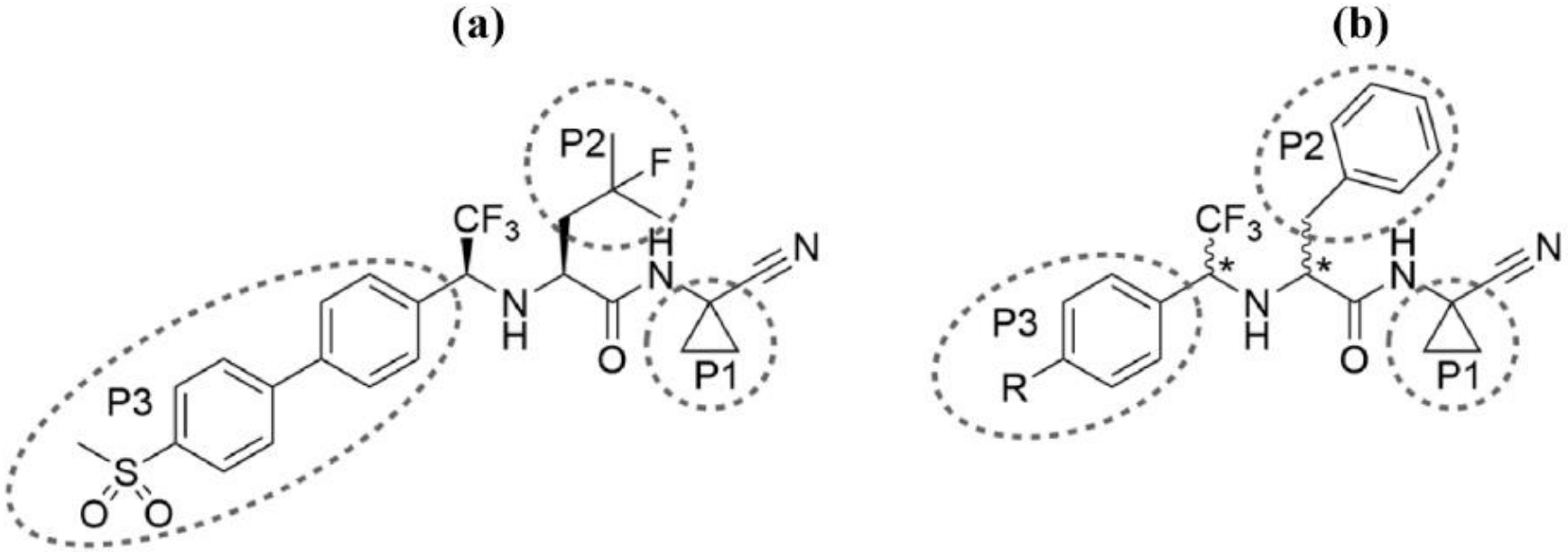






- More than 500 chemicals were synthesized and characterized in our research group, with a potency range from micro to nanomolar against cathepsin L.

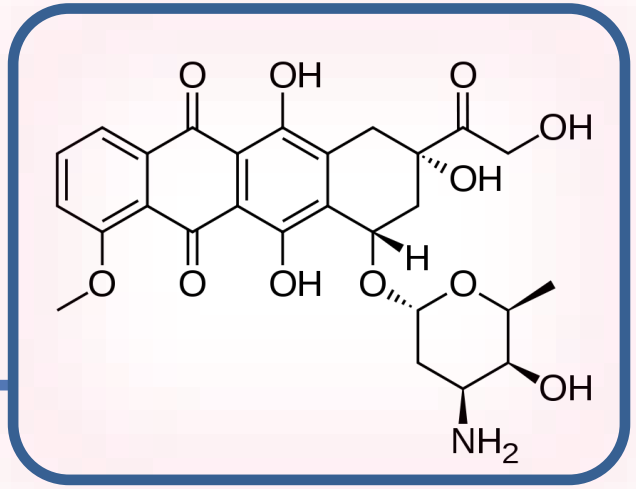
Chemicals based on the odanacatib (a) scaffold simplification (b)



Introduction

The approach

- Previous studies made by us with a series of cathepsin L inhibitors pointed out that they could work as anticancer chemicals against the triple-negative breast cancer cell line (MDA-MB-231) in combination with doxorubicin (unpublished work).



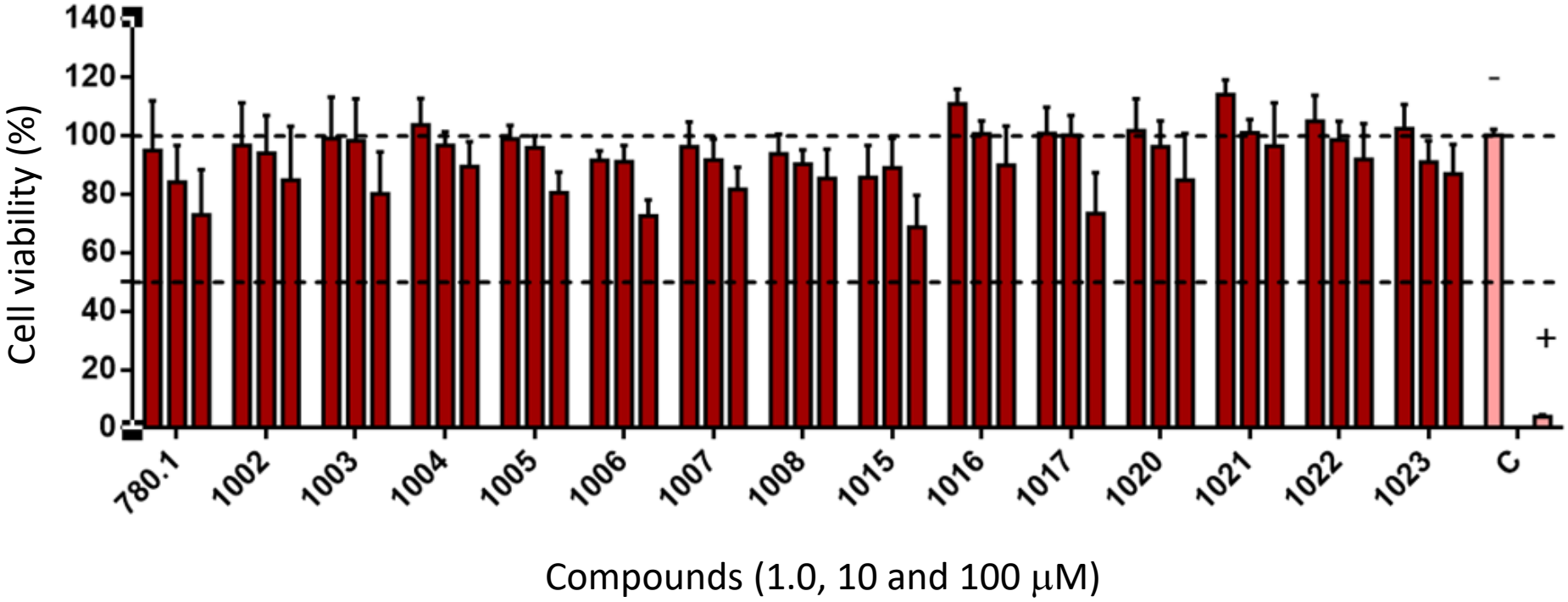
- Therefore, a combination therapy model was developed to evaluate novel cathepsin L inhibitors with doxorubicin.

Materials and methods

- The MTT assay was used in this work
- The cell lines (MDA-MB-231, MCF-7, and Balb/3T3 cloneA31) were cultured in an incubator with 90% humidity and 5% CO_2 atmosphere
- 100 mL of cells at $1.0x10^5$ cells/mL were pipetted in the wells
- After 24 h the medium was replaced by a new one with the samples
- Chemicals were incubated for 72 h
- The supernatant was removed and 1.0 mg/mL MTT solution was added
- The solution was incubated for 3 h
- The readout was made at 570 nm with a plate reader

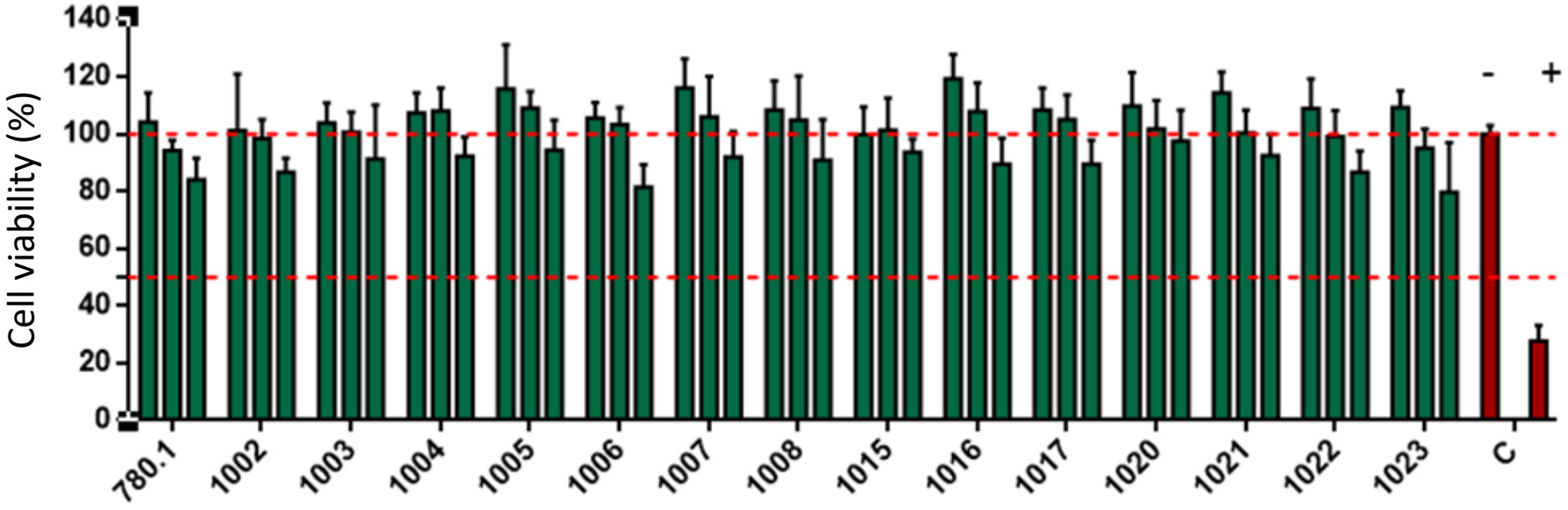
Results and discussion

Initial screening results – new chemicals (MDA-MB-231)



Results and discussion

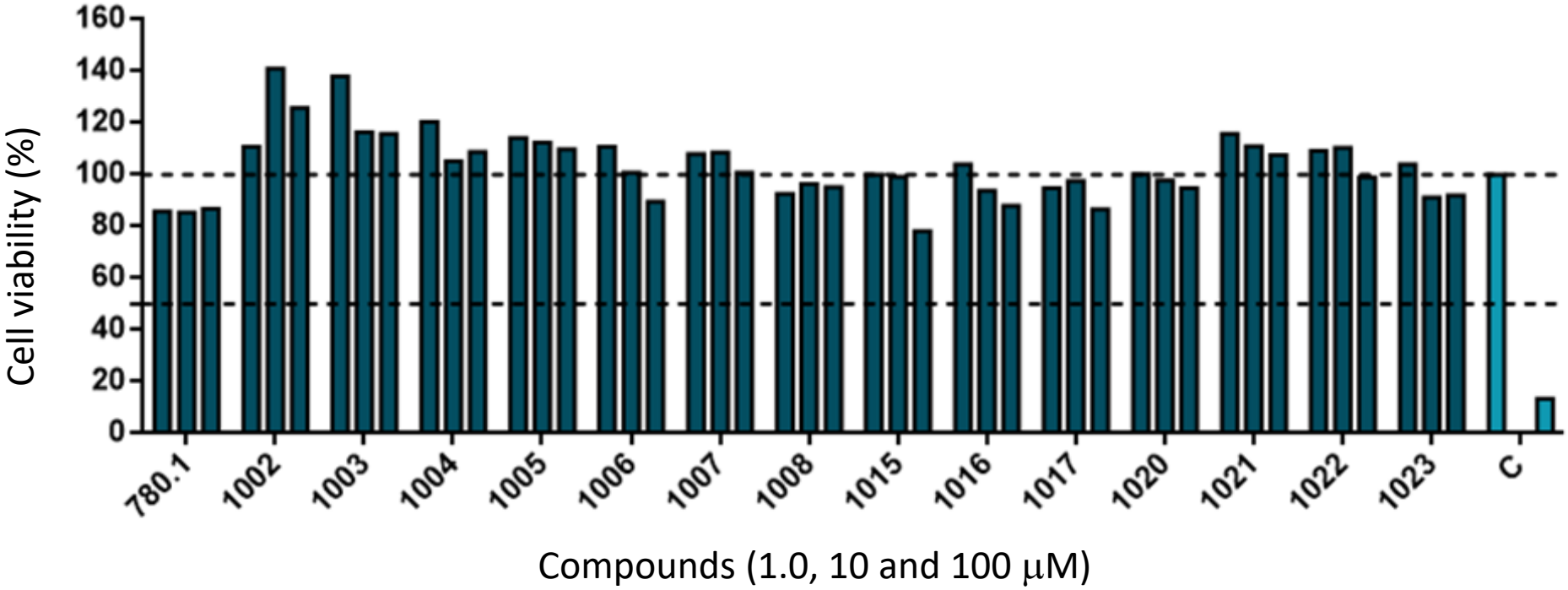
Initial screening results – new chemicals (MCF-7)



Compounds (1.0, 10 and 100 μM)

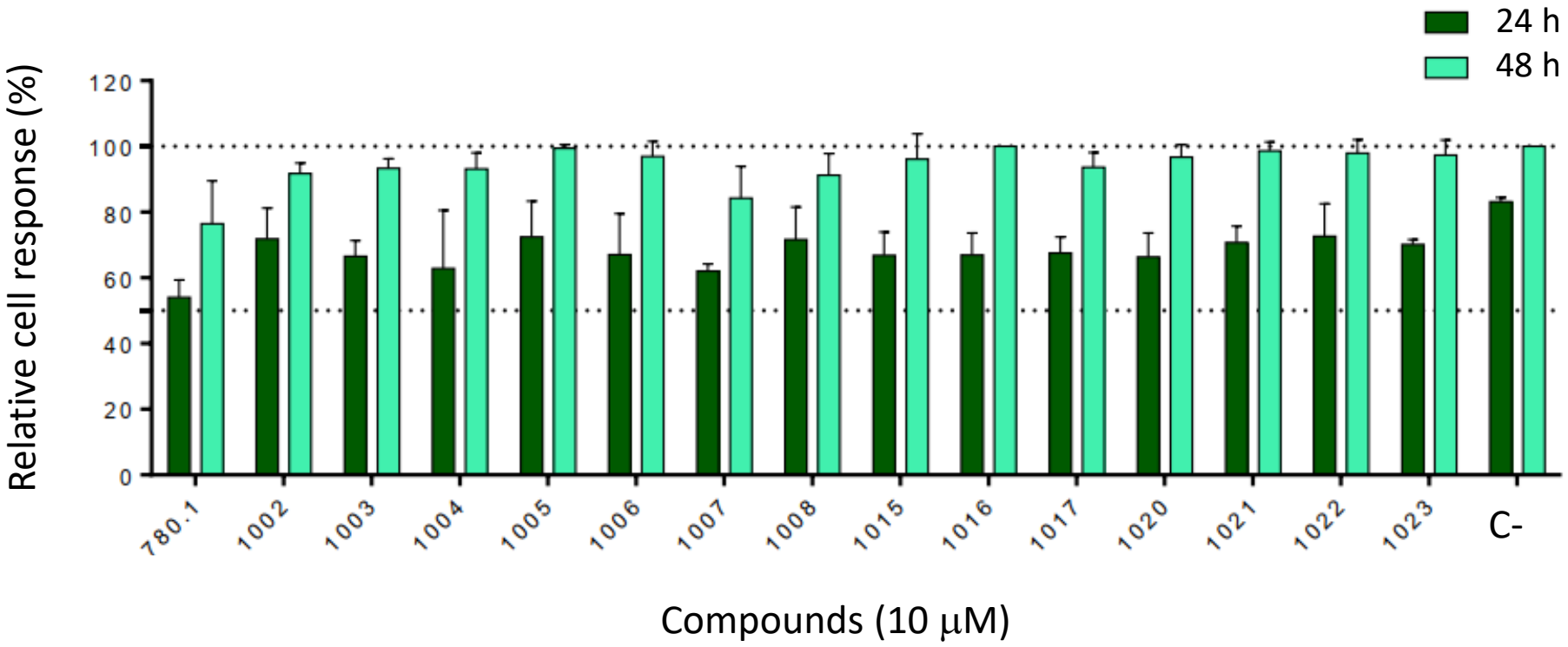
Results and discussion

Initial screening results – new chemicals (Balb/3T3 clone A31)



Results and discussion

Cell motility: scratch healing for new chemicals (MDA-MB-231)



Results and discussion

Cell motility: scratch healing for new chemicals (MDA-MB-231)

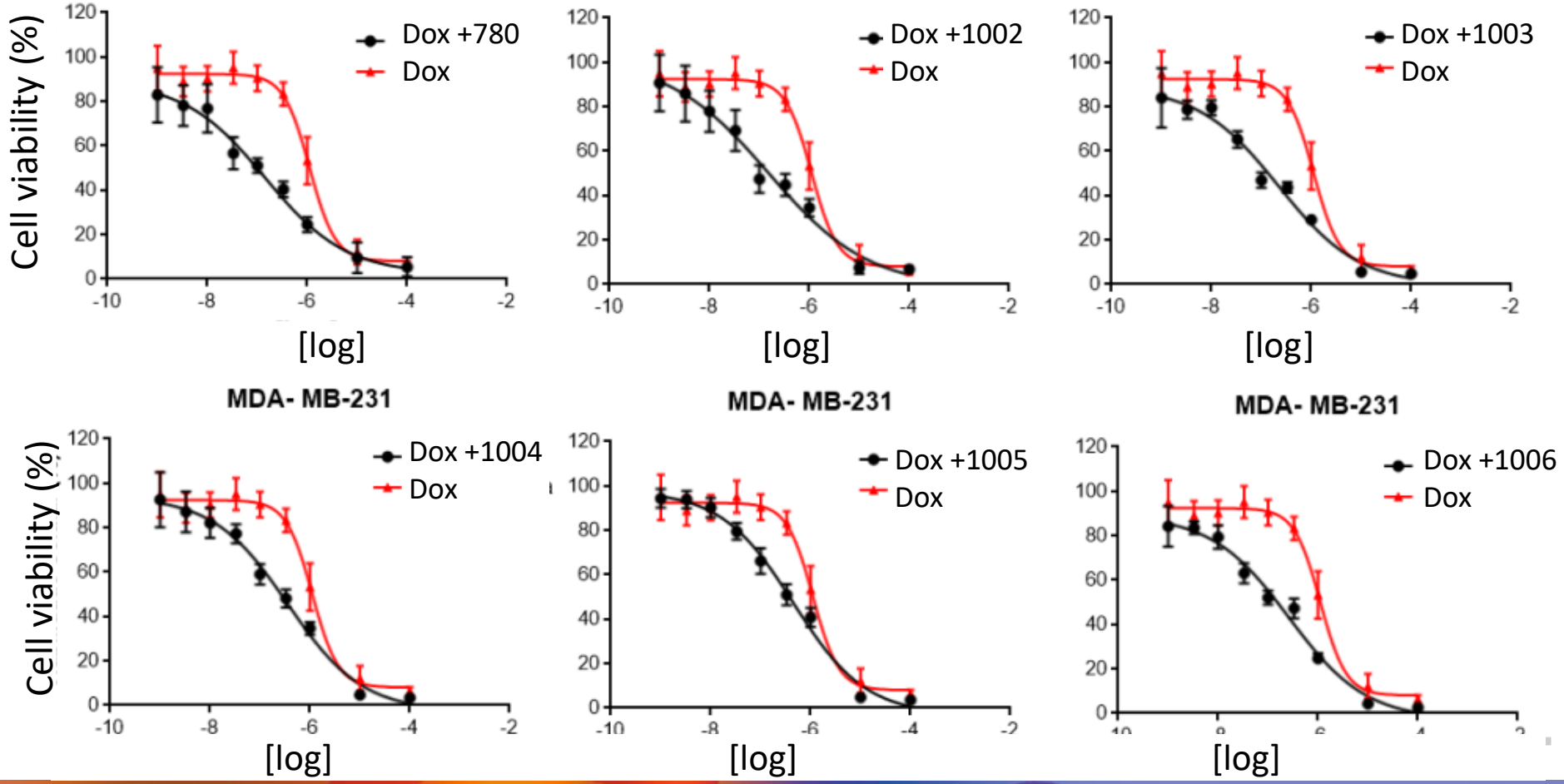
Sample	Relative response (%) 24 h	48 h	Sample	Relative response (%) 24 h	48 h
780.1	65	76	**1015**	79	96
1002	86	91	**1016**	79	100
1003	79	93	**1017**	81	93
1004	75	93	**1020**	79	96
1005	87	99	**1021**	84	98
1006	81	96	**1022**	87	97
1007	74	84	**1023**	84	97
1008	85	91			

Relative response based on the negative controls for 24 h and 48 h

Results and discussion

Combination therapy

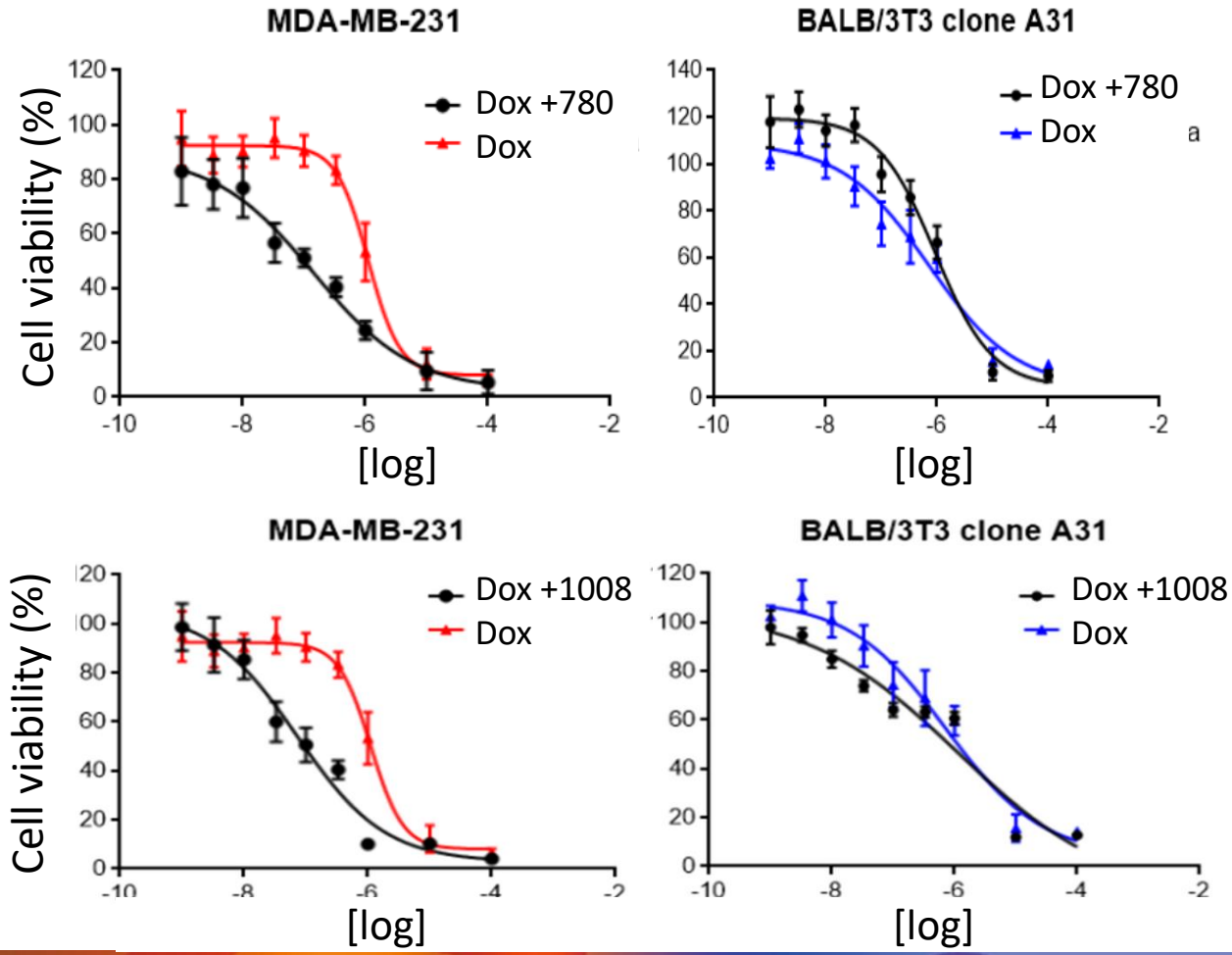
Concentration-dependence response (MDA-MB-231)



Results and discussion

Combination therapy

Concentration-dependence response (MDA-MB-231 *vs*. BALB/3T3 clone A31)



Results and discussion

Data compilation – combination therapy (BALB/3T3 clone A31)

Sample	IC_{50} ±SE (µM)	R^2/S	IC_{50} ratio
Doxorubicin (Doxo)	0,720 ± 0,19	0,93/8,95	-
Doxo + Neq780.1	0,923 ± 0,03	0,97/8,01	0,78
Doxo + Neq1002	3,23 ± 0,66	0,95/8,96	0,22
Doxo + Neq1003	1,55 ± 0,45	0,90/14,3	0,46
Doxo + Neq1004	1,43 ± 0,26	0,93/11,2	0,50
Doxo + Neq1005	1,94 ± 0,39	0,92/12,8	0,37
Doxo + Neq1006	1,93 ± 0,33	0,95/11,1	0,37
Doxo + Neq1007	2,46 ± 0,52	0,93/9,85	0,29
Doxo + Neq1008	1,15 ± 0,83	0,93/7,96	0,63
Doxo + Neq1015	1,94 ± 0,39	0,92/12,8	0,37
Doxo + Neq1016	0,308 ± 0,06	0,92/12,1	2,3
Doxo + Neq1017	0,682 ± 0,11	0,91/10,1	1,1
Doxo + Neq1020	0,929 ± 0,16	0,95/6,94	0,78
Doxo + Neq1021	1,069 ± 0,12	0,96/6,15	0,67
Doxo + Neq1022	0,934 ± 0,26	0,92/8,92	0,77
Doxo + Neq1023	0,642 ± 0,16	0,93/8,22	1,1

Results and discussion

Data compilation – combination therapy (MDA-MB231)

Sample	IC_{50} ±SE (μM)	R^2/S	IC_{50} ratio
Doxorubicin (Doxo)	1,11 ± 0,08	0,95/7,11	-
Doxo + Neq0780.1	0,142 ± 0,03	0,92/8,07	7,8
Doxo + Neq1002	0,152 ± 0,05	0,92/8,58	7,3
Doxo + Neq1003	0,221 ± 0,04	0,95/6,24	5,0
Doxo + Neq1004	0,359 ± 0,06	0,96/6,66	3,1
Doxo + Neq1005	0,448 ± 0,05	0,98/4,93	2,5
Doxo + Neq1006	0,261 ± 0,05	0,96/5,58	4,3
Doxo + Neq1007	0,361 ± 0,07	0,95/7,40	3,1
Doxo + Neq1008	0,0726 ± 0,01	0,94/8,38	15
Doxo + Neq1015	0,350 ± 0,04	0,95/7,91	3,2
Doxo + Neq1016	0,250 ± 0,03	0,96/6,26	4,4
Doxo + Neq1017	0,566 ± 0,10	0,93/8,53	2,0
Doxo + Neq1020	0,663 ± 0,09	0,91/9,25	1,7
Doxo + Neq1021	0,404 ± 0,07	0,94/8,41	2,7
Doxo + Neq1022	0,542 ± 0,05	0,96/6,27	2,0
Doxo + Neq1023	0,564 ± 0,06	0,96/6,12	2,0

Conclusions and perspective

- We identified *in vitro* the first promising combination therapy involving the application of cathepsin L inhibitors together with doxorubicin against a triple-negative breast cancer cell
- The combination therapy is selective for MDA-MB-231 in relation to other cell lines
- It is still unknown why cathepsin L inhibitors sensitize the cells to doxorubicin
- Mechanistic studies are being made by us to identify the reason for such a response
- Spheroids are also under study to identify whether this combination therapy would also work in a 3D setting

Acknowledgments

MEDICINAL & BIOLOGICAL CHEMISTRY GROUP - NEQUIMED

Prof. Dr. Carlos Alberto Montanari

Prof. Dr. Andrei Leitão

Prof. Dr. Fernanda Canduri

Dr. Fabiana Rosini Technitian

Ph.D. students

Talita Alvarenga
Hélio Franco
Vinícius Bonatto
Diandra Alencar
Thiago Matos
Tatiane Janku
Rebeka de Oliveira
Rafael Lameiro
Arthur Pereira
Sabrina Botelho
Felipe Martins

Master students

Bruna de Melo
Isabela Marques
Wellington Souza

Undergraduates

Nathália Wolf
Debora Magnani
Anderson Francelino
Guilherme Carlos

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Brazilian

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- ✓ Prof. Dr. Lúcio Freitas Jr. - ICB-USP
- ✓ Prof. Dr. Carolina Moraes - UNIFESP
- ✓ Prof. Dr. Luiz Juliano Neto - UNIFESP
- ✓ Prof. Dr. Jerônimo Lameira - UFPA
- ✓ Prof. Dr. João A. M. Neto - ICB-USP
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- ✓ Prof. Dr. Manu Platt GeorgiaTech/Univ. Emory - USA