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Insight into P-glycoprotein activity of Royleanones from *Plectranthus* spp.

Vera M. S. Isca[1,2], Epole Ntungwe[1,3], Eva Domínguez-Martín[1,3], Jaime A. S. Coelho[1], Ricardo J. Ferreira[2,4], Jelena Dinic[5], Milica Pesic[5], Daniel J. V. A. dos Santos[2], Nuno R. Candeias[6,7], Carlos A. M. Afonso[2], Patrícia Rijo[1,2*]

[1]CBIOS-Universidade Lusófona Research Center for Biosciences & Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal
[3]Department of Biomedical Sciences, Faculty of Pharmacy, University of Alcalá, Campus Universitario, 28871 Alcalá de Henares, Spain
[4]Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade, Despota Stefana 142, 11060 Belgrade, Serbia
[5]LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
*Corresponding author: patricia.rijo@ulusofona.pt

FACULDADE DE FARMÁCIA UNIVERSIDADE DE LISBOA

Insight into P-glycoprotein activity of Royleanones from *Plectranthus* spp.

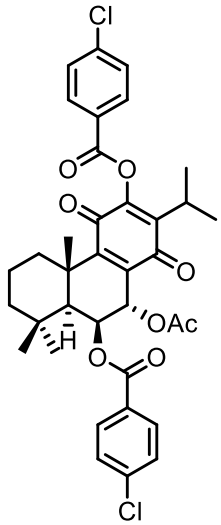
Graphical Abstract

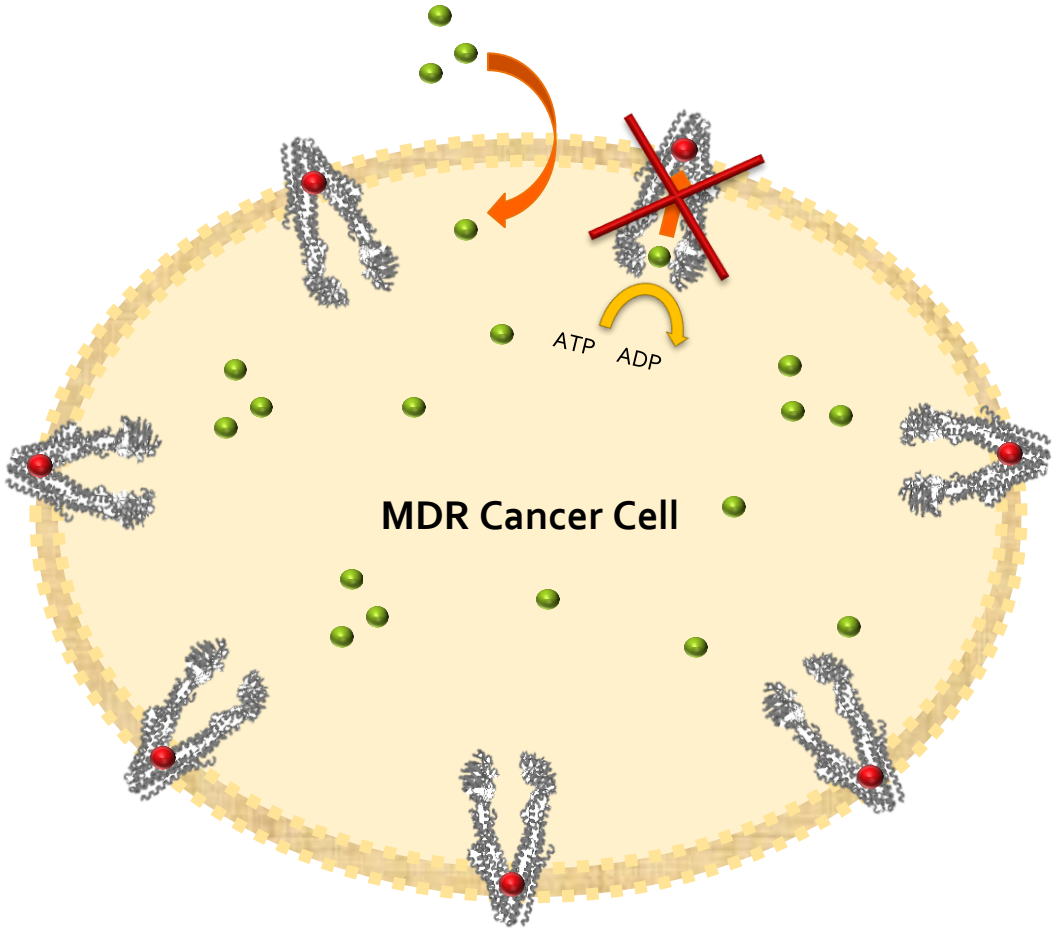
Chemoterapeitic Drug = Doxorubicin

P-gp

Phospholipid bilayer

P-gp inhibitor =





Abstract:

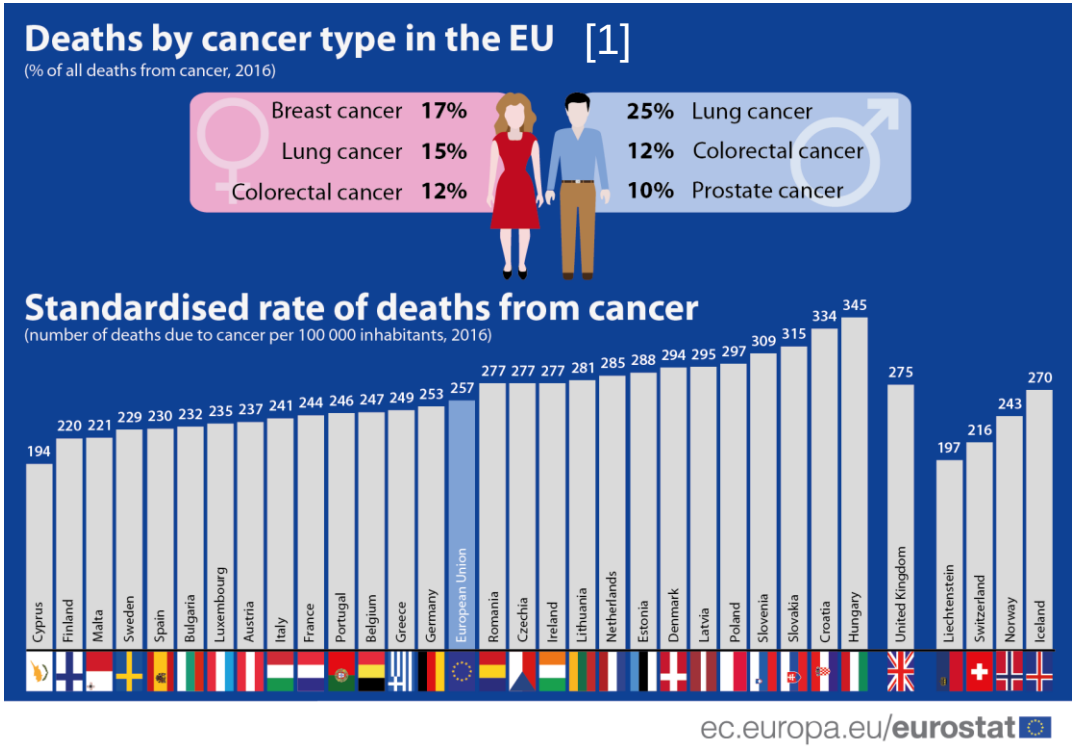
The development of multidrug resistance (MDR) often associated with overexpression of P-glycoprotein (P-gp) is a major cause of failure in cancer chemotherapy. The growing incidence of cancer and the development of MDR drive the search for novel and more effective anticancer drugs [1-3]. In this context, we use *Plectranthus* plants as potential sources of antitumoral compounds [4]. Moreover, we found that natural diterpenoids obtained from *Plectranthus* spp., namely 6,7-dehydroroyleanone (**1**) [5], 7α-acetoxy-6β-hydroxyroyleanone (**2**) [4] and 7α,6β-dihydroxyroyleanone (3) [6], displayed promising cytotoxic activity.

In this work, we synthesized a small library of compounds derived from royleanones **1** and **2** and evaluated their ability to modulate P-gp activity in human non-small cell lung carcinoma NCI-H460 and its MDR counterpart NCI-H460/R. Furthermore, molecular docking and molecular dynamic studies were performed to elucidate the mechanisms by which these derivatives may exert their inhibitory P-gp activity. These studies indicate that derivatives bearing aromatic moieties exhibit increased binding affinity towards P-gp, most likely acting as non-competitive efflux modulators when bound to the M-site. Remarkably, one of these derivatives showed the ability to sensitize the resistant NCI-H460/R cells to doxorubicin, and consequently could be considered as a novel P-gp inhibitor useful in combination with classic chemotherapeutics.

Keywords: cytotoxic activity; multidrug resistance; P-glycoprotein; Plectranthus; royleanones

Introduction

Cancer is among leading causes of death worldwide



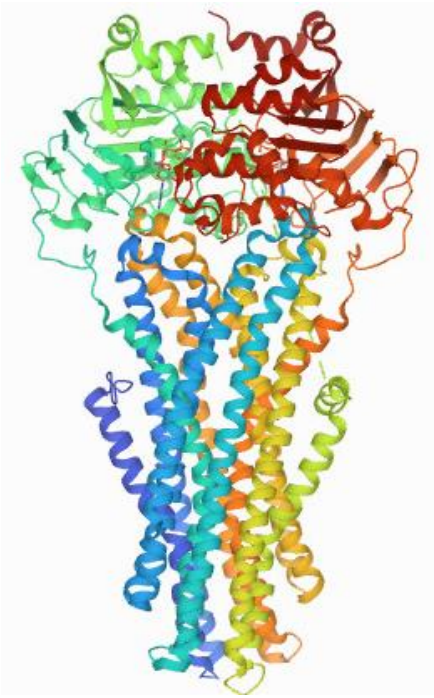
☢ Resistance to clinical drugs in use

Multidrug resistance (MDR) is one of the main challenges in cancer treatment [2, 3].

[1]. https://ec.europa.eu/eurostat/web/products-eurostat-news/-/EDN-20200204-1
[2]. Nanayakkara A. K. et al. (2018), Sci. Rep. 8 (967).
[3]. Isca VMS, Ferreira RJ, Garcia C, Monteiro CM, Rijo P *et al*. (2020) ACS Med Chem Letters 11, 5, 839–845.

Introduction

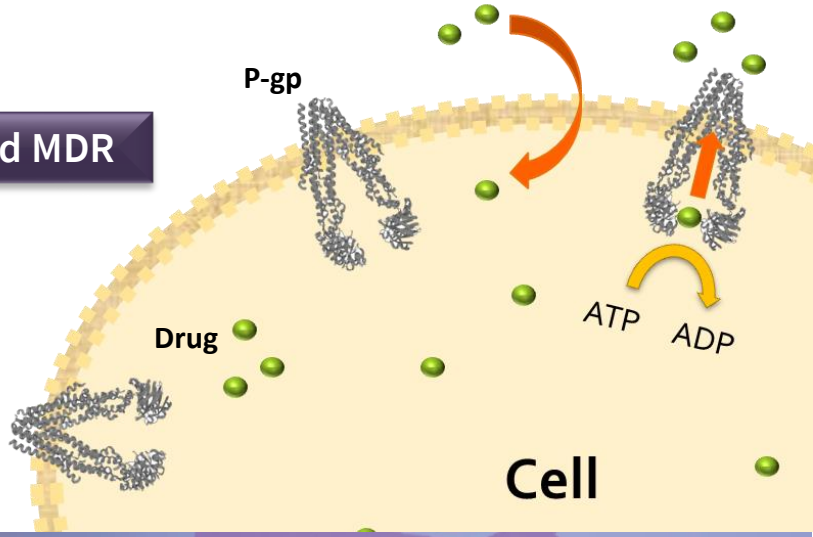
P-glycoprotein (P-gp) - Multidrug resistance protein



P-gp [4]

- ATP-dependent efflux pump → **Removing cytotoxic agents outside of the cell**
- P-gp reduces the efficacy of some drugs
- Cancer cells overexpress P-gp → **MDR**

P-gp-mediated MDR



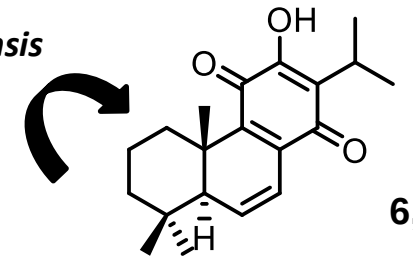
[3]. Isca VMS, Ferreira RJ, Garcia C, Monteiro CM, Rijo P *et al*. (2020) ACS Med Chem Letters 11, 5, 839–845.
[4] https://www.rcsb.org/structure/6C0V.

Introduction

Plectranthus genus (Lamiaceae)

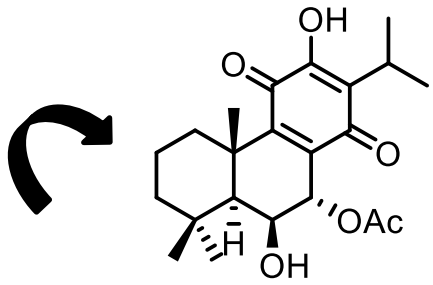
Source of Lead cytotoxic compounds

***P. madagascariensis* (Pers.) Benth [4]**



6,7-dehydroroyleanone (1)

***P. grandidentatus* Gürke [5]**



7α-acetoxy-6β-hydroxyroyleanone (2)

[4]. Garcia C, Silva CO, Monteiro CM, Nicolai M, Rijo P *et al.* (2018). Future Med Chem, 1(10): 1177-1189.
[5]. Matias D, Nicolai M, Saraiva L, Pinheiro R, Rijo P *et al*. (2019). ACS Omega, 4(5): 8094-8103.

Introduction

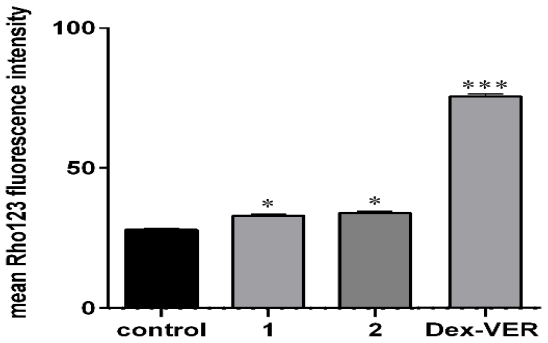
Effect of Natural royleanones 1 and 2 on P-gp inhibition:

Table 1. Cell growth inhibition thought MTT method: IC_{50} values (µM)

	NCI-H460	NCI-H460/R	MCR-5
1 [4]	14.06±2.34	11.21±1.45	30.0±4.0
2 [5]	2.7 ± 0.4	3.1 ± 0.4	8.6 ± 0.4
Positive control (paclitaxel)	0.0006±0.0001	0.12±0.01	0.008±0.001

Drug exposure during 72 h
NCI-H460: Sensitive non-small cell lung cancer cell lines
NCI-H460/R: Non-small cell lung cancer cell lines Multidrug-resistant counterpart
MRC-5: Normal human embryonal bronchial epithelial cells

- Cytotoxic activity against lung cancer cell lines
- Cytotoxic activity against MDR lung cancer cell lines
- Not a Pg-p substract



- Slight P-gp inhibition: **not promising**

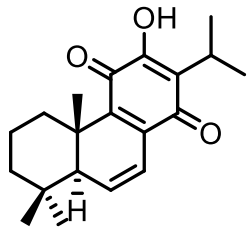
[4]. Garcia C, Silva CO, Monteiro CM, Nicolai M, Rijo P et al. (2018). Future Med Chem, 1(10): 1177-1189.
[5]. Matias D, Nicolai M, Saraiva L, Pinheiro R, Rijo P et al. (2019). ACS Omega, 4(5): 8094-8103.

Strategy

Enhanced P-gp inhibition potential to overcome MDR

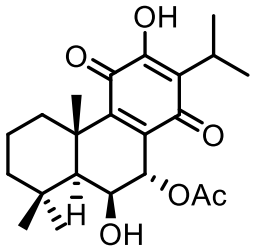
P. madagascariensis

P. grandidentatus



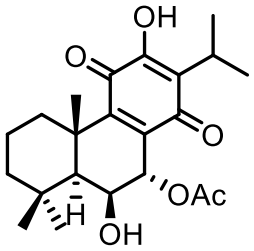
1

or



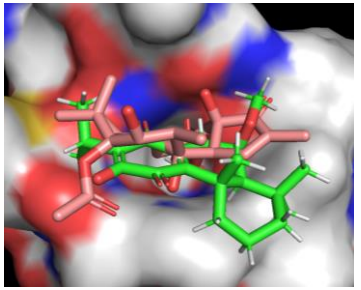
2

Derivatization



3 to **7**

Cytotoxic Evaluation

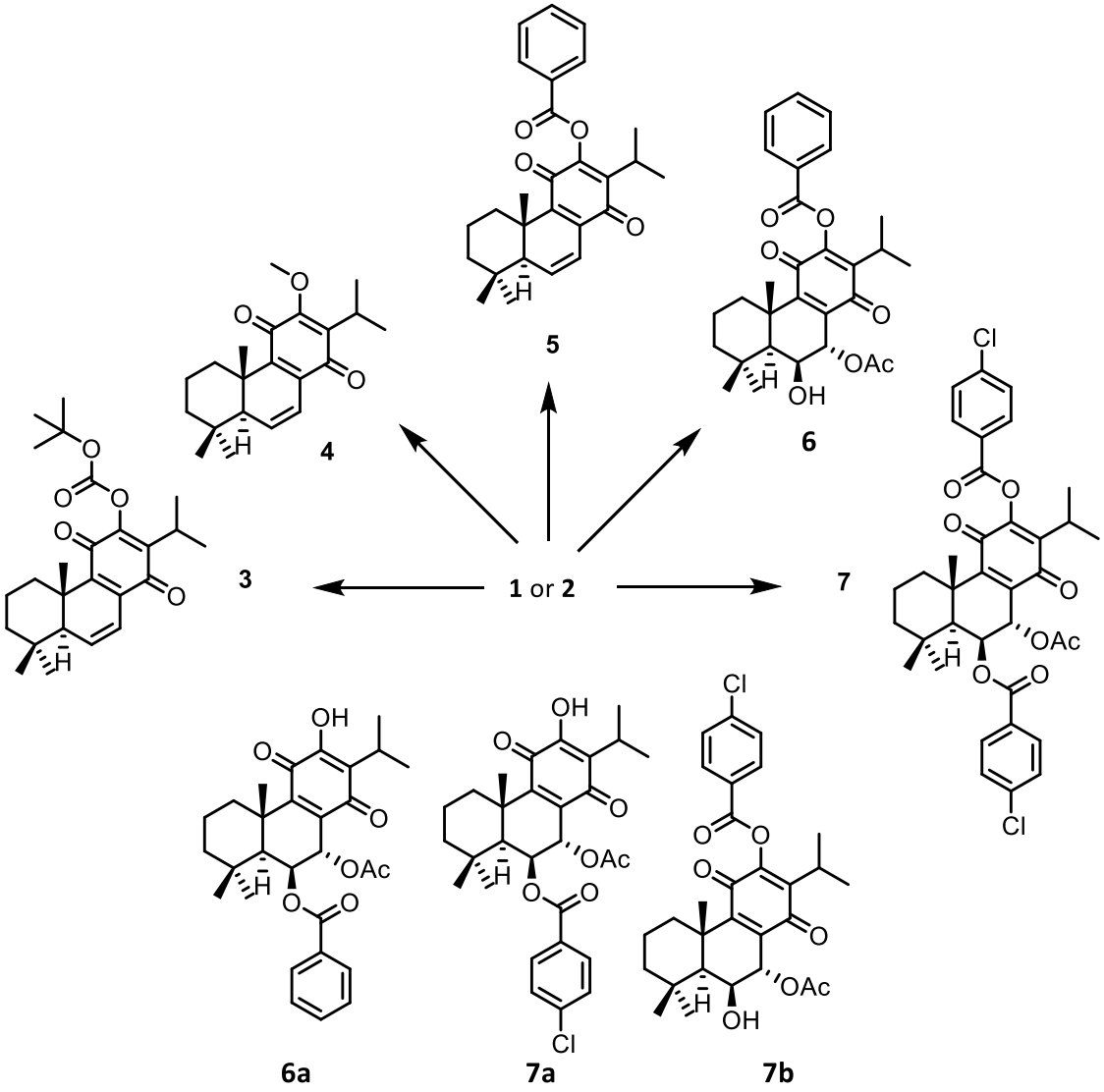


***In silico* studies**

Results and discussion

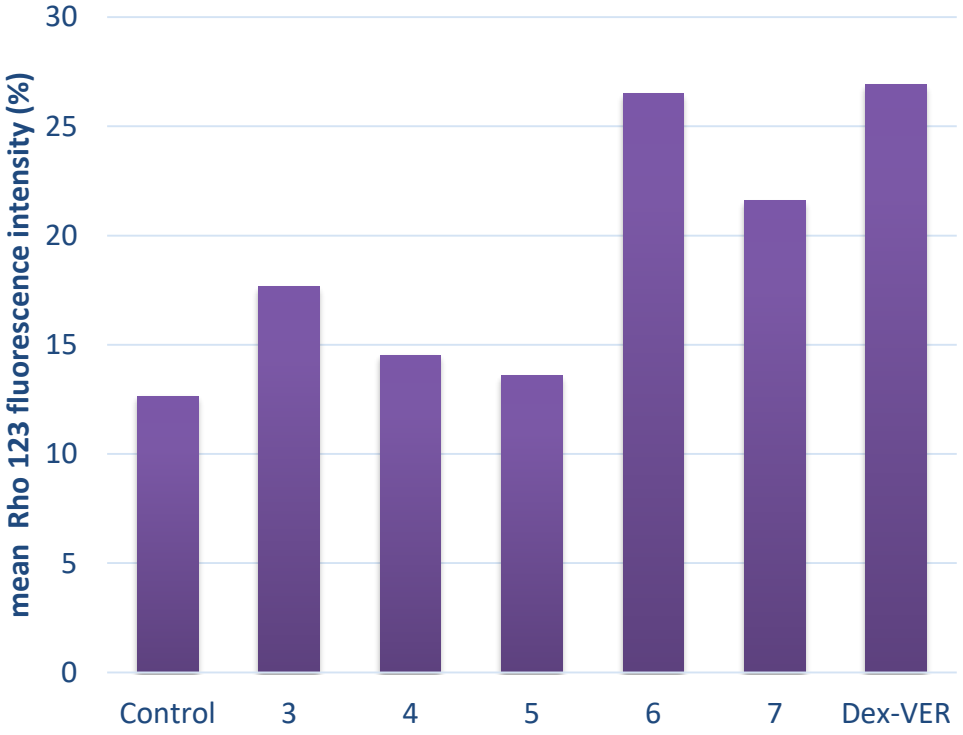
Derivatives in study:

- **Compounds 3** to **7** were obtained through hemi-synthesis from **1** and **2**
- **Compounds 6a**, **7a** and **7b** are theoretical derivatives used for the *in silico* studies

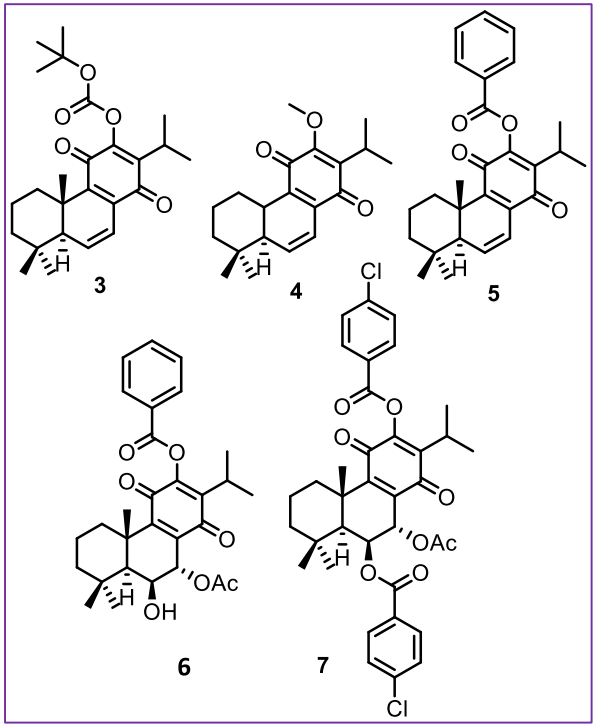


Results and discussion

P-gp inhibition in MDR cancer cell line of derivatives 3 to 7:



Graphic 1. Compounds 3-7 P-gp inhibition in an MDR cancer cell line



- Compound **6** displayed inhibition potential similar to **Dex-Ver** (positive control)
- Benzoyloxy substituent in position 12 is important for P-gp inhibition
- -OAc group in position 7 seems important for P-gp inhibition

Results and discussion

Molecular docking with a murine P-gp:

Table 2. Docking results, protein-ligand contacts for the tested compounds obtained by LigPlot

	Top-ranked affinity kcal.mol^{-1}			CIc
	H-site	R-site	M-site	
1	--	-8.8	-8.6	M
2	--	-8.7	-9.2	W
6	--	-9.3	-9.9	S
6a	-9.2	-10.4	-10.7	S
7	-10.5	-11.6	-10.4	S
7a	-9.2	-10.2	-10.3	S
7b	--	-9.3	-9.2	M

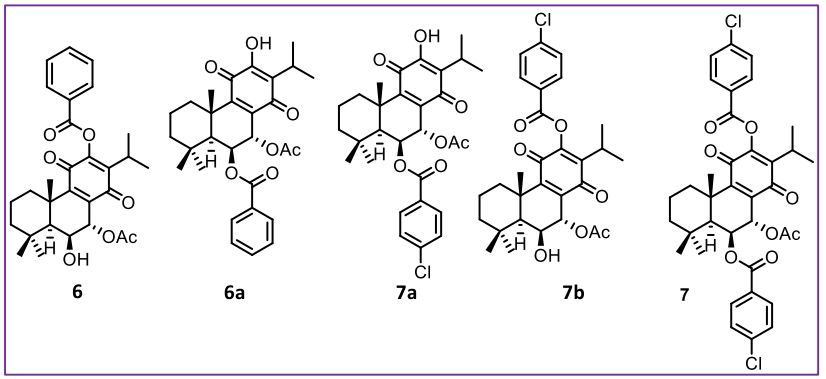
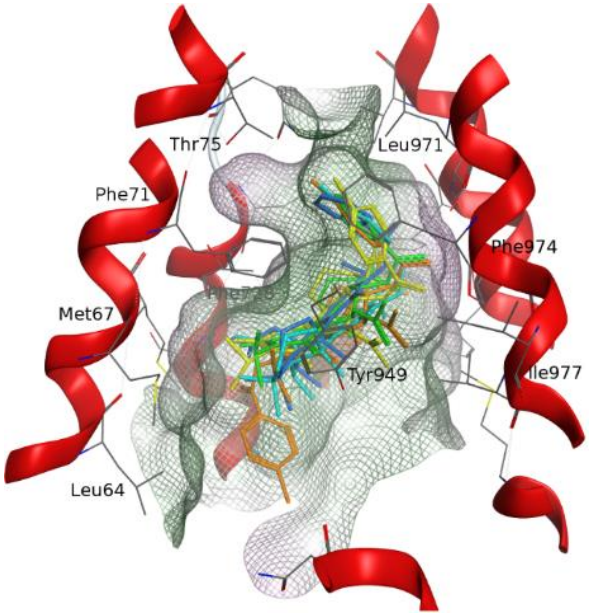


Figure A. Top-ranked docking pose at M site for compounds 3 (orange), 4a (yellow), 4b (cyan), 5 (blue) and 5a (green)



- Compounds **6** and **7** may act as non-competitive inhibitors
- One Benzoyloxy substituent seems important for P-gp inhibition

Molecular Dynamics (MD):

- The presence of a second benzoyloxy moiety does not significantly contribute to the binding affinity towards P-gp
- The presence of a -*para* substitution decreases the calculated ΔG_{bind}

Results and discussion

Derivative 7 sensitizes the NCI-H460/R cell line to doxorubicin:

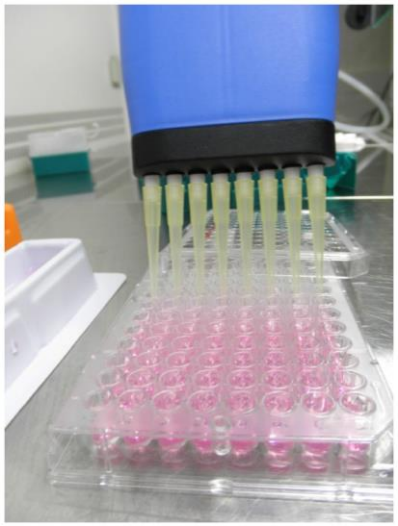
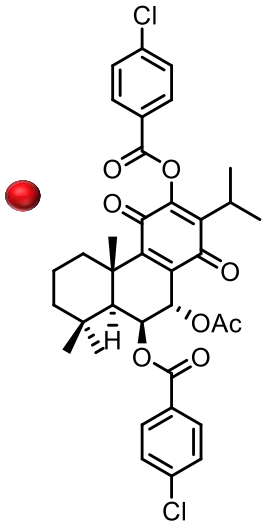


Table 3. Derivative 7 sensitizes the NCI-H460/R cell line to doxorubicin

Combined Treatments	IC_{50} for DOX (μM)	Relative Reversal Factor
DOX	2.774 ± 0.025	--
7 (0.5 μM) + DOX	0.823 ± 0.016	3.37
7 (1.0 μM) + DOX	0.594 ± 0.017	4.67
7 (2.0 μM) + DOX	0.608 ± 0.020	4.56

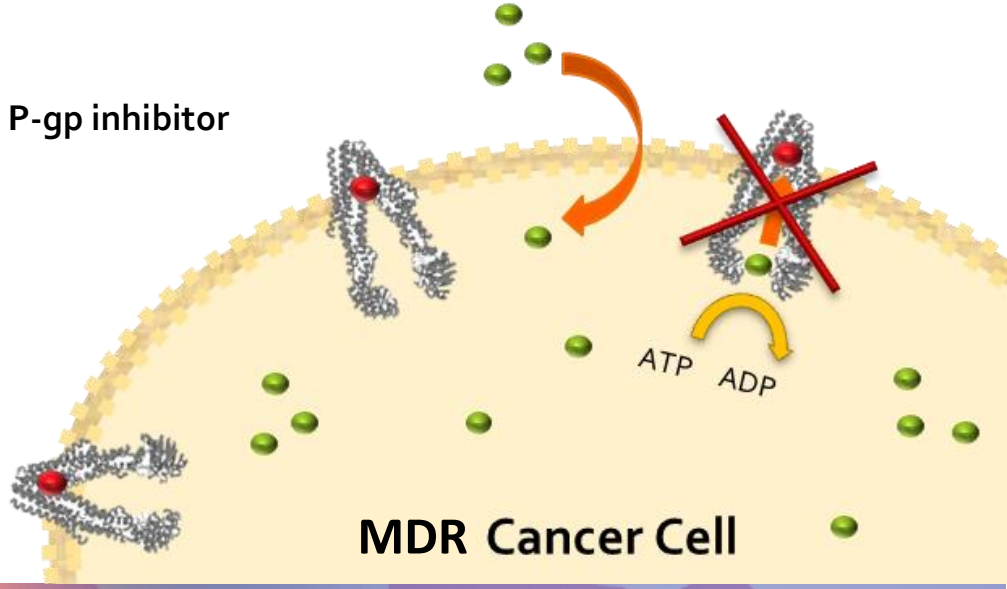


7 = P-gp inhibitor

Doxorubicin

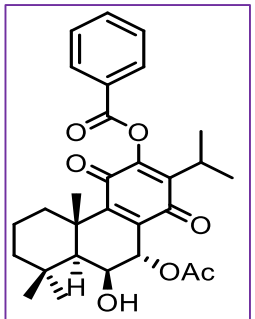
P-gp

Phospholipid bilayer

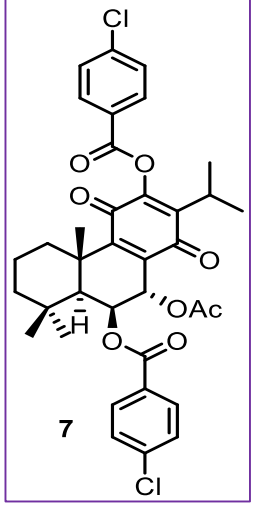


Conclusions

- Compounds **6** and **7** have significant *in vitro* P-gp inhibitory potential, were **6** is comparable to **Dex-Ver** (positive control).
- Derivative **6**: Benzoyloxy moiety (position 12) and -OAc group (position 7) seemed important for P-gp inhibition.
- MD and docking predictions suggest that compounds **6** and **7** (and its theoretical derivatives **6a**, **7a** and **7b**) may act as a non-competitive efflux modulators.
- MD and docking predictions also suggest that one benzoyloxy substituent (position 12) is important for P-gp inhibition.
- Derivative **7**, showed the ability to sensitize the resistant NCI-H460/R cells to doxorubicin. This diterpene could be considered as a novel P-gp inhibitor useful in combination with classic chemotherapeutics.



6



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Acknowledgments

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