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Inhibition of P-glycoprotein activity to overcome multidrug resistance in cancer with new diterpene royleanones from *Plectranthus* spp.

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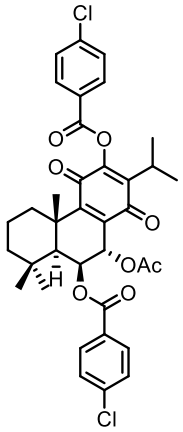
Inhibition of P-glycoprotein activity to overcome multidrug resistance in cancer with new diterpene royleanones from *Plectranthus* spp.

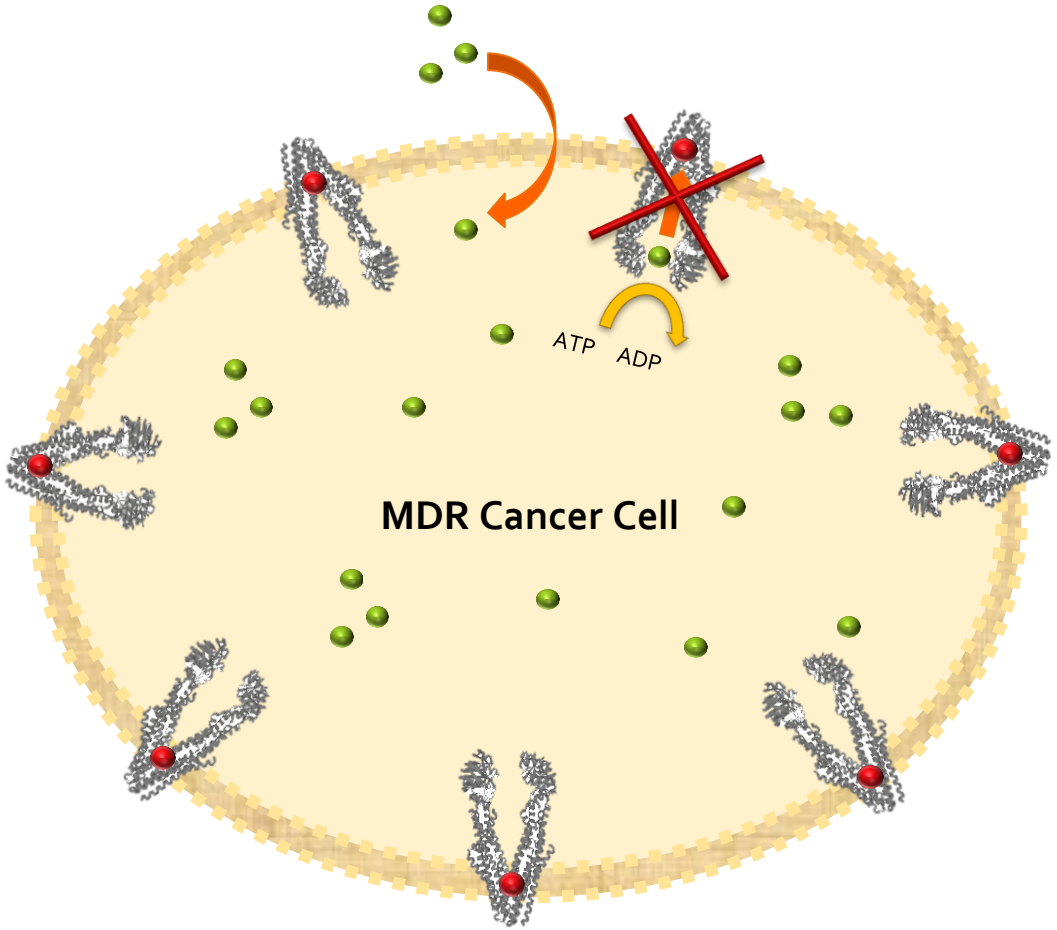
Chemoterapeitic Drug = Doxorubicin

P-gp

Phospholipid bilayer

P-gp inhibitor =





Abstract:
Multidrug resistance (MDR) is one of the major obstacles in cancer chemotherapy. MDR is often associated with overexpression of the efflux pump, P-glycoprotein (P-gp). The growing incidence of cancer and the development of MDR drive the search for novel and more effective anticancer drugs. In this context, we have recognized *Plectranthus* plants as potential sources of lead compounds. Accordingly, two natural diterpenoids, 6,7-dehydroroyleanone (1) and 7α-acetoxy-6β-hydroxyroyleanone (2) obtained from *Plectranthus* spp., exhibited promising cytotoxic activity.
In this work, the reactivity of 1 and 2 was studied to synthesize a library of new derivatives with P-gp inhibitory potential. The ability to inhibit P-gp activity was evaluated in human non-small cell lung carcinoma NCI-H460 and its MDR counterpart NCI-H460/R. Furthermore, molecular docking and molecular dynamics studies were conducted to explain the molecular interaction of royleanones with P-gp.
Royleanones 1 and 2 showed similar cytotoxic activity against cancer cell lines and MDR cancer cell lines. Two benzoylated derivatives displayed improved P-gp inhibition activity comparing to the natural ones (1 and 2). Interestingly, one of these derivatives also displayed the ability to sensitize the resistant NCI-H460/R cells to doxorubicin and therefore could be considered as a novel P-gp inhibitor suitable in combination with classic anticancer drugs.

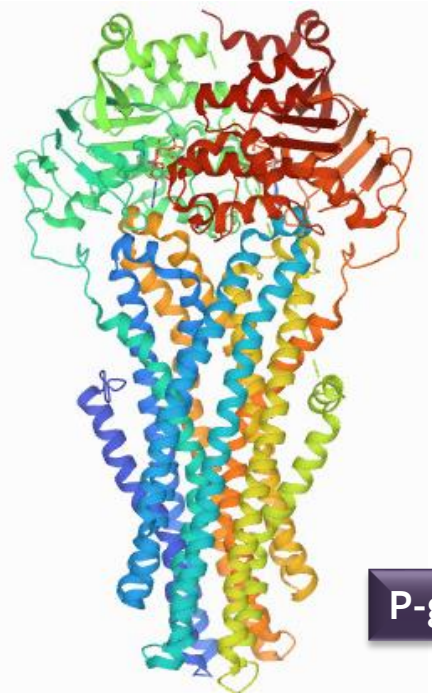
Keywords: Cancer; Multidrug resistance; P-glycoprotein activity; *Plectranthus*; Royleanone derivatives

Introduction

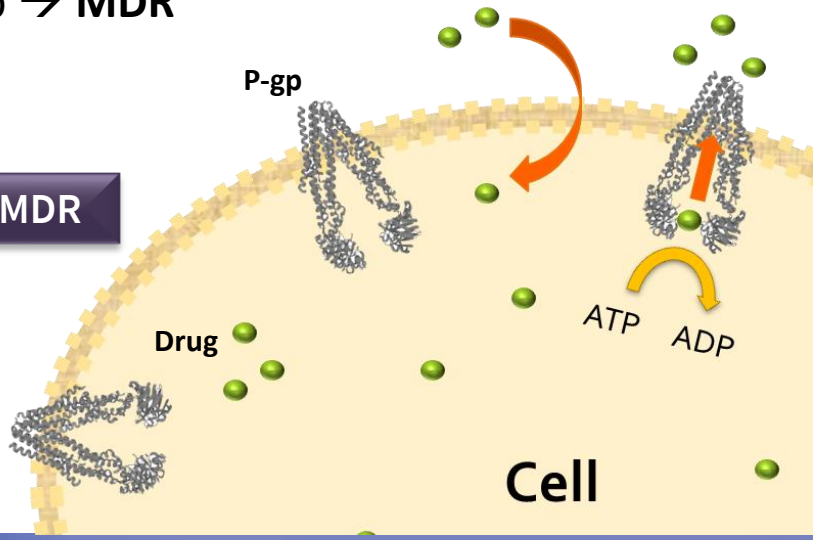
Multidrug resistance (MDR): one of the main challenges in cancer treatment [1].

P-glycoprotein (P-gp) – MDR protein

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



P-gp [2]



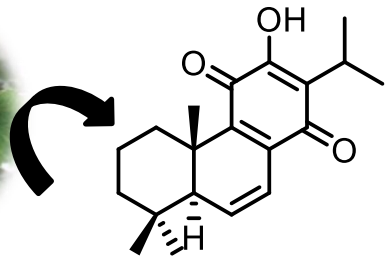
P-gp-mediated MDR

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

Introduction

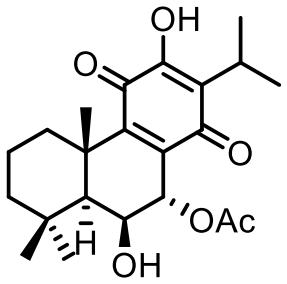
Plectranthus genus (Lamiaceae): Source of Lead cytotoxic compounds

P. madagascariensis (Pers.) Benth [3]



6,7-dehydroroyleanone (1)

P. grandidentatus Gürke [4]



7α-acetoxy-6β-hydroxyroyleanone (2)

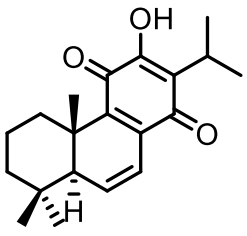
1 and 2:

- Cytotoxic activity against **NCI-H460** lung cancer cell lines
- Cytotoxic activity against **NCI-H460/R** MDR lung cancer cell lines
- Not a Pg-p substracte
- Slight P-gp inhibition

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

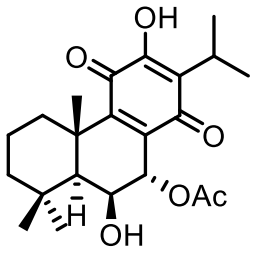
Strategy

Enhanced P-gp inhibition potential to overcome MDR:



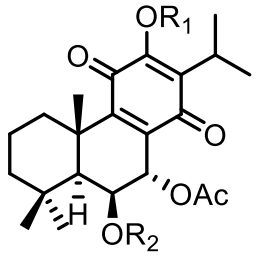
1

or



2

Derivatization

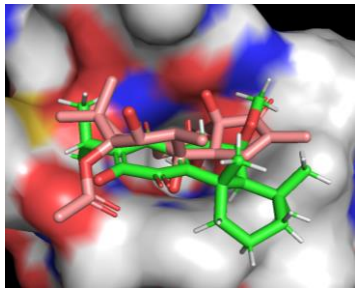


3 to **17**

NCI-H460
NCI-H460/R
MRC-5

Cytotoxic Evaluation

***In silico* studies**



Results and discussion

Molecular docking with a murine P-gp:

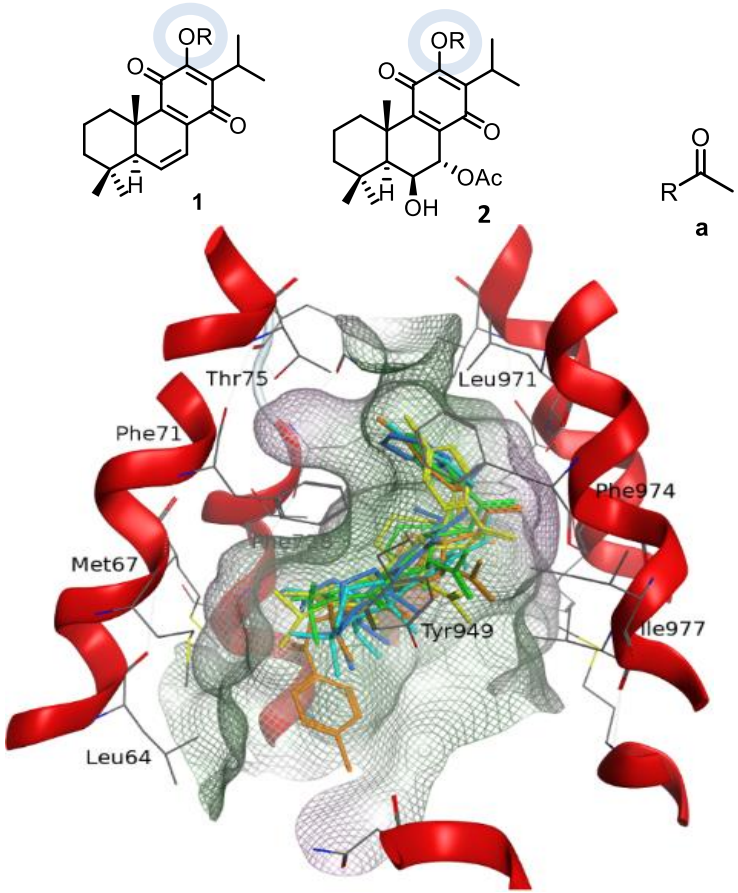
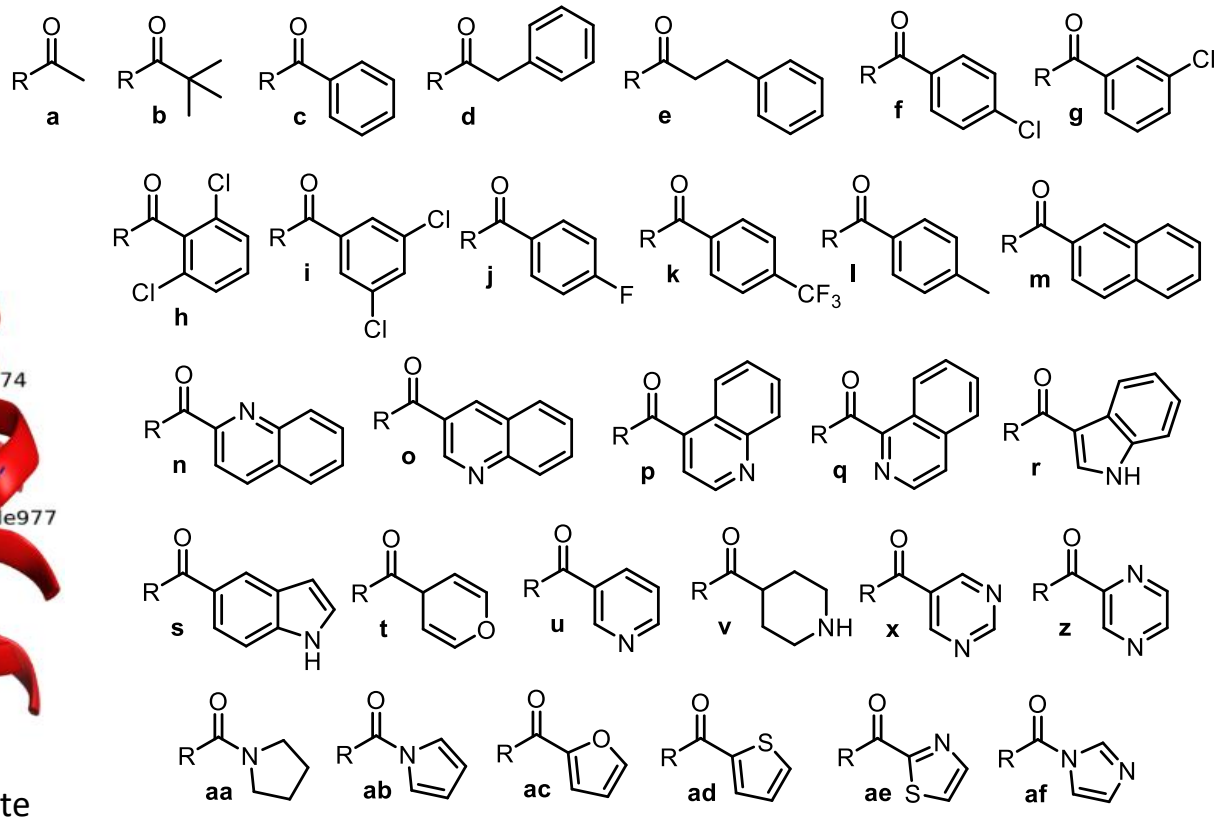


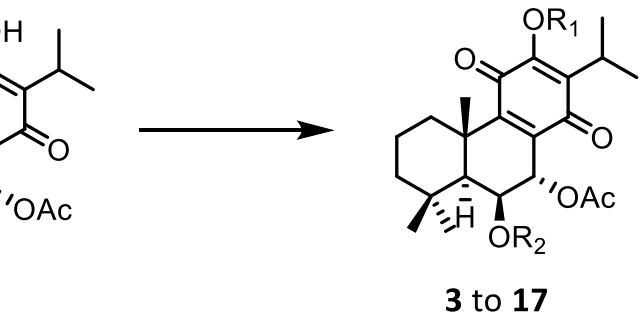
Figure. Top-ranked docking pose at M site for several derivatives

New compound library:

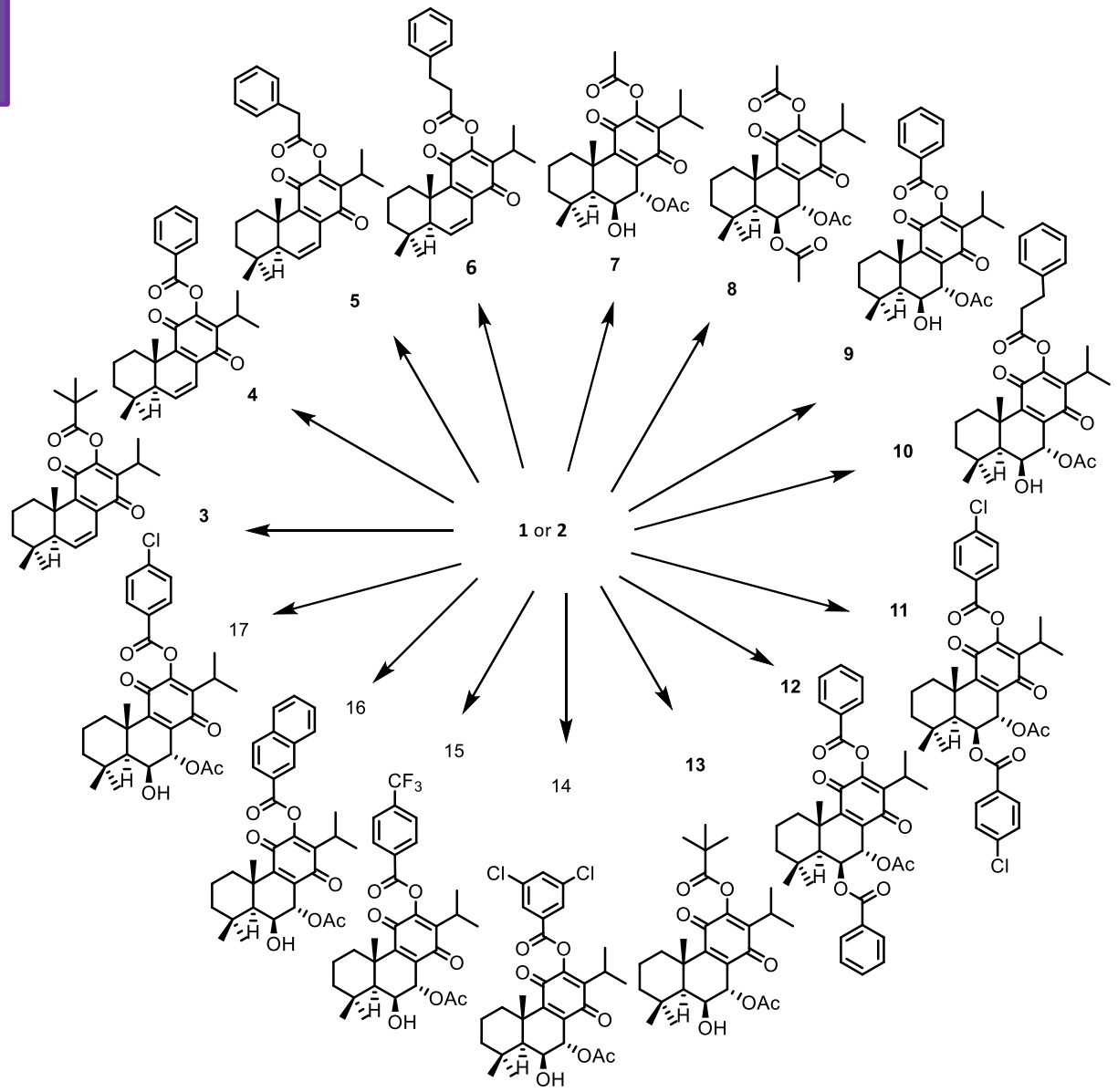


Results and discussion

Derivatives prepared:

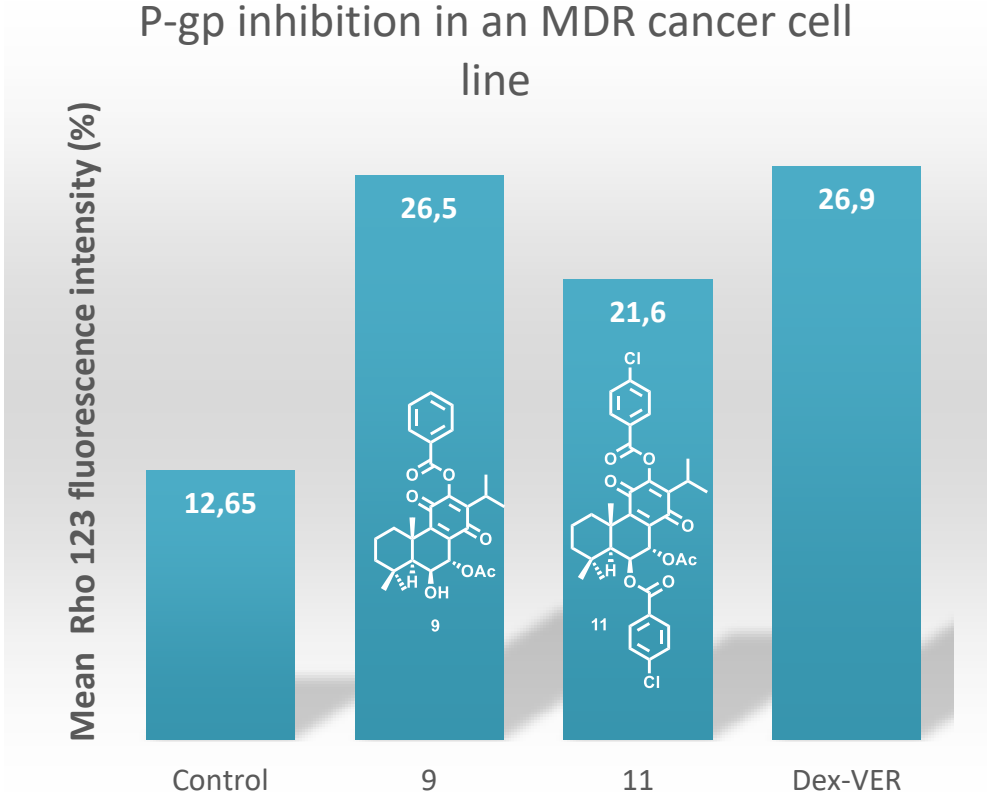


3 to **17**



Results and discussion

Rhodamine 123 accumulation assay



Experiments were performed in triplicates (n=3). Significant difference compared to control: * $p < 0.05$, *** $p < 0.001$

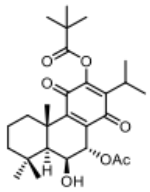
Evaluation of derivatives 3-11:

- Compounds **9** and **11** exhibit promising P-gp inhibition potential
- Compound **9** displayed inhibition potential similar to Dex-Ver

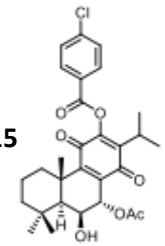
Evaluation of derivatives 12-17 is currently on going:

- **13**, **15**, **17** seem promising

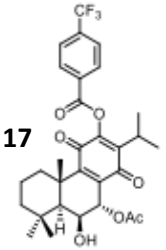
13



15



17



Isca VMS, Rijo P et al. (2020) ACS Med Chem Letters 11, 5, 839–845.

Results and discussion

Derivative 11 sensitizes the NCI-H460/R cell line to doxorubicin:

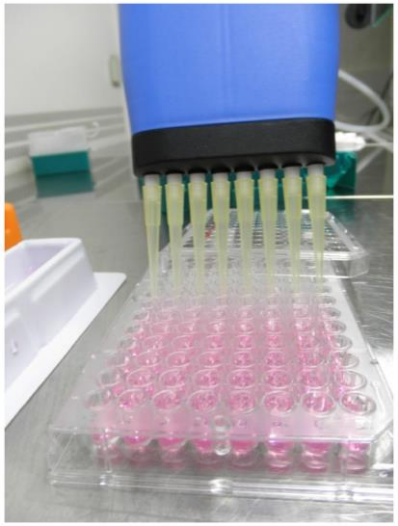
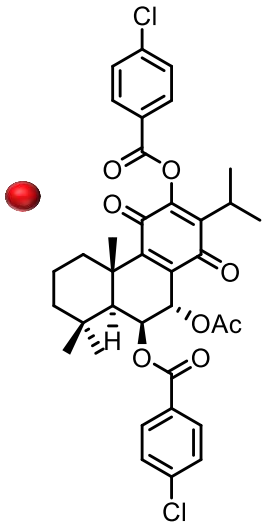


Table. Derivative 7 sensitizes the NCI-H460/R cell line to doxorubicin

Combined Treatments	IC_{50} for DOX (μM)	Relative Reversal Factor
DOX	2.774 ± 0.025	--
11 (0.5 μM) + DOX	0.823 ± 0.016	3.37
11 (1.0 μM) + DOX	0.594 ± 0.017	4.67
11 (2.0 μM) + DOX	0.608 ± 0.020	4.56

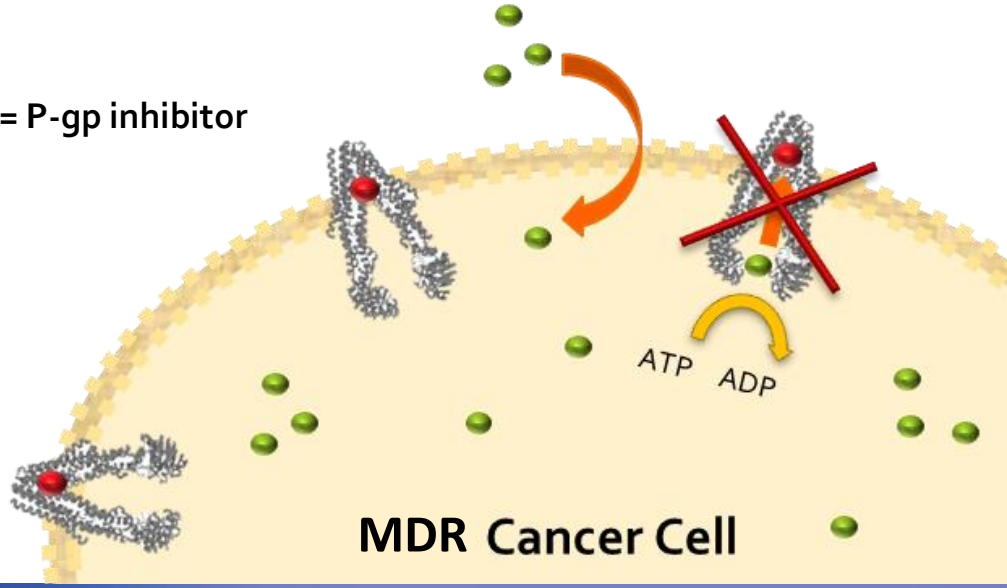


11 = P-gp inhibitor

Doxorubicin

P-gp

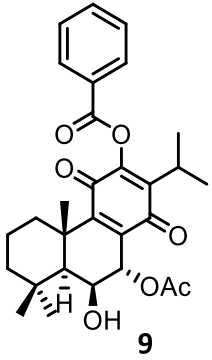
Phospholipid bilayer



Conclusions

☢ **MD and docking predictions:**

- One aromatic moiety increase affinity to P-gp
- Selection of the hit compounds to synthetize

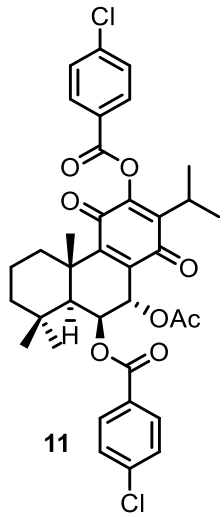


9

☢ ***In vitro* study:**

- Derivatives **9** and **11:** Increased P-gp inhibition potential
- **9**: Similar to **Dexverapamil**
- **11**: Ability to sensitize the MDR cancer cells to doxorubicin

Novel P-gp inhibitor useful in combination with classic chemotherapeutics.



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Acknowledgment

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