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.
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
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

[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 substrate
- Slight P-gp inhibition

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Strategy

Enhanced P-gp inhibition potential to overcome MDR:

1 or 2

Derivatization

3 to 17

In silico studies

Cytotoxic Evaluation

NCI-H460
NCI-H460/R
MRC-5
Results and discussion

Molecular docking with a murine P-gp:

New compound library:

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

Derivatives prepared:

3 to 17
Results and discussion

Rhodamine 123 accumulation assay

P-gp inhibition in an MDR cancer cell line

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 ongoing:

- 13, 15, 17 seem promising

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

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

<table>
<thead>
<tr>
<th>Combined Treatments</th>
<th>IC$_{50}$ for DOX (µM)</th>
<th>Relative Reversal Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX</td>
<td>2.774 ± 0.025</td>
<td>--</td>
</tr>
<tr>
<td>11 (0.5 µM) + DOX</td>
<td>0.823 ± 0.016</td>
<td>3.37</td>
</tr>
<tr>
<td>11 (1.0 µM) + DOX</td>
<td>0.594 ± 0.017</td>
<td>4.67</td>
</tr>
<tr>
<td>11 (2.0 µM) + DOX</td>
<td>0.608 ± 0.020</td>
<td>4.56</td>
</tr>
</tbody>
</table>

Phospholipid bilayer

Doxorubicin

P-gp

11 = P-gp inhibitor

MDR Cancer Cell
**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

**MD and docking predictions:**
- One aromatic moiety increase affinity to P-gp
- Selection of the hit compounds to synthetize

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