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Royleanone analogues from *Plectranthus* spp. demonstrate P-gp inhibition and PKC modulation

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pharmaceuticals MDPI

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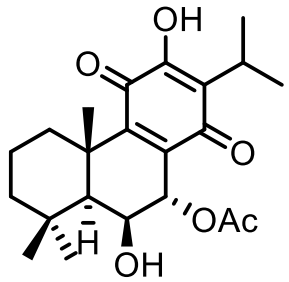
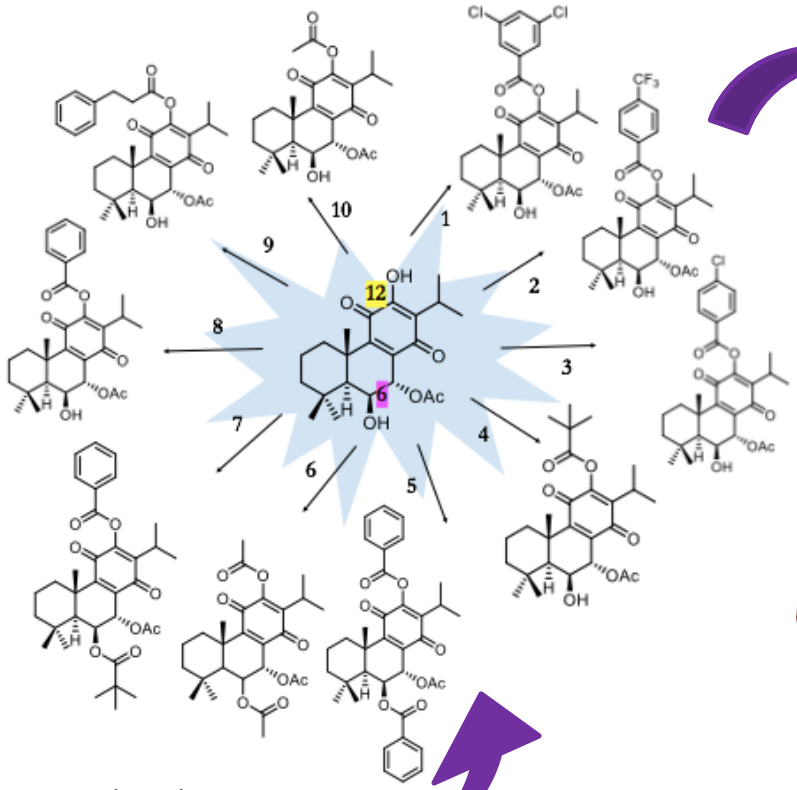
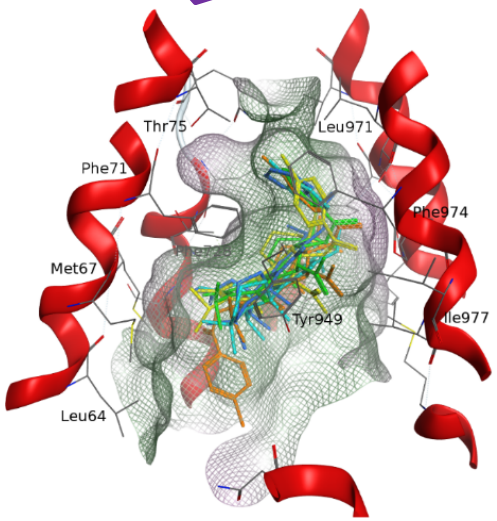
Royleanone analogues from *Plectranthus* spp. demonstrate P-gp inhibition and PKC modulation

***P. madagascariensis* (Pers.) Benth**



7α-acetoxy-6β-hydroxyroyleanone (**Roy**)





Abstract:

The number of multidrug resistant (MDR) cancer cases across the globe is continuing to rise, such that the search for novel anti-cancer therapeutics is paramount. However, in MDR cancers, such as the overexpression of membrane transport proteins like P-glycoprotein (P-gp) or the modulation of Protein Kinases C (PKC) isoforms, continues to be a major impediment to effective therapy. Known for their medicinal properties, species from *Plectranthus* have reported cytotoxicity against various cancer cell lines, due to diterpenes, such as 7α-acetoxy-6β-hydroxyroyleanone (**Roy**) and 6,7-dehydroroyleanone (**DeRoy**). Based on molecular docking simulations, 10 semi-synthetic derivates of **Roy** that displayed strong P-gp interactions *in silico* were prepared. The antitumoral activity was evaluated in resistant human cancer cell lines NCI-H460/R and DLD1-TxR, showing three derivatives having the most prominent selectivity towards cancer cells, compared to normal lung fibroblasts MRC5. Moreover, they showed a reduction in P-gp activity in Rho123 accumulation and indicated P-gp inhibition in the DOX accumulation assay using the same resistant cell lines. Overall, it was demonstrated that three abietane diterpenoid derivatives induced P-gp inhibition in MDR cancer cell lines. As for the PKC activity, further analogues were tested as PKC (α, βI, δ, ε and ζ) modulators; one benzoylated derivative showed the ability to selectively activate PKC-δ, while the natural compound **DeRoy** displayed improved PKC activity, compared with the positive control, in all tested isoforms. Further investigations are ongoing to prepare analogues of other biological active diterpenoids to obtain potential hits as P-gp and PKC modulators.

Keywords: Abietanes; anticancer; derivatives; P-gp; PKC; royleanone

Introduction

- Multi-drug resistance (MDR) cancers continue to be a serious concern
- *Plectranthus* species renowned for their medicinal properties
- Diterpenes 7α-acetoxy-6β-hydroxyroyleanone (**Roy**) and 6,7-dehydroroyleanone (**DeRoy**) showed cytotoxic activity against various cancer cell lines
- Anti-cancers targets: P-glycoprotein (P-gp) - (overexpression of membrane transport proteins) or Protein Kinases C (PKC) isoforms

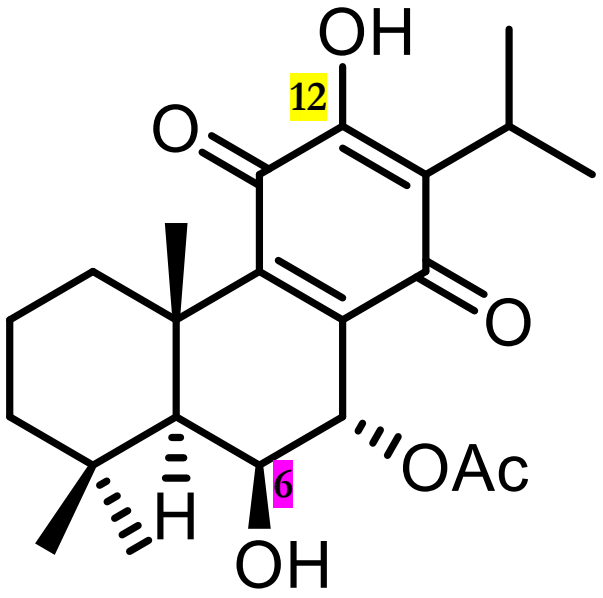


Fig. 1. Structure of 7α-acetoxy-6β-hydroxyroyleanone (**Roy**) isolated from *Plectranthus* spp.

Methods

- 10 semi-synthetic derivates of **Roy**, based on *in silico* molecular docking studies of P-gp interactions, were prepared
- Antitumoral activity of the compounds assessed in sensitive and resistant human cancer cell lines **NCI-H460**/R and **DLD1**/R
- P-gp activity assessed by Rho 123 and DOX accumulation assays
- PKC activity assessed by yeast-based assay

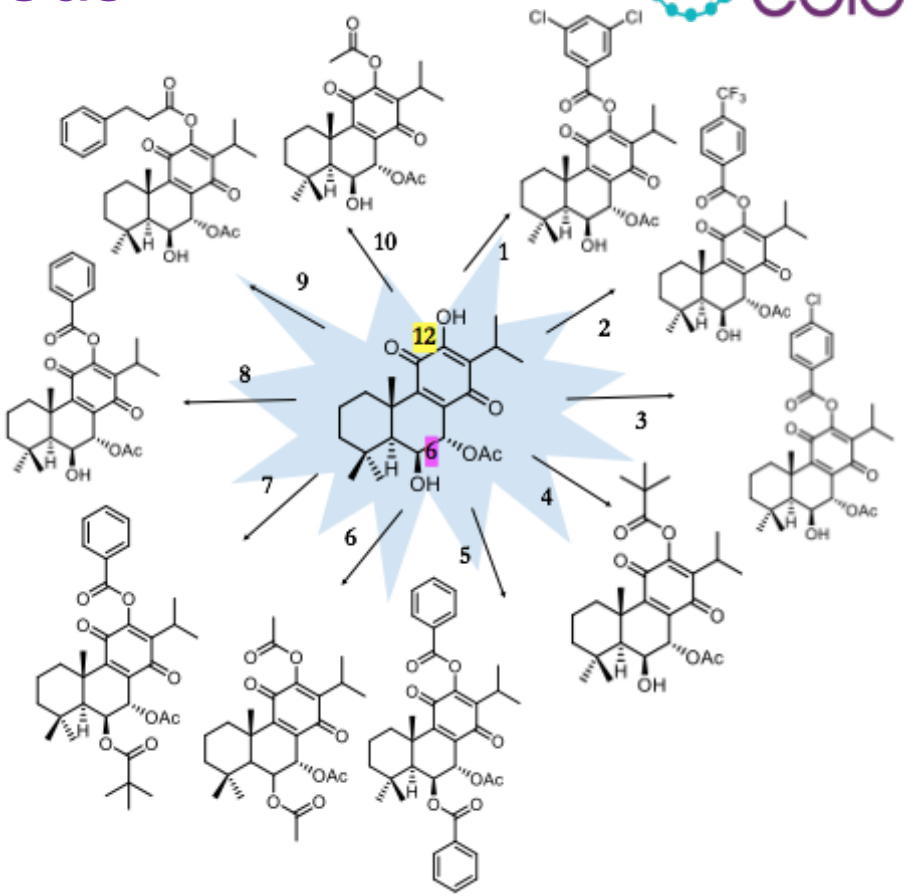


Fig. 2. Structures of 10 Semi-synthetic derivatives of 7α-acetoxy-6β-hydroxyroyleanone (**Roy**) prepared for anti-cancer evaluation

Results and Discussion

- All tested derivatives **reduced cell viability after 72h** in a dose dependant manner
- **2**, **3** and **4** had the most prominent **selectivity** (2.7, 2.3 and 2.6 times, respectively) towards cancer cells, compared to MRC5

Derivative	IC_{50} [μM]				
	H460	RH460	DLD1	RDLD1	MRC-5
2	7.48±2.82	7.05±2.18	6.06±2.13	6.05±1.77	17.65±5.26
3	2.86±0.75	3.87±1.25	3.50±1.52	2.77±0.83	8.33±3.86
4	10.91±5.00	5.55±1.49	5.51±2.08	4.88±1.44	15.62±4.45

Table 1. IC_{50} values for lung (H460) and colon (DLD1) sensitive cancer cell lines, resistant counterparts (RH460 and RDLD1, respectively) and normal human fibroblasts (MRC5)

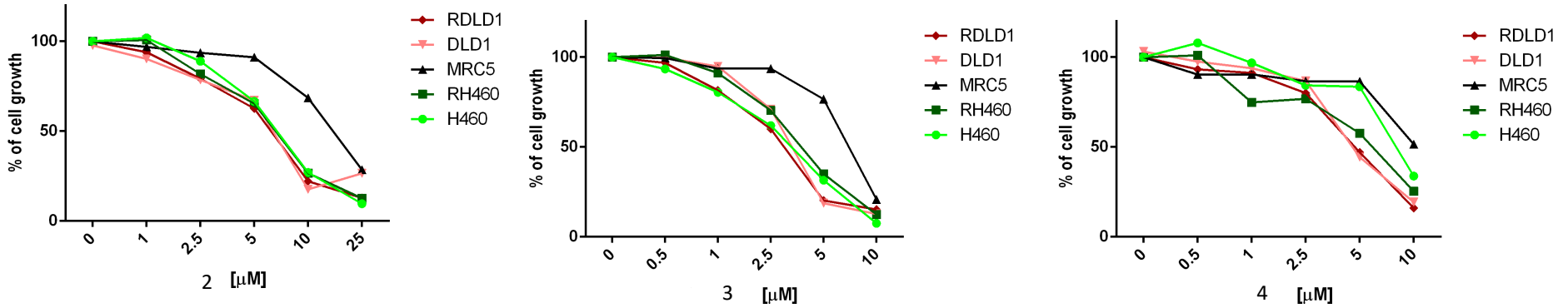


Fig. 3. Antiproliferative activity of derivatives **2**, **3** and **4** by MTT assay

Results and Discussion

- **2**, **3** and **4** showed **reduction in P-gp activity** in Rho123 assay and indicated P-gp **inhibition** in DOX

Derivative	P-gp *in silico* Modulation Prediction	FAR RH460	SI RH460	FAR RDLD1	SI RDLD1
1	++++	1.11	4.98	1.09	6.02
2	++++	1.78	**7.96**	1.41	**7.85**
3	++	2.64	**11.81**	4.28	**23.72**
4	+	3.78	**16.93**	9.31	**51.66**
5	++++	1.06	4.73	1.01	5.59
6	Not tested	1.09	4.88	1.27	7.02

Table 2. P-gp inhibition in MDR cell lines of derivatives **1**-**6**

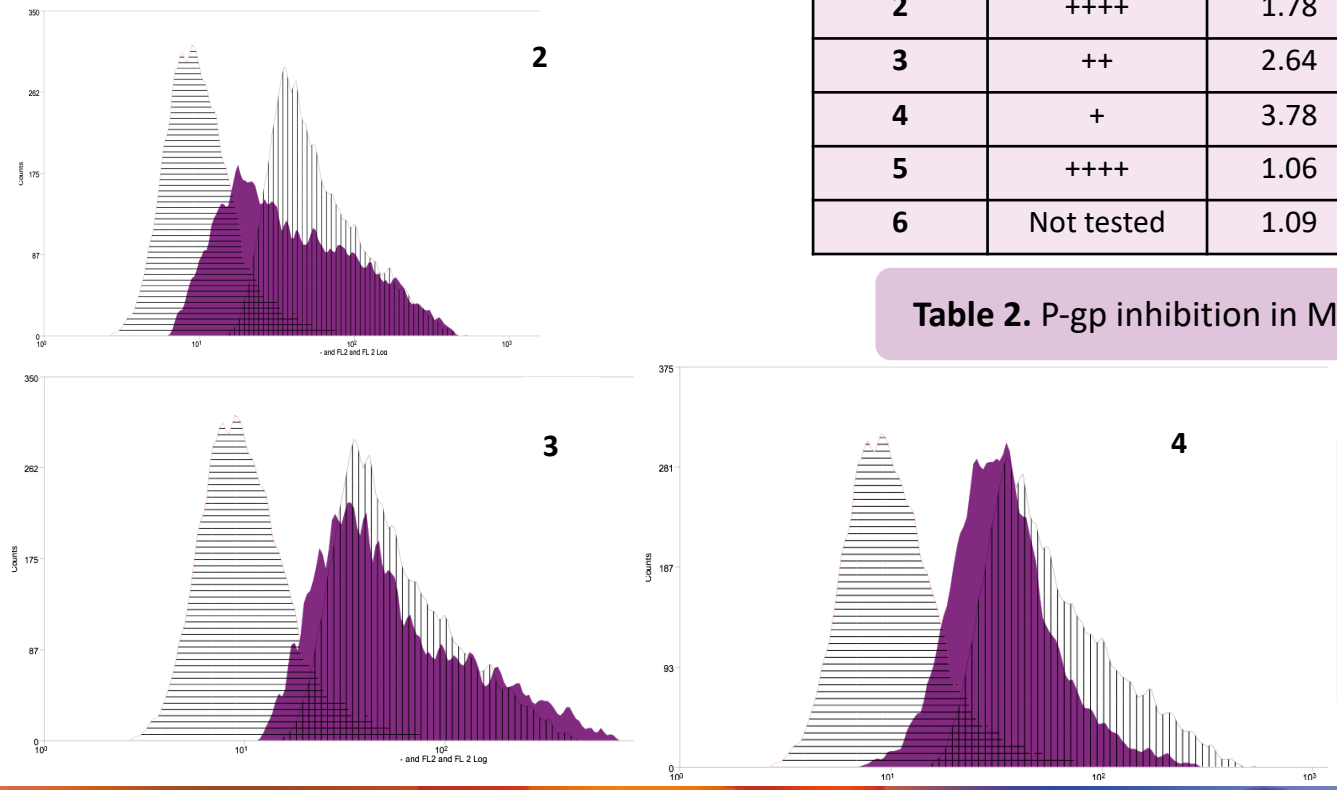


Fig. 4. Increased Rho123 accumulation in RH460 cell line (purple) when treated with derivatives **2**, **3** and **4**, when compared to untreated RH460 cell line (horizontal), using sensitive H460 (vertical) using as control.

Results and Discussion

- One benzoylated derivative selectively activated PKC-δ, while the natural compound **DeRoy** displayed improved PKC activity, compared with the positive control, in all tested isoforms.

cpds	EC_{50} (nM)				
	PKCα	PKCβI	PKCδ	PKCε	PKCζ
PMA	111.6 ± 18.4	243.2 ± 69.1	573,8 ± 36.7	1678 ± 46.48	-
ARA	-	-	-	-	205.4 ± 32.6
DeRoy	15 ± 1.9	0.97 ± 4.34	3.1 ± 60	5.8 ± 0.70	43.8 ± 2.32
Roy	350 ± 42	423 ± 67	ND	994 ± 63	4113 ± 159
Roy-Bz	ND	ND	107.53[5]	ND	ND
Roy-Pr	195 ± 16	229 ± 21	325 ± 49	770 ± 46	ND

Table 3. EC_{50} values of compounds and analogues tested on individual PKC isoforms

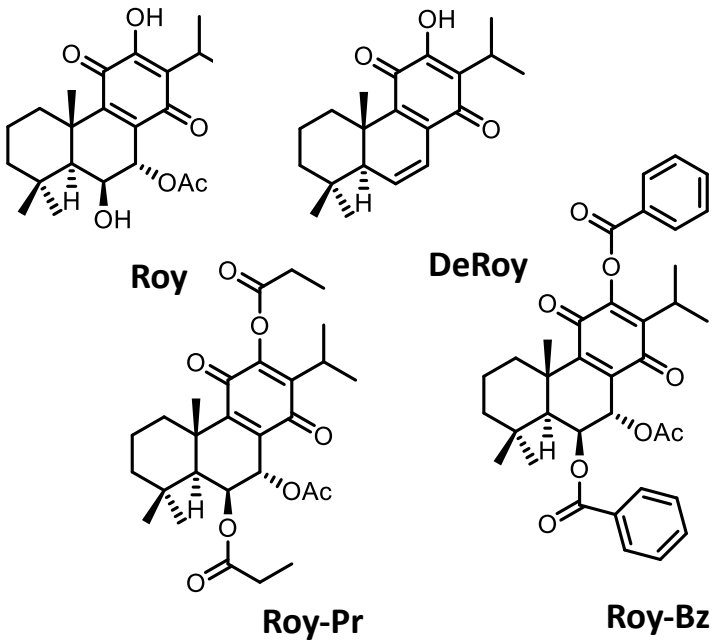
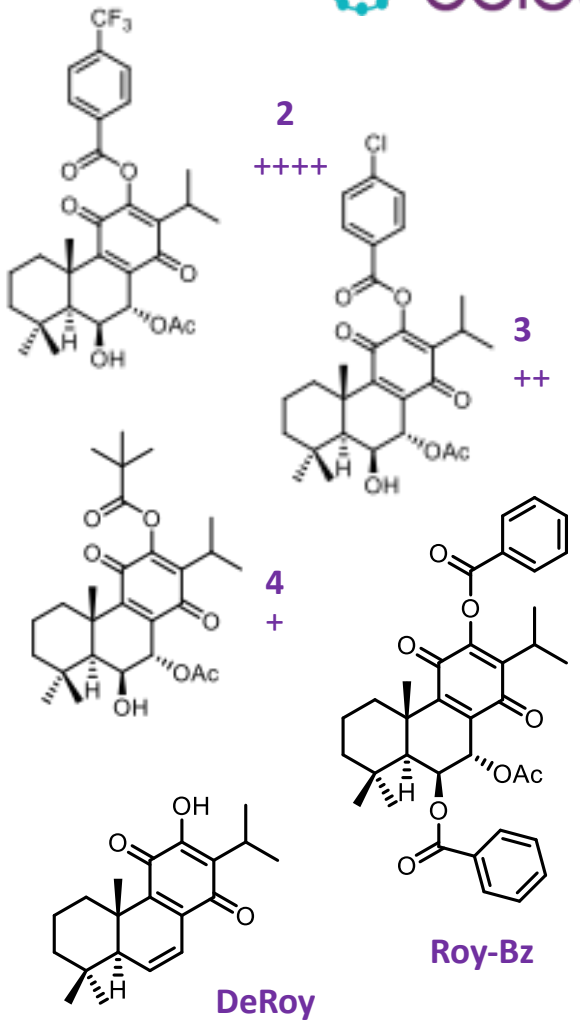


Fig 5. Structures of two natural compounds **Roy** and **DeRoy** and two derivatives tested in PKC isoforms

Conclusions

- In Rho123 assay **2**, **3** and **4 reduced P-gp activity**
- The **DOX assay** showed increased accumulation of DOX with **2**, **3** and **4** in *both* resistant cell lines, indicating **P-gp <u>inhibition</u>**
- P-gp modulation predictions were not completely coherent
- The **Roy-Bz** derivative selectively activated PKC-δ
- **DeRoy** displayed improved PKC activity, compared with the positive control, in all tested isoforms



Current and Future work

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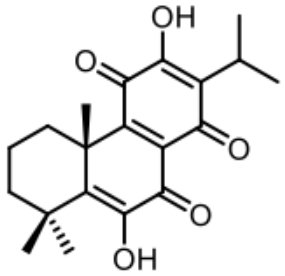
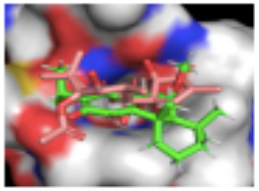
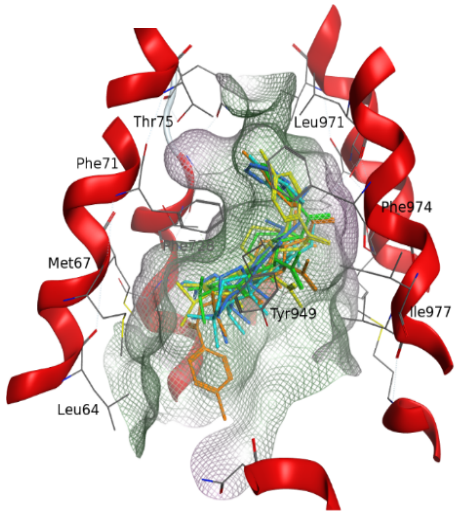


Coleon-U-Quinone



***In silico* studies**



- Complete structural elucidation
- SAR
- Refine MD
- Analogues
- Nanoparticle dev.

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