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7α-acetoxy-6β-hydroxyroyleanone as lead compound: from extraction optimization to hybrid nanoparticles for breast cancer therapy

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pharmaceuticals MDPI

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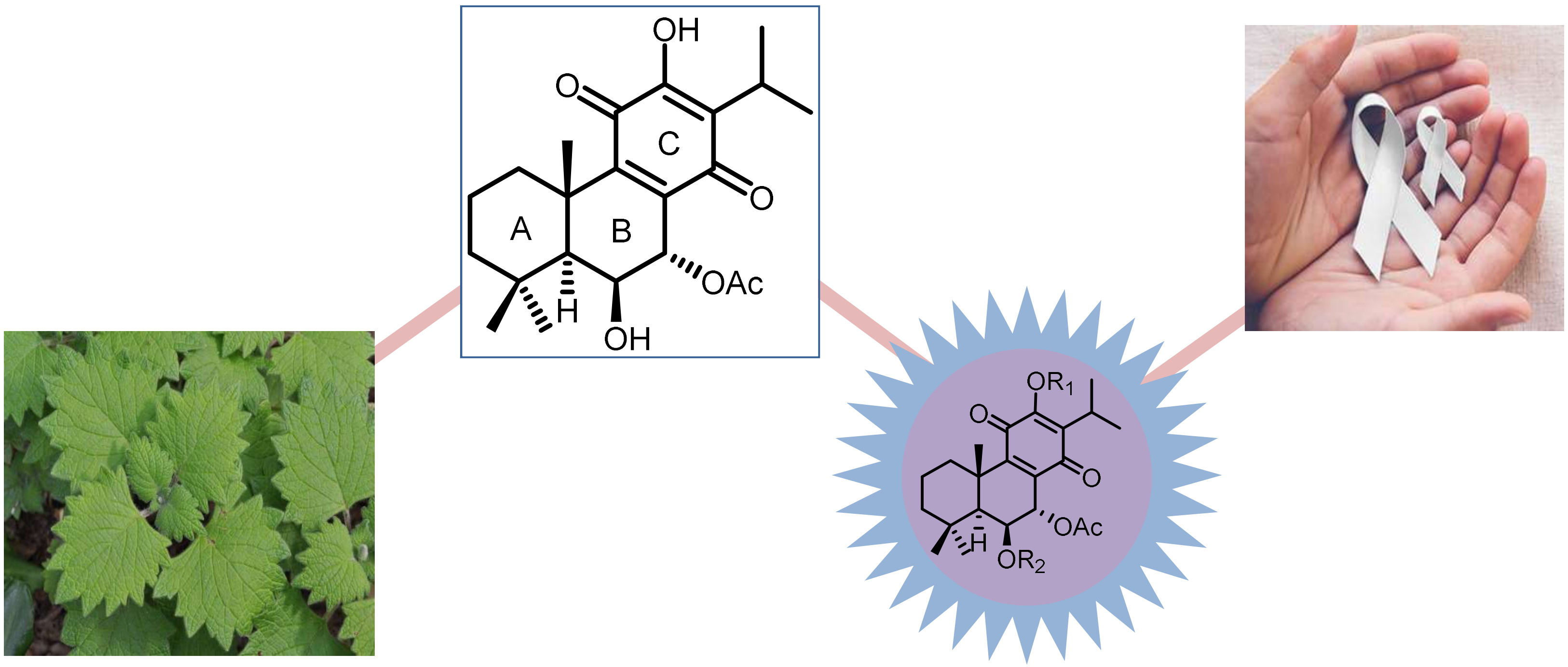
7α-acetoxy-6β-hydroxyroyleanone as drug lead: from extraction optimization to hybrid nanoparticles for breast cancer therapy

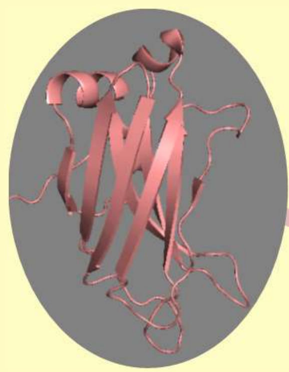
Graphical Abstract



Abstract: Breast cancer is the most prevalent cancer worldwide. *Plectranthus* spp. have been used in traditional medicine for various ailments, including cancer, and its bioactive components have been investigated for their potential anticancer effects. In particular, the compound 7α-acetoxy-6βhydroxyroyleanone (Roy, **1**) displayed promising antiproliferative activity against several cancer cell lines. However, Roy **1** is highly hydrophobic and consequently has low water solubility, limiting its therapeutic use. Nanoformulations offer a potential solution. Accordingly, Roy **1** was investigated as a lead compound for the development of new antitumoral drugs through extraction optimization from P. grandidentatus, study of reactivity, evaluation of aqueous stability, and synthesis of Roy **1** gold nanoparticles (NPs). The acetonic ultrasound assisted extraction method was optimized by performing three cycles of 30 minutes each, which improved the isolation yield (46.8 ± 11.25 µg.mg-1). The reactivity of Roy **1** was explored to prepare new bioactive esters. Consequently, a new (**4**) and three known (**2**, **3**, and **5**) ester derivatives were prepared. Considering the stability study, compounds **1**, **2**, and **3** were evaluated and results indicate that all of them were completely water stable (concentration of 0.1 mM, pH 7.4, and 37°C, for 10 days). Moreover, **1_NPs** were successfully synthetized and exhibit promising physicochemical characteristics and an impressive 74.9% drug conjugation efficiency. Additionally, natural **1**, derivatives **2**-**5**, and **1_NPs** system exhibit significant activity against the aggressive breast cancer cell line, MDA-MB-231. These findings represent a significant advancement in our ongoing efforts to develop novel therapeutic agents and drug delivery platforms.

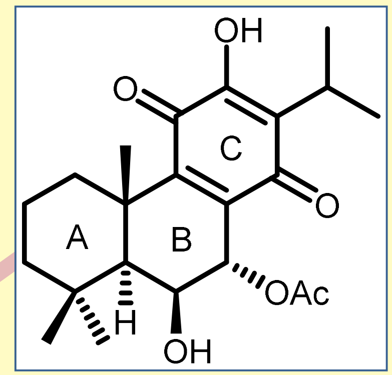
Keywords: Plectranthus; royleanone; derivatives; stability; antitumoral activity; nanosystem

Introduction



PKC activation

Plectranthus spp.



7α-acetoxy-6β-hydroxyroyleanone (Roy **1**)

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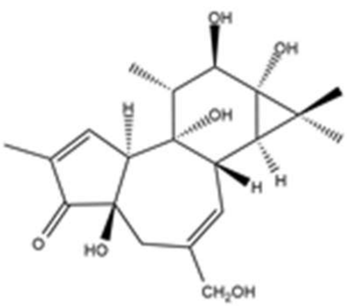
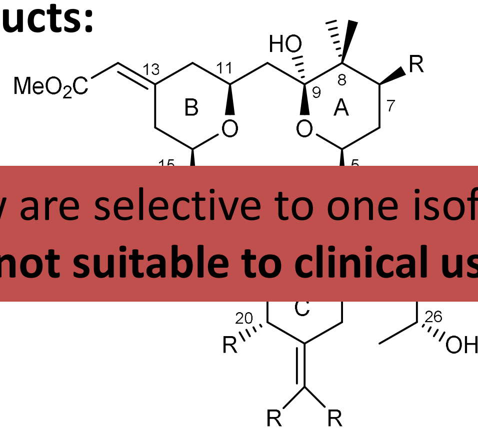
Protein kinase C (PKC)

- Associated with proliferation, migration, invasion, tumorigenesis, and metastasis
- **Target of many natural products:**

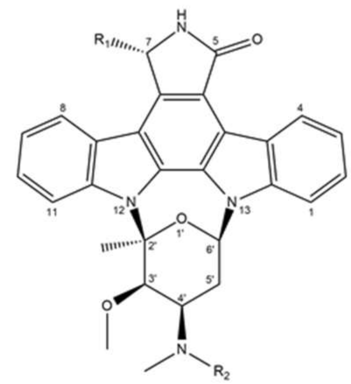


Phorbol Esters



Few are selective to one isoform:
not suitable to clinical use

Bryostatins



Staurosporine Analogues

Breast cancer therapy: PKC-α activation

Breast cancer caused 685 000 deaths globally in 2020.

Colon cancer therapy: PKC-δ activation

Colorectal cancer is the third most common cancer worldwide.

Matias D, Bessa C, Simões MF, Reis CP, Saraiva L, Rijo P. Natural Products as Lead Protein Kinase C Modulators for Cancer Therapy, in: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, 2016, pp. 45–79.
https://www.who.int/

Plectranthus genus

Plantae

Magnoliophyta

Magnoliopsida

Lamiales

Lamiaceae

Plectranthus

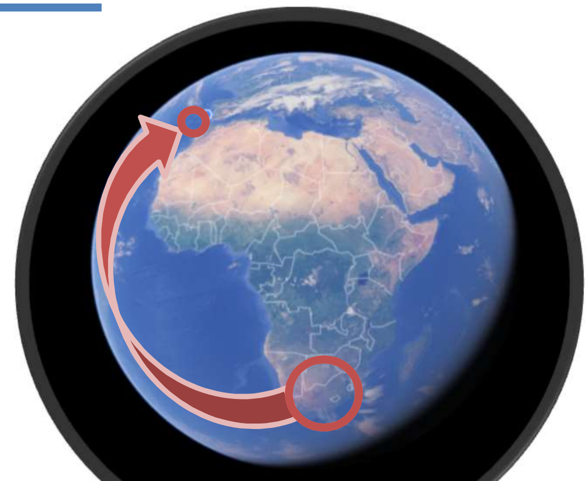
Includes more than 300 species

Basil

Rosemary

Mint

Oregano



Plants obtained from South Africa and cultured in Portugal (Instituto Superior de Agronomia de Lisboa)

Ladeiras D, Monteiro CM, Pereira F, Reis CP, Afonso CAM , Rijo P. (2016) Curr Pharm Des, 22(12), 1682–1714.
Rice LJ, Brits GJ, Potgieter CJ, Van Staden J (2011) S Afr J Bot 77: 947-959.
Lukhoba CW, Simmonds MSJ, Paton AJ (2006) J Ethnopharmacol 103:1-24.

Plectranthus genus

Current uses:

→ **Ornamental**
→ **Ethnobotanical**
→ **Household**

P. ecklonii

Unusual applications:

P. ciliatus → Used to wash clothes
P. hadiensis → Fish poison; Charm
P. laxiflorus → Mosquito repellent
P. neochilus → Air purifier
P. unguentarius → Pomade

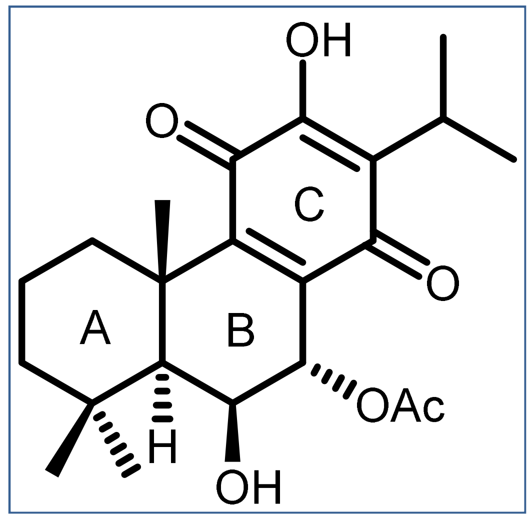
Food and Food additives:

Nine species reported to be edible and three used as food additives

P. esculentus

Rice LJ, Brits GJ, Potgieter CJ, Van Staden J (2011) S Afr J Bot, 77: 947-959.

P. grandidentatus Gürke



7α-acetoxy-6β-hydroxyroyleanone **(Roy 1)**

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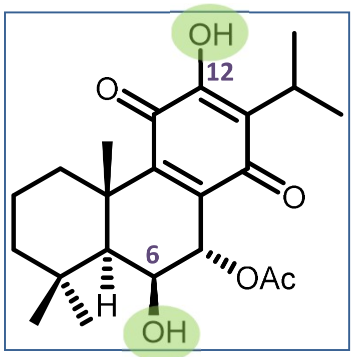
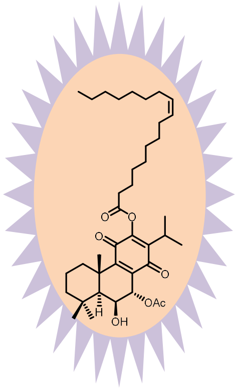
Cytotoxic abietane diterpenes



Roy (1)

Poorly water-soluble compounds

Nanotechnology:
- Drug solubility
- Targeted delivery



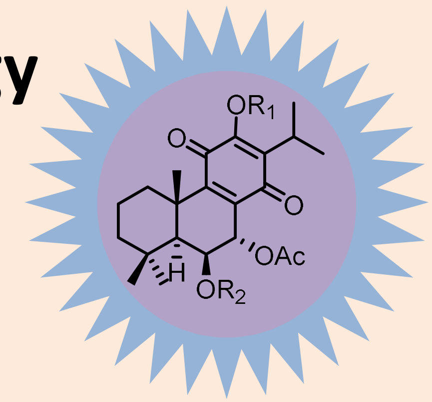
Self-assembly nanosystem: low cytotoxicity on Vero-E6 cells; may act as a prodrug

Matias D, Nicolai M, Saraiva L, Pinheiro R et al., Rijo P (2019) ACS Omega, 4(5): 8094-8103
Ntungwe E, Domínguez-Martín E, Bangay G, Garcia C, et al., Rijo P (2022) Int J Mol Sci, 22(19): 10210

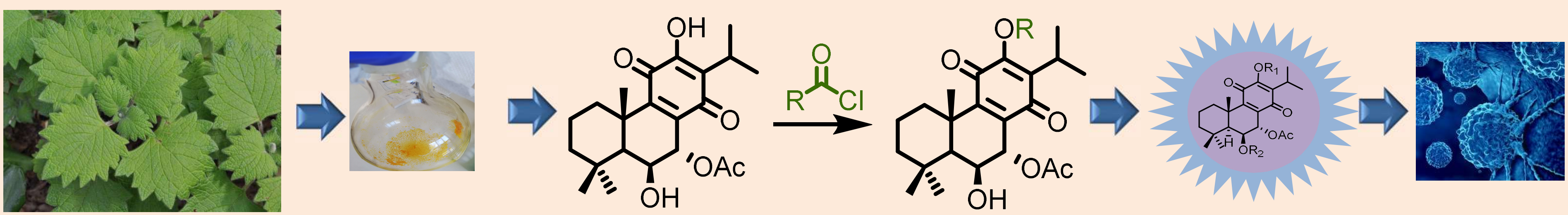
Objectives

Enhance cytotoxic activity of Roy 1 for anticancer therapy

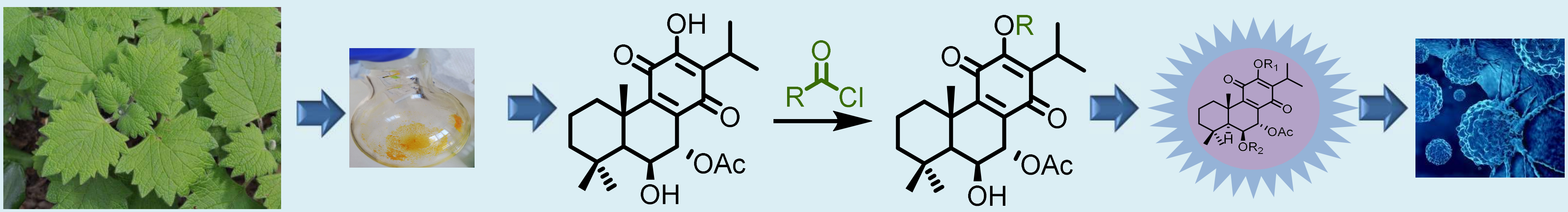
Methodology



- Extraction and isolation
- Reactivity study
- Stability study
- Nanoparticles syntesis
- Effect on triple negative human breast cancer cell lines



Results and discussion



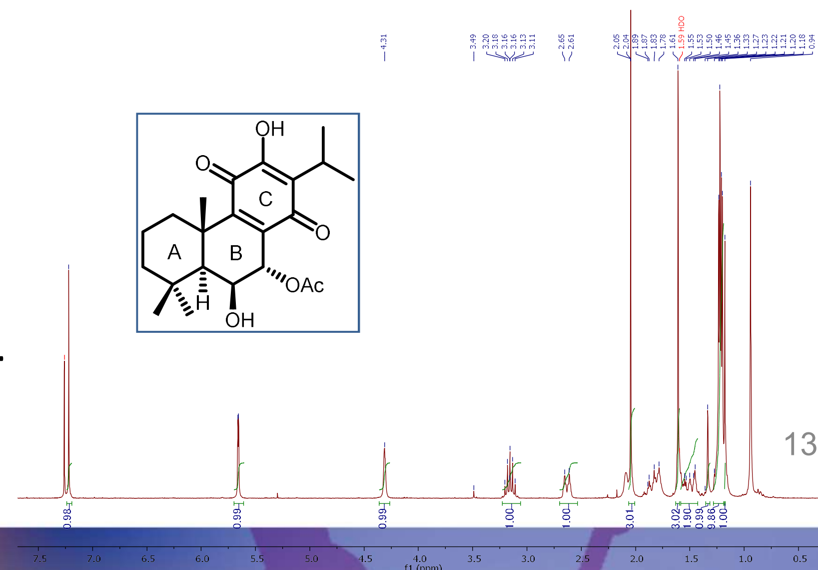
Extraction and isolation

2.464 Kg *P. grandidentatus* dry aerial parts

Ultrasound assisted-extraction with acetone: 30 minutes in ultrasound (3x)

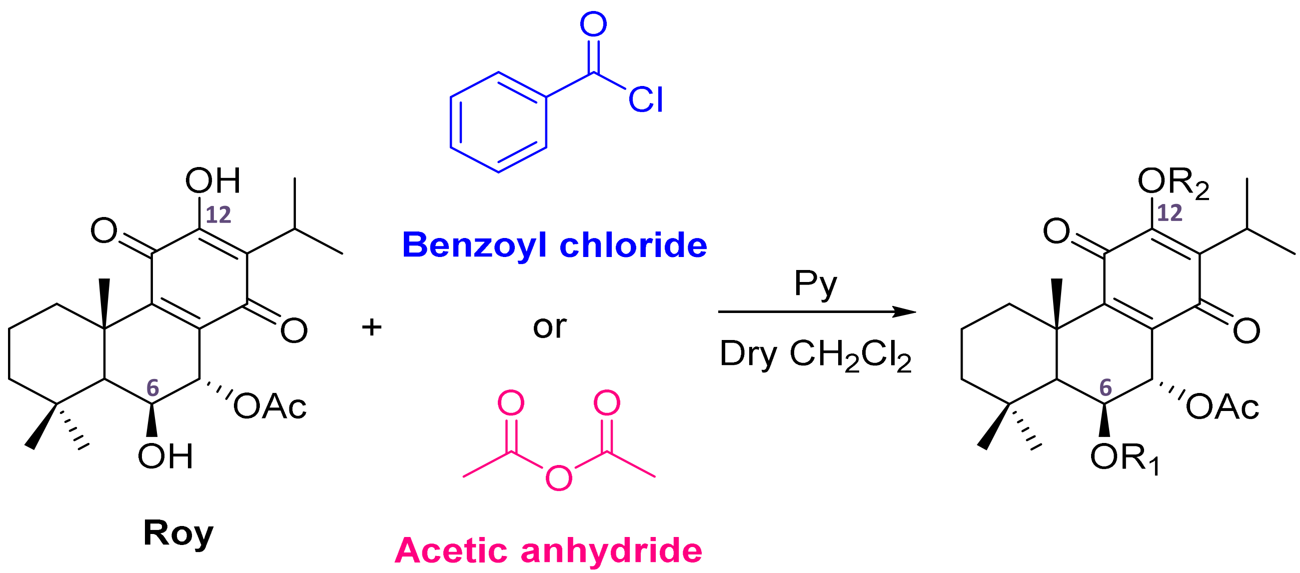
57.41 g of extract (**2.3% w/w**)

- **46.8 µg.mg^{-1}** of **1** in the extract (**0.1 % w/w**)
- Isolation in dry flash chromatography
- Recrystallization from *n*-Hexane to afford pure **1**
- Structure characterization by NMR



Roy Reactivity study

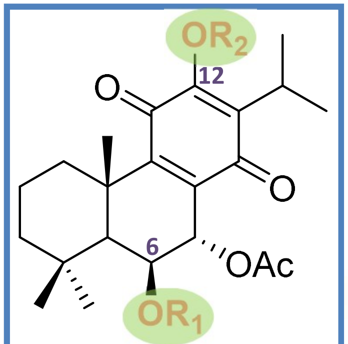
Optimization of Roy esterification:



- Amount of reagent
- Amount of pyridine
- Base
- Temperature
- Time
- Work up

Roy Reactivity study

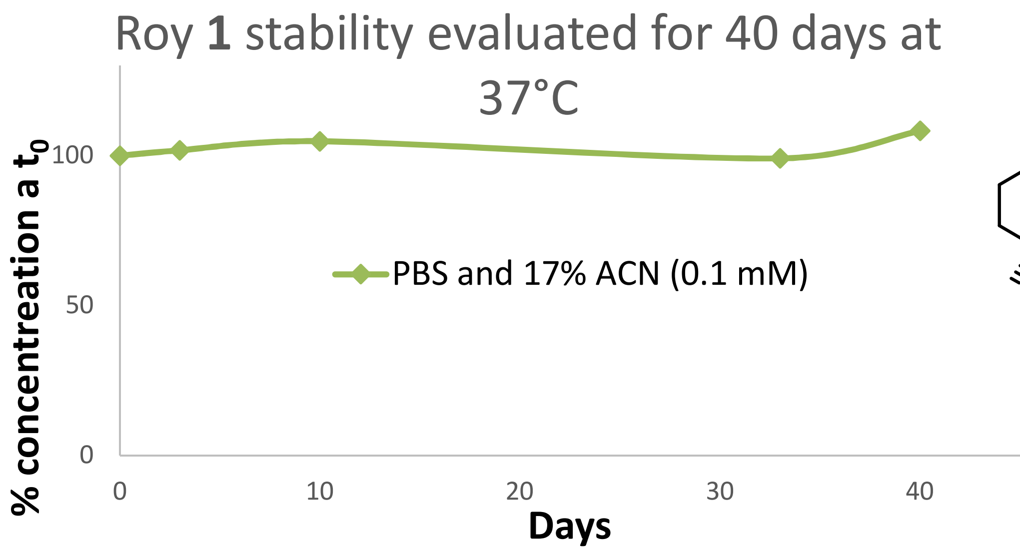
Entry	Reagent / eq	Pyridine (mL)	Temp (°C)	Time (min)	Product/ yield (%)
1	**Benzoyl Chloride** 15	catalytic	Rt	60	**2** 69 %
2	**Benzoyl Chloride** 4	catalytic	0	60	**2** 58 %
3	**Benzoyl Chloride** 100	1	50	120	**3** 28 %
4	**Benzoyl Chloride** 100	0.5	50	Overnight	**3** 79 %
5	**Acetic Anhydride** 1	0.5	Rt	15	**4** 77 %
6	**Acetic Anhydride** 8	0.5	0	15	**4** 86 %
7	**Acetic Anhydride** 100	1	50	Overnight	**5** 48 %

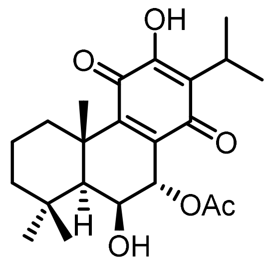


2: R_1= H; R_2=Bz
4: R_1= H; R_2=Ac
3: R_1= R_2=Bz
5: R_1= R_2=Ac

- 12-OH → Mild conditions
- Both 12 and 6-OH → 50ºC, overnight, pyridine as solvent

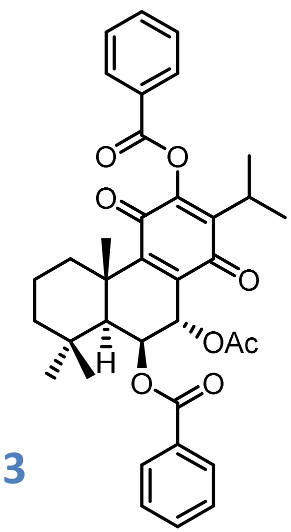
Stability study



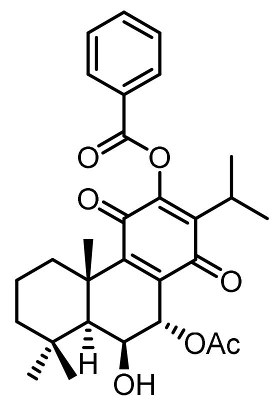


1

❖ **2** and **20** also dysplayed stability in aqueous medium



3

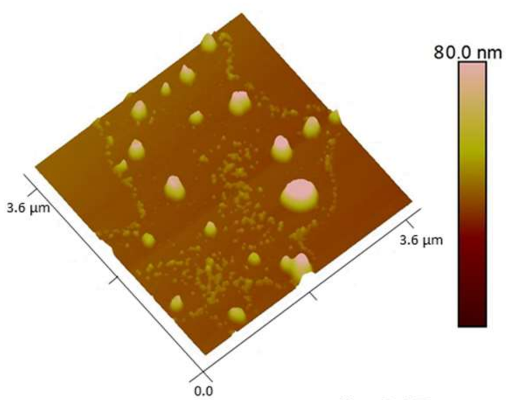


2

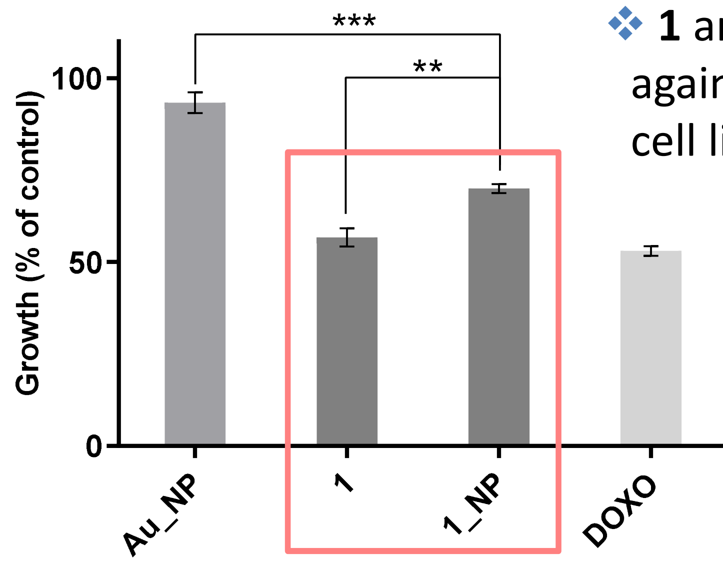
Synthesis of Nanoparticles (NPs)

NPs / Concentration	Size (nm)	PI	ZP	Loading efficiency (%)
1_NPs 0.02 mM	50.17	0.244	-8.02	-
1_NPs 0.1mM	209.13	0.283	-3.10	74.9%
1_NPs 5mM	53.99	0.355	-8.71	22.6%

- Promising physicochemical characteristics
- 75% loading efficiency



1_NPs



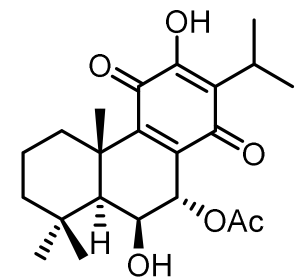
- **1** and **1_NPs** cytotoxic against breast cancer cell line MDA-MB-231

Conclusions

P. grandidentatus

Phytochemistry:

- Extraction yield of 2.3 % (w/w)
- 46.8 µg·mg^{-1} Roy **1** in extract → Optimization of the ultrasound acetonic extraction
- Isolation of 1.0 g of Roy **1** for hemi-synthesis

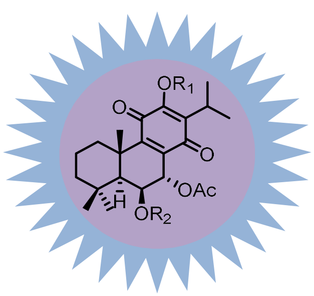


1

Optimization of esterification conditions:

- 12-OH seems the most reactive
- For both positions derivatization, high temperature (50 °C), excess of reagents, and higher reaction time

Nanoformulation:



- **1_NPs** system exhibit promising physicochemical characteristics and 75 % drug conjugation efficiency
- **1** and **1_NPs** cytotoxic against aggressive breast cancer cell line MDA-MB-231.

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