The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)

01–30 NOVEMBER 2021 | ONLINE

Self-assembly nanoparticles of natural bioactive abietane diterpenes

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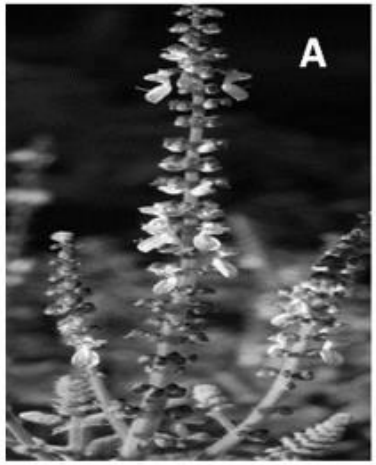
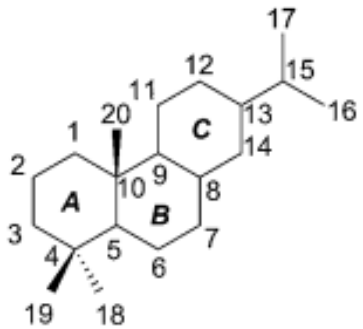
Graphical Abstract



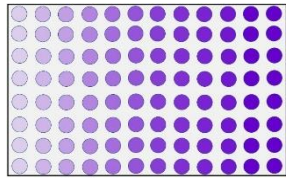
A. P. hadiensis
B. *P. madagascariensis*

Cytotoxic Royleanones



I, abietane skeleton

Self assembly nanoparticles



Cytotoxicity study by MTT

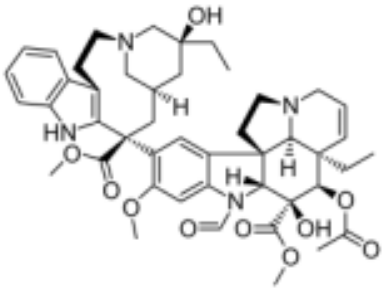
Abstract:
Self-assembly nanoparticle (NP) is one of the most promising methods in drug delivery. This method allows nanoparticles to be generated using an inducer that can assemble spontaneously by electrostatic interactions or noncovalent interactions. [1]. Natural products remain a good source of bioactive compounds for the treatment of diseases including cancer [2]. In this work 7α-acetoxy-6β-hydroxyroyleanone (Roy), 7α-acetoxy-6β-hydroxy-12-benzoyloxyroyleanone (12BzRoy), and 6,7-dehydroroyleanone (DHR) from *Plectranthus* species with cytotoxicity were used as lead compounds for the synthesis of self-assembled conjugates. These royleanones were conjugated to squalene (sq), oleic acid (OA), and/or 1-bromododecane (BD) self-assembly inducers. Roy-OA, DHR-sq, and 12BzRoy-sq conjugates were successfully synthesized and characterized. Their nanoassemblies were characterized by DLS. Roy-OA NP and DHR-sq NP assemblies show promising size, Pdl, zeta potential, and spherical morphology from SEM. More so, Roy-OA NPs had a low release of Roy at physiological pH 7.4 after 24 h. The cytotoxic effect of DHR-sq, Roy-OA, and their corresponding NPs was also assessed in different cancer cell lines. The cytotoxicity of DHR-sq NPs and Roy-OA NPs were lower when compared with DHR and Roy respectively. Our results, therefore, suggest that the nanoassemblies can act as prodrugs for the release of cytotoxic lead molecules.

Keywords: Nanoparticle ; royleanones; Self-assembly

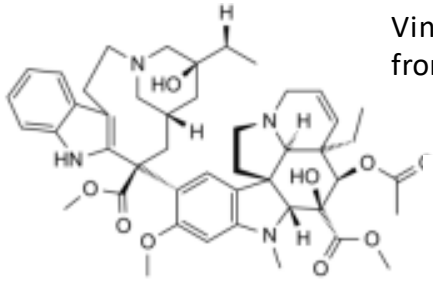
Natural products: Source of Lead molecules

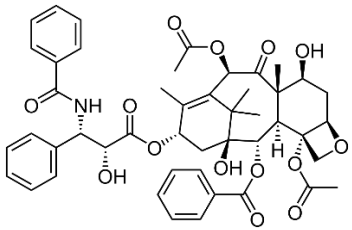
From medicinal plants

Anticancer



Vinblastine and Vincristin
from *Catharanthus roseus*





Paclitaxel
(*Taxus baccata*)

Drugs in clinical use:

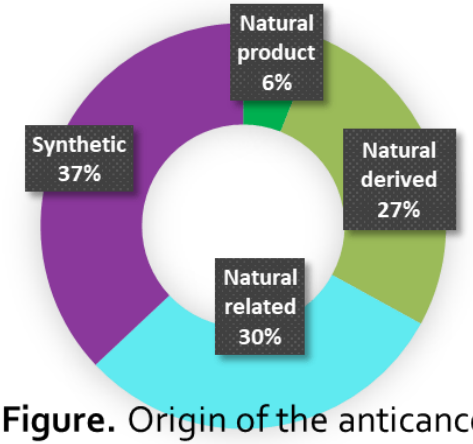


Figure. Origin of the anticancer compounds with approved clinical application in the period 1930-2014.

Newman DJ, Cragg GM; Natural Products as Sources of New Drugs from 1981 to 2014; J Nat Prod. 2016 Mar 25;79(3):629-61.

Plectranthus genus as a valuable source of bioactive compounds

- ***Plectranthus* genus** (*Lamiaceae* family)
  - e.g. *Salvia officinalis* L. (Sage)
  - *Rosmarinus officinalis* L. *(Rosemary)*

- **Source** of **bioactive natural products**, namely **diterpenoids**

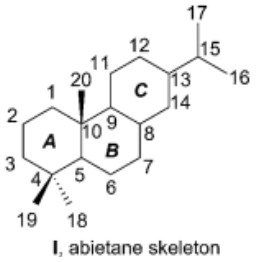
- **Traditionally used**:
  - Tropical Africa, Asia and Austrália
  - Introduced in the New World, following the Portuguese Discoveries (XVI century): Africa and Brasil

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

C. Garcia, C. Teodósio, C. Oliveira, C. Oliveira, A. Díaz-Lanza, C. P. Reis, N. Duarte, P. Rijo. Naturally occurring *Plectranthus*-derived abietane diterpenes with antitumoral activities.

Naturally occurring *Plectranthus*-derived abietane diterpenes with antitumoral activities

- Phytochemical studies: *Plectranthus* extracts and/or isolated compounds
- Extensive chemical diversity (alkaloids, flavonoids, etc…)
- **TERPENES**



I, abietane skeleton

…Namely, **DITERPENES**

acyclic

tricyclic

bicyclic

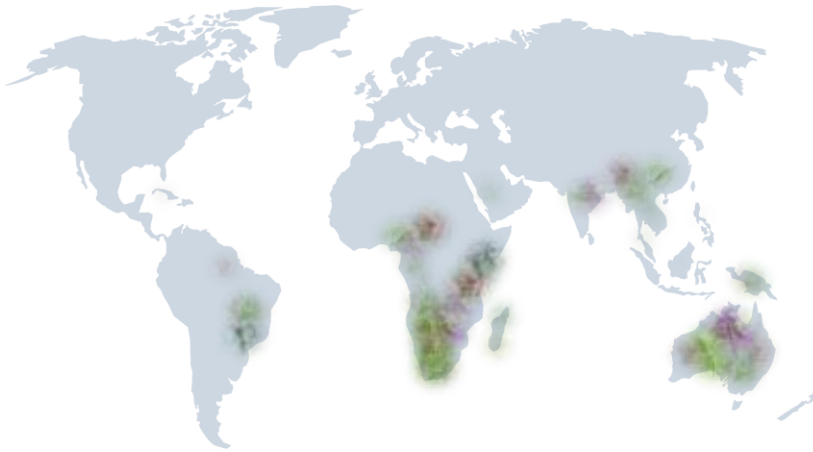
tetracyclic

pentacyclic

Macrocyclic

Abietanes

Bioactive



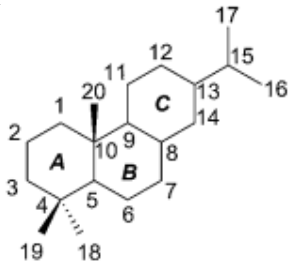
Plectranthus genus distribution

Garcia C. et al., Current Pharmaceutical Design, 2019, 24(36): 4207 - 4236

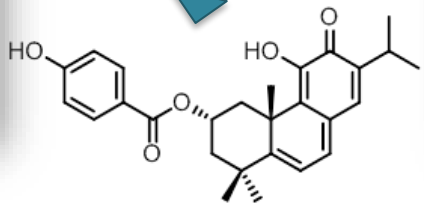
Bioactive diterpenoids from *Plectranthus* genus

Cytotoxic abietanes

Plectranthus ecklonni Benth.

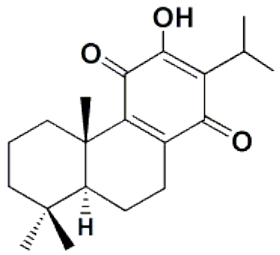


I, abietane skeleton

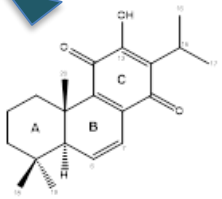


Parvifloron D

P. madagascariensis



Royleanone



6,7-dehydroroyleanone

Very low water-soluble compounds

Garcia C, et al Rijo P. Naturally Occurring Plectranthus-derived Diterpenes with Antitumoral Activities. Curr Pharm Des. 2018;24(36):4207-4236.

Strategies to promote a targeted delivery of these drugs to cancers cells

Development of novel nanosystems for anti-tumor therapy

- ✓ promising targeted delivery system
- ✓ enhance drug solubility
- ✓ Enhance permeability
- ✓ Retention effect at tumor site

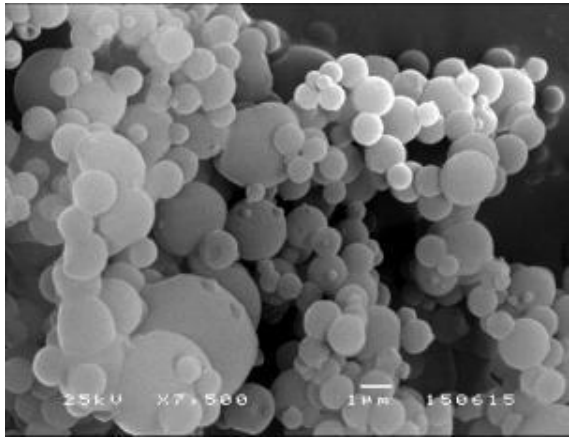


Figure: particle morphology by scanning electron microscopy Polymeric lactic acid (PLA) microparticles through spray-drying formulation

Natural Products with antiproliferative properties: studies for cancer treatment

Abietane diterpenoids:

- Carnosol (*Rosmarinus officinalis*)
- Coleon U (*Plectranthus sp.*)
- Ingenol (*Euphorbia sp.*)

Phenolic compounds:

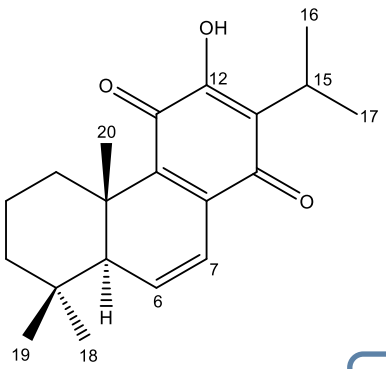
- Curcumin (*Curcuma longa*)
- Resveratrol (several sources)

Flavonoids:

- Quercetin

Matias et al. Chapter 2.Studies in Natural Products Chemistry, Vol. 50 (2017)

Another example:
Assembly of 6,7-dehydroroyleanone (DHR) with hybrid nanoparticles



6,7-dehydroroyleanone (DHR)

P. madagascariensis essential oil

- ***P. madagascariensis:***
  - Traditional use: skin and respiratory ailments
  - Cytotoxic compounds:
    - *7α-acetoxy-6β-hydroxyroyleanone*
    - *coleon U*

Catarina Garcia, PhD

- **Optimized extraction**:
  - High pressure extractions (yields= 0.45 to 5.90%)
  - Clevenger apparatus (yield=18%)

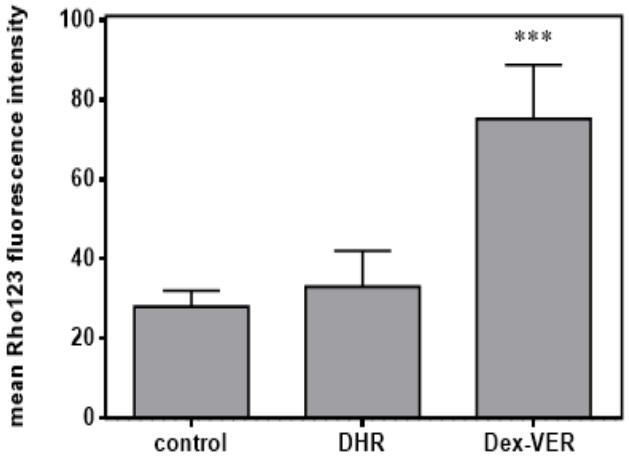
Clevenger apparatus

Garcia C. et al., Future Medicinal Chemistry, 2018, 10(10), 1177-1189

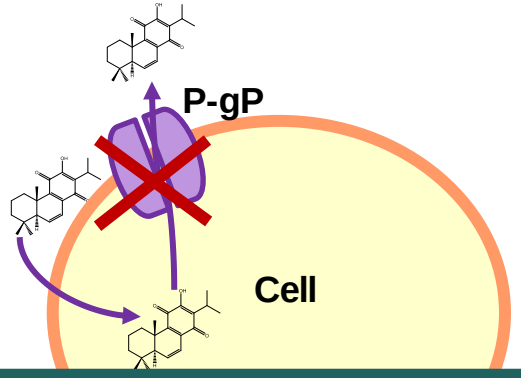
Anticancer properties of the abietane diterpene 6,7-dehydroroyleanone (DHR)

DHR cellular target evaluation

6,7-dehydroroyleanone target: P-glycoprotein (P-gP)



DHR effect on Rho 123 accumulation in P-gp overexpressing cells.

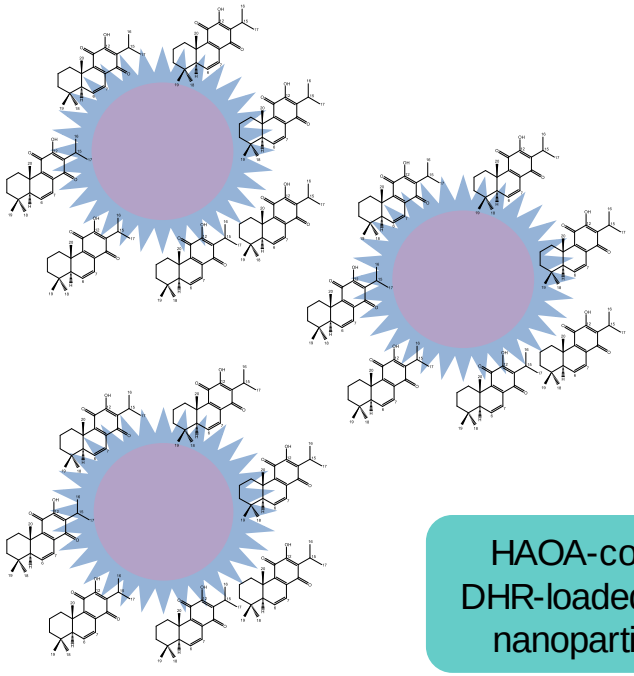


- Similar IC_{50} between sensitive and resistant cell lines
- Ability to **evade** the activity of **P-glycoprotein** responsible for drugs extrusion
- Not a substrate of P-gP

Garcia C. et al., Future Medicinal Chemistry, 2018, 10(10), 1177-1189

Assembly of 6,7-dehydroroyleanone (DHR) with hybrid nanoparticles

DHR hybrid nanoparticles: cytotoxic evaluation



HAOA-coated DHR-loaded gold nanoparticles

Potentiate the effect of DHR in cancer cells

- Production of hybrid nanoparticles
  - CE (%): 98.57±0.23%
  - Spherical form
  - Mean size: 281.1nm
  - PI: 0.1
- Cytotoxic evaluation of DHR and DHR hybrid-nanoparticles

Garcia C. et al., Future Medicinal Chemistry, 2018, 10(10), 1177-1189

Assembly of 6,7-dehydroroyleanone (DHR) with hybrid nanoparticles

Polymeric Coated Gold Nanoparticles (GNPs) Loaded with 6,7-dehydroroyleanone (DeRoy)

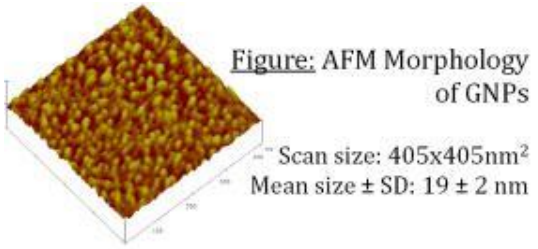


Figure: AFM Morphology of GNPs

Scan size: 405x405nm²
Mean size ± SD: 19 ± 2 nm

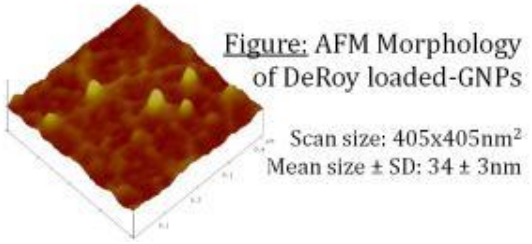


Figure: AFM Morphology of DeRoy loaded-GNPs

Scan size: 405x405nm²
Mean size ± SD: 34 ± 3nm

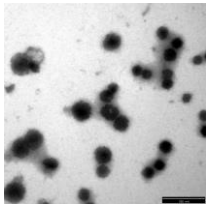


Figure: TEM analysis of Hybrid polymeric-gold nanoparticles GNPs

- The empty nanoparticles exert no cytotoxic
- Enhance targeting action:
  - increasing effect is due to targeted delivery of DHR

Sample	IC_{50} (µg/mL)	
	NCI-H460	NCI-H460/R
DeRoy	4.10 ± 0.61	3.18 ± 0.32
GNPs	> 50	> 50
DeRoy loaded-GNPs	0.53 ± 0.06	0.65 ± 0.18

Comparison of IC_{50} values in human NSCLC sensitive and MDR cells

Improve the cytotoxicity effect of DeRoy

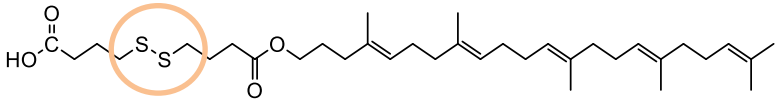
Cytotoxicity: Conjugation with H-NPS increases 5 to 8 fold

Silva C. et al. (WO2017095251) NANOSSISTEMA DE OURO COM REVESTIMENTO BIOPOLIMÉRICO E GAMA DE ABSORÇÃO NO INFRAVERMELHO PRÓXIMO E MÉTODO PARA A SUA PREPARAÇÃO. PCT/PT2016/000016.

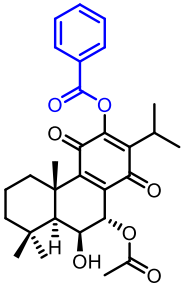
Self-Assembly Nanoparticles of Royleanones

Epole Ntungwe
PhD student

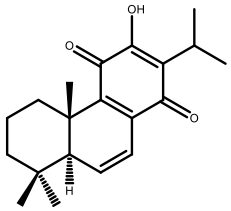
Squalene



Drug-conjugates:

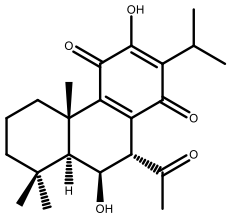


12RoyBz

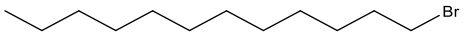


DHR

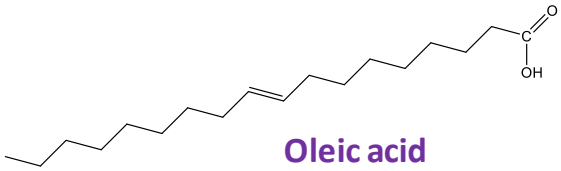
Royleanones



Roy



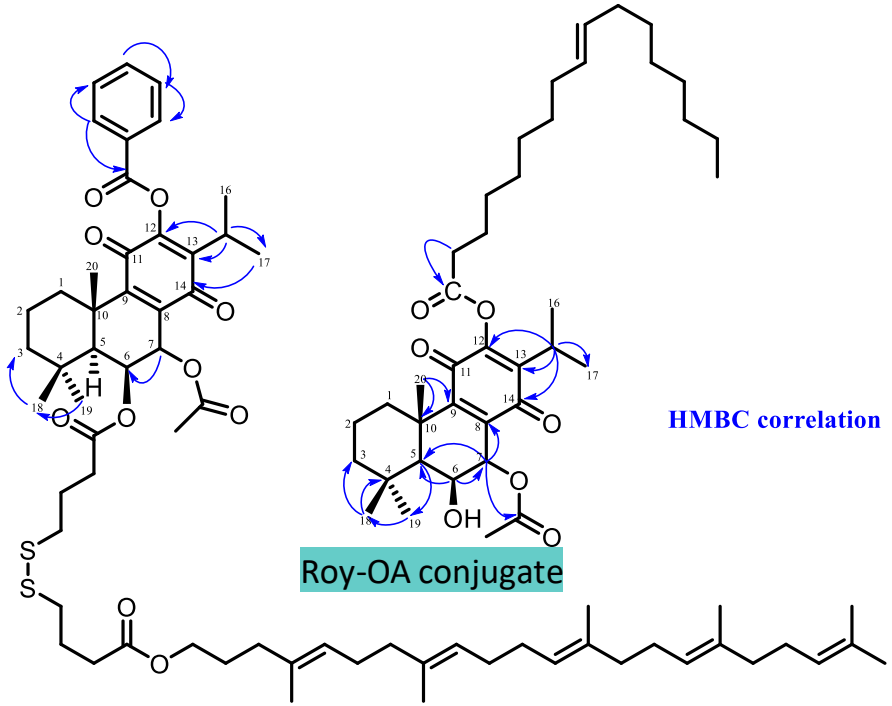
1-bromododecane

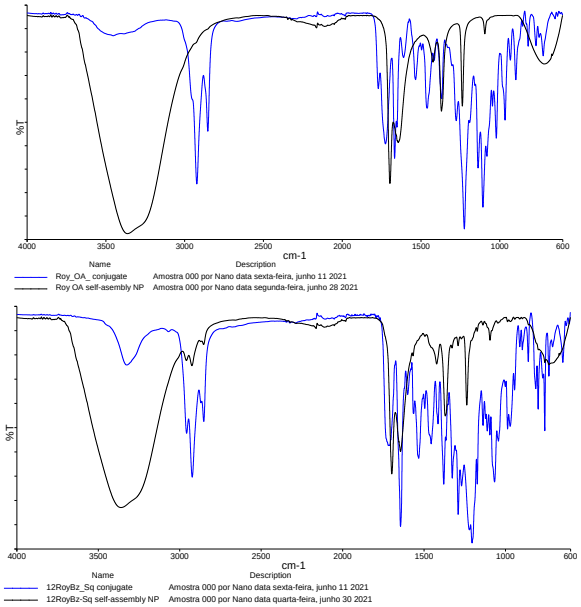


Oleic acid

Ntungwe, E.; et al. Self-Assembly Nanoparticles of Natural Bioactive Abietane Diterpenes. Int. J. Mol. Sci. 2021, 22, 10210. https://doi.org/10.3390/ijms221910210.

Self-Assembly Nanoparticles of Royleanones





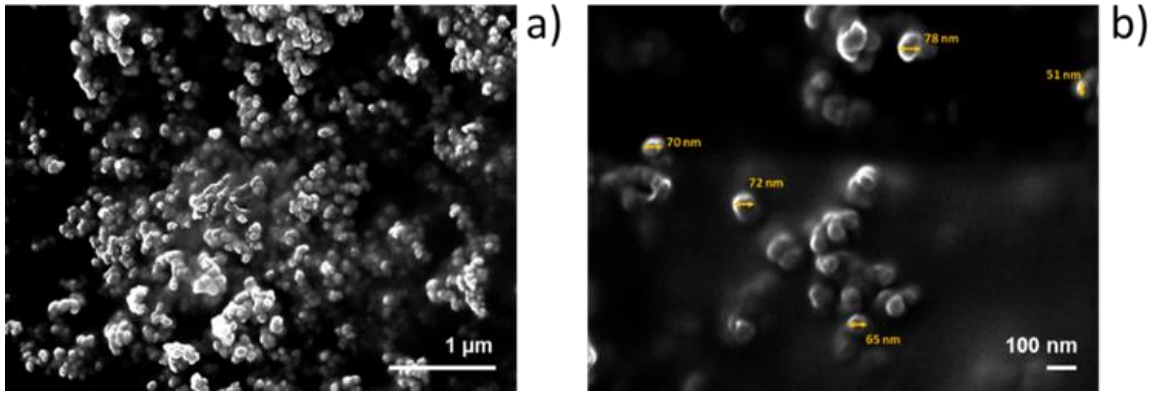
A. FTIR of Roy-OA conjugate, 5 (blue) and its Self-assembled NPs (**black**)

B. FTIR of 12BzRoy-Sq conjugate, 7 (blue) and its Self-assembled NP (**black**)

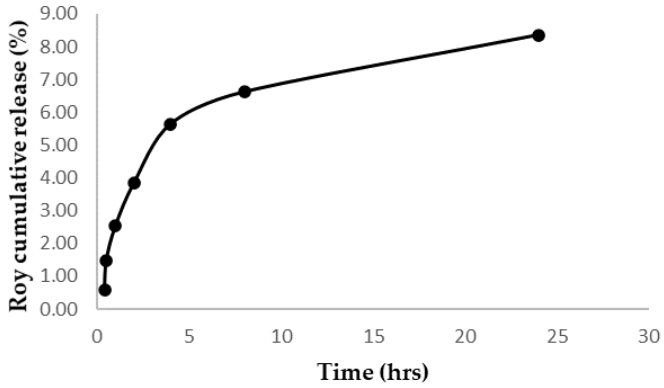
Ntungwe, E.; et al. Self-Assembly Nanoparticles of Natural Bioactive Abietane Diterpenes. Int. J. Mol. Sci. 2021, 22, 10210. https://doi.org/10.3390/ijms221910210.

Self-Assembly Nanoparticles of Royleanones

Sample	Size (d.nm)	PdI	Zeta Potential (mV)
ROY-OA NP Assembly	509.33 ± 4.29	0.249 ± 0.012	- 46.2 ± 0.4
12BzRoy-Sq NP Assembly	2739.33 ± 100.50	0.731 ± 0.187	- 28.9 ± 1.2



Scanning electron microscopy (SEM) images of Roy-OA NPs at a) lower (x25,000) and b) higher (x70,000) magnifications



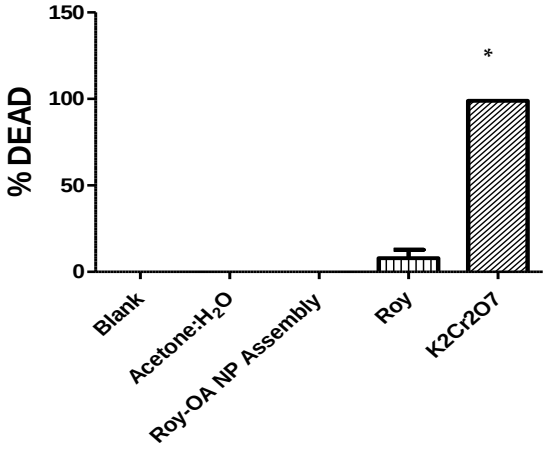
Cumulative release of Roy from Roy-OA NP in phosphate buffer saline adjusted to pH 7.4

Roy showed a slow release:
8.35 % was released from the NPs after 24 hrs

Ntungwe, E.; et al. Self-Assembly Nanoparticles of Natural Bioactive Abietane Diterpenes. Int. J. Mol. Sci. 2021, 22, 10210. https://doi.org/10.3390/ijms221910210.

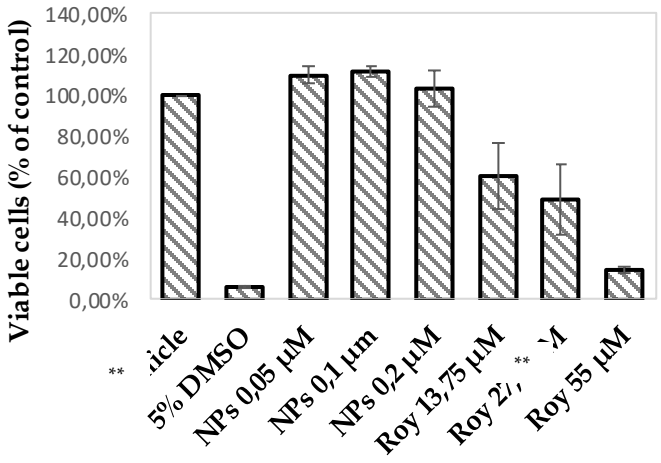
Self-Assembly Nanoparticles of DHR, 12BzRoy and Roy

Preliminary toxicity assay



- **Roy-OA NPs did not decrease cell viability high concentration of 0.2 µM**

- **Roy-OA NPs may act as a prodrug for the release of the cytotoxic Roy**



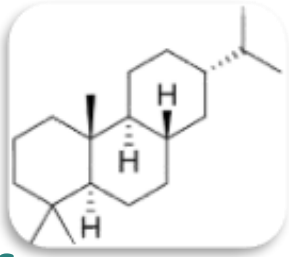
Brine shrimp lethal (BSLA) activity (%) against Roy and Roy-OA NP after 24 h expo-sure

Inhibition of cell viability assayed by MTT in Vero-E6 cells. **P-value <0.001

Ntungwe, E.; et al. Self-Assembly Nanoparticles of Natural Bioactive Abietane Diterpenes. Int. J. Mol. Sci. 2021, 22, 10210. https://doi.org/10.3390/ijms221910210.

Conclusions

- ✓ Natural products: source of potential new drug candidates
  - ✓ *Plectranthus* spp.: Bioactive Abietane diterpenoids
  - ✓ Low absorption and, unspecific toxicity



- ✓ Nanotechnology Strategies - Roy-OA NP:
  - ✓ promote a targeted delivery to cancers cells
  - ✓ strategy to deliver Roy:
  - ✓ increasing the water solubility:
    - ✓ suspension of self-assembly nanoparticles of Roy
  - ✓ In vitro release profile of Roy in NPs showed a delay on the relea
    - ✓ Important issue for a potential therapeutic application of Roy

Acknowledgments

Epole Ntungwe
Eva Dominguez
Andreia Rosatella
Gabrielle Bangay
Catarina Garcia
Iris Guerreiro
(Portugal)

Catarina Reis
M. Manuela Gaspar
Carlos A.M. Afonso
Noélia Duarte
Tânia S. Ferreira Gonçalves
(Portugal)

Daniele Passarella
Gaia Fumagalli
Eleonora Colombo
(Italy)

Lucília Saraiva
Cláudia Bessa
(Portugal)

Funding

UIDP/04567/2020

UIDB/04567/2020

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Aknowledgments

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