New Microbial Natural Products Targets Cancer Stem Cells and Stops Pancreatic Cancer Progression by Inhibiting MEK2-dependent Cell Signaling

Carmen Ramos[*1], Gloria Crespo[1], Nuria de Pedro[1], Carmen Griñán-Lisón[2], Caridad Díaz[1], Víctor González-Menéndez[1], Juan A. Marchal[2], Rosario Fernández-Godino[1], Olga Genilloud[1] and Fernando Reyes[1].*

* Carmen.ramos@medinaandalucia.es

[1]Fundación MEDINA, Parque Tecnológico de Ciencias de la Salud, Avda. del Conocimiento 34, 18016 Granada, Spain.

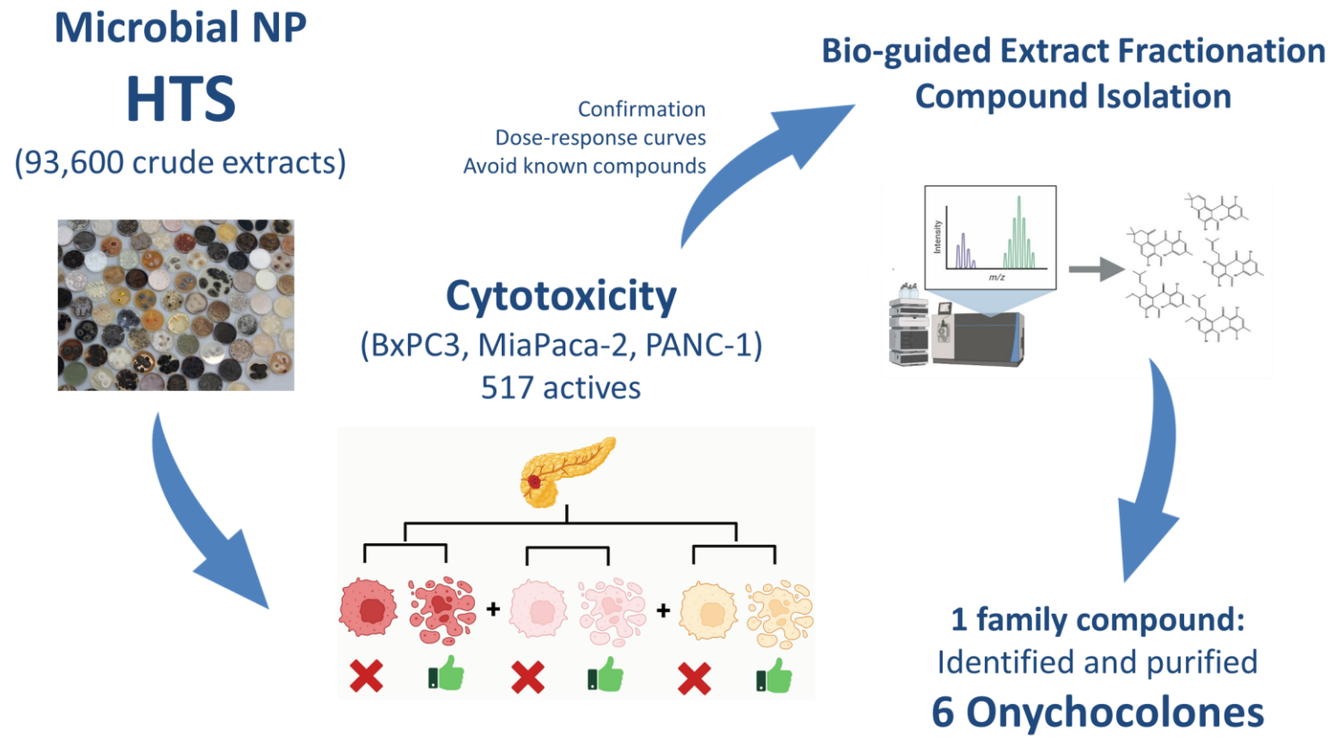
[2] Biosanitary Research Institute of Granada (ibs. GRANADA), University Hospitals of Granada-University of Granada, Granada, Spain.

www.medinadiscovery.com

Introduction

- **Natural Products (NPs)** are one of the main sources for cancer drug discovery. In the past 30 years, the percentage of NP or **NP-inspired new chemical entities (NCEs)** has risen to 74 %, in the antitumor arena [1].
- **Fundación MEDINA** owns one of the largest and most diverse microbial NP libraries in the world (>190.000 strains, >200.000 extracts) [2,3], which has been successfully used to identify bioactive compounds in different High-throughput Screening (HTS) campaigns.
- **Pancreatic cancer (PC)** shows a high fatality rate [4]. and has no cure. Thus, the identification of efficient chemotherapeutic agents is crucial.
- **In this study, we have identified and isolated 6 new benzophenone derivatives, onychocolones A-F from the fungus Onychocola sp with antitumoral activity in pancreatic Cancer Stem Cells (CSC) in vitro and in vivo.**

Compound identification and isolation



Hit selection

6 Onychocolones →

Confirmation anti-tumoral activity

Compound	MIA PaCa-2 (ED_{50} µM)*	BxPC-3 (ED_{50} µM)*	PANC-1 (ED_{50} µM)*	
1	4.3[4.1-4.7]	5.1 [4.9-5.3]	7.4[6.6-8.3]	✓
2	13.3[12.0-14.8]	16.5[15.7-17.3]	16.0[14.2-17.8]	✓
3	10.0[8.3-12.0]	20.3[18.8-22.0]	26.2[22.1-31.1]	✓
4	5.1[4.6-5.6]	4.5[4.2-4.7]	5.0[4.6-5.5]	✓
5	23.5[20.5-27.0]	28.8[26.6-31.2]	38.0[30.9-46.8]	X
6	> 50	> 50	> 50	X

Hit selection (pro-apoptotic activity)

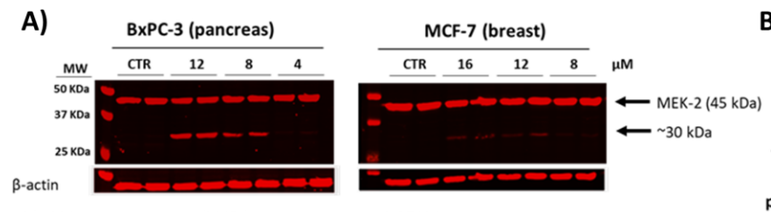
Compound	SubG0 (% of control)**	Annexin V (% of control)**	Caspase 3/7 activation (IC50 µM)	
1	13.3	68.3	25.2 [23.2-27.2]	✓
2	2.9	21.0	>50	X
3	3.0	36.2	18.4 [16.0-21.0]	X
4	12.3	32.4	36.1 [33.9-38.5]	X

** Cells treated with 10 µM of compounds

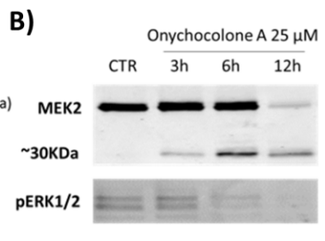
→ **Onychocolone A**

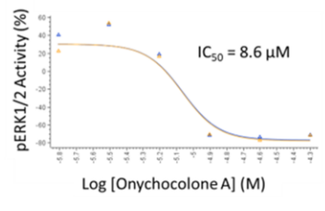
Mechanism of Action

Onychocolone A decreases MEK2 and ERK1/2 in pancreatic cells specifically



A) Western blot (WB) analysis of MEK2 shows an additional shorter band (30 kDa)* corresponding to a truncated MEK2 in pancreatic cancer cells but not breast cancer cells treated with onychocolone A.

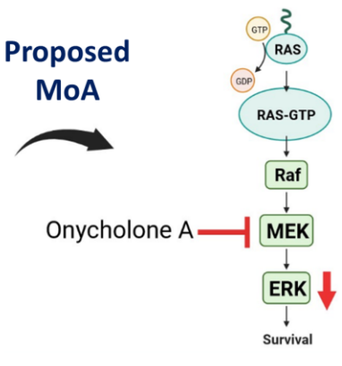




B) WB Validation of decreased levels of MEK2 and its substrate ERK1/2 in MIA PaCa-2 cells treated with 25 µM onychocolone A.

C) Dose-response curve shows a decreased activity of the MEK2 substrate, ERK1/2, in MIA PaCa-2 cells treated with onychocolone A for 24 h.

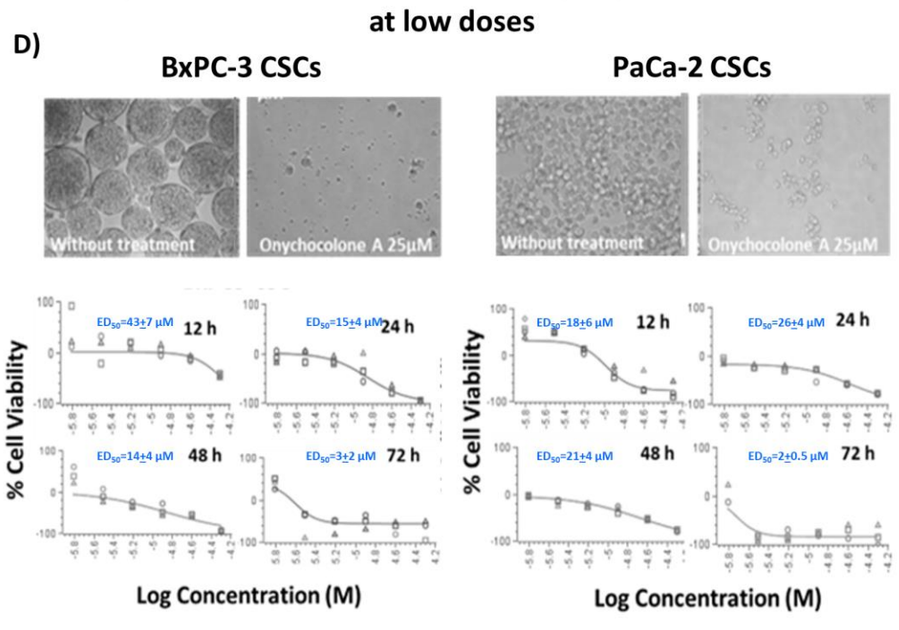
Proposed MoA



Effect on Cancer Stem Cells (CSC)

In vitro

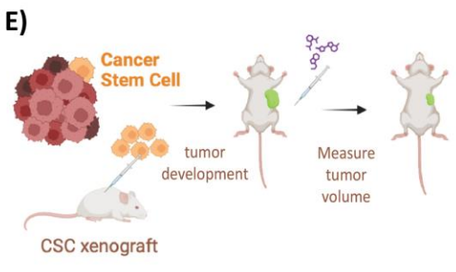
Onychocolone A reduces cell viability of pancreatic CSCs at low doses

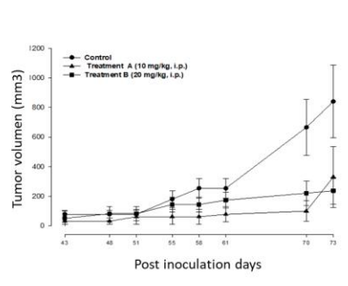


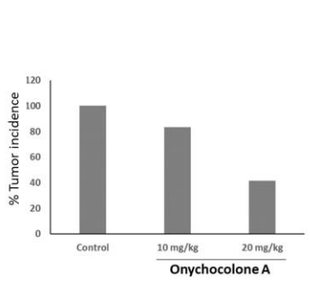
D) BxPC-3 and MIA PaCa-2 CSCs were generated using conditioned medium [5] and low attached culture plates. Next CSCs were treated with onychocolone A in dose and time-response curves. Cytotoxicity was measured by MTT.

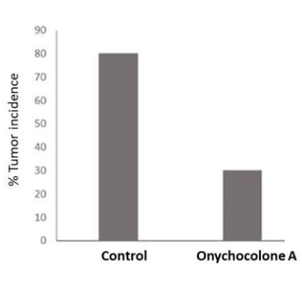
In vivo

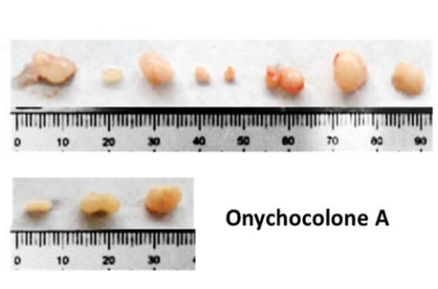
Onychocolone A in PBS or sesame oil reduced tumor growth of pancreatic CSCs in tumor xenograft mouse model

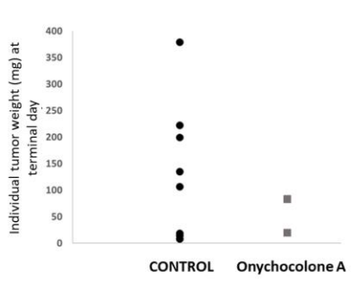












E) Experimental design; F) Tumor size and **G) Tumor incidence** diminished over time in nude mice injected with CSCs BxPC-3 and treated with 10 or 20 mg/kg of **Onycholone A in PBS; H) Tumor incidence**, **I) size**, and **J) weight** diminished in nude mice injected with CSCs BxPC-3 and treated with 2.5 mg/kg of **Onycholone A in sesame oil.**

Conclusions

- ❑ **Onychocolone A** showed a cytotoxic effect on pancreatic CSCs mediated by the **inhibition of the MEK onco-signaling pathway**.
- ❑ The ***in vivo*** efficacy of Onychocolone A was demonstrated by the **reduction of tumor growth** in a heterotopic pancreatic xenograft mice model generated by CSC.
- ❑ The data support that Onychocolone A is a **promising new small molecule** for hit-to-lead phase for the development of a **new treatment of pancreatic cancer**.

References

[1] *J Nat Prod*. 2020;83:770–803. DOI: 10.1021/acs.jnatprod.9b01285
[2] *Sci Rep*. 2018 Jun 27;8(1):9729. DOI: 10.1038/s41598-018-28192-5
[3] *J Biomol Screen*. 2016 Jul;21(6):567-78. DOI: 10.1177/1087057116635517
[4] *World J Oncol* [Internet]. 2019;10:10–27. Available from: http://www.wjon.org/index.php/WJON/article/view/1166
[5] WO/2016/020572

MSD · Universidad de Granada · Junta de Andalucía · Andalucía se mueve con Europa · Unión Europea Fondo Social Europeo

- Ayudas a infraestructuras y equipamientos de I+D+i para entidades de carácter privado convocada, en régimen de concurrencia competitiva, en el ámbito del Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020) y de la Estrategia de Innovación de Andalucía (RIS3 Andalucía). IEPR-0031.
- Spanish Ministry of Science and Innovation under grant agreement INP-2011-0016-PCT-010000-ACT7– 2011.
- Ministerio de Ciencia e Innovación, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica y el Fondo Europeo de Desarrollo Regional (FEDER).PCT_300000-2009-0016; PCT-010000-2010-3; INP-2011-0016-PCT-010000-ACT7.