



# The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)

01-30 NOVEMBER 2022 | ONLINE

## Encapsulating Fenretinide into Nanoparticles: Where we are and Where we are going

Chaired by **DR. ALFREDO BERZAL-HERRANZ**;  
Co-Chaired by **PROF. DR. MARIA EMÍLIA SOUSA**



*pharmaceuticals*



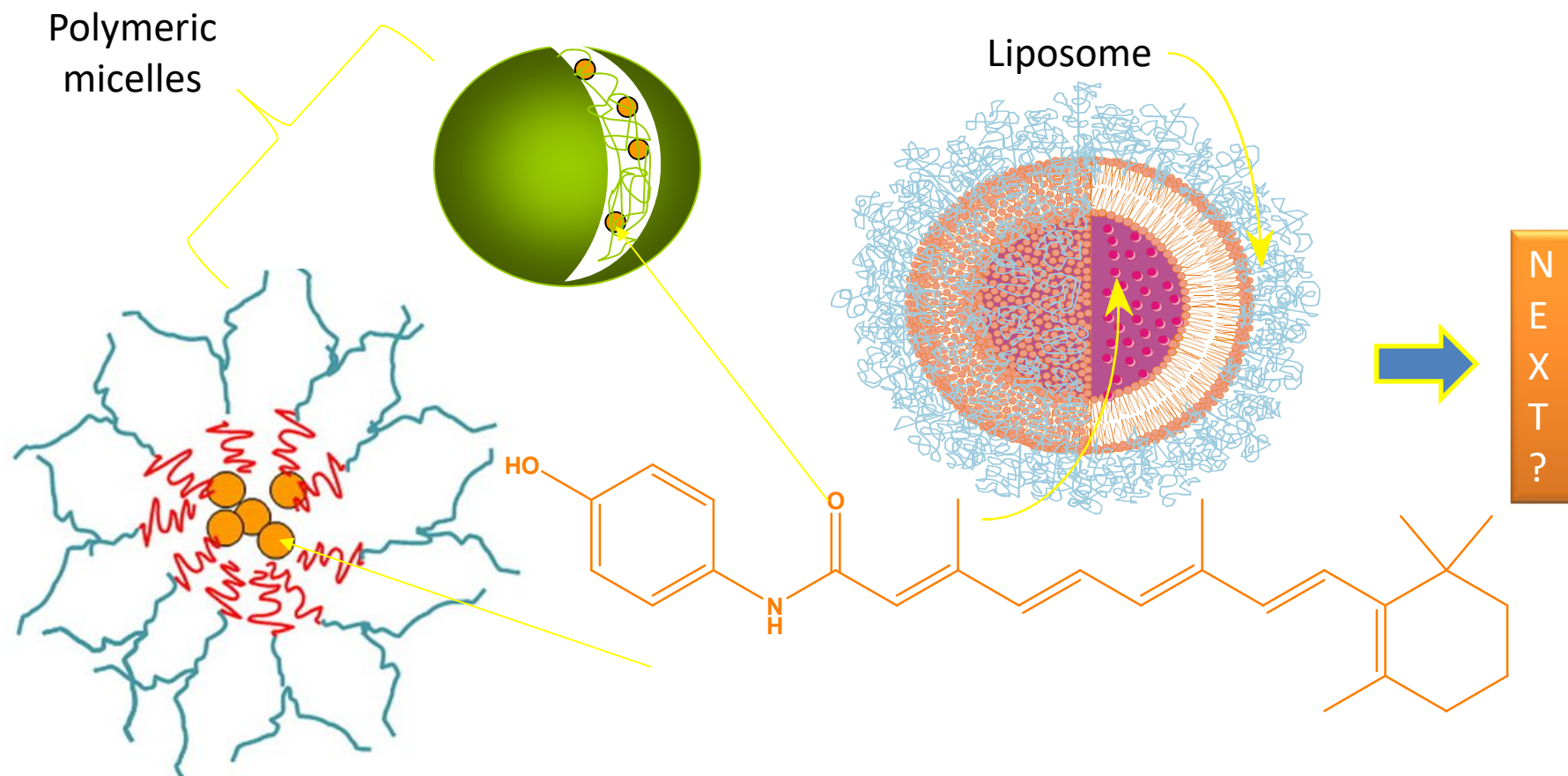
**Guendalina Zuccari<sup>1,\*</sup>, Carla Villa <sup>1</sup>, and Eleonora Russo <sup>1</sup>**

<sup>1</sup> Department of Pharmacy, University of Genoa, Viale Cembrano 4, 16145 Genoa, Italy.

\* Corresponding author: [guendalina.zuccari@unige.it](mailto:guendalina.zuccari@unige.it)



# Encapsulating Fenretinide into Nanoparticles: Where we are and Where we are going



ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

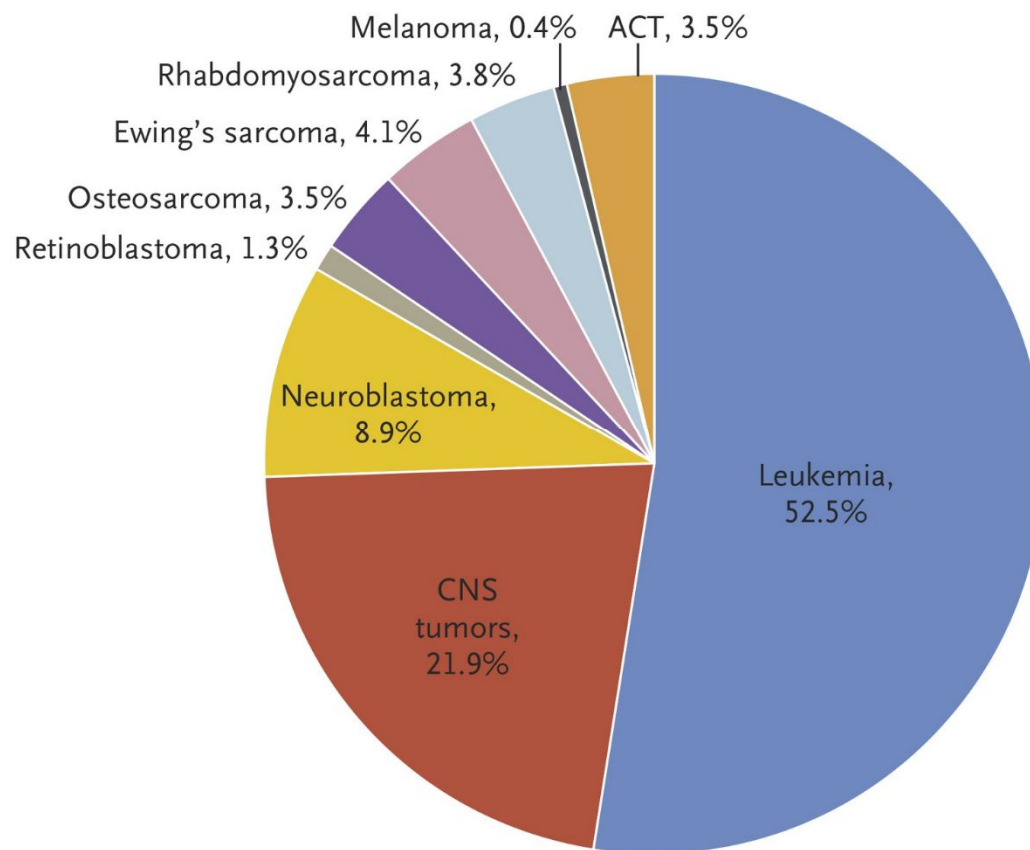
**Abstract:** Fenretinide (N-(4-hydroxyphenyl)-retinamide, 4-HPR) is a synthetic retinoid with fewer adverse effects than natural retinoids, effective against ovarian, prostate, small cell lung, brain, neuroectodermal-derived tumors. Clinical responses in adult and pediatric patients are often partial, revealing a limited activity of 4-HPR against existing disease. The underlying causes of this slight therapeutic efficacy consist in 4-HPR poor water solubility, low bioavailability and high first-pass hepatic effect. To overcome these drawbacks, nanomedicine could represent a valid alternative. We have already developed nanostructured drug delivery systems able to encapsulate 4-HPR. Indeed, polymeric micelles made of branched polyethylene glycol or amphiphilic dextrin have been prepared and investigated for their effectiveness both in vitro and in vivo. We have also designed a liposomal 4-HPR endowed with an active targeting moiety. Recently, we have focused our attention on a more physiological and not immunogenic drug delivery system. With this in mind 4-HPR-loaded mesenchymal stem cells-derived extracellular vesicles have been prepared. The drug amount encapsulated into the vesicles was determined by HPLC. Briefly, prior 4-HPR quantification an extraction procedure was optimized and, to estimate the analyte recovery an internal standard was employed. Since for this purpose, N-(4-ethoxyphenyl)-retinamide (4-EPR) has been reported, we developed a new operator-friendly one-step procedure to synthesize highly pure 4-EPR in quantitative yield. Studies aim to establish the best drug loading conditions are ongoing.

**Keywords:** Nanomedicine; Fenretinide; Drug Delivery Systems; Cancer; Retinoids



# Frequency of Paediatric Cancer Types

1 in 7,000 children

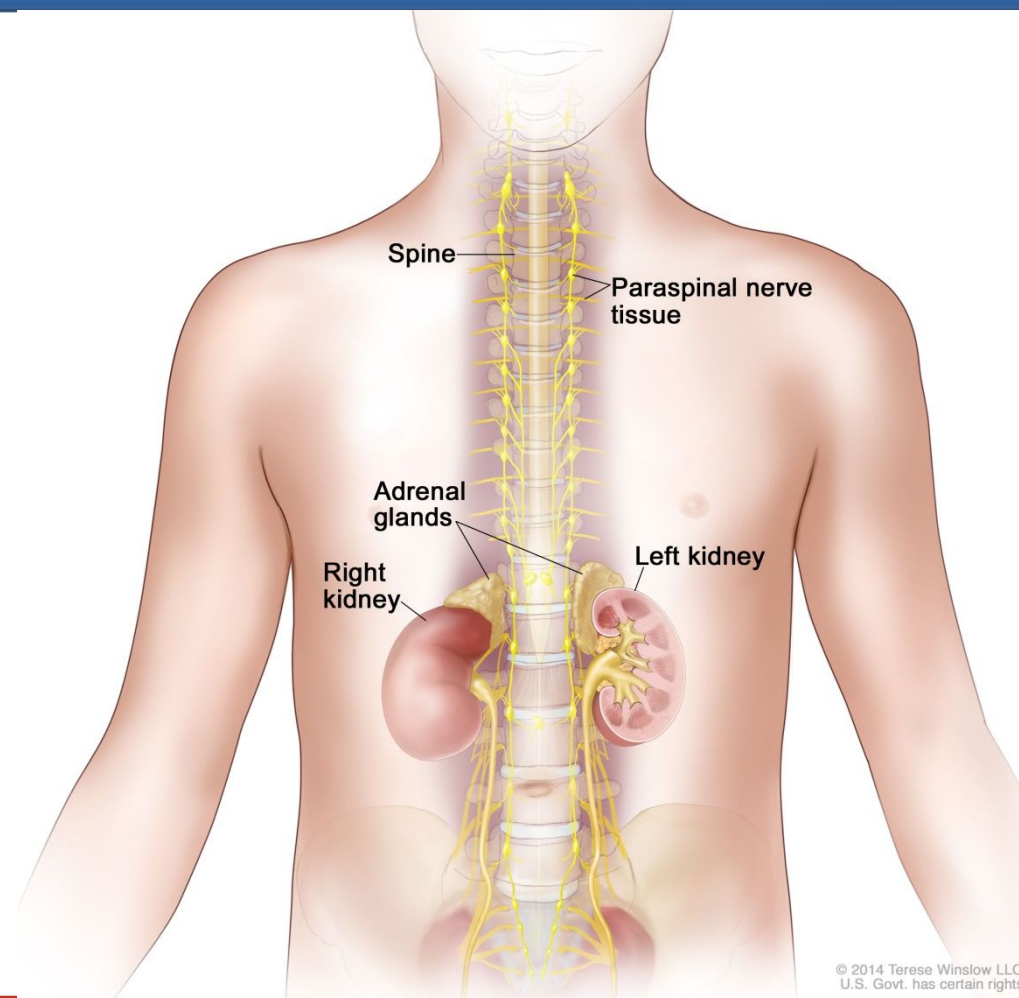


ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE



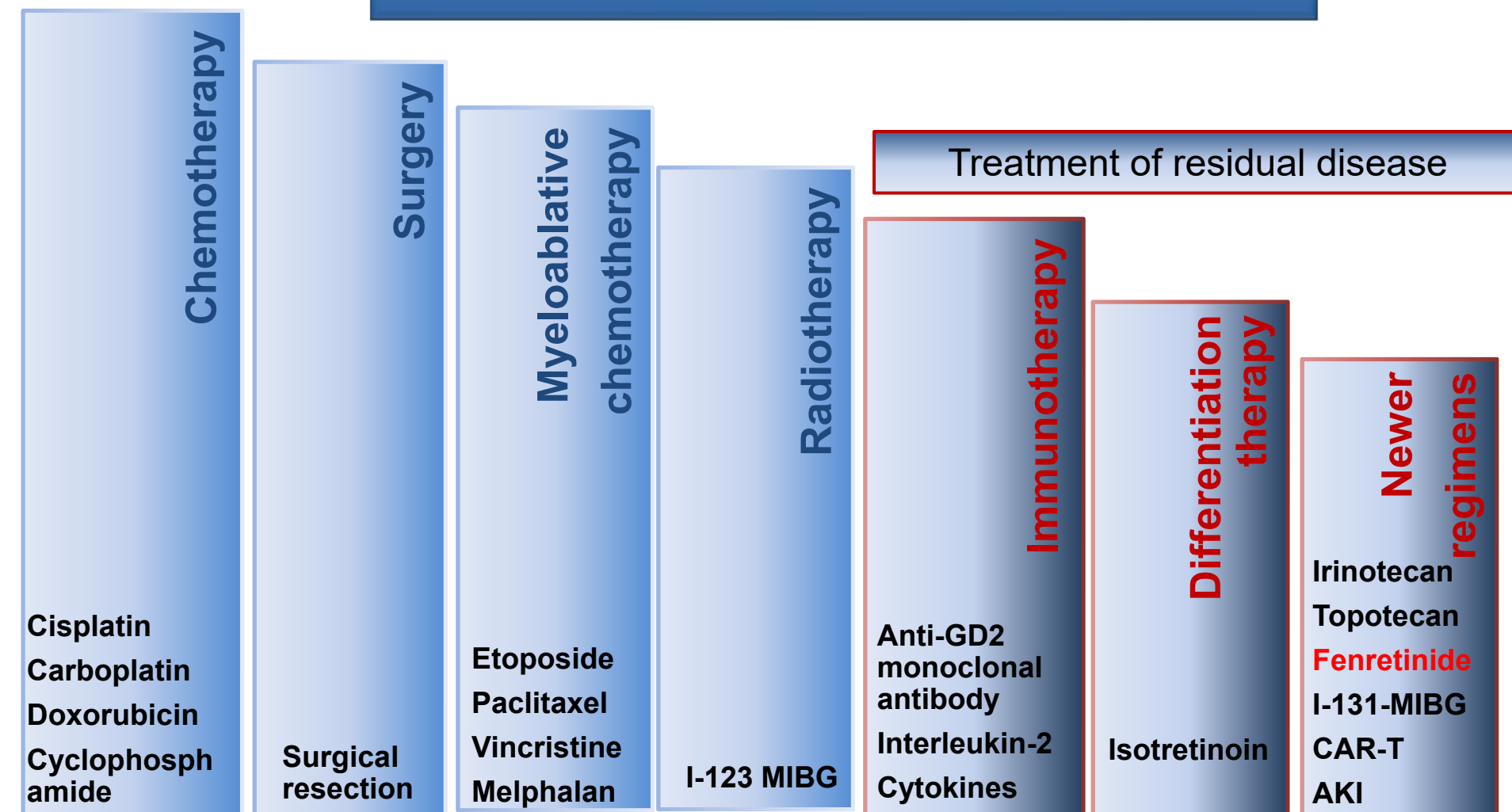
# Primary distribution of Neuroblastoma



**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**  
01-30 NOVEMBER 2022 | ONLINE

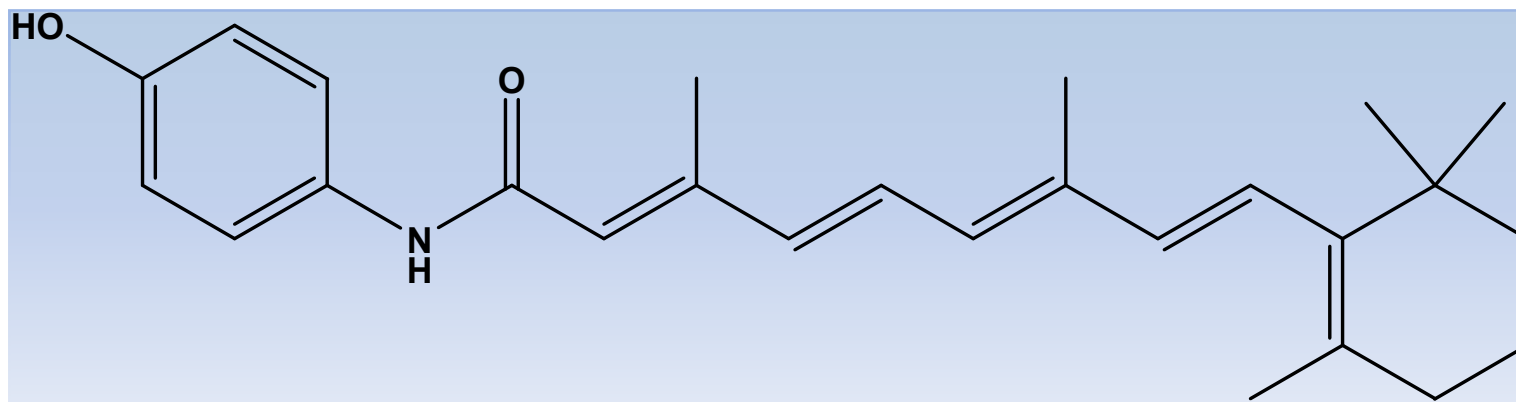
# Therapy for Neuroblastoma



ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Fenretinide



**N-(4-hydroxyphenyl)retinamide (4-HPR)**

PROS	CONS
HIGH ANTITUMOR ACTIVITY	LOW SOLUBILITY
FAVORABLE TOXICOLOGICAL PROFILE	POOR BIOAVAILABILITY
NO INDUCTION OF RESISTANCE	CLINICAL TRIALS WITH HIGH VARIABILITY IN RESULTS

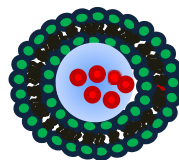
**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**  
01-30 NOVEMBER 2022 | ONLINE

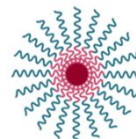
# Nanomedicines for Neuroblastoma

## Nanostructured Drug Delivery Systems

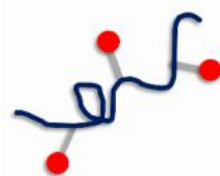
Liposomes



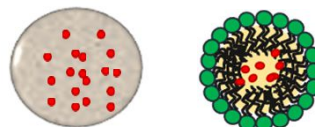
Polymeric Micelles



Polymer-Drug Conjugates



Polymeric/Lipid Nanoparticles



Complexes



ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE





BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine



nanomedjournal.com

Research Article

## Novel micelles based on amphiphilic branched PEG as carriers for fenretinide

Isabella Orienti, PhD<sup>a,\*</sup>, Guendalina Zuccari, PhD<sup>a</sup>, Mirella Falconi, MD, PhD<sup>b</sup>, Gabriella Teti, PhD<sup>b</sup>, Nicola A. Illingworth, PhD<sup>c</sup>, Gareth J. Veal, PhD<sup>c</sup>

JPP

Journal of Pharmacy  
And Pharmacology

## Enhanced anti-neuroblastoma activity of a fenretinide complexed form after intravenous administration

Roberta Carosio<sup>a</sup>, Vito Pistoia<sup>a</sup>, Isabella Orienti<sup>b,\*</sup>, Franca Formelli<sup>c</sup>, Elena Cavadini<sup>c</sup>, Salvatore Mangraviti<sup>d</sup>, Paolo G. Montaldo<sup>a</sup>, Emanuela Ognio<sup>e</sup>, Laura Emionite<sup>e</sup> and Guendalina Zuccari<sup>b,\*</sup>



Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: [www.elsevier.com/locate/jconrel](http://www.elsevier.com/locate/jconrel)



## Enhanced anti-tumor and anti-angiogenic efficacy of a novel liposomal fenretinide on human neuroblastoma

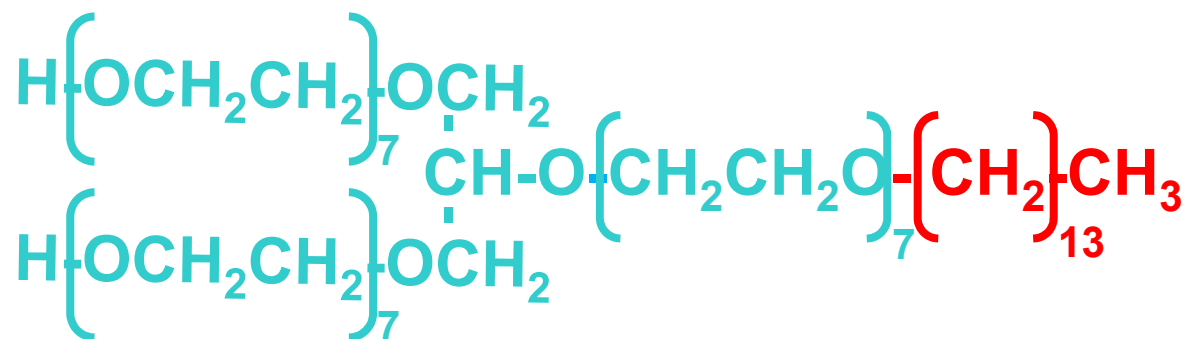


Daniela Di Paolo<sup>a,1</sup>, Fabio Pastorino<sup>a,\*,1</sup>, Guendalina Zuccari<sup>b</sup>, Irene Caffa<sup>a,2</sup>, Monica Loi<sup>a</sup>, Danilo Marimpietri<sup>c</sup>, Chiara Brignole<sup>a</sup>, Patrizia Perri<sup>a</sup>, Michele Cilli<sup>d</sup>, Beatrice Nico<sup>e</sup>, Domenico Ribatti<sup>e</sup>, Vito Pistoia<sup>c</sup>, Mirco Ponzoni<sup>a,3</sup>, Gabriella Pagnan<sup>a,\*,3</sup>

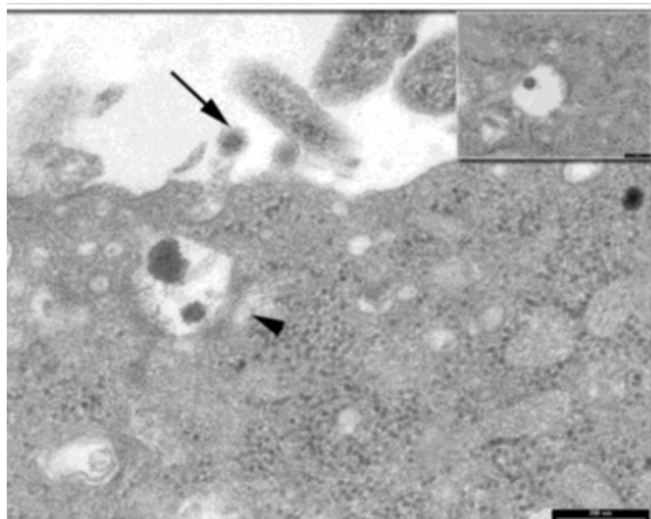
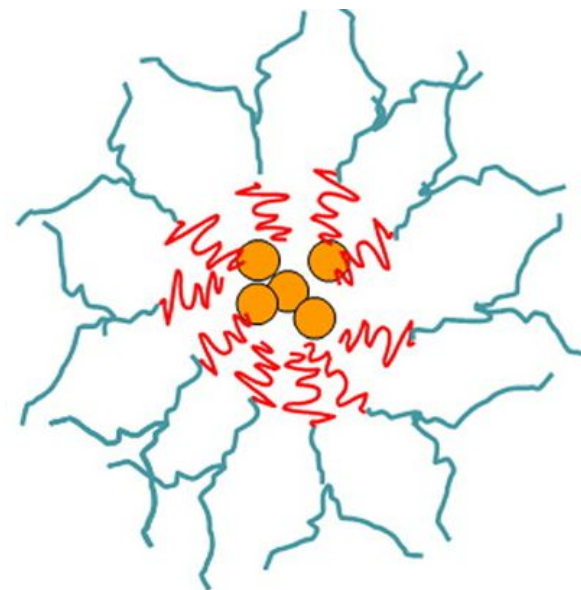
ECMC  
2022

01-30 NOVEMBER 2022 | ONLINE




# Our Previous Results-1



Glycerol ethoxylate linked to myristyl chain



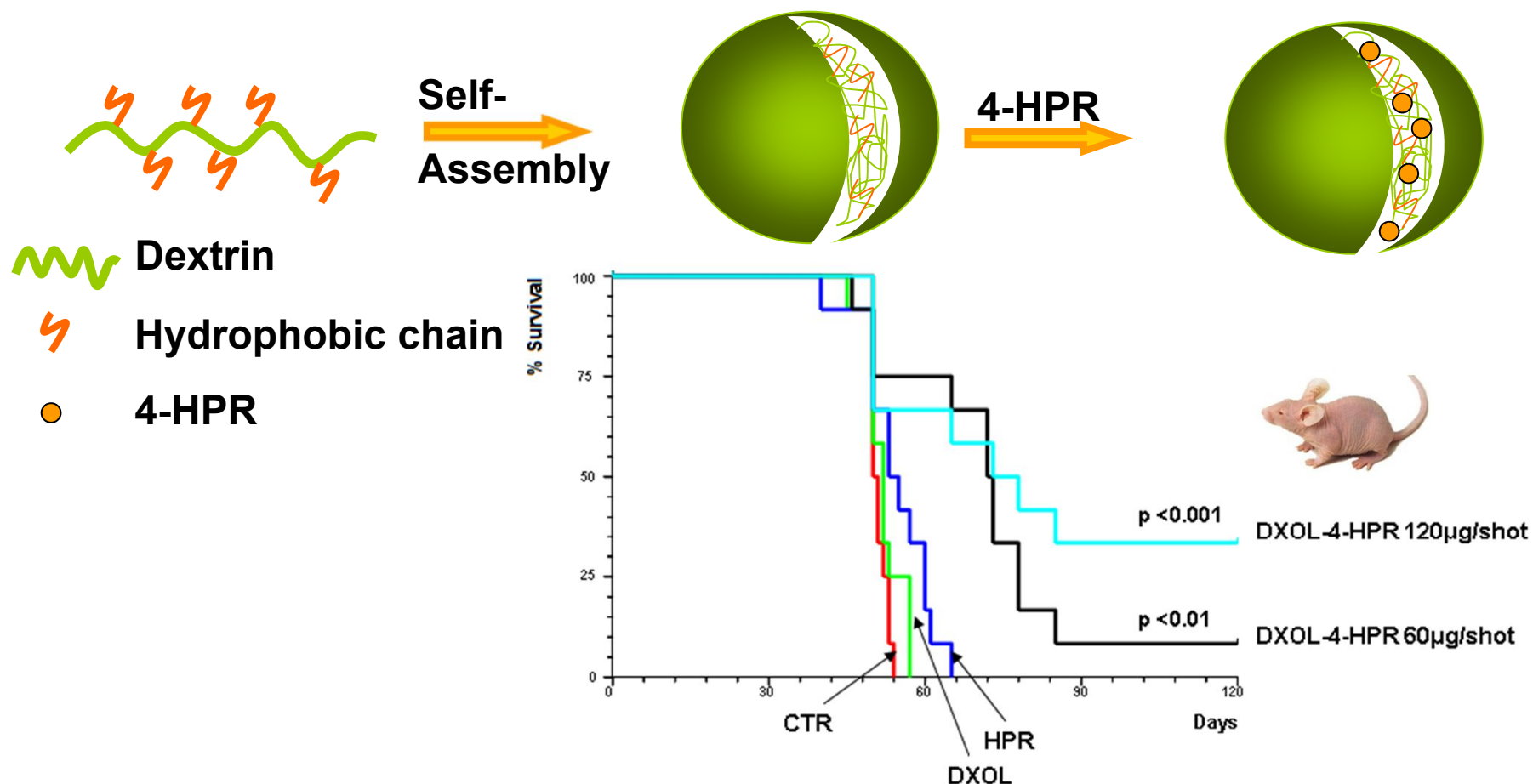
Micelles  
entering a  
neuroblastoma  
SH-SY5Y cell

-  Branched PEG
-  Hydrophobic chain
-  4-HPR

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

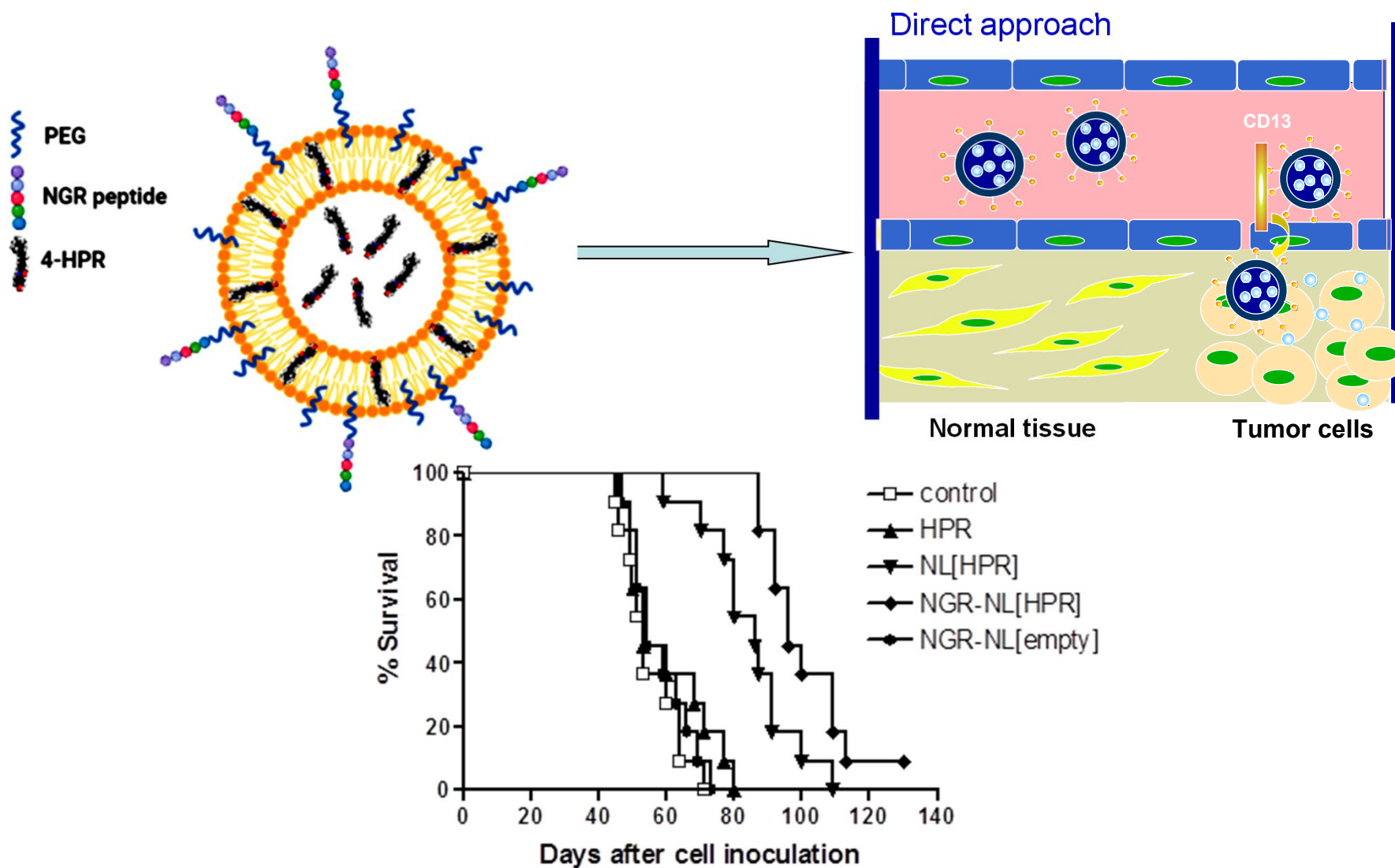
## Our Previous Results-2



ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

## Our Previous Results-3



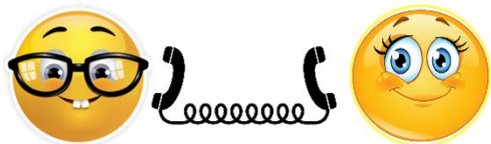


# Extracellular Vesicles

**Microvesicles  
(100 nm- 1 $\mu$ m)**

**Exosomes (30-150 nm)**

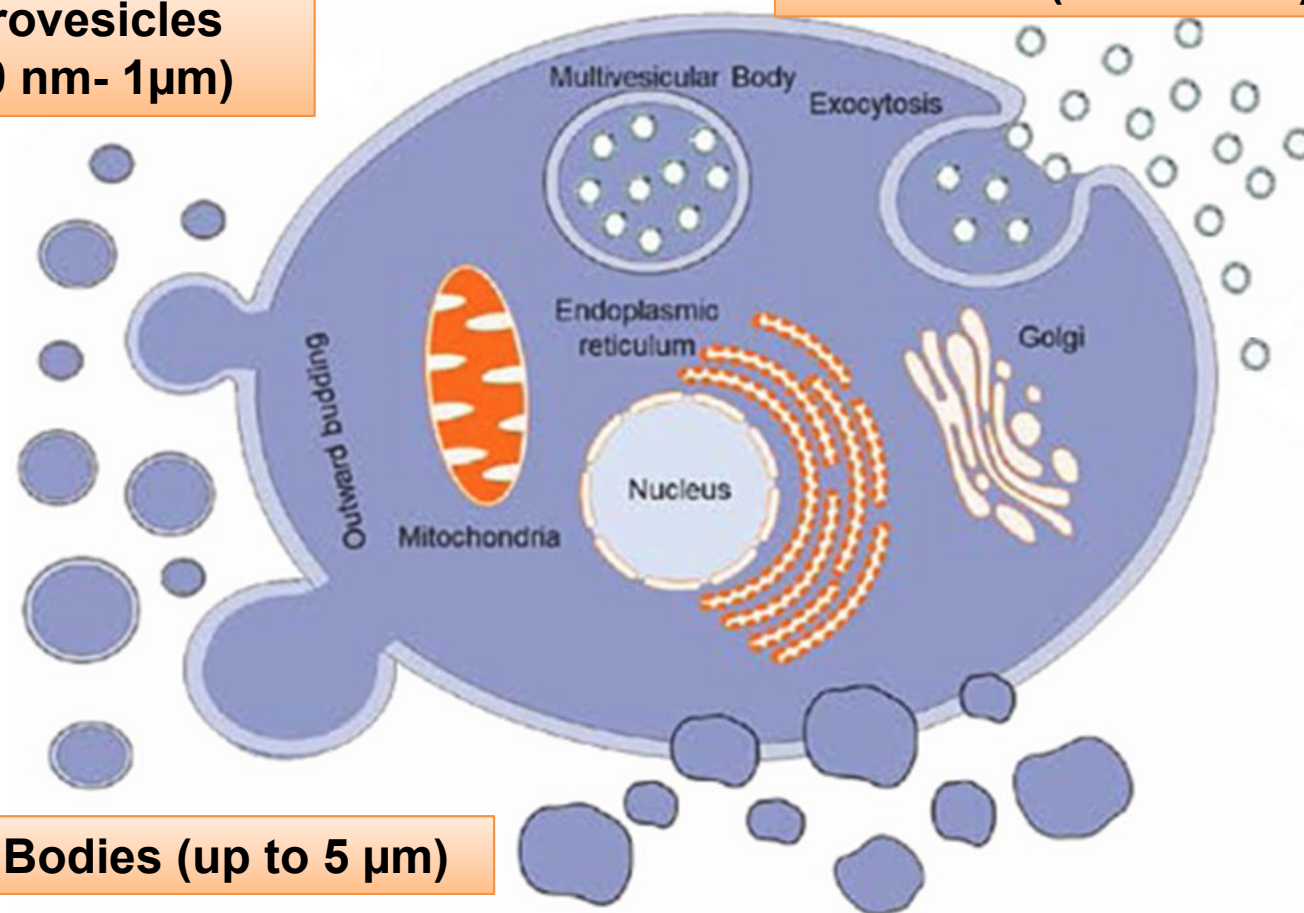
CROSS-TALK



Peptides  
RNA  
Lipids  
Proteins



**Apoptotic Bodies (up to 5  $\mu$ m)**

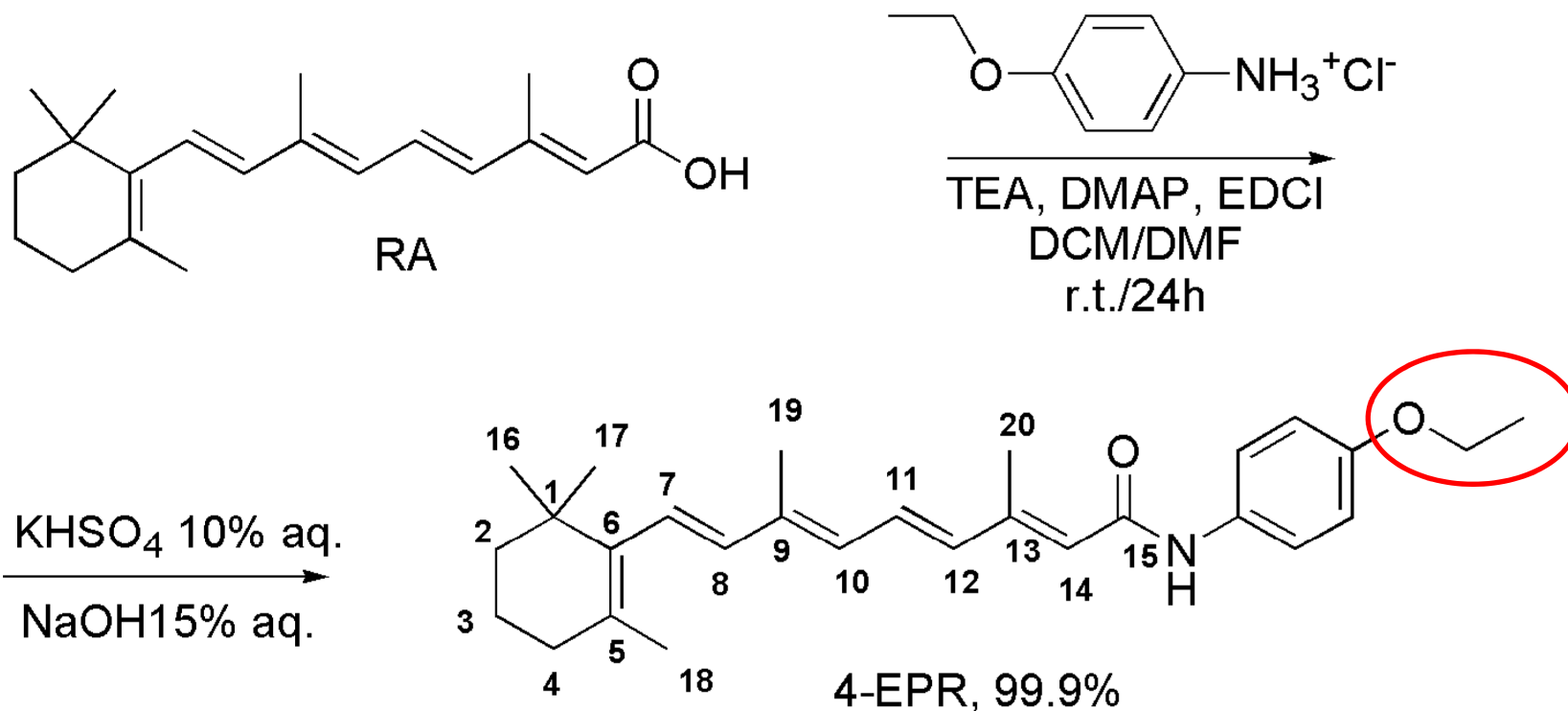


ECMC  
2022

**The 8th International Electronic  
Conference on Medicinal Chemistry**

01-30 NOVEMBER 2022 | ONLINE

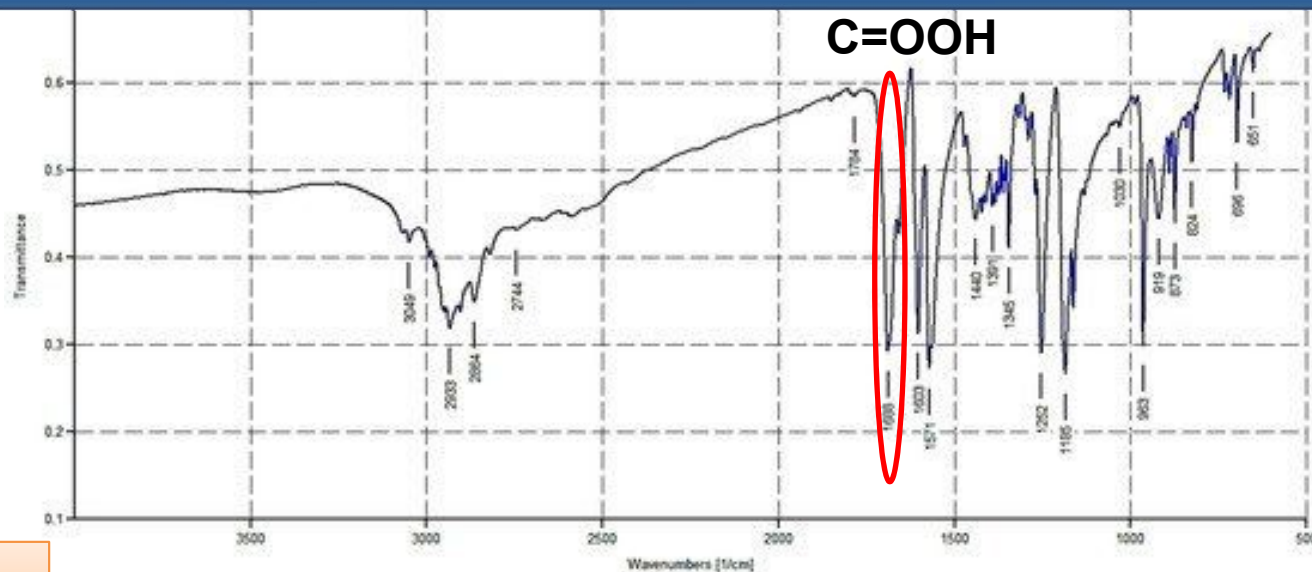
# Synthesis of the Internal Standard N-(4-ethoxyphenyl)-retinamide (4-EPR)



RA = retinoic acid; TEA = triethylamine; DMAP = 4-dimethylaminopyridine;  
 EDCI = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; DCM = dichloromethane;  
 DMF = *N,N*-dimethylformamide

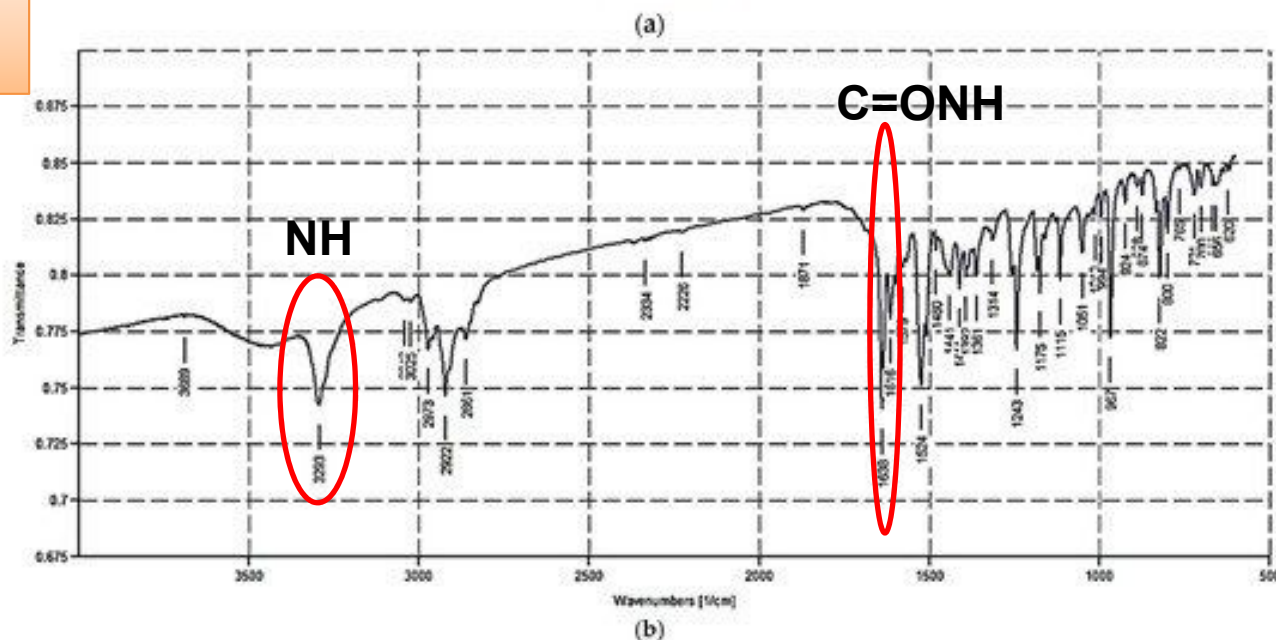
Alfei S, Zuccari G. *Molecules* 2022.

# Synthesis of the Internal Standard *N*-(4-ethoxyphenyl)-retinamide (4-EPR)



RA

✓ FTIR  
spectra

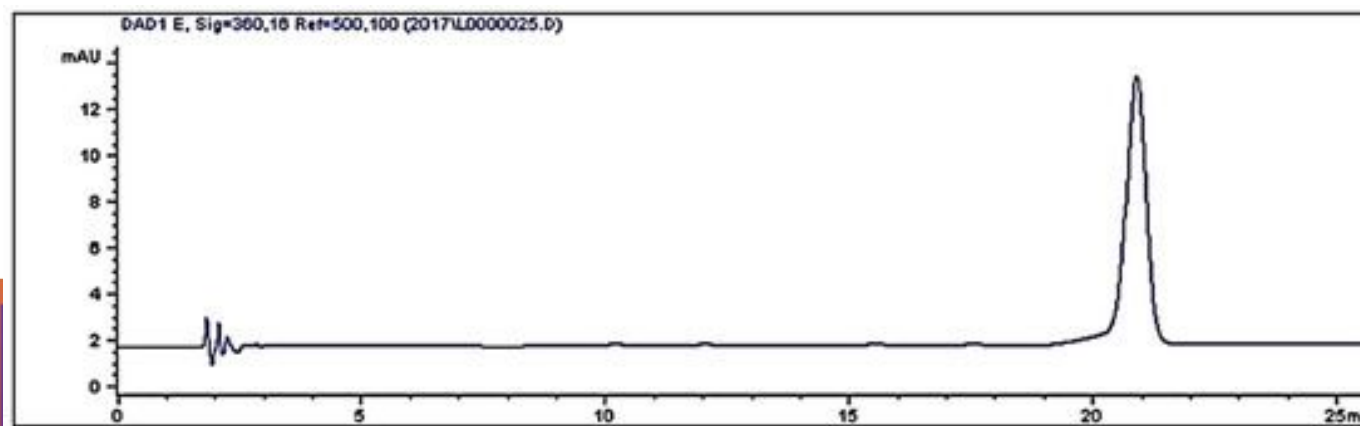
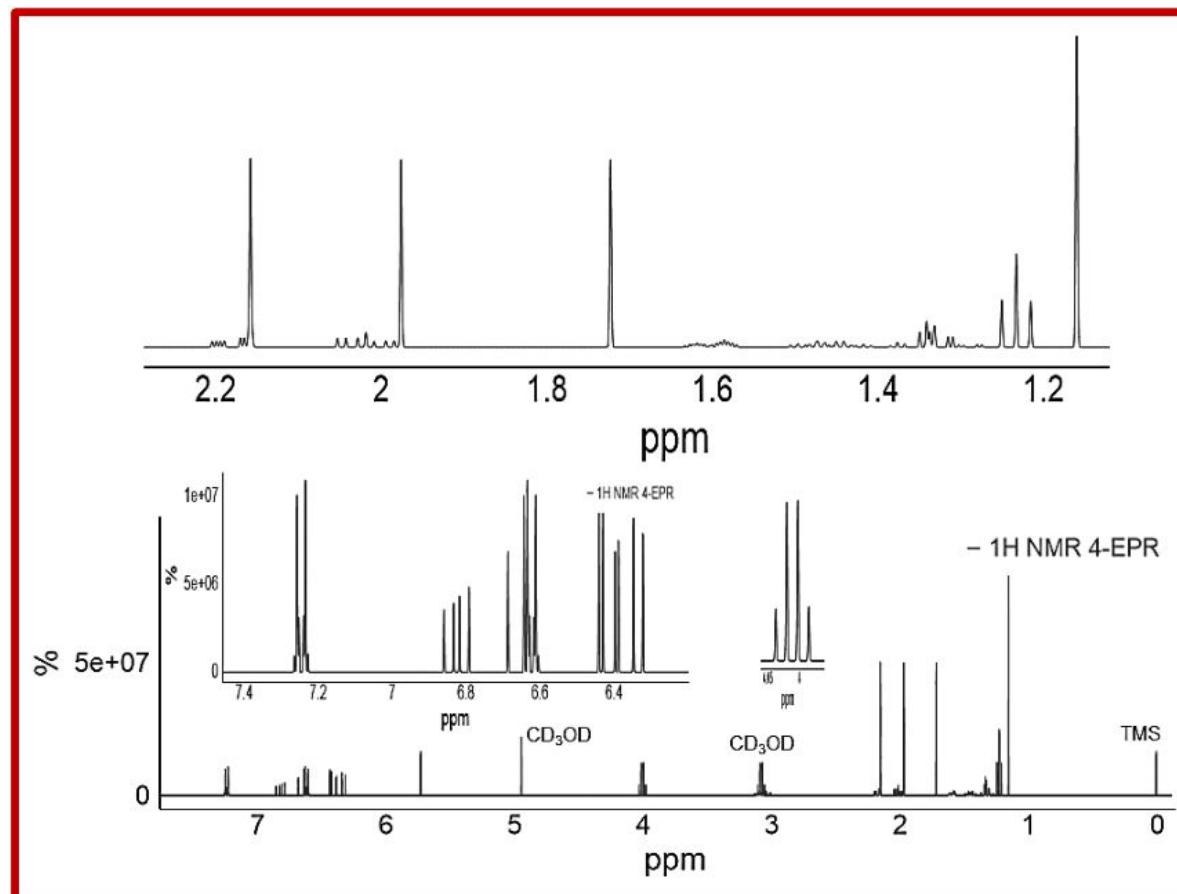


4-EPR

ECMC  
2022



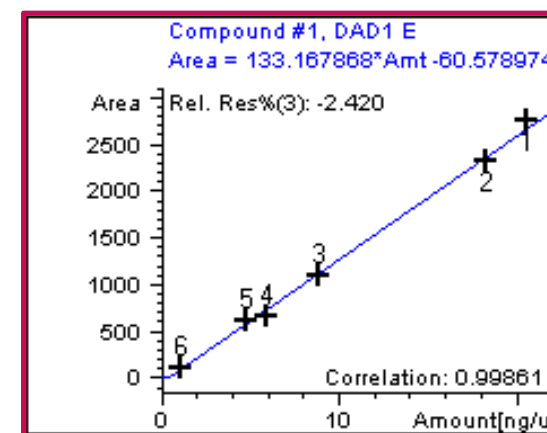
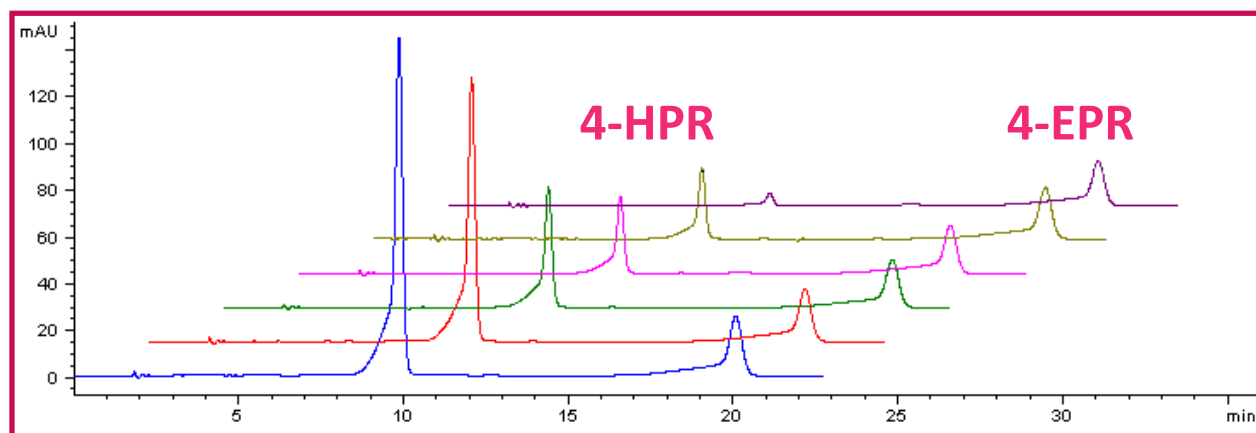
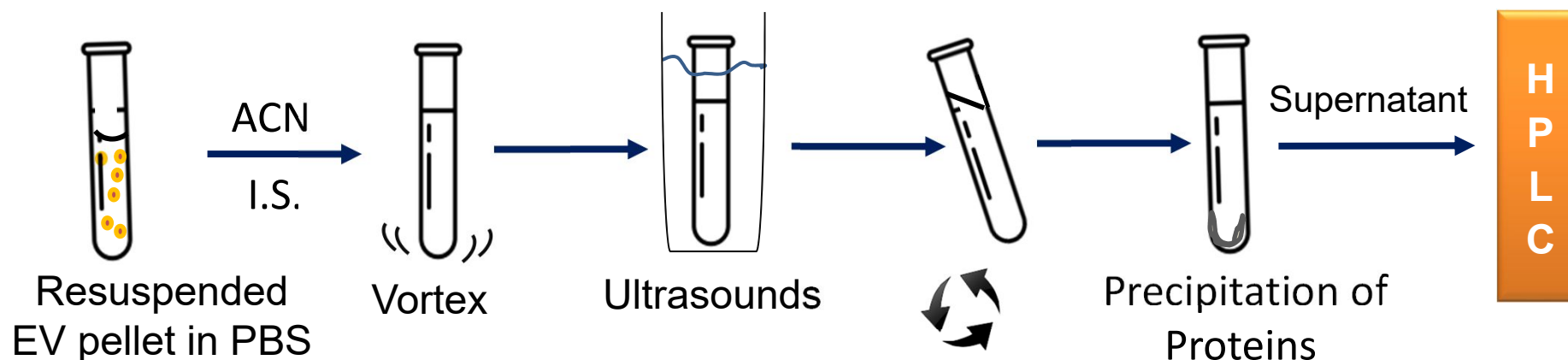
✓  $^1\text{H}$  NMR Spectrum



✓ RP HPLC-DAD analysis of 4-EPR



# Vesicle Cargo Measurement by HPLC-1



ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Vesicle Cargo Measurement by HPLC-2

Treatment of MSCs with 4-HPR		
Concentration	Time	$\mu\text{mol}$ 4-HPR per EV
10 $\mu\text{M}$	48 h	$1.68 \pm 0.43 \text{ E-14}$
20 $\mu\text{M}$	48 h	$5.93 \pm 0.32 \text{ E-14}$
25 $\mu\text{M}$	48 h	$6.48 \pm 0.51 \text{ E-14}$

ECMC  
2022

**The 8th International Electronic  
Conference on Medicinal Chemistry**  
01-30 NOVEMBER 2022 | ONLINE

## Conclusions

- ✓ A method for the extraction of Fenretinide from Extracellular Vesicles and its detection by HPLC was optimized

## Future Perspectives

- ✓ Experiments for the evaluation of uptake, apoptosis, cell cycle are on going
- ✓ In vivo experiments in metastatic mouse model to evaluate the effectiveness against minimal residual disease
- ✓ Use of bioreactors to scale up the production

**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**  
01-30 NOVEMBER 2022 | ONLINE



**Department of Pharmacy:**

**Prof. Eleonora Russo**

**Prof. Carla Villa**

**Prof. Silvana Alfei**



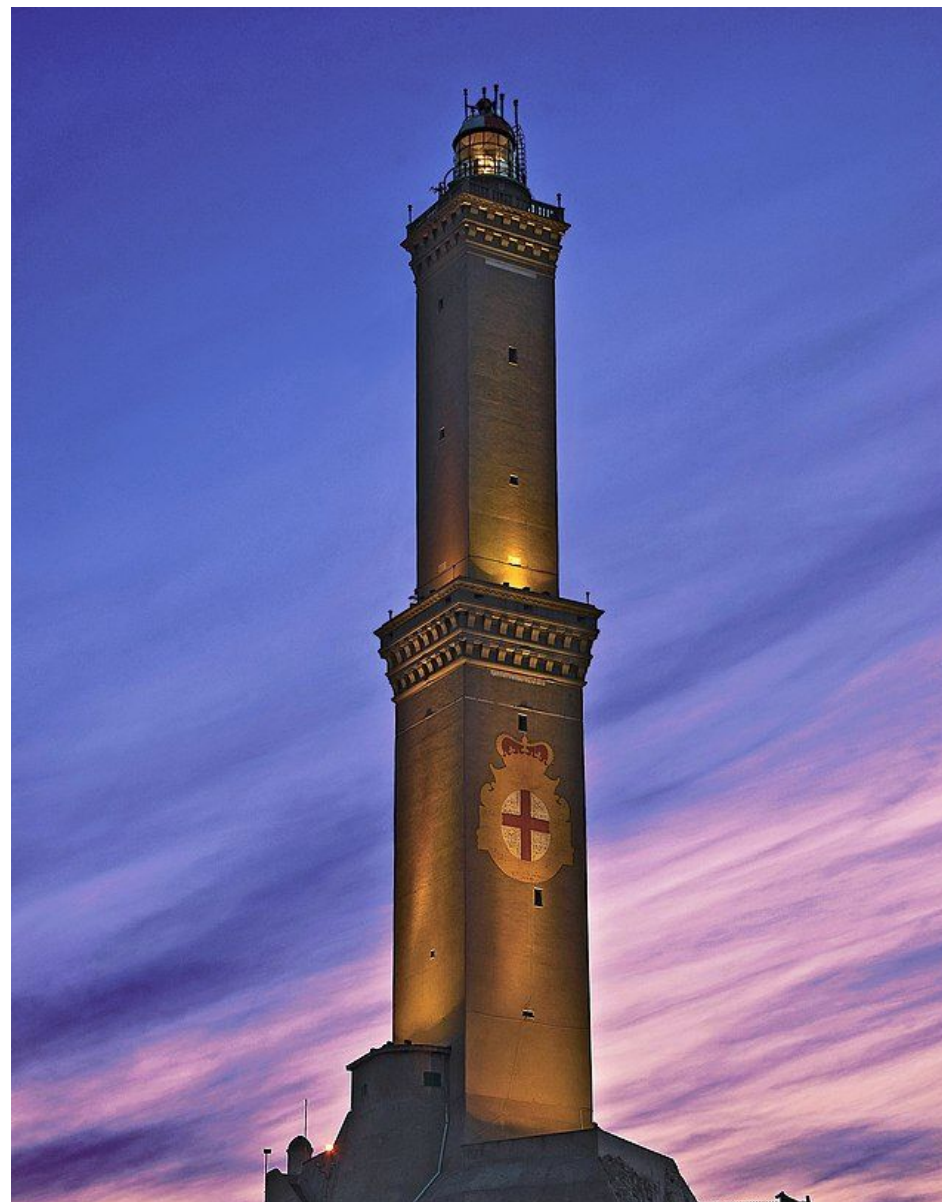
**ISTITUTO GIANNINA GASLINI**

ISTITUTO PEDIATRICO  
DI RICOVERO E CURA  
A CARATTERE SCIENTIFICO

**Cell Factory:**

**Dr. Danilo Marimpietri**

**Dr. Alessia Zorzoli**



**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**

**01-30 NOVEMBER 2022 | ONLINE**