The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Resveratrol-loaded glycosylated liposomes for targeting bacteria

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

pharmaceuticals MDPI

Cecilia Bombelli[1,*], Livia Pagano[2], Foteini Gkartziou[3], Stefano Aiello[2], Beatrice Simonis[1,2], Francesca Ceccacci[1], Simona Sennato[4], Alessia Ciogli[5], Spyridon Mourtas[6], Iris Spiliopoulou[7], Sophia G. Antimisiaris[3,6],Francesca Bugli[8,9], Cecilia Martini[9], Riccardo Torelli[8,9], Maurizio Sanguinetti[8,9], Giovanna Mancini[10]

[1]CNR-Institute for Biological Systems (ISB), Secondary Office of Rome-Reaction Mechanisms c/o Chemistry Dpt, Sapienza University, Rome, Italy; [2]Chemistry Dpt Sapienza University, Rome, Italy; [3]Institute of Chemical Engineering Sciences, FORTH/ICE-HT, Platani, 26504 Patras, Greece; [4]CNR-Institute of Complex Systems (ISC)-Sede Sapienza c/o Physics Dpt, Sapienza University,Rome, Italy; [5]Dpt of Chemistry and Technology of Drug, Sapienza University, Rome, Italy; [6]Lab Pharm Technology, Department of Pharmacy, School of Health Sciences, University of Patras, Greece; [7]Dpt of Microbiology, School of Medicine, University of Patras, Greece; [8]Dpt di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy; [9]Dpt di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie,UCSC, Rome, Italy; [10]CNR- Institute for Biological Systems (ISB), Area della Ricerca RM1, Monterotondo, Italy

* Corresponding author: cecilia.bombelli@cnr.it

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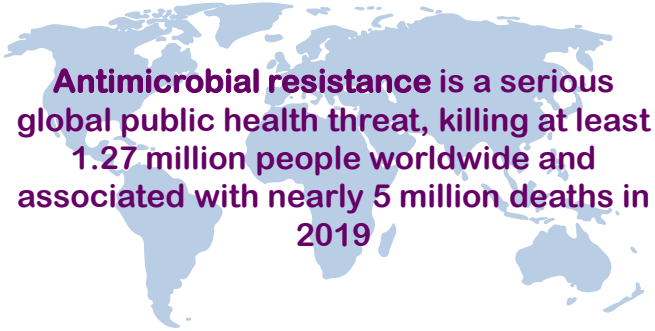
ISB

Introduction - Bacterial diseases: a global health problem

Antimicrobial Resistance (AMR)

-bacteria no longer respond to drugs

-infections are harder to treat



Antimicrobial resistance is a serious global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019

Introduction - Bacterial diseases: a global health problem

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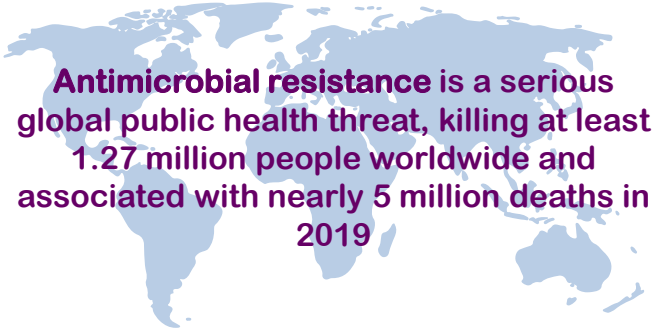

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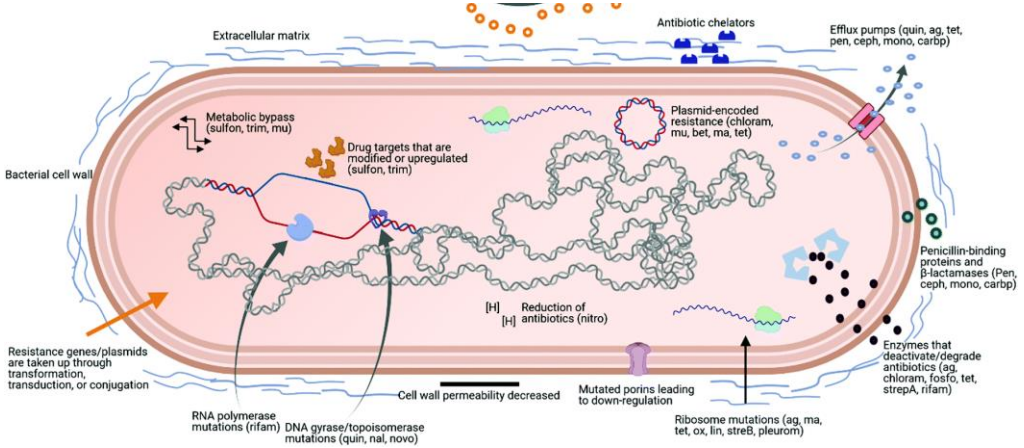
Mechanisms of bacterial resistance

-target modification

-drug inactivation

-drug transport

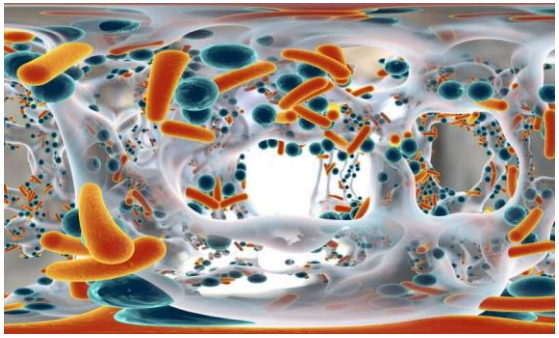


Gan, B. H., Gaynord, J., Rowe, S. M., Deingruber, T., & Spring, D. R. (2021) Chemical Society Reviews, 50(13), 7820-7880.

Introduction - Bacterial diseases: a global health problem

Biofilm: a contribution to AMR

"A microbial community of cells attached to a substratum or to each other, embedded in a self-produced polymeric matrix; bacteria in the biofilm exhibit an altered phenotype with respect to growth rate and gene transcription"



Biofilm advantages for bacteria

-A physical barrier

-Persister cells

-Transfer of genetic material

-Quorum sensing

→

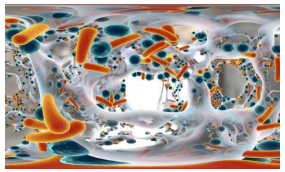
- Responsible for 60-85% of bacterial infections in west countries
- Sensibility to drugs 1000 times lower

R.M. Donlan, J.W. Costerton, Biofilm survival mechanism of clinically relevant microorganism, Clinical Microbiol. Rev. 15 (2002) 167–193

Introduction - Bacterial diseases: a global health problem

AMR

Biofilm



Increased morbidity and mortality

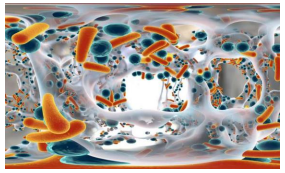
Need of new approaches

- Search for new drugs
- Modification of conventional drugs
- Natural bioactive molecules
- Quorum sensing inhibition
- Matrix disruptive drugs
- Biofilm formation prevention
- Mechanical removal of biofilm
- Use of nanotechnology

Introduction - Bacterial diseases: a global health problem

AMR

Biofilm



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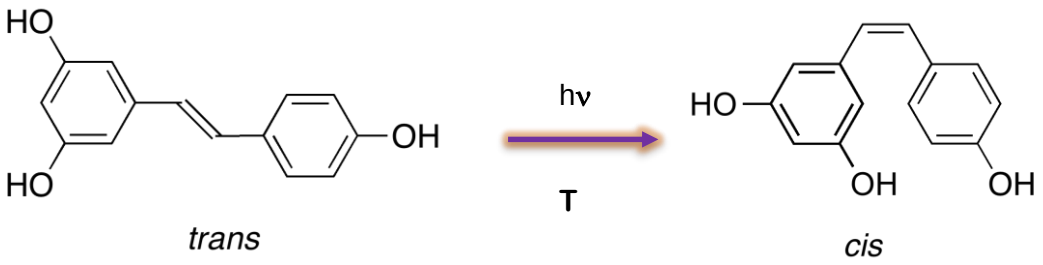
Introduction – Natural bioactive compounds: Resveratrol



Biological activities:

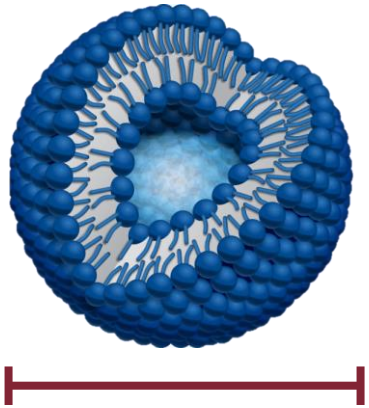
- Antitumoral
- Cardioprotective
- Antioxidant
- Antimicrobial

Limitation for *in vivo* administration:

- Low bioavailability
- Low solubility
- Fast metabilization
- Low specificity for bacteria
- Chemical instability (isomerization)

→ Drug Delivery System

Introduction – Liposomes to target bacteria

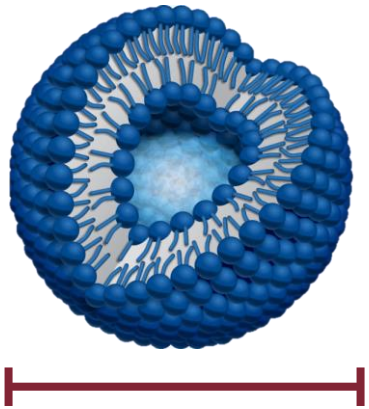


Diameter: 50 nm – 10 µm

- Tunable physico-chemical properties
- Loading of both hydrophilic and lipophilic compounds
- Circumventing some of the mechanisms of AMR
- Protection of the drug from degradation
- Controlled and sustained release
- Prolonged plasma circulation
- Reduced toxicity
- Functionalizable surface (targeting)

Ferreira M. et al. "Liposomes as antibiotic delivery systems: A promising nanotechnological strategy against antimicrobial resistance." Molecules (2021): 2047

Aim of the work – Liposomes to target bacteria



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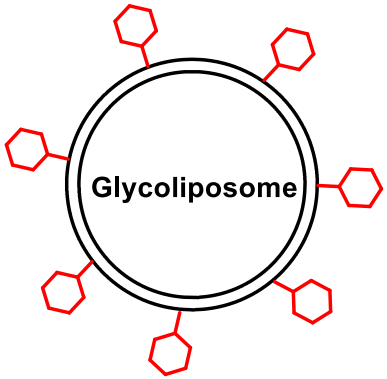

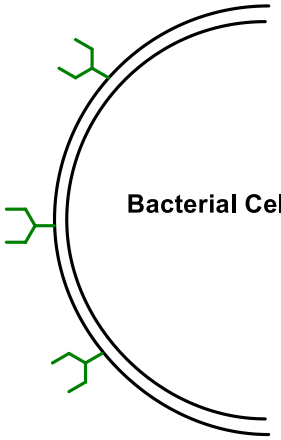

Carbohydrate Mojety

Carbohydrate Receptors (Lectins)

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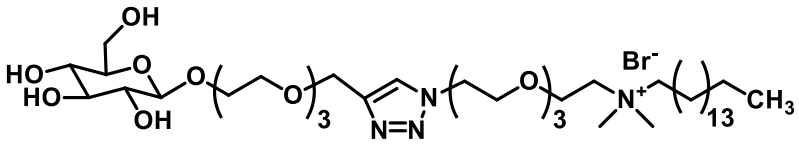
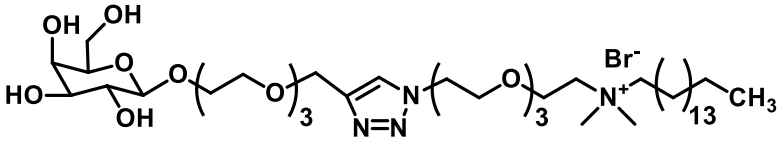
Aim of the work – Liposomes to target bacteria

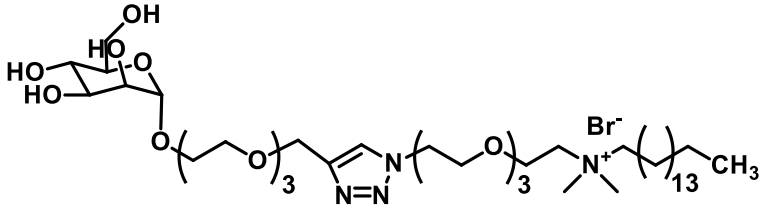
Development of glycoliposomes loaded with resveratrol for the treatment of biofilm enhanced bacterial infections



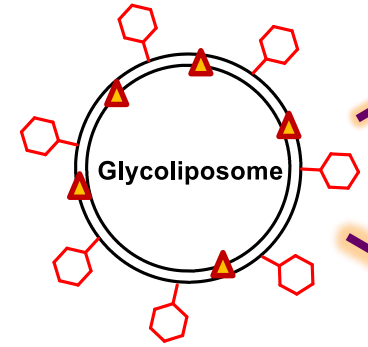
GL4

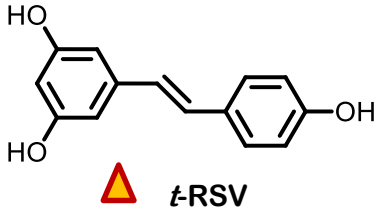


GLT1



MAN1



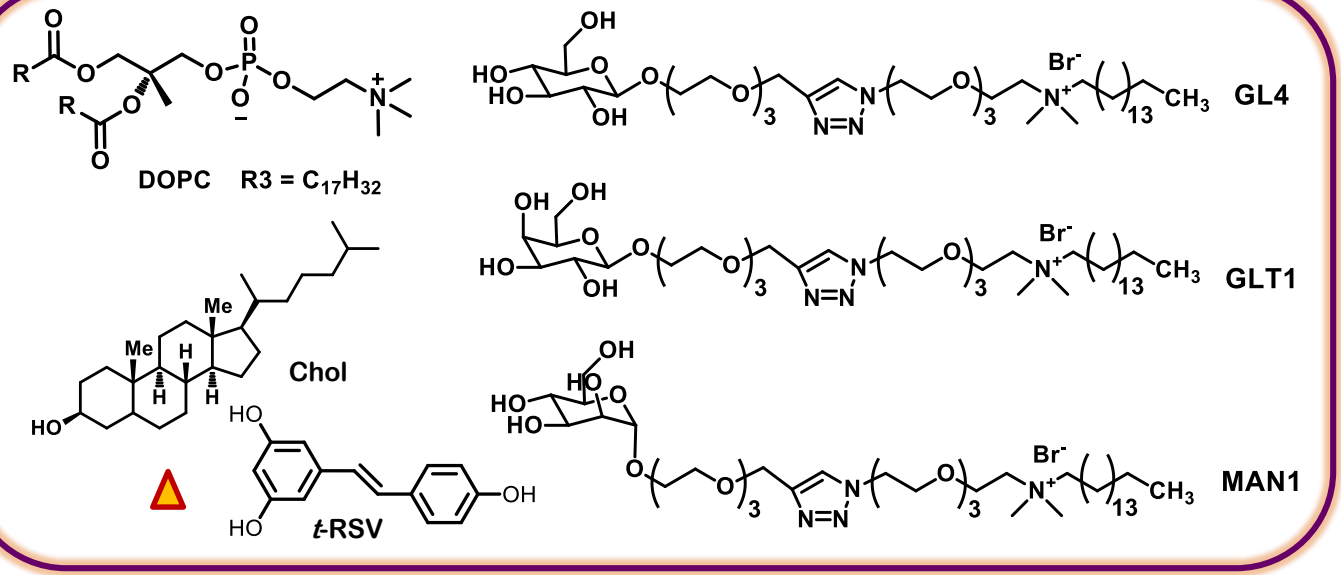


Biofilm disruption experiments on *methicillin resistant S. Aureus* (MRSA)

Growth Inhibition Experiments on two strains of *S. epidermidis* Slime+/-

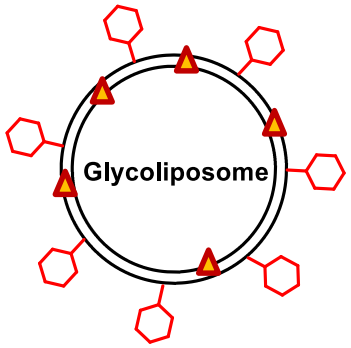
Aiello, S., et al. 2021. Mannosyl, glucosyl or galactosyl liposomes to improve resveratrol efficacy against Methicillin Resistant Staphylococcus aureus biofilm. Colloids Surfaces A Physicochem. Eng. Asp. 617, 126321 and reference therein.

Results and discussion – glycoliposomes for MRSA biofilm



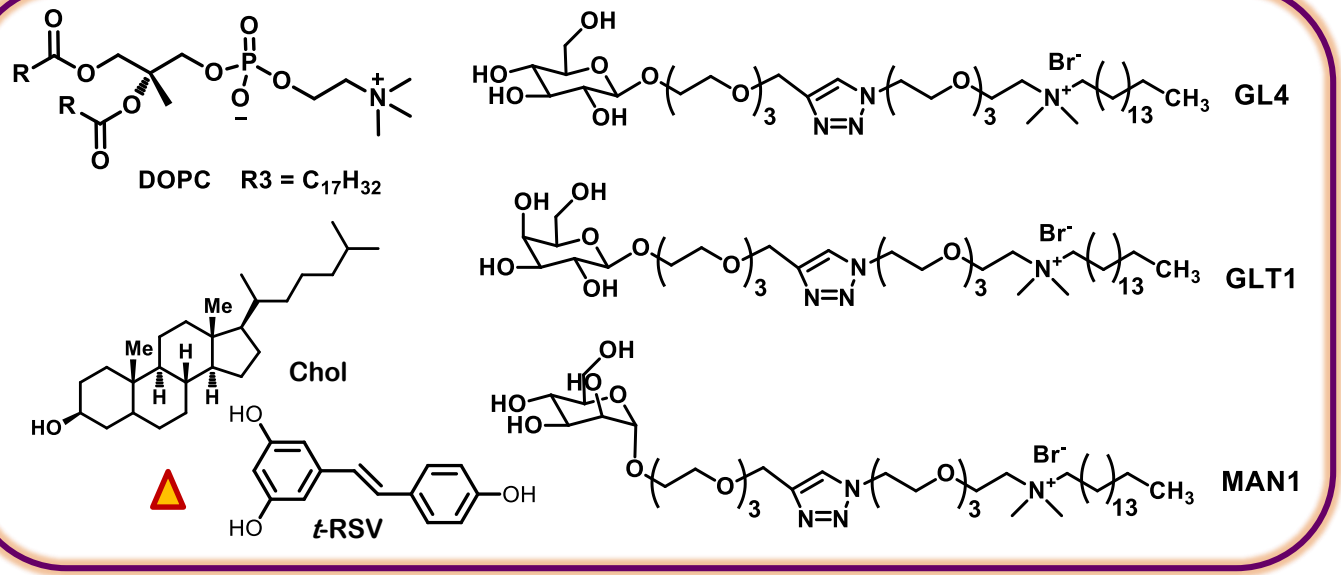
[total lipids]=20 mM
RSV/lipids =1:8
[Glycolipid] = 5% of total lipids
PBS buffer

RSV passive loading
(extrusion technique)



Liposomes characterization	
size	DLS
ζ- Potential	Electrophoretic mobility
RSV EE%	HPLC
RSV stability	UV (ABTS)
Disruption of MRSA Biofilm	CV, SEM

Results and discussion – glycoliposomes for MRSA biofilm



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Formulation	Lipid ratio	D_H (nm)	ζ-Potential (mV)	EE (%)	RSV (mM)
DOPC/Chol	80:20	106±1	-14.8±1.2	65	1.17±0.01
DOPC/Chol/GL4	75:20:5	91±2	19.5±1.8	89	2.27±0.04
DOPC/Chol/MAN1	75:20:5	101±6	26.2±1.6	79	2.08±0.04
DOPC/Chol/GLT1	75:20:5	98±3	27.1±3.3	90	2.03±0.043

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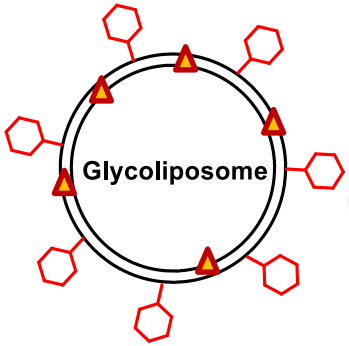
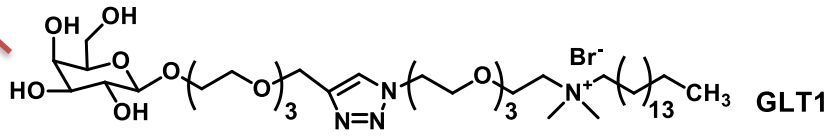
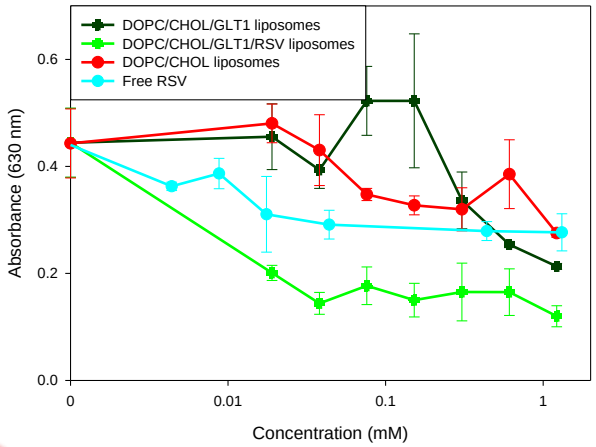


Mature biofilm of a clinical isolate of di MRSA
MIC RSV 1.2 mM
RSV= 0.018 mM - 1.2 mM (free and liposome-loaded)
Incubation:12h at 37°C

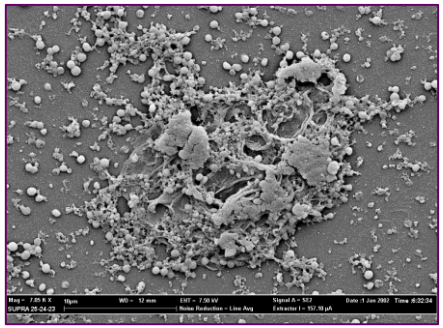


GLT1

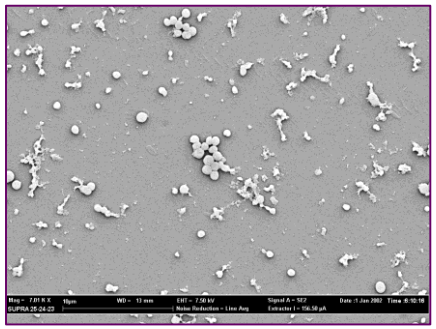
Crystal Violet test



SEM



RSV= 0.5 mM



DOPC/CHOL/GLT1
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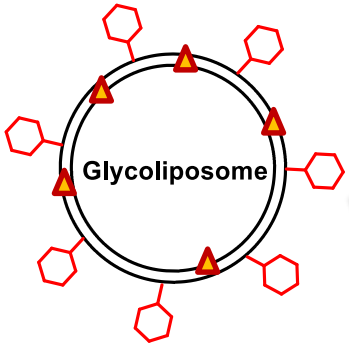

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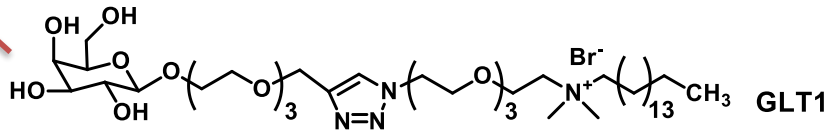

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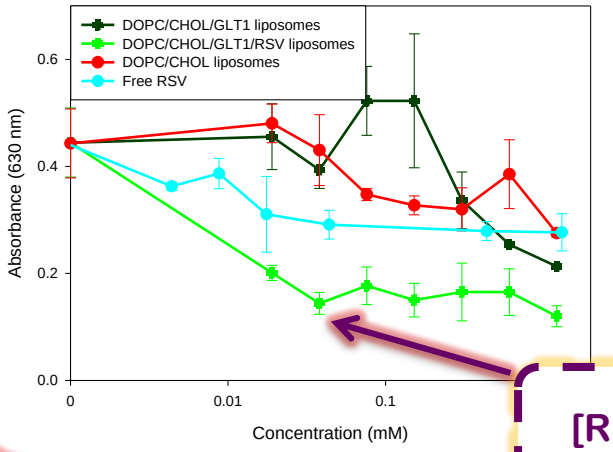
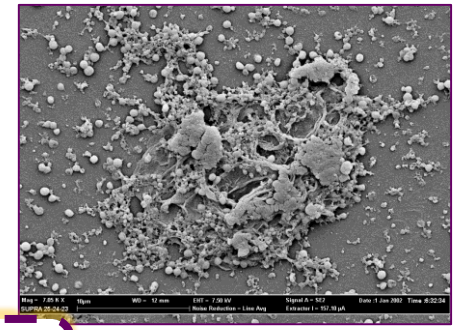
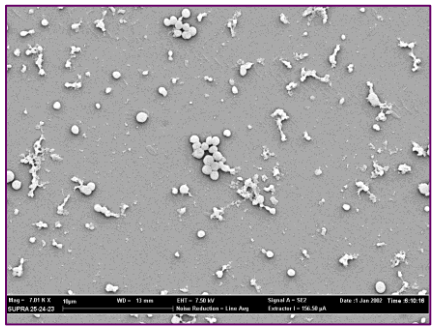


[RSV] < < MIC

SEM



V= 0.5 mM



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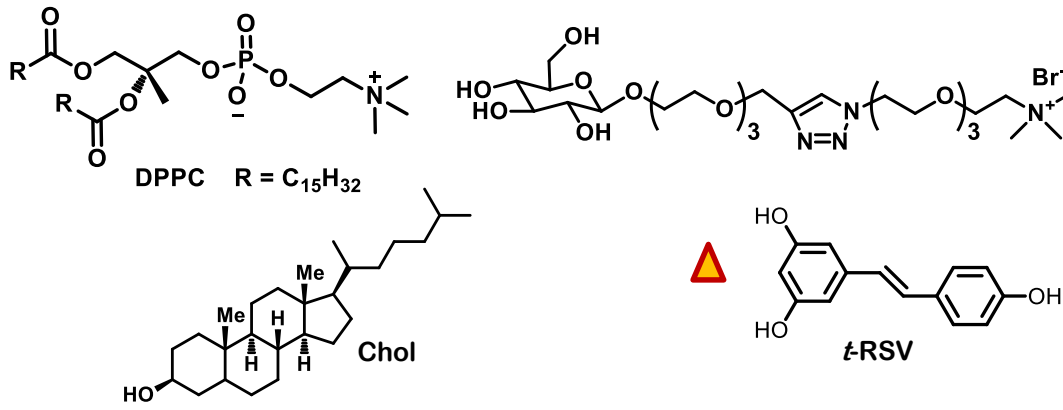
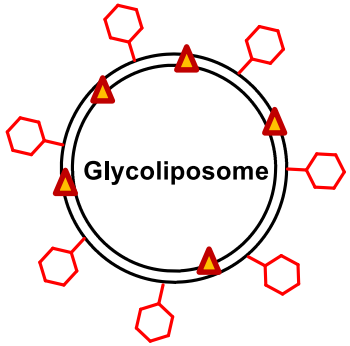
Results and discussion – glycoliposomes for *S. epidermidis*



[total lipids]=10-20-40 mM
RSV/lipids =1:8
[Glycolipid] = 5% of total lipids
PBS buffer

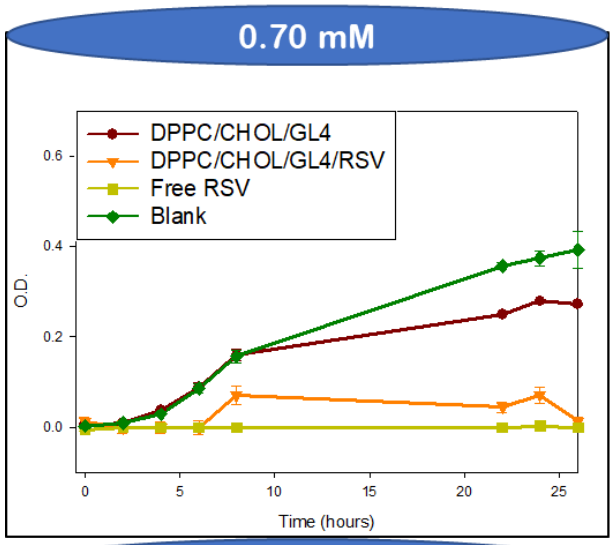
Passive loading
extrusion *vs* sonication

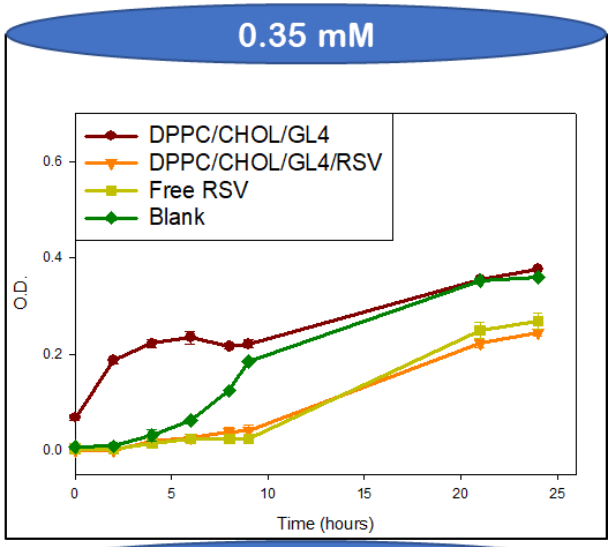


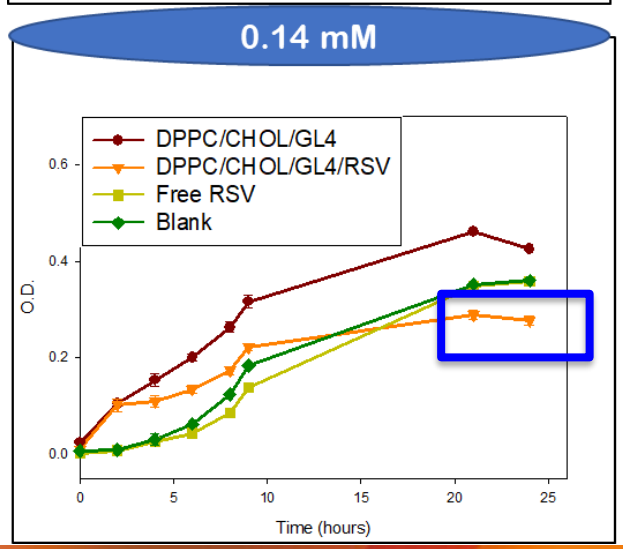
t-Resveratrol EE % (HPLC)		
Loading technique	*EE(%)*	*RSV/lipids*
extrusion	50-98	1:16-1:8
sonication	6-66	1:9-1:8

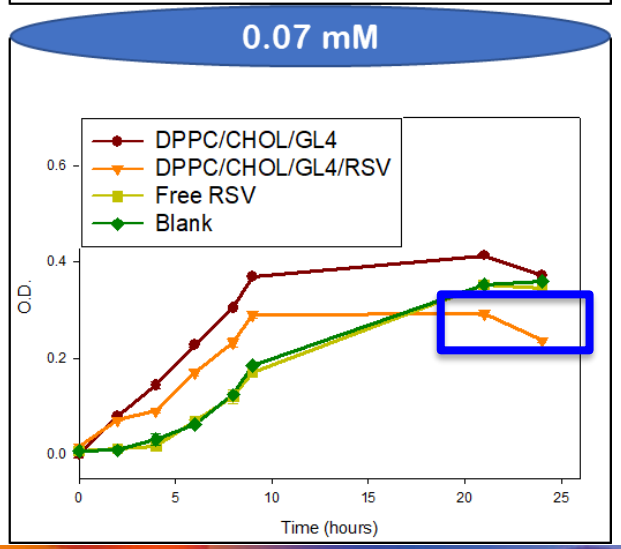
Pagano L. et al. Chemistry and Physics of Lipids 243 (2022) 105174

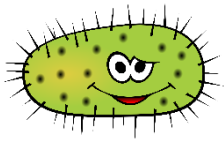
Results and discussion – glycoliposomes for *S. epidermidis*









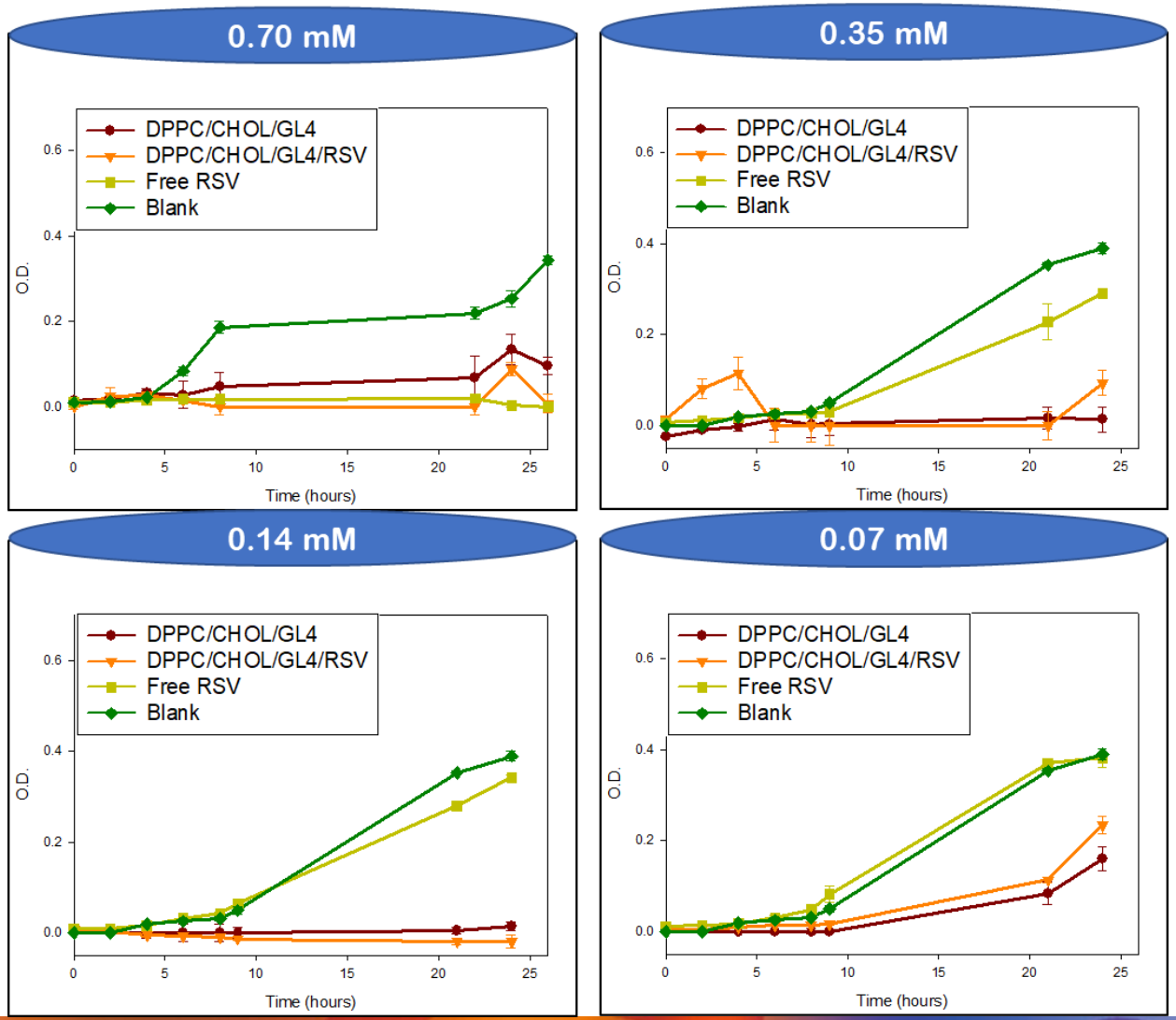


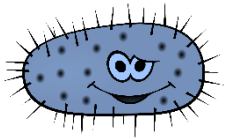
SLIME +
ATCC 35984

MIC RSV 0.7 mM
O.D. at 590 nm every 2 h
Incubation at 37 °C

[RSV] < < MIC

Results and discussion – glycoliposomes for *S. epidermidis*





SLIME –
ATCC 12228

MIC RSV 0.7 mM
O.D. at 590 nm every 2 h
Incubation at 37 °C

Antibacterial activity of empty liposomes

Conclusions

- Several resveratrol-loaded liposomal formulations were prepared using glycosylated amphiphiles and characterized in terms of size, polydispersity, stability over time and RSV entrapment efficiency

- The resveratrol-loaded formulation containing amphiphile GLT1 was able to disrupt preformed MRSA biofilm better than free resveratrol even at very low concentration

- Biological evaluation of resveratrol-loaded liposomal formulations shows that the formulation containing amphiphile GL4 was able to inhibit the growth of *S. epidermidis* even at concentration equal to 1/10 of RSV MIC.

Acknowledgments

Consiglio Nazionale
delle Ricerche

Cecilia Bombelli
Francesca Ceccacci
Giovanna Mancini

Simona Sennato

Francesca Bugli
Riccardo Torelli
Maurizio Sanguinetti
Cecilia Martini

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UNIVERSITÀ DI ROMA

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