"PETRU PONI" INSTITUTE OF MACROMOLECULAR CHEMISTRY

Centre of Advanced Research in Bionanoconjugates and Biopolymers

IntelCentru

SCIENTIFIC REPORT

PN-III-P1-1.1-PD-2021-0606, Contract No. PD 37 / 2022

Squalenoylation and micellar encapsulation as an effective approach for enhancing the biological properties of the antitumoral and antimicrobial drugs *(Acronym: Drug-ReSQue)*

Stage II (*January 1st, 2023 – December 31st, 2023*)

Design, synthesis, and characterization of a squalenoylated drugs series (flucytosine and glicine-curcumin hybrid). Design, synthesis, and characterization of a PEGylated squalene-commercial drug nanotherapeutics series (flucytosine and glicine-curcumin hybrid). *In vitro* testing of the obtained modified drugs.

The details of the activities carried out in stage 2 are shown in the table below:

Implementation plan of the Drug-ReSQue project. Stage 2023.

Stage II	Included activities	Results
Design, synthesis, and characterization of a squalenoylated drugs series (flucytosine and glicine-curcumin hybrid). Design, synthesis, and characterization of a PEGylated squalene-commercial drug nanotherapeutics series (flucytosine and glicine-curcumin hybrid). In vitro testing of the obtained modified drugs. ***Deliverables:*** • Research report • 1 Open Access scientific article (ISI journal Q1 or Q2, with high impact factor • Attending to two conferences	**A2.1.** Synthesis of squalene aldehyde	**1 Gold Open Access scientific article published in Polymers journal (Q1, IF: 5)** **Participation at 3 conferences** **Scientific report for stage II** **Updating the project Web page**
	A2.2. Synthesis of squalenic acid	
	A2.3. Synthesis of PEGylated squalene via imine or amide linkage	
	A2.4. Structural characterization of squalene aldehyde, squalenic acid and PEGylated squalene	
	A2.5. Morphological characterization of PEGylated squalene derivatives	
	A2.6. Determination of the critical micellar concentration of PEGylated squalene derivatives	
	A2.7. Synthesis of new therapeutics by squalenoylation of commercial drugs (flucytosine and glicine-curcumin hybrid)	
	A2.8. Synthesis of new nanotherapeutics by encapsulating commercial drugs (flucytosine and glicine-curcumin hybrid) in PEGylated squalene micellar assemblies	
	A2.9. Structural characterization of squalenoylated drugs (flucytosine and glicine-curcumin hybrid).	
	A2.10. Determination of the encapsulation degree of drugs (flucytosine and glicine-curcumin hybrid) in PEGylated squalene nanoassemblies	
	A2.11. Morphological characterization of new nanotherapeutics	
	A2.12. Determination of physiological drug release profiles (flucytosine and glicine-curcumin hybrid) from nanotherapeutics	
	A2.13. *In vitro* cytotoxicity determination of the obtained nanotherapeutics on normal cell lines	
	A2.14. Evaluation of the in vitro antimicrobial activity on different microbial cultures of the obtained nanotherapeutics	

Stage II – 2023 of the ***Drug-ReSQue*** project was dedicated to obtaining, physico-chemical characterization and evaluation of the biological properties of anti-bacterial nanotherapeutic systems based on squalene derivatives and commercial drugs (flucytosine ***FLU***, curcumin ***CRC*** and its derivative ***hCRC***) as it follows:

Activities ***A2.1.*** – ***A2.5.*** were accomplished by the synthesis and physicochemical characterization of squalene derivatives (squalene aldehyde ***SQ-CHO***, squalenic acid ***SQ-COOH***, and PEGylated squalene ***SQ-PEG***).

Within the activity ***A2.6.*** studies were carried out to determine the critical micellar concentration (**CMC**) of SQ-PEG and the results obtained showed that in PBS solution with a pH of 7.4 SQ-PEG has a ***CMC*** value of ***0.151 mg/mL***.

At activities ***A2.7.*** and ***A2.8.*** two new systems were obtained by squalenoylation of ***FLU*** and ***CRC*** drugs (***SQ-FLU*** and ***SQ-CRC***) and three new systems by encapsulating the drugs in micellar formations of SQ-PEG (***SQ-PEG-(FLU)***, ***SQ-PEG-(CRC)*** and ***SQ-PEG-(hCRC)***). The obtaining of the squalenoylated drugs was demonstrated by proton and carbon *NMR* spectroscopy, *FTIR* and *ESI-MS* (***A2.9.***).

Activity ***A2.10.*** was accomplished by determining the degree of encapsulation of ***FLU***, ***CRC***, and ***hCRC*** drugs in ***SQ-PEG*** micellar structures using UV-Vis spectroscopy. The obtained results showed encapsulation efficiencies of **~83%** for ***FLU***, **~91%** for ***CRC*** and **~74%** for ***hCRC***.

Within the ***A2.11.*** activity, the new nanotherapeutics were morphologically characterized by STEM and DLS, and the results obtained from these studies showed that the three nanotherapeutics obtained have spherical morphology with nanometric dimensions and low aggregation tendencies. Moreover, by recording the zeta potentials, negative values between **-25.6** and **-21.86 mV** were obtained, which indicates a high colloidal stability.

At ***A2.12.*** activity, which involved the determination of drug release profiles under physiological conditions, remarkable results were obtained which demonstrates that drug encapsulation in SQ-PEG micelles achieves controlled release of the drugs over 72 hours.

Activities ***A1.13.*** and ***A1.14.*** were carried out by carrying out *in vitro* studies of cytotoxicity (*normal HGF cells*) and antibacterial efficiency (*on 10 reference strains*) of the three nanotherapeutics obtained. The results of these studies showed that by encapsulating the proposed drugs in SQ-PEG micelles, the biological properties are improved as follows: cytotoxicity decreases, and antibacterial efficiency is improved in the case of the nanotherapeutic with ***FLU*** on 4 out of 5 yeasts tested. *The results obtained during this stage were disseminated in the form of a scientific report, an oral communication and two posters presented at national and international conferences. Also at this stage, a scientific article was published in the journal Polymers (Q1, IF: 5) in the Gold Open Access regime (**Craciun, B.F.**; Sandu, I.-A.; Peptanariu, D.; **Pinteala, M.**; Polymers, **2023**, 15, 4225, doi: https://doi.org/10.3390/polym15214225).*