"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 I (*April 1st, 2022 – December 31st, 2022*)

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

The details of the activities carried out in stage 1 are shown in the table below:

Implementation plan of the Drug-ReSQue project. Stage 2022.

Stage I	Included activities	Results
Design, synthesis, and characterization of a squalenoylated drugs series (methotrexate and cytarabine). Design, synthesis, and characterization of a PEGylated squalene-commercial drug nanotherapeutics series (methotrexate and cytarabine). In vitro testing of the obtained modified drugs. ***Deliverables:*** • Research report • Attending to a conference	**A1.1.** Synthesis of squalene aldehyde	**Participation at 1 conference** **Scientific report for stage I** **Web page**
	A1.2. Synthesis of squalenic acid	
	A1.3. Synthesis of PEGylated squalene via imine or amide linkage	
	A1.4. Structural characterization of squalene aldehyde, squalenic acid and PEGylated squalene	
	A1.5. Morphological characterization of PEGylated squalene derivatives	
	A1.6. Determination of the critical micellar concentration of PEGylated squalene derivatives	
	A1.7. Synthesis of new therapeutics by squalenoylation of commercial drugs (methotrexate and cytarabine)	
	A1.8. Synthesis of new nanotherapeutics by encapsulating commercial drugs (methotrexate and cytarabine) in PEGylated squalene micellar assemblies	
	A1.9. Structural characterization of squalenoylated drugs (methotrexate and cytarabine).	
	A1.10. Determination of the encapsulation degree of drugs (methotrexate and cytarabine) in PEGylated squalene nanoassemblies	
	A1.11. Morphological characterization of new nanotherapeutics	
	A1.12. Determination of physiological drug release profiles (methotrexate and cytarabine) from nanotherapeutics	
	A1.13. *In vitro* cytotoxicity determination of the obtained nanotherapeutics on normal cell lines	
	A1.14. Evaluation of the *in vitro* efficiency on tumour cell lines of the obtained nanotherapeutics	

Stage I – 2022 of the ***Drug-ReSQue*** project was dedicated to obtaining, physicochemical characterization and evaluation of the biological properties of antitumor nanotherapeutic systems based on squalene derivatives and commercial drugs (methotrexate ***MTx*** and cytarabine ***Cit***) according to the activities provided for this stage:

Thus, activities ***A1.1.*** – ***A1.5.*** 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 ***A1.6.*** studies were performed to determine the critical micellar concentration (**CMC**) of SQ-PEG using pyrene as a fluorophore and fluorescence spectroscopy to determine changes in emission spectra. The obtained results showed that in aqueous solutions SQ-PEG has a ***CMC*** value of ***0.154 mg/mL***.

At activities ***A1.7.*** and ***A1.8.*** two new systems were obtained by squalenoylation of Cyt and MTx drugs (SQ-Cyt and SQ-MTx) and two new systems by encapsulating the drugs in micellar formations of SQ-PEG (SQ-PEG-(Cyt) and SQ-PEG-(MTx)). The obtaining of the squalenoylated drugs was demonstrated by proton and carbon NMR spectroscopy, FT-IR and ESI-MS (***A1.9.***).

Activity ***A1.10.*** was accomplished by determining the degree of encapsulation of **MTx** and **Cyt** drugs in SQ-PEG micellar structures using UV-Vis spectroscopy. The results obtained showed encapsulation efficiencies of ~54% for MTx and ~45% for Cit, with drug loadings of ~4% and ~3.5%, respectively.

Within the ***A1.11.*** activity, the new nanotherapeutics were characterized by *STEM* and *DLS* techniques from a morphological point of view. The obtained results from these studies showed that both *SQ-PEG-(MTx)* and *SQ-PEG-(Cyt)* have spherical morphology with nanometric dimensions with reduced tendency for agregation, but the nanotherapeutic *SQ-PEG-(Cyt)* showed larger hydrodynamic diameters than its MTx-based counterpart at both studied pH values. Moreover, by recording the zeta potentials, negative values between -3 and +3 mV were obtained indicating a low colloidal stability, as expected.

At ***A1.12.*** activity, which involved the determination of drug release profiles under physiological and tumour conditions, remarkable results were obtained demonstrating that by encapsulating drugs in SQ-PEG micelles, a controlled release dictated by pH value and temperature is achieved.

Activities ***A1.13.*** and ***A1.14.*** were carried out by performing *in vitro* cytotoxicity studies (normal cells) and antitumor efficiency (tumour cells) of the two nanotherapeutics obtained. The results of these studies showed that encapsulating ***MTx*** and ***Cit*** drugs in SQ-PEG micelles, the biological properties are improved as follows: cytotoxicity decreases, and antitumor efficiency is improved on both cell lines without any selectivity. *The results obtained during this stage were disseminated in the form of a scientific report and a poster presented at a national conference with international participation.*