"PETRU PONI" INSTITUTE OF MACROMOLECULAR CHEMISTRY

Centre of Advanced Research in Bionanoconjugates and Biopolymers

IntelCentru

SUMMARY OF THE FINAL 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)*

Implementation period (*April 1st, 2022 – May 31st, 2024*)

The details of the activities carried out during the implementation period are presented below:

Implementation plan of the Drug-ReSQue project.

Stage I (2022)	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 II (2023)	**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-	**A2.1.** Synthesis of squalene aldehyde	**1 Gold Open Access scientific article published in Polymers journal (Q1, IF: 5)** **Participation at 3 conferences**
	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)	

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.	**A2.8.** Synthesis of new nanotherapeutics by encapsulating commercial drugs (flucytosine and glicine-curcumin hybrid) in PEGylated squalene micellar assemblies	**Scientific report for stage II** **Updating the project Web page**
	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 III (2024)	**Included activities**	**Results**
In vitro* testing of the obtained modified drugs, interpretation of the results and their dissemination** ***Deliverables: • Research report. • 1 scientific article (ISI journal Q1 or Q2, with high impact factor).	**A3.1.** Completion of the *in vitro* antimicrobial tests started in stage 2	**1 scientific article submitted for publication to the journal *Bioconjugate Chemistry* (Q2, IF: 4.7)** **Final Scientific Report** **Update the WEB page of the project**
	A3.2. Correlation of the data obtained in stage 2	
	A3.3. Writing a scientific article based on the results from the second stage	

The objective of the ***Drug-ReSQue*** project was to obtain, physicochemically characterize, and assess the biological properties of nanotherapeutic systems based on commercial drugs (cytarabine ***Cit***, flucytosine ***FLU***, curcumin ***CRC***, and its derivative ***hCRC***) and squalene derivatives.

Squalene derivatives (squalene aldehyde ***SQ-CHO***, squalenic acid ***SQ-COOH***, and PEGylated squalene ***SQ-PEG***) were synthesized and physicochemically characterized in order to complete activities from ***A1.1*** to ***A1.5*** and from ***A2.1*** to ***A2.5***.

The critical micellar concentration (***CMC***) of ***SQ-PEG*** was determined at activity ***A1.6*** and ***A2.6***. The CMC values obtained in PBS solution with a pH of 7.4 were ***0.154*** and ***0.151 mg/mL*** respectively.

By squalenoylation the drugs ***MTx***, ***Cit***, ***FLU***, and ***CRC***, four novel systems (***SQ-MTx, SQ-Cit***, ***SQ-FLU***, and ***SQ-CRC***) were obtained at activities ***A1.7*** and ***A2.7***. The FTIR, ESI-MS, and proton and carbon NMR spectroscopy were used to demonstrate the obtaining of squalenoylated drugs (***A1.9*** and ***A2.9***).

Five novel systems, ***SQ-PEG-(MTx)***, ***SQ-PEG-(Cit)***, ***SQ-PEG-(FLU)***, ***SQ-PEG-(CRC)***, and ***SQ-PEG-(hCRC)***, were obtained during activities ***A1.8*** and ***A2.8*** by encapsulating drugs in ***SQ-PEG*** micelles.

Activities ***A1.10*** and ***A2.10*** were accomplished by determining the degree of encapsulation of ***MTx***, ***Cit***, ***FLU***, ***CRC***, and ***hCRC*** in ***SQ-PEG*** micellar structures using UV-Vis spectroscopy. The obtained results showed encapsulation efficiencies: **54%** (***MTx***), **45%** (***Cit***), **83%** (***FLU***), **91%** (***CRC***), and **74%** (***hCRC***).

STEM and DLS were used in activities ***A1.11*** and ***A2.11*** to characterize the morphology of the new nanotherapeutics. The findings of these investigations demonstrated that the five nanotherapeutics had spherical morphology, nanometric dimensions, and minimal aggregation tendencies. Additionally, by recording the zeta potentials, the obtained values proved to be dependent on the type of the encapsulated drug. As a result, the nanotherapeutics based on ***MTx*** and ***Cit*** showed values between **-3** and **+3 mV**, indicating low colloidal stability, whereas the ones based on ***FLU***, ***CRC***, and ***hCRC*** demonstrated increased colloidal stability with values between **-25.6** and **-21.86 mV**.

Remarkable results were obtained at activities ***A1.12*** and ***A2.12***, which involved determining the drug release profiles under simulated conditions (tumor and physiological). These results showed that the drugs could be encapsulated in ***SQ-PEG*** micelles to achieve a temperature and pH-dependent controlled release for ***MTx*** and ***Cit***, and sustained release over 72 hours for ***FLU***, ***CRC***, and ***hCRC*** (physiological conditions).

The *in vitro* cytotoxicity (normal cells) and anti-tumor efficaciousness (tumor cells) of nanotherapeutics based on ***MTx*** and ***Cit*** were investigated in activities ***A1.13*** and ***A1.14***. Following these investigations, it was shown that by encapsulating drugs in ***SQ-PEG*** micelles their biological properties were enhanced, reduced cytotoxicity, and increased anti-tumor efficacy on both cell lines.

Within the activities ***A2.13***, ***A2.14***, and ***A3.1***, the other three nanotherapeutics were subjected to *in vitro* investigations of their cytotoxicity (on normal HGF cells) and anti-bacterial efficiency (on 10 reference strains). Moreover, considering these investigations, it was shown that encapsulating the commercial drugs in SQ-PEG micelles, their biological properties were enhanced as follows: on four of the five examined yeasts, the nanotherapeutic containing ***FLU*** exhibited improved antibacterial efficacy and decreased cytotoxicity.

The results obtained within the Drug-ReSQue project during the implementation period between 01 April 2022 and 31 May 2024 were disseminated in the form of three scientific reports (two intermediate and one final), an oral communication and three posters presented at national and international conferences . Also the obtained results were the subject of two scientific articles in ISI journals Q1 and Q2 with high impact factor.

Participation in national and international conferences:

B.F. Craciun, D. Peptanariu, **M. Pinteala**. *PEGylated squalene micelles as amphiphilic nano-carrier for in vitro enhanced delivery of methotrexate to MCF-7 tumoral cells.* ***XXXVIth National Chemistry Conference (CNCHIM-2022)***, Călimănești – Căciulata, Vâlcea, Romania, ***04-07 Octombrie 2022***;

B.F. Craciun și **M. Pinteala**. *Enhanced in vitro effectiveness of conventional drugs using PEGylated Squalene micelles as nanocarriers.* ***5th Edition of "Faculty of Chemistry Conference <5-MIT IasiCHEM 2023>"***, Iasi, Romania, ***26-27 Octombrie 2023***;

B.F. Craciun și **M. Pinteala**. *PEGylated squalene-based nanotherapeutics: enhanced in vitro antitumor activity of commercial drugs.* ***15th Edition of the Conference "New Trends in Chemistry Research"***, Timișoara, Romania, ***21-22 Septembrie 2023***;

B.F. Craciun și **M. Pinteala**. *Novel nanotherapeutic systems based on PEGylated squalene for improving the in vitro activity of commonly used antitumor drugs.* ***25th International Conference "Materials, Methods & Technologies 2023"***, Burgas, Bulgaria, ***17-20 August 2023***.

Scientific papers in ISI rated journals:

B.-F. Craciun*, I.-A. Sandu, D. Peptanariu, **M. Pinteala***. *Novel Nanotherapeutic Systems Based on PEGylated Squalene Micelles for Enhanced In Vitro Activity of Methotrexate and Cytarabine*. ***Polymers* 2023**, 15, 4225. https://doi.org/10.3390/polym15214225 (Gold OA) (**IF = 5.0, Q1**).

B.-F. Craciun*, I. Rosca, D. Peptanariu, **M. Pinteala**. *Enhancing the flucytosine antifungal efficiency by encapsulation into PEGylated squalene micellar nanocarrier.* ***Bioconjugate Chemistry*, 2024**, ***Under review*,** Manuscript ID: **bc-2024-00237n**. **(IF = 4.7, Q2**).

More information can be found on the Drug-ReSQue project webpage:

https://icmpp.ro/projects/l1/about.php?id=49