Executive summary of the activities carried out during the implementation period

Project title: **Antiaggregation potential of 6-hydroxy-L-nicotine from Paenarthrobacter nicotinovorans pAO1 against amyloid peptide: in vitro and in vivo studies**

Contract No.: **PCE 49/2022**

Project code: **PN-III-P4-PCE-2021-1692**

Project manager: **Prof. univ. dr. habil. Lucian HRIȚCU**

Timeframe: **02/06/2022 – 31/12/2022**

In phase 1, all proposed activities were completed. Activities began with the evaluation of interactions between 6HLN and Aβ1-42 through molecular docking studies. Subsequently, the production of 6HLN was carried out using biotechnology based on the microorganism *Paenarthrobacter nicotinovorans*. The compound 6-hydroxy-L-nicotine (6HLN) was evaluated from the perspective of its antiaggregation and disaggregation potential against mature Aβ1-42 fibrils, as well as in cell cultures.

The obtained results demonstrated that 6HLN exhibits a binding energy to Aβ1-42 similar to that of nicotine. Moreover, compared to Aβ1-40 and Aβ25-35, 6HLN exhibits a stronger binding energy compared to nicotine (Fig. 1).

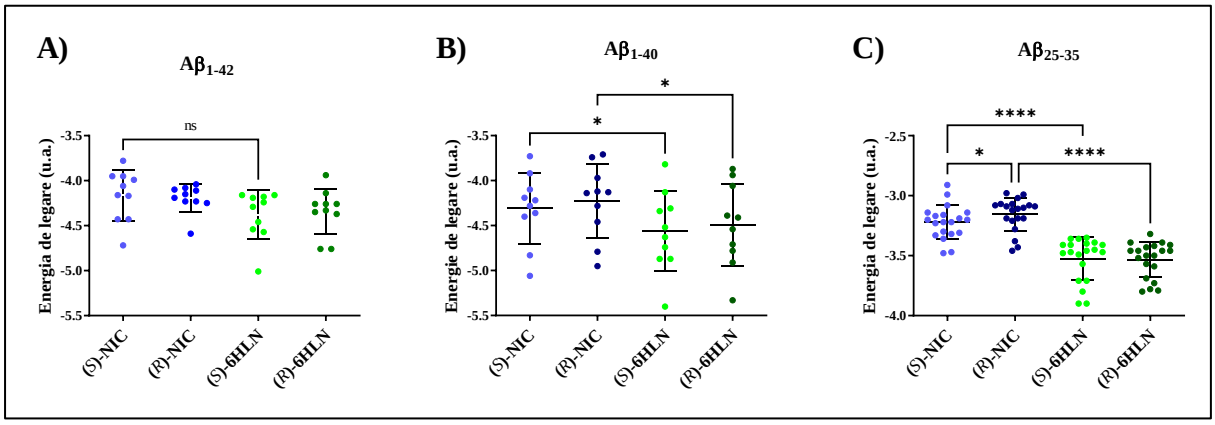

Fig. 1. Binding potential of nicotine (NIC) and 6-hydroxy-L-nicotine (6HLN) to Aβ1-42 (A), Aβ1-40 (B) and Aβ25-35 (C) receptors. Values are expressed as means ± S.E.M (n=10 conformations/receptor for Aβ1-42 and Aβ1-40 and n=20 conformations/receptor for Aβ25-35). ANOVA analysis identified overall significant differences for B) $F(1.693; 15.23)=10.44$; $p=0.002$ and C) $F(1.593; 30.26)=101.9$; $p<0.0001$. For Tukey post hoc analyzes – **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns – non-significant.

6HLN was produced using biotechnology based on the microorganism *Paenarthrobacter nicotinovorans*. Thus, an amount of ~30 mg of 6HLN was recovered from 2 L of culture medium supplemented with 1000 mg of nicotine resulting in a 3% yield (**Fig. 2**).

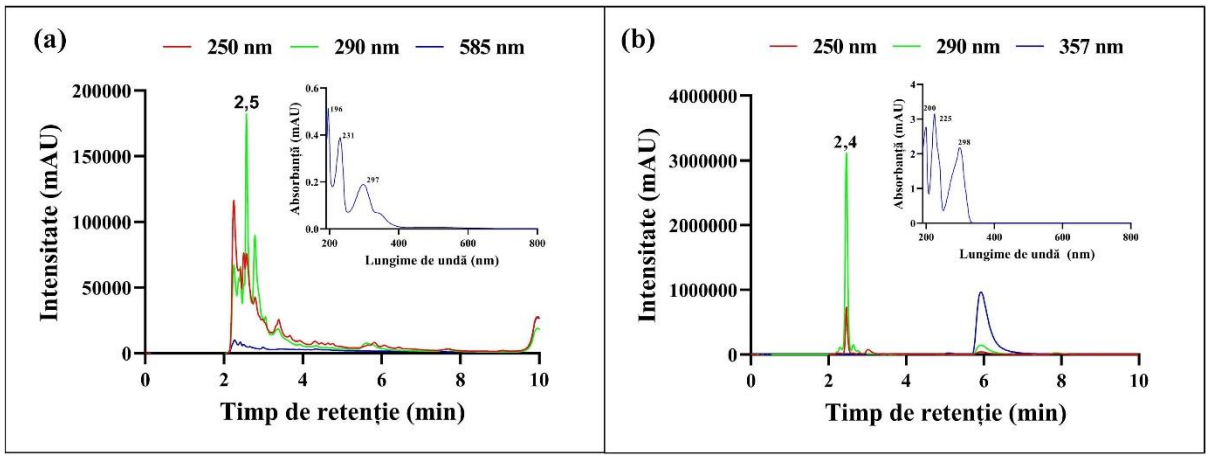

Fig. 2. HPLC analysis of the culture medium of the *P. nicotinovorans* strain after 27 h of cultivation (a) and 6HLN purified from the culture medium (b). The retention time of 6HLN is 2.5 ± 0.1 minutes. The absorption spectra of 6HLN are illustrated in the background of the graphs.

At 24 hours of incubation, in the A549 cell line, compound 6HLN (25nM, 100nM and 1000nM) significantly inhibited the aggregation of Aβ1-42 (1μM). Similar effects were also observed in the MCF7 cell line, where 6HLN (100nM and 1000nM) significantly inhibited Aβ1-42 (1μM) aggregation. At 48 hours of incubation, in the A549 cell line, 6HLN (100nM and 1000nM) significantly inhibited the aggregation of Aβ1-42 (1μM and 5μM), while at the concentration of 25nM, 6HLN significantly reduced only the aggregation capacity of Aβ1- 42 (1 μM). In MCF7 cells, 6HLN (1000nM) had a significant inhibitory effect on Aβ1-42 (1μM and 5μM) aggregation and 6HLN (100nM) significantly inhibited only Aβ1-42 (1μM) aggregation. At 72 h incubation, in A549 cells, 6HLN (1000nM) maintains its inhibitory effects on Aβ1-42 (1μM and 5μM) aggregation, while 6HLN (100nM) only inhibits Aβ1-42 (1μM) aggregation. Alternatively, in MCF7 cells, the aggregation capacity of Aβ1-42 (1μM) is significantly reduced by 6HLN (25nM and 100nM), whereas the aggregation capacity of Aβ1-42 (5μM) is significantly decreased by 6HLN (100nM) (**Fig. 3**).

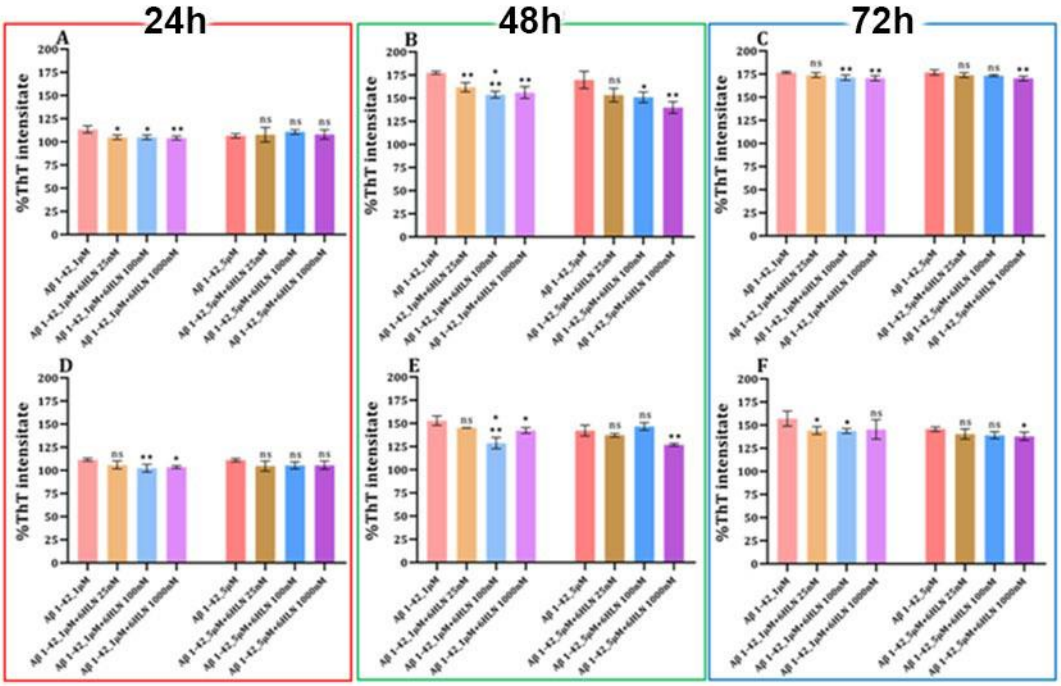

Fig. 3. Effects of 6HLN on the aggregation of Aβ1-42 used in the monomeric state in the A549 cell line (A, B, C) and in the MCF7 cell line (D, E, F).

In the A549 cell line, 6HLN (25nM and 100nM) disaggregates Aβ1-42 (1μM) fibrils, 24 hours after incubation.

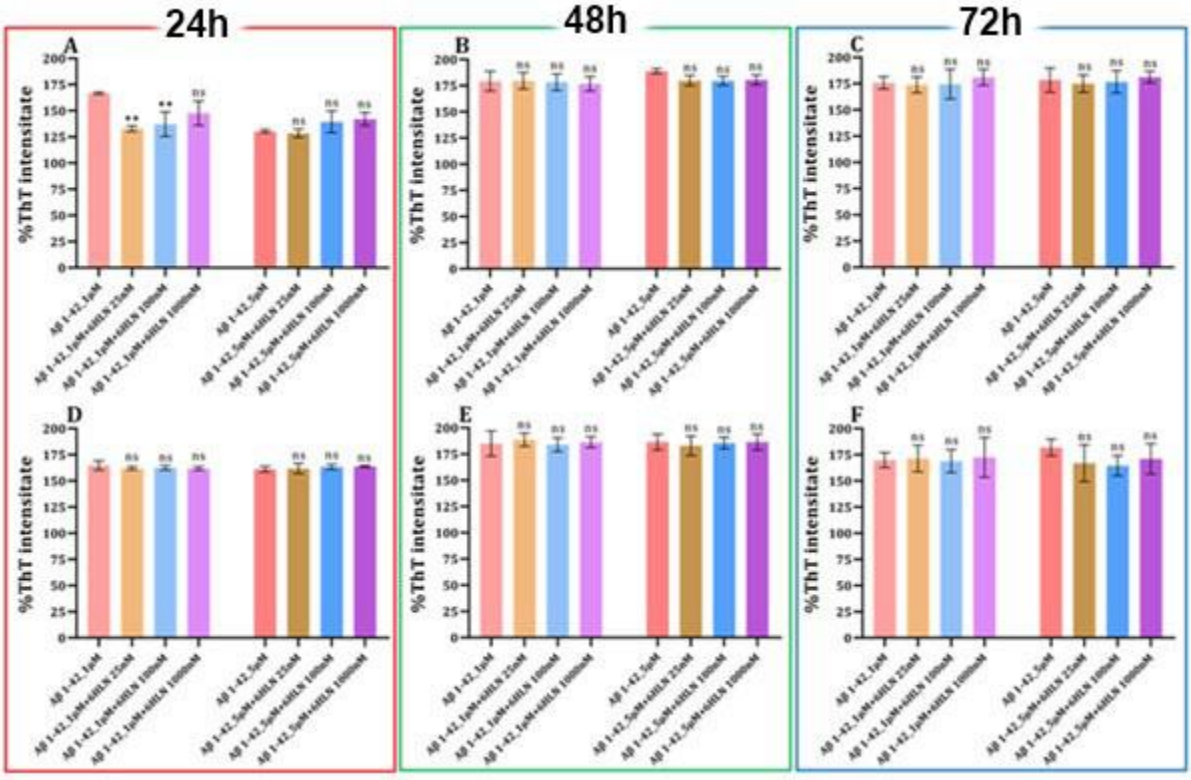

Fig. 3. Effects of 6HLN on the disaggregation process of Aβ1-42 used in the fibrillar state in the A549 cell line (A, B, C) and in the MCF7 cell line (D, E, F).

Disseminating the results

In the 1st stage of the project, the scientific results generated were presented in 3 conferences (2 international and 1 national) in the form of 3 oral presentations supported by the project director (Prof. dr. habil. Lucian Hrițcu). Participation in the conferences, together with announcements regarding the vacant positions put up for competition, were permanently published and disseminated on the project's web page: http://cercetare.bio.uaic.ro/grupuri/bioactive/content/grants/PCE2022_hl.html.

Two drafted manuscripts are currently under review at ISI journals.