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Self-assemblies of azacitidine prodrugs: a promising strategy of treatment for myelodysplastic syndromes

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Introduction

5-Azacitidine, a cytidine analogue and a hypomethylating agent, is a staple drug being in the treatment of myelodysplastic syndromes^[1]. However, after administration, it exhibits several limitations including restricted diffusion and cellular internalization due to its hydrophilicity, and rapid enzymatic degradation by deaminases. **The aim of this research is to improve the drug diffusion and protect it from metabolic degradation via the synthesis of amphiphilic prodrugs and their potential self-assembly.** Azacitidine was conjugated to two different omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The carboxylic acid group of the omega-3 fatty acids was conjugated to the amine group of azacitidine, yielding an amphiphilic prodrug. Next, the nanoprecipitation of the obtained prodrugs was performed and self-assemblies were successfully obtained for both prodrugs with a diameter of 200 nm, a polydispersity index below 0.2 and a positive zeta potential. Both self-assemblies had an IC₅₀ close to azacitidine. AzaEPA showed a slow and gradual internalization. This strategy would allow protection while increasing azacitidine's specificity and bioavailability. This prodrug should be cleaved by cathepsin B [2], an enzyme overexpressed in cancerous cells [3], thus increasing the specificity of the drug.

1. Prodrug synthesis

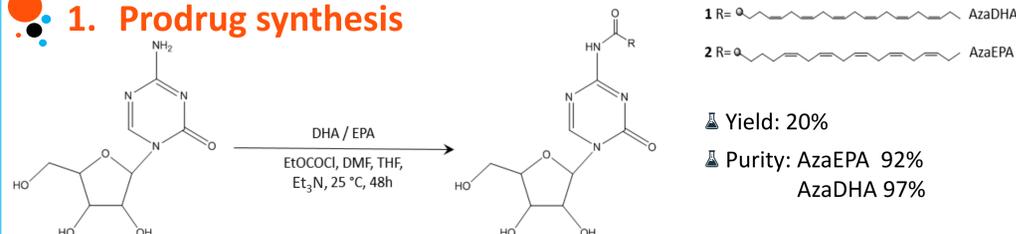


Figure: Synthesis of N⁴-azacitidine DHA (AzaDHA, 1) and N⁴-azacitidine EPA (AzaEPA, 2).

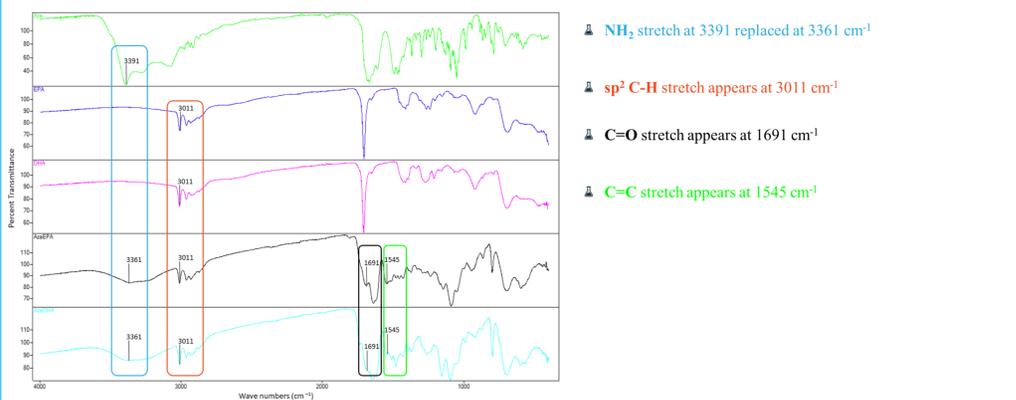


Figure: Fourier-Transform InfraRed spectroscopy curves and wave numbers of interest between 4000 cm⁻¹ and 600 cm⁻¹

Sample	Formula	Calculated %			Found%		
		C	H	N	C	H	N
AzaEPA	C ₂₈ H ₄₀ N ₄ O ₆	63.62	7.63	10.6	61.66	7.63	9.67
AzaDHA	C ₃₀ H ₄₂ N ₄ O ₆	64.96	7.63	10.1	63.2	7.71	9.31

Table: Elemental analysis of AzaDHA and AzaEPA

3. Cryo-TEM and MTT studies

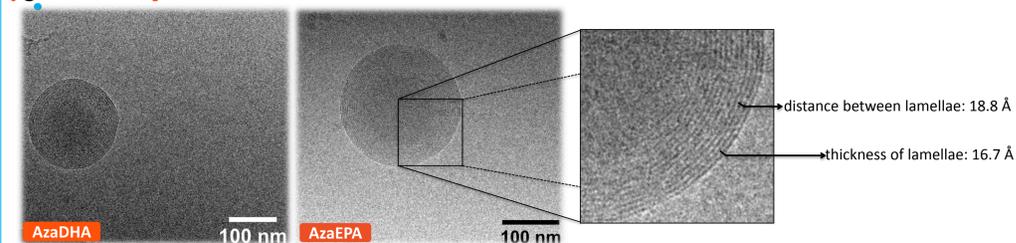


Figure: Cryo-TEM images of the prodrugs self-assemblies; forming multilamellar vesicles (MLVs).

IC ₅₀	Azacitidine	DHA	EPA	AzaDHA	AzaEPA
6 hours	6.5 μM	100.2 μM	196.7 μM	27.5 μM	33.7 μM
24 hours	1.0 μM	92.8 μM	115.9 μM	11.3 μM	16.6 μM
48 hours	1.4 μM	44.5 μM	103.8 μM	10.2 μM	13.7 μM

Table: The half maximal inhibitory concentration determined by a MTT assay on HL-60 cells, after treatment by azacitidine, fatty acids and self-assemblies at different time points.

Conclusion

The synthesis of two amphiphilic conjugates was successfully performed and verified. Their self-assembly in water was successful and confirmed using the fluorescent probe pyrene method to determine the critical aggregation concentration. The formed nanoassemblies were then characterized presenting a hydrodynamic diameter of 190 nm and a positive surface charge. The self-assemblies attain a multilamellar vesicle supramolecular structure. The obtained formulations had a low IC₅₀ comparable to that of free azacitidine, allowing them to achieve a close cytotoxicity. Additional in vitro studies on the HL-60 cell line (human acute myeloid leukemia) will be conducted to compare the efficacy of the self-assemblies compared to the parent drug.

References:

[1] Issa, J.-P. J. The myelodysplastic syndrome as a prototypical epigenetic disease. *Blood* 121, 3811–3817 (2013)

[2] Dheer, D., Nicolas, J. & Shankar, R. Cathepsin-sensitive nanoscale drug delivery systems for cancer therapy and other diseases. *Advanced Drug Delivery Reviews* (2019)

[3] Alatrash, G. et al. Cathepsin G is broadly expressed in acute myeloid leukemia and is an effective immunotherapeutic target. *Leukemia* 31, 234 (2016)

2. Critical aggregation concentration & nanoprecipitation

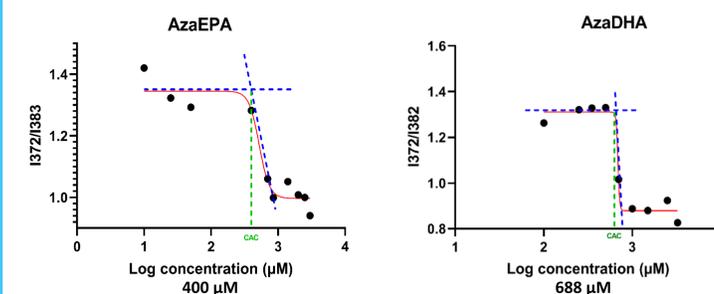


Figure: Boltzmann-type sigmoid obtained for the AzaEPA and AzaDHA suspensions in water showing the CAC value, corresponding to the first sharp decrease point.

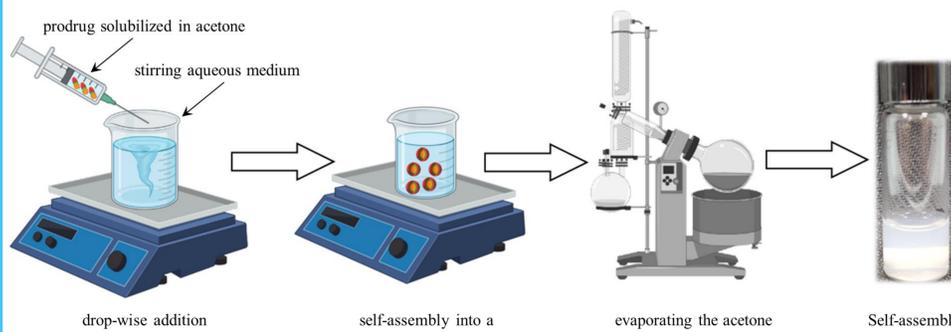


Figure: Formulation of self-assemblies via nanoprecipitation. The conjugates are dissolved in an organic solvent, then added drop-wise to a stirring aqueous medium allowing for the spontaneous formation of self-assemblies. The organic solvent is then evaporated using a rotary evaporator, thus yielding an opalescent aqueous suspension.

4. Perspectives

- Release study of azacitidine under the action of cathepsin B
- Protection from deamination study
- DOSY and SAXS experiments to further verify the obtained MLVs
- Stability of azacitidine vs. the self-assemblies in water
- Pharmacokinetics of drug release from MLVs
- Determining the internalization pathway
- Cell cycle studies
- Apoptosis studies
- Post-treatment cell morphology studies using Mgg coloration
- LINE-1 global methylation studies