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Self-assemblies of azacitidine prodrug: An innovative therapy against myelodysplastic syndromes

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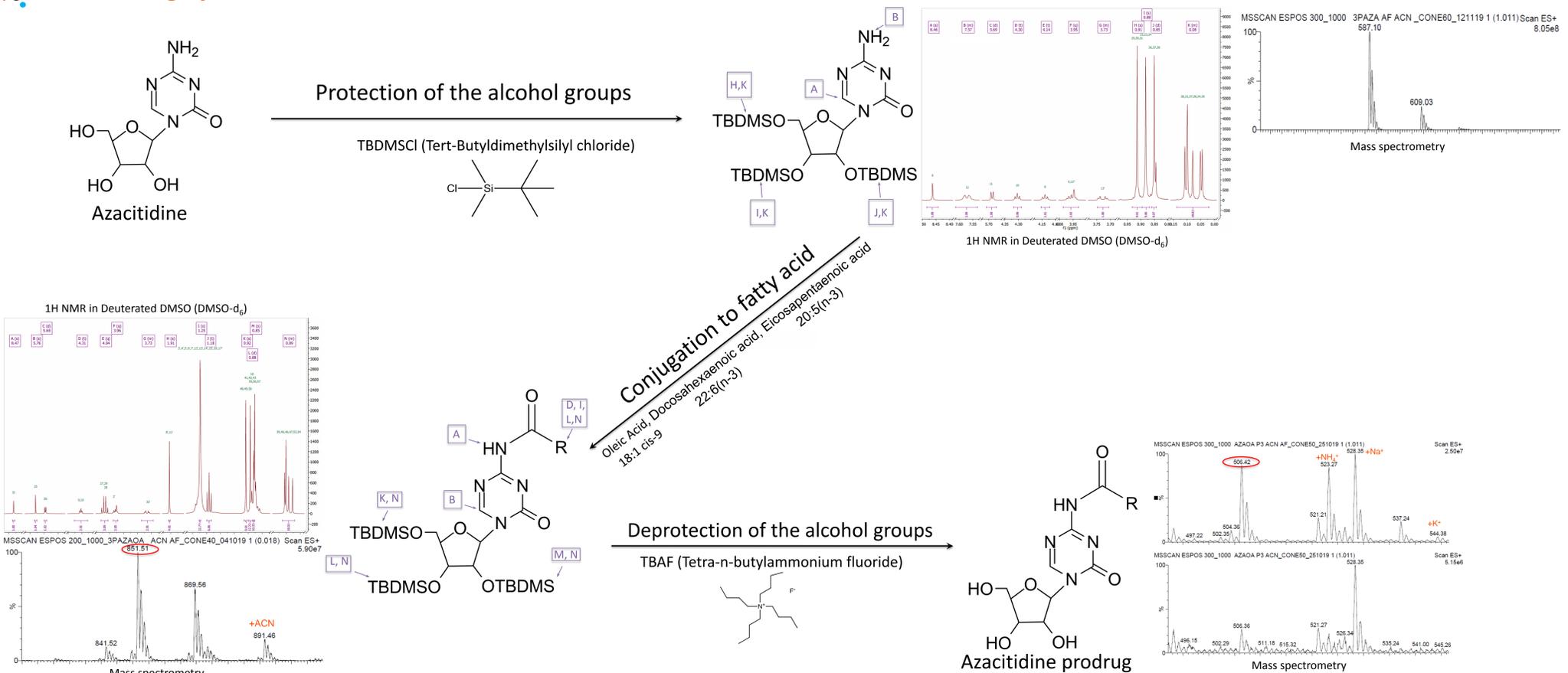
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Introduction

5-azacitidine, a cytidine analogue and a hypomethylating agent, is one of the main drugs being used for 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. **Our objective is to improve the drug diffusion and to protect it from metabolic degradation via the formulation of an amphiphilic prodrug and its self-assembly into a nanoparticle.** Following the conjugation of the fatty acid to the azacitidine, the obtained prodrug was solubilized in acetone and mixed with water at different ratios to obtain self-assemblies by nanoprecipitation, thus protecting the active molecule from enzymatic degradation. 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



2. Self-assembly and characterization

Further Experiments :
Determining size, shape and zeta potential:

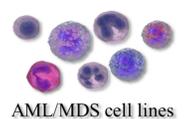
- Critical Aggregation Concentration (CAC) } To determine size and zeta potential
- Dynamic Light Scattering (DLS) } To determine size and concentration
- Nanoparticle Tracking Analysis (NTA) } To determine shape
- Transmission electron cryo-microscopy (cryo-TEM) } To determine shape

Nanoprecipitation: the prodrug is solubilized in an organic solvent (acetone) then mixed with water at different ratios, leading to the formation of the self assemblies. Following that, the solvent is evaporated to obtain the self-assembled nanoparticles suspended in water. The formation of the self-assemblies is characterized by the observation of an opalescence in the solution.

3. Next step: in vitro studies

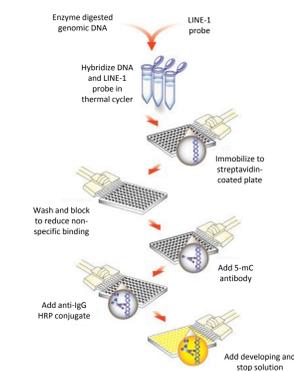
The *in vitro* studies will be performed on several acute myeloid leukemia cell lines with varying sensitivity to azacitidine:

- HI-60
- HEL
- Azacitidine resistant SKM1
- Molm 13
- SKM1



Studies to be performed:

- Cell viability studies (MTT, LDH)
- Cellular uptake studies
- Cell cycle study (FACS)
- Methylation quantification (LINE -1, whole DNA methylome analysis)



The Global DNA Methylation – LINE-1 is an ELISA-based assay that is specially formulated for the detection of methylation in Long Interspersed Nucleotide Element 1 (LINE-1) repeat elements of human genomic DNA. LINE-1 methylation serves as a surrogate for global DNA methylation levels and can be utilized to compare relative changes in 5-methylcytosine (5-mC) levels across different sample types

Conclusion

An azacitidine prodrug was achieved following a multi-step reaction to conjugate azacitidine to a fatty acid. The obtained prodrug will undergo a process of self-assembly to produce nanoparticles, that will be fully characterized. Finally, *in vitro* studies will be performed to determine the biological efficacy of these nanoparticles.

References:

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