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Introduction to the versatility of biomimetic membranes to characterize modulators of Aβ₁₋₄₂ fibrillization with potential interest in Alzheimer's disease treatment

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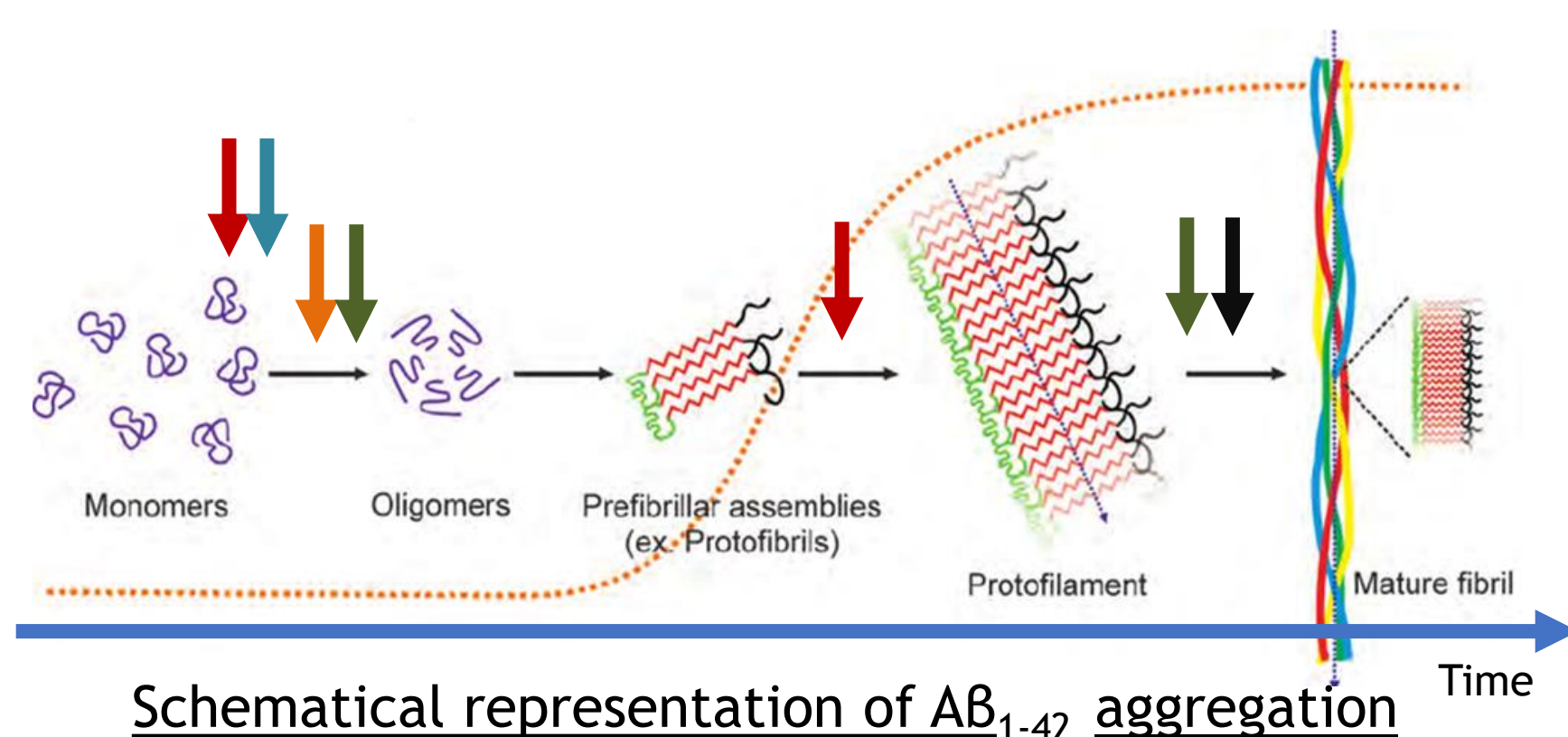
INTRODUCTION

Alzheimer's disease (AD) is the most widespread form of senile dementia worldwide and represents the leading socioeconomic problem in healthcare. The onset and the progression of this neurodegenerative disease is associated with the aggregation of the amyloid-β peptide (Aβ) through a fibrillization process. Hence, an attractive therapeutic strategy against this disorder is the development of molecules able to interfere at specific steps of this fibrillization pathway¹, *i.e.* able to inhibit the formation of the soluble toxic oligomeric Aβ aggregates.

To identify such promising therapeutic molecules, experimental methods are required *in vitro* to precisely follow and characterize the state (monomer/oligomers/fibrils) and the conformation of Aβ peptide during its fibrillization process. In the same time, these methods must be simple enough to remain compatible with the High Throughput Screening (HTS) of new compounds with potential interest in AD treatment.

In the present study, we propose to combine experimental methods to allow a multiparameter characterization of potential modulators of Aβ₁₋₄₂ fibrillization. These kinetic studies are carried out in presence of liposomes as biomimetic neuronal membranes. Indeed, it has been established that lipids of the neuronal membrane are an important factor in the cascade of events that leads to the formation of the amyloid fibers.

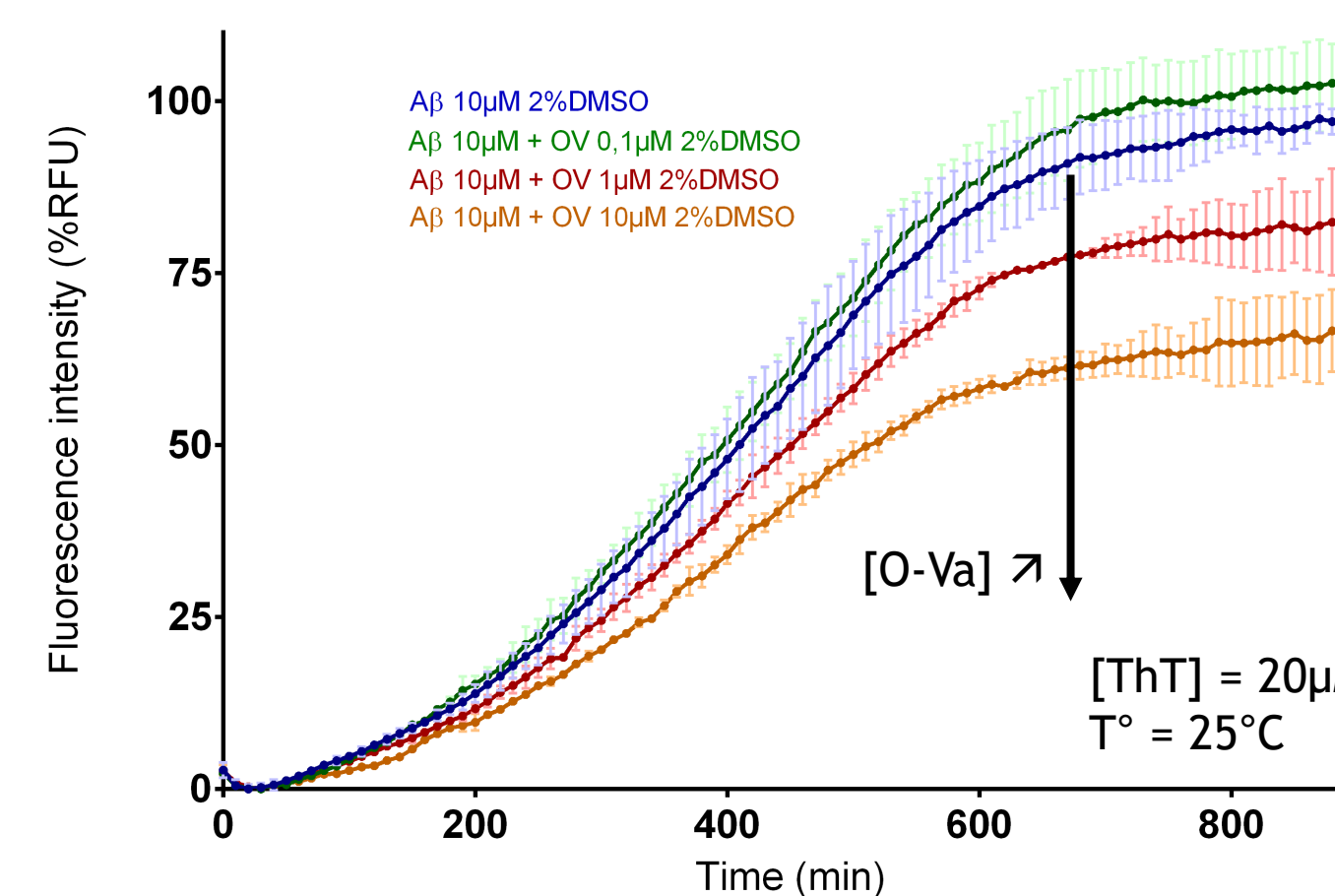
Adapalene Bexarotene Indometacin O-Vanillin Chicago sky blue 6B



We used ThT to monitor³ the effect of these molecules on the aggregation of the peptide in their presence at different concentrations.

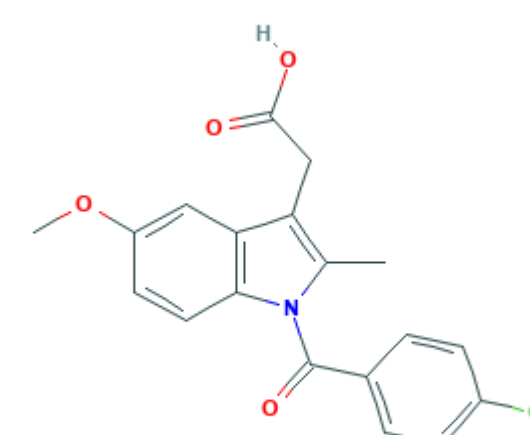
Kinetic study of Aβ₁₋₄₂ aggregation

In order to be able to characterize new compounds we have selected 5 molecules whose anti-aggregative properties are already described in the scientific literature.^{1,2}



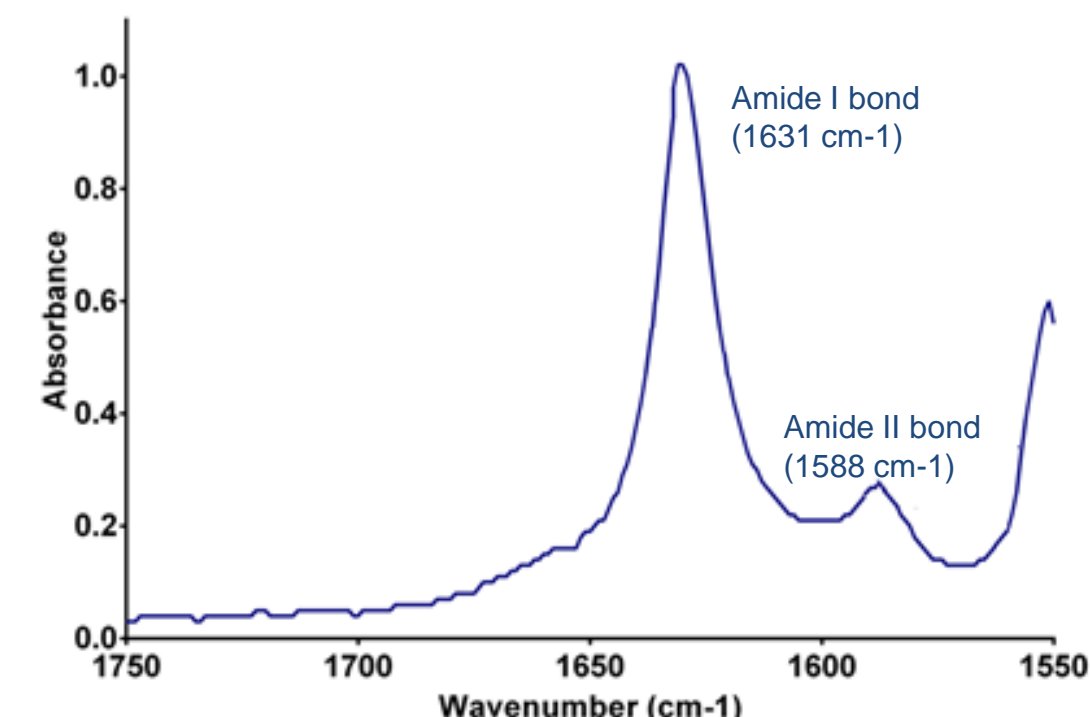
Aβ₁₋₄₂ kinetic in presence of o-vanillin

Quantitative effect observed in the presence of o-vanillin. In addition to inhibiting the formation of oligomers, it also inhibits the formation of fibers.



Conformational study of Aβ₁₋₄₂ aggregation

In parallel, considering that the fibers formed by Aβ are mainly organized into β-sheets, assays were correlated with the secondary structure analysis of the peptide using ATR-FTIR spectroscopy.⁵

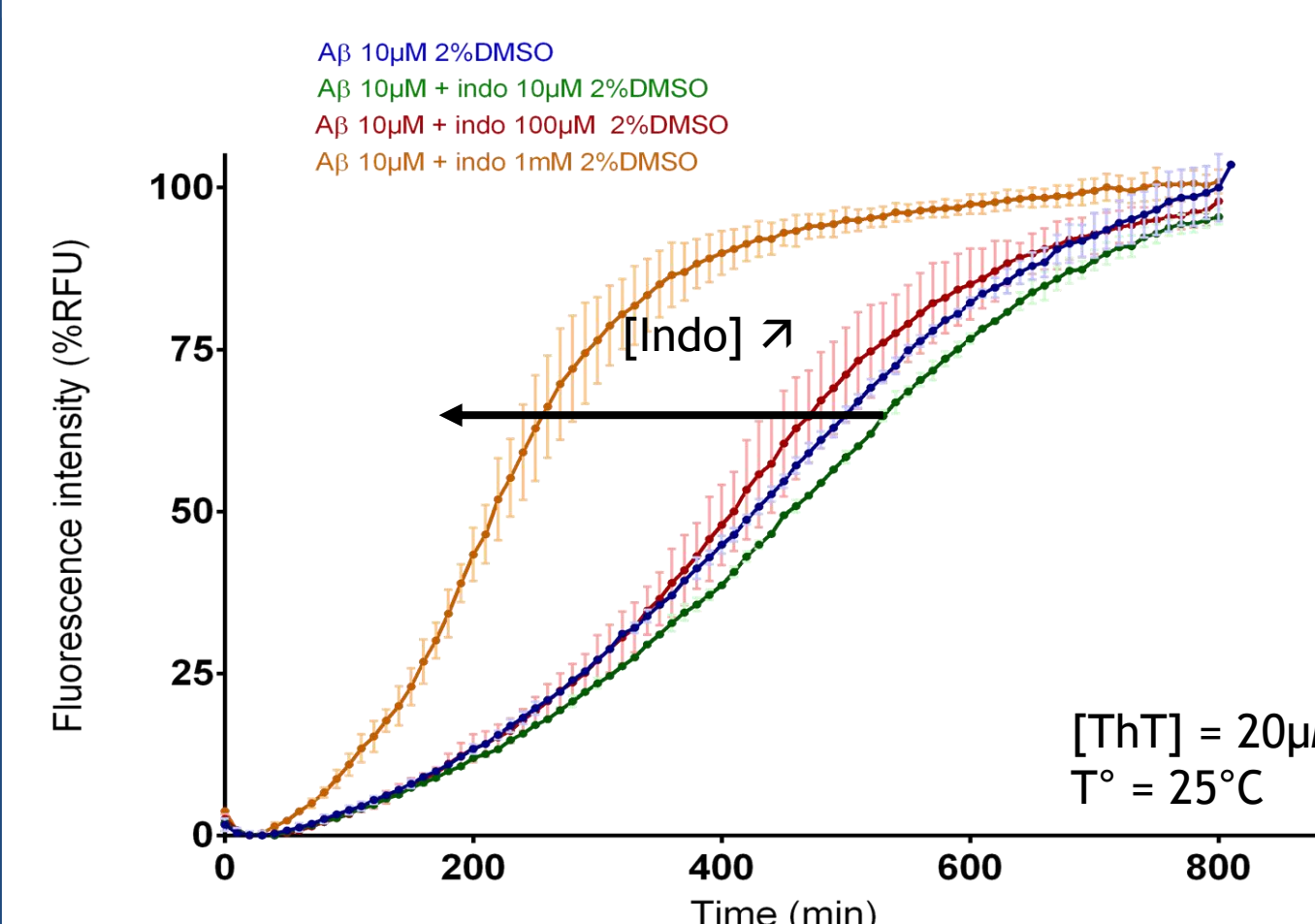


ATR-FTIR spectra of aggregated Aβ₁₋₄₂

Structure	Amide I frequency (cm ⁻¹)
random coil	1630-1650
α-helix	1650-1670
β-sheet	1620-1640
Aggregated strands	1610-1630

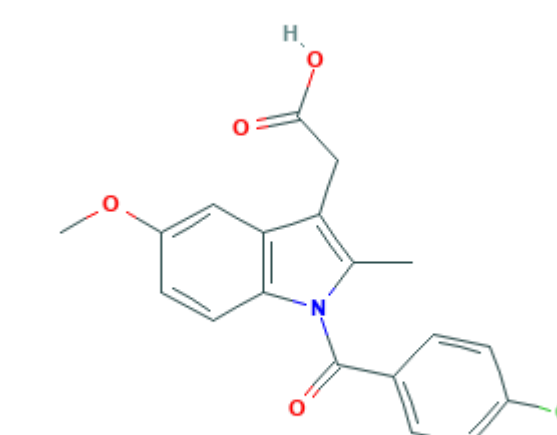
ATR-FTIR spectra of aggregated Aβ₁₋₄₂ at 10 μM. The amide band I position is characteristic of the β sheets.

Using a reference (peptide alone), it will be possible to make relative comparisons and to study possible effects of the molecules of interest on the peptide structuration.



Aβ₁₋₄₂ kinetic in presence of indometacin

Kinetic effect observed in the presence of indometacin. Inhibiting the formation of oligomers (critical step of aggregation) results in an acceleration of the aggregation.



It is therefore possible to discriminate different types of molecule actions thanks to ThT monitoring, which is a simple and effective method that is perfectly adapted to screening.

However, experiments will be performed on variants of Aβ₁₋₄₂⁴ to confirm our results and support our conclusions.

Molecule/membrane interactions

In order to take into account the role of the membrane in the peptide aggregation but also because the molecules tested will be potential drug candidates, it is essential to study the interactions between the molecules and the membranes.

First, using a complex model of liposomes (SPC/CHOL/PG₆₀:20:20) to mimic neuronal membranes, we have transposed a methodology of the scientific literature⁶ to study of interactions between modulators of Aβ₁₋₄₂ aggregation and membranes.

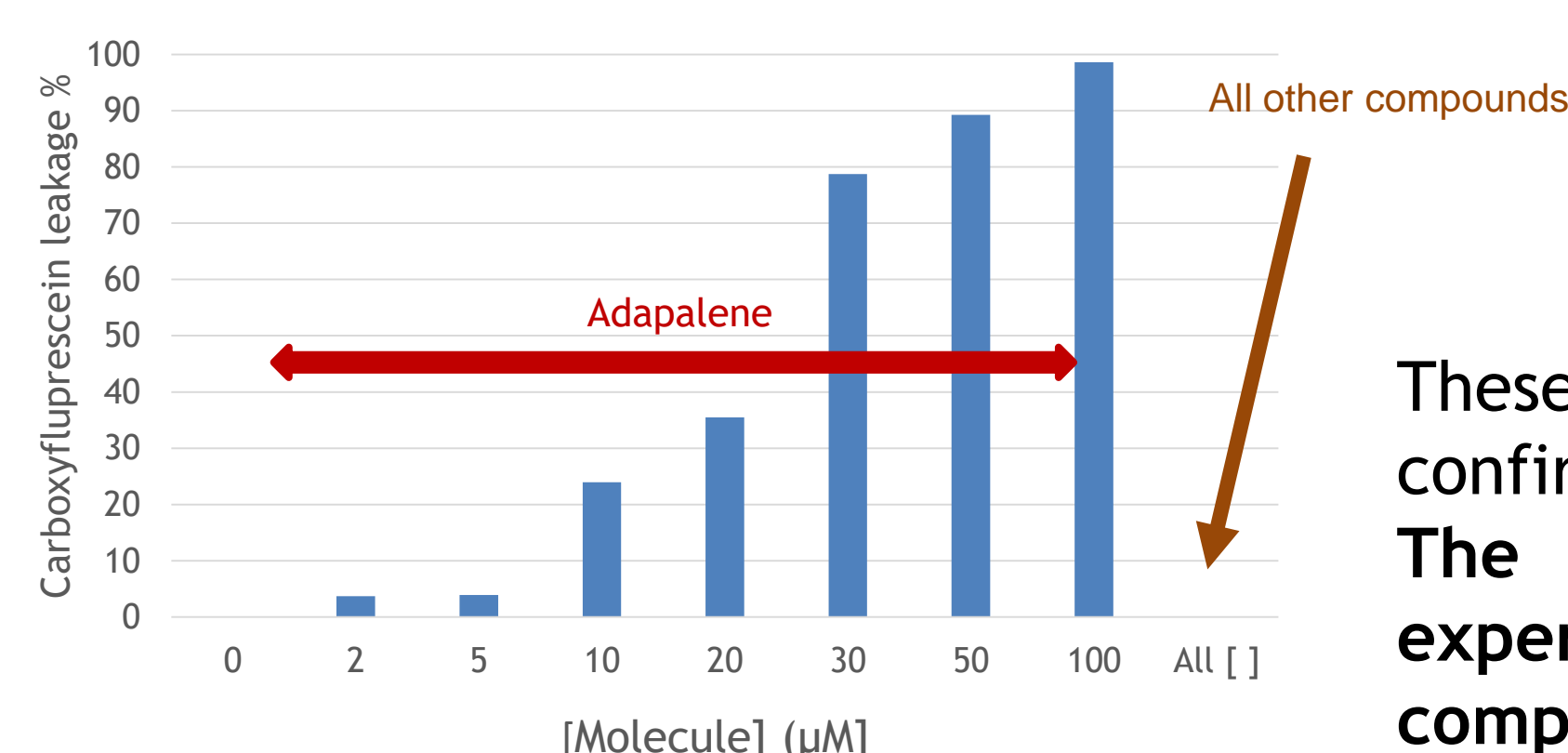
Only one of the compounds demonstrated interaction with the liposome membranes.

These results were correlated with toxicity studies where adapalene is also the only compound to have exerted a membrane destabilization.

In order to study the toxicity, we once again used liposomes using the liposome leakage assay⁷ method.

	Adapalene	Bexarotene	Chicago SB6B	Indometacin	o-Vanillin
Structure					
Log D _{7,4} (MarvinSketch)	3,9	3,82	-4,95	0,27	1,86
Log K _p	3,6	<1	<1	<1	<1

Partition coefficient determination of aggregation modulators



Liposome leakage by aggregation modulators

These are preliminary results and confirmation remains necessary. The combination of these two experiments allows a more complete prediction of the interaction of molecules with membranes.

CONCLUSION

From results obtained to date, it appears that the tested modulators present a range of effects that can affect defined stages of the aggregation of the Aβ₁₋₄₂ peptide. Conformational studies with ATR-FTIR spectroscopy will give us the possibility to analyse if any molecules have an effect on the secondary structure. Especially, on anti-parallel β-sheets structure that characterizes the presence of toxic oligomeric species⁸.

In the long term the results will make it possible to characterize the kinetic effects of the reference molecules and may be applied to original molecules synthesized at the CERMN.

This study highlights also some advantages of the use of liposomes to characterize and discriminate newly Aβ aggregation modulators synthesized by chemists and could even be expanded to other amyloid diseases.

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