Synthesis and physico-chemical characterization of fatty esters with an aromatic part.

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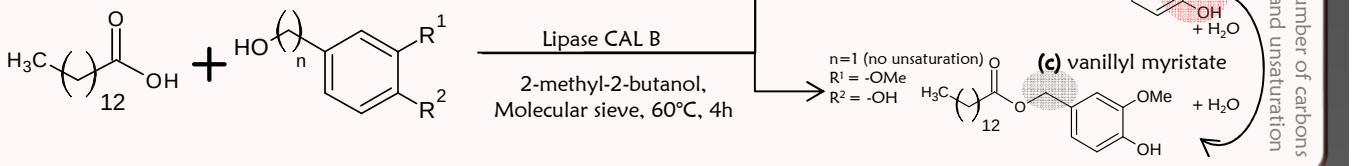
Interests

Specific antioxidant molecules (e.g. phenolic compounds) help to prevent oxidation reactions of the cell membrane. Grafting a long alkyl chain onto these compounds should improve their interfacial properties and their ability to interact with membrane lipids.

Objectives

A structure-function relationship study was undertaken to identify the structural patterns affecting the interfacial and membrane interaction properties. A computational approach was used to predict the interactions with a lipid and membrane model. The toxicity was evaluated *via* a cell viability assay.

1st step: Synthesis *via* an enzymatic route



2nd step: Biophysical Characterization

Interfacial properties

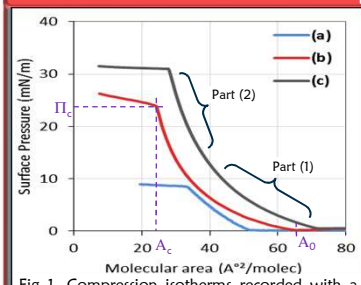


Fig 1. Compression isotherms recorded with a Langmuir film balance (KSV, Finland). Spreading of pure compounds (25µl, 2mM) on milliQ water at 25°C. The aromatic reagents were not able to form stable monolayers.

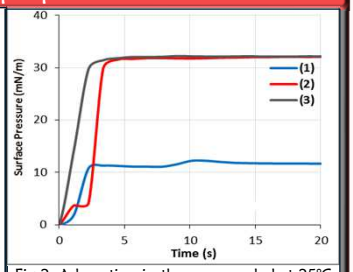


Fig 2. Adsorption isotherms recorded at 25°C with a Langmuir film balance (KSV, Finland). Injection of pure compounds solutions into milliQ water (final concentration of about 6 µM)

Table 1: Characteristic parameters of the monolayers determined by compression isotherms at air/water interface and by molecular modelling

	A_0 (Å²/molecule)	A_c (Å²/molecule)	Π_c (mN/m)	Compressibility (m/N)	$A_{Modelling}$ (Å²/molecule)
(a)	48,6± 1,6	32,4± 0,9	8,2± 0,6	58,5	56,4± 1,7
(b)	64,9± 1,0	23,2± 1,6	26,9± 4,0	82,1 (1) 27,6 (2)	57,3± 3,5
(c)	70,9± 2,6	26,9± 1,3	31,4± 0,4	54,7 (1) 19,4 (2)	67,0± 3,7

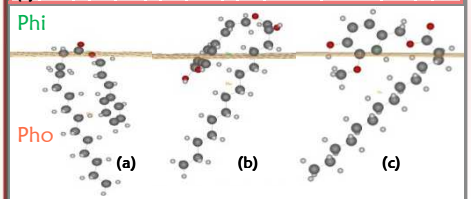


Fig 3. Organization of the molecules at an hydrophobic/hydrophilic interface determined *in silico* (Structure Tree method[1]).

Discussion

The addition of an alkyl chain confers interfacial properties to the phenolic compounds. **(a)**, **(b)** and **(c)** molecules have an affinity for the air/water interface; weak for **(a)** but stronger and similar for **(b)** and **(c)**. They are able to form monolayers with increasing stabilities: **(a)**<**(b)**<**(c)**. We observe $A_{0(a)} < A_{0(b)} < A_{0(c)}$: molecular areas increase to maximize the amount of polar groups in the hydrophile phase.
When injected into the subphase, molecules absorbed immediately onto the interface.

In Silico prediction of Interactions with membranes

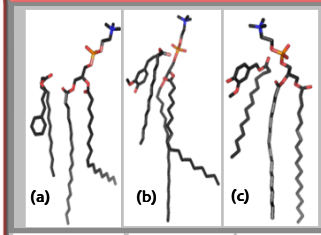


Fig 4. Molecular assembly of **(a)**, **(b)**, and **(c)** with POPC, a lipid representative of eucaryotic cell membranes. Calculated according to the Hypermatrix method[2].

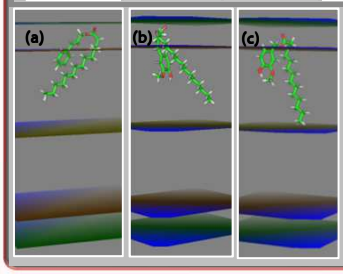


Fig 5. *In Silico* prediction of the interactions of **(a)**, **(b)** and **(c)** with a model membrane calculated according to the IMPALA method[3].

Discussion

For all molecules, the ester bond neighbours POPC's ester bond and the alkyl chains are close and nearly parallel. **(a)**, **(b)** and **(c)** insert into the external sheet of the membrane. The hydrophobic chains are tilted. Polar phenolic groups of **(b)** and **(c)** remain in the hydrophobic region and can probably cause membrane perturbations.

Toxicity evaluation

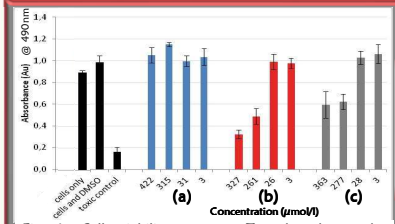


Fig 6. Cell viability assay . Test based on the bioreduction of MTS into formazan (G5421, Promega)

Discussion

Molecule **(a)**: non toxic.
Molecules **(b)** and **(c)**: toxicity dependant on concentrations and similar.

Conclusion

The addition of a fatty chain on aromatic molecules enhances their interfacial properties. All the tested molecules showed an affinity for the air/water interface. It increased with substitution of the aromatic ring with OH and OMe groups. They spontaneously penetrate membranes. Their interaction with the membrane could be at the origin of cell toxicity due to a phenomenon of membrane destabilization. The toxicity of phenolic fatty esters is concentration-dependent. In the future, their *in-vivo* antioxidant ability at low-concentration will be studied.

References

[1] Lins L., Biochemical Pharmacology, 52, 1155-1168, 1996. [2] Brasseur, R., CRC Press: Boca Raton, FL, Vol. 1, 203–219, 1990. [3] Ducarme, Ph., Proteins: Structure, Function, and Genetics, 30, 357-371, 1998.

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