Novel phenolic glycolipids: antioxidant activity and effect on membrane models.

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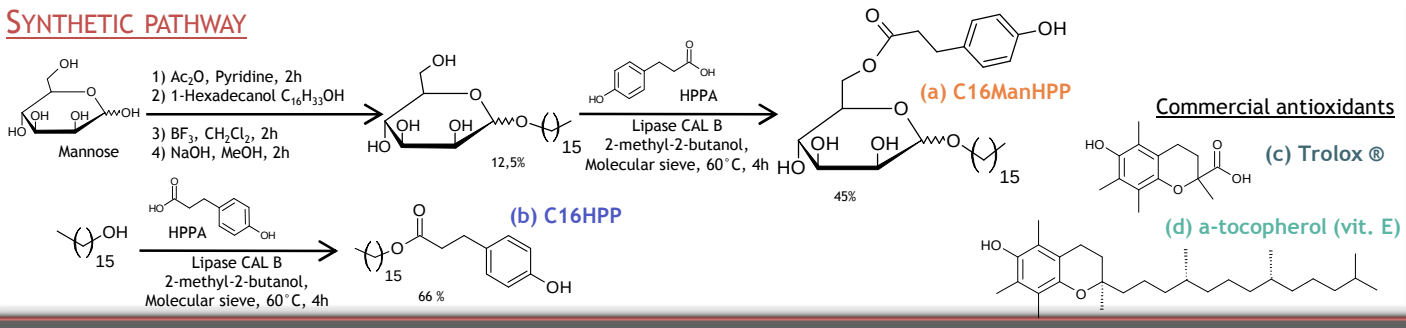
Background

Specific antioxidant (AO) molecules (e.g. phenolic compounds) help to prevent oxidation reactions of the cell membrane [1]. Grafting a long alkyl chain onto these compounds should improve their ability to interact with membrane lipids [2]. The presence of a sugar unit could also be useful to target specific cells [3].

Strategy

In this study, a novel phenolic glycolipid and its derivative without sugar unit were synthesized. Their cytotoxicity and their effect on cell viability when an oxidative stress is induced were tested. The cumulative effect of both kind of compounds should bring a higher cell viability than the one observed without the antioxidant. In parallel, their interaction with cell models (liposomes) was studied through membrane permeability experiments.

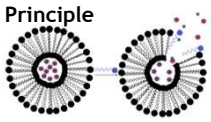
Synthetic pathway



Do studied compounds permeabilize liposomes?

Permeability Assay

Principle



Test based on the measurement of the fluorescence induced by the release of the fluorescent probe out of liposomes.

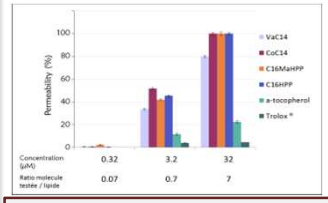
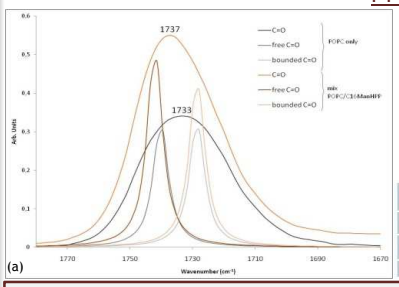


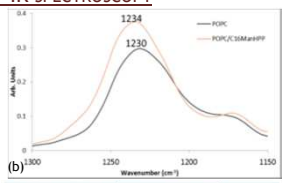
Fig 1.: Permeability percentage in function of the ratio lipid/ studied compounds.

At a ratio AO/lipid around 1, both synthesized compounds have an action on liposomes and reference AO not.

Do studied compounds interact with lipids at molecular level? If yes, with which part of them ?

FT-IR spectroscopy





Vibration wavelenght (cm^{-1})	C=O Ester		P=O Ester
	free	bounded	
POPC	1739	1728	1230
POPC/C16ManHPP	1741	1728	1234
POPC/C16HPP	1740	1726	1234

Fig 2.: C=O (a) and P=O (b) stretching bands of lipid POPC polar head.

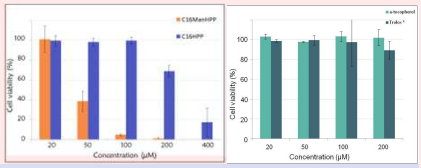
The shift of the bands characterizing the phospholipid confirms the interaction of both compounds with biomimetic bilayers. More particularly, the polar head of the phospholipid is involved. No significant shift of alkyl bands was observed indicating that the fatty chain is probably less implicated in the interaction lipid-compound.

Cellular Viability Assay

Principle

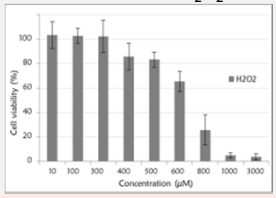
Test based on the bioreduction of MTS into formazan by cellular dehydrogenase enzyme present in alive cells.

Step 1 : What is the maximum non toxic AO concentration ?
=> incubation of AO for 24 h



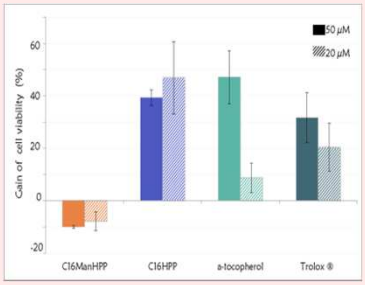
Results: Working AO concentration= 20 µM

Step 2 : What is the minimum toxic oxidant (H_2O_2) concentration ?
=> incubation of H_2O_2 for 3h



Results: Working concentration = 1000 µM

Step 3 : What is the effect on cells of oxidant/antioxidant combination?
=> incubation of AO for 24h then H_2O_2 for 3h



Results: Commercial AO and C16HPP improve the cell viability regarding the negative effect of H_2O_2. The compound bearing a mannose unit increase the cell toxicity induced by H_2O_2.

Conclusions

The two synthesized compounds are able to interact with membrane lipids and more particularly with the polar head of phospholipids. These interactions induce permeabilization of the bilayer at high concentration and could explain their cytotoxicity. The C16HPP is able to lower the negative effect of an oxidant agent (H_2O_2), but the C16ManHPP increases the negative effect of this agent and causes damages leading to cell death even at low concentration.

References

[1] Kemeleva, *Russian Journal of Bioorganic Chemistry*, **2008**, 34, 4, 499-509. [2] Lopez-Giraldo, *OCL*, **2007**, 14, 1, 51-59. [3] Lopez-Herrera, *Biochimica et Biophysica Acta*, **2005**, 1741, 55- 64.

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