Pharmacomodulation of ellagic acid, a promising antiplasmodial agent, to improve its bioavailability.

Gilles Degotte[1,2] ; Annabelle Halleux[2]; Aurore Hans[2] ; Olivia Jansen[1] ; Allison Ledoux[1] ; Pauline Desdemoustier[1] ; Bernard Pirotte[2] ; Pierre Francotte[2] ; Michel Frederich[1].

[1] *Laboratoire de Pharmacognosie, Centre Interdisciplinaire de Recherches sur le Médicament (CIRM), ULiège – Quartier Hôpital - B36 Tower 4, Avenue Hippocrate 15, 4000 Liège, Belgium.*

[2] *Laboratoire de Chimie Pharmaceutique, Centre Interdisciplinaire de Recherches sur le Médicament (CIRM), ULiège – Quartier Hôpital - B36 Tower 4, Avenue Hippocrate 15, 4000 Liège Belgium.*

Email: gdegotte@doct.uliege.be

Context

Vector-borne diseases represent more than 17 percent of all infectious sicknesses and affect millions of people each year.[1] Among them, malaria is the most important causing more than 400 000 deaths and 216 million of cases. This parasitosis is caused by a protozoan belonging to the *Plasmodium* genus. More than 90% of cases and deaths following malaria are linked to *P. falciparum* which is transmitted thanks to mosquitoes from *Anopheles* family.[2]

While many efforts are consent to develop a vaccine, chemotherapy remains the most valid therapeutic strategy, nowadays. Combination of artemisinin derivatives (artemether, artesunate) and a long acting agent such as lumefantrine, amodiaquine, mefloquine… are particularly recommended. Unfortunately, as for antibiotics, resistance to antimalarials emerge from their use. Considering this resistance and the prevalence of malaria, development of new antiplasmodials represents an urgent need.[3]

Objectives

Nature remains an important source of medicinal compounds[4]. Screening of natural molecules identified from plants used in traditional medicine leads to identification of metabolites which are active against *Plasmodium*'s strains, including the one which are resistant to modern antiplasmodials.

Among the candidates, ellagic acid (EA, **1**) seems to be one of the most promising compound thanks to its proven *in vitro* efficacy (105-330 nM) and *in vivo* activity after intraperitoneal injection. Interestingly, no toxic effect could be demonstrated at >5 g/kg (*per os*) or 1g/kg/d (IP). This polyphenolic compound seems thus very attractive for malaria treatment.[5] However, EA suffers from a poor oral bioavailability, partly explained by a reduced water solubility (9.7 µg/mL) itself linked to strong intermolecular bounds[6].

The aim of our work is the design and the preparation of crowded analogues and prodrugs with enhanced solubility and by extension, improved bioavailability which would permit its oral use.

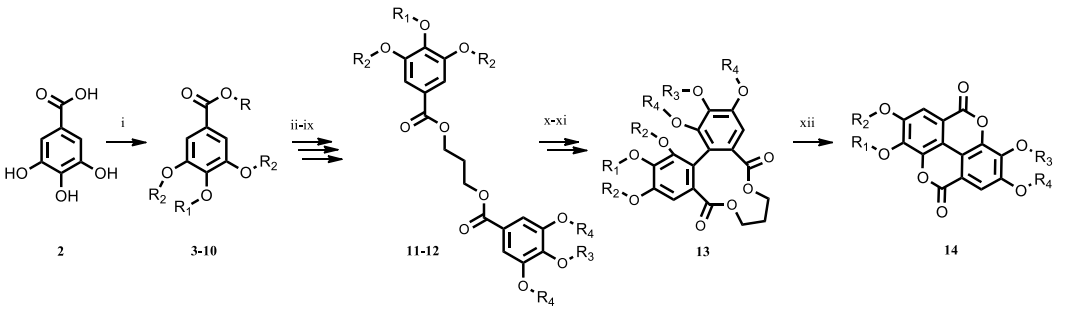
Pharmacomodulation

As described in Fig.1 , taking advantage of phenolic functions, we plan to insert bulky/polar chains on selected positions. This should lead to crowded analogues of EA. The planned pharmacomodulations should also give insights about the pharmacophore of this polyphenol.

Fig. 1 Pharmacomodulation opportunities

Synthesis

Considering issues encountered following a direct synthesis approach, a total synthesis strategy is preferred in order to obtain the desired molecules.[7,8]



i CH_3OH, H_2SO_4 ii Ac_2O, H_2SO_4 iii BnBr, KI, K_2CO_3, acetone iv K_2CO_3, CH_3OH, H_2O v NaH, MOMCl, DMF vi LiOH, CH_3OH, THF, H_2O vii 1,3-propanediol-PMB, DMAP, EDCI-HCl, CH_2Cl_2 viii DDQ, Sorenson's buffer, CH_2Cl_2 ix DMAP, EDCI-HCl, CH_2Cl_2 x THF, IPA/HCl xi $CuCL_2$, *n*-$BuNH_2$, CH_3OH xii LiOH, CH_3OH, THF, H_2O

Antiplasmodial effect

In order to explore the effect of substituted dervivatives on malaria parasite, final compounds and all the intermediates are submitted in an *in vitro* assay to determine their inhibitory effect, expressed as IC_{50} (µM), on 3D7 strain of *Plasmodium falciparum* (chloroquin-sensitive). This evaluation follows the procedure established by Frederich et *al.* (2002)[8] and is based on colorimetric measure of *Plasmodium* lactate deshydrogenase activity[9] realized at least in triplicates.

Conclusion & Prospects

As observed during the pharmacological assay, some intermediates showed a higher effect (< 125 µM) than gallic acid (**2**). Interestingly, this inhibitory activity clearly increases when there is a linker between 2 gallate moieties. This observation suggests the importance to look like as a dimer to observe the same rank of potency of EA.

We can also observe that substituants on the phenolic functions seem to negatively impact this effect (**11 vs 12**).

The next step will be testing against 3D7 to confirm pharmacological results and the final substituted compound. Moreover, other mono- and disubstituted derivatives of EA's scaffold will be synthetized. Another part of the future work will consist to determine the aqueous solubility of the new compounds vs EA.

Product (*n*=)	R	R_1/R_3	R_2/R_4	IC_{50} (µM)
1	/	H	H	IP
2 (3)	H	H	H	125,19±5,76
3 (3)	CH_3	H	H	118,14±16,62
4 (2)	CH_3	CH_3CO	CH_3CO	47,29±17,98
5 (3)	CH_3	$BnCH_2$	CH_3CO	33,27±12,86
6 (3)	CH_3	$BnCH_2$	H	54,11±13,02
7 (1)	CH_3	$BnCH_2$	MOM	43,83
8 (3)	H	$BnCH_2$	MOM	58,74±28,25
9 (4)	$CH_2CH_3CH_2OPMB$	$BnCH_2$	MOM	19,89±4,16
10 (2)	$CH_2CH_3CH_2OH$	$BnCH_2$	MOM	82,02±15,64
11 (2)	$CH_2CH_3CH_2$	$BnCH_2$	MOM	17,19±5,23
12 (2)	$CH_2CH_3CH_2$	$BnCH_2$	H	3,67±0,19
13	$CH_2CH_3CH_2$	$BnCH_2$	H	IP
14	/	$BnCH_2$	H	IP

IP = In Progress

Thanks to FNRS, Fondation Léon Fredericq and University of Liège for financial support.

References :

1. World Health Organisation. *World Malaria Report 2017*. (2017). doi:http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/
2. WHO. *World malaria report 2016*. *World Health Organization* (2016). doi: ISBN 978 92 4 1564403
3. Lubell, Y. *et al.* Artemisinin resistance – modelling the potential human and economic costs. *Malar. J.* **13,** 452 (2014).
4. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod*. 2016;79(3):629-661. doi:10.1021/acs.jnatprod.5b01055
5. Soh, P. N. *et al.* In vitro and in vivo properties of ellagic acid in malaria treatment. *Antimicrob. Agents Chemother.* **53,** 1100–1106 (2009).
6. Bala, I., Bhardwaj, V., Hariharan, S. & Kumar, M. N. V. R. Analytical methods for assay of ellagic acid and its solubility studies. *J. Pharm. Biomed. Anal.* **40,** 206–210 (2006).
6. Hirokane, T., Hirata, Y., Ishimoto, T., Nishii, K. & Yamada, H. A unified strategy for the synthesis of highly oxygenated diaryl ethers featured in ellagitannins. *Nat. Commun*. **5,** (2014).
7. Gokcen, T., Gulcin, I., Ozturk, T. & Goren, A. C. A class of sulfonamides as carbonic anhydrase I and II inhibitors. *J. Enzyme Inhib. Med. Chem.* **31,** 180–188 (2016).
8. Frédérich M, Jacquier M-J, Thépenier P, et al. Antiplasmodial Activity of Alkaloids from Various Strychnos Species. doi:10.1021/np020070e
9. Makler MT, Ries JM, Williams JA, et al. Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am J Trop Med Hyg. 1993;48(6):739-741. doi:10.4269/ajtmh.1993.48.739