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Modifications of the chemical structure of terpenes in antiplasmodial and antifungal drug research

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Abstract—Pure natural monoterpenes were evaluated in vitro for their antiplasmodial activities against *Plasmodium falciparum*. Chemically modified terpenes were also tested to see whether the introduction of an alkyne, a cyclopropane, a diene, or a cyclopentenone moiety had an influence on the biological activity. The IC_{50} obtained on a chloroquine-resistant strain of *Plasmodium* (FcM29-Cameroon) showed moderate activity, but with the alkyne and the cyclopentenone derivatives showing a promising enhancement of activity compared with the parent molecules. On the contrary, no antifungal activity was found in vitro using *Candida albicans*. Given the observed antiplasmodial activity of some of these modified monoterpenes, new monoterpene derivatives could be the basis for new antimalarial drugs to be researched.

Malaria remains the major tropical disease worldwide, with more than 300 million people being infected, and 3 million deaths annually.[1] Moreover, the development of opportunistic pathogens such as *Candida albicans*, the most frequently isolated human fungal pathogen that causes infections in immunologically compromised people, has recently exacerbated the situation.[2] The search for new antimalarial drugs from plant source constitutes a promising strategy as exemplified by the use of quinine and artemisinin.[3] Among the huge diversity of chemical structures found in nature, the large terpene family has provided numerous examples of antimalarial compounds including mono-,[4] sesqui-,[5] di-,[6] or triterpenoids.[7,8] Monoterpenes are abundant natural C-10 compounds that meet the non-toxicity and low-cost criteria required for new drug candidates. Antiparasitic activities have already been reported for pure monoterpenes.[9–11] Furthermore, many plant extracts and essential oils, rich in monoterpenes, have therefore been tested against *Plasmodium*[7,12,13] or *Leishmania*[10] as well as against *C. albicans*.[14,15] In these studies, the biological activity of plant extracts was correlated with their composition, principally with the presence of specific abundant components such as terpenes.

This prompted us to evaluate the biological activity of several pure monoterpenes against *Plasmodium falciparum* and *C. albicans*, as well as new monoterpenes

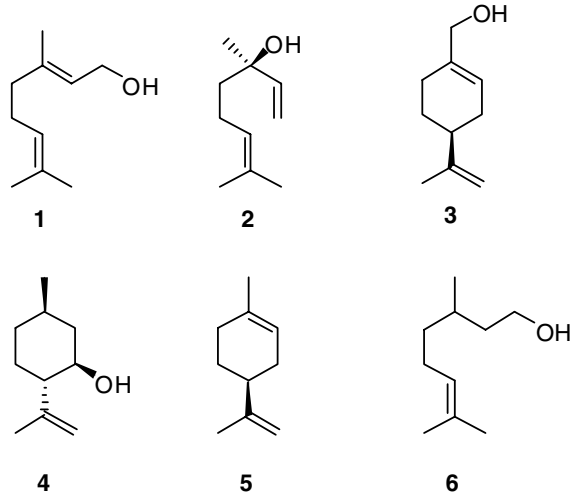

Figure 1. Structure of monoterpenes **1**–**6**.

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Table 1. In vitro effects of pure monoterpenes on the FcM29-Cameroon strain of *Plasmodium falciparum* (IC_{50} in μM)

Tested compound	IC_{50} in μM (SE)[a]
1 Geraniol (GER)	52 (7)
2 Linalool (LIN)	519 (50)
3 Perillyl alcohol (PA)	184 (18)
4 Isopulegol (ISOP)	282 (4)
5 Limonene	66 (0)
6 Citronellol	192 (9)
Artemisinin	0.007 (0.002)
Chloroquine	0.291 (0.006)

[a] Standard error.

derivatives, obtained through selective chemical reactions, to establish whether structural modifications of the monoterpenes could improve the biological activity.

We focused our study on several monoterpenes: geraniol **1**, (−)-linalool **2**, (−)-perillyl alcohol **3**, (−)-isopulegol **4**, (−)-limonene **5**, and (±)-citronellol **6**[16] (Fig. 1). Linalool **2**, perillyl alcohol **3**, and limonene **5** were chosen first since their biological activities have already been reported.[9,11,17,18] They were compared with other oxygenated monoterpenes similar in structure: geraniol **1** and citronellol **6**, two acyclic terpenes, and isopulegol **4**, a cyclic one. All the compounds were tested on the FcM29-Cameroon strain of *P. falciparum*.[19–23] Table 1 gives the IC_{50} values (in μM) for the pure monoterpenes **1**–**6** on *P. falciparum*.[24,25] All the pure monoterpenes tested gave IC_{50} ranging from 52 μM to 519 μM. These results are in line with those already published by other researchers.[4,9,11] Geraniol **1** (52 μM) and limonene **5** (66 μM) were the more active terpenes in our study. The control drugs chloroquine and artemisinin gave IC_{50} of 291 nM and 7 nM, respectively. Compared to both these reference compounds, the monoterpenes values are too high to be of pharmaceutical interest.

Structural modifications on these pure monoterpenes were then carried out with a view to enhancing the biological activity (Fig. 2). Starting from monoterpenes **1**–**4** bearing an alcohol function, a terminal alkyne moiety was introduced, leading to the corresponding O-tethered enynes **1a**–**4a**.[26] An alkyne was added to the monoterpenes to obtain enynes from which a large variety of structures are accessible (cyclopropane, cyclopentenone, or diene) through metal-catalyzed reactions. These enynes were then involved in metal-catalyzed reactions leading to a cyclopropane moiety as in the perillyl alcohol derivative **3b**; a diene as in the isopulegol derivative **4b**; a cyclopentenone moiety as in the linalool derivative **2b**.[26] The lactone **4c** derived from isopulegol as previously reported[27] was also included in this study. The antiplasmodial activities of the chemically modified monoterpenes were then evaluated and the ratio of the IC_{50} of the pure monoterpene to the IC_{50} of its corresponding derivative is given in Table 2. Notably, it appears that the introduction of an alkyne leads to an increase in the activities of perillyl alcohol (**3a** 5.6 times more active than **3**), isopulegol (**4a** 9.1 times more active than **4**), and linalool (**2a** 13 times more active than **2**),

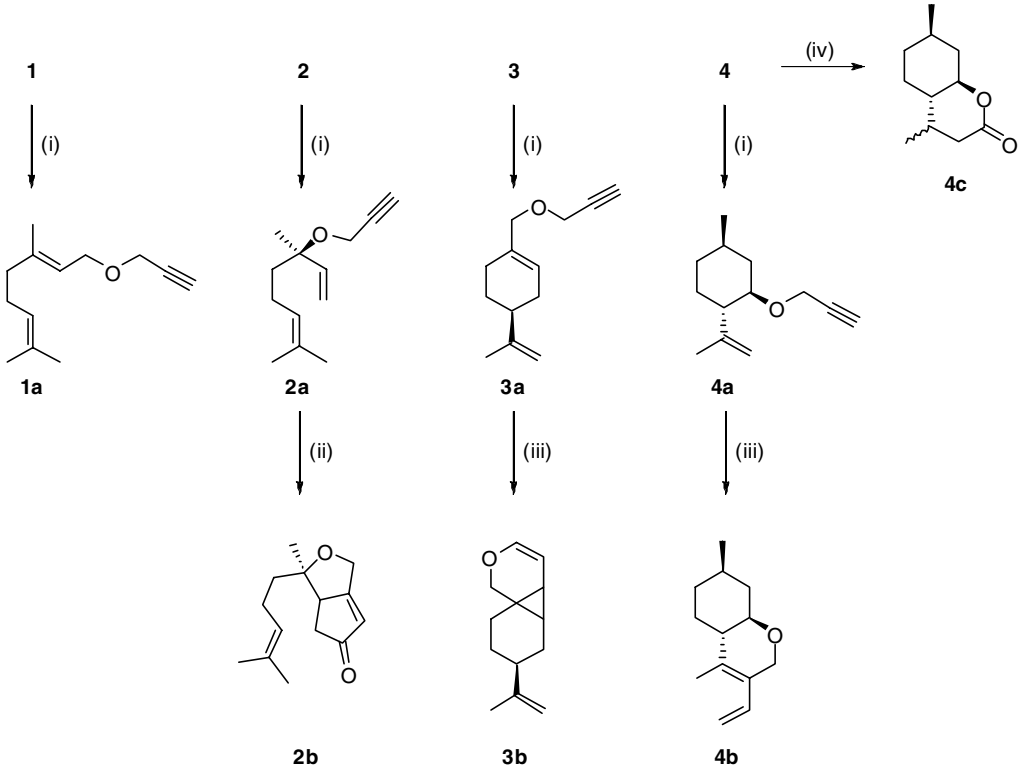

Figure 2. Preparation of monoterpenes derivatives from **1** to **4**. Reagents and conditions: (i) 2 mol equiv NaH, THF, 40 °C, 15 h then 1.2 mol equiv propargylbromide, THF, reflux, 4 h; (ii) 1.2 mol equiv $Co_2(CO)_8$, THF, RT, 3 h then 10 mol equiv; TMANO, THF, 3 h; (iii) 0.05 mol equiv $PtCl_2$, toluene, 80 °C, 2 h; (iv) see Ref. 27.

Table 2. Influence of the chemical modifications on the antiplasmodial activity

Tested compound	IC_{50} in μM (SE)[a]	Relative activity[b]
GERalkyne **1a/1**	122 (48.1)	0.4
LINalkyne **2a/2**	39 (4)	13
PAalkyne **3a/3**	33 (28)	5.6
ISOPalkyne **4a/4**	31 (7)	9.1
LINcyclopentenone **2b/2**	1.8 (0.1)	290
PAcyclopropane **3b/3**	>100	<1.8
ISOPdiene **4b/4**	>100	<2.7
ISOPlactone **4c/4**	470 (2)	0.6

[a] Standard error.
[b] The ratio of the IC_{50} of the pure monoterpene to the IC_{50} of its derivative.

but not for geraniol (**1a** less active than **1**). The specificity of geraniol remains unclear and should be further examined.

The most notable enhancement of the activity was obtained for the cyclopentenone derivative prepared from linalool: **2b** was found to be 290 times more active than **2**, reaching an IC_{50} value of 1.8 μM and thus making this compound the most interesting hit in the study. Similar modifications to other monoterpenes should be soon achieved to assess the versatility of this pharmacophore. On the contrary, both the cyclopropane and the diene moieties were not of interest as far as antiplasmodial activity is concerned, since derivatives **3b** and **4b** were less than 3 times more active than the corresponding terpenes **3** and **4**, respectively. Similarly, the lactone **4c** was found to be less active than the corresponding terpene isopulegol **4**.

Studies on the ATCC 90028 *C. albicans* strain were aimed at evaluating any potential antifungal activity.[28,29] The results show that for all the pure monoterpenes and their derivatives, all the MIC were systematically higher than 200 μg/mL for both 24 h and 48 h incubation times (data not shown). Comparatively, the 5-fluorocytosine used as a reference in this test showed a MIC of 0.75 μg/mL for the 48 h incubation time, similar to the ATCC reference values for this ATCC 90028 strain, that range from 0.5 to 2 μg/ml with this molecule. We can thus conclude that these compounds are not toxic for organisms other than *Plasmodium*. The previous observed activity of these compounds seems to be specific to *Plasmodium*.

A possible mode of action of monoterpenes on *Plasmodium* has been recently proposed[11]: the chemical structure of monoterpenes resembles that of intermediates of the *P. falciparum* isoprenoid pathway and thus makes them potential inhibitors of isoprenoid biosynthesis. The identification of the key enzymes involved in this process in the parasites should help us to understand the mechanisms of synthesis and allow the design of new potential drugs.

In summary, in this study, we report that both alkyne and cyclopentenone pharmacophores added on to a monoterpene skeleton increased the in vitro antimalarial activity and thus should be further studied through the preparation and evaluation of a larger family of new terpenoids based on these structural features. New monoterpene derivatives could be the basis of a new class of antimalarial compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.056.

References and notes

1. Snow, R. W.; Guerra, C. A.; Noor, A. M.; Myint, H. Y.; Hay, S. I. *Nature* **2005**, *434*, 214.
2. Calderone, R. A. *Candida* and Candidiasis; ASM Press: Washington, DC, 2002.
3. Schwikkard, S.; van Heerden, F. R. *Nat. Prod. Rep.* **2002**, *19*, 675.
4. Portet, B.; Fabre, N.; Roumy, V.; Gornitzka, H.; Bourdy, G.; Chevalley, S.; Sauvain, M.; Valentin, A.; Moulis, C. *Phytochemistry* **2007**, *68*, 1312.
5. Klayman, D. L.and included references *Science* **1985**, *228*, 1049.
6. Schrével, J.; Sinou, V.; Grellier, P.; Frappier, F.; Guénard, D.; Potier, P. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8472.
7. Benoit-Vical, F.; Imbert, C.; Bonfils, J. P.; Sauvaire, Y. *Phytochemistry* **2003**, *62*, 747.
8. Suksamrarn, S.; Panseeta, P.; Kunchanawatta, S.; Distaporn, T.; Ruktasing, S.; Suksamrarn, A. *Chem. Pharm. Bull. (Tokyo)* **2006**, *54*, 535.
9. Moura, I. C.; Wunderlich, G.; Uhrig, M. L.; Couto, A. S.; Peres, V. J.; Katzin, A. M.; Kimura, E. A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2553.
10. do Socorro, S. R. M. S.; Mendonca-Filho, R. R.; Bizzo, H. R.; de Almeida Rodrigues, I.; Soares, R. M.; Souto-Padron, T.; Alviano, C. S.; Lopes, A. H. *Antimicrob. Agents Chemother.* **2003**, *47*, 1895.
11. Rodrigues Goulart, H.; Kimura, E. A.; Peres, V. J.; Couto, A. S.; Aquino Duarte, F. A.; Katzin, A. M. *Antimicrob. Agents Chemother.* **2004**, *48*, 2502.
12. Valentin, A.; Pelissier, Y.; Benoit, F.; Marion, C.; Kone, D.; Mallie, M.; Bastide, J. M.; Bessiere, J. M. *Phytochemistry* **1995**, *40*, 1439.
13. Milhau, G.; Valentin, A.; Benoit, F.; Mallié, M.; Bastide, J. M. *J. Essent. Oil. Res.* **1997**, *9*, 329.
14. D'Auria, F. D.; Tecca, M.; Strippoli, V.; Salvatore, G.; Battinelli, L.; Mazzanti, G. *Med. Mycol.* **2005**, *43*, 391.
15. Duarte, M. C. T.; Figueira, G. M.; Sartoratto, A.; Rehder, V. L. G.; Delarmelina, C. *J. Ethnopharmacol.* **2005**, *97*, 305.
16. All monoterpenes were purchased from Sigma (France) and used without any purification.
17. Xu, M.; Floyd, H. S.; Greth, S. M.; Chang, W. C.; Lohman, K.; Stoyanova, R.; Kucera, G. L.; Kute, T. E.; Willingham, M. C.; Miller, M. S. *Toxicol. Appl. Pharmacol.* **2004**, *195*, 232.
18. Ji, J.; Zhang, L.; Wu, Y. Y.; Zhu, X. Y.; Lv, S. Q.; Sun, X. Z. *Leuk. Lymphoma* **2006**, *47*, 2617.
19. *Drug testing*. The molecules tested were dissolved in dimethyl sulfoxide to obtain initial solutions ranging from 2 mg/mL to 20 mg/mL, and then successive dilutions were carried out in RPMI 1640 (Gibco Invitrogen, USA). In

parallel, two antimalarial compounds: chloroquine (Bufa, Holland) and artemisinin (Sigma, France) and one antifungal compound, 5-fluorocytosine (Sigma, France), were tested and used as controls. Only chloroquine was directly dissolved in RPMI 1640 to give an initial solution at 1 mg/mL.

20. *Plasmodium falciparum strain and in vitro culture*. The FcM29-Cameroon strain of *P. falciparum* that is chloroquine-resistant with an IC_{50} for chloroquine of 291 nM was continuously cultured according to Trager and Jensen taking into account the modifications described by Van Huyssen and Rieckman. In order to ensure the homogeneity of the study, chloroquine-sensitivity tests are regularly carried out on this strain. The parasites were maintained in vitro in O+ human red blood cells (French blood bank). The culture medium was RPMI 1640 (Gibco Invitrogen, USA) supplemented with 5% human serum (French blood bank) and containing 25 mM HEPES and L-Glutamine. The culture was performed at 37 °C with a hematocrit of 2–4% and in an atmosphere of 5% CO_2.
21. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
22. Van Huyssen, W.; Rieckmann, K. H. *Trop. Med. Parasitol.* **1993**, *44*, 329.
23. Benoit-Vical, F.; Lelievre, J.; Berry, A.; Deymier, C.; Dechy-Cabaret, O.; Cazelles, J.; Loup, C.; Robert, A.; Magnaval, J.; Meunier, B. *Antimicrob. Agents Chemother.* **2007**, *51*, 1463.
24. *Evaluation of antiplasmodial activity.* The radioisotopic micromethod described by Desjardins et al. was used to evaluate the activity of the tested molecules on *P. falciparum*. Tests of the drug activity were performed in 96-well culture plates (TPP, 9296, Switzerland) with cultures at asexual stages at 1% parasitaemia and 2% hematocrit. The asexual erythrocytic stages were cultured in the plates for 48 h. For each test, the parasite culture was incubated with the drugs at various concentrations. Parasite growth was estimated by [^{3}H] hypoxanthine incorporation (Perkin-Elmer, USA). The [^{3}H] hypoxanthine incorporated in the presence of drugs was compared with that incorporated by parasites without any test compounds. Using this method, the IC_{50} (50% inhibitory concentration) values were determined graphically by plotting concentrations versus percentage inhibition. The IC_{50} values reported are the mean of 2–4 independent experiments.
25. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
26. See Supplementary Material section.
27. Lenoble, G.; Naigre, R.; Chenal, T.; Urrutigoïty, M.; Daran, J.-C.; Kalck, P. *Tetrahedron Asym.* **1999**, *10*, 929.
28. *Testing the antifungal susceptibility with Candida albicans.* The *C. albicans* American Type Culture Collection 90028 (ATCC 90028) was used in this study. In order to evaluate the antifungal activity of the natural monoterpenes and their synthetic derivatives, a microdilution method adapted from the National Committee for Clinical Laboratory Standards (NCCLS-M27A) was used. The culture medium used was RPMI 1640 (Sigma, France) with L-Glutamine but without sodium bicarbonate and containing 0.165 M morpholinopropanesulfonic acid (MOPS) buffer (Sigma, France). The yeasts were grown on Sabouraud dextrose agar (SDA) and were maintained in an incubator at 35 °C until they were tested. Prior to testing, the isolate was subcultured and was incubated at 35 °C. Inocula were prepared by suspending the yeast in 1.0 mL of a saline sterile solution and adjusting to a final concentration of 2.5×10^6 yeasts/mL. Each well of 96-well plates received 100 μL of this suspension and 100 μL of various concentrations of drugs, and the plates incubated for 24 h at 35 °C. The results were determined using a spectrophotometer (Elx 808, Vetra Microplate Reader, Avantec) at a wavelength of 550 nm. The terpene derivatives were only tested with fresh dilutions of the ATCC 90028 *C. albicans* strain. Two readings were necessary at 24 h and 48 h growth to interpret the results and determinate the MIC (Minimal Inhibitory Concentration). These MIC were determined graphically by plotting concentrations of tested drugs versus percentage inhibition.
29. NCCLS. Approved standard M27-A Na. Com. LS, Wayne, PA. **1997**.