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Antimalarial and cytotoxic potential of four quassinoids from *Hannoa chlorantha* and *Hannoa klaineana*, and their structure–activity relationships

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Abstract

Hannoa chlorantha and *Hannoa klaineana* (Simaroubaceae) are used in traditional medicine of Central African countries against fevers and malaria. Four stem bark extracts from *H. klaineana* and four quassinoids from *H. chlorantha* were examined in vitro against *Plasmodium falciparum* NF 54. The extracts displayed good activities, while the quassinoids were highly active, with IC_{50} values well below 1 $\mu g\,ml^{-1}$, those of chaparrinone and 15-desacetylundulatone being much lower than 0.1 $\mu g\,ml^{-1}$ (0.037 and 0.047 $\mu g\,ml^{-1}$, respectively). Chaparrinone is five times more active than 14-hydroxychaparrinone against *P. falciparum*, indicating that the hydroxyl function at C-14 is unfavourable for antiplasmodial activity. As 14-hydroxychaparrinone has a seven-times higher cytotoxic activity against P-388 cells than chaparrinone, the latter compound has the better antiplasmodial therapeutic index. All four quassinoids were evaluated in vivo in a standard 4-day test as well. 15-Desacetylundulatone was proven to be the most active compound, almost totally suppressing the parasitaemias of OF1 mice for at least 7 days, while both chaparrinone and 14-hydroxychaparrinone were active for at least 4 days. Quassinoids have ED_{50} values much lower than 50 $mg\,kg^{-1}$ body weight day^{-1} and none of them caused obvious side effects. The keto function at C-2 in 15-desacetylundulatone is apparently of crucial importance for its high activity. 6-α-Tigloyloxyglaucarubol was not active at all. Chaparrinone is considered the most interesting of the investigated quassinoids and its in-vivo antimalarial potential will be examined further.

Key words: *Hannoa chlorantha*; *Hannoa klaineana*; Simaroubaceae; Quassinoids; Malaria; *Plasmodium falciparum* in vitro; *Plasmodium berghei* in vivo; Cytotoxicity; P-388 cells

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Table 1
In vitro inhibitory activities of extracts and quassinoids from *Hannoa klaineana* and *Hannoa chlorantha* against *Plasmodium falciparum*[a] and P-388[b] cells

Extracts and quassinoids	IC_{50} ($\mu g\,ml^{-1}$)	
	P. falciparum	P-388
H. klaineana, stem bark		
CH_2Cl_2	2.400	N.D.
Petroleum ether (35–70°C)	8.833	N.D.
MeOH	0.672	N.D.
H_2O-decoction	1.658	N.D.
H. chlorantha, quassinoids		
Chaparrinone	0.037	0.34
14-Hydroxychaparrinone	0.188	0.05
15-Desacetylundulatone	0.047	N.D.
6-α-Tigloyloxyglaucarubol	0.257	N.D.
Reference drugs		
Doxorubicin	—	0.02
Artemisinin	0.039	—
Mefloquine	0.026	—
Quinine	0.063	—

[a]Asexual erythrocytic forms of the NF 54 strain, clone A1A9.
[b]Murine leukemia cells.
N.D. = not done.

Table 2
Antimalarial activities of four quassinoids from *Hannoa chlorantha* against *Plasmodium berghei* Anka in OF1 mice

Treatment	Parasitaemias (%)	
	Day 4	Day 7
Control	1.12 (0.64–1.60)	5.14 (3.31–6.97)
	—	—
	(0†/6)	(1†/6)
Chaparrinone	0.11 (0.51–0.17)	5.30 (3.85–6.75)
	$P<0.001$	$P>0.050$
	(0†/6)	(0†/6)
14-Hydroxy-chaparrinone	0.18 (0.12–0.24)	4.18 (2.70–5.66)
	$P<0.001$	$P>0.050$
	(0†/6)	(0†/6)
15-Desacetyl-undulatone	0.023 (0.004–0.042)	0.37 (0.00–0.78)
	$P<0.001$	$P<0.001$
	(0†/6)	(0†/6)
6-α-Tigloyloxy-glaucarubol	1.62 (0.45–2.80)	5.88 (0.00–15.73)
	$P>0.050$	$P>0.050$
	(0†/6)	(3†/6)

† = dead.

tain a number of quassinoids biogenetically related to degraded triterpenoids [18]. Several of them display activities against blood forms of *P. falciparum* in vitro [19, 20], against tumour cell lines [21], or against rodent malaria in vivo [22, 23]. The good activities displayed here (Table 1) indicate that *H. klaineana* or related species possibly contain secondary metabolites that have not yet been investigated for their antiplasmodial activities. Consequently, it seemed worthwhile exploring this potential in detail, by examination of selected quassinoids from *H. chlorantha*. Two of these quassinoids, 15-desacetylundulatone and 6-α- tigloyloxyglaucarubol, have been isolated previously from the root bark of *H. klaineana* [7].

The in-vitro activities of *H. chlorantha* quassinoids against *P. falciparum* blood stages are considerable (Table 1), although somewhat lower than the reported activities of certain other quassinoids in a comparable experimental model [18, 24]. Two of these activities (chaparrinone and 15-desacetylundulatone), however, fall within the range of activities of frequently used antimalarials such as artemisinin, mefloquine, and quinine (Table 1). Of the four quassinoids examined here, only the in-vitro antiplasmodial activity of chaparrinone has been evaluated by other authors [25]. Their findings are in agreement with ours.

Several of the previously described quassinoids displaying hydroxylation at C-14, such as the WST-63 quassinoid from *Brucea sumatrana* [26], bruceins D and E, and their derivatives [27, 28], eurycomanone, and eurycomanol [29], are also hydroxylated at C-15. Therefore, examination of these compounds does not allow discrimination between the biological effects related to each type of hydroxylation. Direct comparison between chaparrinone and 14-hydroxychaparrinone, however, permits elucidation of the specific role of the 14-OH function. Chaparrinone ($IC_{50} = 0.037\,\mu g\,ml^{-1}$) is about five times more active against erythrocytic *P. falciparum* forms (Table 1) than 14-hydroxychaparrinone ($IC_{50} = 0.188\,\mu g\,ml^{-1}$). This indicates that hydroxylation at C-14 leads to a significant

reduction of antiplasmodial activity. On the other hand, the five-fold difference in antiplasmodial activities of 15-desacetylundulatone and 6-α-tigloyloxyglaucarubol (IC_{50} values 0.047 and 0.257 $\mu g\,ml^{-1}$, respectively), quassinoids with an identical type of esterification at C-6, is probably due to the conversion of the alcohol function at C-2 into a keto group in the case of 6-α-tigloyloxyglaucarubol.

All investigated quassinoids are active against *P. falciparum* in vitro, although none is esterified at C-15, and this factor has been described by others as important for high antiplasmodial activity [30].

Both chaparrinone and 14-hydroxychaparrinone displayed in-vitro antiproliferative activities against P-388 murine leukemia cells (Table 1). The latter compound was especially active and its IC_{50} value (0.05 $\mu g\,ml^{-1}$) approached that of doxorubicin (0.02 $\mu g\,ml^{-1}$). Interestingly, hydroxylation at C-14 resulted in a seven-fold increase in cytotoxic activity, an effect opposite to that obtained with the antiplasmodial activities of chaparrinone and 14-hydroxychaparrinone (Table 1). This implies a 34-fold decrease in antiplasmodial therapeutic index (9.19 vs 0.27 for chaparrinone vs 14-hydroxychaparrinone, respectively).

The cytotoxic activities of 15-desacetylundulatone and 6-α-tigloyloxyglaucarubol against P-388 cells were not examined here, since their in-vivo effects against mouse tumours have been analysed before. Only 15-desacetylundulatone was demonstrated to be active against both P-388 leukemia (CDF1 mice) and colon 38 adenocarcinoma (B6D2F2 mice) [7].

Based upon the encouraging in-vitro antiplasmodial results, the curative antimalarial potential of all four quassinoids was examined in vivo, by administering them to *P. berghei*-infected OF1 mice (Table 2). Only one, 6-α-tigloyloxyglaucarubol, did not suppress the parasitaemia. The ED_{50} values of the other three compounds are apparently much lower than 50 $mg\,kg^{-1}$ body weight day^{-1}.

Application of 50 mg of 15-desacetylundulatone $kg^{-1}\,day^{-1}$ in the 4-day test resulted in a 98% reduction of the parasitaemia at day 4 that was sustained during the full course of the experiment (92% at day 7) (Table 2). Experiments designed to check whether slightly increased doses of this compound could lead to a total clearance of *P. berghei* parasites are in preparation. No obvious signs of toxicity were observed at the administered dose of 50 $mg\,kg^{-1}\,day^{-1}$ of 15-desacetylundulatone and the mean survival time of the treated mice was not different from that of the control group (data not shown). Lumonadio et al. [7], however, demonstrated toxic effects for doses of 60 $mg\,kg^{-1}\,day^{-1}$ and higher.

The difference in antimalarial activities between 15-desacetylundulatone and 6-α-tigloyloxyglaucarubol (Table 2) supports the above hypothesis that the keto function at C-2 of 15-desacetylundulatone is essential for its high activity, also in vivo. A comparable example (6-α-seneciolyloxychaparrinone vs 6-α-seneciolyloxychaparrin) has been described by other authors [31].

Chaparrinone and 14-hydroxychaparrinone both strongly suppressed the parasitaemias at day 4 (90% and 84%, respectively) (Table 2). Considering the temporary aspect of this suppression, it would be interesting to increase the administered dose of these two quassinoids in a new series of experiments, provided that no toxic effects can be detected.

In summary, it has been confirmed that *H. chlorantha*- and *H. klaineana*-derived quassinoids have potential as antimalarial drugs. 15-Desacetylundulatone is the most potent of the compounds investigated, but the fact that the doses needed to obtain high in-vivo activity are close to the toxic range must be considered a drawback. This could possibly be overcome by applying different treatment regimens wherein the daily dose is split into several lower doses or by the use of sustained-release delivery systems [32]. Although chaparrinone and 14-hydroxychaparrinone are equally active against *P. berghei* in vivo and no toxicity has been demonstrated, the former compound appears to be the most interesting since it has a higher in-vitro therapeutic index than the latter. The antimalarial characteristics of chaparrinone will, therefore, further be explored in the near future.

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