In vitro antiplasmodial activity of prenylated chalcone derivatives of hops (Humulus lupulus) and their interaction with haemin

Sonja Frölich¹, Carola Schubert¹, Ulrich Bienzle² and Kristina Jenett-Siems¹*

¹Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Königin-Luise-Str. 2–4, D-14195 Berlin, Germany; ²Institut für Tropenmedizin, Medizinische Fakultät der Charité, Humboldt-Universität, Spandauer Damm 130, D-13086 Berlin, Germany

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Objectives: There is an urgent need to discover new antimalarials, due to the spread of chloroquine resistance and the limited number of available drugs. Chalcones are one of the classes of natural products that are known to possess antiplasmodial properties. Therefore, the in vitro antiplasmodial activity of the main hop chalcone xanthohumol and seven derivatives was evaluated. In addition, the influence of the compounds on glutathione (GSH)-dependent haemin degradation was analysed to determine its contribution to the antimalarial effect of chalcones.

Methods: In vitro antiplasmodial activity was evaluated against the chloroquine-sensitive strain poW and the multiresistant clone Dd2 using a [3H]hypoxanthine-incorporation assay. Inhibition of GSH-dependent haemin degradation was analysed by a multiwell plate assay at 11 mM.

Results: Of the eight compounds tested, four possessed activity with IC₅₀ values < 25 μM against at least one of the two strains of Plasmodium falciparum. The main hop chalcone, xanthohumol, was most active with IC₅₀ values of 8.2 ± 0.3 (poW) and 24.0 ± 0.8 μM (Dd2). Three of these compounds were additionally active in the haemin-degradation assay.

Conclusions: The results demonstrate for the first time the ability of chalcone derivatives to interfere with the haemin-degradation process of P. falciparum. This effect might contribute to their antiplasmodial activity. Nevertheless, as one compound showed inhibition of P. falciparum without being able to interact with GSH-dependent haemin degradation, other modes of action must add to the observed antiparasitic activity of hop chalcones.

Keywords: Plasmodium falciparum, xanthohumol, chalcones, GSH-dependent haemin degradation

Introduction

When blood stages of the malarial parasite Plasmodium falciparum enter human erythrocytes, they feed from enzymatic degradation of haemoglobin. Haemoglobin is ingested from the host cell and digested inside the parasite’s food vacuole.¹ The by-product of this digestion is toxic haemin, or ferrirprotoporphyrin IX, which is detoxified by forming an insoluble polymer, malaria pigment or haemozoin.² Recently, an alternative haem detoxification mechanism has been described. Non-polymerized haemin exits the food vacuole into the parasite’s cytosol, where it is degraded by glutathione (GSH).³ Quinoline antimalarials—like chloroquine—have been shown to interfere with both detoxification pathways, thus leading to parasite death.⁴⁻⁵

In order to test the ability of compounds to interfere with either pathway, different in vitro assays have been developed, thus allowing the possible modes of action of antiplasmodial compounds to be investigated.⁶⁻⁸

The hops plant, Humulus lupulus L., of the family Cannabinaeae is a large, dioecious climber often cultivated. Secretory glands on the surface of the female flowers contain a volatile oil and also a resin consisting of bitter compounds, such as humulones and lupulones. In addition, polyphenols like flavonoids

*Corresponding author. Tel: +49-30-838-53720; Fax: +49-30-838-53729; E-mail: kjsiems@zedat.fu-berlin.de

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and chalcones are present, with, in particular, the prenylated chalcone derivative xanthohumol (XN) as a main constituent. Therapeutically, hop cones are used as mild sedatives, normally in combination with valerian (Valeriana officinalis L.). Numerous biological activities of different hop constituents have been reported, e.g. antimicrobial, antioxidant and cytotoxic activities. 8-Prenylfluorogin has been identified as a potent phytoestrogen. Recently, the possible cancer chemopreventive activity of xanthohumol has been described, based on its ability to modulate the activity of enzymes involved in carcinogen metabolism and detoxification. 

As different chalcones are known to possess antiparasitic properties, we were prompted to evaluate the in vitro antiplasmodial activity of the main hop chalcone, xanthohumol, and seven natural or semi-synthetic derivatives, against two different strains of P. falciparum, as well as their interaction with GSH-dependent haemin degradation.

Materials and methods

RPMI 1640 medium was purchased from Gibco-BRL. GSH and diethylenetriamine-penta-acetic acid (DETPAC) were obtained from Lancaster. Haemin, HEPES, Na phosphates, NaHCO	extsubscript{3} and DMSO were obtained from Roth. [3H]Hypoxanthine was purchased from Amersham. Flat-bottomed 96-well plates were obtained from Neolab.

Test compounds were kindly supplied by Prof. Dr Rudolf Hänsel, Institut für Pharmazie, Freie Universität Berlin and analysed for structure and impurities. The purity of the substances (>95%) was checked by HPLC and thin layer chromatography (TLC).

In vitro antiplasmodial assay

P. falciparum strains poW (IC	extsubscript{50} of chloroquine = 0.015 μM) and Dd2 (IC	extsubscript{50} = 0.14 μM) were maintained in continuous culture in human red blood cells (A*) diluted to 5% haematocrit in RPMI 1640 medium supplemented with 25 mM HEPES, 30 mM NaHCO	extsubscript{3} and 10% human A* serum. Extracts and substances were dissolved in DMSO (20 mg/mL) and diluted in medium to final concentrations between 100 and 1.56 μg/mL. Antiplasmodial activity tests were performed in 96-well culture plates (Corning	extsuperscript{TM}, Sigma-Aldrich) as described by Desjardins et al. Briefly, aliquots of 150 μL of parasitized culture (2.5% haematocrit, 0.5% parasitaemia) were exposed to two-fold dilutions of test substances. After incubation in a candle jar for 24 h, 0.5 μCi of [3H]hypoxanthine (1 mCi/mL) was added to each well and the plates incubated for a further 18 h. Cells were harvested onto glass fibre filters (Wallac) with a cell harvester (Inotech) and incorporated radioactivity was determined by a liquid scintillation counter (1450 Microbeta plus). All tests were performed in triplicate. The percentage of growth inhibition was calculated as: [(1−cpm in drug treated cultures/cpm in untreated cultures)]×100. The concentration at which growth was inhibited by 50% (IC	extsubscript{50}) was estimated by interpolation.

Multiwell plate GSH–haemin interaction assay

The GSH–haemin interaction assay was performed as described by Steele et al. In brief, three stock solutions were prepared: 1 mM DETPAC in 10 mM Na phosphate pH 7.0; 2 mM haemin in DMSO (prepared fresh daily); 100 mM GSH, 1 mM DETPAC, 10 mM Na phosphate pH 6.8. For the experiments, working solutions were as follows: ‘A’, 4 vol. of DETPAC/phosphate stock +1 vol. of ethanol; ‘B’, 5 μL of haemin stock solution per mL of solution ‘A’; and ‘C’, 0.15 mL of GSH stock solution per mL of solution ‘A’.

Assays were carried out in 96-well (400 μL) flat-bottomed plates. Solution A (100 μL) was added, followed by drug (2 μL of 2 mM drug stock in DMSO) or solvent control in eight parallel samples. Solution B (200 μL) was then added to all wells followed by 50 μL of solution C. Final concentrations of drug and haemin were 11 and 5.7 μM, respectively. The absorbance at 360 nm (A	extsubscript{360}) was measured after 1 and 30 min with a plate reader (Spectrafluor Tecan) to determine the ΔA	extsubscript{360}. The effect of the haemin-binding compounds was evaluated as the percentage decrease compared with control absorbance. Mean and SD for the eight parallel samples of at least three independent experiments were calculated.

Results

The in vitro antiplasmodial activity was evaluated against the chloroquine-sensitive strain poW and the multiresistant clone Dd2 using a [3H]hypoxanthine-incorporation assay. Of the eight compounds tested (Figure 1), four were active with IC	extsubscript{50} values < 25 μM against at least one of the two strains (Table 1). The main hop chalcone, xanthohumol (1), was most active, revealing IC	extsubscript{50} values of 8.2 μM (poW) and 24.0 μM (Dd2), respectively. 2′,3′-Dihydroxanthohumol (2) gave an IC	extsubscript{50} value of 12.9 μM against poW, and also two pyrano-derivatives (3, 4), where the prenyl residue forms an additional ring, possessed antiplasmodial activity with IC	extsubscript{50} values of 16.4 and 23.7 μM (poW), respectively. 6′-Desmethylxanthohumol (6), on the other hand, displayed only a moderate effect [IC	extsubscript{50} values: 42.4 μM (poW); 92.1 μM (Dd2)]. Compounds 5 (2′,4′-trimethylxanthohumol) and 7 (2′,4′,4-trimethyl-6′-desmethylxanthohumol) were inactive, showing IC	extsubscript{50} values > 100 μM against both strains, whereas the flavanone derivative 8 revealed moderate activity against the multiresistant clone Dd2 (IC	extsubscript{50} value 55.3 μM), but no effect against poW (IC	extsubscript{50} value > 100 μM).

In the haemin-degradation assay, compounds 1, 2 and 4 displayed >60% inhibition at a concentration of 11 μM, compared with 82% for chloroquine (Figure 2). Compounds 6 and 8 were weakly active, inhibiting haemin degradation by 36 and 24%, respectively, whereas the remaining derivatives showed no inhibition.

Discussion

In this work, we evaluated the in vitro antiplasmodial activity of the major hop chalcone xanthohumol (1) as well as seven natural or semi-synthetic derivatives. The best activity was displayed by xanthohumol (1) itself. This is only the second time that prenylated natural chalcones were proven to possess antiplasmodal properties, but in contrast to 1, the prenyl residue of licochalcone A, which was isolated from Chinese liquorice roots, is attached due is cyclized to an additional ring, also show antiplasmodial activity. Compared with 1, desmethylxanthohumol (6), which only differs by the lack of the methyl residue at the C-6′-hydroxy group, is four to five times less active. This might be due to its higher hydrophilicity, which makes it more difficult to...
Table 1. IC\textsubscript{50} values of chalcone derivatives tested against poW and Dd2 strains of \textit{P. falciparum}

<table>
<thead>
<tr>
<th>Compound</th>
<th>poW IC\textsubscript{50} (\mu M)a</th>
<th>Dd2 IC\textsubscript{50} (\mu M)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.2 ± 0.3</td>
<td>24.0 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>12.9 ± 0.6</td>
<td>17.4 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>16.4 ± 0.9</td>
<td>10.7 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>23.7 ± 1.5</td>
<td>35.0 ± 2.9</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 126</td>
<td>&gt; 126</td>
</tr>
<tr>
<td>6</td>
<td>42.4 ± 0.3</td>
<td>92.1 ± 2.4</td>
</tr>
<tr>
<td>7</td>
<td>&gt; 131</td>
<td>&gt; 131</td>
</tr>
<tr>
<td>8</td>
<td>&gt; 147</td>
<td>55.3 ± 2.1</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.015 ± 0.002</td>
<td>0.14 ± 0.012</td>
</tr>
</tbody>
</table>

Values are given as means of three experiments ± SD.

Figure 1. Chemical structures of chalcone and flavanone derivatives evaluated in this study.

Figure 2. GSH–haemin interaction assay. The effect of chalcone derivatives (11 \mu M) and chloroquine (11 \mu M) as reference compound on the interaction of 1 mM GSH with 5.7 \mu M haemin is given as % inhibition of haemin degradation compared with drug-free control. Values are the means of four assays ± SD.
reach the site of action inside the parasite. Interestingly, the semi-synthetic methylated derivatives 5 and 7 are totally inactive, thus, as synthetic 2′,4′-dimethoxychalcones revealed good antiplasmodial activity in an extensive structure–activity relationship study, the methylation of the hydroxy group at position 4 in particular seems to be less favourable.

When comparing our results with those obtained with human cells, \textit{P. falciparum} is slightly more sensitive to XN (1) than different cancer cell lines and also macrophages. Real cytotoxic activity can only be observed at 100 \(\mu\)M, although antiproliferative effects become visible at lower concentrations depending on the cell line used. In the case of \(\text{XN}\), this cytotoxicity is not due to an oestrogen-mimicking activity, since in contrast to the flavanone derivative 8-prenyllaringenin, \(\text{XN}\) does not display oestrogenic effects; instead, inhibition of DNA synthesis is discussed. Nevertheless, in order to develop \(\text{XN}\) as a new antiplasmodial lead compound, efforts to separate antiplasmodial and cytotoxic bioactivities have to be undertaken.

The exact mechanism of action of antiplasmodial chalcones is not known, although they are often considered to be cytoxic protease inhibitors. Nevertheless, natural congeners characterized by a carbonyl moiety in position 9 and a free hydroxy group. In the case of cyclized chalcones such as flavanones and flavones, binding to haemin might not be possible via a 2′-methoxy group (5, 7) or a 2′,3′-pyran ring system (3) instead of a free hydroxy group, on the other hand, were only weakly active despite their free hydroxy group. In the case of cyclized chalcones such as flavanones and flavones, binding to haemin might not be possible via this structural feature because of the lesser flexibility of the skeleton. Finally, desmethyloxanthohumol (6) is more easily isomerized to the analogous flavanone derivative in aqueous solutions, thus explaining its poor activity.

Our results demonstrate for the first time the ability of chalcone derivatives to interfere with the haemin degradation process of \textit{P. falciparum}. This effect might contribute to their antiplasmodial activity. Nevertheless, as compound 3 showed inhibition of \textit{P. falciparum} without being able to interact with GSH-dependent haemin degradation, other modes of action must contribute to the observed antiparasitic activity of hop chalcones. Thus, studies evaluating their possible inhibition of cysteine proteases are currently under way.

Acknowledgements

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References


