Plants as Sources of Antimalarial Drugs: In Vitro Antimalarial Activities of Some Quassinoids

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Fourteen quassinoids, obtained from simaroubaceous plants, were tested for in vitro antimalarial activity. All of these inhibited the incorporation of [3H]hypoxanthine into Plasmodium falciparum in vitro at concentrations below 0.41 μg ml−1. The two most potent quassinoids, bruceantin and simikalactone D, showed 50% inhibitory concentration values of 0.0008 and 0.0009 μg ml−1, respectively. The results are compared with the antiamoebic, antileukemic, and cytotoxic activities of these compounds reported in the literature.

In the 1960s, the appearance in Southeast Asia and South America of strains of Plasmodium falciparum showing resistance to chloroquine heralded the need for alternative antiplasmodial therapy. Several hundreds of millions of people suffer from malaria, and all currently used antimalarial drugs now show limitations in their spectra of activity (17). Resistance of P. falciparum to available antimalarial drugs is an increasing world problem (10). Therefore, it is crucial that mechanistically novel antimalarial agents be added to our chemotherapeutic armamentarium as soon as possible.

Certain quassinoids, obtained from simaroubaceous plants, are known to possess a variety of biological activities, including antitumor (13), antiviral (11), antifeedant (12), antiamoebic (5), and antiinflammatory (7) activities. More recently, some of these compounds, namely, bruceantin (6), simikalactone D (15), glaucarubinone (16), soularubinone (16), and sergeolide (4), have been found to show high activity against P. falciparum in vitro. Sergeolide also markedly reduces virulence of experimentally induced P. berghei in mice (4); however, it unfortunately also shows high toxicity. In our present study, we monitored the in vitro anti-P. falciparum activities of a series of 14 quassinoids which were made available from isolations performed under the auspices of the National Cancer Institute, Bethesda, Md. Our results are considered in the light of published data for other biological activities of these quassinoids.

(This report is part 2 of a study on plants as sources of antimalarial drugs. For part 1, see reference 9.)

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MATERIALS AND METHODS

P. falciparum strain. A chloroquine-resistant strain (K-1) of P. falciparum, which was originally obtained from Thailand (14) and has been cryopreserved at the London School of Hygiene and Tropical Medicine, was used throughout.

Maintenance of cultures. Cultures of P. falciparum were maintained in vitro in human blood cells (O+ve) diluted to 5% hematocrit with RPMI 1640 medium (10% human O+ serum) by published techniques (2, 3, 15).

Test protocol. The test procedure was based upon the method of Desjardins et al. (2). The quassinoids were dissolved in ethanol and diluted with RPMI 1640 medium before testing. The concentration of ethanol in the test never exceeded 0.1%, and controls demonstrated that there was no effect on [3H]hypoxanthine incorporation. Portions (50 μl) each of diluted quassinoids were dispensed into 96-well microtiter trays so as to yield final test concentrations of 50, 5, 0.5, 0.05, 0.005, and 0.0005 μg ml−1. More accurate determination of 50% inhibitory concentration (IC50) values was achieved with 12 2-fold dilutions at concentrations around the range of the value obtained by 10-fold dilutions. All tests were performed in duplicate. To each well was added 50 μl of human erythrocytes (O+ve, diluted to 5% hematocrit) with 1% parasitemia (dilutions to 1% parasitemia were made with uninfected washed erythrocytes). Two series of controls were performed, one with parasitized blood without quassinoid and another with uninfected erythrocytes without quassinoid. The IC50 value for chloroquine was determined during each experiment. After incubation in a 3% O2–4% CO2–95% N2 gas phase for 18 h at 37°C, 5 μl of [3H]hypoxanthine (40 μCi ml−1; Amersham Corp., United Kingdom) was added to each well, and incubation was continued for a further 18 to 24 h.

Harvesting. Erythrocytes were washed from the wells with normal saline with a Titertek cell harvester (Flow Laboratories, Inc., McLean, Va.) through a glass fiber membrane predampened with saline. The glass fiber membrane was flushed with distilled water for 20 s to lyse erythrocytes and then flushed with saline for 20 s to remove remaining traces of hemoglobin. After being washed further with distilled water and saline (20 s with each), the membrane was dried, and the glass fiber disc for each well was pushed out into polypropylene scintillation vials (4-ml volume). To each vial, 4 ml of scintillation fluid (Packard toluene scintillator) was added, and the counts per minute were determined for 10 min at about 30% efficiency.

Analysis of results. Counts per minute were converted to disintegrations per minute by using an external standard, and the percentage of inhibition was calculated from the following equation: percent inhibition = 100 − [(disintegrations
per minute in infected erythrocytes plus quassinoid) –
disintegrations per minute in uninfected erythrocytes) \times
100)/(disintegrations per minute in infected erythrocytes –
disintegrations per minute in uninfected erythrocytes).
Concentration-versus-percent inhibition curves were interpreted
by linear regression analysis, from which the IC50 values and
their 95% confidence intervals were determined.

Sources of quassinoids. The compounds used in the study
were isolated from species of the family Simaroubaceae and
were obtained from the repository of the National Cancer
Institute, Bethesda, Md. (Fig. 1, compounds 1 to 14).

RESULTS
The results are presented in Table 1. Of the 14 quassinoids
tested, all showed in vitro antimalarial activity, having IC50
values below 0.41 \( \mu \text{g ml}^{-1} \). Of these quassinoids, 10,
from ailanthinone to simalikalactone D (Table 1), possessed IC50
values of less than 0.02 \( \mu \text{g ml}^{-1} \). Chloroquine diphosphate
showed an IC50 value of 0.21 \( \mu \text{g ml}^{-1} \) in the same test.

DISCUSSION
The in vitro antimalarial activities in our study for
simalikalactone D (IC50, 0.0009 \( \mu \text{g ml}^{-1} \)) and glaucarubinone
(IC50, 0.004 \( \mu \text{g ml}^{-1} \)) are of the same order as those reported
by Trager and Polonsky (16), namely, 0.002 \( \mu \text{g ml}^{-1} \) for
simalikalactone D and 0.006 \( \mu \text{g ml}^{-1} \) for glaucarubinone.
The results reported in Table 1 show the importance for
activity of the presence of an ester function at C-15;
glaucarubinone is about three times more active than chaparrin
and about eight times more active than glaucarubol. Changes
in the nature of this ester function produce marked alterations
in activity; glaucarubinone is about twice as potent as
holancanthe, whereas bruceantin is more than twice as
active as bruceantinol and more than three times as active as
brusatol. A comparison of the activities of glaucarubinone
and 6α-senecioxyloxychaparrinone suggests that an ester
function at C-15 improves activity over one at C-6. Also, if
the C-15 is already esterified, additional esterification at C-6
appears to offer little enhancement in activity, as shown by
the IC50 values of holancanthe and undulatone. The A-ring
substitution pattern is apparently crucial to activity;
glaucarubinone, having an \( \alpha, \beta \)-unsaturated keto function in
ring A, is over 10 times more active than glaucarubin. There
is no obvious overall difference in activities of compounds
with a C-20 to C-11 oxygen bridge or a C-20 to C-13 oxygen
bridge.

The in vitro antimalarial activities of the quassinoids in the
present study did not always parallel their other biological
activities, as reported in the literature. Table 1 lists reported
data for the antileukemic, antiamoebic, and cytotoxic activ-
ities of some of the quassinoids tested for in vitro antimal-
arial activity in this study. Trager and Polonsky (16) reported
that the in vitro antimalarial activities of the five quassinoids
they tested (viz., simalikalactone D, glaucarubinone,
soularubinone, simarolide, and chaparrinone) paralleled
their antileukemic activities. In our study, the in vitro
antimalarial activities of the quassinoids did not exactly
parallel their in vivo murine lymphocyte leukemia (P-388)
optimal test-to-control survival values or optimal doses
reported by Cassady and Suffness (1) and Suffness (unpub-
lished data). However, the compounds with the highest
in vitro antimalarial activity in our test, bruceantin (IC50,
0.0008 \( \mu \text{g ml}^{-1} \)) and simalikalactone D (IC50, 0.0009 \( \mu \text{g ml}^{-1} \)), rate among the best antileukemic quassinoids re-
ported (Table 1).

Bruceantin and simalikalactone D were also found (5) to
possess the highest in vitro amoebicidal activity of a series of
17 quassinoids examined. Bruceantin had an IC50 of 0.018 \( \mu \text{g ml}^{-1} \),
and simalikalactone D had an IC50 of 0.047 \( \mu \text{g ml}^{-1} \) in
the latter test. Apart from this, there is little similarity
between in vitro antimalarial and antiamoebic activities
of the quassinoids; glaucarubinone was about twice as active as
ailanthinone in the antimalarial test but only half as active as
ailanthinone in the antiamoebic test. Also, glaucarubin,
which has only relatively modest in vitro antimalarial activity
(IC50, 0.055 \( \mu \text{g ml}^{-1} \)), was active in the antiamoebic test
(IC50, 1.57 \( \mu \text{g ml}^{-1} \)), whereas some quassinoids with high in
vitro antimalarial activities, viz., holancanthe, undulatone,
6α-senecioxyloxychaparrinone, brusatol, bruceantinol, and
soularubinone, were not active in the antiamoebic test at 2 \( \mu \text{g ml}^{-1} \), the highest concentration tested. It is worth noting that
chloroquine sulfate (antiamoebic IC50, 85 \( \mu \text{g ml}^{-1} \)) also
displayed little antiamoebic activity.

Some quassinoids are known to be toxic to mammalian
cells (1), and, in the present study, 6α-senecioxyloxychap-
arrinone, bruceantin, and simalikalactone D proved to be
the most potent quassinoids tested against KB cells (Table 1).
Apart from ailanthinone, 6α-senecioxyloxychaparrinone, and
simalikalactone D, all of the quassinoids tested were active...
in the in vitro antimalarial test at concentrations well below their toxic concentrations against 9KB human tumor cells in vitro. In both tests, the presence of an \( \alpha,\beta \)-unsaturated keto function in ring A is of overriding importance for activity; chaparrin, glaucarubol, and glaucarubin are about 20 times less cytotoxic in the 9KB cell test than undulatone, the least toxic of the compounds, which has an \( \alpha,\beta \)-unsaturated keto group in ring A. However, apart from this similarity, in vitro antimalarial activity does not parallel cytotoxicity against 9KB cells. In the first place, a C-15 ester function, which is important for antimalarial activity, is of less significance to cytotoxicity in the 9KB cell test. Chaparrin, glaucarubol, and glaucarubin, which have markedly different activities in the antimalarial test, show very similar toxicities against 9KB cells. However, when a C-15 ester group is present, changes in the nature of this ester function produce distinct alterations in the activities in both tests. Different criteria for the ester function appear to operate in the two tests. For antimalarial activity, the 2-hydroxy-2-methylbutyric ester at C-15, as in glaucarubinone, is about twice as active as the C-15 acetate as in holacanthone, whereas glaucarubinone is about five times more active than holacanthone against 9KB cells. Also, bruceantin, containing a 3,4-dimethyl-2-pentenoic chain at C-15, is over 3 times more active than brusatol (senecioic moiety at C-15) in the antimalarial test but is over 10 times more toxic than brusatol against 9KB cells. Another difference in structural requirements for activity in the two tests is illustrated by comparing the activities of glaucarubinone, which is esterified at C-15, and \( 6\alpha \)-senecioyoxychaparrinone, which is esterified at C-6; glaucarubinone is about twice as active as \( 6\alpha \)-senecioyoxychaparrinone in the antimalarial test but is almost five times less active against 9KB cells. These important differences in the activities of compounds in the two tests suggest that it may be possible to find a quassinoid with good antimalarial activity and low mammalian cytotoxicity.

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LITERATURE CITED