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IN VITRO STUDIES ON THE MODE OF ACTION OF QUASSINOIDS WITH ACTIVITY AGAINST CHLOROQUINE-RESISTANT *PLASMODIUM FALCIPARUM*

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Abstract—Using the incorporation of [^{3}H]isoleucine or [^{3}H]hypoxanthine into acid-insoluble products as indices of protein- and nucleic acid-synthetic activity, respectively, it was shown that seven plant-derived quassinoids with differing chemical substitutions all inhibited protein synthesis more rapidly than nucleic acid synthesis in human erythrocytes infected with *Plasmodium falciparum, in vitro*. Five quassinoids (ailanthinone, bruceantin, bruceine B, glaucarubinone and holacanthone) were effective within 30 min at doses 10 times their 48 hr *in vitro* IC_{50} values. Chaparrin and glaucarubol differed in that they did not inhibit protein synthesis during the time course of these experiments when applied at 10 times their *in vitro* IC_{50} values. When these compounds were used at 209 and 114 times their respective IC_{50} values, their observed effects were identical to those of the other quassinoids studied. The time (t_{50}) at which nucleic acid synthesis was reduced to 50% of control was directly proportional to the t_{50} for protein synthesis, suggesting that failure of nucleic acid synthesis is a consequence of inhibition of protein synthesis. It is concluded that in the malaria parasite, as in eukaryote models, quassinoids are rapid and potent inhibitors of protein synthesis, and that this is most likely due to effects upon the ribosome, rather than upon nucleic acid metabolism.

Since the appearance in south-eastern Asia and South America in the 1960s of strains of *Plasmodium falciparum* showing resistance to chloroquine, the need to develop new antimalarial drugs has become critical [1]. Because most of the drugs currently used against malaria now show limitations in their spectra of activity [2], it is crucial that mechanistically novel agents be made available as alternatives in the fight against this disease.

Certain species of simaroubaceous plants, notably *Brucea* and *Simarouba*, are used in traditional medicine to combat a number of diseases, including malaria [3]. In many cases their biological activity has been attributed to the quassinoids ('bitter principles') they contain. Some such plant-deprived quassinoids have been shown to possess anti-amoebic [4, 5], antifeedant [6], anti-inflammatory [7], antitumour [8] and antiviral [9] properties. More importantly, quassinoids such as bruceantin [10], simalikalactone D, glaucarubinone and soularubinone [11], and sergeolide [12] have been shown to be highly active against chloroquine-resistant strains of *P. falciparum, in vitro*. Similarly, the virulence of experimentally-induced *P. berghei* infections in mice is markedly reduced by treatment with sergeolide [12], but the compound is also extremely toxic. For a number of other quassinoids found to be active against malaria, both *in vitro* and *in vivo* [13–15], it was noted that the rank order of their efficacies as anti-plasmodial agents did not always reflect their relative cytotoxic activities in mammalian systems, suggesting a degree of selectivity for the malaria parasite [13].

It has been concluded from studies of eukaryotic cells and cell-free systems that bruceantin [16] and other quassinoids [17–21] act primarily as inhibitors of protein synthesis, although considerable evidence exists from both *in vivo* and *in vitro* studies using the P-388 murine leukaemia model system [22–24] that some (though not all) quassinoids tested exert effects upon many other processes crucial to cellular function. Most notably, inhibitory effects upon enzymes regulating nucleic acid metabolism and purine synthesis, inhibitory effects upon oxidative phosphorylation, and the blocking of aerobic respiration have been reported. The present study was carried out to determine whether quassinoids also inhibit protein synthesis in the malaria parasite. Experiments were designed to assess indirectly whether the effects of quassinoids are likely to be more significant at the level of the ribosome, or at an earlier stage, involving nucleic acid metabolism. From the quassinoids we have previously found to possess anti-plasmodial activity [13–15], seven compounds with differing chemical substitutions (see Fig. 1) and differing *in vitro* IC_{50}¶ values (see Table 1) were selected, and examined for their capacity to inhibit protein synthesis (inhibition of tritiated isoleucine incorporation [25]) or nucleic acid synthesis

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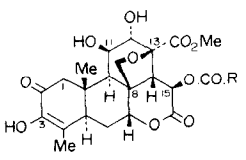
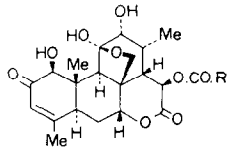
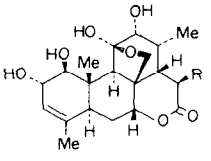
¶ Abbreviations: [^{3}H]hyp, tritiated hypoxanthine; [^{3}H]ile, tritiated isoleucine; IC_{50}, concentration of drug causing 50% inhibition of parasite growth in an *in vitro* screening test; t_{50}, time at which incorporation of isotope represents 50% of the amount incorporated by drug-free controls.



R
1. CH=C(Me)CH(Me)$_2$
2. Me



R
3. CH(Me)Et
4. Me
5. C(Me)(OH)Et



R
6. H
7. OH

Fig. 1. Structures of quassinoids. 1, Bruceantin; 2, Bruceine B; 3, Ailanthinone; 4, Holacanthone; 5, Glaucarubinone; 6, Chaparrin; 7, Glaucarubol.

(inhibition of tritiated hypoxanthine incorporation [26]) by *P. falciparum*, *in vitro*. Actinomycin D and cyclohexmide were used as controls for nucleic acid synthesis and protein synthesis, respectively.

MATERIALS AND METHODS

Drugs. The procedures whereby the quassinoids used were isolated and identified, with their physical and chemical properties have previously been reported [14, 15]. Actinomycin D, chloroquine diphosphate and cycloheximide were obtained from Sigma Chemical Co. (Poole, U.K.). Solution of the more polar quassinoids was facilitated by addition of a small amount of absolute ethanol (BDH, Poole, U.K.), the final concentration of which, at test, was less than 0.1%. For use, stock solutions were diluted in glucose-enriched RPMI 1640 culture medium (RPMI) to give concentrations 350 times the desired final concentration. The addition of 10 μl of drug solution to 3.5 ml of cell suspension gave a final drug concentration ten times the *in vitro* IC_{50} value (see Table 1) previously determined [13]. In making this final dilution no significant change in volume was deemed to have occurred.

Labelled precursors. L-[4,5-^{3}H]Isoleucine ([^{3}H]ile; sp. act. 754 mCi/mg) and [G-^{3}H]hypoxanthine ([^{3}H]hyp; sp. act. 43.3. mCi/mg) were purchased from Amersham International Ltd (Amersham, U.K.). The radioactive concentration of [^{3}H]ile, supplied as an aqueous solution containing 2% ethanol, was 1.0 mCi/ml; this was diluted in RPMI to give 40 μCi/ml. Lyophilised [^{3}H]hyp was dissolved in RPMI to give 40 μCi/ml. These solutions were aliquoted and stored at −20°.

Parasite cultivation and sampling. The chloroquine- and pyrimethamine-resistant K1 (Thailand) strain of *P. falciparum* [27] was cultured in human A$^+$ erythrocytes suspended in RPMI 1640 supplemented with D-glucose and 10% human A$^+$ serum [28, 29]. For incorporation experiments vigorously growing cultures with a predominance of late or large trophozoites were selected. The cells were washed with RPMI and the parasitaemia adjusted to 5% with uninfected red cells. The cell suspension was diluted to an haematocrit of 5% and divided into 3.5 ml aliquots in 15 ml screw-capped plastic tissue culture tubes (NUNC, Denmark). [^{3}H]ile or [^{3}H]hyp was added to give a final radioactive concentration of 2 μCi/ml. Tubes were preincubated at 37° for 30 min, after which drugs were added and the tubes reincubated. At the desired time intervals duplicate 200-μl samples of suspension were taken, after mixing, and pipetted into microcentrifuge tubes containing 1 ml ice-cold 0.9% (w/v) saline, and stored on melting ice until processed.

Sample preparation and determination of radioactivity. Techniques were modified from those previously used in these laboratories [25], and based

Table 1. Concentrations of drugs used, together with their effects upon protein and nucleic acid synthesis

Compound tested	Antimalarial activity *in vitro* IC_{50} (M)	Concentration at test ($\times$ IC_{50}) (M)	Protein synthesis mean t_{50}ile (min)	N	Nucleic acid synthesis mean t_{50}hyp (min)	N	t_{50} ratio ile/hyp
Bruceantin	1.46×10^{-9}	10	122	3	233	2	0.52
Glaucarubinone	8.10×10^{-9}	10	108	4	343	2	0.31
Holacanthone	1.61×10^{-8}	10	66	5	195	2	0.33
		302	44	1	138	1	0.32
Ailanthinone	1.88×10^{-8}	10	71	5	184	2	0.38
		3114	50	1	145	1	0.34
Bruceine B	2.29×10^{-8}	10	73	4	299	3	0.24
Chaparrin	4.74×10^{-7}	10	inact	3	—	nt	—
		209	93	1	257	1	0.36
Glaucarubol	1.03×10^{-6}	10	inact	3	—	nt	—
		114	239	1	833	1	0.29
Chloroquine diphosphate	$2\text{–}3 \times 10^{-7}$	10	inact	1	—	nt	—
Cycloheximide	7.27×10^{-8}	423	70	3	184	1	0.38
Actinomycin D	nd	10^{-5}	149	1	64	1	2.33

inact = inactive; nt = not tested; nd = not determined, but estimated at $<4.17 \times 10^{-10}$ M.

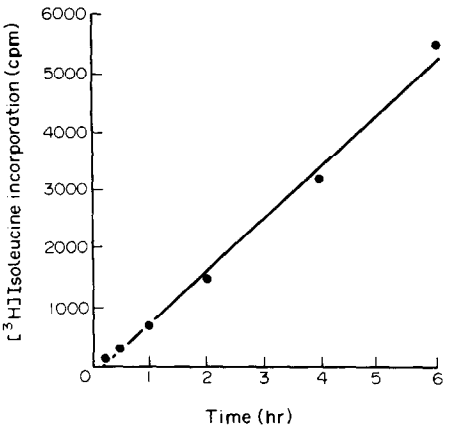


Fig. 2. Incorporation of [^{3}H]ile into protein by *P. falciparum*-infected human erythrocytes, in a preliminary experiment, over a period of 6 hr. The cpm illustrated are the means from cells harvested from duplicate 200 μl aliquots of culture suspension (5% parasitaemia, 5% haematocrit). The equation of the straight line fitted to the data points [[^{3}H]ile incorporation (cpm) = 920.05 × incubation time (hr) − 237.36; r = 0.997; Eqn (1)] was determined from linear regression analysis, using the method of least-squares.

upon the method of Neame [30]. Samples were removed from the ice and spun in a microcentrifuge (MSE Micro Centaur) at 10,000 *g* for 5–10 sec. After discarding the supernatants the pellets were washed by resuspending in 400 μl ice-cold 0.9% saline and centrifuging once more. Pelleted cells were then lysed with 200 μl distilled water and the extracts treated with an equal volume of 10% (v/v) trichloroacetic acid (Sigma). Precipitates were washed with 800 μl distilled water, and the acid-insoluble material recovered by centrifuging at 10,000 *g* for 2 min. After discarding the supernatants, pellets were redissolved in 200 μl undiluted formic acid (90% w/w; Hopkin & Williams) and transferred to 7 ml plastic scintillation counting vials (Packard). A volume of 150 μl of a freshly-prepared bleaching solution containing equal amounts of glacial acetic acid, hydrogen peroxide ('100 volumes' or 30% w/v H_2O_2) and ethyl acetate (all from BDH) was added; vials were tightly closed and heated in a water bath at 100° for 15–20 min. After cooling, 3.5 ml of Emulsifier Scintillator 299 (Packard) were added and the vials were shaken. Counting for tritium activity was carried out in a Packard Tri-Carb Scintillation Spectrometer (Model 574). Counting efficiency was monitored using the external standard, which had been calibrated using a tritiated hexadecane standard (Amersham).

RESULTS

Effects of drugs upon protein synthesis

A preliminary experiment (Fig. 2) showed that a linear relationship, for this experiment

$$[^3\text{H}]\text{ile incorporation (cpm)} = 920.05 \times \text{incubation time (hr)} - 237.36; r = 0.997, \quad (1)$$

exists between incubation time and [^{3}H]ile incorporation by drug-free parasitized erythrocyte controls. Such a relationship continued for up to 6 hr in some experiments (c.f. Figs 2 and 3a). Since the onset of the inhibitory effects of quassinoids was very rapid, it was generally unnecessary to continue incubation beyond 160–180 min. Where incorporation of radioisotope ceased to be a linear function of time the cultures were considered to be no longer viable, and data obtained thereafter were rejected.

Cycloheximide (3×10^{-5} M [25]) caused clear inhibition of isotope incorporation, apparent within 30 min of addition of the drug (Fig. 4a). Of the seven quassinoids studied (Fig. 1), ailanthinone, bruceantin, bruceine B, glaucarubinone and holacanthone rapidly inhibited incorporation of radioisotope at final molar concentrations 10 times their *in vitro* IC_{50} concentrations. The effects of three of these quassinoids are shown in Figs 3a and 5a.

When tested at this concentration, neither chaparrin nor glaucarubol had any obvious effect upon

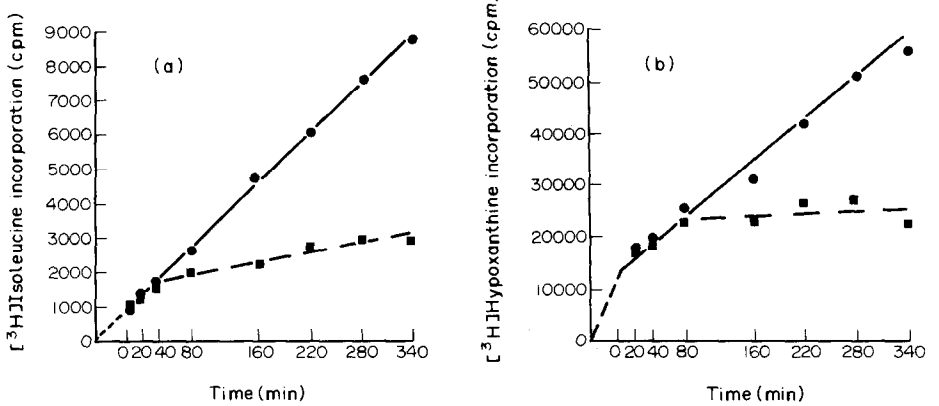


Fig. 3. (a) Incorporation of [^{3}H]ile into protein and (b) incorporation of [^{3}H]hyp into nucleic acids by *P. falciparum*-infected human erythrocytes, over 6 hr, in a single experiment. Controls denoted by (●—●); bruceantin (1.46×10^{-8} M; ■—■) was added at t = 0, and clear inhibition of [^{3}H]ile incorporation (a) was apparent within 80 min of addition. [^{3}H]hyp incorporation (b) was less markedly affected by bruceantin at 80 min, but was considerably reduced at 160 min and thereafter. The cpm illustrated are the means from cells harvested from duplicate 200-μl aliquots of culture suspension (5% parasitaemia, 5% haematocrit).

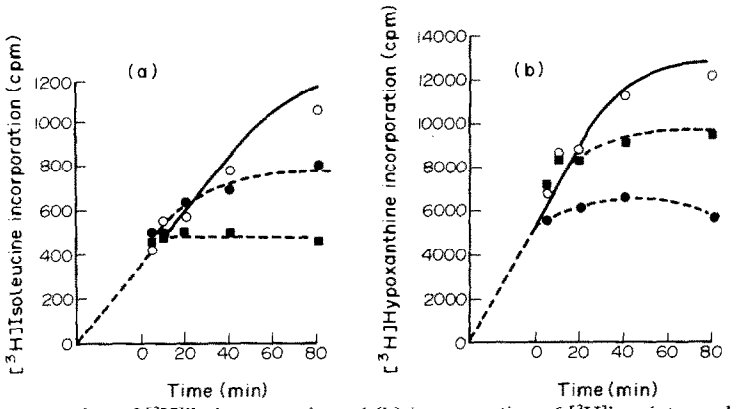


Fig. 4. (a) Incorporation of [^{3}H]ile into protein and (b) incorporation of [^{3}H]hyp into nucleic acids by *P. falciparum*-infected human erythrocytes, in a single experiment. Drugs were added at $t = 0$. Controls denoted by ○—○; cycloheximide (3×10^{-5} M: ■--■) inhibited [^{3}H]ile incorporation (a) within 20 min of addition, but actinomycin D (10^{-5} M; ●--●) had no marked effect until 80 min after adding the drug. Conversely, [^{3}H]hyp incorporation (b) was more sensitive to actinomycin D than to cycloheximide. The cpm illustrated are the means from cells harvested from duplicate 200-μl aliquots of culture suspension (5% parasitaemia, 5% haematocrit).

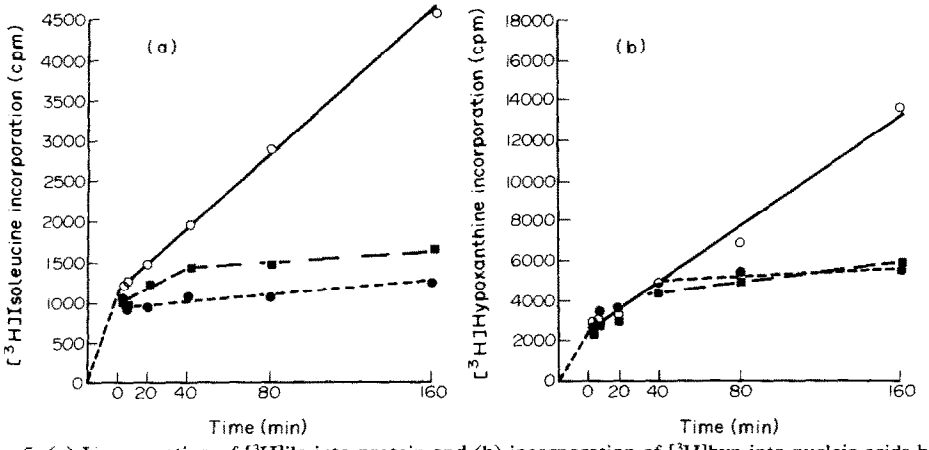


Fig. 5. (a) Incorporation of [^{3}H]ile into protein and (b) incorporation of [^{3}H]hyp into nucleic acids by *P. falciparum*-infected human erythrocytes, in a single experiment, and showing typical effects of quassinoids. Drugs were added at $t = 0$; controls denoted by ○—○. Both ailanthinone (1.88×10^{-7} M; ●--●) and glaucarubinone (8.10×10^{-8} M; ■—■) inhibited [^{3}H]ile incorporation (a) within a very short period of incubation, but their effects upon [^{3}H]hyp incorporation (b) were not apparent until 80 min after adding the drug. The cpm illustrated are the means from cells harvested from duplicate 200-μl aliquots of culture suspension (5% parasitaemia, 5% haematocrit).

[^{3}H]ile incorporation, over a comparable span of time. However, when applied at 209 and 114 times their respective *in vitro* IC_{50} values, clear inhibition of incorporation was observed (Fig. 6a), similar to, though less complete than, the effects of other quassinoids, over the same period of time. Increasing the concentrations of ailanthinone (~3000 × IC_{50}) or holacanthone (~300 × IC_{50}) did not enhance their effects upon [^{3}H]ile incorporation (Table 2).

Actinomycin D (10^{-5} M [31]) also inhibited [^{3}H]ile incorporation, but the effect was considerably later in onset than that caused by cycloheximide (Fig. 4a) or quassinoids (e.g. Fig. 5a).

Incorporation of [^{3}H] did not differ from control when parasitized cells were incubated with chloroquine diphosphate (3×10^{-6} M; 10 times the IC_{50} value for this strain).

Effects of drugs upon nucleic acid synthesis

In non-drug treated controls isotope incorporation again increased with time (e.g. Figs 3b and 5b). The most profound effects upon [^{3}H]hyp incorporation were those caused by actinomycin D (Fig. 4b). Inhibition of [^{3}H]hyp incorporation by actinomycin D was markedly more rapid in onset than the effect caused by cycloheximide (Fig. 4b) or any of the quassinoids tested (Figs 3b, 5b and 6b).

Inhibition of nucleic acid synthesis by the higher concentrations of chaparrin and glaucarubol was far less pronounced than their inhibitory effect upon protein synthesis, and was less complete than the effect upon [^{3}H]hyp incorporation of any of the other quassinoids tested, or of cycloheximide.

Correlation of data

For each drug tested the percentage inhibition of control (non drug-treated) incorporation of radioisotope was calculated and plotted as a function of the logarithm (to the base 10) of the sample time (Fig. 7). A straight line was fitted to the data points,

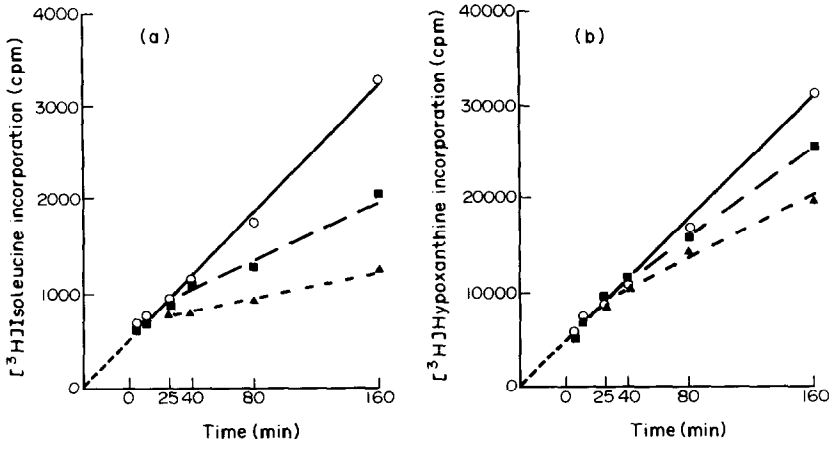


Fig. 6. Incorporation of [^{3}H]ile into protein by *P. falciparum*-infected human erythrocytes. Drugs were added at $t = 0$; controls denoted by O—O. (a) Neither chaparrin nor glaucarubol inhibited protein synthesis at 10 times their *in vitro* IC_{50} values, but, when used at much higher concentrations (chaparrin 9.90×10^{-5} M, ▲--▲; glaucarubol 1.17×10^{-4} M, ■—■), both showed inhibitory actions upon protein synthesis comparable to those of the other quassinoids. (b) Delayed inhibition of nucleic acid synthesis was also observed as an effect of these higher concentrations of glaucarubol or chaparrin, in the same experiment. The cpm illustrated are the means from cells harvested from duplicate 200-μl aliquots of culture suspension (5% parasitaemia, 5% haematocrit).

Table 2. Comparison of the inhibitory effects upon [^{3}H]ile incorporation of low and high concentrations of the quassinoids ailanthinone and holacanthone

Sample time (min)	Control cpm	Ailanthinone 1.88×10^{-7} M ($10 \times IC_{50}$) cpm	Ailanthinone 5.85×10^{-5} M ($3114 \times IC_{50}$) cpm	Holacanthone 1.61×10^{-7} ($10 \times IC_{50}$) cpm	Holacanthone 4.86×10^{-6} M ($302 \times IC_{50}$) cpm
5	621	434	471	526	511
10	653	439	449	518	532
25	726	435	492	563	528
40	945	459	512	578	541
80	1468	353	535	664	522
160	2327	608	436	661	523
220	4266	449	476	828	529

The cpm presented are the means from cells harvested from duplicate 200 μl aliquots of culture suspension (5% parasitaemia, 5% haematocrit).

where a linear relationship was observed, using the method of least-squares. This allowed the determination of the time (in minutes) from addition of drug at which the incorporation of isotope represented 50% of control (t_{50}). Results are shown in Table 1. When, for each drug, the calculated t_{50} value for [^{3}H]hyp incorporation was plotted against that for [^{3}H]ile incorporation, a linear relationship ($r = 0.954$; $P < 0.001$) was obtained (Fig. 8). The equation of the straight line fitted to the data points shows that

$$t_{50(\text{hypoxanthine})} = 3.45 \times t_{50(\text{isoleucine})} - 41.99. \quad (2)$$

The $t_{50(\text{isoleucine})}/t_{50(\text{hypoxanthine})}$ ratio for each quassinoid and for cycloheximide was always less than 1; in the case of actinomycin D this ratio was 2.33.

DISCUSSION

The present study demonstrates an effect of quassinoids upon the malaria parasite which suggests a primary disruptive action upon protein synthesis, followed by inhibition of nucleic acid synthesis. It is not possible to conclude from these experiments whether inhibition of protein synthesis *per se* is the sole, or most important, mode of action of quassinoids as antimalarial compounds, since other significant potential sites of action [22–24] have yet to be studied in the malaria parasite. However, previous reports [17–21] indicate that quassinoids selectively inhibit protein synthesis in a wide range of eukaryote cells and cell-free model systems where they have been shown to be biologically effective, and this property often correlates significantly with, for example, antileukaemic or antineoplastic efficacy.

Irreversible inhibition of protein synthesis was shown in rabbit reticulocytes and reticulocyte lysates treated with bruceantin [19]; secondary inhibition of DNA synthesis ensued, but RNA synthesis was minimally affected. Similarly, the inhibition of protein, DNA and RNA synthesis in HeLa cells

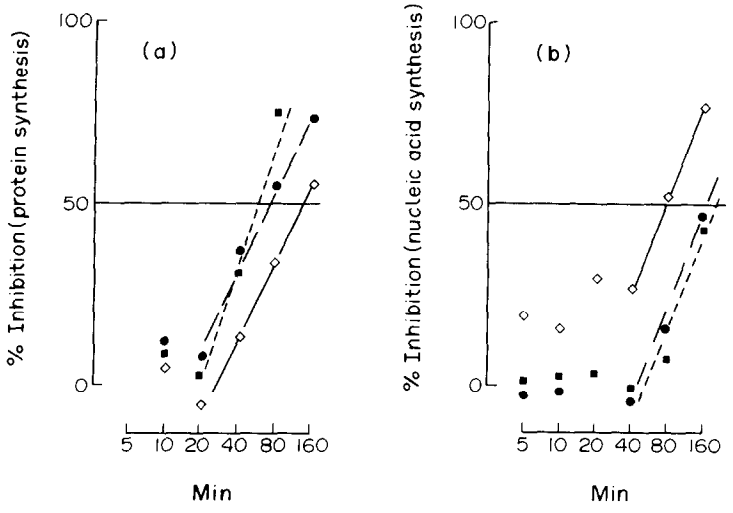


Fig. 7. For each drug tested, the percentage inhibition of control incorporation of (a) [^{3}H]ile or (b) [^{3}H]hyp was calculated and plotted against time on a logarithmic scale. A straight line was fitted to the linear part of each curve, using the method of least-squares. The time at which isotope incorporation represented 50% of control (t_{50}) was determined from linear regression analysis. Actinomycin D, 10^{-5} M (◇—◇); cycloheximide, 3×10^{-5} M (●—●); holacanthone, 1.61×10^{-7} M (■—■).

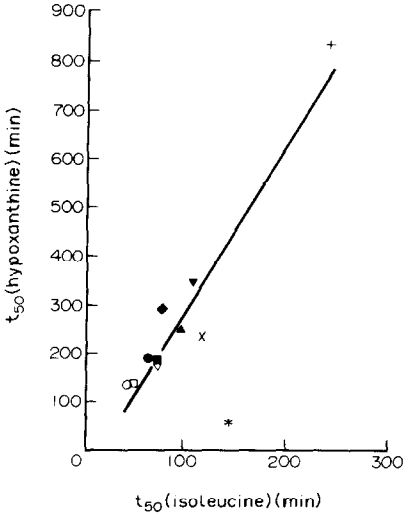


Fig. 8. Mean t_{50} values for [^{3}H]hyp incorporation were plotted as a function of mean t_{50} values for [^{3}H]ile incorporation, and were found to be directly related. A straight line was fitted to the data points using the method of least-squares [$t_{50(\text{hypoxanthine})} = 3.45 \times t_{50(\text{isoleucine})} - 41.99$; $r = 0.954$; $P < 0.001$; Eqn (2)]. Actinomycin D was clearly different from the quassinoids and cycloheximide in that $t_{50(\text{hypoxanthine})}$ was less than $t_{50(\text{isoleucine})}$. (×) bruceantin, 1.46×10^{-6} M; (▼) glaucarubinone, 8.10×10^{-8} M; (●) holocanthone, 1.61×10^{-7} M; (○) holocanthone, 4.86×10^{-5} M; (■) ailanthinone, 1.88×10^{-7} M; (□) ailanthinone, 5.85×10^{-5} M; (◆) bruceine B, 2.29×10^{-7} M; (▲) chaparrin, 9.95×10^{-5} M; (+) glaucarubol, 3.37×10^{-4} M; (▽) cycloheximide, 3.00×10^{-5} M; (*) actinomycin D, 10^{-5} M).

treated with bruceantin (2 μM) was 90%, 60% and 15%, respectively [17]. From studies with rabbit reticulocytes [20] it was concluded that disruption of protein synthesis by the quassinoid brusatol results from inhibition of ribosomal peptidyl transferase activity, leading to the termination of chain elongation.

In our experiments depression of nucleic acid synthesis by any given quassinoid occurred after the effects upon protein synthesis. Since the temporal relationship between the two effects was constant [Fig. 8; Eqn (2)], irrespective of whether the quassinoid was a rapid (e.g. ailanthinone) or a more delayed (e.g. chaparrin) inhibitor of protein synthesis (see Table 1), the evidence suggests that disruption of protein synthesis is the more direct response, to which the inhibition of nucleic acid synthesis is secondary. Furthermore, the time courses of the effects of quassinoids and actinomycin D (a potent and specific inhibitor of DNA-directed RNA synthesis [16, 32]) upon both [^{3}H]ile and [^{3}H]hyp incorporation were quite different. As expected, actinomycin D had a marked inhibitory effect upon nucleic acid synthesis in our experiments, which was considerably more rapid in onset than that caused by cycloheximide or the quassinoids we tested. Conversely, the effect of actinomycin D upon protein synthesis was not apparent until well after the time when incorporation of [^{3}H]hyp had been substantially reduced by the drug.

Experimental conditions were standardized by using "equipotent" doses of the different quassinoids, based upon their 48 hr *in vitro* antimalarial IC_{50} values. Chaparrin and glaucarubol differed from the other quassinoids in that they did not, in the time scale of these experiments, inhibitit protein synthesis at concentrations 10 times those previously shown to be effective in the 48 hour drug-sensitivity screen [13–15]. By markedly increasing the dose of those compounds it was possible to demonstrate that they too inhibit protein synthesis in the malaria parasite before any effect upon nucleic acid synthesis is apparent.

The importance of A-ring substitution and oxidation status in determining antimalarial efficacy and

cytotoxicity has already been discussed [13], and the present results do not contradict this structure–activity requirement. Chaparrin and glaucarubol differ from the other quassinoids tested in the nature of their A-ring, both having a diol substitution, rather than the α,β-unsaturated ketol found in the other compounds tested. Both compounds are also unesterified at C-15, whilst the other quassinoids have varying ester functions at this position. It is, therefore, difficult to distinguish whether the differences apparent in the activities of chaparrin and glaucarubol, compared with other quassinoids, are due to the modification of the A-ring, or result from a reduced uptake due to the lack of an ester function at C-15 [33]. The apparent 'concentration-dependence' of the more acute effect of these two compounds upon protein synthesis, contrasted with the data obtained from the 48 hr *in vitro* antimalarial screening test, suggests that rate of uptake of individual quassinoids might be an important factor in determining their antimalarial efficacy. Alternatively, it is possible, though yet to be demonstrated, that an A-ring glycol system can be oxidised to a ketol configuration by red cell or parasite redox mechanisms, resulting in production, *in situ*, of a more active compound. It is significant that these two compounds show less antileukaemic activity *in vivo*, [c.f. 13, 33], are inactive against *Entamoeba histolytica in vitro* [4], but are considerably less cytotoxic than most other quassinoids examined so far [13].

More recent studies [21] suggest that, as protein synthesis inhibitors, quassinoids show some 'selectivity' for cancer cells (P-388 lymphocytic leukaemia, Ehrlich and hepatoma carcinoma and L-1210 lymphoid leukaemia), as well as for certain 'normal' tissues, such as lymphocytes, and that other cell and tissue types may be relatively unaffected. This may stem either from differences in permeability of individual quassinoids, or from inherent differences at the ribosomal level [21]. Such findings lend support to the idea that a quassinoid may be found which shows selective toxicity for malaria parasites [13].

From our experiments we conclude that quassinoids inhibit protein synthesis in the malaria parasite, and that there is a subsequent inhibitory effect upon nucleic acids. Since chloroquine did not alter [^{3}H]ile incorporation in our experiments, in agreement with the lack of effect upon protein synthesis previously reported in *P. berghei* [34], quassinoids may be presumed to act upon the malaria parasite through a fundamentally different mechanism, and so warrant further investigation as potentially useful agents in the treatment of chloroquine-resistant malaria. Further investigations are also necessary before it can be concluded whether inhibition of protein synthesis, or inhibition of other cellular processes underlies the specific mode of action of quassinoids as antimalarial agents.

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