

## RESEARCH NOTE

# INTERACTIONS BETWEEN ANTIPLASMODIAL 3,6-DIAMINO-1'-DIMETHYL-9-ANILINOACRIDINE AND HEMATIN AND CONCANAMYCIN A

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**Abstract.** Antiplasmodial 9-anilinoacridine derivatives exert their effects either by inhibiting DNA topoisomerase (topo) II or by interfering with heme crystallization within the parasite acidic food vacuole. Previous studies have shown that analogs of 9-anilinoacridine containing 3,6-diamino substitutions (in the acridine ring) inhibit *Plasmodium falciparum* DNA topo II *in situ*, whereas those with a 3,6-diCl substitution act by inhibiting beta-hematin formation, a property also seen with 3,6-diamino-1'-dimethyl-9-anilinoacridine (DDAA). To understand this seemingly anomalous property of DDAA, studies of its interaction with hematin and localization within the parasite food vacuole were undertaken. A weak interaction with hematin was demonstrated spectroscopically. Antagonism of DDAA inhibition of *Plasmodium falciparum* growth in culture by concanamycin A, a macrolide antibiotic inhibitor of vacuolar H<sup>+</sup>-ATPase derived from *Streptomyces* sp, was equivocal.

## INTRODUCTION

With over 500 million new cases of malaria annually, a million deaths from infection with *Plasmodium falciparum*, particularly in children in sub-Saharan Africa, and the likelihood of emergence of parasites resistant to all currently employed antimalarials (Greenwood *et al*, 2005), there is an urgent need to identify new antiplasmodial compounds with the potential of being developed into effective and affordable drugs. The effectiveness of quinoline and acridine compounds, such as

chloroquine and quinacrine, is due to their abilities to inhibit the process of heme crystallization to produce hemozoin, which occurs within the malaria parasite acidic food vacuole as a means of detoxifying the membranolytic property of free heme released following digestion of host hemoglobin (Tilley *et al*, 2001).

A number of derivatives of 9-anilinoacridine have shown promising antiplasmodial activity *in vitro* (Gamage *et al*, 1994). These compounds act either by inhibiting malaria parasite DNA topoisomerase II or by binding with hematin similar to that of chloroquine, thereby compromising hemozoin biogenesis. Analogs of 9-anilinoacridine containing 3,6-diamino substitutions (in the acridine ring) with both 1'-electron donating and 1'-electron withdrawing groups (in the aniline ring) inhibit DNA

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topoisomerase II *in situ* as evidenced by their ability to produce DNA-protein adducts (Auparakkitanon and Wilairat, 2000). However, when inhibition of  $\beta$ -hematin (structurally identical to hemozoin) formation *in vitro* was compared among the antiplasmodial 9-anilinoacridines, analogs with a 3,6-diCl substitution together with an electron-donating group in the 1'-anilino position were better inhibitors than those with 3,6-diamino substitutions (Auparakkitanon *et al*, 2003).

The potent antiplasmodial activity of 3,6-diamino-1'-dimethyl-9-anilinoacridine (DDAA) ( $IC_{50}$  value of 34 nM in chloroquine-resistant *P. falciparum* K1 in culture) has been attributed to its ability to inhibit DNA topoisomerase II (Auparakkitanon and Wilairat, 2000), but, paradoxically, it can also inhibit  $\beta$ -hematin formation (with an  $IC_{50}$  value similar to chloroquine) although showing poor binding with hematin (Auparakkitanon *et al*, 2003). To clarify this situation, binding studies of DDAA with hematin using both spectrophotometric and spectrofluorometric methods were conducted. Localization of DDAA within the malaria parasite acidic food vacuole was also evaluated by examining whether its antiplasmodial function could be antagonized in the presence of concanamycin A, a macrolide antibiotic inhibitor of vacuolar  $H^+$ -ATPase derived from *Streptomyces* sp (Drose *et al*, 1993).

## MATERIALS AND METHODS

### Parasite culture and *in vitro* inhibitory activity assay

*P. falciparum* K1 strain was maintained under "candle jar" conditions described by Trager and Jensen (1976) and *in vitro* inhibition was assessed using the [ $^3H$ ]-hypoxanthine incorporation method as previously described (Auparakkitanon and Wilairat, 2006). The  $IC_{50}$  values (50% inhibition of parasite incorporation of radioactivity) were obtained from dose-response curves. In drug combination stud-

ies,  $IC_{50}$  values of one drug (DDAA) in the presence of a series of fixed concentrations of the other drug (concanamycin A) were measured. The fractional inhibitory concentrations (FICs), defined as  $IC_{50}$  of a given compound in a mixture/ $IC_{50}$  of that compound alone, for each fixed concentration were plotted as an isobologram. If the mean sums of FIC = 1, the drug combination is considered as being additive; if the sum of FIC < 0.5, there is synergism; and if the sum of FIC > 4, it is antagonistic.

### DDAA-hematin interaction assay

Interaction between DDAA and hematin was performed employing a continuous variation technique (Job's plot) as described previously (Auparakkitanon and Wilairat, 2006). In brief, solutions containing the following 14 DDAA:hematin (molar) combinations were prepared in 2 mM sodium phosphate, pH 6, containing 40% (v/v) dimethyl sulfoxide: 0:1, 1:9, 1:4, 3:7, 2:3, 1:1, 3:2, 5:3, 13:7, 27:13, 7:3, 4:1, 9:1, 1:0; the final combined concentration of hematin plus DDAA in the mixtures was 10  $\mu$ M. Absorption spectra of hematin and fluorescence spectra of DDAA were recorded in a Shimadzu UV-250 IPC spectrophotometer between 240-700 nm at a speed of 0.5 nm/minute and in a Shimadzu RF-5310 PC spectrofluorometer between 420 and 700 nm at an excitation wavelength of 414 nm. The absorbance peak of hematin at 400 nm was chosen for use in the Job's plot, and the difference between the measured absorbance and expected value based on concentration added (absorbance change  $\times -1$ ) was plotted against the DDAA:hematin molar ratio.

## RESULTS

Employing changes in hematin absorbance at 400 nm, the Job's plot of the interaction between DDAA and hematin shows weak interaction, as indicated by the broad shape of the curve, with a stoichiometry of

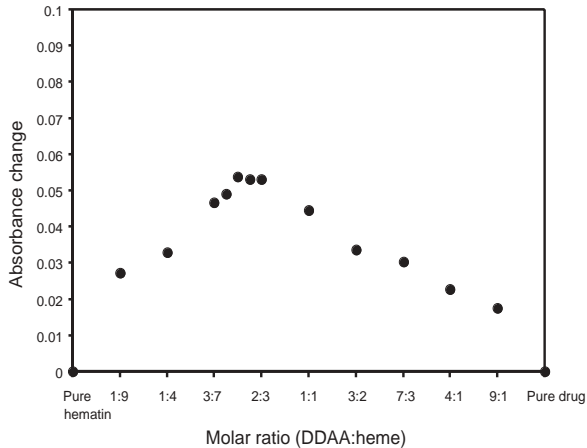


Fig 1–Job's plot of DDAA binding to hematin. The total concentration of the two components was 10  $\mu$ M in 40% aqueous dimethyl sulfoxide with mole fractions varying from 0 to 1. Absorbance was measured at 400 nm at 25°C.

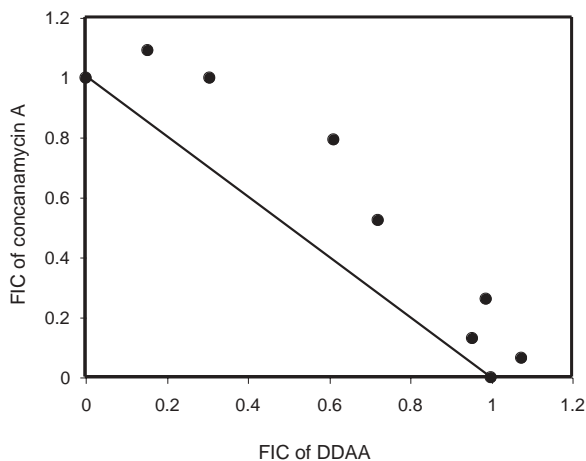


Fig 2–Isobologram of concanamycin A and DDAA. The solid line indicates an isobole where the two drugs act additively. The  $IC_{50}$  value for concanamycin A and DDAA was 0.2 and 34 nM respectively. FIC: fractional inhibitory concentration.

hematin: DDAA of 2:1 (Fig 1). Similar experiments conducted by following the emission fluorescence of DDAA at 480 nm failed to show any changes in the fluorescence intensity (data not shown).

If DDAA interferes with hemozoin forma-

tion *in situ*, it should accumulate in the acidic food vacuole of the parasite where the heme target is located and thus be antagonized by concanamycin A, an inhibitor of vacuolar  $H^+$ -ATPase. The isobologram obtained from FIC measurements of DDAA and concanamycin A combinations shows an additive effect, or a very mild antagonism (indifference) at best (sum of FICs ranging from 1.09-1.41) (Fig 2).

## DISCUSSION

Analogs of 9-anilinoacridine exert their antiparasmodial effects either by inhibiting parasite DNA topoisomerase II (Auparakkitanon and Wilairat, 2000) or by interfering with  $\beta$ -hematin formation (Auparakkitanon *et al*, 2003). Their target specificity is governed, in part, by the type of side chains at the 3,6-positions of the acridine ring: the amino group directs specificity to DNA topoisomerase II and chloro group to hematin.

However, 3,6-diamino-1'-dimethyl-9-anilinoacridine (DDAA) demonstrates the DNA topoisomerase II inhibitory property *in situ* (Auparakkitanon and Wilairat, 2000), but it is also able to inhibit *in vitro*  $\beta$ -hematin production as efficiently as chloroquine (Auparakkitanon *et al*, 2003). This study showed a weak interaction of DDAA with hematin using both absorbance and fluoroscopic spectroscopic approaches, and the demonstration of its location within the malaria parasite acidic food vacuole was equivocal. As a Mannich base with two amino moieties in the acridine ring (Gamage *et al*, 1994), DDAA has the requisite property permitting accumulation in an intracellular acidic compartment. The lack of an antagonistic effect by concanamycin A on DDAA-induced parasite growth inhibition may be due to the low concentrations of concanamycin A ( $IC_{50}$  = 0.2 nM) used in the drug combination tests, as a 300-fold increase in concentration is needed to produce alkalization of the *P. falciparum* food vacuole

(Saliba *et al*, 2003). Inhibition of  $\beta$ -hematin formation needs not require binding to hematin, and thereby preventing its involvement in the crystallization process, but conceivably can be achieved by blocking crystal growth through binding to the growing crystal lattice face as has been recently demonstrated for the action of artemisinin, chloroquine, diethylaminoalkoxyxanthenes and quinine (Solomonov *et al*, 2007). The ability of antiplasmodial drugs to have more than one target should make them attractive for future drug development as this would delay the onset of parasite drug resistance, a strategy underpinning current combination drug therapies.

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