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In vitro efficacy of 7-benzylamino-1-isoquinolinamines against *Plasmodium falciparum* related to the efficacy of chalcones

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ABSTRACT

A series of 1,7-diaminoisoquinolinamines, that are expected to mediate antimalarial activity by the same mechanism employed by the chalcones, were produced. Six 7-benzylamino-1-isoquinolinamines were found to be submicromolar inhibitors in vitro of drug-resistant *Plasmodium falciparum*, with the best possessing activity comparable to chloroquine. Despite being developed from a lead that is a DHFR inhibitor, these compounds do not mediate their antimalarial effects by inhibition of DHFR.

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Each year, more than 1 million people die from malaria.[1] The organism responsible for most of these deaths, *Plasmodium falciparum*, has developed resistance to most available drugs.[2,3] Thus, there is an urgent need for novel and affordable new products.[4] Chalcones (1,3-diphenyl-2-propen-1-ones) and their carbocyclic and heterocyclic analogs display a wide range of biological activities including antiprotozoal, antibacterial, antifungal and antiproliferative activity.[5] Several groups have studied the relationship between the structure of substituted chalcones and their antimalarial activity.[6–14] Chalcones possessing submicromolar efficacy against drug-resistant strains of *P. falciparum* in vitro have been identified.[6,15] The emerging SAR does not correlate with the SAR of known antimalarial targets, implying that the mechanism by which the chalcones act is distinct from established mechanisms.[7–9] Both reports that propose possible mechanisms for chalcone action involve their modification of the erythrocyte membrane: either by inhibiting the permeation pathways induced by the parasite in the erythrocyte membrane,[8] or by transformation of normal erythrocytes into echinocytes, which creates conditions unfavorable for parasite growth.[9] Several of these compounds have been tested in animal models of malaria (*Plasmodium yoelii*- or *Plasmodium berghei*-infected mice)[6,12,14,16] and some possess significant in vivo efficacy.[12,14,16] However, in vitro and in vivo efficacies often do not correlate.[6,16] Although possibly due to the difference in Plasmodium species employed between in vitro and in vivo assays, we suspect that rapid compound metabolism also contributes because the chalcones tested by us were all rapidly digested in vitro by microsome preparations.[6]

We therefore wished to identify a compound series structurally distinct from the chalcones, but that mediates antimalarial activity via the same mechanism. We aimed to achieve this through use of a 3D-pharmacophore that we had developed using CATALYST software which models the structural and electronic features required by compounds to effect antimalarial activity via this novel chalcone-mechanism.[17] The pharmacophore was used in an in silico screening of the collection of compounds at the Walter Reed Army Institute of Research. It predicted that 6-(3′-bromobenzylamino)-2,4-quinazolinediamine (**1**) should mediate antimalarial effects by a chalcone-like mechanism (Fig. 1).

Such quinazolinetriamines are known to exert potent antimalarial activity by inhibition of dihydrofolate reductase (DHFR).[18] Unfortunately, acquired drug resistance to DHFR inhibitors rapidly develops.[19] We, therefore, sought to design compounds instead that exhibit antimalarial activity via a chalcone-like mechanism and have minimal DHFR activity. Published data suggests that the N^1- and the 2-amino groups are important in mediating the inhibition of DHFR by 6-benzylamino-quinazolinetriamines.[20–22]

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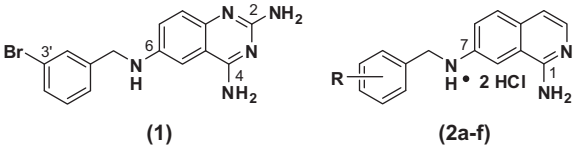


Figure 1. Structures of the in silico screening hit (**1**) and compounds designed from it (**2a**–**f**).

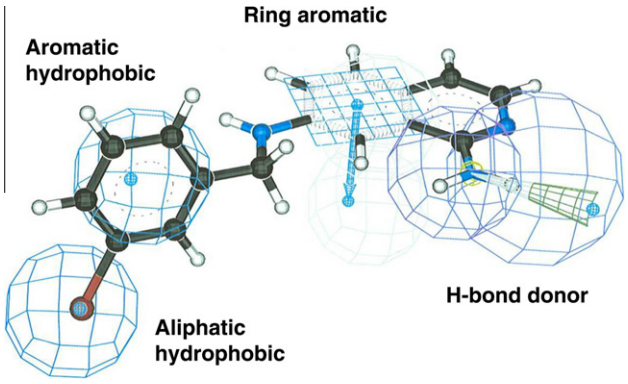
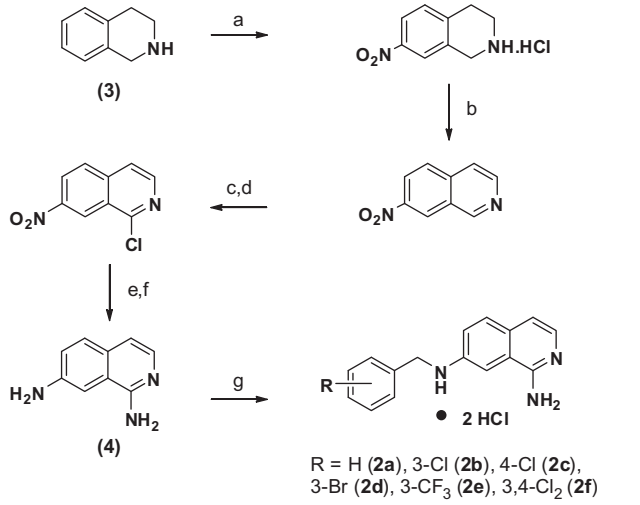


Figure 2. The four structural features required for antimalarial activity via the chalcone-mechanism, represented by the geometric shapes labeled according to type, overlaid with the structure of compound **2d**.

We proposed that 7-benzylamino-1-isoquinolinamines (**2a**–**f**), which lack these functional groups, should inhibit DHFR to a considerably lesser extent than do the 6-benzylamino-quinazolinetriamines (Fig. 1). Such isoquinolinamines are not described in the literature, so have not previously been assessed as antimalarial agents. Our pharmacophore model predicted that the bromo-substituted 7-benzylamino-1-isoquinolinamines (**2d**) would be a submicromolar inhibitor of *P. falciparum*. In Figure 2 the structure of this isoquinolinamine is overlaid upon that of the pharmacophore. The latter comprises the four structural features required for inhibition via the chalcone-mechanism, and is shown by the geometric shapes.[17] The overlay shows that the isoquinolinamine possesses all four of these structural features.

The seven-step synthetic sequence, shown in Scheme 1, was developed by which the 7-benzylated 1,7-diaminoisoquinolines

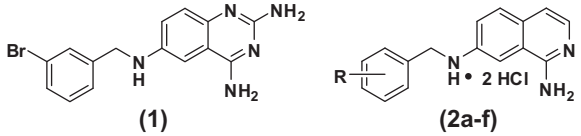


Scheme 1. (a) KNO_3 (aq), H_2SO_4 (aq); HCl, EtOH. (b) $(KSO_3)_2NO$, Na_2CO_3 (aq). (c) *m*-CPBA. (d) *p*-TsCl, pyr. (e) (1) Ethanolamine. (f) H_2, Pd/C. (g) *R*-C_6H_5CHO; $NaBH_3CN$; HCl, EtOH.

(**2a**–**f**) were prepared. The route commences with commercially available 1,2,3,4-tetrahydroisoquinoline (**3**), and proceeds via 1,7-isoquinolinediamine (**4**).[23,24] We have previously reported that this sequence was used successfully to produce 1,7-isoquinolinediamine (**4**) on about a 1 mmol-scale.[25] Subsequent efforts to scale-up the synthesis by about 10-fold resulted in significantly reduced yields in several of the steps. Significant optimization of the synthesis would, therefore, be needed to produce the final compounds in significant quantities. This finding, together with the length of the synthetic sequence and the expense of the oxidant employed in the second step, $(KSO_3)_2NO$, give us concern that it may not be possible to bring down the production cost of the isoquinolinamines sufficiently to be acceptable for their intended use.

The in vitro antimalarial activities of the novel 1,7-diaminoisoquinolines (**2a**–**f**) were determined by measuring their ability to inhibit [^{3}H]-hypoxanthine uptake by *P. falciparum*.[26] The results against the mefloquine-resistant, chloroquine-sensitive D6 strain; the Southeast Asian multi-drug resistant isolate TM91C235; and the chloroquine-resistant, mefloquine-sensitive W2 strain; are shown in Table 1. The compounds were found to inhibit parasite

Table 1
Analogs synthesized with in vitro efficacies[a] against *P. falciparum* D6, W2 and TM91C235 and resistance indices[b,c] calculated relative to D6



Compound number	R	*P. falciparum* D6 IC_{50}[a] (μM)	*P. falciparum* TM91C235 IC_{50}[a] (μM)	*P. falciparum* W2 IC_{50}[a] (μM)	Resistance index[b] TM91C235/D6	Resistance index[c] W2/D6
Chloroquine		**0.0134**	**0.0712**	**0.397**	5.30	29.5
Mefloquine		0.0201	0.0374	0.00601	1.85	0.289
1	—	<0.000142	0.0208	0.00208	147	14.6
2a	H	0.212	0.486	1.40	2.29	6.59
2b	3-Cl	0.0648	0.158	0.359	2.44	5.55
2c	4-Cl	0.0736	0.205	0.417	2.78	5.67
2d	3-Br	0.103	0.142	0.421	1.38	4.09
2e	3,4-DiCl	0.0691	0.111	0.455	1.60	6.58
2f	3-CF_3	0.0501	0.0719	0.260	1.44	5.20

[a] Inhibition of [^{3}H]-hypoxanthine uptake by *P. falciparum*; values from one experiment.
[b] IC_{50} against TM91C235/IC_{50} against D6.
[c] IC_{50} against W2/IC_{50} against D6.

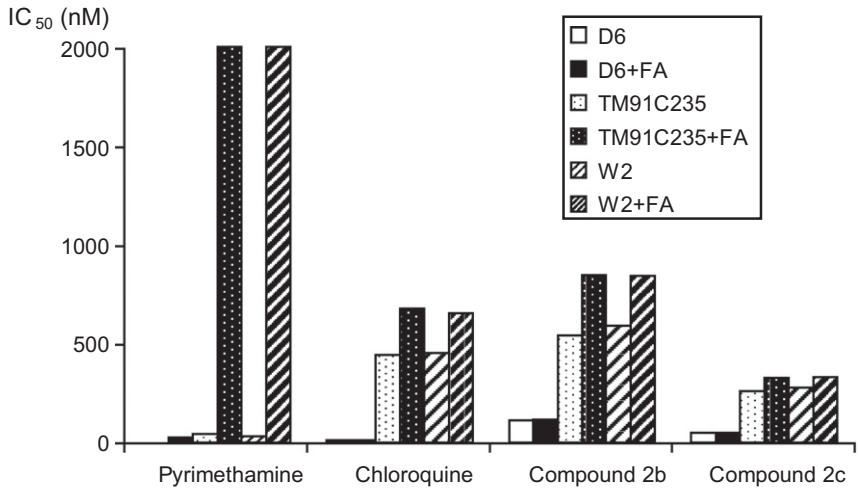


Figure 3. The effect of folinic acid, which can circumvent DHFR inhibition, on in vitro antimalarial efficacy. The IC_{50} of pyrimethamine (a DHFR inhibitor) increases significantly in the presence of 1 mM folinic acid (+FA); in contrast, the IC_{50} of chloroquine (not a DHFR inhibitor) and of compounds **2b** and **2c**, are not significantly altered.

growth, generally at submicromolar concentrations. The best possess potencies comparable to chloroquine. The data suggest that both the 3- and the 4-halo substituents on the benzyl group contribute to potency. Potencies against the TM91C235 isolate on average were twofold higher than against the D6 strain, with the average for the W2 strain being almost sixfold higher. These results suggest that 1,7-diaminoisoquinolines may not generally have significant cross-resistance; but do display a modest reduction in sensitivity, particularly to the 4-aminoquinoline, chloroquine.

In order to ascertain that the antimalarial properties of the 1,7-diaminoisoquinolines are not due to their inhibition of DHFR, we tested two such compounds in an assay which employs folinic acid to circumvent inhibition of parasite growth by inhibition of DHFR.[27] This method compares the IC_{50} for a test compound in the presence, or the absence, of 1 mM folinic acid (+FA). When the DHFR inhibitor pyrimethamine is tested with either pyrimethamine-sensitive (D6), or pyrimethamine-resistant (TM91C235, W2) parasites, the IC_{50} of pyrimethamine increases significantly in the presence of 1 mM folinic acid, as shown in Figure 3. In contrast, folinic acid does not cause a significant change in the IC_{50} of chloroquine, which is not a DHFR inhibitor. That folinic acid does not cause a significant change in the IC_{50} of the 1,7-diaminoisoquinolines **2b** or **2c** suggests that they, like chloroquine but unlike pyrimethamine, do not mediate their antimalarial effects by inhibition of DHFR.

A 3D-pharmacophore model developed from the antimalarial activities of a series of chalcones was used to design a series of 1,7-diaminoisoquinolinamines that are expected to mediate antimalarial activity by a novel chalcone-like mechanism. As predicted by the model, substituted 1,7-diaminoisoquinolinamines were found to be submicromolar inhibitors of drug-resistant *P. falciparum*, with the best possessing activity comparable to chloroquine. Despite being developed from a lead that is a DHFR inhibitor,[18] we have demonstrated that the 1,7-diaminoisoquinolinamines do not mediate their antimalarial effects by inhibition of DHFR. Future studies will include testing representative compounds against Pf DHFR and in vivo efficacy testing.

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