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In vitro and in vivo efficacy and in vitro metabolism of 1-phenyl-3-aryl-2-propen-1-ones against *Plasmodium falciparum*

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Abstract—Investigation of a series of 1-phenyl-3-aryl-2-propen-1-ones resulted in the identification of nine inhibitors with submicromolar efficacy against at least one *Plasmodium falciparum* strain in vitro. These inhibitors were inactive when given orally in a *Plasmodium berghei* infected mouse model. Significant compound degradation occurred upon their exposure to a liver microsome preparation, suggesting metabolic instability may be responsible for the lack of activity in vivo.

Each year, more than 1 million people die from malaria. The organism responsible for most of these deaths, *Plasmodium falciparum*, has developed resistance to most available drugs.[1] Thus, there is an urgent need for novel and affordable products. 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.[2–7] Interest in their antimalarial properties began with the report that the natural product Licochalcone A completely cleared *Plasmodium yoelii* from infected mice when dosed subcutaneously, thus protecting the mice from parasite-induced death.[2] A related synthetic analog, 2,4-dimethoxy-2′-*n*-butoxy-chalcone, possessed substantial oral activity in mice infected with drug-resistant strains of *Plasmodium berghei*. Moreover, this compound did not show toxicity in human peripheral blood lymphocytes at concentrations 30 times greater than the effective dose.[3] A recent comprehensive study described the in vitro efficacy of 92 oxygenated chalcone analogs, including 12 with good activity against *P. falciparum* K1 ($IC_{50} < 10$ μM). Several analogs, when dosed intraperitoneally in *P. berghei* infected mice, were able to prolong mouse lifespan relative to control, with two being as effective as chloroquine. Significantly, the authors report a lack of correlation between in vitro and in vivo efficacy.[4] Further development of the chalcones would benefit from an understanding of this observation, as well as a determination of their mechanism of antimalarial action, which is under investigation by several groups.[5]

Our study was built on an earlier collaboration between the Walter Reed Army Institute of Research and the Rosenthal group which led to several chalcones with submicromolar activity against both *P. falciparum* W2 (a chloroquine-, quinine-, and pyrimethamine- resistant strain) and D6 (mefloquine resistant).[6] At that time an in vivo efficacy study of these inhibitors was not performed. In this paper, we will describe the resynthesis of some of these compounds along with novel related analogs. Following determination of their in vitro activity, the in vivo activity and in vitro stability of selected compounds in the series were assessed.

Initial attention was focused on 2-propen-1-ones substituted at the 3-position with a fused aromatic ring since such analogs are well represented in those reported to possess in vivo efficacy,[4,7] our ultimate goal. Compounds in which the fused aromatic ring was either an

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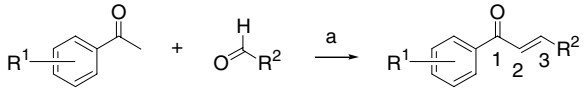
all-carbon naphthalene or a nitrogen-containing quinoline were prepared, since it was not clear from previous reports which contributed more to antimalarial activity.[4] A potential advantage of heterocyclic compounds is reduced lipophilicity, which should improve aqueous solubility—important since the modest water solubility of the chalcone scaffold may be an obstacle to future drug development.[5] Substitution on the 1-phenyl ring is known to significantly influence activity—dimethoxy and especially dichloro substitution patterns are well represented in the more active analogs synthesized to date.[4,6,7] This study therefore focused on such structures, including novel analogs containing methoxy and halo-substituents in the same compound. Analogs substituted at the 4-position of the 1-phenyl ring were of particular interest since this site appears to be especially prone to metabolic oxidation.[8]

Compounds were prepared by the condensation of substituted methyl ketones with substituted aldehydes as shown in Scheme 1. The optimum conditions for this condensation depended upon both the nature of the aldehyde and the substituents on the ketone. For all the naphthaldehyde-derived compounds, standard conditions of sodium hydroxide in aqueous ethanol (Method A)[9] gave good yields. For condensations involving quinoline carboxaldehydes, barium hydroxide in methanol (Method B) was used, since Method A typically failed to provide the desired propenones. For a small number of reactions involving dihalogenated methyl ketones with quinoline carboxaldehydes, both Methods A and B did not furnish the desired propenones. Usually in these cases condensation could be mediated using diethylamine in pyridine (Method C).[10] In a few instances, mainly involving 2-quinolines, none of the methods described provided the desired product. The structures of the compounds prepared, and results from their biological testing, are shown in Table 1. All compounds were assayed in vitro against *P. falciparum*, and selected compounds in a *P. berghei*-infected mice model and for predicted metabolic stability, using protocols that have been reported previously.[11–13]

Most of the compounds were active in vitro against both strains of *P. falciparum* tested ($IC_{50} < 10$ μM). One determination of each IC_{50} was made; in other studies, when multiple determinations were made standard deviations were generally small, such that a fourfold difference in activity is likely to be significant. Efficacies in the chloroquine-, quinine-, and pyrimethamine-resistant W2 strain and in the mefloquine-resistant D6 strain were similar, a preliminary indication that the compounds are devoid of significant cross-resistance (resistance indices ≈1). Both the 3-aryl and the 1-phenyl substituents (R^1- and R^2-, respectively) impact activity, as reported



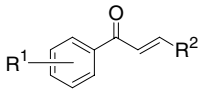
Scheme 1. Reagents and conditions: (a) excess 50% aq NaOH, EtOH (Method A) or 1.2 equiv $Ba(OH)_2 \cdot 8H_2O$, MeOH (Method B) or excess Et_2NH, pyridine (Method C).

previously.[4,6,7] The initial series of compounds prepared (**1**–**33**) contain either a carbocyclic naphthalene or a heterocyclic quinoline as the R^2-substituent, with either one, two or three methoxy groups as the R^1-substituent(s). Most of the naphthalene-derivatives were moderately active (IC_{50} in the range 1–10 μM). In all cases 1-naphthyl compounds appear to be superior to their corresponding 2-naphthyl isomers (comparison of compounds **1** and **2**, **6** and **7**, **11** and **12**, **15** and **16**, **19** and **20**, **23** and **24**, **26** and **27**, and **31** and **32**). In these naphthalene series the various di- and tri-methoxy compounds tested had similar activities, and were more active than the corresponding mono-methoxy analogs.

As noted above, it was not clear from previous studies whether the presence of a nitrogen in the R^2-substituent always enhanced activity.[4] In this study, the nitrogen-containing 3-quinolines appear more active than their corresponding carbocyclic analogs, the 2-naphthalenes (comparison of **4** and **2**, **9** and **7**, **13** and **12**, **17** and **16**, **21** and **20**, **25** and **24**, and **29** and **27**), with just one exception (**33** and **32**). However, this was not the case with 4-quinolines and their corresponding carbocyclic analogs, the 1-naphthalenes (**10** and **6**, and **22** and **19** favor the quinoline, whereas **14** and **11**, **18** and **15**, and **30** and **26** favor the naphthalene). Antimalarial activities of the 2-quinoline-derivatives compare favorably with those of their 3- and 4-quinoline, and 2-naphthalene analogs, a finding that has not been described previously. In the 2-, 3-, and 4-quinoline series, dimethoxy compounds were more active than the corresponding mono- and tri-methoxy analogs. When the naphthalene was preferred over the quinoline, that difference in activity appears no greater than threefold. However, examples were found where the activity of the quinoline was tenfold better than that of the naphthalene. Based on these results a second series of compounds, designed to explore halo and halomethoxy R^1-substituents, focused on phenyl-disubstituted quinolines (**34**–**58**). Whereas just one dimethoxy-substituted compound with submicromolar efficacy against at least one of the *P. falciparum* strains tested was identified (**22**), three such dihalosubstituted compounds (**42**, **43**, and **45**) were found, building upon reported results.[6,7] Five such compounds were identified amongst the monohalo, monomethoxy-compounds prepared (**50**, **51**, **55**, **56**, and **58**), structures for which there is no literature precedence. In some halogen-containing compounds there was little difference in activity between the chloro- and fluoro-analogs (comparison of **35** and **40**, and **54** and **57**), in one case the fluoro-analog appears more active (**38** and **44**), whereas in other cases the chloro-analog appears more active (**36** and **42**, **37** and **43**, **39** and **45**). The nine submicromolar inhibitors included both 3- and 4-quinolines, in contrast to previous findings favoring 3-quinolines,[4] and possessed either 2,5- or 3,4-disubstitution on the 1-phenyl substituent. Preliminary pharmacophore modeling using CATALYST software suggests that the 1-phenyl ring contributes as an aromatic hydrophobe and one of the R^1-substituents (either methoxy or halo) as an aliphatic hydrophobe.

Of these nine most active compounds, five were selected for in vivo efficacy and in vitro metabolic stability

Table 1. Analogs synthesized with in vitro efficacies against *Plasmodium falciparum* W2 and D6; with efficacy against *Plasmodium berghei* in infected mice, in vitro metabolic half life, and calculated log *P* data for select compounds



Compound	R^1	R^2	Synthetic method	*P. f.* W2 IC_{50}[a] (μM)	*P. f.* D6 IC_{50}[a] (μM)	Resistance index[b]	In vivo activity at 640 mg/kg[c]	Metabolic half life (h)[d]	log*P*[e]
Chloroquine				**0.24**	**0.013**	18.5	Curative	—	—
1	4-MeO–	1-Naphthyl-	A	10	9.1	1.1	—	—	—
2	4-MeO–	2-Naphthyl-	A	>17	>17	—	—	—	—
3	4-MeO–	2-Quinolinyl-	B	>17	4.7	—	—	—	—
4[f]	4-MeO–	3-Quinolinyl-	B	3.3	1.8	1.8	—	—	—
5[f]	4-MeO–	4-Quinolinyl-	B	10	5.4	1.9	—	—	—
6[f]	2,4-DiMeO–	1-Naphthyl-	A	4.6	4.9	0.94	—	—	—
7	2,4-DiMeO–	2-Naphthyl-	A	>16	8.7	—	—	—	—
8	2,4-DiMeO–	2-Quinolinyl-	B	1.2	2.0	0.60	—	—	—
9[f]	2,4-DiMeO–	3-Quinolinyl-	B	3.3	4.8	0.70	—	—	—
10[f]	2,4-DiMeO–	4-Quinolinyl-	B	2.0	3.0	0.67	—	—	—
11	2,5-DiMeO–	1-Naphthyl-	A	2.8	2.9	0.97	—	—	—
12	2,5-DiMeO–	2-Naphthyl-	A	5.0	5.5	0.91	—	—	—
13	2,5-DiMeO–	3-Quinolinyl-	B	2.7	2.0	1.4	—	—	—
14	2,5-DiMeO–	4-Quinolinyl-	B	7.3	3.0	2.4	—	—	—
15	2,6-DiMeO–	1-Naphthyl-	A	3.1	3.2	0.97	—	—	—
16	2,6-DiMeO–	2-Naphthyl-	A	3.3	3.4	0.97	—	—	—
17	2,6-DiMeO–	3-Quinolinyl-	B	1.1	2.2	0.53	—	—	—
18	2,6-DiMeO–	4-Quinolinyl-	B	5.7	13	0.44	—	—	—
19	3,4-DiMeO–	1-Naphthyl-	A	5.0	5.2	0.96	—	—	—
20	3,4-DiMeO–	2-Naphthyl-	A	7.7	9.8	0.79	—	—	—
21	3,4-DiMeO–	3-Quinolinyl-	B	2.2	3.4	0.65	—	—	—
22	3,4-DiMeO–	4-Quinolinyl-	B	**0.50**	**0.88**	0.57	Inactive	0.26	4.12
23	3,5-DiMeO–	1-Naphthyl-	A	4.3	4.5	0.96	—	—	—
24	3,5-DiMeO–	2-Naphthyl-	A	9.5	7.4	1.3	—	—	—
25	3,5-DiMeO–	3-Quinolinyl-	B	4.3	11	0.39	—	—	—
26	2,3,4-TriMeO–	1-Naphthyl-	A	5.8	4.3	1.3	—	—	—
27	2,3,4-TriMeO–	2-Naphthyl-	A	>14	>14	—	—	—	—
28	2,3,4-TriMeO–	2-Quinolinyl-	B	3.3	2.9	1.1	—	—	—
29[f]	2,3,4-TriMeO–	3-Quinolinyl-	B	2.8	6.7	0.42	—	—	—
30[f]	2,3,4-TriMeO–	4-Quinolinyl-	B	>14	2.6	—	—	—	—
31	3,4,5-TriMeO–	1-Naphthyl-	A	4.8	5.2	0.92	—	—	—
32	3,4,5-TriMeO–	2-Naphthyl-	A	6.0	6.6	0.91	—	—	—
33	3,4,5-TriMeO–	3-Quinolinyl-	B	14	21	0.67	—	—	—
34	2,4-DiF–	2-Quinolinyl-	B	1.3	2.3	0.57	—	—	—
35	2,4-DiF–	3-Quinolinyl-	B	6.2	5.0	1.2	—	—	—
36	2,5-DiF–	3-Quinolinyl-	B	7.8	5.6	1.4	—	—	—
37	2,5-DiF–	4-Quinolinyl-	C	8.6	3.7	2.3	—	—	—
38	3,4-DiF–	3-Quinolinyl-	B	5.8	8.5	0.68	—	—	—
39	3,4-DiF–	4-Quinolinyl-	B	5.7	9.4	0.61	—	—	—
40	2,4-DiCl–	3-Quinolinyl-	B	6.3	7.9	0.80	—	—	—
41	2,4-DiCl–	4-Quinolinyl-	C	18	6.2	2.9	—	—	—
42	2,5-DiCl–	3-Quinolinyl-	B	**1.7**	**0.62**	2.7	Inactive	0.41	4.78
43[g]	2,5-DiCl–	4-Quinolinyl-	C	**0.23**	**0.19**	1.2	Inactive	ND	4.78
44	3,4-DiCl–	3-Quinolinyl-	B	12	3.1	3.9	—	—	—
45[g]	3,4-DiCl–	4-Quinolinyl-	B	**0.80**	**1.7**	0.47	Inactive	ND	5.31
46	2-F, 4-MeO–	3-Quinolinyl-	B	3.1	2.1	1.5	—	—	—
47	2-F, 4-MeO–	4-Quinolinyl-	B	4.5	2.4	1.9	—	—	—
48	2-MeO-4-F–	3-Quinolinyl-	B	1.5	1.1	1.4	—	—	—
49	2-MeO, 4-F–	4-Quinolinyl-	B	6.2	6.0	1.0	—	—	—
50	2-MeO, 5-F–	2-Quinolinyl-	B	**0.67**	**3.8**	0.18	—	—	—
51	2-MeO, 5-F–	3-Quinolinyl-	B	**1.1**	**0.46**	2.4	—	—	—
52	2-MeO, 5-F–	4-Quinolinyl-	B	4.1	5.1	0.80	—	—	—
53	3-F, 4-MeO–	2-Quinolinyl-	B	5.3	1.3	4.1	—	—	—
54	3-F, 4-MeO–	3-Quinolinyl-	B	2.7	1.6	1.7	—	—	—
55	3-F, 4-MeO–	4-Quinolinyl-	B	**3.2**	**0.93**	3.4	Inactive	0.51	4.25
56	2-MeO, 5-Cl–	3-Quinolinyl-	B	**0.59**	**0.95**	0.62	—	—	—
57	3-Cl, 4-MeO–	3-Quinolinyl-	B	1.6	2.3	0.70	—	—	—

Table 1 (*continued*)

Compound	R^1	R^2	Synthetic method	*P. f.* W2 IC_{50}[a] (μM)	*P. f.* D6 IC_{50}[a] (μM)	Resistance index[b]	In vivo activity at 640 mg/kg[c]	Metabolic half life (h)[d]	log*P*[e]
58	3-Cl, 4-MeO–	4-Quinolinyl-	B	**0.95**	**1.8**	0.53	—	—	—

[a] Inhibition of [^{3}H] hypoxanthine uptake by *P. falciparum*; values from one experiment; where efficacy in at least one strain is submicromolar results are in bold.
[b] IC_{50} against W2/IC_{50} against D6.
[c] Activity determined by measurement of survival of five *P. berghei*-infected mice treated with compound (640 mg/kg, po, 5 days) compared to five untreated control mice.
[d] Half life of compound upon exposure to mouse liver microsomes.
[e] Calculated using Advanced Chemistry Development ACD/log*D* sol suite.
[f] In vitro efficacy against *P. falciparum* K1 reported previously.[4]
[g] In vitro efficacy against *P. falciparum* W2 and D6 reported previously.[6]

testing. Structural variability was a selection criteria, though compounds substituted at the 4-position on the 1-phenyl ring were preferred due to the likelihood for biotransformation at this site.[9] The in vivo efficacy study showed no compound was toxic at the highest dose tested of 640 mg/kg/dose (mice survived the first 5 days of the assay). However, the compounds failed to enhance mouse longevity (mice died during days 6–8 of the assay, as did the untreated control mice, consistent with parasite-induced death). The results of in vitro metabolic stability testing of selected analogs revealed two problems with this series of compounds. Compounds **22**, **42**, and **55** were rapidly metabolized. Determination of the half life for **43** and **45** was prevented by their low solubility in the aqueous assay solvent. This issue, which has been reported by others in a structurally related series,[5] possibly affects in vivo efficacy due to poor bioavailability-indeed all five compounds have relatively high calculated log*P* values.

In conclusion, nine 1-phenyl-3-aryl-2-propen-1-ones with submicromolar efficacy against drug-resistant strains of *P. falciparum* in vitro were identified. In all cases the 1-phenyl substituent was either 2,5- or 3,4-disubstituted with chloro and/or methoxy substituents, and the 3-aryl substituent was either a 3- or 4-quinoline. Five such compounds demonstrated low toxicity, but failed to show efficacy in vivo in a malaria-infected mouse model. The lack of correlation between in vitro and in vivo efficacy, though possibly due to the difference in Plasmodium species, is likely influenced by rapid compound metabolism, evidenced by rapid in vitro microsome digestion. Metabolically resistant analogs are currently being prepared, since a solution to this problem will be critical for future development of the chalcones against both malaria and other diseases.

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11. In vitro efficacy was determined by a modified version of Desjardins' method, in which parasites were pre-exposed to test compound prior to measurement of their [^{3}H]-hypoxanthine uptake, as reported previously.[6]
12. In vivo efficacy was determined by inoculating mice intraperitoneally with 1.0×10^6 *Plasmodium berghei* P-line-infected red blood cells (day 0). The test compound was dissolved in DMSO, diluted with 0.5% hydroxyethylcellulose and 0.1% Tween, and administered orally at 40, 160, and 640 mg/kg/dose once daily for 5 days (days 3–7) to five mice, as described in Jiang, S.; Zeng, Q.; Gettayacamin, M.; Tungtaeng, A.; Wannaying, S.; Lim,

A.; Hansukjariya, P.; Okunji, C. O.; Zhu, S.; Fang, D. *Antimicrob. Agents Chemother.* **2005**, *49*, 1169.

13. To predict metabolic stability, a 25 μM solution of test compound was prepared in a mixture containing pooled human liver microsomes (0.5 mg/mL total protein from BD Gentest) and 0.1 M sodium phosphate buffer (pH 7.4) with an NADPH-regenerating system (1.25 mM $\beta NADP^+$, 3.3 mM glucose-6-phosphate, and 3.3 mM $MgCl_2$). Glucose-6-phosphate dehydrogenase (1 U/mL final concentration) was added to initiate reaction. The amount of parent compound remaining at various time points was determined using LC-MS analysis; half life was calculated assuming first-order decay, as described in Shearer, T. W.; Kozar, M. P.; O'Neil, M. T.; Smith, P. L.; Schiehser, G. A.; Jacobus, D. P.; Diaz, D. S.; Yang, Y.-S.; Milhous, W. K.; Skillman, D. R. *J. Med. Chem.* **2005**, *48*, 2805.