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Dibenzylideneacetone analogues as novel *Plasmodium falciparum* inhibitors

Rahul Balasaheb Aher [a,*], Gajanan Wanare [a], Neha Kawathekar [a], Ravi Ranjan Kumar [b,†], Naveen Kumar Kaushik [b], Dinkar Sahal [b], Virander Singh Chauhan [b]

[a] Department of Pharmacy, Shri G.S. Institute of Technology and Science, 23 Park Road, Indore 452003 (MP), India
[b] Malaria Research Laboratory, International Center for Genetic Engineering and Biotechnology, New Delhi 110067, India

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ABSTRACT

A series of dibenzylideneacetones (**A1**–**A12**) and some of their pyrazolines (**B1**–**B4**) were synthesized and evaluated in vitro for blood stage antiplasmodial properties in *Plasmodium falciparum* culture using SYBR-green-I fluorescence assay. The compound (**1E**, **4E**)-1,5-bis(3,4-dimethoxyphenyl)penta-1,4-dien-3-one (**A9**) was found to be the most active with IC_{50} of 1.97 μM against chloroquine-sensitive strain (**3D7**) and 1.69 μM against chloroquine-resistant field isolate (**RKL9**). The MTT based cytotoxicity assay on **HeLa** cell line has confirmed that **A9** is selective in its action against malaria parasite (with a therapeutic index of 166). Our results revealed that these compounds exhibited promising antiplasmodial activities which can be further explored as potential leads for the development of cheaper, safe, effective and potent drugs against chloroquine-resistant malarial parasites.

The emergence and spread of parasites resistant to most of the clinically used antimalarials and their combinations have aroused an imperative need to develop new drugs against malaria. The *Plasmodium falciparum* malaria resistance remains the worst parasitic burden in developing countries. The development of resistance by the parasite against first line as well as second line antimalarial drugs has drawn attention to develop new drugs to alleviate the disease burden. Half of the world's population is at a risk of malaria, and according to the WHO report of 2009, an estimated 243 million cases led to nearly 863 thousands deaths in 2008, most of them being children under 5 years of age.[1]

Since a vast majority of those affected with malaria are the poor sections of modern societies, the new drugs crying to come out of the discovery pipe line must be potent, safe and affordable. To be widely useful, they must reach developing countries at a cheaper rate with proper distribution. Moreover, the absence of an effective vaccine for protection and the availability of artemisinin and its derivatives as the only option has made the situation rather serious. Indeed, most recent reports indicate the emergence of clinical resistance against artemisinin in the form of prolonged parasite clearance time.[2,3] As a result, there is an urgent need for new efficient antimalarial agents.

The α,β-unsaturated ketones like chalcone, curcumin have been studied as antimalarial agents.[4,5] Here in this study, antimalarial activity of dibenzylideneacetone derivatives has been investigated. As shown in Figure 1, these compounds are chalcones with an additional carbon–carbon double bond, and lacks the moiety containing active methylene (–CH_2–) and carbonyl (C=O) groups of curcumin (indeed the presence of which decreases the stability of curcumin).[6] So, a chemically diversed series of molecules with probable new mechanism of action, that is, dibenzylideneacetone derivatives were synthesized and evaluated for their antimalarial potency against *P. falciparum*, chloroquine-sensitive (**3D7**) and chloroquine-resistant (**RKL9**) strains. Alterations in dibenzylideneacetone were made to study the effect of different substituents (with varied lipophilic, steric and electronic properties) on antimalarial potency. Some of the analogs were further converted into their corresponding pyrazolines to test the activity enhancement by pyrazoline ring as found in curcumin–pyrazoline derivatives ($IC_{50}^{MP\text{-}14}$ = 0.45 μM).[4]

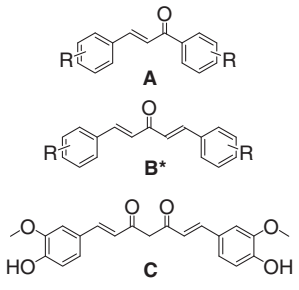

Figure. 1. Structures of Chalcone (**A**), Dibenzylideneacetone (**B***) and Curcumin (**C**)*: synthesized analogues.

* Corresponding author. Tel.: +91 9730962361.
E-mail addresses: rahulba26@yahoo.co.in, rahulba26@gmail.com (R.B. Aher).
† Author contributed equally.

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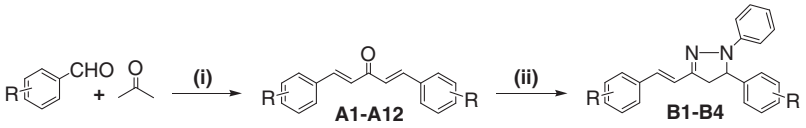

Scheme 1. Synthesis of dibenzylideneacetones (**A1**–**A12**) and their pyrazoline derivatives (**B1**–**B4**). Reagents and conditions: (i) 10% NaOH, EtOH, H_2O; (ii) Phenylhydrazine, CH_3COOH, 8 h reflux.

The compounds **A1**–**A12** were prepared from acetone and substituted benzaldehydes in a base catalyzed aldol reaction (Scheme 1) as described by Kiran et al.[7] Further, some of the dibenzylideneacetone derivatives were converted to pyrazolines using a reported method.[4] Synthetic methods used were chosen based on their simple procedures with better yields of products along with their cost effectiveness. The purity and structures of all synthesized compounds were confirmed by TLC and spectroscopic techniques, respectively. The IR spectra of all the dibenzylideneacetones displayed desired characteristic absorption bands in their respective regions like α,β-unsaturated ketone (1650–1680 cm^{-1}) and *trans* C=C (970–990 cm^{-1}). All the pyrazolines (**B1**–**B4**) displayed their characteristic absorption bands due to C=N and C–N in regions 1590–1600 and 1310−1330 cm^{-1}, respectively. The absence of an absorption bands of α,β-unsaturated ketone (at 1650–1680 cm^{-1}) and N–H stretching in pyrazoline compounds (**B1**–**B4**) confirms the cyclization of pyrazoline ring. The ^1H NMR spectra of all derivatives exhibited a characteristic multiplet for aromatic protons and two CH=CH protons at 6.49–8.05 ppm. The coupling constants (J = 16 Hz) for the two protons attached to the double bonds confirms the stereochemistry of synthesized compounds as *E*-form. The remaining protons exhibited the expected δ values. The mass spectra of compounds displayed molecular ions which confirmed their molecular weights.

The synthesized compounds were screened for in vitro antimalarial activity against chloroquine-sensitive **3D7** strain and chloroquine-resistant field isolate **RKL9** of *P. falciparum* using SYBR-green-I fluorescence assay.[8] Chloroquine diphosphate and artemisinin were used as positive controls. The results are shown in Table 1. Screening against *P. falciparum* **3D7** strain revealed that the lead unsubstituted dibenzylideneacetone (**A1**) lacks significant potency (IC_{50} of 213.41 μM) while its derivatives **A5**, **A6** and **A9** are significantly active with single digit micromolar IC_{50} values. Among the halogenated derivatives (**A2**–**A6**), the chloro derivatives (**A5**, IC_{50} = 6.26 μM, **A6**, IC_{50} = 9.94 μM, **A3**, IC_{50} = 92.35 μM) were the active ones, while the fluoro derivative (**A2**, IC_{50} = 133.2 μM) and the bromo derivative (**A4**, IC_{50} = 221.89 μM) lacked significant antimalarial activity. This is in accord with presence of the chloro group in various active antimalarial molecules including chloroquine, pyronaridine, acridinedione and others.[9–11] It is observed from results that, *ortho* chloro enhanced the antimalarial potency appreciably (IC_{50} = 6.26 μM), but chloro substitution at both *ortho* and *para* positions caused a marginal decrease in potency (**A6**, IC_{50} = 9.94 μM). There may be effective interactions of the compound with target protein(s) when chloro is *ortho* substituted. The presence of *para* chloro in **A6** may be preventing the effective binding of *ortho* chloro in active site of target protein(s). The decrease in potency of *para* substituted chloro compound (**A3**) may be due to inappropriate orientation of chloro to fit in binding site.

The introduction of methoxy group in an effort to increase electron density, has decreased the potency of unsubstituted derivative (IC_{50} = 213.41 μM) when methoxy is at *para* position (**A7**, IC_{50} = 254.8 μM); while it has improved the same when it is at *ortho* position (**A8**, IC_{50} = 74.74 μM). Interestingly, dual substitution of methoxy group at meta and *para* position leads to a dramatic increase in the potency (**A9**, IC_{50} = 1.97 μM). This may be due to the favourable steric interaction of these groups.

Both electron withdrawing (**A10**, nitro, IC_{50} = 38.54 μM) and electron releasing (**A11**, methyl, IC_{50} = 114.35 μM and **A12**, methylated amine, IC_{50} = 156 μM) substituents were found to improve the potency of unsubstituted lead. The electronic effect in the case of **A12** may be compromised by the steric hindrance caused by the bulky dimethyl substitution.

Unlike the potent curcumin–pyrazoline analogs, the heterocyclization of lead dibenzylideneacetone (**A1**) to pyrazoline analog (**B1**) did not increase the antimalarial potency. To know, whether substitutions may result in restoration of potency, derivatization

Table 1
The in vitro antimalarial activity against chloroquine-sensitive strain (**3D7**) and chloroquine-resistant field isolate (**RKL9**) of *P. falciparum* and their cytotoxicity

Compd	R	IC_{50} (μM)		Resistance index ($IC_{50}^{RKL9}/IC_{50}^{3D7}$)	TC_{50}^{HeLa} (μM)	Therapeutic index
		3D7	**RKL9**			
A1	H	213.41 ± 2.1	n.d.	—	n.d.	—
A2	4-F	133.2 ± 2.0	n.d.	—	n.d.	—
A3	4-Cl	92.35 ± 1.2	n.d.	—	n.d.	—
A4	4-Br	221.89 ± 4.5	n.d.	—	n.d.	—
A5	2-Cl	6.26 ± 0.09	6.26 ± 0.85	1	230.8 ± 3	37
A6	2,4-Di-Cl	9.94 ± 0.2	10.75 ± 0.23	1.08	>268.76	>25
A7	4-MeO	254.80 ± 3.7	n.d.	—	n.d.	—
A8	2-MeO	74.74 ± 1.2	n.d.	—	n.d.	—
A9	3,4-Di-MeO	1.97 ± 0.03	1.69 ± 0.033	0.85	282.16 ± 3	166
A10	3-NO_2	38.54 ± 0.7	n.d.	—	n.d.	—
A11	4-Me	114.35 ± 1.5	n.d.	—	n.d.	—
A12	4-NMe_2	156 ± 2.7	n.d.	—	n.d.	—
B1	H	>308.25	n.d.	—	n.d.	—
B2	4-F	9.10 ± 0.22	11.09 ± 0.24	1.21	>277.47	>25
B3	4-MeO	>130	n.d.	—	n.d.	—
B4	4-Me	>141.85	n.d.	—	n.d.	—

n.d.: not done.

Table 2
Drug likeness properties of the active molecules

Compd	Mol wt	H_A	H_D	Mol log *P*	Mol PSA (Å^2)	Mol vol (Å^3)
A5	302.03	1	0	5.97	13.24	297.90
A6	369.95	1	0	7.4	13.24	332.44
A9	354.15	5	0	3.99	45.43	392.32
B2	362.16	2	0	6.79	17.19	348.58

H_A: Hydrogen acceptor; H_D: Hydrogen donor; PSA: Polar surface area.

of **B1** with fluoro substitution on phenyl rings regained and improved the potency (**B2**, IC_{50} = 9.10 μM) where as methoxy (**B3**, IC_{50} >130 μM) and methyl (**B4**, IC_{50} >141.85 μM) substitutions exhibited moderate potency.

The derivatives possessing significant antimalarial activity in **3D7** strain were further tested against chloroquine-resistant field isolate **RKL9** of *P. falciparum*. We found that **A5**, **A6**, **A9** and **B2** were equipotent against **RKL9** with resistance indices ($IC_{50}^{RKL9}/IC_{50}^{3D7}$) of ~1 (Table 1). Indeed the most active compound, **A9** with a resistance index of 0.85 is more potent against the chloroquine resistant strain than the sensitive one. Finally the active compounds (**A5**, **A6**, **A9** and **B2**) showing IC_{50} up to 10 μM were further analyzed for their cytotoxic behavior against **HeLa** cell lines by the colorimetric MTT assay.[12] Therapeutic indices ($TC_{50}^{HeLa}/IC_{50}^{RKL9}$) with values ranging between 25 and 150 indicated that the most active compounds are also relatively nontoxic and selectively act against the malarial parasite. The results are shown in Table 1.

Further, the compounds were analyzed for the drug likeness using online server. The structures of active compounds (**A5**, **A6**, **A9** and **B2**) were drawn in the Java molecular editor (JME), an online server for predicting the drug likeness properties.[13] The results are summarized in Table 2. The in silico druggablity for the active compounds (**A5**, **A6**, **A9** and **B2**) are in accord with Lipinski's rule of five for druggable compounds. Most of the compounds are very lipophilic and thus require major improvements in order to be tested in vivo models. Although these compounds are Michael acceptors (similar to different drugs, food products including flavonoids, curcumin), they can be further optimized for antimalarial potency and can be explored for their underlying mechanism of action.

In summary, this study allows us to conclude that desirable improvement of antimalarial activity in symmetrical dibenzylideneacetones requires (a) electron donating group (methoxy) at the *meta* and *para* positions; (b) electron withdrawing (chloro) group at the *ortho* position. Although this initial study involved only a limited number of symmetrical dibenzylideneacetones, it can be further extended for detailed SAR of symmetrical as well as unsymmetrical dibenzylideneacetones. Due to the straightforward and single step synthesis, these low molecular weight dibenzylideneacetones represent a novel antimalarial scaffold, and a potential starting point for the development of new, safe, effective, cheap and potent inhibitors/drugs against malaria.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.037.

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