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Original article

Reinvestigation of structure–activity relationship of methoxylated chalcones as antimalarials: Synthesis and evaluation of 2,4,5-trimethoxy substituted patterns as lead candidates derived from abundantly available natural β-asarone

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ABSTRACT

We have examined the antimalarial structure–activity relationship of a series of methoxylated chalcones (**A**–CH=CH–CO–**B**) against *Plasmodium falciparum* (3D7 strain) using fluorescence-based SYBR Green assay. Our study has revealed that electron releasing methoxy groups on ring **A** and electron withdrawing groups on ring **B** increases antimalarial potency while the positional interchange of these groups causes a decrease. In particular, 2,4,5-trimethoxy substitution pattern at ring **A** provided potent analogues which were easily derived from abundantly available natural β-asarone rich *Acorus calamus* oil. Cytotoxic evaluation indicated that the most active compounds **27** (IC_{50}: 1.8 μM) and **26** (IC_{50}: 2 μM) were also relatively non-toxic. Furthermore, compound **12** showed excellent resistance index of 1.1 against chloroquine resistant Dd2 strain of *P. falciparum*.

1. Introduction

Malaria is currently one of the most endemic diseases that affects more than 500 million people per year, with an associated 2.5 million deaths [1–3]. The continual tendency of the malarial parasite to develop resistance [4,5] to newly developed drugs causes a great concern and makes it urgent to find suitable new therapeutics. Although, artemisinin, a natural antimalarial product [6,7] isolated from the Chinese plant *Artemesia annua* has been a miraculous entry for the treatment of multidrug-resistant *Plasmodium falciparum* malaria, however, its poor solubility, short plasma half life, neurotoxicity and high cost are major problems [8,9]. Consequently, various derivatives of artemisinin [10–12] like artemether [10], arteether [10] and sodium artesunate [11,12] having better physiochemical properties have been prepared. However, low percentage of artemisinin in plants and complexities of its total synthesis still remained a challenge [13–15]. Furthermore, the emerging clinical resistance against artemisinins noticed in South Asia [16–20] has motivated the scientists to look for alternate synthetic and cost effective medicines for the treatment of malaria.

In this context, chalcones (1,3-diaryl-2-propen-1-ones), being structurally simple class of compounds have attracted much recent attention due to wide ranging biological profiles [21] such as antioxidant, antileishmanial, antitumor, antibacterial besides antimalarial activity. After the first report on the antimalarial activity of licochalcone A (IC_{50}: 4.1 μM), a natural product isolated from Chinese liquorice root [22], extensive efforts have been made for its modification and antimalarial structure–activity correlation of other novel chalcones [23–25]. On the other hand, various heterocyclic analogues of chalcones [26–30] possessing sulfonamide [28], phenyl urenyl [29], ferrocenyl moieties [30] besides other nitrogen and sulfur containing compounds [31–34] have also been reported to possess antimalarial activity. However, a majority of above heterocyclic lead candidates involve delicate reaction conditions while there is a vast scope for exploring the antimalarial potency of core skeleton of chalcones having simple substituents. In addition, it would be doubly beneficial if such simple chalcone scaffolds could be derived from easily available natural precursors [35].

In the course of our efforts for exploring the basic chalcone moiety for antimalarial activity, we report that the presence of 2,4,5-trimethoxy substitution on ring **A** of chalcone significantly favors the antimalarial activity in contrast to previous reports [23,25], wherein, ring **A** with electron deficient groups have

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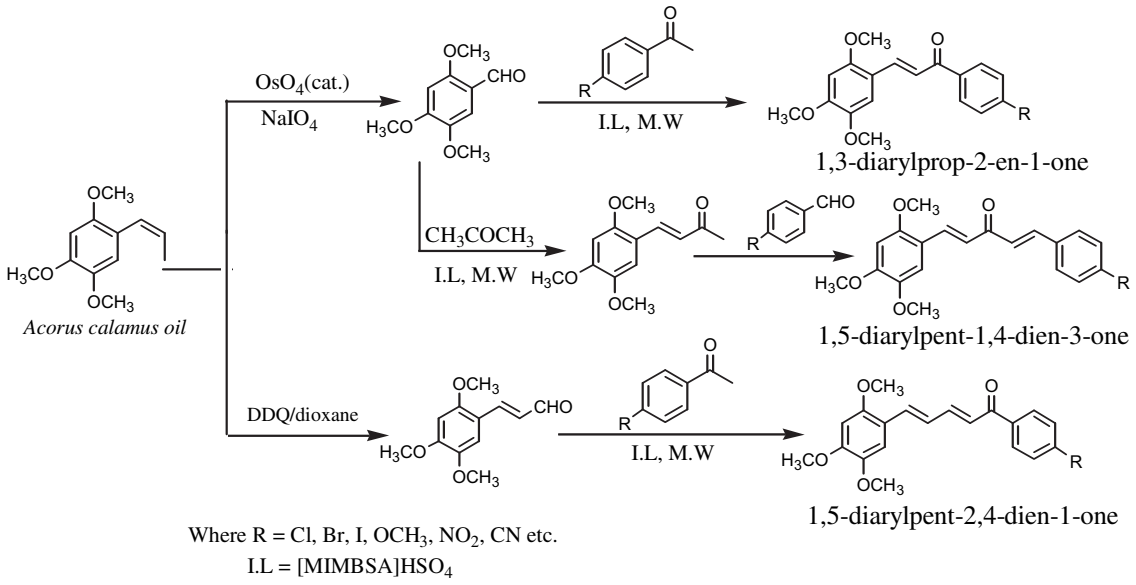


Scheme 1. General procedure for the synthesis of 2,4,5-trimethoxy-substituted chalcone derivatives from β-asarone rich *Acorus calamus* oil.

Table 1
Screening of chalcones for antimalarial activity against *P. falciparum* 3D7 strain.

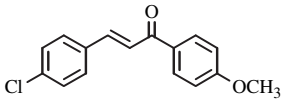
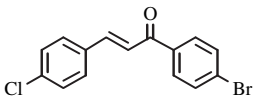
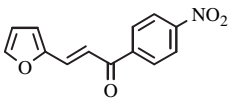
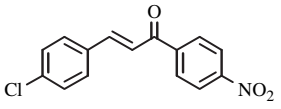
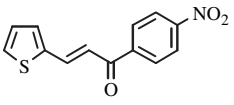
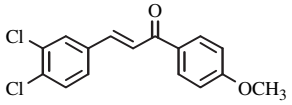
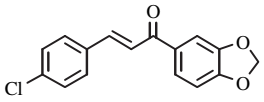
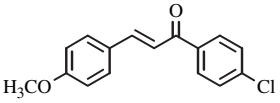
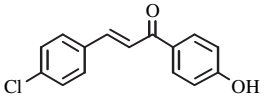
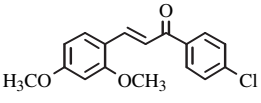
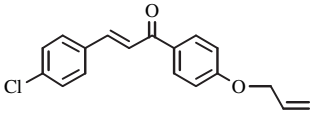
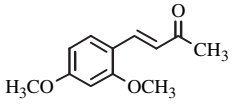
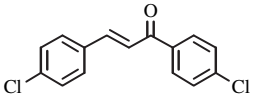
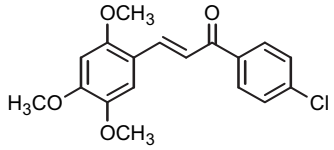
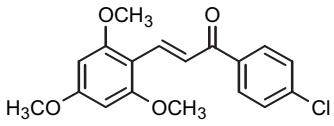
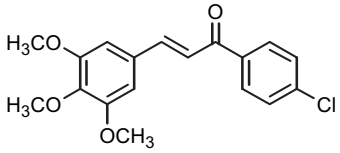
S. No.	Structure	IC_{50} (μM)	S. No.	Structure	IC_{50} (μM)
1		88.0	8		50
2		>200	9		78
3		169.9	10		>200
4		97.9	11		28.8
5		30	12		6
6		75	13		48.5
7		65			

Table 2
Effect of position of trimethoxy substitution on antimalarial activity against *P. falciparum* 3D7 strain.

S. No.	Structure	IC_{50} (μM)
14		4
15		8
16		4.6

displayed potent activity. Moreover, the lead candidates are easily derivable from inexpensive and naturally abundant β-asarone rich *A. calamus* oil [36–39].

2. Results and discussion

We have been working on development of synthetic methodologies [40–43] for bioactive compounds using microwave, ionic liquids besides utilizing innocuous natural precursors [36–39], which are some of the tools of green chemistry [44]. In the present study, abundantly available β-asarone (a natural phenylpropene, present up to 96% yield in *A. calamus* oil) was oxidized with $NaIO_4$/OsO_4 to provide 2,4,5-asaronaldehyde [36] under microwave which upon condensation with ketones (acetophenone or acetone) in ionic liquid [MIMBSA]HSO_4 [45] provided a cost effective route to chalcone (1,3-diarylprop-2-en-1-one) or conjugated chalcone (1,5-diarylpent-1,4-dien-3-one). Similarly, β-asarone upon oxidation with DDQ (2,3-Dichloro-5,6-dicyanobenzoquinone) provided 2,4,5-trimethoxycinnamaldehyde [46] which was condensed with 4-bromoacetophenone to obtain conjugated chalcone (1,5-diarylpent-2,4-dien-1-one) (Scheme 1). All the synthesized compounds were well characterized by NMR (^1H and ^{13}C) spectra. For the sake of discussion, the aromatic rings in the final chalcones are designated as **A** (from aldehydes) and **B** (from acetophenone) respectively.

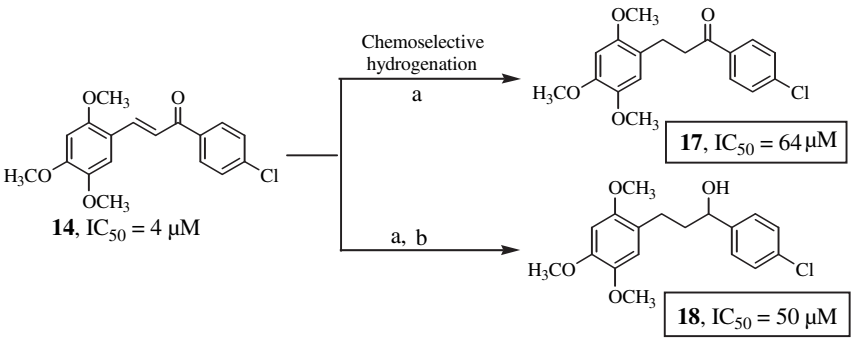
In view of the well known antimalarial potency of various chlorinated compounds [47–49] like chloroquine, pyronaridine, acridinedione etc., we initially desired to explore some chlorinated chalcones using 3-(4-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**1**, Table 1) as a representative compound. Our screening of the comparative efficacies of the molecules described in this work was based on the validated, micro titer plate based high through put format SYBR green fluorescence technique [50]. This method is based on the fact that in the mature human red blood cells (which lack DNA), the quantitative estimation of SYBR green fluorescence acts as an index of the growth of the malaria parasite allowing precise estimation of IC_{50} value for each compound. However, screening against *P. falciparum* 3D7 strain revealed that compound **1** with an IC_{50}: 88.0 μM lack significant potency. Thereafter, molecules with different substituents on each of the two rings were synthesized and evaluated for antimalarial activity.

It is clear (Table 1) that introduction of 3,4-methylenedioxy (**4**), hydroxy (**5**), allyl (**6**), chloro (**7**), bromo (**8**) or nitro (**9**) group at ring **B** did not lead to significant activity. On the other side, replacement of 4-chloro on ring **A** with 3,4-dichloro (**10**) drastically reduced the antimalarial activity (IC_{50}: >200 μM). Also, chalcones with furan (**2**) and thiophene ring (**3**) proved futile for enhancing the antimalarial activity. Till this stage a majority of above discussed chalcones were having electron deficient substituent (Cl) in their aryl ring **A**.

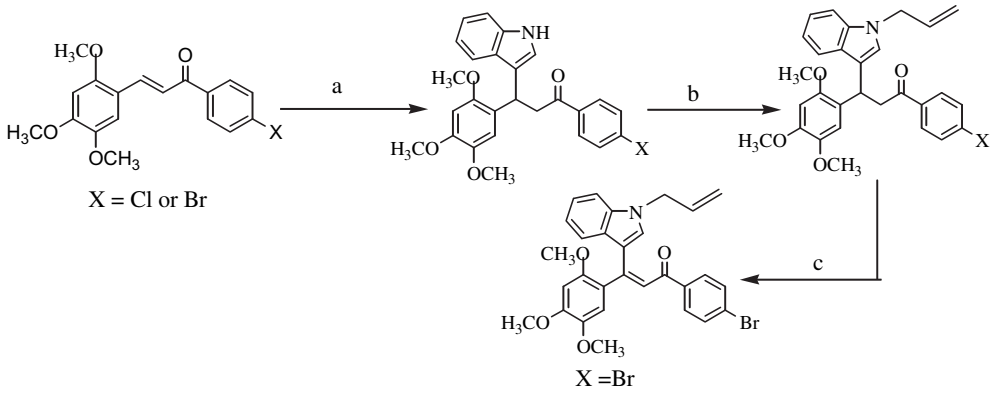
To our surprise, a reversal of substituents between rings **A** and **B** of **1** (IC_{50}: 88.0 μM) led to a three fold increase in activity (IC_{50}: 28.8 μM) of resulting **11**. Thereafter, we thought to increase the electron density on ring **A** keeping chloro substitution constant on ring **B**. To our pleasure, compounds with 2,4-dimethoxy (**12**, Table 1) and 2,4,5-trimethoxy groups (**14**, Table 2) on ring **A** exhibited progressively better antimalarial potential with IC_{50} values of 6 μM and 4 μM respectively. These observations clearly indicate that increase in the electron density on ring **A** significantly enhanced the antimalarial activity which is evidently in contrast to earlier reports linking potent antimalarial activity with electron deficient ring **A** of chalcones [23,25]. In addition, **13**, with **B** ring absent, showed drastic reduction in activity (**13**, IC_{50}: 48.5 μM) as compared to **12**, thus underlining the importance of both aryl rings (**A** and **B**) of chalcone for activity.

2.1. Effect of changing the position of trimethoxy substitution on ring A

After recognizing the association of electron releasing methoxy functional groups at ring **A** (**14**) for good activity, we next ventured to evaluate the positional importance of the three methoxy groups



Scheme 2. General method for the reduction of enone moiety of chalcone **14** to dihydro derivatives. Reagents and conditions: (a) $PdCl_2$/HCOOH/MeOH/H_2O, microwave; (b) $NaBH_4$, MeOH, r.t.



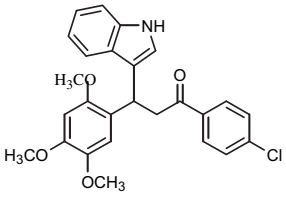
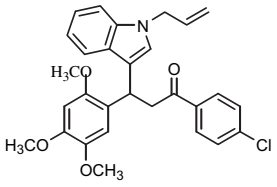
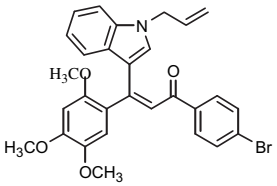
Scheme 3. General procedure for the addition of indole moiety on chalcone. Reagents and conditions: (a) indole, PTSA (cat.), acetonitrile, r.t.; (b) allyl bromide, KOH, TBAB, r.t.; (c) DDQ, dioxane, r.t. overnight.

on ring **A** for further optimization of antimalarial activity. Consequently, some more trimethoxy chalcone derivatives were (**15**–**16**, Table 2) screened with SYBR Green assay. The SAR analysis showed that 2,4,5-trimethoxy-substituted chalcone (**14**, IC_{50}: 4 μM) exhibited significantly to marginally better antimalarial potency as compared to its 2,4,6 (**15**, IC_{50}: 8 μM) and 3,4,5 (**16**, IC_{50}: 4.6 μM) counterparts. The fact that activity is markedly affected by changing the position of trimethoxy substituents at the phenyl ring suggests that 2,4,5-trimethoxy substitution makes a special contribution which may be due to its orientation and binding ability with the malarial parasite proteins.

2.2. Reduction of α,β-unsaturated ketone unit abrogates antimalarial activity

In order to evaluate the role of α,β-unsaturated ketone moiety, the reduction of double bond of active compound **14** was carried out. However, resulting compound **17** (IC_{50}: 64 μM) showed significantly reduced activity. Similarly, reduction of both double bond and keto group (**18**) also proved futile (Scheme 2).

Table 3
Effect of incorporation of heterocyclic moiety on antimalarial activity of chalcone against *P. falciparum* 3D7 strain.

S. No.	Structure	IC_{50} (μM)
19		8.6
20		8.4
21		17.5

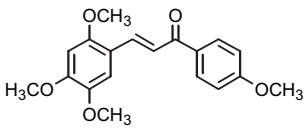
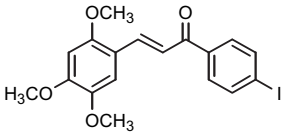
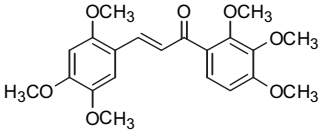
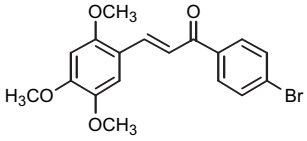
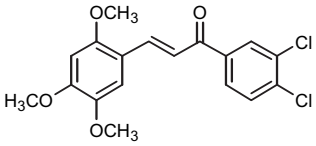
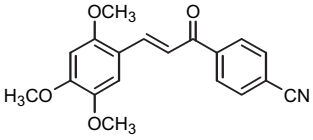
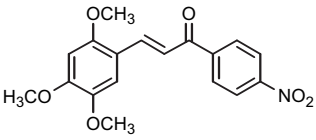
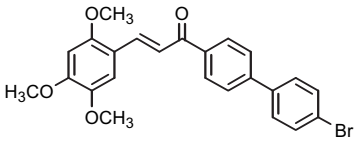
2.3. Effect of incorporation of heterocyclic moiety

Since indole derivatives are well known to be antimalarials [31], we became interested to see the effect of Michael addition of indole moiety on the lead structure (**14**). Thus addition of indole on **14** (Scheme 3) yielded a saturated compound **19** (IC_{50}: 8.6 μM, Table 3) which, however, was found to be less active than the parent **14**. Further, *N*-allylation of **19** slightly improved the activity (**20**, IC_{50}: 8.4 μM). Hence, it was realized that introduction of a double bond on **20** might increase the activity. However, oxidation of **20** (Cl substituted **B** ring) with DDQ to regenerate the double bond was found problematic due to formation of numerous side products. Interestingly, the corresponding bromo derivative **21** (Table 3) could be easily prepared but its antimalarial activity didn't improve as compared to **14**.

2.4. Effect of substituents on ring *B*

After realizing the crucial role of 2,4,5-position and double bond of enone moiety of chalcone for potent activity, we next ventured to evaluate the effect of various substituents on ring **B** keeping 2,4,5-trimethoxy group constant on ring **A** (**22**–**29**, Table 4). The structure–activity analysis demonstrated that ring **B** having 4-methoxy (**22**, IC_{50}: 11.5 μM) or 2,3,4-trimethoxy (**23**, IC_{50}: 7.8 μM) substitution provided compounds with lower activity as compared to **14** which indicated the importance of electron deficient ring **B**. Consequently, various other chalcones with electron withdrawing groups like NO_2 (**25**), I (**26**), Br (**27**) or CN (**28**) on ring **B** were prepared and screened for activity. The compounds **25** and **28** possessing NO_2 and CN substituents respectively were clearly inactive (Table 4). However, a two fold increase in activity was observed with I or Br substituents as both **26** and **27** showed good antimalarial potential with IC_{50} value of 2 μM and 1.8 μM respectively as compared to 4 μM in case of Cl (**14**, Table 2). Surprisingly, the presence of multiple electron withdrawing substituents (3,4-dichloro substituted **B** ring, **24**) did not lead to significant activity. On the other hand, replacement of ring **B** of **27** with 4-bromobiphenyl moiety produced lesser active compound **29** (IC_{50}: 47 μM), which emphasizes that size characteristics of ring **B** also play an important role in antimalarial activity.

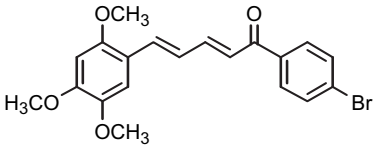
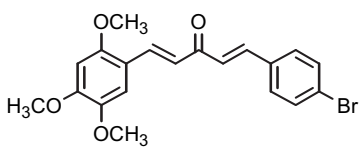
Table 4
Effect of substitution on ring **B** of chalcones for antimalarial activity against *P. falciparum* 3D7 strain.

S. No.	Structure	IC_{50} (μM)	S. No.	Structure	IC_{50} (μM)
22		11.5	26		2
23		7.8	27		1.8
24		>20	28		>100
25		>200	29		47

2.5. Effect of extended conjugation

After studying a series of chalcones (**1**–**29**), we planned to extend the conjugation in the most active chalcone **27**. Consequently, condensation of 2,4,5-trimethoxycinnamaldehyde [46] with 4-bromoacetophenone furnished the multiconjugated 1,5-diarylpent-2,4-dien-1-one (**30**, Table 5), which upon antimalarial evaluation showed reduced activity than **27**. Thereafter, another multiconjugated chalcone derivative **31**, an analogue of bioactive curcumin [51,52], was synthesized by condensation of asaronaldehyde with acetone followed by further condensation with 4-bromobenzaldehyde. However, the above compound also depicted reduced activity (IC_{50}: 18 μM), thereby, suggesting the critical role of basic chalcone nucleus for potent antimalarial activity.

Table 5
Effect of conjugation on antimalarial activity against *P. falciparum* 3D7 strain.

S. No.	Structure	IC_{50} (μM)
30		12.2
31		18

2.6. Evaluation of cytotoxicity and resistant index for identified lead candidates

The identified lead chalcones were also tested against chloroquine resistant Dd2 strain of *P. falciparum* (Table 6). Against a Resistance Index (IC_{50} Dd_2/IC_{50} 3D7) of ~4 for Chloroquine, these indices for the potent chalcones (**12**, **14**, **16**, **26** and **27**) were found to be 1.1, 1.7, 2.5, 3.7 and 2.8 respectively (Supplementary Fig. 1). Finally, the active compounds showing IC_{50} up to 4 μM (**12**, **14**, **16**, **26** and **27**) were analyzed for their cytotoxic behavior against two mammalian cell lines viz. HeLa and fibroblast L929 (Supplementary Fig. 1). Selectivity indices (IC_{50} HeLa cell line/IC_{50} *Pf* 3D7) (Table 6) with values ranging between 3 and >50 indicated that the most active compounds (**26** and **27**) were also relatively non-toxic.

In order to find the antimalarial target of chalcones, we resorted to examination of the possible inhibitory effect of the potent chalcones in a Hemoglobin degradation assay. In this assay, freshly prepared parasite extract was used to digest the human hemoglobin (Sigma) and inhibition of degradation in presence of

Table 6
Resistance and selectivity indices for potent chalcones.

Compound no	SYBR green assay IC_{50} (μM)		Resistance index IC_{50} Dd2/IC_{50} 3D7	Selectivity index	
	Pf 3D7	*Pf* Dd2		TC_{50} HeLa/ IC_{50} 3D7	TC_{50} L929/ IC_{50} 3D7
12	6	6.8	1.1	>16.6	14.6
14	4	6.9	1.7	9	8.3
16	4.6	11.5	2.5	3	2.8
26	2	7.5	3.7	50	>50
27	1.8	5	2.8	31.7	55.6
Chloroquine	40 nM	170 nM	4.2	>200	>200

chalcones (100 μM) was monitored using SDS PAGE. However none of the tested chalcones showed inhibition in this assay suggesting that these molecules do not exert their antimalarial action via inhibition of the proteolytic pathways of hemoglobin degradation.

3. Conclusion

We have identified a basic chalcone scaffold (having 2,4,5-trimethoxy substitution on aryl ring **A**) which is crucial for antimalarial activity. This is the first SAR study which shows that chalcones which are methoxylated in their aryl ring **A** and electron deficient at ring **B** are better antimalarials than those in which these groups are interchanged. Moreover, synthesis of lead candidates from abundantly available natural precursor i.e. β-asarone (2,4,5-trimethoxyphenyl propene) rich *A. calamus* oil offers a good potential to develop cost effective antimalarial drugs. The strong dependence of antimalarial activity on specific substitution of rings **A** and **B** as well as possibility of further modification on above identified unit would be helpful for design of new therapeutic antimalarials. Further work regarding modifications on identified bioactive chalcone moiety (**27**) for enhancing the antimalarial potency is currently under progress.

4. Experimental section

4.1. General procedure for the synthesis of substituted chalcones (**1**–**5** and **7**–**13**, Tables 1 and **15**–**16**, Table 2)

To a solution of substituted benzaldehyde (3 mmol) and appropriate acetophenone (3 mmol) in methanol (15 ml), 10% aqueous NaOH (6 mmol) was added. The reaction mixture was stirred till completion of starting material. The obtained precipitates were washed with dilute HCl, excess of water, methanol, dried in air and finally recrystallized with methanol to obtain pure chalcones whose structure were confirmed by NMR spectra as discussed below:

4.1.1. 3-(4-Chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**1**) [45]

Creamy solid (88%), m. p. 128–131 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.97 (2H, d, J = 9.2 Hz), 7.69 (1H, d, J = 16.1 Hz), 7.50 (2H, d, J = 8.0 Hz), 7.46 (1H, d, J = 17.7 Hz), 7.31 (2H, d, J = 8.1 Hz), 6.92 (2H, d, J = 8.7 Hz), 3.81 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.4, 163.5, 142.4, 136.1, 133.6, 130.8, 129.5, 129.2, 122.3, 113.9 and 55.5.

4.1.2. 3-(Furan-2-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**2**)

Yellow solid (76%), m. p. 139–142 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.27 (2H, d, J = 8.4 Hz), 8.09 (2H, d, J = 8.8 Hz), 7.58–7.49 (2H, m), 7.35 (1H, d, J = 15.3 Hz), 6.73 (1H, d, J = 4.0 Hz), 6.48 (1H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.1, 151.2, 150.0, 145.6, 142.9, 132.1, 129.3, 123.8, 118.3, 117.7 and 113.0. HRMS-ESI: m/z $[M + H]^+$ for $C_{13}H_9NO_4$, calculated 244.0604; observed 244.0615.

4.1.3. 1-(4-Nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (**3**)

Yellow solid (73%), m. p. 171–173 °C, ^{1}H NMR (300 MHz, DMSO); δ 8.34–8.30 (4H, m), 7.99 (1H, d, J = 15.0 Hz), 7.83 (1H, s), 7.73 (1H, s), 7.57 (1H, d, J = 15.0 Hz), 7.21 (1H, s); ^{13}C NMR (75.4 MHz, DMSO); δ 188.3, 150.2, 142.8, 139.9, 138.7, 134.2, 131.8, 130.2, 129.3, 124.3 and 120.5. HRMS-ESI: m/z $[M + H]^+$ for $C_{13}H_9O_3NS$, calculated 260.0376; observed 260.0372.

4.1.4. 1-(3,4-Dioxymethylene)-3-(4-chlorophenyl)prop-2-en-1-one (**4**)

White solid (85%), m. p. 164–167 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.68 (1H, d, J = 15.7 Hz), 7.57 (1H, d, J = 8.0 Hz), 7.49–7.44 (3H, m), 7.40 (1H, d, J = 15.7 Hz), 7.31 (2H, d, J = 8.4 Hz), 6.82 (1H, d, J = 8.0 Hz), 5.98 (2H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 187.9, 151.8, 148.3, 142.7, 136.2, 133.5, 132.8, 129.5, 129.2, 124.7, 122.1, 108.4, 107.9 and 101.9. HRMS-ESI: m/z $[M + H]^+$ for $C_{16}H_{11}ClO_3$, calculated 287.0470; observed 287.0469.

4.1.5. 3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**5**) [53]

White solid (54%), m. p. 178–179 °C, ^{1}H NMR (300 MHz, CD_3COCD_3); δ 9.31(1H, s), 8.10 (2H, s), 7.85–7.73 (4H, m), 7.48 (2H, s), 6.98 (2H, s); ^{13}C NMR (75.4 MHz, CD_3COCD_3); δ 188.4, 163.4, 142.7, 136.7, 135.7, 132.5, 131.5, 130.4, 124.2 and 116.7.

4.1.6. 1,3-Bis(4-chlorophenyl)prop-2-en-1-one (**7**) [54]

Off white solid (91%), m. p. 108–111 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.98 (2H, d, J = 7.6 Hz), 7.79 (1H, d, J = 16.6 Hz), 7.59 (2H, d, J = 8.4 Hz), 7.50–7.39 (5H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.1, 144.1, 139.7, 137.0, 136.7, 133.6, 130.2, 130.0, 129.7, 129.4 and 122.3.

4.1.7. 1-(4-Bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (**8**)

White solid (89%) m. p. 164–168 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.88–7.74 (3H, m), 7.64–7.58 (4H, m), 7.41–6.99 (3H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.7, 143.5, 136.4, 132.9, 131.7, 129.7, 129.3, 129.0, 128.5, 127.7 and 121.6.

4.1.8. 3-(4-Chlorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**9**) [53]

Yellow solid (73%); m. p. 158–161 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.37 (2H, d, J = 7.2 Hz), 8.16 (2H, d, J = 7.2 Hz), 7.82 (1H, d, J = 15.6), 7.61 (2H, d, J = 8.2 Hz), 7.49–7.41 (3H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.4, 149.8, 144.9, 142.5, 136.9, 132.5, 130.7, 129.1, 128.8, 123.6 and 121.4. HRMS-ESI: m/z $[M + H]^+$ for $C_{15}H_{10}ClNO_3$, calculated 288.0422; observed 288.0425.

4.1.9. 3-(3,4-Dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**10**)

Pale yellow solid (89%), m. p. 129–131 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.05 (2H, d, J = 9.3 Hz), 7.70–7.64 (2H, m), 7.54–7.42 (3H, m), 7.00 (2H, d, J = 9.1 Hz), 3.90 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.3, 164.0, 141.3, 135.6, 134.5, 133.6, 131.2, 131.1, 130.0, 127.8, 123.8, 114.3 and 55.9. HRMS-ESI: m/z $[M + H]^+$ for $C_{16}H_{12}Cl_2O_2$, calculated 307.0287; observed 307.0280.

4.1.10. 1-(4-Chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**11**) [24]

Creamy solid (87%), m. p. 120–124 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.02–7.97 (2H, m), 7.86 (1H, d, J = 16.6 Hz), 7.66–7.62 (2H, m), 7.52–7.48 (2H, m), 7.44 (1H, d, J = 16.6 Hz), 7.00–6.96 (2H, m), 3.89 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.4, 162.2, 145.5, 139.3, 137.2, 130.7, 130.2, 129.2, 127.8, 119.5, 114.8 and 55.7.

4.1.11. 1-(4-Chlorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**12**)

Yellow solid (92%), m. p. 124–126 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.09 (1H, d, J = 15.7 Hz), 7.97–7.93 (2H, m), 7.58 (1H, d, J = 8.4 Hz), 7.53–7.44 (3H, m), 6.55 (1H, d, J = 7.1 Hz), 6.48 (1H, s), 3.90 (3H, s), 3.86 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.1, 163.6, 160.9, 141.4, 138.9, 137.5, 131.4, 130.2, 129.1, 120.2, 117.3, 105.9, 98.8, 55.9 and 55.8. HRMS-ESI: m/z $[M + H]^+$ for $C_{17}H_{15}O_3Cl$ calculated 303.0783; observed 303.0783.

4.1.12. 4-(2,4-Dimethoxyphenyl)but-3-en-2-one (**13**)

Pale yellow solid (81%), m. p. 62–64 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.73 (1H, d, J = 16.1 Hz), 7.39 (1H, d, J = 8.4 Hz), 6.59 (1H, d, J = 17.3 Hz), 6.42 (1H, d, J = 8.5 Hz), 6.35 (1H, s), 3.76 (3H, s), 3.73 (3H, s), 2.2 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 199.0, 163.0, 159.8,

138.7, 129.8, 125.4, 116.3, 105.5, 98.3, 55.5 and 27.0. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{12}H_{14}O_3$, calculated 207.1016; observed 207.1011.

*4.1.13. 1-(4-Chlorophenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**15**)*

Yellow solid (88%), m. p. 155–157 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.29 (1H, d, *J* = 15.5 Hz), 7.96 (2H, d, *J* = 8.2 Hz), 7.85 (1H, d, *J* = 15.3 Hz), 7.45 (2H, d, *J* = 8.6 Hz), 6.13 (2H, s), 3.91 (6H, s), 3.86 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 191.1, 163.7, 162.2, 138.5, 138.0, 136.9, 130.2, 128.6, 121.6, 106.8, 90.9, 56.2 and 55.7. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{17}O_4Cl$, calculated 333.0888; observed 333.0886.

*4.1.14. 1-(4-Chlorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**16**)*

Pale yellow solid (91%), m. p. 108–110 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.05 (2H, d, *J* = 9.1 Hz), 7.82 (1H, d, *J* = 15.3 Hz), 7.55 (2H, d, *J* = 8.0 Hz), 7.47 (1H, d, *J* = 15.7 Hz), 6.94 (2H, s), 4.00 (9H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.6, 154.2, 146.1, 141.4, 139.7, 137.2, 130.8, 130.6, 129.5, 121.5, 106.6, 61.6 and 56.9. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{17}O_4Cl$, calculated 333.0888; observed 333.0883.

Alternatively, above chalcone derivatives were also prepared by using ionic liquid [MIMBSA]HSO_4 under focused microwave irradiations [55]. Thus, it was observed that condensation of electron-rich benzaldehydes with various acetophenones in ionic liquid occurred very efficiently to provide the chalcone derivatives in good yield. Furthermore, addition of methanol (as co-solvent) in ionic liquid decreased the reaction time beside ease in purification of chalcone (after completion of reaction almost all the chalcones got precipitated in methanol).

Therefore, the most potent compounds, i.e. chalcones with 2,4,5-trimethoxy substitution were easily accessed in good yield from abundantly available β-asarone using ionic liquid [MIMBSA]HSO_4 under focused microwave irradiation (2–10 min) as mentioned below:

*4.2. General procedure for green synthesis of 2,4,5-trimethoxy-substituted chalcone derivatives from β-asarone in ionic liquid under microwave irradiation (**14**, Tables 2 and **22**–**29**, Table 4)*

A mixture of β-asarone (0.31 g, 1.5 mmol), $NaIO_4$ (1.17 g, 5.5 mmol), OsO_4 (0.0004 g, 0.0015 mmol), and benzyltriethylammonium chloride (0.01 g, 0.04 mmol) were dissolved in H_2O-THF (3 ml, 4:1) and irradiated under focused microwave (150 W, 100 °C). After completion of starting material, the reaction mixture was extracted with ethyl acetate (3 × 15 ml) and vacuum evaporated to give a crude mixture, which on column chromatography with silica gel (60–120 mesh size) using hexane–ethyl acetate (9:1), provided 2,4,5-trimethoxybenzaldehyde in 83% yield. Subsequently, the above benzaldehyde (0.24 g, 1.22 mmol) was taken in methanol (1 ml) and subjected to Claisen–Schmidt condensation with appropriate acetophenone (1.22 mmol) using ionic liquid [MIMBSA]HSO_4 (1 g) under focused microwave irradiation (100 W, 75 °C) for 2–10 min. The obtained precipitates were washed with excess of water and recrystallized from methanol to obtain 2,4,5-trimethoxy-substituted chalcone derivatives whose NMR are given below:

*4.2.1. 1-(4-Chlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**14**) [56]*

Yellow solid (87%), m. p. 141–142 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.12 (1H, d, *J* = 15.9 Hz), 7.97 (2H, d, *J* = 8.6 Hz), 7.49 (2H, d, *J* = 8.4 Hz), 7.45 (1H, d, *J* = 16.2 Hz), 7.13 (1H, s), 6.54 (1H, s, (3.96 (3H, s), 3.92 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.18, 155.2, 155.1, 143.7, 141.0, 139.0, 137.5, 130.2, 129.1, 120.1, 115.7, 112.0, 97.2, 57.0, 56.7 and 56.4.

*4.2.2. 1-(4-Methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**22**)*

Yellow solid (84%), m. p. 121–123 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.09–7.99 (3H, m), 7.49 (1H, d, *J* = 16.6 Hz), 7.11 (1H, s), 6.96 (2H, d, *J* = 8.0 Hz), 6.50 (1H, s), 3.91 (3H, s), 3.87 (6H, s), 3.84 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.6, 163.4, 154.9, 152.7, 143.6, 139.6, 132.0, 130.8, 120.4, 116.1, 114.0, 111.9, 97.4, 57.3, 56.9 and 56.7. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{19}H_{20}O_5$, calculated, 329.1384; observed 329.1380.

*4.2.3. 1-(2,3,4-Trimethoxyphenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**23**)*

Yellow solid (86%), m. p. 90–94 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.01–7.93 (1H, m), 7.46–7.27 (2H, m), 7.14–7.11 (1H, m), 6.78–6.72 (1H, m), 6.53–6.51 (1H, m), 3.94–3.83 (18H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 191.5, 156.5, 154.4, 153.3, 152.4, 143.3, 142.1, 138.6, 127.3, 125.4, 124.6, 115.7, 111.2, 107.2, 97.0, 62.0, 60.9, 56.5, 56.3 and 56.0. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{21}H_{24}O_7$, calculated 389.1595; observed 389.1587.

*4.2.4. 1-(3,4-Dichlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**24**)*

Yellow solid (87%), m. p. 131–134 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.13–8.07 (2H, m), 7.83 (1H, d, *J* = 8.1 Hz), 7.57 (1H, d, *J* = 6.8 Hz), 7.38 (1H, d, *J* = 15.6 Hz), 7.11 (1H, s), 6.52 (1H, s), 3.96 (3H, s), 3.91 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.9, 155.4, 153.4, 143.7, 141.7, 138.9, 137.0, 133.4, 130.9, 130.8, 127.9, 119.5, 115.5, 112.1, 97.2, 57.0, 56.7 and 56.5. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{16}O_4Cl_2$, calculated 367.0498; observed 367.0490.

*4.2.5. 1-(4-Nitrophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**25**)*

Brick red solid (82%), m. p. 187–189 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.35–8.31 (2H, m), 8.13–8.09 (3H, m), 7.44 (1H, d, *J* = 15.7 Hz), 7.12 (1H, s), 6.54 (1H, s), 3.98 (3H, s), 3.96 (3H, s), 3.92 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.4, 154.9, 153.0, 149.5, 143.6, 143.1, 141.9, 129.1, 123.4, 119.2, 114.6, 111.3, 96.4, 56.3, 56.0 and 55.8. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{17}O_6N$, calculated 344.1129; observed 344.1149.

*4.2.6. 1-(4-Iodophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**26**)*

Yellow solid (92%), m. p. 183–185 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.12 (1H, d, *J* = 15.7 Hz), 7.87 (2H, d, *J* = 8.7 Hz), 7.74 (2H, d, *J* = 8.7 Hz), 7.42 (1H, d, *J* = 16.1 Hz), 7.12 (1H, s), 6.53 (1H, s), 3.96 (3H, s), 3.91 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.7, 155.2, 153.2, 143.7, 141.1, 138.5, 138.1, 130.3, 120.1, 115.7, 112.0, 100.2, 97.2, 57.0, 56.7 and 56.4. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{17}O_4I$, calculated 425.2439; observed 425.2440.

*4.2.7. 1-(4-Bromophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**27**)*

Fluorescent yellow solid (94%), m. p. 157–158 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.01 (1H, d, *J* = 15.9 Hz), 7.77 (2H, d, *J* = 9.15 Hz), 7.52 (2H, d, *J* = 9.7 Hz), 7.32 (1H, d, *J* = 15.5 Hz), 7.00 (1H, s), 6.41 (1H, s), 3.84 (3H, s), 3.80 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.2, 155.2, 153.2, 143.7, 141.0, 137.9, 132.1, 130.3, 127.6, 120.0, 115.7, 112.0, 97.2, 57.0, 56.7 and 56.4. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{17}O_4Br$, calculated 377.0383; observed 377.0379.

*4.2.8. 1-(4-Cynophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**28**)*

Bright yellow solid (84%), m. p. 197–199 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.13–8.04 (3H, m), 7.79 (2H, d, *J* = 8.4 Hz), 7.42 (1H, d, *J* = 15.1 Hz), 7.11(1H, s), 6.53 (1H, s), 3.92 (3H, s), 3.90 (3H, s), 3.87

(3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.2, 155.7, 153.8, 143.9, 142.8, 142.5, 132.9, 129.4, 119.9, 118.7, 115.9, 115.5, 112.2, 97.3, 57.1, 56.8 and 56.6. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{19}H_{17}O_4N$, calculated 324.1230; observed 324.1261.

*4.2.9. 1-[(4-(4-Bromophenyl)phenyl)]-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (**29**)*

Bright yellow solid (96%), m. p. 137–139 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.16–8.07 (3H, m), 7.67–7.48 (7H, m), 7.15 (1H, s), 6.53 (1H, s), 3.95 (3H, s), 3.91 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.6, 155.1, 153.1, 144.0, 143.7, 140.6, 139.3, 138.2, 132.4, 129.5, 129.1, 127.3, 122.8, 120.4, 115.9, 112.0, 97.3, 57.0, 56.7 and 56.4. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{24}H_{21}O_4Br$, calculated 453.0696; observed 453.0766.

*4.3. General procedure for the synthesis of 3-(4-chlorophenyl)-1-[4-(prop-2-en-1-yloxy)phenyl] prop-2-en-1-one (**6**)*

To the solution of chalcone **5** (0.5 g, 1.9 mmol) in dry acetone (20 ml), allyl bromide (0.46 g, 3.8 mmol) and anhydrous K_2CO_3 (0.52 g, 3.8 mmol) were added. The reaction mixture was refluxed for 6 h. After consumption of starting chalcone (monitored on TLC), reaction mixture was filtered to remove K_2CO_3. The filtrate was vacuum evaporated and washed with hexane to remove excess of allyl bromide. The obtained crude solid was recrystallized with methanol to obtain **6** as a white solid in 69% yield, m.p. 119–120 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.05 (2H, d, J = 8.0 Hz), 7.77 (1H, d, J = 16.2 Hz), 7.58–7.49 (3H, m), 7.40 (2H, d, J = 8.9 Hz), 7.02 (2H, d, J = 8.04 Hz), 6.14–6.01 (1H, m), 5.48–5.31 (2H, m), 4.64 (2H, d, J = 5.1 Hz); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 188.7, 162.9, 142.7, 136.5, 134.0, 132.9, 131.4, 131.2, 129.8, 129.5, 122.7, 118.5, 115.0 and 69.3. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{15}ClO_2$, calculated 299.0833; observed 299.0833.

*4.4. General procedure for synthesis of 1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)propan-1-one (17) and 1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)-propan-1-ol (**18**)*

Compound **17** was prepared by the chemoselective hydrogenation of **14** using silica-supported $PdCl_2$ as catalyst and a combination of MeOH/HCOOH/H_2O [57] as a source of hydrogen. Product **17** was isolated in 82% yield as a viscous liquid. Further, reduction of **17** with $NaBH_4$ [58] afforded the corresponding alcohol (**18**) in 85% yield. The NMR data (^{1}H & ^{13}C) of **17** and **18** are given below:

*4.4.1. 1-(4-Chlorophenyl)-3-(2,4,5-trimethoxyphenyl)propan-1-one (**17**)*

Viscous liquid, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.92 (2H, d, J = 8.5 Hz), 7.43 (2H, d, J = 8.5 Hz), 6.76 (1H, s), 6.53 (1H, s), 3.89 (3H, s), 3.83 (3H, s), 3.81 (3H, s), 3.23 (2H, t, J = 7.2 Hz), 3.00 (2H, t, J = 7.60 H); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 199.3, 152.1, 148.7, 143.4, 139.8, 135.8, 130.1, 129.3, 121.2, 115.1, 98.3, 57.2, 56.8, 56.7, 39.9 and 25.9. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{18}H_{19}ClO_4$, calculated 335.1045; observed 335.1041.

*4.4.2. 1-(4-Chlorophenyl)-3-(2,4,5-trimethoxyphenyl)-propan-1-ol (**18**)*

Viscous liquid, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.35–7.25 (4H, m), 6.70 (1H, s), 6.53 (1H, s), 4.60–4.55 (1H, m), 3.88 (3H, s), 3.83 (3H, s), 3.82 (3H, s), 2.79–2.62 (2H, m), 2.01–1.89 (2H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 151.4, 147.9, 143.3, 132.7, 128.3, 127.2, 125.8, 121.3, 114.4, 98.1, 72.5, 56.7, 56.3, 56.2, 39.7 and 25.8.

*4.5. General procedure for synthesis of 1-(4-Chlorophenyl)-3-(1H-indol-3-yl)-3-(2,4,5-trimethoxyphenyl)propan-1-one (**19**)*

To a solution of chalcone **14** (1.05 mmol, 0.35 g) in acetonitrile (15 ml), catalytic amount of *P*-toluenesulfonic acid (20 mol%, 0.038 g) and indole (1.26 mmol, 0.147 g) were added. The reaction mixture was stirred at room temperature till completion of starting material. After evaporation of acetonitrile, the obtained crude mixture was purified through recrystallization with methanol and water to afford **19** as a white solid in 81% yield, m.p. 149–150 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 8.05 (1H, br, s), 7.97 (2H, d, J = 8.2 Hz), 7.45 (3H, d, J = 8.6 Hz), 7.36 (1H, d, J = 7.8 Hz), 7.19 (1H, d, J = 6.7 Hz), 7.14–7.12 (1H, m), 7.05–7.00 (1H, m), 6.69 (1H, s), 6.56 (1H, s), 5.37 (1H, t, J = 7.3 Hz), 3.90 (3H, s), 3.86 (3H, s), 3.69 (2H, d, J = 7.50 Hz), 3.65 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 198.2, 151.1, 148.4, 143.2, 139.3, 136.7, 135.5, 129.8, 128.9, 127.0, 124.0, 122.2, 122.0, 119.7, 119.5, 118.5, 113.5, 111.2, 97.9, 56.9, 56.5, 56.3, 44.9 and 32.5. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{26}H_{24}O_4$ ClN, calculated 450.1467; observed 450.1467.

*4.6. General procedure for synthesis of 1-(4-chlorophenyl)-3-[1-(prop-2-en-1-yl)-1H-indol-3-yl]-3-(2,4,5-trimethoxyphenyl) propan-1-one (**20**)*

To a solution of **19** (0.67 mmol, 0.3 g) in dry THF (15 ml), KOH (2 mmol, 0.112 g), allyl bromide (1.34 mmol, 0.161 g) and CTAB (20 mol%, 0.048 g) were added. The reaction mixture was stirred till completion of starting material. After evaporation of THF, the crude reaction mixture was washed with hot water and then hexane to remove the excess of allyl bromide. The obtained crude product on recrystallization with diethyl ether afforded **20** as a white solid in 79% yield, m. p. 126–127 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.94 (2H, d, J = 8.2 Hz), 7.42–7.40 (3H, m), 7.28 (1H, d, J = 7.3 Hz), 7.19–7.14 (1H, m), 7.03–7.00 (2H, m), 6.70 (1H, s), 6.54 (1H, s), 6.02–5.95 (1H, m), 5.35 (1 H, t, J = 7.1 Hz), 5.19 (1H, d, J = 10.0), 5.05 (1H, d, J = 17.3 Hz), 4.70 (2H, s), 3.88 (3H, s), 3.84 (3H, s), 3.65 (5H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 198.4, 151.3, 148.6, 143.4, 139.5, 137.1, 135.9, 134.1, 130.0, 129.1, 127.8, 126.0, 124.4, 122.1, 120.1, 119.3, 117.6, 117.2, 113.7, 109.8, 98.2, 57.1, 56.7, 56.5, 49.0, 45.2 and 32.7. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{29}H_{28}O_4ClN$, calculated 490.1780; observed 490.1780.

The above two procedures i.e. Sections 4.5 and 4.6, were also applied on chalcone **27** to provide the Michael adduct i.e. 1-(4-Bromophenyl)-3-[1-(prop-2-en-1-yl)-1H-indol-3-yl]-3-(2,4,5-trimethoxyphenyl)-propan-1-one (**27c**) in overall 64% yield. Further, dehydrogenation of **27c** into **21** was performed as mentioned below:

*4.7. General procedure for synthesis of 1-(4-bromophenyl)-3-[1-(prop-2-en-1-yl)-1H-indol-3-yl]-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**21**)*

To a solution of **27c** (0.56 mmol, 0.3 g) in dry dioxane (15 ml), DDQ (0.67 mmol, 0.153 g) was added and stirred at room temperature for overnight. The reaction mixture was extracted with ethyl acetate (3 × 15 ml). The combined organic phases were washed successively with water (2 × 10 ml), brine (2 × 5 ml), dried over anhydrous Na_2SO_4 and finally evaporated in vacuo. The obtained crude mixture after passing through a small bed of neutral alumina, recrystallized with diethyl ether to afford **21** as a reddish solid in 71% yield, m. p. 145–147 °C, ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.89 (1H, d, J = 7.6 Hz), 7.82 (2H, d, J = 8.4 Hz), 7.55 (2H, d, J = 8.2 Hz), 7.44 (1H, s), 7.40 (1H, d, J = 8.6 Hz), 7.28–7.23 (2H, m), 6.99 (1H, s), 6.73 (1H, s), 6.51 (1H, s), 6.01–5.90 (1H, m), 5.25 (1H, d, J = 10.2 Hz), 5.15 (1H, d, J = 17.2 Hz), 4.71 (2H, d, J = 4.9 Hz), 3.93 (3H, s), 3.75 (3H, s), 3.60 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 190.7, 151.3, 149.8, 146.9, 143.0, 138.8, 137.7, 132.8, 132.7, 131.5, 130.0, 126.6, 126.3, 122.9, 121.4, 121.2, 120.8, 118.8, 118.1, 114.8, 110.6, 98.0, 56.7, 56.1 and 49.3. HRMS-ESI: *m/z* $[M + H]^+$ for $C_{29}H_{26}O_4BrN$, calculated 532.1112; observed 532.1115.

4.8. General procedure for synthesis of 1-(4-bromophenyl)-5-(2,4,5-trimethoxyphenyl)-pent-2,4-dien-1-one (**30**)

To the solution of β-asarone (1.92 mmol, 0.4 g) in dry dioxane (20 ml), acetic acid (1 drop) and DDQ (3.84 mmol, 0.87 g) was added. The reaction mixture was sonicated for 30 min. Filter the reaction mixture to remove solid $DDQH_2$. The filtrate was evaporated and taken in ethyl acetate (50 ml) and washed with water (3 × 10 ml), 10% NaOH (2 × 2 ml), brine (3 × 10 ml), dried over Na_2SO_4. The filtrate was evaporated to afford a crude yellow liquid, which was purified on neutral alumina with hexane–ethyl acetate (2:3) mixture to provide the corresponding 2,4,5-trimethoxycinnamaldehyde [46] in 85% yield. Subsequently, above cinnamaldeyde (1.63 mmol, 0.363 g) was subjected to Claisen–Schmidt condensation with 4-bromoacetophenone (1.63 mmol, 0.324 g) using ionic liquid $[MIMBSA]HSO_4$ providing **30** as orange yellow solid in 70% yield, m. p. 146–149 °C, ^1H NMR (300 MHz, $CDCl_3$); δ 7.79 (2H, d, $J = 8.4$ Hz), 7.63–7.54 (3H, m), 7.32 (1H, d, $J = 15.3$ Hz), 6.99 (1H, s), 6.94–6.85 (2H, m), 6.45 (1H, s), 3.88 (3H, s), 3.85 (3H, s), 3.83 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.9, 153.7, 152.1, 147.4, 144.0, 138.0, 132.3, 130.4, 127.9, 125.5, 123.7, 117.5, 110.8, 97.8, 57.1, 57.0 and 56.6. HRMS-ESI: m/z $[M + H]^+$ for $C_{20}H_{19}O_4Br$, calculated 403.0539; observed 403.0528.

4.9. General procedure for synthesis of 1-(4-bromophenyl)-5-(2,4,5-trimethoxyphenyl)penta-1,4-dien-3-one (**31**)

2,4,5-trimethoxybenzaldehyde (1.53 mmol, 0.3 g) (obtained from the oxidation of β-asarone) was allowed to react with excess of acetone (5 ml) in ionic liquid $[MIMBSA]HSO_4$ (1.5 g) under focused microwave irradiation (100 W, 80 °C) to obtain the corresponding 4-(2,4,5-trimethoxyphenyl)but-3-en-2-one as a deep yellow solid in 67% yield. Subsequently, the above enone (1.03 mmol, 0.242 g) was further subjected to Claisen–Schmidt condensation with 4-bromobenzaldehyde (1.03 mmol, 0.189 g) using 10% aqueous sodium hydroxide to afford **31** as an orange yellow solid in 82% yield, m. p. 152–154 °C, ^1H NMR (300 MHz, $CDCl_3$); δ 8.10 (1H, d, $J = 16.0$ Hz), 7.68 (1H, d, $J = 15.8$ Hz), 7.53–7.48 (4H, m), 7.17–7.10 (2H, m), 6.98 (1H, d, $J = 16.0$ Hz), 6.52 (1H, s), 3.96 (3H, s), 3.89 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$); δ 189.3, 154.8, 153.1, 143.7, 141.3, 139.0, 134.4, 132.5, 130.0, 126.1, 124.7, 124.4, 115.5, 111.1, 97.1, 56.8, 56.7 and 56.4. HRMS-ESI: m/z $[M + H]^+$ for $C_{20}H_{19}O_4Br$, calculated 403.0539; observed 403.0536.

4.10. Biological assays

4.10.1. Measurement of inhibition of P. falciparum growth in culture

In this study chloroquine sensitive 3D7 and resistant Dd2 strains of *P. falciparum* were used in *in-vitro* culture. Parasite strains were cultivated by the method of Trager and Jensenm [59] with minor modifications. Cultures were maintained in fresh O^{+ve} human erythrocytes at 4% hematocrit in complete medium (RPMI 1640 with 0.2% sodium bicarbonate 0.5% Albumax, 45 μg/L hypoxanthine and 50 μg/L gentamicin) at 37 °C under reduced O_2 (gas mixture 5% O_2, 5% CO_2, and 90% N_2).

Stock solutions of Chloroquine were prepared in water (miliQ grade) and test compounds were dissolved in DMSO. All stocks were then diluted with culture medium to achieve the required concentrations (In all cases the final concentration contained 0.4% DMSO, which was found to be non-toxic to the parasite). Drugs and test compounds were then placed in 96-well flat-bottom tissue-culture grade plates to yield triplicate wells with drug concentrations ranging from 0 to 10^{-4} M in a final well volume of 100 μl. Chloroquine was used as a positive control in all experiments. Parasite culture was synchronized at ring stage with 5% sorbitol. Synchronized culture was aliquoted to drug containg 96-well plate at 2% hematocrit and 1% parasitemia. After 72 h of incubation under standard culture conditions, plates were harvested and read by the SYBR Green I fluorescence-based method [59] using a 96-well fluorescence plate reader (Victor, Perkin Elmer), with excitation and emission wavelengths at 497 and 520 nm, respectively. The fluorescence readings were plotted against drug concentration, and IC_{50} values obtained by visual matching of the drug concentration giving 50% inhibition of growth.

4.10.2. Measurement of cytotoxic activity against mammalian cell lines in culture

Animal cell lines (Hela and fibroblast L929) were used to determine drug toxicity by using MTT assay for mammalian cell viability assay as described by Mosmann 1983 [59] using Hela and fibroblast L929 cells cultured in complete RPMI containing 10% fetal bovine serum, 0.2% sodium bicarbonate, 50 μg/ml gentamycin. Briefly, Cells (10^4 cells/200 μl/well) were seeded into 96-well flat-bottom tissue-culture plates in complete culture medium. Drug solutions were added after overnight seeding and incubated for 24 h in a humidified atmosphere at 37 °C and 5% CO_2. DMSO (final concentration 10%) was added as +ve control. An aliquot of a stock solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml in 1× phosphate-buffered saline) was added at 20 μl per well, and incubated for another 4 h. After spinning the plate at 1500 RPM for 5 min, supernatant was removed and 100 μl of the stop agent DMSO was added. Formation of formazon, an index of survival was read at 570 nm. The 50% cytotoxic concentration (TC_{50}) was determined by analysis of dose–response curves. Selectivity index was calculated as TC_{50}/IC_{50}.

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Appendix. Supplementary information

Supplementary information associated with this article can be found in the on-line version, at doi:10.1016/j.ejmech.2010.08.049.

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