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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro SAR evaluation of natural vanillin-based chalcones tethered quinolines as antiplasmodial agents

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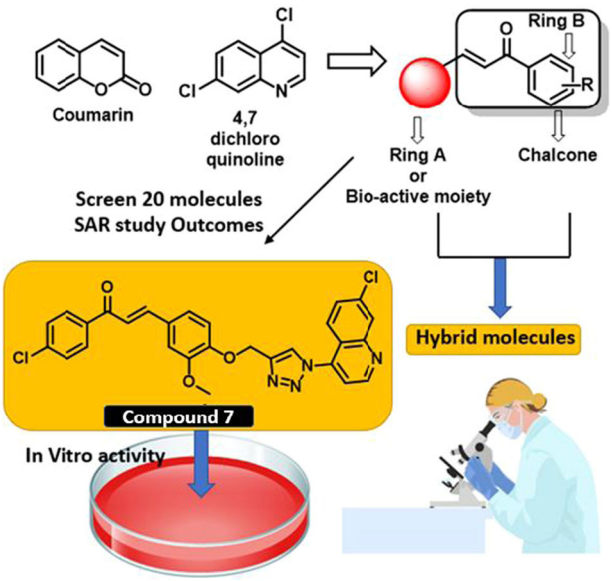
Abstract

A series of novel chalcone derivatives were synthesized and investigated against the chloroquine-sensitive *P. falciparum* 3D7 (*Pf*3D7) strain and chloroquine-resistant *P. falciparum* K1 strain to establish their structure-activity relationship. In this study, compound **7** was found most active as well as less cytotoxic ($IC_{50} = 4.12\ \mu M$ and $3.14\ \mu M$ for *Pf*3D7 *and Pf*K1 respectively; $CC_{50} = 46.18\ \mu M$). Compound 7 was studied for effect on parasite growth and the microscopic examination showed excessive DNA damage in the trophozoite stage. The parasite recovery after drug removal was poor due to the dramatic genotoxic effect of compound **7**. It suggested that 7-chloro quinoline and triazole linkage were crucial for antimalarial potential.

Graphical abstract



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Keywords Natural vanillin · Chalcone-hybrids · Antiplasmodial agents · Multidrug-resistant strain.

Introduction

Malaria is a mosquito-borne disease, which is associated with high morbidity and mortality [1]. Although diverse potent antimalarial agents including quinolones [2–4] (e.g. chloroquine) and endoperoxides [3a] (e.g., Artemisinin and its derivatives) [3b] are available, the emergence of resistance against front-line drugs has raised a major health concern. Thus, the search for newer efficacious drugs as well as new molecular frameworks possessing antimalarial activity remains a vital goal towards achieving control over malaria [5]. Among various antimalarial molecules, the design and development of novel hybrid molecules also represent a logical approach that has the potential to overcome the rapid development of drug resistance, decrease parasite burden, and reduce both the cost and the risk of drug-drug interactions compared to cocktails or multicomponent drugs [6, 7]. In view of encouraging efficacies with good bioavailability and minimized toxicity; the next generation drugs may be hybrid molecules reducing the possibility of resistance. This was demonstrated by the compounds such as tetraoxaquine **1a**, trioxaquine, trioxaferroquine and stilbene-chalcone hybrid **1b** [8–12] (Fig. 1).

Chalcone (1,3-diaryl-2-propen-1-one), coumarin, triazole, and quinoline are major classes of naturally occurring compounds that have been reported to possess several effective therapeutic properties [11, 13–15]. These pharmacophores are derived from both natural and synthetic routes, each with a distinct mechanism of action, and could be beneficial in malaria treatment as well as minimize the chances of getting drug resistance. Ratifying this approach, various research groups have reported hybrid molecules by coupling chalcone, coumarin, curcumin, flavone, and quinoline with different other bioactive molecules, like resveratrol, maleimide, and alpha-lipoic acid [16–20] to address the problems with hybrid molecules, their solubility, and toxicity. Our group has reported chalcone, chalcone-stilbene hybrids, and distyrylbenzenes for their antimalarial activities [21, 22]. In line with our earlier report on the stilbene-chalcone hybrid with the antimalarial property envisioned that a hybrid of the chalcone molecules with quinolines and coumarin may exhibit potential antimalarial activities [8, 23]. Hence, we designed chalcone-quinoline and coumarin-based novel hybrid molecules and studied its structure-activity relationship (SAR) for the antimalarial activity, and also studied the effect of the active molecule on the growth of the human malaria parasite, *Plasmodium falciparum*.

Results and discussion

Chemistry

Earlier, we studied the antiplasmodial activity of allylated chalcone based on the licochalcone **A** against chloroquine (CQ) sensitive *Pf3D7* and CQ resistant *PfINDO* strains of *P. falciparum* [8]. Among them particular, 1-(4-Chlorophenyl)-3-[3-methoxy-4-(prop-2-en-1-yloxy)phenyl]-prop-2-en-1-one was the most potent (IC_{50}: 3.0 µM) against *Pf3D7* with resistance indices of 1.2 and 6.6 against *PfDd2*, and *PfINDO* strains, respectively. These results fascinated us to develop a SAR around this molecule by the synthesis of its derivatives with diverse functionalities to enhance the antiplasmodial activity. In this regard, chalcone (**4**) was synthesized by the condensation of 4-Chloroacetophenone (**2**) and vanillin (**3**) in presence of sodium hydroxide. Furthermore, propargylated chalcone (**6**) was obtained by the reaction of chalcone (**4**) with propargyl bromide. This further reacts with 4-azido-7-chloroquinoline and gives compound (**7**) by applying copper-catalyzed click chemistry (Scheme 1). However, allylated chalcone (**5**) was also synthesized from chalcone (**4**) by the reaction of allyl bromide. While compound (**8**) was synthesized from compound (**4**) following the Mannich reaction condition. Furthermore, compound **9/**was obtained by the reaction of allyl bromide with compound **8** in the presence of K_2CO_3 (Scheme 1).

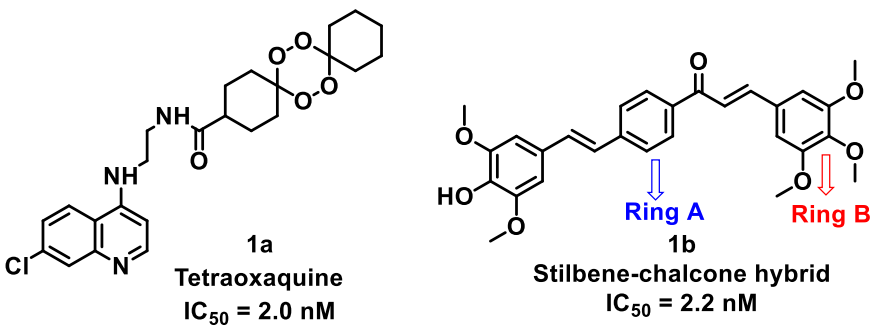
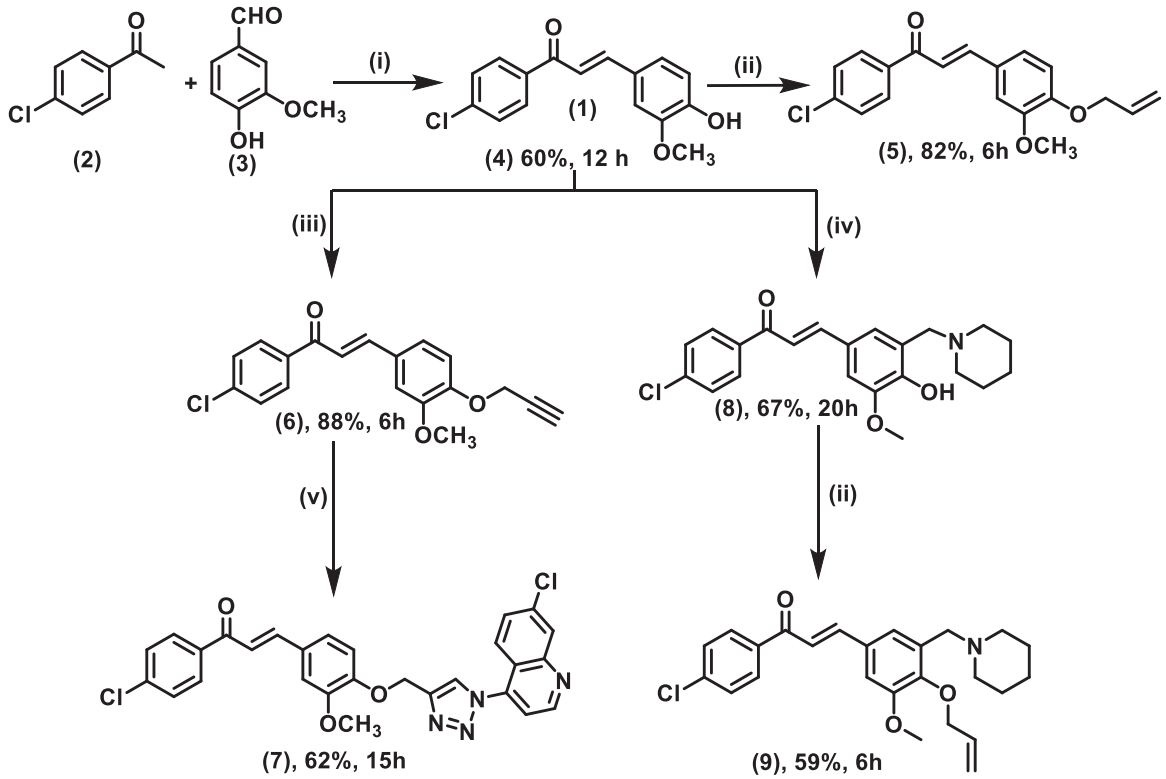


Fig. 1 Hybrid molecules showing antimalarial potential



Scheme 1 Synthesis of 4-chloro-chalcone derivatives. **Reaction conditions**: (i) Methanol, aq. NaOH, rt.; (ii) allyl bromide, K_2CO_3, DMF, rt.; (iii) Propargyl bromide, K_2CO_3, DMF, rt.; (iv) paraformaldehyde, piperidine; DMF, 60 °C. (v) 4-azido-7-chloroquinoline, Sodium ascorbate, $CuSO_4.5H_2O$, DMF, 60 °C

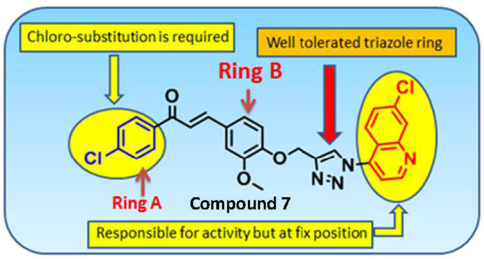
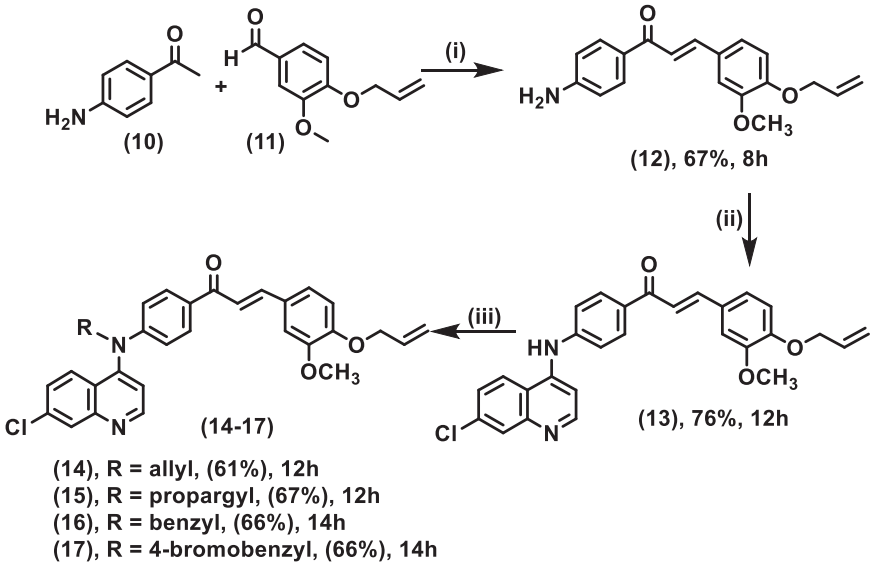


Fig. 2 Represents the structure-activity relationship with compound **7**

We next shifted our attention to check the reliability of the 4-chloro substituent in ring **A** of compound **7** (**See** Fig. 2). So we have replaced chloro substituent with 7-chloro quinoline in ring **A** and synthesized (E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)prop-2-en-1-one (**13**) and its derivatives (**14-17**). These were synthesized by the reaction between intermediate **12** and 4,7-dichloroquinoline and this was followed by alkylation as shown in (Scheme 2).

Notably, 7-chloroquinoline containing chalcone (**7**) was the most active but when we attached 7-chloroquinoline in ring **A**, surprisingly total activity was lost (**22-29**, Table 1). Furthermore, in other attempts, our attention shifted to replace the 4-chloro ring **A** of compound **7** with coumarin **22** and **23** (Scheme 3), which have been reported to possess good pharmacokinetic properties. Henceforth, 3-acetylcoumarins (**20** and **21**) were synthesized by the reaction of 2-hydroxybenzaldehydes (**18** and **19**) respectively, with ethyl acetoacetate in presence of piperidine as a base, which was further reacted with vanillin (**3**) via Claisen–Schmidt condensation reaction to get coumarin-chalcone **24 and 26** (Scheme 3) in acidic methanol (acetyl chloride in/methanol), at room temperature for 3 h. After completion of the reaction, the mixture was filtered to collect the precipitate, and purified by recrystallization affording the pure hydroxylated and methoxylated coumarin-chalcone hybrids (**22** and **24**) in 70% and 58% yield respectively. Considering the significance of *O*-allyl groups, these coumarin-chalcone hybrids were *O*-allylated (**23** and **25**) by using allyl bromide and K_2CO_3 in DMF. Additionally, *O*-propargylated coumarin-chalcone (**26**) was also synthesized by using propargyl bromide in presence of K_2CO_3 in DMF. Further keeping in mind, chalcone quinolone hybrid (**7**) (Scheme 1) was most active. We performed a reaction of propargylated coumarin-chalcone (**26**) with 4-azido-7-chloroquinoline in the presence of $CuSO_4.5H_2O$, sodium ascorbate in DMF at 60 °C to get triazole-linked coumarin-chalcone-quinoline hybrid (**27**). On the other side, chloroacetylchloride was reacted with



Scheme 2 Synthesis of 7-chloroquinoline-chalcones hybrids. Reaction conditions: (i). aq. NaOH, ethanol; (ii) 4,7 dichloroquinoline; THF, reflux; (iii) allylbromide/ propargylbromide/benzyl bromide/4-bromobenzyl bromide, KOH, THF, CTAB, stir it

Table 1 Evaluation of antimalarial activity and selectivity index of compounds (6-29)

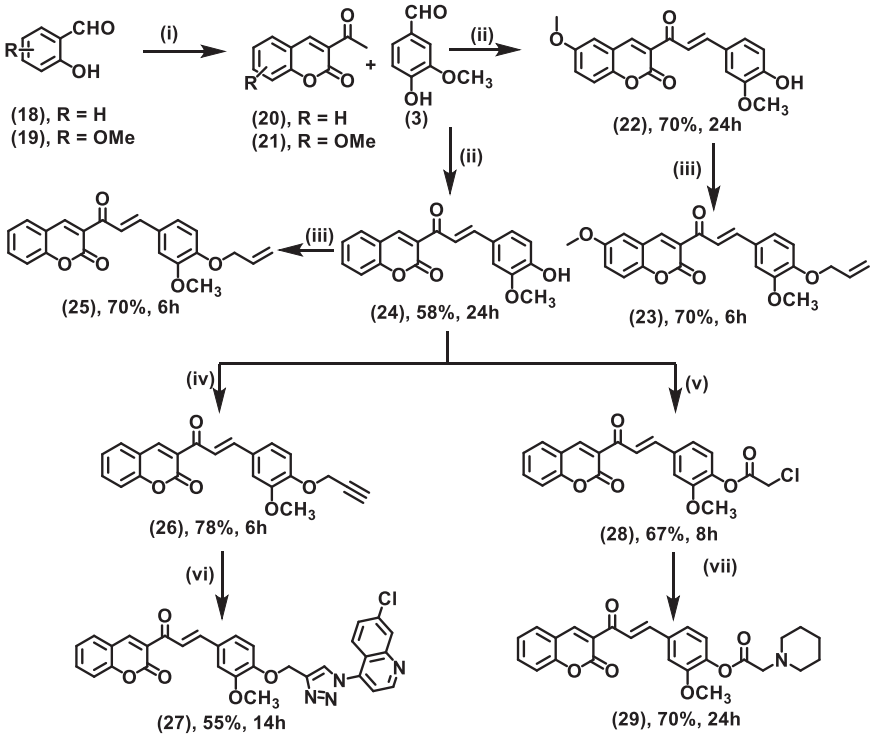
Compound No	IC_{50} (μM) *Pf3D7*	IC_{50} (μM) *PfK1*	CC_{50} (μM)	Selectivity index *Pf3D7*	Selectivity index *PfK1*
6	>5	>5	90.26	ND	ND
7	4.12 ± 0.75	3.55 ± 1.0	46.18 ± 10.0	11.2	13.0
8	>5	>5	ND	ND	ND
9	>5	>5	ND	ND	ND
15	>5	ND	ND	ND	ND
16	4.13 ± 1.0	>5	23.97 ± 5.0	5.8	ND
22	>5	>5	ND	ND	ND
23	>5	ND	ND	ND	ND
24	>5	ND	ND	ND	ND
25	>5	ND	ND	ND	ND
27	>5	>5	>200	ND	ND
28	>5	ND	ND	ND	ND
29	>5	>5	ND	ND	ND
CQ- diphosphate	0.005 ± 0.0015	0.258 ± 0.050	125 ± 25	25000	484

IC_{50} and CC_{50} are the half-maximal inhibitory and cytotoxic concentrations, respectively. IC_{50} and CC_{50} values were determined 72 h after the treatment of compounds. CQ (IC_{50} 5 nM) and podophyllotoxin (CC_{50} 5.2 μM) were used as reference compounds for IC50 and CC50 assays, respectively. The compounds which showed $IC_{50} \leq 5$ μM were only taken forward for assessment of IC_{50} values in chloroquine-resistant (K1 strain) and CC_{50} in VERO cells. The selectivity index (SI) is the ratio of CC_{50}/IC_{50}

coumarin-chalcone (**24**) to get chloroacetylated coumarin-chalcone (**28**) which was further reacted with secondary amine piperidine to get the compound (**29**)/(Scheme 3).

Antimalarial activity

All the synthesized hybrid-compounds were tested for anti-malarial activity against chloroquine-sensitive *P. falciparum* 3D7 (*Pf*3D7) strain and chloroquine-resistant *P. falciparum* K1 strain (Table 1) by SYBR-Green-I assay [24]. The fluorescence readout gives an indication of parasite growth in infected RBCs. SYBR green-based fluorescence plotted with respect to drug concentration gives a precise estimation of parasite inhibitory concentrations. Interestingly, the most active compound i.e. (E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one ($IC_{50} = 2.5$ μM) was selected from our previous reports for further hybridization which is considered as the basis of the study and used as a precursor for further elaboration of new chemical entities in antimalarial activity[8]. Thereafter, the structure-activity



Scheme 3 Synthesis of coumarin-chalcone hybrids. **Reaction conditions:** (i) ethyl acetoacetate, Methanol, piperidine, rt.; (ii) Methanol, acetyl chloride, rt; (iii) allyl bromide, K_2CO_3, DMF, rt.; (iv) Propargyl bromide, K_2CO_3, DMF, rt; (v) Chloroacetyl chloride, K_2CO_3, DMF, rt; (vi) 4-azido-7-chloroquinoline, NaN_3, Sodium ascorbate, $CuSO_4.5H_2O$, DMF, 60 °C; (vii) secondary amine, K_2CO_3, DMF, rt

relationship studies were carried out by changing the substitutions on ring **B** keeping the 4-chloro substituent constant on ring **A**. Amino methyl, allyl, and propargyl substituent on ring **B** exhibited no activity against both the strains but compound (**7**) was quite efficient in killing both chloroquine-sensitive and resistant strain. This clearly shows the benefit of the addition of triazole-linked 7-Chloro quinoline to the chalcone. We next ventured to evaluate the positional importance of the amino 7-chloroquinoline group on ring **A** (Table 1).

It is known that activity was markedly affected by *p*-substitution of *O*-allyl group[8]. So, allylated vanillin substitution at the ring **B** was kept unchanged, and subsequently, the effect of changing the nature of the *N*-substituent (H, allyl, phenyl, C_4H_9, and $CH_2C_6H_4Br$) was evaluated, and reduced activity was observed in each instance (Table 1**, 15,16**) except, *N*-allylated chalcone (**14**) but lacked selectivity. It was observed that there was no further enhancement in antimalarial activity for any of the coumarin-chalcone hybrids (Table 1). Heteroaryl-substitution is an appealing strategy for desirable activity and several inspiring reports on the antimalarial activity of heterocyclic containing chalcone derivatives [25] boost us to synthesize such analogues. We designed chalcones by the condensation of benzaldehydes with different heterocyclic carbonyls like 7-chloroquinoline, and coumarin [26]. However, in each case, the antimalarial potential was not found, although, the 7-chloroquinoline is considered an excellent lead prototype for the development of antimalarial drugs [27, 28].

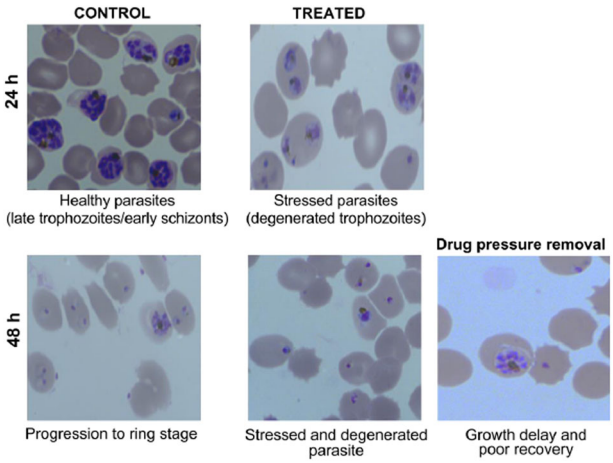


Fig. 3 Microscopic examination of Giemsa-stained blood smears of *P. falciparum* (3D7, 6–8% parasitemia) treated with 10 µM of compound 7 at 24 h and 48 h. After 24 h, both control and treated sample were washed and the parasite was cultured without the drug for another 24 h

Microscopic examination of antimalarial activity

Compound (**7**) was studied for microscopic examination which revealed that the compound treatment caused drastic effects on parasite growth in comparison to control. After 24 h, the ring-stage parasite progressed into the late trophozoite and schizont stage in control, whereas, the compound-treated sample showed delayed parasite growth and they were arrested in the early trophozoite stage. After 48 h, healthy schizonts in the control sample

progressed into a new infection cycle and parasites were predominantly in the ring stage. While, the treated parasite showed mainly stressed trophozoite with reduced staining of parasite DNA, probably due to excessive DNA damage. We also performed experiments to check whether the parasite is able to recover after the removal compound (**7**). Due to dramatic genotoxic effect of compound (**7**), the parasite shows poor recovery from stress even after drug removal (Fig. 3).

Conclusion

The study revealed that among all synthesized hybrid molecules vanillin-quinoline hybrid molecules showed better antimalarial potency against *Pf*3D7 and *Pf*K1, respectively) in comparison to vanillin-coumarin hybrid molecules ((IC_{50} of greater than 5 μM) as well as natural licochalcone (IC_{50} of 4.1 μM). Microscopic examination studies of compound (**7**) showed a drastic effect on parasite growth even after removal of the compound. The study suggests that hybrid molecules may exhibit promising activities, and their economical route of synthesis may provide useful leads towards future antimalarial drug discovery.

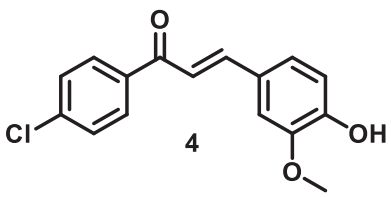
Experimental section

Chemistry

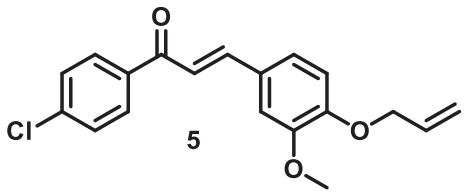
Chemical and reagents

All the reagents were obtained from commercial sources (Merck or Acros). The solvents used for isolation/purification of compounds were obtained from commercial sources (Merck) and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-400 spectrometer. TMS was used as an internal reference for ^{1}H NMR. HRMS-ESI spectra were determined using micro mass Q-TOF ultima spectrometer.

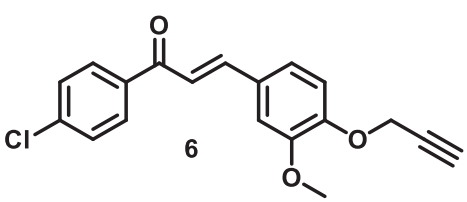
Procedure for the synthesis of 4-chloro substituted chalcones (4,5)



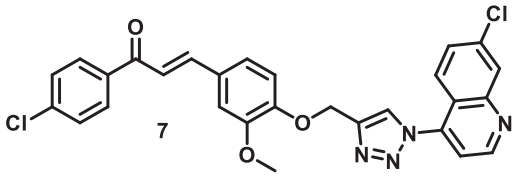
(*E*)-1-(4-Chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (4) To the solution of 4-chloroacetophenone **2** (3 mmol), and vanillin **3** (3 mmol) in ethanol (20 mL), KOH (4 mmol) was added. Reaction progress was monitored with TLC. After the completion reaction mixture was concentrated and washed with water, taken in ethyl acetate, and dried over sodium sulfate. The desired compound **4** was obtained after recrystallization in methanol and characterized by ^{1}H &^{13}C NMR and HRMS data. Bright yellow solid (Yield 60%) m.p. 110-115 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.95 (d, $J = 8.6$ Hz, 2H), 7.75 (d, $J = 15.6$ Hz, 1H), 7.46 (d, $J = 8.6$ Hz, 2H), 7.32 (d, $J = 15.6$ Hz, 1H), 7.21 (dd, $J = 8.2$, 1.6 Hz, 1H), 7.12 (d, $J = 1.6$ Hz, 1H), 6.96 (d, $J = 8.2$ Hz, 1H), 6.09 (1H, s), 3.95 (3H, s); ^{13}C NMR ($CDCl_3$,100 MHz): δ (ppm) 189.4, 148.6, 146.9, 145.8, 139.0, 136.8,129.9, 128.9, 127.3, 123.5, 119.2, 115.0, 110.2, 56.1. HRMS-ESI: m/z $[M + H]^+$ for $C_{16}H_{14}ClO_3$, calculated 289.0631; observed 289.0625.



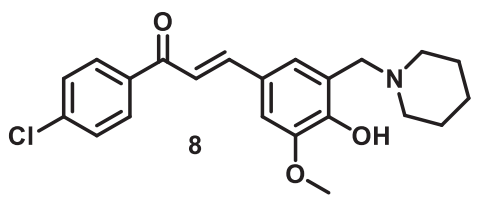
(E)-1-(4-Chlorophenyl)-3-[3-methoxy-4-(prop-2-en-1-yloxy) phenyl]prop-2-en-1-one (5) Compound **4** (0.99 mmol) was treated with allyl bromide (1.05 mmol) in presence of K_2CO_3 (1.99 mmol) in DMF (5 mL) at rt. for 6 h. Reaction mixture was diluted with water and desired compound **5** was obtained by filtration and recrystallization with methanol and characterized by ^{1}H &^{13}C NMR and HRMS data. Pale yellow solid (Yield 82%) m.p. 90-93 °C, ^{1}H NMR ($CDCl_3$,400 MHz): (ppm) 7.95 (d, $J = 8.6$ Hz, 2H), 7.75 (d, $J = 15.6$ Hz, 1H), 7.47 (d, $J = 8.6$ Hz, 2H), 7.33 (d, $J = 15.6$ Hz, 1H), 7.20 (d, $J = 8.4$, 1.9 Hz, 2H,), 7.16 (d, $J = 1.9$ Hz, 1H), 6.90 (d, $J = 8.3$ Hz, 1H), 6.14-6.04 (1H, m), 5.43 (dd, $J = 17.2$, 1.4 Hz, 1H,), 5.32 (d, $J = 10.5$, 1.3 Hz, 1H), 4.68 (m, 1H), 3.95 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 189.3, 150.7, 149.7, 145.5, 139.0, 136.8, 132.7, 129.9, 128.9, 127.9, 123.1, 119.6, 118.5, 113.0, 110.7, 69.8, 56.1. HRMS-ESI: m/z $[M + H]^+$ for $C_{19}H_{18}ClO_3$, calculated 329.0944 observed 329.0945.



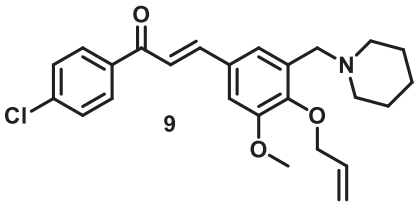
(E)-1-(4-Chlorophenyl)-3-[3-methoxy-4-(prop-2-yn-1-yloxy) phenyl]prop-2-en-1-one (6) Compound **4** (0.99 mmol) was treated with propargyl bromide (1.05 mmol) in the presence of K_2CO_3 (1.99 mmol) in DMF (4 mL) at rt. for 6 h. The reaction mixture was diluted with water and desired compound **6** was obtained by filtration and recrystallization with methanol and characterized by ^1H &^{13}C NMR and HRMS data. Pale yellow solid (Yield 88%) m.p. 90-93 °C, ^1H NMR ($CDCl_3$,400 MHz): δ (ppm) 7.95 (d, $J = 8.6$ Hz, 2H), 7.76 (d, $J = 15.6$ Hz, 1H), 7.47 (d, $J = 8.6$ Hz, 2H), 7.35 (d, $J = 15.6$ Hz, 1H), 7.24 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.17 (d, $J = 1.9$ Hz, 1H), 7.06 (d, $J = 8.3$ Hz, 1H), 4.82 (d, $J = 2.4$ Hz, 2H), 3.95 (3H, s), 3.59 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 189.2, 149.8, 149.3, 145.3, 139.1, 136.7, 129.9, 128.9, 122.7, 120.0, 113.8, 110.8, 77.9, 76.3, 56.7, 56.1. HRMS-ESI: m/z $[M + H]^+$ for $C_{19}H_{16}ClO_3$, calculated 327.0788 observed 327.0781.



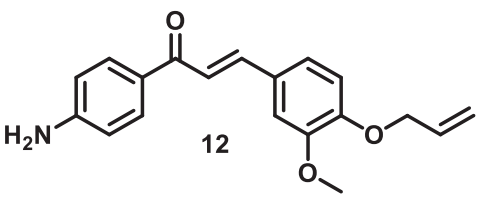
(E)-1-(4-Chlorophenyl)-3-(4-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)prop-2-en-1-one (7) Compound **6** (0.61 mmol) was treated with 4-azido-7-chloroquinoline (0.61 mmol) in the presence of copper sulfate (0.12 mmol), sodium ascorbate (0.25 mmol) in DMF (5 mL) at rt. for 15 h. Reaction mixture was diluted with water and desired compound **7** was obtained by filtration and recrystallization with chloroform/methanol and characterized by ^1H &^{13}C NMR and HRMS data. Pale yellow solid (Yield 62%) m.p. 90–93 °C, ^1H NMR ($CDCl_3$,400 MHz): δ (ppm) 9.20 (s, 1H), 9.00 (s, 1H), 8.31 (s, 1H), 8.19 (d, $J = 8.5$ Hz, 2H), 8.02 (d, $J = 9.1$ Hz, 1H), 7.90 (dd, $J = 7.7$ 4.2 Hz, 1H), 7.83 (dd, $J = 6.0$, 9.2 Hz, 2H), 7.76 (d, $J = 15.6$, 1H), 7.64 (d, $J = 8.5$ Hz, 2H), 7.60 (s, 1H), 7.47 (d, $J = 6.4$ Hz, 1H), 7.34 (d, $J = 8.3$ Hz, 1H), 5.41 (s, 1H), 3.89 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 188.5, 152.8, 150.4, 149.8, 145.4, 143.7, 140.8, 138.4, 136.9, 135.9, 130.8, 129.5, 129.3, 128.6, 128.6, 127.7, 125.9, 125.8, 124.4, 120.1, 117.7, 113.9, 111.7, 56.2. HRMS-ESI: m/z $[M + H]^+$ for $C_{28}H_{21}Cl_2N_4O_3$, calculated 531.0991 observed 531.0987.



(E)-1-(4-chlorophenyl)-3-(4-hydroxy-3-methoxy-5-(piperidin-1-ylmethyl)phenyl)prop-2-en-1-one (8) Compound **4** (0.69 mmol) was treated with paraformaldehyde (1.38 mmol), piperidine (1.38 mmol) in DMF (3 mL) at 60° C for 20 h. Reaction mixture was diluted with water and desired compound **8** was obtained by filtration and recrystallization in methanol. This was fully characterized by ^1H &^{13}C NMR and HRMS data. Yellow solid (Yield 67%) m.p. 109–112 °C, ^1H NMR ($CDCl_3$,400 MHz): δ (ppm) 8.17 (d, $J = 8.0$ Hz, 2H), 7.76 (d, $J = 15.4$ Hz, 1H), 7.68 (d, $J = 15.2$ Hz, 1H), 7.63 (d, $J = 8$ Hz, 2H), 7.47 (s, 1H), 7.26 (s, 1H), 3.87 (s, 3H), 3.65 (s, 2H), 3.61 (t, $J = 4.0$ Hz, 4H), 2.47 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ(ppm) 189.1, 150.2, 148.3, 145.7, 138.9, 136.8, 129.8, 128.8, 126.0, 122.7, 120.9, 118.8, 110.4, 66.1, 61.4, 56.0 and 52.8. HRMS-ESI: m/z $[M + H]^+$ for $C_{22}H_{25}ClNO_3$, calculated 386.1523 observed 386.1535.

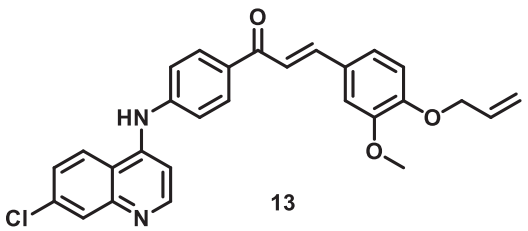


(E)-3-(4-(allyloxy)-3-methoxy-5-(piperidin-1-ylmethyl)phenyl)-1-(4-chlorophenyl)prop-2-en-1-one (9) Compound **8** (0.99 mmol) was treated with allyl bromide (1.05 mmol) in the presence of K_2CO_3 (1.99 mmol) in DMF (5 mL) at rt. for 6 h. The reaction mixture was diluted with water and desired compound **10** was obtained by filtration followed by column purification with hexane: ethyl acetate (8:2) which was characterized by ^1H &^{13}C NMR and HRMS data. Yellow oil (Yield 59%), ^1H NMR ($CDCl_3$, 400 MHz): δ(ppm) 7.97 (d, $J = 8$ Hz, 1H), 7.72 (d, $J = 16$ Hz, 1H), 7.49 (d, $J = 8$ Hz, 2H), 7.37 (d, $J = 16$ Hz, 1H), 7.30 (s, 1H), 7.09 (s, 1H), 6.15-6.08 (m, 1H), 5.41-5.36 (m, 1H), 4.59 (d, $J = 4.0$ Hz, 2H), 3.92 (s, 3H), 3.65 (s, 2H), 3.72-3.70 (t, $J = 4$ Hz, 4H), 3.50 (s, 2H), 2.50 (m, 4H), 1.26 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ(ppm) 189.3, 150.2, 150.0, 145.9, 139.3, 137.2, 133.1, 130.2, 129.3, 128.3, 123.5, 119.9, 118.9, 113.4, 111.1, 70.2, 59.2, 57.0, 54.1, 28.7 and 25.4. HRMS-ESI: m/z $[M + H]^+$ for $C_{25}H_{29}ClNO_3$, calculated 426.1836 observed 426.1848.



Procedure for the synthesis of (E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-aminophenyl)prop-2-en-1-one (Compound 12) To the solution of 4-aminoacetophenone **10** (3 mmol) and 4-allyloxyvanillin 11 (3 mmol) in ethanol (20 mL), KOH (4 mmol) was added. The reaction mixture was stirred for 8 h. Reaction progress was monitored by thin-layer chromatography and after completion of the reaction, the reaction mixture was concentrated and washed with (3 × 20 mL) water and recrystallized in methanol to give compound **6**. Which was further used for the next reaction.

Procedure for the synthesis of 7-chloroquinoline-chalcones and its derivatives (Compounds 13-17)

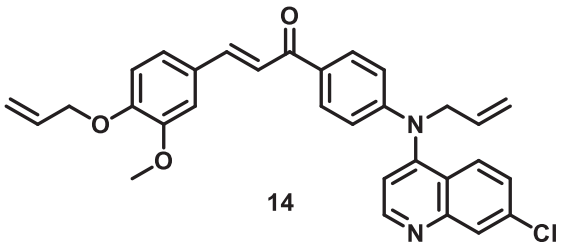


13

(E)-3-(4-(Allyloxy)-3-methoxyphenyl)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl) prop-2-en-1-one (13) Compound **12** was further treated with 4,7-dichloroquinoline in DMF at rt. for overnight to give compound **13**. Reaction progress was monitored with TLC. After completion reaction mixture was concentrated and washed with water, recrystallized in methanol and characterized by ^{1}H &^{13}C NMR and HRMS data. Yellow solid (Yield 76%) m.p. 168–171 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): (ppm) 8.78 (d, $J = 9.0$ Hz, 1H), 8.63 (d, $J = 6.3$ Hz, 1H), 8.31 (d, $J = 8.4$ Hz, 2H), 8.14 (s, 1H), 7.89 (d, $J = 15.5$ Hz, 1H) 7.82 (d, $J = 9.0$ Hz, 1H), 7.73 (d, $J = 15.5$ Hz, 1H), 7.65 (d, $J = 8.4$ Hz, 2H), 7.58 (s, 1H), 7.39(d, $J = 8.3$ Hz, 1H), 7.17 (d, $J = 6.4$ Hz, 1H), 7.04 (d, $J = 8.4$ Hz, 1H) 6.11-6.02 (m, 1H), 5.42 (dd, $J = 17.3$, 1.52 Hz, 1H), 5.28 (dd, $J = 10.5$, 1.2 Hz, 1H), 4.64 (d, $J = 5.24$ Hz, 1H), 3.89, (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 180.0, 150.6, 149.7, 147.0, 144.9, 143.3, 143.1, 137.6, 135.1, 134.0, 130.8, 128.2, 127.4, 126.3, 124.3, 123.2, 122.6, 120.0, 118.4, 117.9, 113.5, 112.6, 111.6, 103.0, 69.4, 56.3.HRMS-ESI: m/z $[M + H]^+$ for $C_{28}H_{24}ClN_2O_3$, calculated 471.6449; observed 471.1464.

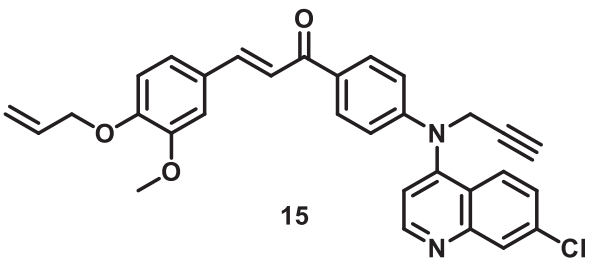
General procedure for the synthesis 7-chloroquinoline-chalcone derivative (14-17)

To the solution of compound **13** (2.7 mmol) in dry tetrahydrofuran (15 mL), potassium hydroxide (13.5 mmol), allyl/propargyl/benzyl/4-bromobenzyl bromide (5.5 mmol), and cetyltrimethylammonium bromide (CTAB) (0.7 mmol) was added. The contents were stirred at room temperature for 12–14 h till the starting disappeared on TLC. After the completion of the reaction, the reaction mixture was partitioned between ethyl acetate (70 mL) and water (15 mL). The ethyl acetate layer was washed with water till neutral, dried over sodium sulfate, and evaporated. The obtained residue was purified by column chromatography in hexane: ethyl acetate (7:3 v/v) to afford the desired compounds (**14-17**) whose structure was confirmed through NMR and mass spectrometry.



14

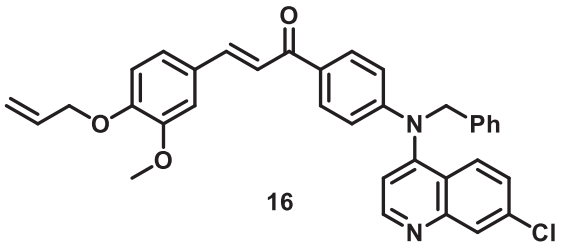
(E)-1-(4-(Allyl(7-chloroquinolin-4-yl)amino)phenyl)-3-(4-(allyloxy)-3-methoxy phenyl) prop-2-en-1-one (14) Orange-yellow viscous liquid (Yield 61%), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.40 (d, $J = 8.3$ Hz, 2H), 6.92 (d, $J = 7.8$ Hz, 2H), 6.73 (s, 1H), 6.56 (s, 1H), 6.49-6.30 (m, 4H), 6.06 (s, 1H), 6.05 (d, $J = 7.8$ Hz, 1H), 5.90 (d, $J = 4.9$ Hz, 1H), 5.70 (d, $J = 8.4$ Hz, 1H), 4.80-4.75 (m, 2H), 4.19-4.02 (m, 1H), 3.40 (d, $J = 5.1$ Hz, 2H), 3.30 (d, $J = 5.1$ Hz, 2H), 2.60 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ (ppm) 188.9, 151.4, 151.0, 150.0, 149.5, 147.7, 145.0, 144.7, 143.7, 137.6, 136.0, 135.0, 133.6, 130.7, 128.6, 127.7, 125.1, 124.0, 123.7, 122.7, 120.2, 118.4, 116.2, 113.7, 111.5, 103.4, 70.0, 56.3 and 44.5. HRMS-ESI: m/z $[M + H]^+$ for $C_{31}H_{28}ClN_2O_3$, calculated 511.6759; observed 511.6782.



15

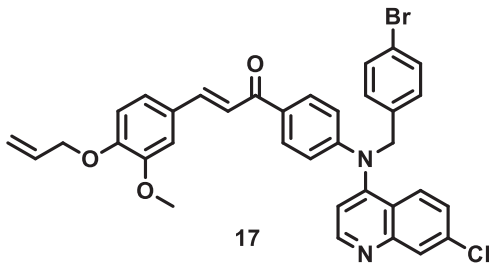
(E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-((7-chloroquinolin-4-yl)(prop-2-yn-1-yl)amino)phenyl)prop-2-en-1-one (15) Yellow solid (Yield 67%) m.p. 79-81 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.00 (d, $J = 4.0$ Hz, 1H), 8.20 (d, $J = 2.0$ Hz, 1H), 7.78 (s, 1H), 7.76 (d, $J = 5.2$ Hz, 1H), 7.48-7.38 (m, 1H), 7.21 (d, $J = 8.0$ Hz, 1H), 7.16 (d, $J = 2.0$ Hz,

1H), 6.92 (d, $J = 8.0$ Hz, 1H), 6.88 (d, $J = 8.0$ Hz, 1H), 6.11 (m, 1H), 5.46 (dd, $J = 16.0$, 1.2 Hz, 1H), 5.35 (dd, $J = 10.5$, 1.2 Hz, 1H), 4.68 (d, $J = 5.4$ Hz, 2H), 4.62 (d, $J = 2.0$ Hz, 2H), 3.94 (s, 3H), 2.39 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 188.3, 152.2, 151.1, 150.6, 150.4, 150.3, 149.6, 144.0, 136.0, 132.7, 130.8, 130.3, 129.1, 128.2, 128.1, 128.0, 125.1, 123.8, 122.7, 119.7, 118.5, 118.4, 116.0, 113.0, 110.5, 78.2, 74.1, 69.7, 56.0 and 42.5. HRMS-ESI: m/z $[M + H]^+$ for $C_{31}H_{26}ClN_2O_3$, calculated 509.1632; observed 509.1626.



16

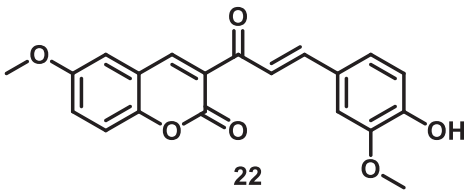
(E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-(benzyl(7-chloroquinolin-4-yl)amino)phenyl)prop-2-en-1-one (16) Yellow solid (Yield 66%) m.p. 224–226 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.8 (d, $J = 6.0$ Hz, 1H), 8.24 (d, $J = 6.0$ Hz, 1H), 8.06 (d, $J = 16.0$ Hz, 1H), 7.86 (s, 1H), 7.68-7.58 (m, 3H), 7.16–7.36 (m, 8H), 6.92 (d, $J = 8.0$ Hz, 1H), 6.79 (d, $J = 8.0$ Hz, 2H), 6.56 (d, $J = 8.0$ Hz, 1H), 6.09 (m, 1H), 5.42 (dd, $J = 16.0$, 1.2 Hz, 1H), 5.29 (dd, $J = 10.5$, 1.2 Hz, 1H), 4.68 (d, $J = 2.0$ Hz, 2H), 4.62 (s, 2H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 188.7, 155.2, 152.5, 150.9, 149.6, 147.9, 145.1, 138.9, 135.1, 133.4, 130.8, 128.5, 127.5, 126.7, 124.1, 123.9, 122.7, 120.2, 118.6, 113.7, 111.5, 103.4, 70.1, 59.9 and 56.0. HRMS-ESI: m/z $[M + H]^+$ for $C_{35}H_{30}ClN_2O_3$, calculated 561.1945; observed 561.1932.



17

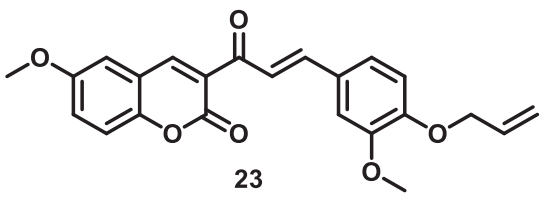
(E)-3-(4-(allyloxy)-3-methoxyphenyl)-1-(4-((4-bromobenzyl)(7-chloroquinolin-4-yl)amino)phenyl)prop-2-en-1-one (17) Yellow solid (Yield 66%) m.p. 232–235 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.54 (d, $J = 8.0$ Hz, 1H), 8.11 (d, $J = 8.0$ Hz, 1H), 8.00 (d, $J = 16.0$ Hz, 1H), 7.71 (s, 1H), 7.52–7.46 (m, 6H), 7.35 (d, $J = 8.0$ Hz, 1H), 7.20–7.12 (m, 4H), 6.92 (d, $J = 8.0$ Hz, 1H), 6.72 (d, $J = 8.0$ Hz, 2H), 6.48 (d, $J = 8.0$ Hz, 1H), 6.08 (m, 1H), 5.40 (dd, $J = 16.0$ 1.2 Hz, 1H), 5.24 (dd, $J = 10.5$, 1.2 Hz, 1H), 4.66 (d, $J = 2.0$ Hz, 2H), 4.61 (s, 2H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 189.9, 156.3, 153.7, 151.0, 149.8, 147.7, 146.1, 138.0, 133.1, 131.2, 130.9, 128.9, 127.5, 124.1, 123.9, 122.7, 121.1, 118.6, 115.2 114.7, 111.5, 69.8, 57.0 and 56.3. HRMS-ESI: m/z $[M + H]^+$ for $C_{35}H_{29}BrClN_2O_3$, calculated 639.1050; observed 639.1039.

Procedure for the synthesis of coumarin-chalcones and its derivatives (Compounds 22-29)



22

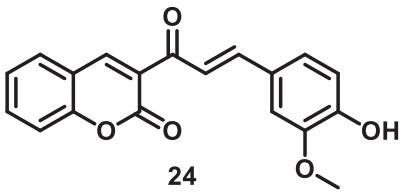
(E)-3-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)-6-methoxy-2H-chromen-2-one (22) Acetyl chloride (3 mL) was added drop wise to ice cold methanol (25 mL) with stirring and after 5 min compound **19** was added resulted 3-acetyl-6-methoxy-2H-chromen-2-one **21** (0.01 mmol) was obtained. Compound **21** and vanillin **3** (0.01 mmol) were stirred for 24 h at rt in the presence of NaOH in ethanol. The reaction mixture was concentrated and washed with water. The desired compound **22** was recrystallized in methanol and characterized by ^{1}H & ^{13}C NMR and HRMS data. Yellow solid (Yield 70%) m.p. 148–151 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.58 (s, 1H), 7.82 (d, $J = 16.0$ Hz, 1H), 7.69 (d, $J = 8.0$ Hz, 1H), 7.17 (m, 2H), 7.02 (d, $J = 16.0$ Hz, 1H), 6.99 (d, $J = 8.0$ Hz, 1H), 6.87 (s, 1H), 6.79 (d, $J = 8.0$ Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 183.6, 159.8, 155.6, 149.9, 148.2, 145.7, 134.5, 126.0, 125.3, 124.1, 122.3, 119.0, 118.9, 117.0, 113.2, 111.1, 57.0 and 55.8. HRMS-ESI: m/z [M + H] + for C20H17O6, calculated 353.1025; observed 353.1043.



23

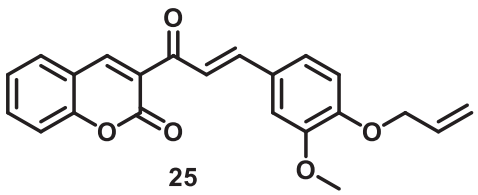
(E)-3-(3-(4-(allyloxy)-3-methoxyphenyl)acryloyl)-6-methoxy-2H-chromen-2-one (23) Compound **22** (0.62 mmol) was treated with allyl bromide (0.62 mmol) in presence of

K_2CO_3 (0.74 mmol) in DMF at rt. for 6 h. Reaction mixture was diluted with water and desired compound **23** was obtained by filtration and recrystallization with methanol/DCM and characterized by ^1H & ^{13}C NMR and HRMS data. Yellow solid (Yield 70%) m.p. 178–181 °C, ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.53 (s, 1H), 7.81 (d, $J = 16.0$ Hz, 2H), 7.79 (d, $J = 8.0$ Hz, 1H), 7.21–7.13 (m, 2H), 7.02 (d, $J = 16.0$ Hz, 1H), 6.99 (d, $J = 7.4$ Hz, 1H), 6.76 (m, 1H), 6.06 (m, 1H), 5.41 (dd, $J = 17.2$, 10.3 Hz, 2H), 4.68 (s, 2H), 3.95 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 182.0, 159.1, 156.6, 147.2, 145.7, 142.0, 134.5, 133.1, 127.9, 125.6, 123.3, 122.3, 118.7, 111.0, 70.1, 56.0 and 55.6. HRMS-ESI: m/z $[M + H]^+$ for C23H21O6, calculated 393.1338; observed 393.1333.



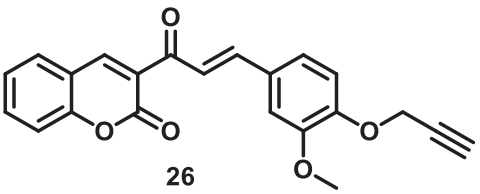
24

(E)-3-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)-2H-chromen-2-one (24) Acetyl chloride (3 mL) was added drop wise to ice cold methanol (25 mL) with stirring and after 5 min. 3-acetyl-2H-chromen-2-one **20** (0.01 mmol) was added and stirred for 4–5 min. To this solution vanillin **3** (0.01 mmol) was added and stirred for 24 h at rt. The reaction mixture was concentrated and washed with water. The desired compound **24** was recrystallized in methanol and characterized by ^1H & ^{13}C NMR and HRMS data. Yellow solid (58% yield), m.p 186–288 °C, ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.58 (s, 1H), 7.86–7.77 (m, 2H), 7.66 (dd, $J = 7.9$, 10.2 Hz, 1H), 7.41–7.34 (m, 2H), 7.24–7.19 (m, 2H), 6.95 (d, $J = 8.1$ Hz, 1H), 5.97 (s, 1H), 3.97 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 186.3, 159.5, 155.2, 148.7, 147.8, 146.8, 145.6, 134.1, 130.0, 127.5, 125.6, 125.0, 124.6, 121.5, 118.7, 116.7, 114.8, 109.9, 56.1; HRMS-ESI: m/z $[M + H] +$ for $C_{19}H_{15}O_5$, calculated m/z 323.0919; observed 323.0909.



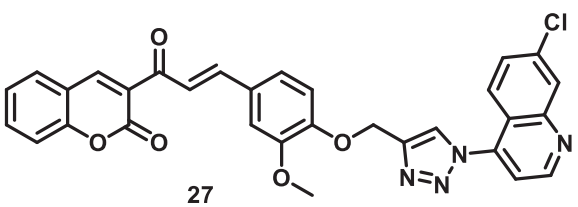
25

3-{(2E)-3-[3-Methoxy-4-(prop-2-en-1-yloxy)phenyl]prop-2-enoyl}-2H-chromen-2-one (25) Compound **24** (0.62 mmol) was treated with allyl bromide (0.62 mmol) in presence of K_2CO_3 (0.74 mmol) in DMF at rt. for 6 h. Reaction mixture was diluted with water and desired compound 25 was obtained by filtration and recrystallization with methanol/DCM and characterized by ^1H & ^{13}C NMR and HRMS data. Yellow solid (Yield 70%) m.p. 148-151 °C, ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.57 (s, 1H), 7.82 (d, $J = 2.2$ Hz, 2H), 7.68–7.63 (m, 2H), 7.40 (d, $J = 8.2$ Hz, 1H), 7.35 (dd, $J = 7.6$, 7.6 Hz, 1H), 7.23 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.20 (d, $J = 1.9$ Hz, 1H), 6.89 (d, $J = 8.3$ Hz, 1H), 6.14–6.04 (m, 1H), 5.43 (dd, $J = 17.2$, 1.4 Hz, 1H), 5.32 (dd, $J = 10.5$, 1.3 Hz, 1H), 4.67 (d, $J = 5.4$ Hz, 2H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 186.3, 159.4, 155.2, 150.8, 149.6, 147.8, 145.3, 134.1, 132.7, 130.0, 128.1, 125.6, 125.0, 123.8, 121.9, 118.6, 118.5, 116.7, 112.9, 110.8, 69.8, 56.0; HRMS-ESI: m/z $[M + H]^+$ for $C_{22}H_{19}O_5$, calculated 363.1232; observed 363.1220.



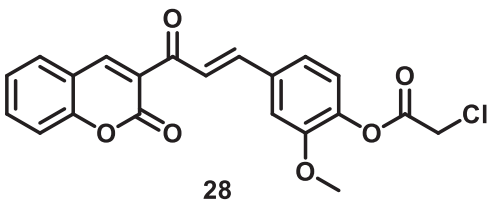
26

©-3-(3-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)acryloyl)-2H-chromen-2-one (26) Compound **24** (0.62 mmol) was treated with propargyl bromide (0.62 mmol) in presence of K_2CO_3 (0.74 mmol) at rt. for 6 h. Reaction mixture was diluted with water and desired compound **26** was obtained by filtration and recrystallization with methanol and characterized by ^1H & ^{13}C NMR and HRMS data. Yellow solid (Yield 78%) m.p. 185–187 °C, ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.58 (s, 1H), 7.83 (s, 2H), 7.68–7.64 (m, 2H), 7.40 (d, $J = 8.2$ Hz, 1H), 7.36 (dd, $J = 6.6$, 0.9 Hz, 1H), 7.24 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.22 (d, $J = 1.9$ Hz, 1H), 7.06 (d, $J = 8.32$ Hz, 1H), 4.82 (d, $J = 2.4$ Hz, 2H), 3.94 (s, 3H), 2.55 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 186.3, 159.4, 155.2, 149.8, 149.4, 147.9, 145.1, 134.2, 130.0, 129.0, 125.5, 125.0, 123.4, 122.4, 118.6, 116.7, 113.6, 110.9, 78.0, 76.3, 56.6, 56.0; HRMS-ESI: m/z $[M + H]^+$ for $C_{22}H_{17}O_5$, calculated 361.1076; observed 361.1074.



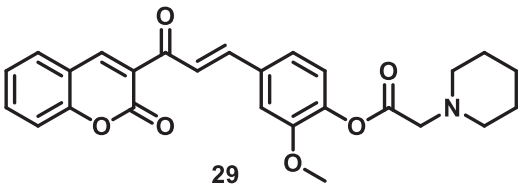
27

(E)-3-(3-(4-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl) methoxy)-3-methoxyphenyl)acryloyl)-2H-chromen-2-one (27) Compound **26** (0.14 mmol) was treated with 4-azido-7-chloroquinoline (0.14 mmol) in presence of copper sulfate (0.028 mmol), sodium ascorbate (0.036 mmol) in DMF (5 mL) at rt. for 14 h. Reaction mixture was diluted with water and desired compound **27** was obtained by filtration and recrystallization with methanol and characterized by ^{1}H & ^{13}C NMR and HRMS data. Yellow solid (Yield 55%) m.p. 222–224 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.07 (d, $J = 4.6$ Hz, 1H), 8.58 (s, 1H), 8.25 (d, $J = 1.9$ Hz, 1H), 8.16 (s, 1H), 7.97 (d, $J = 9.1$ Hz, 1H), 7.83 (s, 2H), 7.69–7.65 (m, 2H), 7.60 (dd, $J = 9.1$, 2.07 Hz, 1H), 7.50 (d, $J = 4.6$ Hz, 1H), 7.41–7.36 (m, 2H), 7.34–7.29 (m, 1H), 7.23 (d, $J = 1.6$ Hz, 1H), 7.15 (d, $J = 8.3$ Hz, 1H), 5.50 (s, 2H), 3.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 186.3, 159.5, 155.2, 151.4, 150.2, 150.0, 149.8, 148.0, 144.9, 144.7, 140.8, 137.0, 134.2, 130.0, 129.6, 129.1, 129.0, 125.4, 125.0, 124.9, 124.5, 123.6, 122.5, 120.6, 118.6, 116.7, 116.1, 113.7, 110.9, 62.9, 56.0; HRMS-ESI: m/z $[M + H]^+$ for $C_{31}H_{22}N_4ClO_5$, calculated 565.1279; observed 565.1288.



28

(E)-2-methoxy-4-(3-oxo-3-(2-oxo-2H-chromen-3-yl)prop-1-en-1-yl)phenyl 2-chloroacetate (28) Compound **24** (1.5 mmol) was drop wise treated with 1-chloroacetyl chloride (4.6 mmol) in presence of K_2CO_3 (6.2 mmol) in DMF (4 mL) at rt. for 8 h. Reaction mixture was diluted with water and desired compound **28** was obtained by filtration and recrystallization with methanol and characterized by 1H & ^{13}C NMR and HRMS data. Yellow solid (Yield: 67%) m.p. 157-160 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.61 (s, 1H), 7.92 (d, $J = 16.0$ Hz, 1H), 7.83 (d, $J = 16.0$ Hz, 1H), 7.69 (t, $J = 8.0$ Hz, 1), 7.43–7.37 (m, 2H), 7.31–7.28 (m, 2H), 7.13 (d, $J = 8.0$ Hz, 1H), 4.37 (s, 2H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 186.3, 165.1, 159.4, 155.2, 151.1, 148.3, 145.6, 144.0, 141.3, 134.4, 130.1, 125.0, 124.4, 122.9, 122.1, 118.5, 116.7, 112.0, 56.0 and 40.5. HRMS-ESI: m/z $[M + H]^+$ for $C_{21}H_{16}ClO_6$, calculated 399.0635; observed 399.0613.



29

(E)-2-methoxy-4-(3-oxo-3-(2-oxo-2H-chromen-3-yl)prop-1-en-1-yl)phenyl 2-(piperidin-1-yl)acetate (29) Compound **28** (0.67 mmol) was treated with piperidine (1.2 equiv.) in DCM in presence of K_2CO_3 (2 equiv.) at rt. for 24 h. Reaction mixture was concentrated and washed with water and recrystallized in methanol to give compound **29** which was characterized by 1H &^{13}C NMR and HRMS data. Yellow solid (Yield 70%) m.p. 148–151 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.61 (s, 1H), 7.94 (d, $J = 16.0$ Hz, 1H), 7.83 (d, $J = 16.0$ Hz, 1H), 7.69 (t, $J = 8.0$ Hz, 2H), 7.43-7.37 (m, 2H), 7.31–7.28 (m, 2H), 7.13 (d, $J = 8.0$ Hz, 1H), 3.95 (s, 3H), 3.37 (s, 2H), 2.45 (m, 4H) 1.53-1.59 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 186.6, 159.8, 155.6, 151.2, 149.9, 148.2, 145.7, 134.5, 133.1, 130.2, 128.5, 126.0, 125.3, 124.1, 122.3, 119.0, 118.9, 117.0, 113.2, 111.1, 70.1 and 56.4. HRMS-ESI: m/z $[M + H]^+$ for $C_{26}H_{26}NO_6$, calculated 448.1760; observed 448.1433.

Biology: Materials and method

Evaluation of antimalarial activity

Chloroquine-sensitive strain 3D7 and multidrug-resistant strain K1 (resistant to chloroquine, sulfadoxine-pyrimethamine, chlorproguanil) of *P. falciparum* were maintained at 6-8% parasitemia and 2% hematocrit in RPMI complete medium (RPMI 1640 supplemented with HEPES, 0.2% sodium bicarbonate, 0.2% D-glucose, 0.5% albumax, 45 mg/L hypoxanthine, 0.25 mg/L fungi zone and 50 mg/L gentamycin) at 37 °C in a humidified CO_2 incubator. The antimalarial activity was determined using SYBR Green–I based fluorescence assay (Smilkstein et al., 2004). Chloroquine (C-6628, Sigma) was used as a reference drug. Parasite inhibition experiments were conducted at 0.8% parasite maintained at 1% hematocrit in RPMI medium. Ring stage parasites were treated with different dilutions of compounds in a 96-well plate (37 °C, 72 h). Control experiments included untreated parasites (infected-RBCs), DMSO treated (infected-RBCs) and non-infected RBCs. The stock solution of compounds (1 mM) is prepared in

DMSO. To minimize the contribution of DMSO on parasite growth, the highest concentration of 5 µM was used, followed by two-fold serial dilutions of compound (seven-point evaluation) in RPMI media. In parallel, parasite culture was maintained in 60 mm dish without any drug to monitor the parasite growth (37 °C, 72 h). After the 72 h, 100 µl lysis buffer [20 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.008% saponin, and 0.08% Triton X-100] containing 2× SYBR Green dye (S7585) was added in each well of 96-well plate and incubated (37 °C, 1 h). The fluorescence was recorded in an FLX800, BIOTEK instrument (excitation at 480 nm, emission at 520 nm). Data were analyzed to obtain inhibitory concentration (IC_{50}) values. The compounds which showed $IC_{50} \leq 5$ µM were only taken forward for assessment of IC_{50} values in chloroquine-resistant (K1 strain) and CC_{50} in VERO cells.

Cytotoxicity (CC_{50}) was evaluated in VERO cells (C 1008; monkey kidney epithelial cells) using the MTT assay. VERO cells were maintained in RPMI media supplemented with HEPES, 0.2% sodium bicarbonate, 0.2% D-glucose, 10% FBS, fungi zone (0.25 mg/L) and gentamycin (50 mg/L) at 37 °C in a humidified CO_2 incubator. VERO cells (104/well) were seeded in a 96 well plate and cells were treated with different dilutions of compounds (16–18 h post-seeding). Podophyllotoxin (P4405, Sigma) was used as the positive control. After 72 h, 25 µl of MTT (M2128, Sigma) (stock 5 mg/ml) was added to each well and incubated for 2 h in a CO_2 incubator. The supernatant was removed carefully without disturbing the cells and 150 µl DMSO was added to each well to dissolve the purple precipitate. Absorbance was recorded at 540 nm using an ELISA plate reader and data was analyzed to determine 50% cytotoxic concentration (CC_{50}). For microscopic examination, 3D7 was synchronized with 5% sorbitol. The ring-stage parasite was maintained at 6–8% parasitemia with 2% haematocrit and treated with compound 7 at 10 µM concentration. After 24 h and 48 h of treatment, thin blood smears of both control and treated culture were prepared. Smears were fixed and stained with methanol and Giemsa, respectively. In a parallel experiment, after 24 h of treatment, the culture was washed (twice) with RPMI media to remove the drug and was further incubated without the drug to check the revival of the parasite after drug removal.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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