Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

NOVEL QUINAZOLINE-CHALCONE HYBRIDS AS ANTIPLASMODIUM AGENTS: SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR DOCKING

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ABSTRACT

Development of new antiplasmodial molecular scaffold is an urgent need to overcome the problem of resistance against the malaria parasite. A series of quinazoline-chalcone hybrids, covalently linked by amine linkage were synthesized in a convenient way starting from commercially available 2-amino benzoic acid in a five step process. Synthesized hybrids evaluated for *in vitro* antimalarial activity against CQ-sensitive (MRC-2) and CQ-resistant (RKL-9) *P. falciparum* strains. Nine out of sixteen compounds found to be more potent to chloroquine against CQ-sensitive strain (MRC-2) and six compounds observed to be more or equipotent to chloroquine against CQ-resistant strain RKL-9. The *in vitro* cytotoxicity study performed on the human HepG2 cell line and the selectivity index found in ranges from 4 to 203 against CQ-resistant strain RKL-9. *In vitro* heme crystallization inhibition assay was carried out where most of the synthesized hybrids showed considerable inhibition of β-hematin formation suggesting that compounds are strongly interfering with the hemozoin formation in parasites. Further, molecular docking study against the *cysteine protease falcipain-2* was performed to explore the binding affinity and orientations between between the hybrids and the target enzyme. ASN21 and CYS22 amino acids of protein act as hydrogen bond donor region facilitate hydrogen bonding with ligand. Overall, findings support the antimalarial potential of quinazoline-chalcone hybrids.

Keywords: Quinazoline, *Cysteine protease falcipain-2,* Cytotoxicity assay, Heme crystallization inhibition assay, Docking.

1. INTRODUCTION

Malaria enforces a severe social burden that has delayed economic development in regions where it is endemic. The World Health Organization (WHO) estimated that 228 million malaria cases globally with 405,000 malaria-related fatal outcomes worldwide, where India and 15 sub-Saharan African countries bear the largest share of burden across the world in 2019. Over the past decades, *Plasmodium falciparum*, has developed resistance mechanisms to almost all existing anti-malarial drugs with a significant impact on malaria control [1, 2].

The situation is gradually worsening mainly due to non-availability of effective drugs and development of drug resistance in areas where malaria is frequently transmitted. Therefore, an urgent effort is necessary for the development of structurally novel and effective antimalarial drugs to overcome the problem of resistance. In the past decade, multi-target drugs have raised considerable interest among researchers due to their advantages in the treatment of diseases with complex patho-mechanisms and health disorders linked to issues of drug resistance [3]. This approach may be beneficial in overcoming drug resistance such as in antimicrobial chemotherapy [3, 4]. Covalent bi therapy or double drug is a rational chemistry-based approach which involves the covalently linking two molecules, each with their own mode of action, to produce a single hybrid molecule with dual activity. Literature postulates that hybrid molecules not only exhibit improved therapeutic response and low risk of drug-drug interaction but also cost effective which in turn reduces overall drug pressure of the treatment [5].

Based on the above facts and an exhaustive literature survey, we aim to synthesize novel hybrid molecules through direct coupling between the structural scaffolds of quinazoline-chalcone. Considering all the scaffolds of heterocyclic ring, quinazoline is the most commonly encountered heterocyclic compound in medicinal chemistry have received considerable attention in the recent years due to their diverse pharmacological activities [6]. Several bio-active natural products, such as febrifugine and isofebrifugine, are quinazoline

alkaloids isolated from the roots of Chinese herb *Dichroa febrifuga* as the active component against malaria but its use has declined due to severe toxicity [7, 8]. The potent antimalarial activity of febrifugine has been stimulated medicinal chemists to pursue compounds derived from febrifugine, which may act as valuable lead for novel drugs. Over the last few decades, there has been an extensive focus of research towards the investigation of the antiplasmodial potential of the quinazoline ring with promising results [9-12].

The second pharmacologically active substance, chalcone remains an attraction among synthetic chemistry scholars because of its distinctive potential analogues with a variety of substitutions, which can be synthesized unambiguously by the cost-efficient Claisen-Schmidt condensation [13]. The antimalarial activity of chalcones was apparent first time as inhibitors of proteases after the first report on, "licochalcone A" extracted from the Chinese liquorice roots [14]. Later, many more potential analogues of licochalcone A with different substitution have been reported for substantial anti-malarial activity. In addition to this, there has been an extensive focus of research towards the investigation of antiplasmodial potential of the chalcones for various infectious diseases including malaria. Variety of chalcones viz. alkoxylated, prenylated, hydroxylated, quinolinated, oxygenated were derived from either syntheses or natural sources have been assessed for antiplasmodial activity with promising outcomes [15-21]. Several mechanisms have been postulated for anti-malarial activity of various chalcones [22-26], the exact mode of action still remains unclear. Based on molecular modeling studies the linear and planer structural features of chalcones enables them to fit appropriately within the active site of *Plasmodium* and *Trypanosoma* cysteine proteases where through they mostly supposed to prevent host haemoglobin degradation [27].

In continuation of our research program focused on the design and synthesis of heterocyclic compounds for infectious diseases [28-30], this time we intended to combine quinazoline and chalcone in one molecule (Fig. 1), expecting that quinazoline-chalcone hybrids may present improved biological activities compared to the isolated parental structures. In the present study, the designing of synthesized molecules were based on the results of molecular modeling studies (i.e. QSAR and pharmacophore mapping) and further screened through docking, molecular dynamic simulation and *in silico* ADME tool which was reported previously [29]. Finally, sixteen computationally driven molecules were selected for their experimental verification.

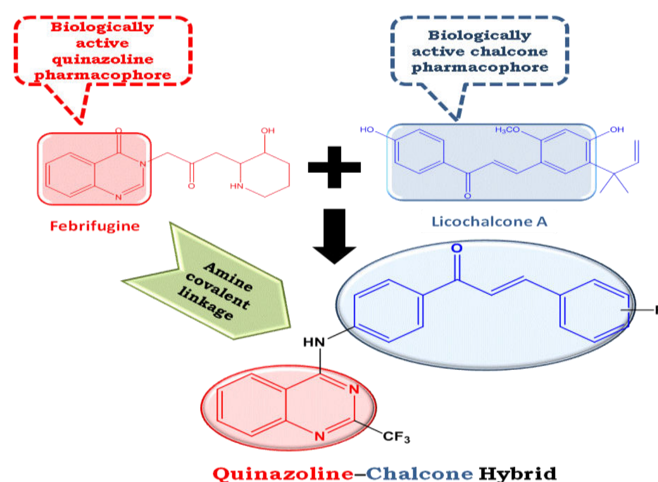


Fig. 1: Designed quinazoline-chalcone hybrid molecule

2. EXPERIMENTAL SECTION

2.1. General

All the chemical reagents were obtained from Sigma Aldrich chemical Co, USA. All the organic solvents used were of high purity unless otherwise stated. Melting points were determined in one end open capillary tubes on a Buchi 530 apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Bruker Alpha-I FTIR Spectrometer in KBr. ^1H and ^{13}C NMR spectra were recorded on Bruker DRX-400 MHz spectrometer in DMSO/$CDCl_3$. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a micro TOF-Q II 10330 intrument. Elemental analysis was undertaken with Elemental vario EL III Carlo Erba 1108 analyzer. All reactions as well as the purity of the compounds were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh) using solvent system of n-hexane: ethyl acetate (9:1). The spots were developed in iodine chamber and visualized under ultra violet lamp.

2.1.1. Synthesis of 2–trifluoromethyl-4H- benzo [d][1,3]oxazin-4-one (A)

2-Amino benzoic acid (0.05 mol, 6.85g) was dissolved in 13 ml of trifluoroacetic anhydride in a flat bottom flask. The mixture was stirred and refluxed for 2 h at 40°C under anhydrous conditions. Thereafter, acetic anhydride (10 ml) was added and the mixture was further refluxed for 2 h at 110°C. The reaction mixture

was then cooled and subjected to vacuum evaporation to obtain off white solid of 2-(trifluoromethyl)-4H-benzo[d] [1,3] oxazin-4-one (A) and recrystallized with absolute ethanol.

Light brown solid, yield: 86%; mp: 174-176°C; FTIR (KBr) ν: 1033 cm^{-1} (C-O-C symmetric stretching), 1231 cm^{-1} (C-O-C asymmetric stretching), 930 cm^{-1} (oxazine ring). ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm) 8.46 (d, J=7.84Hz,1H, het-Ar-H), 7.98-7.95 (m, 1H, het-Ar-H) 7.58-7.54 (m,1H, het-Ar-H), 7.14-7.11 (m,1H, het-Ar-H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm):168.4, 169.4, 116.3, 122.4, 124.2, 133.9, 130.9 and 140.8 (C_2, C_4, C_5, C_6, C_7, C_8, C_9 and C_{10} of quinazoline ring), 119.8 (trifluoromethyl C); ESI-MS (m/z): 215.0 (M^+); Anal. calcd. for $C_9H_4F_3NO_2$: C, 50.25; H, 1.87; N, 6.51. Found: C, 50.19; H, 1.78; N, 6.53.

2.1.2. Synthesis of compound 2–trifluoromethyl-3H-quinazolin-4-one (B)

The 2-trifluoro methyl-4*H*-benzo[*d*][1,3]oxazin-4-one (A) (0.05 mol, 10.76g), was dissolved in formamide (12 ml) with 2 or 3 drops of glacial acetic acid. The mixture refluxed for 4 hr and completion of reaction was monitored by TLC. The solid obtained was filtered and the crude product 2-trifluromethyl-3H-quinazolin-4-one (B) thus formed was washed with petroleum ether and dried.

Light brown solid, yield: 85%; mp: 165-167°C; FTIR (KBr) ν: 3328cm^{-1} (N-H), 3108 cm^{-1} (Ar-H), 1728 cm^{-1} (C=O), 1641 cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm) 7.32-7.35 (m, 4H, het-Ar-H) 8.0 (s,1H, N-H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 173.1, 175.0, 125.0, 149.1, 134.9, 154.9, 132.2 and 162.2 (C_2, C_4, C_5, C_6, C_7, C_8, C_9 and C_{10} of quinazoline ring), 120.8 (trifluoromethyl C); ESI-MS (m/z): 214.1 (M^+); Anal. calcd. for $C_9H_5F_3N_2O$: C, 50.48; H, 2.35; N, 13.08. Found: C, 50.45; H, 2.38; N, 13.09.

2.1.3. Synthesis of 4-chloro-2–trifluoromethyl-3,4-dihydroquinazoline (C)

The mixture of 2–trifluoromethyl-3H-quinazolin-4-one (B) (0.05 mol, 10.7g), and phosphorous oxychloride (0.2 mol, 20 mL) were stirred and refluxed until reaction completed at 115-120°C. Excess of phosphorous oxychloride was removed by distillation under reduced pressure. The content was extracted three times with ethyl acetate and washed with the solution of sodium bicarbonate (10%). The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The formed solid was recrystallized by ethanol.

Yellowish brown solid; yield: 72%; mp: 170-173°C; FTIR (KBr) ν:3112 cm^{-1} (Ar-H), 1637cm^{-1}(C=N), 1169 cm^{-1} (C-F), 745 cm^{-1} (C-Cl); ^{1}H NMR (400MHz, DMSO-*d6*, δ ppm)8.17 (d, J=2.5 Hz, 1H, het-Ar-H) 7.86 (d, J=2.5 Hz, 1H, het-Ar-H) 7.25 (t, J=2.5, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 169.8, 134.7, 119.5, 119.6, 131.7, 138.0, 121.6 and 154.2 (C_2, C_4, C_5, C_6, C_7, C_8, C_9 and C_{10} of quinazoline ring), 117.2 (trifluoromethyl C); ESI-MS (m/z): 234.3 (M^+); Anal. calcd. for $C_9H_6ClF_3N_2$: C, 46.08; H, 2.58; N, 11.94. Found: C, 46.04; H, 2.62; N, 11.93.

2.1.4. Synthesis of of 1-(4-(2-(trifluoromethyl) quinazolin-4-ylamino)phenyl)ethanone (D)

4-chloro-2–trifluromethyl-3,4-dihydroquinazoline (**C**) (0.05 mol, 11.63g) and 4-amino acetophenone (0.05 mol, 6.76g) was mixed with ethanol (15 ml) and refluxed at 130-150°C for 6h and completion of reaction was monitored by TLC. The reaction mixture was cooled at room temperature and neutralized with 10% sodium bicarbonate solution. The resulting organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The solid product was dried and purified by column chromatography by using silica gel as adsorbent eluting with n-hexane/ethyl acetate (9:1) to afford the corresponding products.

Yellowish brown solid; yield:82%; mp:180-183°C; FTIR (KBr) ν:3323 cm^{-1} (N-H), 3111 cm^{-1} (Ar-H), 2918 cm^{-1} (C-H), 1690 cm^{-1} (C=O), 1657 cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm) 9.49 (s, 1H, NH), 8.26 (d, J=8.0Hz, 2H, het-Ar-H) 8.05-8.03 (m, 2H, het-Ar-H), 7.72-7.66 (m, 2H, Ar-H), 7.36 (t, J=7.5Hz, 2H, Ar-H), 2.97 (s, 3H, CH_3);^{13}C NMR (100 MHz, DMSO-*d6*, δ ppm): 161.2, 170.9, 111.1, 129.4, 129.8, 132.0, 132.5 and 152.4 (C_2, C_4, C_5, C_6, C_7, C_8, C_9 and C_{10} of quinazoline), 110.9 (trifluoromethyl C), 141.4, 125.7,131.0, 126.0, 130.5 and 121.7 (C_{12}, C_{13}, C_{14}, C_{15}, C_{16} and C_{17} of benzene), 189.9 (C_{18} of C=O) 30.2 (C_{19} of CH_3); ESI-MS (m/z): 331.1 (M^+); Anal. calcd. for $C_{17}H_{12}F_3N_3O$: C, 61.63; H, 3.65; N, 12.68. Found: C, 61.58; H, 3.70; N, 12.66.

2.1.5. General Synthesis of (E)-3-phenyl-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino) phenyl) prop-2-en-1-one **(1-16)**.

To the 250ml round bottom flask 2-(trifluoromethyl) quinazolin-4-ylamino) phenyl) ethanone (D) (0.01mol,

3.31g) was dissolved in ethanol, followed by addition of various substituted benzaldehyde (0.01 mol) & sodium hydroxide (10%). The mixture was stirred and refluxed and completion of reaction monitored through TLC. After cooling mixture was acidified with dilute HCl. The resulting precipitate was collected by filtration, washed with water, diethyl ether & and recrystallized from 75% ethanol to afford pure compound.

2.1.5.1. (E)-3-(4-fluorophenyl)-1-(4-(2-(trifleoromethyl) quinazolin-4-ylamino) phenyl)prop-2-en-1-one (1)

Yellowish brown; Yield: 77%; mp: 192-194°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.8 (s, 1H, NH), 8.61 (d, J=4.4 Hz, 1H, het-Ar-H), 8.36 (t, J=9.2 Hz, 3H, het-Ar-H), 7.72 (d, J=15.6 Hz, 1H, vinylic-H), 7.52 (d, J=8.0 Hz, 4H, Ar-H), 7.23 (d, J=7.2 Hz, 4H, Ar-H), 7.05 (d, J=15.6 Hz, 1H, vinylic-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm):189.0, 158.77, 149.14, 148.65, 138.12, 138.82, 138.26, 137.72, 137.61, 137. 42, 136.27, 136.25, 136.15, 135.41, 134.96, 129.01, 123.96, 123.74, 116.44, 114.82; ESI-MS (m/z):437.1 (M^+); Anal. calcd. for $C_{24}H_{15}F_4N_3O$:C, 65.90; H, 3.46; N, 9.61. Found: C, 65.80; H, 3.66; N, 9.51.

2.1.5.2. (E)-3-(2-fluorophenyl)-1-(4-(2-(trifluoromethyl) quinazolin-4-ylamino)phenyl)prop-2-en-1-one (2)

Yellowish brown; Yield: 71%; mp 195-197°C;^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.15 (s, 1H, NH), 8.73 (d, J=15.6 Hz, 1H, vinylic-H), 8.50 (t, J=8.8 Hz, 1H, het-Ar-H), 8.44 (d, J=7.6 Hz, 2H, Ar-H), 8.27 (t, J=8.4 Hz, 1H, het-Ar-H), 8.17 (d, J=7.6 Hz, 1H, het-Ar-H), 7.98 (d, J=8.4 Hz, 2H, Ar-H), 7.94 (d, J=7.2 Hz, 1H, het-Ar-H), 7.82 (d, J=15.6 Hz, 1H, vinylic-H), 7.22 (t, J=8.0 Hz, 1H, Ar-H), 7.66-7.54 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.95, 160.28, 151.96, 146.85, 145.66, 137.42, 134.15, 134.05, 133.27, 133.0, 132.61, 132.08, 131.51, 131.0, 130.02, 126.50, 125.30, 124.02, 115.61, 114.96; ESI-MS (m/z): 437.1 (M^+); Anal. calcd. for $C_{24}H_{15}F_4N_3O$: C, 65.90; H, 3.46; N, 9.61. Found: C, 65.86; H, 3.58; N, 9.71.

2.1.5.3. (E)-3-(4-chlorophenyl)-1-(4-(2-(trifluoromethyl) quinazolin-4-ylamino)phenyl) prop-2-en-1-one (3)

Pale Yellow solid; Yield: 72%; mp 198-199°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.21 (s, 1H, NH), 8.20 (d, J=8.0 Hz, 2H, het-Ar-H), 7.92 (d, J=15.6 Hz, 1H, vinylic-H), 7.86 (d, J=7.6 Hz, 3H, Ar-H), 7.76 (d, J=15.6 Hz, 1H, vinylic-H), 7.62 (d, J=8.4 Hz, 3H, Ar-H), 7.56 (t, J=8.0 Hz, 2H, het-Ar-H), 7.15 (d, J=8.4 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.95, 160.28, 151.08, 143.15, 141.66, 133.85, 131.13, 130.82, 130.26, 130.0, 129.61, 129.42, 129.27, 129.15, 127.95, 122.50, 122.30, 121.02, 114.96, 113.74; ESI-MS (m/z): 453.9 (M^+); Anal. calcd. for $C_{24}H_{15}ClF_3N_3O$: C, 63.51; H, 3.33; N, 9.26. Found: C, 63.54; H, 3.24; N, 9.31.

2.1.5.4. (E)-3-(3-bromophenyl)-1-(4-(2-(trifluoromethyl) quinazolin-4-ylamino)phenyl) prop-2-en-1-one (4)

Brown solid; Yield: 78%; mp 220-223°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.65 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 7.97 (t, J=8.4 Hz, 3H, het-Ar-H, Ar-H), 7.77 (d, J=15.6 Hz, 1H, vinylic-H), 7.67 (d, J=8.8 Hz, 2H, het-Ar-H), 7.36 (d, J=9.6 Hz, 2H, Ar-H), 7.27 (d, J=8.4 Hz, 2H, Ar-H), 7.18 (d, J=8.4 Hz, 2H, Ar-H), 6.62 (d, J=15.6 Hz, 1H, vinylic-H);^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 189.0, 158.99, 155.0, 148.62, 145.99, 143.04, 142.0, 139.50, 138.99, 137.88, 136. 0, 134.75, 132.37, 132.0, 131.78, 130.40, 129.95, 127.41, 124.71, 121.84, 117.0, 114.22;ESI-MS (m/z): 497.4 (M^+); Anal. calcd. for $C_{24}H_{15}BrF_3N_3O$: C, 57.85; H, 3.03; N, 8.43. Found: C, 57.68; H, 3.27; N, 8.14.

2.1.5.5. (E)-3-(4-(trifluoromethyl)phenyl)-1-(4-(2-(trifluoro-methyl)quinazolin-4-ylamino) phenyl)prop-2-en-1-one (5)

Yellowish brown solid; Yield: 67.9%; mp 210-214°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.38 (s, 1H, NH), 8.13-7.95 (m, 1H, het-Ar-H), 7.92 (d, J=15.6 Hz, 1H, vinylic-H), 7.86 (d, J=8.0 Hz, 2H, het-Ar-H), 7.81 (d, J=8.0 Hz, 2H, Ar-H), 7.76 (d, J=3.2 Hz, 2H, Ar-H), 7.72 (d, J=4.0 Hz, 2H, Ar-H), 7.62-7.52 (m, 1H, het-Ar-H), 7.51-7.40 (m, 1H, Ar-H), 7.38-7.25 (m,1H, Ar-H), 7.10 (d, J=15.6 Hz, 1H, vinylic-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.95, 156.56, 153.81, 145.0, 142.36, 139.84, 137.0, 135.28, 134.0, 131.79, 131.0, 130.09, 129.89, 129.51, 129.0, 127. 83,125.20, 124.76, 122.11, 121.0, 117.0; ESI-MS (m/z): 487.1 (M^+); Anal. calcd. for $C_{25}H_{15}F_6N_3O$:C, 61.61; H, 3.10; N, 8.62. Found: C, 61.54; H, 3.34; N, 8.51.

2.1.5.6. (E)-3-(3-(trifluoromethyl)phenyl)-1-(4-(2-(trifluoro-methyl)quinazolin-4-ylamino)phenyl)prop-2-en-1-one (6)

Yellowish brown solid; Yield: 77%; mp 205-208°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.17 (s, 1H, NH), 8.34 (d, J=7.6 Hz, 2H, het-Ar-H), 8.28 (d, J=14.4 Hz, 1H, vinylic-H), 8.14 (s, 1H), 7.85 (d, J=7.2 Hz, 2H, Ar-H), 7.81 (d, J=4.0 Hz, 2H, Ar-H), 7.78 (d, J=7.6

Hz, 2H, Ar-H), 7.69 (d, J=14.4 Hz, 1H, vinylic-H), 7.57 (t, J=6.8 Hz, 1H, het-Ar-H), 7.49 (t, J=7.2 Hz, 2H, het-Ar-H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.96, 146.81, 141.21, 138.56, 135.81, 134.0, 132.55, 130.50, 130.37, 130.25, 130.0, 129.75, 129. 52, 129.34, 129.21, 129.0, 128.99, 128.92, 128.26, 127.82, 122.85, 122.63, 119.0; ESI-MS (m/z): 487.1 (M^+); Anal. calcd. for $C_{25}H_{15}F_6N_3O$: C, 61.61; H, 3.10; N, 8.62. Found: C, 61.55; H, 3.32; N, 8.43.

2.1.5.7. (E)-3-(4-nitrophenyl)-1-(4-(2-(trifluoromethyl) quinazolin-4-ylamino)phenyl)prop-2-en-1-one (7)

Orange solid; Yield: 81%; mp >250°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.07 (s, 1H, NH), 8.24 (d, J=8.4 Hz, 2H,Ar-H), 8.05 (d, J=9.6 Hz, 2H, Ar-H), 7.98 (d, J=8.8 Hz, 1H, het-Ar-H), 7.82 (d, J=15.6 Hz, 1H, vinylic-H), 7.78 (d, J=8.8 Hz, 2H, Ar-H), 7.72 (d, J=8.0 Hz, 2H, Ar-H), 7.64 (d, J=7.6 Hz, 1H, het-Ar-H), 7.57 (d, J=14.4 Hz, 1H, vinylic-H), 7.50 (t, J=7.6 Hz, 2H, het-Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 189.00, 151.45, 150.90, 143.21, 140.79, 138.80, 137.62, 135.38, 134.89, 133.74, 133.50, 133.0, 132.69, 131.36, 128.85, 128.0, 125.90, 120.72, 117.0, 114.20; ESI-MS (m/z): 464.1 (M^+); Anal. calcd. for $C_{24}H_{15}F_3N_4O_3$: C, 62.07; H, 3.26; N, 12.06. Found: C, 62.17; H, 3.46; N, 12.10.

2.1.5.8. (E)-3-m-tolyl-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl)prop-2-en-1-one (8)

Pale Yellow solid ; Yield: 56%; mp 235-238°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.63 (s, 1H, NH), 8.25 (d, J=7.2 Hz, 1H, het-Ar-H), 7.98 (d, J=7.6 Hz, 2H, het-Ar-H), 7.92 (d, J=7.6 Hz, 3H, Ar-H), 7.82 (d, J=15.2 Hz, 1H, vinylic-H), 7.68 (d, J=15.2 Hz, 1H, vinylic-H), 7.63 (d, J=8.4 Hz, 3H, Ar-H), 6.74 (d, J=8.0 Hz, 2H, Ar-H), 6.60 (s, 1H, Ar-H), 2.36 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.99, 157.88, 153.78, 147.21, 144.86, 141.26, 140.0, 137.65, 137.48, 136.42, 135.61, 134.85, 133.83, 133.19, 133.08, 132.78, 128.99, 126.95, 123.22, 120.52, 117.86, 113.0, 29.70; ESI-MS (m/z): 433.1 (M^+); Anal. calcd. for $C_{25}H_{18}F_3N_3O$: C, 69.28; H, 4.19; N, 9.69. Found: C, 69.47; H, 4.25; N, 9.54.

2.1.5.9. (E)-3-(4-ethylphenyl)-1-(4-(2-(trifluoromethyl) quinazolin-4-ylamino)phenyl)prop-2-en-1-one (9)

Pale Yellow solid; Yield: 62%; mp 221-223°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.18 (s, 1H, NH), 8.32 (t, J=8.4 Hz, 1H, het-Ar-H), 8.10 (t, J=8.4 Hz, 1H, het-Ar-H), 7.97 (d, J=8.8 Hz, 2H, het-Ar-H), 7.89 (d, J=8.8 Hz, 2H, Ar-H), 7.83 (d, J=8.8 Hz, 1H, Ar-H), 7.76 (d, J=15.6 Hz, 1H, vinylic-H), 7.59 (d, J=6.8 Hz, 1H, Ar-H), 7.58 (d, J=5.2 Hz, 1H, Ar-H), 7.55-7.31 (m, 1H, Ar-H), 7.04 (d, J=15.6 Hz, 1H, vinylic-H), 6.72 (d, J=8.4 Hz, 2H, Ar-H), 2.49 (q, J=8.0 Hz, 2H, CH_2), 1.09 (t, J=7.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 189.00, 152.60, 144.68, 141.99, 138.76, 137.37, 135.65, 135.12, 133.78, 132.31, 131.99, 131.67, 130.49, 129.0, 126.44, 125.79, 124.25, 120.64, 117.79, 113.0, 24.40, 14.25; ESI-MS (m/z): 447.6 (M^+); Anal. calcd. for $C_{26}H_{20}F_3N_3O$: C, 69.79; H, 4.51; N, 9.39. Found: C, 69.55; H, 4.59; N, 9.34.

2.1.5.10. (E)-3-(3-hydroxyphenyl)-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl)prop-2-en-1-one (10)

Light brown solid; Yield: 65%; mp 215-217°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 11.58 (s, 1H, OH), 9.94 (s, 1H, NH), 8.27 (d, J=15.6 Hz, 1H, vinylic-H), 8.18 (d, J=8.4 Hz, 1H, het-Ar-H), 7.96 (d, J=8.0 Hz, 1H, het-Ar-H), 7.88 (d, J=8.4 Hz, 2H, Ar-H), 7.83-7.23 (m, 1H, het-Ar-H), 7.68 (d, J=15.2 Hz, 1H, vinylic-H), 7.53-7.41 (m, 1H, het-Ar-H), 7.36 (d, J=7.6 Hz, 2H, Ar-H), 7.28 (t, J=9.2 Hz, 1H, Ar-H), 7.18 (d, J=8.4 Hz, 1H, Ar-H), 6.65 (d, J=8.4 Hz, 1H, Ar-H), 6.61 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.95, 156.0, 147.0, 144.20, 141.12, 138.74, 135.85, 130.82, 130.62, 130.26, 130.0, 129.82, 129.61, 129.42, 129.27, 129.25, 129.15, 128.76, 128.11, 121.01, 117.36, 116.01; ESI-MS (m/z): 435.2 (M^+); Anal. calcd. for $C_{24}H_{16}F_3N_3O_2$: C, 66.21; H, 3.70; N, 9.65. Found: C, 66.44; H, 3.83; N, 9.56.

2.1.5.11. (E)-3-(4-methoxyphenyl)-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl) prop-2-en-1-one (11)

Pale Yellow solid; Yield: 71%; mp 240-244°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.78 (s, 1H, NH), 8.34 (d, J=8.4 Hz, 1H, het-Ar-H), 8.15 (d, J=8.8 Hz, 2H, Ar-H), 8.08 (d, J=8.8 Hz, 1H, het-Ar-H), 7.93 (d, J=15.6 Hz, 1H, vinylic-H), 7.87 (d, J=8.8 Hz, 2H, Ar-H), 7.83 (d, J=8.0 Hz, 1H, Ar-H), 7.75 (d, J=7.6 Hz, 1H, Ar-H), 7.66 (d, J=15.6 Hz, 1H, vinylic-H), 7.58 (t, J=10.8 Hz, 2H, het-Ar-H), 6.69 (d, J=7.6 Hz, 2H, Ar-H), 3.28 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.91, 152.75, 148.38, 140.64, 140.25, 139.99, 139.09, 138.98, 138.15, 138.0, 134.12,

133.81, 132.91, 132.55, 130.25, 130.11, 129.41, 125.31, 116.91, 116.30, 55.88; ESI-MS (m/z): 449.4 (M^+); Anal. calcd. for $C_{25}H_{18}F_3N_3O_2$: C, 66.81; H, 4.04; N, 9.35. Found: C, 66.93; H, 4.23; N, 9.43.

2.1.5.12. (E)-3-(4-(dimethylamino)phenyl)-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl) prop-2-en-1-one (12)

Reddish yellow solid; Yield: 70%; mp 236-238°C; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 9.72 (s, 1H, NH), 8.11 (d, J=15.6 Hz, 1H, vinylic-H), 7.93 (t, J=8.4 Hz, 1H, het-Ar-H), 7.87 (d, J=15.6 Hz, 1H, vinylic-H), 7.84-7.72 (m, 1H, Ar-H), 7.62-7.49 (m, 1H, Ar-H), 7.42 (d, J=8.8 Hz, 2H, het-Ar-H), 7.39 (d, J=8.4 Hz, 1H, Ar-H), 7.36 (d, J=2.4 Hz, 2H, Ar-H), 7.33 (d, J=8.8 Hz, 2H, Ar-H), 6.71 (d, J=4.8 Hz, 2H, Ar-H), 2.53 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.99, 156.99, 152.85, 146.05, 143.35, 142.05, 139.80, 138.98, 137.77, 135.86, 131.85, 130.0, 128.06, 126.11, 125.64, 125.35, 123.64, 122.90, 117.95, 115.0, 41.54; ESI-MS (m/z): 462.7 (M^+); Anal. calcd. for $C_{26}H_{21}F_3N_4O$: C, 67.52; H, 4.58; N, 12.11. Found: C, 67.32; H, 4.78; N, 12.02.

2.1.5.13. (E)-3-(3-(dimethylamino)phenyl)-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl) prop-2-en-1-one (13)

Reddish yellow solid; Yield:72%; mp 245-247°C; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 9.70 (s, 1H, NH), 8.44 (t, J=8.0 Hz, 1H, het-Ar-H), 8.37 (d, J=15.6 Hz, 1H, vinylic-H), 8.20 (d, J=8.8 Hz, 1H, het-Ar-H), 8.11 (d, J=7.2 Hz, 1H, Ar-H), 7.92 (d, J=8.4 Hz, 1H, het-Ar-H), 7.86 (d, J=7.2 Hz, 1H, Ar-H), 7.77 (d, J=15.6 Hz, 1H, vinylic-H), 7.67 (d, J=7.6 Hz, 1H, Ar-H), 7.62 (d, J=8.8 Hz, 2H, Ar-H), 7.56 (t, J=8.0 Hz, 1H, het-Ar-H), 7.50 (t, J=7.6 Hz, 1H, Ar-H), 6.69 (d, J=8.8 Hz, 1H, Ar-H), 6.20 (s, 1H, Ar-H), 2.39 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 189.00, 154.88, 152.09, 145.73, 144.65, 141.92, 139.64, 139.0, 136.52, 135.15, 134.76, 134.20, 134.0, 133.01, 132.32, 130.02, 128.91, 127.0, 124.59, 121.78, 117.0, 114.30, 41.0; ESI-MS (m/z): 462.7 (M^+); Anal. calcd. for $C_{26}H_{21}F_3N_4O$: C, 67.52; H, 4.58; N, 12.11. Found: C, 67.73; H, 4.44; N, 12.05.

2.1.5.14. (E)-3-(3,4-dichlorophenyl)-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl) prop-2-en-1-one (14)

Pale yellow solid; Yield: 72%; mp 206-208°C; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 10.04 (s, 1H, NH), 8.48 (d, J=7.2 Hz, 2H, het-Ar-H), 8.13 (d, J=7.2 Hz, 2H, het-Ar-H), 8.09 (d, J=7.2 Hz, 2H, Ar-H), 7.90 (s, 1H, Ar-H), 7.73 (d, J=15.6 Hz, 1H, vinylic-H), 7.53 (d, J=7.6 Hz, 2H, Ar-H), 7.2 (d, J=8.8 Hz, 2H, Ar-H), 7.06 (d, J=15.6 Hz, 1H, vinylic-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.95, 154.50, 151.75, 143.0, 142.95, 140.64, 140.0, 137.42, 136.0, 135.79, 135.30, 135.0, 133.94, 133.21, 131.0, 129.99, 128.0, 125.51, 122.80, 120.02, 119.30, 118.30; ESI-MS (m/z): 487.5 (M^+); Anal. calcd. for $C_{24}H_{14}Cl_2F_3N_3O$: C, 59.03; H, 2.89; N, 8.61. Found: C, 59.22; H, 3.02; N, 8.54.

2.1.5.15. (E)-3-(4-(diethylamino)phenyl)-1-(4-(2-(trifleoromethyl)quinazolin-4-ylamino)phenyl)prop-2-en-1-one (15)

Yellow solid; Yield: 69%; MP >250°C; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 9.82 (s, 1H, NH), 8.10 (d, J=14.4 Hz, 1H, vinylic-H), 7.88 (d, J=7.6 Hz, 2H, het-Ar-H), 7.72 (d, J=14.4 Hz, 1H, vinylic-H), 7.60 (d, J=8.4 Hz, 4H, Ar-H), 7.53 (t, J=10.0 Hz, 2H, het-Ar-H), 6.77 (d, J=8.4 Hz, 2H, Ar-H), 3.15 (q, J=5.2 Hz, 4H, CH_2), 1.21 (t, J=7.2 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 188.95, 148.30, 145.75, 139.38, 136.81, 130.80, 130.57, 130.35, 130.11, 129.90, 129.78, 129.60, 129.41, 128.95, 128.88, 128.55, 128.26, 127.92, 118.08, 112.48, 41.54, 12.25; ESI-MS (m/z): 490.2 (M^+); Anal. calcd. for $C_{26}H_{20}F_3N_3O$: C, 68.56; H, 5.14; N, 11.42. Found: C, 68.66; H, 5.20; N, 11.30.

2.1.5.16. (E)-3-phenyl-1-(4-(2-(trifluoromethyl)quinazolin-4-ylamino)phenyl)prop-2-en-1-one (16)

Pale yellow solid; Yield: 61%; mp 189-192°C; ^1H NMR (400 MHz, DMSO, δ ppm): 9.09 (s, 1H, NH), 8.27 (d, J=6.8 Hz, 1H, het-Ar-H), 7.97 (d, J=15.6 Hz, 1H, vinylic-H), 7.94 (d, J=5.2 Hz, 2H, het-Ar-H, Ar-H), 7.89 (d, J=6.8 Hz, 1H, Ar-H), 7.81 (d, J=6.0 Hz, 2H, Ar-H), 7.76 (d, J=15.6 Hz, 1H, vinylic-H), 7.74-7.70 (m, 3H, Ar-H), 7.43 (t, J=7.2 Hz, 2H, het-Ar-H), 7.35 (q, J=6.8 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO, δ ppm): 189.02, 165.90, 158.67, 158.47, 149.96, 149.50, 149.0, 138.47, 138.20, 133.55, 132.96, 132.45, 131.99, 131.25, 130.89, 127.50, 127.20, 124.26, 123.78, 123.30, 115.0, 114.89; ESI-MS (m/z): 419.2 (M^+); Anal. calcd. for $C_{24}H_{16}F_3N_3O$: C, 68.73; H, 3.85; N, 10.02. Found: C, 68.53; H, 3.89; N, 10.01.

2.2. *In vitro* schizonts maturation inhibition assay (Trager & Jensen 1976)

The *in vitro* antimalarial activities of the compounds were assessed against CQ sensitive and resistant isolates of *P. falciparum* and compared with clinically used antimalarial drug chloroquine. The half maximal inhibitory concentrations (IC_{50}) were obtained. In brief, the cultures of asynchronous parasites of *P. falciparum* (MRC-2 and RKL-9) were synchronized using 5% aqueous solution of sorbitol. All other stages except rings were degenerated. Degenerated stages had been removed by centrifuge for 5 minutes at 1500 rpm. Parasitemia was adjusted to about 1% for assay by diluting with fresh washed RBCs. The synthesized compounds were dissolved in 100 μL of dimethyl sulfoxide (DMSO) and required dilutions were made with a RPMI-1640 medium. The tests were done in 96 well plate using CQ sensitive and resistant isolates. Different concentrations of synthesized compounds were dispensed in 96 well plates in triplicate. The first well in all the rows was without any drug and considered as control. The synchronized parasites were inoculated to all the wells, including control wells. The plates were incubated in a CO_2 incubator at 37˚C for 24-30 h depending on the maturation of the schizonts, thereafter; smears were prepared from all the wells, fixed with methanol, stained with Giemsa's stain and examined under light microscope, 100 x oil immersion. The growth of parasites in the test wells was compared to that of negative controls and the inhibition of parasite growth was expressed as a percentage. The half maximal inhibitory concentration (IC_{50}) responses were estimated by the probit method.

2.3. Cell cytotoxicity assay

Toxicity is an important consideration in any drug development program; therefore we studied cyto-toxicity of these compounds against HepG2 cell lines using3-(4,5-dimethylthiazol 2-yl)-2,5-diphenyl tetra-zolium bromide (MTT) assay (Hattori & Nakanishi 1995). Briefly, cell cultures were routinely maintained in RPMI 1640 medium supplemented with 10% bovine foetal calf serum (FCS). For cytotoxicity evaluations, 5 x 102 cells in 180 ml medium were seeded in each well of a 96-well plate and incubated at 37˚C under a 5% CO_2 atmosphere for 1 h. Aliquots of 20 μL of serial dilutions of the test compounds (stocks in DMSO) were added to the wells. Untreated wells received 20 μL of culture medium while additional solvent controls were prepared with medium containing DMSO to account for any possible effect of DMSO on cell viability. The plates were then incubated at 37˚C under an atmosphere of 5% CO_2 for seven days and 20 μL MTT (5 mg/ml) was added to each well. The plates were further incubated for an additional 4 h at 37˚C under an atmosphere of 5% CO_2 and then centrifuged for 10 minutes at 800 G. The supernatant was carefully aspirated from each well without disturbing the pellet and the cells were washed with 150 μl of phosphate buffered saline (PBS) followed by centrifugation for 10 minutes at 800 G. The supernatant was again carefully aspirated and the plates were dried at 37˚C for an hour. The 100 μL ethanol was added to each well to solubilise the resultant formazan crystals, aided by a gentle mechanical shacking for 1-2 h. Absorbance were measured on a Universal Microplate Reader (ELx800 UV, Bio-tek Instrument) at a wavelength of 570 nm and the percentage cell growth in drug treated well were calculated and plotted against log drug concentration to determine the corresponding IC_{50} values by non-linear regression analysis.

$$\%Toxicity = \frac{\text{Abs. Control} - \text{Abs. Test Comp.}}{\text{Abs. Control}} \text{ x } 100$$

2.4. *In vitro* heme crystallization inhibition assay

The compounds after Schizont Maturation Inhibition Assay, were further screened for the inhibition of the hemozoin formation by the method reported by Ncokazi and Egan [30]. Heme solution is prepared by dissolving 5.2 mg of haemin chloride (Sigma Chemical Co., USA) in 1 ml of DMSO. Chloroquine and synthesized compounds with different molar equivalents to haemin were prepared by dissolving in DMSO.

The test compounds dissolved in DMSO in doses ranging from 0.12 to 5 M equivalents to haemin chloride in 50 μL of 8 mM and of haemin chloride solution in DMSO, 50 μL DMSO as control were taken for the assay. By adding 100 μL of 8M acetate buffer (pH 5.0) the hemozoin formation was initiated. Culture plates were incubated at 37°C for 18 h, centrifuged and the soluble fraction of un precipitated material was collected. Thereafter, 200 μL of DMSO was added to resuspend the remaining pellet in order to remove unreacted haematin and the plates were centrifuged again, the DMSO soluble fraction was collected and the residual pellet (which consists of pure precipitate of haematin) was dissolved in 200 μL of 0.1M NaOH. Thereafter 75 μL of it was transferred to new tubes and

diluted four times by adding 0.1M NaOH. The amount of haematin was determined spectrophotometrically at 405 nm wavelength. The % inhibition of hemozoin formation was calculated by the below given formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{A (test)}}{\text{A (sample)}} \times 100$$

The percentage inhibition of hemozoin of the standard drug was compared with the control.

2.5. Molecular docking analysis

Docking study was performed to analyze the binding affinity as well as binding orientations of antimalarial inhibitors in *Plasmodium falciparum* proteins using Gold software (https://research.csc.fi/-/gold). The crystallographic structure of *Plasmodium falciparum falcipain 2 protease* was accessed from protein data bank under the PDB-ID 6SSZ at resolution 3.45 Å and R factor= 0.308 (Protein data bank). Initially, hydrogen was added to the crystal structure of the amino acid residues and simultaneously, all crystallographic water molecules were removed, as no water molecules actively participating in a bond interaction between ligand and receptor. The co-crystallised ligand {(E)-3-(1,3-benzodioxol-5-yl)-1-(3-nitrophenyl)prop-2-en-1-one} was removed from the protein structure and the binding site was defined as all atoms within 6Å of the crystallographic ligand. Gold is a genetic algorithm based docking program for docking flexible ligand into the binding site of the ligand. For each ligands10 independent genetic algorithm (GA) run was operated.

The energy of the resulting pose (fitness) consists of three terms: (1) hydrogen-bonding energy, (2) internal energy of the ligand, and (3) steric interaction energy. The output was generated in terms of fitness score using a CHEMPLP (Piecewise Linear Potential) scoring function. The scoring function of the GOLD provides a way to rank the placement of ligands relative to one another. CHEMPLP (Piecewise Linear Potential) has been found to give highest success rates for both pose prediction and virtual screening experiments and therefore is used as scoring function [31].

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic protocol utilized towards the synthesis of the targeted quinazoline-chalcone hybrids adopted the well-established chemistry with varied substituted aldehydes. Overall the synthesis of quinazoline-chalcone hybrids (1-16) was carried out in five steps (Fig. 2) starting from commercially available 2-Amino benzoic acid. In the first step, the nucleophilic attack of the amino group of anthranilic acid on the acyl carbon of trifluoromethyl acetic anhydride introduced trifluoroacetyl group and cyclized in the presence of acetic anhydride to afford the 2-(trifluoromethyl)-4H-benzo[d][1,3]oxazin-4-one (A). In the second step, nucleophilic attack of ammonia on the carbonyl carbon of A resulted in the formation of 2–trifluromethyl-3H-quinazolin-4-one (B). In the third step, chlorination is done at 4th position of (B) to afford 4-Chloro-2-trifluoromethyl quinazoline which was then coupled with terminal amino group of 4-aminoacetophenone to afford 1-(4-((2-(trifluoromethyl) quinazolin-4-yl)amino) phenyl)ethan-1-one (C) in the fourth step. Finally titled compounds (1-16) were synthesized *via* the base catalysed Claisen Schmidt condensation using various substituted aldehydes. Reaction conditions with respect to stirring time at room temperature were non-homogeneous. The reaction conditions for the synthesis of the final product (1-16) were optimized for different times and the results are shown in Table 1. Stirring time in the final step for the preparation of titled compounds (1-16) varied and found greatly influenced by the nature of substituent on aryl aldehyde. It was hypothesized that chalcone is being formed in this step of synthesis *via* an initial anion formation of D with NaOH solution and subsequently attacking on the positively charged carbon of carbonyl group of aryl aldehydes. The electron withdrawing substituents are supposed to improve the electropositive character of carbonyl carbon, which ultimately accelerates the rate of the reaction and hence reducing the time for the final product formation. Compounds 5, 6 and 7 carried out at least stirring time (4-6 h), as they have been formed from highly electron withdrawing substituted benzaldehydes. Compounds 1, 2, 3 and 14 took 6-8 h for complete formation followed by 8, 9, 10, 11 and 16 which carried out at 10-12 h and compounds 12, 13 & 15 were taken longest stirring (18-22 h) for their formation.

Structure of synthesized compounds (1-16) and intermediates were confirmed by their distinct R_f values in TLC analysis, melting point, IR and NMR spectroscopic data. Formation of intermediate A was confirmed by characteristics IR signals 1033 cm^{-1} for C-O-C symmetric stretching & 1231 cm^{-1} for C-O-C asymmetric stretching, showing formation of ring system which was finally confirmed by IR signal at 930 cm^{-1} characteristic of oxazine ring. IR signals at 3328 cm^{-1} for N-H, 1640 cm^{-1} for C=N confirmed the

formation of intermediate B. IR signals at 745 cm^{-1} and disappearance of 1728 cm^{-1} for C=O confirmed replacement of C=O by C-Cl and hence intermediate C formation confirmed. IR signals for alkene C-H at 3017 cm^{-1} and alkene C=C at 1462 cm^{-1} confirmed formation of final product. Also, H-NMR has shown a large coupling constant (14-15 Hz; vicinal coupling constant) indicated the relative *trans* configuration of bond between C19 and C20 in the final product.

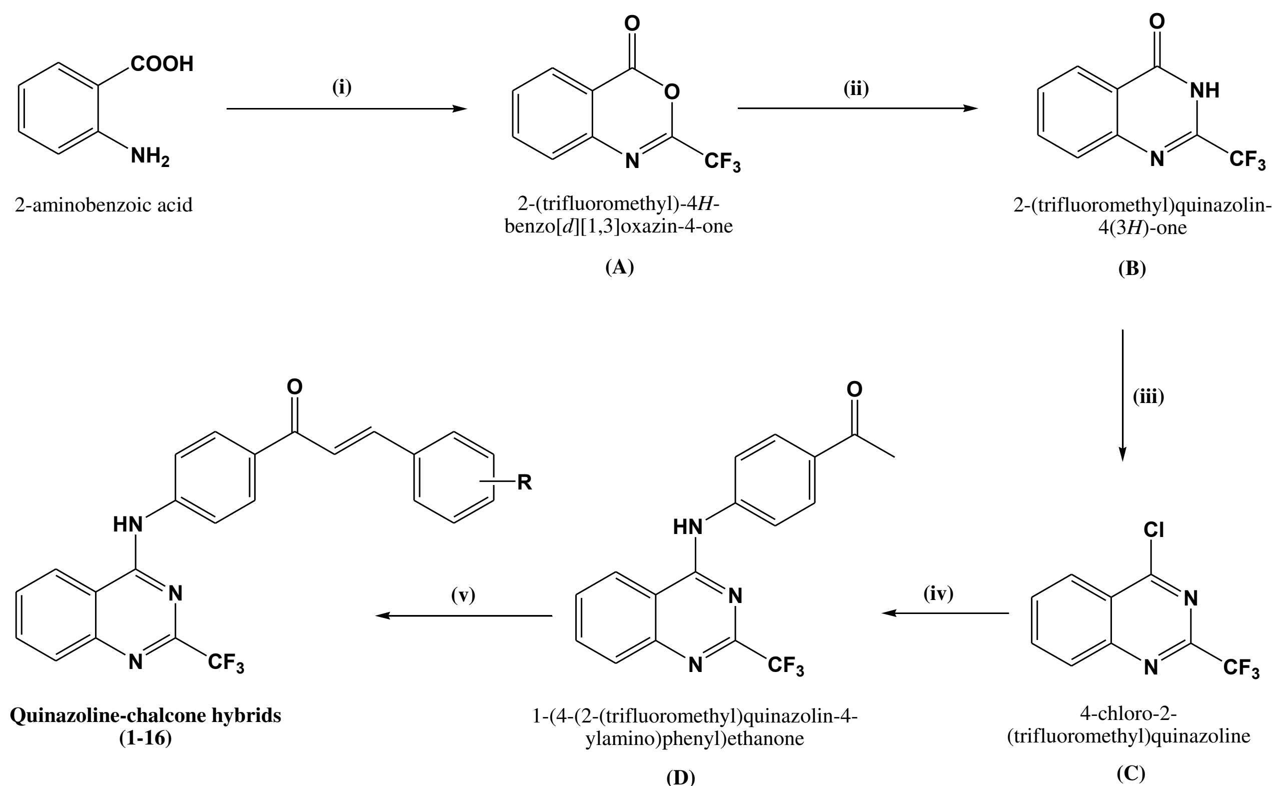


Fig. 2: Scheme for the synthesis of quinazoline-chalcone hybrids (1-16)

3.2. *In vitro* schizonts maturation inhibition assay

In the present study, total sixteen computationally driven molecules were selected for synthesis and evaluated *in vitro* against a Chloroquine (CQ)-sensitive isolate (MRC-2) and CQ-resistant field isolates (RKL-9) of *P. falciparum* malaria parasite. Chloroquine was used as a reference drug in all the experiments for the comparison (MRC-2 IC_{50}=0.05 μM; RKL-9 IC_{50} = 0.40 μM). The inhibitory concentration (IC_{50}) ranged from 0.023 to 1.9 μM for Chloroquine (CQ) -sensitive (MRC-2) and 0.21 to 2.03 μM for CQ-resistant (RKL-9) *P. falciparum* strains. All the synthesized compounds showed significant antimalarial activity against both the strains shown in Table 1 (Fig. 3). The results indicated that synthesized compounds were better worked against CQ-sensitive strain than CQ-resistant strain. While considering the antimalarial activity against CQ-sensitive strain (MRC-2), nine out of sixteen compounds found to be more potent to CQ (**1** = IC_{50} = 0.037 μM; **2**= IC_{50} = 0.039 μM; **3**= IC_{50} = 0.033 μM; **5**= IC_{50} = 0.023 μM; **6**= IC_{50} = 0.038 μM; **7**= IC_{50} = 0.036 μM; **12**= IC_{50} = 0.034 μM; **13**= IC_{50} = 0.032 μM; **14**= IC_{50} = 0.043 μM).

Similarly, in case of chloroquine resistant strain (RKL-9), total six compounds found to be more or equipotent to CQ (**5** = IC_{50} = 0.217 μM; **6**= IC_{50} = 0.383 μM; **7**= IC_{50} = 0.453 μM; **12**= IC_{50} = 0.314 μM; **13**= IC_{50} = 0.362 μM; **14**= IC_{50} = 0.324 μM). Among the series, compound **16,** wherein the terminal phenyl ring was unsubstituted, found to be less active (IC_{50} = 1.934 μM) compared with the rest of the analogs. This signified that the substituted terminal phenyl ring favors the

antimalarial activity. It was observed that the introduction of electron-withdrawing group such as fluorine, chlorine, nitro or trifluoro methyl group has significantly improved the *in vitro* antimalarial profile (except bromine) as compared to electron donating groups such as hydroxyl, methyl, ethyl, methoxy, diethyl amino group. However, compounds **12** & **13** substitution with di-methyl amino ($-(CH_3)_2N$) showed exception to this electron donating trend, were found to be possess potent antimalarial activity. The overall, structure activity relationship study suggested that presence of electron withdrawing group on phenyl ring possess remarkable activity (IC_{50}= 0.02 μM- 0.04 μM for CQ sensitive and IC_{50}= 0.2-0.3 μM for CQ resistant) compared to standard drug chloroquine (IC_{50}= 0.05 μM for CQ sensitive and C_{50}= 0.4 μM for CQ resistant).

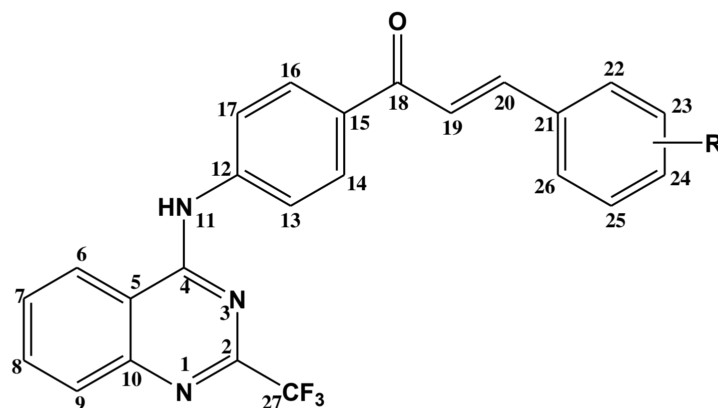


Table 1: *In vitro* schizonts maturation inhibition & cell cytotoxicity assay

S. No.	Comp.	IC_{50} (μM)[a]±SD		Cytotoxicity IC_{50} (μM)[d]	SI[e]
		MRC-2[b]	RKL-9[c]		
1	4-F	0.037±0.003	0.703±0.020	47.83	68.04
2	2-F	0.039±0.020	0.802±0.006	49.72	62.0
3	4-Cl	0.033±0.005	0.540±0.003	44.56	82.52
4	3-Br	0.074±0.002	0.910±0.034	33.21	36.50
5	$4-CF_3$	0.023±0.032	0.217±0.022	56.17	258.90
6	$3-CF_3$	0.038±0.006	0.383±0.017	53.69	140.20
7	$3-NO_2$	0.036±0.001	0.453±0.005	33.61	74.20
8	$3-CH_3$	0.154±0.003	0.548±0.002	52.43	95.70
9	$3-C_2H_5$	0.203±0.007	0.722±0.004	58.54	81.10
10	3-OH	0.442±0.006	0.903±0.018	53.38	59.11
11	$4-OCH_3$	0.232±0.017	0.841±0.040	49.82	59.24
12	$4-(CH_3)_2N$	0.034±0.022	0.314±0.003	51.39	163.70
13	$3-(CH_3)_2N$	0.032±0.010	0.362±0.001	54.82	151.50
14	3,4-Cl	0.043±0.006	0.324±0.005	57.27	176.76
15	$4-(C_2H_5)_2N$	0.464±0.040	0.971±0.001	54.63	56.30
16	H	1.934±0.031	2.034±0.034	57.51	28.30
REF.	Chloroquine	0.050±0.004	0.400±0.003	27.00	67.50

[a] *Concentration corresponding to 50% growth inhibition of the parasite.*

[b]*Chloroquine sensitive strain of P. falciparum, IC_{50}, μM+SD, n=2*

[c]*Chloroquine resistant strain of P. falciparum, IC_{50}, μM+SD, n=2*

[d]*Cytotoxicity against HepG2 cell line. Values are the mean of one experiment in duplicate*

[e]*SI: Selectivity Index (IC_{50} value of Cytotoxicity activity / IC_{50} values of antiplasmodial activity against RKL-9)*

Ref. – Reference compound i.e. chloroquine

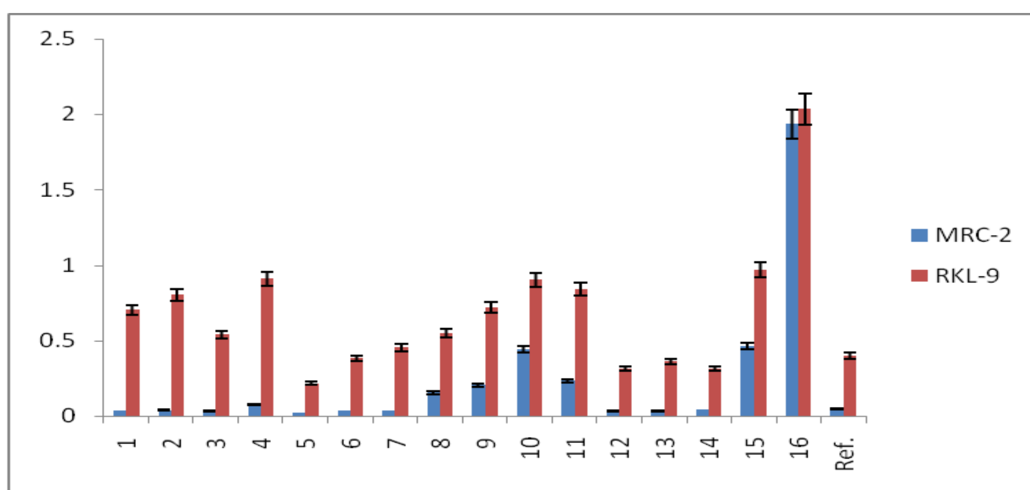


Fig. 3: Graphical presentation of in vitro schizonts maturation inhibition assay of synthesized compounds against CQ-sensitive (MRC-2) and CQ-resistant (RKL-9) *P. falciparum* strains

3.3. Cell cytotoxicity study (IC_{50}) and selectivity index (SI)

Cell cytotoxicity study (IC_{50}) performed on Human Hepatoma Cell Line (HepG2) and determine their selectivity indexes so as to validate their real potential as selective antiplasmodial (**Table 1**). All the synthesized compounds exhibited less cytotoxicity than chloroquine (IC_{50}=27.0μM). Compounds which showed most potent antimalarial activities against *P. falciparum* resistant strain RKL-9 *i.e.* **5**, **6**, **12**, **13** and **14** found almost two fold safer compared to standard drug CQ. Compound **7** having-NO_2 substitution found to be most toxic, while compound **9** having –C_2H_5 substitution exhibited least toxicity in the series. Selectivity indexes for CQ-resistant (RKL-9) *P. falciparum* strains ranging between 28.30 and 258.90 in the series and ten out of sixteen showed better selectivity than CQ against resistant strain RKL-9 of *P. falciparum*. Selectivity ratios of most potent compounds were **5**; S.I.=258.90, **6**; S.I.=140.20, **12**; S.I.=163.70, **13**; S.I.=151.50, and **14**; S.I.=176.76 indicate their selectivity towards antiplasmodial activity.

3.4. *In vitro* heme crystallization inhibition assay

All the synthesized compounds were also studied for their ability to inhibit the crystallization of hematin to β-hematin (the synthetic equivalent of hemozoin) *in vitro*, using a colorimetric β-hematin inhibition assay. The results are given in Table 2. Compounds 8, 9, 16 showed very less (<5%) inhibition of β-hematin formation which might be due to their inability to accumulate significantly inside the parasite digestive vacuole, which is the typical site of hemoglobin catabolism and therefore the site of action of hemozoin inhibitors. Compounds 4, 7 and 10 showed comparable inhibition of β-hematin formation as with CQ. Total nine com-pounds of the series, *e.g.* 1, 2, 3, 5, 6, 11, 12, 13 & 14 showed a better percentage of inhibition (>87%) of β-hematin formation than CQ.

Table 2: *In vitro* heme crystallization inhibition Assay

Comp.	R	% Inhibition of β-hematin Formation
1	4-F	95±0.12
2	2-F	92±0.02
3	4-Cl	88±0.11
4	3-Br	83±0.04
5	4-CF_3	91±0.03
6	3-CF_3	89±0.02
7	3-NO_2	87±0.01
8	3-CH_3	< 5
9	3-C_2H_5	< 5
10	3-OH	83±0.04
11	4-OCH_3	93±0.13
12	4-$(CH_3)_2N$	91±0.05
13	3-$(CH_3)_2N$	90±0.02
14	3,4-Cl	93±0.03
15	4-$(C_2H_5)_2N$	55±0.51
16	H	< 5
REF.	CQ	87±0.01

**Average of duplicate determinations and equivalents of compounds (relative to heamatin)*

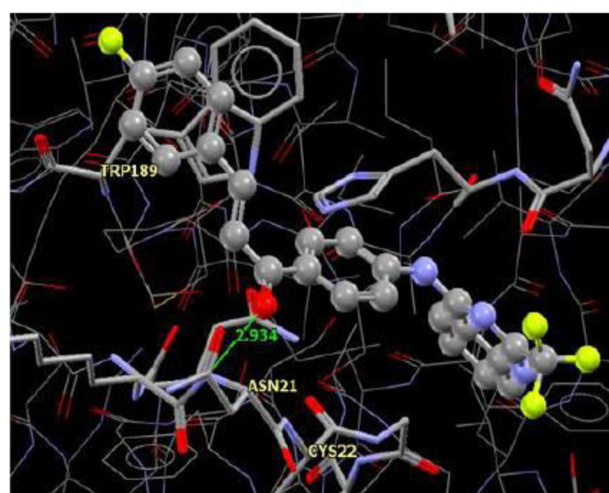
3.5. Molecular docking study

Docking study was executed to explore the binding affinity and binding conformations of synthesized hybrids against *Pf -falcipain 2 protease* enzyme complexed with an *(E)*-chalcone inhibitor (PDB ID: 6SSZ). For protein synthesis, malaria parasites at erythrocytic stage digest amino acids by degradation of hemoglobin. Several enzymes involved in this degradation process [32, 33] including the *cysteine proteases falcipain*-2 [34] and *falcipain-3* [35]. Literature reported that the *cysteine protease* inhibitors have ability to interfere the erythrocytic life cycle of *P. falciparum*, by obstructing the hydrolysis of the host haemoglobin [36-39].

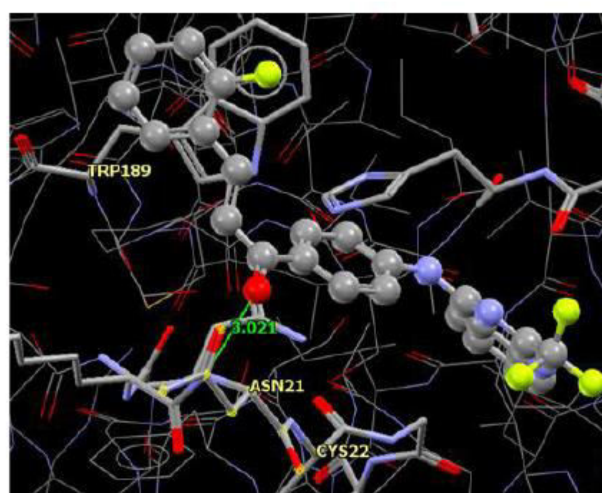
The docking score and fitness function score (Piecewise Linear Potential) of these synthesized hybrids has shown acceptable affinity score towards plasmepin II enzymatic activity. All hybrids possessed higher score compared to co-crystallized ligand (Table 3). The highest dock score (80.79) and PLP fitness score (-82.40) was found to be with substituent di-methyl amino group. However, when binding pose interactions were analyzed, hydrogen bond interaction was observed with only six compounds. The docking poses indicated that the oxygen atom of benzodioxane ring formed hydrogen bond with CYS22 as hydrogen bond donor at a distance 2.875Å. Likewise, similar interactions were observed in top two best docks scored compounds 12 and 13 (-C=O…HN-CYS22) at a bond distance 2.861and 3.050 respectively. Additionally, one more hydrogen bond between the oxygen atom of the carbonyl group and amino acid asparagine (ASN21) was observed with all the best scored compounds (Fig. 4). Furthermore, GOLD software automatically creates fitting points in the binding cavity. These fitting points are exploited during the generation of docking solutions to map ligand atoms into favorable regions of the active site. All the synthesized compounds occupied fitting point generated cavity as well, indicating that all were in a favorable state of the binding active site. The figure 5 shows the fitting point map of the top five best scored poses of synthesized compounds. The overall results suggested that *falcipain- 2* may act as potential target for these novel quinazoline chalcone hybrids.

Table 3: ***Docking results of synthesized compounds and co-crystallised ligand with Pf falcipain 2 protease enzyme***

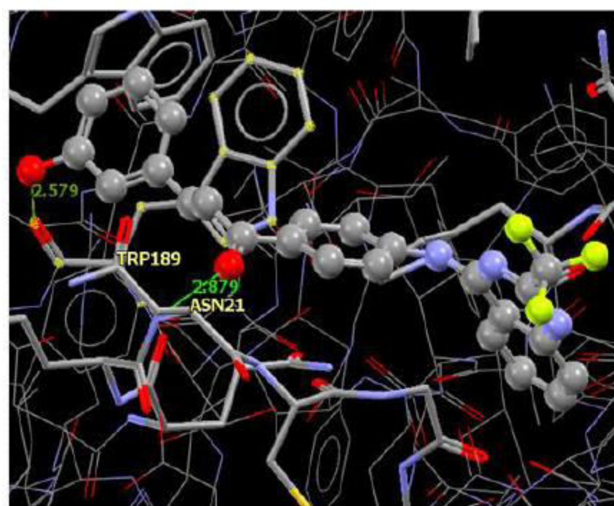
Comp.	R	Docking score	Fitness function (PLP)	H-bond energy (K cal mol^{-1})	Residues (H bond interactions with their distances)
1	4-F	74.64	-75.68	0.76	ASN21 (2.934)
2	2-F	75.46	-77.25	0.42	ASN21 (3.021)
3	4-Cl	73.51	-75.05	0.00	No H bond interaction
4	3-Br	73.44	-75.93	0.00	No H bond interaction
5	4-CF_3	78.56	-80.52	0.00	No H bond interaction
6	3-CF_3	76.51	-77.29	0.00	No H bond interaction
7	4-NO_2	72.26	-75.19	0.00	No H bond interaction
8	3-CH_3	73.81	-76.72	0.00	No H bond interaction
9	4-C_2H_5	75.02	-76.98	0.00	No H bond interaction
10	3-OH	76.62	-74.79	1.86	TRP189 (2.579), ASN21 (2.879)
11	4-OCH_3	73.32	-75.09	0.00	No H bond interaction
12	4-$(CH_3)_2N$	79.38	-80.73	0.80	ASN21 (2.952), CYS22 (2.861)
13	3-$(CH_3)_2N$	80.79	-82.40	0.92	ASN21 (2.891), CYS22 (3.050)
14	3,4-Cl	73.30	-74.70	0.00	No H bond interaction
15	4-$(C2H_5)_2N$	70.21	-78.84	0.94	ASN21 (92.920)
16	H	71.61	-73.02	0.00	No H bond interaction
REF.	Co-Crystallised Ligand	62.55	-54.36	0.42	CYS22(2.875)



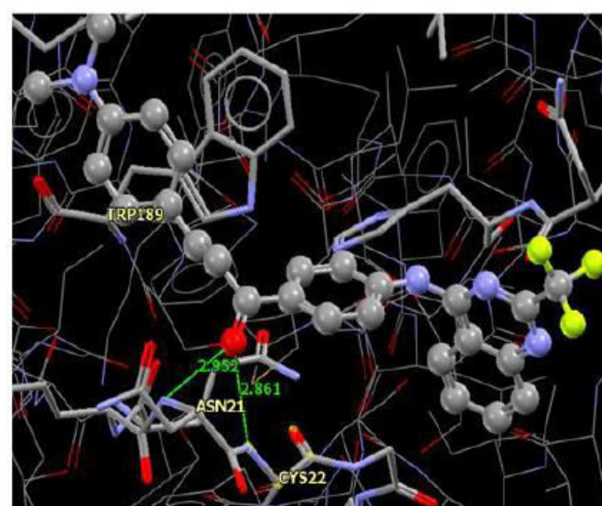
Compound 1



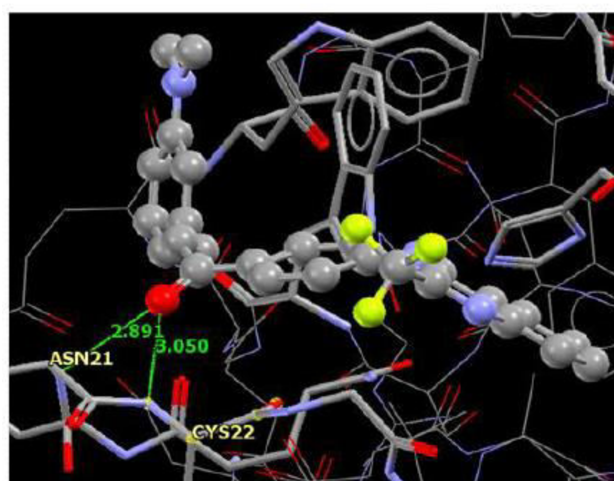
Compound 2



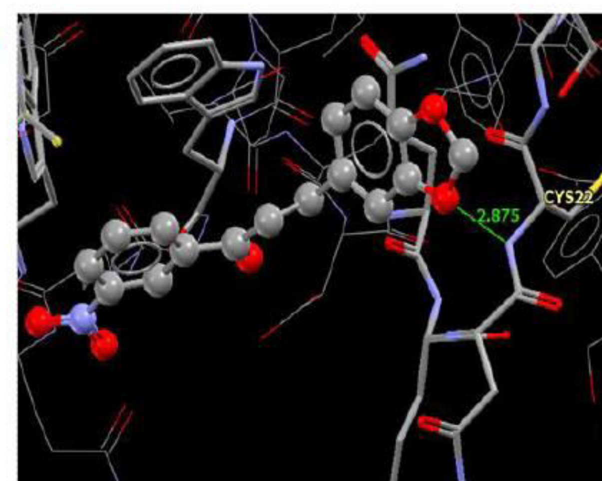
Compound 10



Compound 12



Compound 13



Co-crystallised ligand

Fig. 4: Docking poses of top five best ranked ligands and co-crystallised ligand

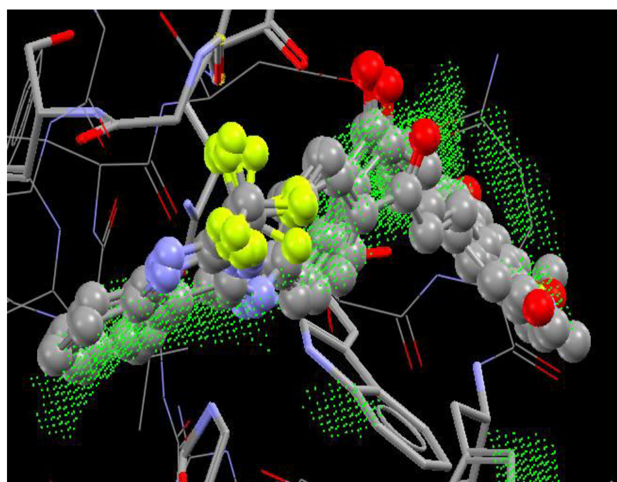


Fig. 5: Fitting point map in binding site of protein 6SSZ

Along with this, all the compounds were docked into the active site of falcifain-2 protease to determine the binding affinity and orientations between ligands and receptor. Where, the oxygen atom of carbonyl group act as a hydrogen bond acceptor region formed a hydrogen bond with ASN21 and CYS22 amino acids of protein. Overall, *in silico* molecular docking also supported the results of *in vitro* studies. Consequently, it is anticipated that these hybrids may act as potential candidate for developing new antimalarial agents.

4. CONCLUSION

Quinazoline-chalcone hybrids covalently linked by amine linkage were synthesized via cost-efficient Claisen-Schmidt condensation reaction and evaluated for their *in vitro* antimalarial activity against chloroquine-sensitive (MRC-2) and chloroquine-resistant (RKL-9) *P. falciparum* strains, cell cytotoxity study, *in vitro* heme crystallization inhibition assay. Compounds **5** (IC_{50}=0.023 μM), **6** (IC_{50}=0.038 μM), **12** (IC_{50}= 0.034 μM), **13** (IC_{50} = 0.032 μM) and **14** (IC_{50} = 0.043 μM) displayed excellent *in vitro* antimalarial activity compared to standard chloroquine against CQ-sensitive strain (MRC-2) as well as CQ-resistant strain (RKL-9). The *in vitro* cytotoxicity study performed on the human HepG2 cell line revealed all the synthesized compounds exhibited less cytotoxicity than chloroquine (IC_{50} = 27.0 μM) and compound **7** having $-NO_2$ substitution found to be most toxic, while compound **9** having $-C_2H_5$ substitution exhibited least toxicity in the series. Selectivity indexes for CQ-resistant (RKL-9) *P. falciparum* strains ranging between 28.30 and 258.90 in the series.

In the *in vitro* heme crystallization inhibition assay, except a few compounds, all the synthesized molecules showed considerable inhibition of β-hematin formation, suggesting that compounds are strongly interfering with the hemozoin formation in parasites. Based on the results of *in vitro* heme crystallization inhibition assay and the selectivity index we hypothesized that hybridization of chalcone with quinazoline acting to interfere with the hemozoin formation pathway thereby a possible mode of action for these hybrid compounds.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

5. ACKNOWLEDGEMENT

Authors are thankful to The Director, National Institute of Malaria Research, New Delhi, India for providing facilities and assistance for *in-vitro* antimalarial bioassay. One of the authors, Mitali Mishra is grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for Senior Research Fellowship.

Funding sources

This work was supported by the Council of Scientific and Industrial Research (CSIR), New Delhi, India [grant number: 09/150(0133)/18-EMR-I].

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