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Synthesis, in vitro antimalarial activity and cytotoxicity of novel 4-aminoquinolinyl-chalcone amides

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ABSTRACT

A series of 4-aminoquinolinyl-chalcone amides **11**–**19** were synthesized through condensation of carboxylic acid-functionalized chalcone with aminoquinolines, using 1,1′-carbonyldiimidazole as coupling agent. These compounds were screened against the chloroquine sensitive (3D7) and chloroquine resistant (W2) strains of *Plasmodium falciparum*. Their cytotoxicity towards the WI-38 cell line of normal human fetal lung fibroblast was determined. All compounds were found active, with IC_{50} values ranging between 0.04–0.5 μM and 0.07–1.8 μM against 3D7 and W2, respectively. They demonstrated moderate to high selective activity towards the parasitic cells in the presence of mammalian cells. However, amide **15**, featuring the 1,6-diaminohexane linker, despite possessing predicted unfavourable aqueous solubility and absorption properties, was the most active of all the amides tested. It was found to be as potent as CQ against 3D7, while it displayed a two-fold higher activity than CQ against the W2 strain, with good selective antimalarial activity (SI = 435) towards the parasitic cells. During this study, amide **15** was thus identified as the best drug-candidate to for further investigation as a potential drug in search for new, safe and effective antimalarial drugs.

1. Introduction

With over three billion people at risk and around 660,000 deaths reported in 2011,[1] malaria poses a major public health threat, globally.[2] Of these reported deaths, 91% were in the African region and 86% were children under the age of five.[1] Malaria is commonly found in tropical and subtropical regions, such as Africa and Asia,[3] and is endemic in 106 countries worldwide.

Although artemisinin combination therapies (ACTs) currently are the preferred treatment method for both uncomplicated and severe malaria,[4] all of the shortcomings of the individual compounds remain problematic, including resistance to the parent molecule. Additionally, alarming reports of *P. falciparum* tolerance to artemisinins in South-Asia and along the Cambodia-Thailand border,[1,5] jeopardize the use of ACTs and signify the urgent need for identifying and developing new therapies that can act on unique targets. An alternative method that has been proposed to overcome the development of resistance is the incorporation of a second pharmacophore via a chemical bond, forming a hybrid drug molecule. Hybrid molecules combine two drugs in a single molecule, with the goal of creating a chemical entity with two or more structural domains, having different biological functions and dual activity that are medically/therapeutically more effective than its individual components.[6]

Despite widespread chloroquine (CQ) resistance in the majority of malaria endemic areas, quinoline based compounds remain an important class of potential antimalarial drugs that require further investigation, mainly as a result of their cheap synthetic cost and diverse applications.[7,8] Additionally, resistance seems to be compound specific and not related to changes in the structure of the drug target. CQ resistance can thus be overcome by hybridization of the parent molecule (quinoline) with other pharmacophores.[9]

Recent advents in genetic sequencing of the *Plasmodium* genome have identified several new unique targets, including the parasite induced permeation pathways and malarial cysteine proteases. These can be specifically targeted with minimal toxicity to the host. Cysteine protease mediates protein hydrolysis through nucleophilic attack on a carbonyl of a susceptible peptide bond. The main function of malarial cysteine protease is the hydrolysis of haemoglobin in the food vacuole.[10] This enzyme is also presumed to be involved in the rupture of the erythrocyte membrane.[11]

A known cysteine protease inhibitor is E64 (Fig. 1), a natural modified peptide, containing an active epoxide functional group. Other inhibitors include natural compounds, such as chalcones (1,3-diaryl propenone) and isatins. The first reported chalcone with antimalarial activity was Licochalcone A (Fig. 1), a natural product

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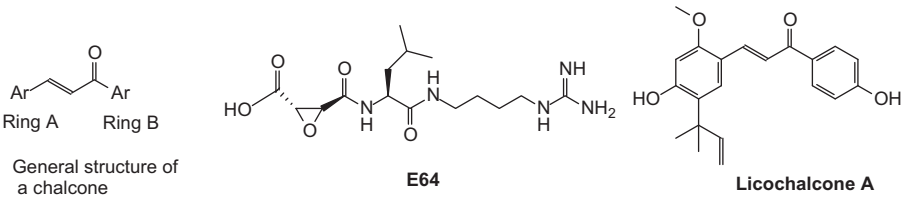


Figure 1. General structure of a chalcone, cysteine protease inhibitor E64 and licochalcone A.

isolated from Chinese liquorice roots, with an IC_{50} value of 6.5 μM against 3D7 clones.[12,13] Ever since, interest in these compounds has ignited. Numerous chalcones have to date been prepared and screened for antiplasmodial activity with varying success.[5,12,14–17] Several chalcone based hybrids have also been reported containing a number of different pharmacophores, such as thiolactone-, isatin-,[18] stilbene-,[19] quinoline-,[20–22] ferrocene-[23,24] and endoperoxide-chalcone[25] based hybrids.

In 2009, Sharma et al.[22] synthesized some of the first reported, substituted quinolinyl-chalcone based hybrids (Fig 2, (A) of which the 4-amino linked compounds showed low to no activity against the NF-54 strain of *P. falciparum*. This disappointing outcome corroborated earlier reports by Chibale et al. (2000)[26] that the length of the methylene spacer between the two nitrogen groups in the side chain of CQ analogues was a major determinant of their antimalarial activities against CQ resistant *P. falciparum*. Sashidhara et al.[20,21] reported the synthesis and biological activity of a number of chloroquine–chalcone based hybrids. Of these keto-enamine chloroquine–chalcone based hybrid compounds (Fig 2, (B) several were equipotent to CQ against 3D7 strains. These compounds also displayed antimalarial activity in vivo against the MDR rodent malaria parasite, *P. yoelii*. Mechanistic studies done by these authors had revealed that these compounds acted through haem polymerisation targets.[20,21] These findings *inter alia* formed the rationale underlying the design of the novel quinolinyl-chalcone hybrids during this study. It also indicated that the combination of chloroquine–chalcone based hybrids may become leading compounds in the future design of more potent antimalarial drugs. Additionally, since both the chalcone and CQ exert their activities in the digestive vacuole, a synergistic effect might be expected.

As part of our program focusing on the discovery of novel antimalarial compounds and in light of the above considerations, we investigated amide compounds formed by conjugating chalcone moiety (with a 5-methylfuran as ring B) to 7-chloroquinoline pharmacophore through various linkers. It is argued that since chalcones are cysteine inhibitors, which specifically target amide bonds, thus, the incorporation of amide bond in the compounds of this study might prove worthy. In this Letter, the synthesis, in vitro antimalarial activity and cytotoxicity of these novel 4-aminoquinolinyl-chalcone amides are reported.

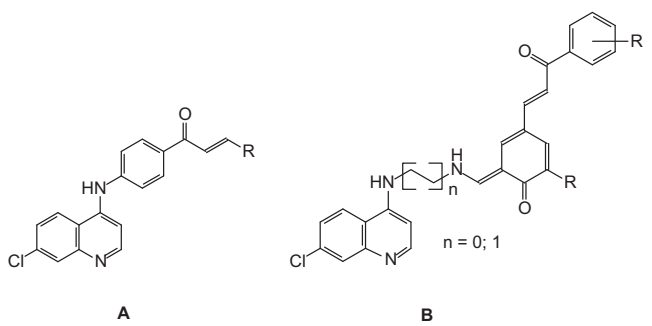


Figure 2. General structures of quinolinyl-chalcones synthesized by Sharma et al.[22] (A) and Sashidhara et al.[21] (B).

2. Materials and methods

2.1. Materials

4,7-Dichloroquinoline, 4-formylbenzoic acid, 2-acetyl-5-methylfuran, hydrochloric acid, sodium hydroxide, 1,1′-carbonyldiimidazole (CDI), ethylene diamine, propylene diamine, butylene diamine, piperazine, 1,2-diamino propane, 1,6-diaminohexane, *N*-methyl-1,3-propylene diamine, 2,2′-(ethylenedioxy)bis(ethylamine), *N*-(3-aminopropyl)-*N*-methylpropane-1,3-diamine, magnesium sulphate and sodium bicarbonate were purchased from Sigma–Aldrich (South-Africa). All solvents used were purchased from Associated Chemical Enterprises (ACE, South Africa). All chemicals and reagents were of analytical grade and were used without further purification. Dichloromethane (DCM) was distilled over calcium hydride and kept on molecular sieves (4 Å) prior to use in reactions.

2.2. General procedures

The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance™ III 600 spectrometer at a frequency of 600 and 150 MHz, respectively, in deuterated dimethyl sulfoxide (DMSO-d_6), deuterated methanol (MeOD), or deuterated chloroform ($CDCl_3$). Chemical shifts are reported in parts per million δ (ppm) with the residual protons of the solvent as reference. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). High Resolution Mass spectrometry (HRMS) was recorded on a Bruker MicroTOF Q II mass spectrometer that had an APCI or an ESI source set at 300 °C, or 180 °C, respectively, using Bruker Compass DataAnalysis 4.0 software. A full scan, ranging between 50–1500 *m*/*z*, was generated at a capillary voltage of 4500 V, an end plate offset voltage of −500 V and a collision cell RF voltage of 100 Vpp.

Infrared (IR) spectra were recorded on a Bruker Alpha-P FTIR instrument. Melting points (mp) were determined with a BÜCHI melting point B-545 instrument and were uncorrected.

Thin layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$), acquired from Merck.

A Shimadzu (Kyoto, Japan) DSC-60 instrument was used to generate DSC thermograms. Samples (3–5 mg) were accurately weighed and sealed in aluminium crimp cells with pierced lids. The samples were heated from 25–300 °C at a 10 C/min rate and a nitrogen gas purge of 35 ml/min.

A Shimadzu (Kyoto, Japan) TGA-60 instrument was used to determine the percentage weight loss (%) of samples during heating. Samples (3–5 mg) were accurately weighed into open aluminium crucibles. The samples were heated from 25–300 °C at a heating rate of 10 °C/min and a nitrogen gas purge of 35 ml/min.

Accelrys Discovery Studio 3.1 was used to calculate ADMET properties of compounds **10**–**19** and **CQ**. ACD/ChemSketch (2000), Version 4.54 was used to calculate log*P* values.

2.3. Biological evaluation

2.3.1. In vitro antimalarial assay

The in vitro antimalarial activity of test samples against the 3D7 and W2 strains of the malaria parasite, *P. falciparum*, was measured by assessing parasite survival after drug exposure, using the parasite lactate dehydrogenase (pLDH) colorimetric enzyme assay.[27] Lactate dehydrogenase is an enzyme found in all the cells and catalyses the formation of pyruvate from lactate by reducing the co-enzyme, nicotinamide adenine dinucleotide (NAD+) to nicotinamide adenine dinucleotide hydrogenase (NADH).

In the pLDH assay, the NAD + analogue, 3-acetylpyridine adenine nucleotide (APAD), is reduced to 3-acetylpyridine adenine nucleotide hydrogenase (APADH) and in turn a yellow, nitro-blue tetrazolium/phenazine ethosulphate (NBT/PES) reagent is converted into purple, formazan crystals. The absorbance was recorded at 620 nm, using a multiwell spectrophotometer (Tecan Infinite F500). Formazan formation is directly proportional to pLDH activity, which in turn is indicative of the number of parasites in the cultures following drug exposure. Assay specificity is ensured by the inability of human LDH, found in the host red blood cells, to use APAD as a co-factor. Compound inhibitory activity was determined by preparing test samples in parasite culture medium in transparent 96-well, flat bottom plates (Greiner Bio-one), with a 100 μM starting concentration in three-fold serial dilutions, to obtain eleven (11) decreasing concentrations ($n = 2$ for each data point). Parasitized red blood cells were added to a final concentration of 1% haematocrit, 2% parasitaemia and the plates incubated for 48 h, before proceeding with the pLDH assay. Percentage parasite survival in each well was calculated relative to control wells that were not exposed to the drug.

Results are presented in Table 2 as the 50% inhibitory concentrations (IC_{50}) of individual compounds calculated from fitted sigmoidal dose-response curves.

2.3.2. In vitro cytotoxicity assay

The cytotoxic effects of the prepared amide compounds were tested by sulforhodamine B (SRB) assay on the WI-38 cell line. The SRB assay was developed by Skehan et al.[28] to measure drug induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye, sulforhodamine B (Acid Red 52), to bind electrostatically in a pH dependent manner to basic protein amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to the fixed cellular protein, while under mild basic conditions it can be extracted from cells and solubilised for measurement.[29] The SRB assay was performed at CSIR in accordance with the protocol of the Drug Evaluation Branch, NCI, and the assay has been adopted for the screening tests compounds of this study.

The WI-38 cell line (normal human fetal lung fibroblast) from ECACC was routinely maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 μg/ml gentamicin. For this screening experiment, the cells (21–50 passages) were inoculated in a 96-well microtiter plates at plating densities of 10 000 cells/well and were incubated for 24 h. After 24 h the cells were treated with the experimental drugs, which had previously been dissolved in DMSO and diluted in medium to produce five concentrations. Neat cells served as control. The blank contained complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm, using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression. The results are summarized in Table 2.

2.4. Synthesis

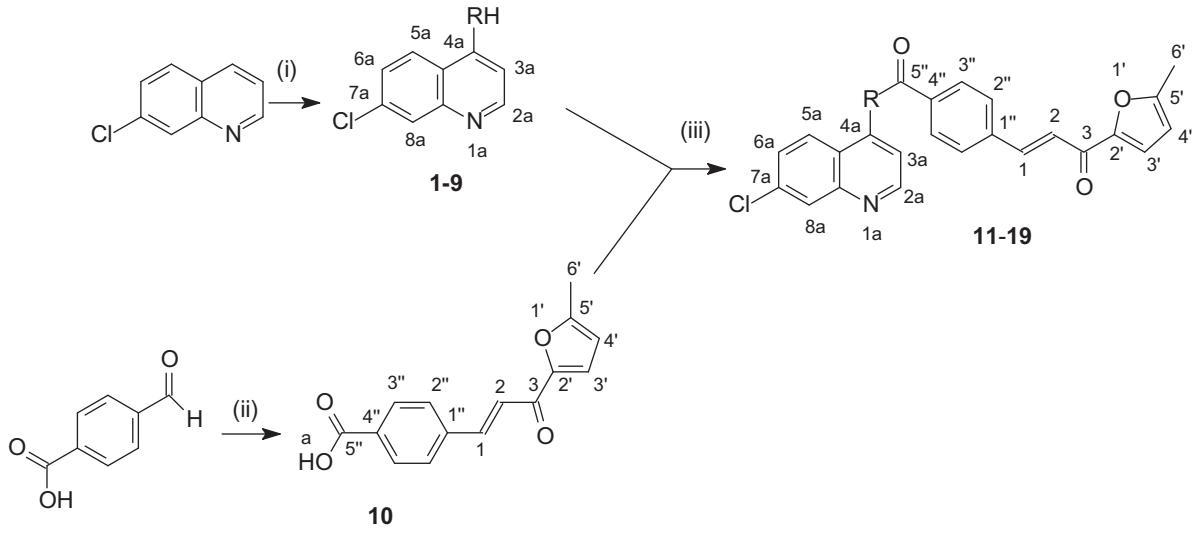
2.4.1. 4-Amino-7-chloroquinolines, 1–9

A combined method, as described by Biot et al.[30] and N'Da et al.[31] were used. 4,7-dichloroquinoline (15.1 mmol, 3 g, 1 equiv) and diamine (0.15 mol, 10 equiv) were reacted for 3–6 h at 80–100 °C [Scheme 1, step (i)]. The reaction mixture was allowed to cool and neutralized with an aqueous solution of 1 M NaOH. The product was extracted with hot ethyl acetate, washed with water, dried over $MgSO_4$ and evaporated to dryness under reduced pressure to produce the desired pure compound in high yields (51–85%), unless stated otherwise.[32] In the reaction involving piperazine and leading to 4-amino-7-chloroquinoline **9**, acetonitrile was used as solvent. The yields, melting points, IR, NMR, and HRMS data are reported.

2.4.1.1. *N*-(2-Aminoethyl)-7-chloroquinolin-4-amine, 1. The reaction of ethylenediamine and 4,7-dichloroquinoline afforded an off-white powder; yield: 85%; mp: 148–150 °C (lit. 143.5–145.4 °C),[32] IR (ATR) ν_{max}/cm^{-1}: 3352, 3298, 2956, 2927, 1610, 1579, 1541, 1426, 1321, 1140, 1082, 807, 770; ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.35 (d, $J = 5.6$ Hz, 1H, H-2a), 8.10 (d, $J = 9.0$ Hz, 1H, H-5a), 7.76 (d, $J = 2.2$ Hz, 1H, H-8a), 7.39 (dd, $J = 9.0$, 2.2 Hz, 1H, H-6a), 6.55 (d, $J = 5.6$ Hz, 1H, H-3a), 3.44 (t, $J = 6.4$ Hz, 2H, H-1′), 2.97 (t, $J = 6.4$ Hz, 2H, H-2′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.82 (C-4a), 152.45 (C-2a), 149.66 (C-9a), 136.33 (C-7a), 127.60 (C-8a), 126.04 (C-6a), 124.32 (C-5a), 118.81 (C-10a), 99.70 (C-3a), 46.27 (C-1b), 40.82 (C-2b); HRMS (APCI) m/z $[M+H]^+$ 222.0789 (Calcd for $C_{11}H_{13}ClN_3$: 222.0798).

2.4.1.2. *N*-(3-Aminopropyl)-7-chloroquinolin-4-amine, 2. The reaction of propylenediamine and 4,7-dichloroquinoline afforded an off-white powder; yield: 83%; mp: 127–129 °C; IR (ATR) ν_{max}/cm^{-1}: 3258, 2936, 2865, 2573, 2233, 1610, 1579, 1537, 1476, 1315, 1220, 1079, 899, 819, 799; ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.33 (d, $J = 5.6$ Hz, 1H, H-8a), 8.06 (d, $J = 9.0$ Hz, 1H, H-5a), 7.76 (d, $J = 2.2$ Hz, 1H, H-8a), 7.37 (dd, $J = 9.0$, 2.2 Hz, 1H, H-6a), 6.51 (d, $J = 5.8$ Hz, 1H, H-3a), 3.41 (t, $J = 7.1$ Hz, 2H, H-1′), 2.80 (t, $J = 7.1$ Hz, 2H, H-3′), 1.96–1.81 (m, 2H, H-2′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.66 (C-4a), 152.42 (C-2a), 149.65 (C-9a), 136.27 (C-7a), 127.58 (C-8a), 125.95 (C-6a), 124.25 (C-5a), 118.76 (C-10a), 99.63 (C-3a), 41.69 (C-1b), 40.23 (C-3b), 31.97 (C-2b); HRMS (APCI) m/z $[M+H]^+$ 236.0950 (Calcd for $C_{12}H_{15}ClN_3$: 236.0955).

2.4.1.3. *N*-(4-Aminobutyl)-7-chloroquinolin-4-amine, 3. The reaction of butylenediamine and 4,7-dichloroquinoline afforded an off-white powder; yield: 70%; mp: 123–125 °C (lit. 122–124 °C);[32] IR (ATR) ν_{max}/cm^{-1}: 3210, 3062, 2942, 2867, 1610, 1574, 1541, 1450, 1427, 1366, 1328, 1281, 1135, 1078, 848, 806; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.37 (d, $J = 5.4$ Hz, 1H, H-2a), 8.30 (d, $J = 9.0$ Hz, 1H, H-5a), 7.76 (d, $J = 2.2$ Hz, 1H, H8a), 7.42 (dd, $J = 9.0$, 2.2 Hz, 1H, H-6a), 6.45 (d, $J = 5.5$ Hz, 1H, H-3a), 3.25 (t, $J = 6.9$ Hz, 2H, H-1′), 2.68 (t, $J = 7.2$ Hz, 2H, H-4′), 1.68 (p, $J = 7.0$ Hz, 2H, H-2′), 1.56 (p, $J = 7.4$ Hz, 2H, H-3′); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 151.92 (C-2a), 150.15 (C-4a), 149.09 (C-9a), 133.38 (C-7a), 127.44 (C-8a), 124.22 (C-6a), 123.99 (C-5a), 117.50 (C-10a), 98.67 (C-3a), 42.04 (C-1b), 39.08 (C-4b), 27.60 (C-2b), 25.01 (C-



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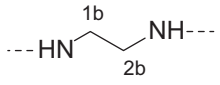
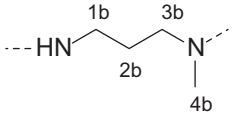
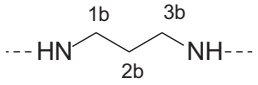
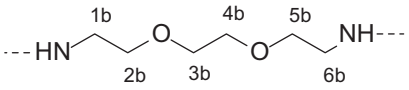
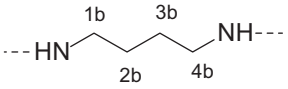
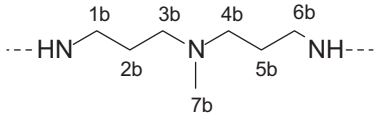
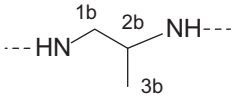
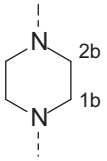
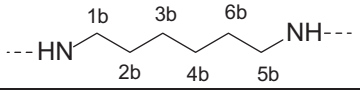
11		16	
12		17	
13		18	
14		19	
15			

Scheme 1. Multi-step synthesis of 4-aminoquinolinyl-chalcone amides **11**–**19**. Reagents and conditions: (i) Liquid diamine/piperazine in acetonitrile, 3–6 h, 80–100 °C; (ii) 2-acetyl-5-methylfuran, MeOH, NaOH, 12 h, rt; (iii) **10**, CDI, DCM, 3 h, rt then **1**–**9**, DMF, 24 h.

3b); HRMS (APCI) *m*/*z* $[M+H]^+$ 250.1104 (Calcd for $C_{13}H_{17}ClN_3$: 250.1111).

2.4.1.4. *N*-(2-Aminopropyl)-7-chloroquinolin-4-amine, 4. The reaction of 1,2-diamine propane and 4,7-dichloroquinoline afforded an off-white powder; yield: 74%; mp: charcoal at 240 °C; IR (ATR) ν_{max}/cm^{-1}: 3257, 2961, 1610, 1575, 1539, 1450, 1368, 1136, 912, 846, 795; ^1H NMR (600 MHz, MeOD) δ (ppm): 8.34 (d, J = 5.6 Hz, 1H, H-2a), 8.11 (d, J = 9.1 Hz, 1H, H-5a), 7.76 (d, J = 2.4 Hz, 1H, H-8a), 7.38 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.55 (d, J = 5.6 Hz, 1H, H-3a), 3.31 (dd, J = 3.7, 2.0 Hz, 1H, H-2′), 3.29–3.26 (m, 1H, H-1′), 1.20 (d, J = 5.4 Hz, 2H, H-3′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.81 (C-4a), 152.40 (C-2a), 149.66 (C-9a), 136.34 (C-7a), 127.59 (C-8a), 126.05 (C-6a), 124.31 (C-5a), 118.77 (C-10a), 99.86 (C-3a), 51.56 (C-1b), 46.54 (C-2b), 20.90 (C-3b); HRMS (APCI) *m*/*z* $[M+H]^+$ 236.0952 (Calcd for $C_{12}H_{15}ClN_3$: 236.0955).

2.4.1.5. *N*-(6-Aminohexyl)-7-chloroquinolin-4-amine, 5. The reaction of hexylenediamine and 4,7-dichloroquinoline afforded a yellow powder after purification by column chromatography eluting with DCM:MeOH (9:1, v/v) then DCM:MeOH:NH_4OH as eluent (9:1:0.1, v/v/v); yield: 51%, mp: 135–138 °C (lit. 133–134 °C)[32]; IR (ATR) ν_{max}/cm^{-1}: 3270, 2918, 2849, 1611, 1574, 1537, 1487, 1367, 1133, 850, 817, 798; ^1H NMR (600 MHz, MeOD) δ (ppm): 8.31 (d, J = 5.6 Hz, 1H, H-2a), 8.13 (d, J = 9.0 Hz, 1H, H-5a), 7.74 (d, J = 2.3 Hz, 1H, H-8a), 7.37 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.48 (d, J = 5.6 Hz, 1H, H-3a), 3.35 (2H, H-1′ peak overlapping H_2O peak), 2.93–2.88 (m, 2H, H-6′), 1.76 (p, J = 7.2 Hz, 2H, H-2′), 1.68 (p, J = 7.5 Hz, 2H, H-5′), 1.53–1.42 (m, 4H, H-3′ and H-4′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.80 (C-4a), 152.07 (C-2a), 149.30 (C-9a), 136.33 (C-7a), 127.23 (C-8a), 125.93 (C-6a), 124.52 (C-5a), 118.65 (C-10a), 99.54 (C-3a), 43.79 (C-1b), 40.82 (C-6b), 29.11 (C-2b), 28.95 (C-5b), 27.69 (C-3b), 27.26 (C-4b); HRMS (APCI) *m*/*z* $[M+H]^+$ 278.1406 (Calcd for $C_{15}H_{21}ClN_3$: 278.1424).

2.4.1.6. {3-[(7-Chloroquinolin-4-yl)amino]propyl}(methyl) amine, 6. The reaction of *N*-methyl-1,3-diamine and 4,7-dichloroquinoline afforded a yellow powder; yield: 62%; mp: 74–80 °C; IR (ATR) ν_{max}/cm^{-1}: 3220, 3103, 3060, 2947, 1610, 1574, 1429, 1133, 853, 822, 772; ^1H NMR (600 MHz, MeOD) δ

(ppm): 8.33 (d, J = 5.6 Hz, 1H, H-2a), 8.05 (d, J = 9.0 Hz, 1H, H-5a), 7.75 (d, J = 2.2 Hz, 1H, H-8a), 7.37 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.50 (d, J = 5.6 Hz, 1H, H-3a), 3.40 (t, J = 7.0 Hz, 2H, H-1′), 2.72 (t, J = 7.2 Hz, 2H, H-3′), 2.42 (s, 3H, H-4′), 1.94 (p, J = 7.2 Hz, 2H, H-2′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.63 (C-4a), 152.41 (C-2a), 149.63 (C-9a), 136.28 (C-7a), 127.58 (C-8a), 125.97 (C-6a), 124.26 (C-5a), 118.75 (C-10a), 99.61 (C-3a), 50.19 (C-1b), 42.02 (C-3b), 35.97 (C-4b), 28.65 (C-2b); HRMS (APCI) m/z [M+H]$^+$ 250.1094 (Calcd for $C_{13}H_{17}ClN_3$: 250.1111).

2.4.1.7. *N*-{2-[2-(2-Aminoethoxy)ethoxy]ethyl}-7-chloroquinolin-4-amine, 7. The reaction of 2,2′-(ethylenedioxy)bis(ethylamine) and 4,7-dichloroquinoline afforded a yellow oil; yield: 83%, IR (ATR) ν_{max}/cm^{-1}: 3265, 2865, 1610, 1576, 1539, 1450, 1427, 1331, 1101, 876, 804; ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.35 (d, J = 5.6 Hz, 1H, H-2a), 8.09 (d, J = 9.0 Hz, 1H, H-5a), 7.77 (d, J = 2.2 Hz, 1H, H-8a), 7.40 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.56 (d, J = 5.6 Hz, 1H, H-3a), 3.78 (t, J = 5.5 Hz, 2H, H-2′), 3.69–3.65 (m, 2H, H-3′), 3.65–3.61 (m, 2H, H-4′), 3.56 (t, J = 5.5 Hz, 2H, H-1′), 3.49 (t, J = 5.3 Hz, 2H, H-5′), 2.74 (t, J = 5.3 Hz, 2H, H-6′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.70 (C-4a), 152.43 (C-2a), 149.66 (C-9a), 136.34 (C-7a), 127.62 (C-8a), 126.04 (C-6a), 124.28 (C-5a), 118.75 (C-10a), 99.81 (C-3a), 73.43 (C-5b), 71.49 (C-3b), 71.31 (C-4b), 69.89 (C-2b), 43.81 (C-1b), 42.01 (C-6b); HRMS (APCI) m/z [M+H]$^+$ 310.1323 (Calcd for $C_{15}H_{21}ClN_3O_3$: 310.1322).

2.4.1.8. (3-Aminopropyl)({3-[(7-chloroquinolin-4-yl)amino]propyl})methylamine, 8. The reaction of *N*-(3-aminopropyl)-*N*-methylpropane-1,3-diamine and 4,7-dichloroquinoline afforded a dark yellow oil after purification by column chromatography, eluting successively with DCM:MeOH (9:1, v/v) and DCM:MeOH:NH$_4$OH (9:1:0.1, v/v/v); yield: 67%; IR (ATR) ν_{max}/cm^{-1}: 3240, 2941, 2848, 2799, 1610, 1576, 1538, 1449, 1366, 1136, 1077, 849, 804; ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.34 (d, J = 5.6 Hz, 1H, H-2a), 8.03 (d, J = 9.0 Hz, 1H, H-5a), 7.77 (d, J = 2.1 Hz, 1H, H-8a), 7.40 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.51 (d, J = 5.7 Hz, 1H, H-3a), 3.39 (t, J = 6.9 Hz, 2H, H-1′), 2.64 (t, J = 7.1 Hz, 2H, H-7′), 2.54 (t, J = 7.2 Hz, 2H, H-3′), 2.46–2.43 (m, 2H, H-5′), 2.28 (s, 2H, H-4′), 1.93 (m, 2H, H-2′), 1.70–1.62 (m, 2H, H-6′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 152.69 (C-4a), 152.47 (C-2a), 149.66 (C-9a), 136.28 (C-7a), 127.62 (C-8a), 125.99 (C-6a), 124.23 (C-5a), 118.76 (C-10a), 99.58 (C-3a), 56.68 (C-3b), 56.57 (C-5b), 42.64 (C-1b), 42.38 (C-4b), 40.95 (C-7b), 30.95 (C-6b), 26.46 (C-2b); HRMS (APCI) m/z [M+H]$^+$ 307.1667 (Calcd for $C_{16}H_{24}ClN_4$: 307.1699).

2.4.1.9. 7-Chloro-4-(piperazin-1-yl)quinoline, 9. The reaction of piperazine and 4,7-dichloroquinoline afforded an off-white powder; yield: 76%; mp: 117–121 °C (lit. 118-120 °C); IR (ATR) ν_{max}/cm^{-1}: 3253, 2939, 2823, 2799, 2746, 1610, 1567,1422, 1377, 1244, 1020, 865, 841, 820, 771; ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.61 (d, J = 5.2 Hz, 1H, H-2a), 8.02 (d, J = 9.0 Hz, 1H, H-5a), 7.89 (d, J = 2.2 Hz, 1H, H-8a), 7.48 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.97 (d, J = 5.1 Hz, 1H, H-3a), 3.21 (dd, J = 6.3, 3.5 Hz, 4H, H-1′), 3.17–3.04 (m, 4H, H-2′); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 159.47 (C-4a), 152.85 (C-2a), 150.54 (C-9a), 136.42 (C-7a), 128.28 (C-8a), 127.29 (C-6a), 127.09 (C-5a), 123.08 (C-10a), 110.24 (C-3a), 54.03 (C-1b), 46.40 (C-2b); HRMS (APCI) m/z [M+H]$^+$ 248.0942 (Calcd for $C_{13}H_{15}ClN_3$: 248.0955).

2.4.2. 4-[(1*E*)-3-(5-Methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid, 10

The chalcone **10** was synthesized by adopting a literature reported method[14] and described as follows: Formylbenzoic acid (10 mmol, 1.5 g, 1 equiv) and 2-acetyl-5-methylfuran (10.3 mmol, 1.3 g, 1.2 ml, 1.03 equiv) were successively added to MeOH (60 ml) upon stirring at room temperature. Sodium hydroxide solution, NaOH (1 M, 20 ml) was added and stirring was continued for 12 h [Scheme 1, step (ii)]. The progress of the reaction was followed by TLC. After completion, the pH of the solution was adjusted to 2 with HCl solution (1 M) upon which an off-white to yellow precipitate formed. The precipitate was subsequently collected by suction filtration and washed with water, then with a 10% MeOH solution, dried and recrystallized from MeOH to yield 2.30 g (90% yield) of the desired compound as an off-white to yellow powder; mp: 221–228 °C; IR (ATR) ν_{max}/cm^{-1}: 3104, 3076, 2982, 2924, 2931, 1684, 1654, 1601, 1509, 1288, 1211, 1065, 1027, 845, 772, 754; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.14 (s, 1H, H-a), 7.97 (d, J = 8.4 Hz, 2H, H-2″), 7.94 (d, J = 8.4 Hz, 2H, H-3″), 7.80 (d, J = 3.5 Hz, 1H, H-3′), 7.75 (d, J = 15.7 Hz, 1H, H-1), 7.72 (d, J = 15.7 Hz, 1H, H-2), 6.44 (d, J = 3.3 Hz, 1H, H-4′), 2.40 (s, 3H, H-6′); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.46 (C-3), 166.86 (C-5″), 158.97 (C-2′), 151.83 (C-5′), 140.75 (C-1), 138.65 (C-4″), 131.98 (C-1″), 129.73 (C-3″), 128.74 (C-2″), 124.16 (C-2), 122.02 (C-3′), 109.64 (C-4′), 13.78 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 257.0890 (Calcd for $C_{15}H_{13}O_4$: 257.0814).

2.4.3. 4-Aminoquinolinyl-chalcone amides, 11–19

Chalcone **10** (3.9 mmol, 1 g, 1 equiv) and CDI (4.9 mmol, 0.8 g, 1.3 equiv) were added to anhydrous DCM (50 ml) and stirred for 3 h at room temperature. Afterwards, aminoquinoline **1–9** (5.9 mmol, 1.5 equiv) was added followed by DMF (10 ml) to enhance dissolution of the quinoline intermediate. The resulting solution was stirred for an additional 24 h. The solution was quenched with the addition of distilled water (50 ml). The organic phase was separated and the aqueous phase was extracted 3 times with DCM. The combined organic layers were washed with water (3 × 50 ml) and then with saturated NaHCO$_3$ solution (50 ml), dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by column chromatography, eluting with DCM:MeOH (9:1, v/v) afforded the pure amide. This procedure was used for the synthesis of all amides **11–19**. NMR, IR and HRMS data are reported below.

2.4.3.1. *N*-{2-[(7-Chloroquinolin-4-yl)amino]ethyl}-4-[(1*E*)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 11. During the extraction, a precipitate formed, which was filtered, washed with water and then with cold methanol to yield 0.29 g (16%) of the desired pure compound as a light yellow powder; mp: 255.7–256.8 °C; IR (ATR) ν_{max}/cm^{-1}: 3338, 1643, 1608, 1579, 1535, 1507, 1297, 1224, 1069, 848, 762; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.82 (t, J = 5.7 Hz, 1H, amide), 8.41 (d, J = 5.4 Hz, 1H, H-2a), 8.20 (d, J = 9.0 Hz, 1H, H-5a), 7.93 (d, J = 8.6 Hz, 2H, H-2″), 7.90 (d, J = 8.5 Hz, 2H, H-3″), 7.80 (d, J = 3.5 Hz, 1H, H-3′), 7.78 (d, J = 2.2 Hz, 1H, H-8a), 7.74 (d, J = 15.7 Hz, 1H, H-1), 7.71 (d, J = 15.7 Hz, 1H, H-2), 7.51 (t, J = 5.6 Hz, 1H, N-H), 7.46 (dd, J = 9.0, 2.3 Hz, 1H, H-6a), 6.64 (d, J = 5.4 Hz, 1H, H-3a), 6.45 (d, J = 3.4 Hz, 1H, H-4′), 3.55 (q, J = 6.3 Hz, 2H, H-2b), 3.48 (q, J = 6.3 Hz, 2H, H-1b), 2.40 (s, 3H, H-6′); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.55 (C-3), 166.21 (C-5″), 158.93 (C-2′), 151.95 (C-2a), 151.85 (C-5′), 150.12 (C-9a), 140.99 (C-1), 137.20 (C-1″), 135.56 (C-4″), 133.46 (C-7a), 128.61 (C-3″), 127.73 (C-2″), 127.54 (C-8a), 124.21 (C-6a), 123.95 (C-5a), 123.53 (C-2), 121.94 (C-3a), 117.49 (C-10a), 109.62 (C-4′), 40.04 (C-1b), 37.97 (C-2b), 13.79 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 460.1390 (Calcd for $C_{26}H_{23}ClN_3O_3$: 460.1428).

2.4.3.2. *N*-{3-[(7-Chloroquinolin-4-yl)amino]propyl}-4-[(1*E*)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 12. During the extraction, a precipitate formed which was filtered, washed with water and then with cold methanol to yield 0.29 g (16%) of the desired pure compound as a light yellow powder; mp: 122.1-122.2 °C; IR (ATR) ν_{max}/cm^{-1}: 3317, 1642, 1609,

1580, 1508, 1370, 1314, 1206, 1068, 792; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.66 (s, 1H, amide), 8.38 (d, J = 5.3 Hz, 1H, H-2a), 8.26 (d, J = 9.1 Hz, 1H, H-5a), 7.91 (m, 4H, 3″ and 4″), 7.78 (m, 2H, H-3′ and H-5a), 7.72 (m, 2H, H-1 and H-2), 7.44 (d, J = 8.9 Hz, 1H, H-6), 7.37 (s, 1H, N-H), 6.48 (d, J = 5.5 Hz, 1H, H-3a), 6.44 (s, 1H, H-4′), 3.72–3.17 (m, 16H, H-1b, H-3b and H_2O), 2.40 (s, 3H, H-6′), 1.93 (m, 2H, H-2b); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.55 (C-3), 165.77 (C-5″), 158.87 (C-2′), 151.84 (C-2a), 150.07 (C-5′), 148.99 (C-9a), 141.03 (C-1), 137.04 (C-1″), 135.78 (C-4″), 133.44 (C-7a), 128.56 (C-3″), 127.68 (C-2″), 127.44 (C-8a), 124.10 (C-6a), 124.02 (C-5a), 123.43 (C-2), 121.88 (C-3′), 117.48 (C-10a), 109.59 (C-4′), 98.69 (C-3a), 40.16 (C-1b), 37.32 (C-3b), 27.81 (C-2b), 13.77 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 474.1543 (Calcd for $C_{27}H_{25}ClN_3O_3$: 474.1543).

2.4.3.3. *N*-{4-[(7-Chloroquinolin-4-yl)amino]butyl}-4-[(1*E*)-3-(5-methylfurfan-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 13.

Light yellow powder; yield 0.43 g (23%); mp: 161.5-169.1 °C; IR (ATR) ν_{max}/cm^{-1}: 3386, 2930, 1641, 1608, 1586, 1551, 1510, 1450, 1325, 1207, 1070, 808, 764; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.65 (t, J = 5.7 Hz, 1H, amide), 8.43 (s, 1H, H-8a), 8.42 (d, J = 4.1 Hz, 1H, H-2a), 8.22 (s, 1H, NH), 7.93–7.89 (m, 4H, H-2″ and H-3″), 7.85 (d, J = 2.3 Hz, 1H, H-5a), 7.82 (d, J = 3.4 Hz, 1H, H-3′), 7.77–7.69 (m, 2H, H-1 and H-2), 7.55 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 6.64 (d, J = 6.1 Hz, 1H, H-3a), 6.46 (d, J = 3.4 Hz, 1H, H-4′), 3.41 (q, J = 6.6 Hz, 2H, H-1b), 3.35 (q, J = 6.4 Hz, 2H, H-4b), 2.41 (s, 3H, H-6′), 1.77–1.63 (m, 4H, H-3b and H-2b); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.55 (C-3), 165.53 (C-5″), 158.88 (C-2′), 152.17 (C-9a), 151.84 (C-5′), 148.27 (C-2a), 141.04 (C-1), 136.98 (C-1″), 135.81 (C-4″), 135.17 (C-7a), 128.54 (C-3″), 127.66 (C-2″), 125.08 (C-6a), 124.79 (C-8a), 124.12 (C-5a), 123.40 (C-2), 121.91 (C-3′), 116.65 (C-10a), 109.60 (C-4′), 98.63 (C-3a), 42.44 (C-1b), 38.87 (C-4b), 26.69 (C-2b), 25.17 (C-3b), 13.78 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 488.1691 (Calcd for $C_{28}H_{27}ClN_3O_3$: 488.1741).

2.4.3.4. *N*-{1-[(7-Chloroquinolin-4-yl)amino]propan-2-yl}-4-[(1*E*)-3-(5-methylfurfan-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 14.

Light yellow crystals; yield 0.39 g (21%); mp: 146.5-148.0 °C; IR (ATR) ν_{max}/cm^{-1}: 3292, 2923, 1644, 1606, 1578, 1507, 1449, 1332, 1206, 1066, 847, 800, 767; ^{1}H NMR (600 MHz, MeOD) δ (ppm): 8.33 (d, J = 5.7 Hz, 1H, H-2a), 8.04 (d, J = 9.1 Hz, 1H, H-5a), 7.82 (d, J = 8.3 Hz, 2H, H-3″), 7.76 (d, J = 8.6 Hz, 2H, H-2), 7.75 (d, J = 6.3 Hz, 1H, H-2″), 7.74 (d, J = 1.9 Hz, 1H, H-8a), 7.59 (d, J = 8.3 Hz, 1H, H-1), 7.53 (d, J = 3.5 Hz, 1H, H-3′), 7.39 (dd, J = 9.0, 2.3 Hz, 1H, H-6a), 6.74 (d, J = 5.9 Hz, 1H, H-3a), 6.35 (d, J = 3.3 Hz, 1H, H-4′), 4.53 (hept, J = 6.8 Hz, 1H, H-2b), 3.60–3.42 (m, 2H, H-1b), 2.43 (s, 3H, H-6′), 1.40 (d, J = 6.8 Hz, 3H, H-3b); ^{13}C NMR (151 MHz, MeOD) δ (ppm): 178.42 (C-3), 169.89 (C-5″), 160.90 (C-2′), 153.50 (C-5′), 153.08 (C-9a), 151.81 (C-2a), 148.97 (C-4a)143.09 (C-1), 139.17 (C-1″), 137.01 (C-4″), 136.66 (C-7a), 129.62 (C-3″), 129.05 (C-2″), 128.16 (C-8a), 127.11 (C-6a), 126.29 (C-5a), 124.27 (C-5a), 122.77 (C-3′), 118.53 (C-10a), 110.80 (C-4′), 99.92 (C-3a), 49.68 (C-2b), 46.44 (C-1b), 18.09 (C-3b), 13.91 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 474.1556 (Calcd for $C_{27}H_{25}ClN_3O_3$: 474.1584).

2.4.3.5. *N*-{6-[(7-Chloroquinolin-4-yl)amino]hexyl}-4-[(1*E*)-3-(5-methylfurfan-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 15.

Light yellow powder; yield 0.5 g (25%); mp:177.1-181.5 °C; IR (ATR) ν_{max}/cm^{-1}: 3560, 3323, 2926, 2858, 1649, 1605, 1598, 1579, 1532, 1507, 1369, 1329, 1299, 1198, 1063, 1014, 807, 769; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.57 (t, J = 5.6 Hz, 1H, amide), 8.37 (d, J = 5.6 Hz, 1H, H-2a), 8.28 (d, J = 9.0 Hz, 1H, H-5a), 7.92–7.85 (m, 4H, H-2″ and H-3″), 7.79 (d, J = 3.5 Hz, 1H, H-3′), 7.77 (d, J = 2.3 Hz, 1H, H-8a), 7.71 (m, 2H, H-1 and H-2), 7.50 (t, J = 5.5 Hz, 1H, NH), 7.44 (dd, J = 9.0, 2.3 Hz, 1H, H-6a), 6.47 (d, J = 5.6 Hz, 1H, H-3a), 6.44 (d, J = 3.4 Hz, 1H, H-4′), 3.30–3.22 (m, 4H, H-6b and H-1b), 2.40 (s, 3H, H-6′), 1.65 (p, J = 7.2 Hz, 2H, H-2b), 1.54 (p, J = 7.2 Hz, 2H, H-5b), 1.38 (m, 4H, H-3b and H-4b); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.63 (C-3), 165.55 (C-5″), 158.99 (C-2′), 151.89 (C-9a), 151.06 (C-2a), 150.71 (C-5′), 148.03 (C-4a), 141.13 (C-1), 137.00 (C-1″), 135.96 (C-4″), 133.91 (C-7a), 128.61 (C-3″), 127.72 (C-2″), 126.63 (C-8), 124.34 (C-6a and C-5a), 123.41 (C-2), 121.99 (C-3′), 117.29 (C-10a), 109.68 (C-4′), 98.66 (C-3a), 42.50 (C-1b), 29.15 (C-6b), 27.78 (C-5b), 26.43 (C-2b), 26.37 (C-3b and C-4b), 13.84 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 516.1997 (Calcd for $C_{30}H_{31}ClN_3O_3$: 516.2054).

2.4.3.6. *N*-{3-[(7-Chloroquinolin-4-yl)amino]propyl}-N-methyl-4-[(1*E*)-3-(5-methylfurfan-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 16.

Yellow crystals; yield 0.70 g (37%); mp: 86.2-88.1 °C; IR (ATR) ν_{max}/cm^{-1}: 3328, 2924, 1653, 1604, 1574, 1507, 1367, 1329, 1206, 1062, 800, 765; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.46 (d, J = 5.6 Hz, 1H, H-2a), 7.99 (d, J = 9.0 Hz, 1H, H-5a), 7.91 (d, J = 2.2 Hz, 1H, H-8a), 7.81 (d, J = 15.7 Hz, 1H, H-1), 7.68 (d, J = 8.0 Hz, 2H, H-2″), 7.45 (d, J = 7.9 Hz, 2H, H-3″), 7.41 (d, J = 15.7 Hz, 1H, H-2), 7.32 (dd, J = 9.0, 2.2 Hz, 1H, H-6a), 7.26 (d, J = 3.5 Hz, 1H, H-3′), 6.92 (d, J = 6.2 Hz, 1H, N-H), 6.39 (d, J = 5.6 Hz, 1H, H-3a), 6.21 (d, J = 3.4 Hz, 1H, H-4′), 3.67 (t, J = 6.2 Hz, 2H, H-3b), 3.42 (t, J = 6.0 Hz, 2H, H-1b), 3.00 (s, 3H, H-4b), 2.43 (s, 3H, H-6′), 1.96 (p, J = 6.1 Hz, 2H, H-2b); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.80 (C-3), 171.94 (C-5″), 158.49 (C-2′), 152.31 (C-5′), 151.26 (C-2a), 150.18 (C-9a), 141.76 (C-1), 137.22 (C-1″), 136.39 (C-4″), 135.13 (C-7a), 128.50 (C-3″), 127.82 (C-8a), 127.43 (C-2″), 125.45 (C-6a), 122.63 (C-5a), 122.16 (C-2), 119.97 (C-3′), 117.51 (C-10a), 109.50 (C-4′), 98.16 (C-3a), 44.42 (C-3b), 38.73 (C-1b), 37.47 (C-4b), 24.75 (C-2b), 14.20 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 488.1741 (Calcd for $C_{28}H_{27}ClN_3O_3$: 488.1741).

2.4.3.7. *N*-[2-(2-{2-[(7-Chloroquinolin-4-yl)amino]ethoxy}ethoxy)ethyl]-4-[(1*E*)-3-(5-methylfurfan-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 17.

Dark yellow platelets; yield 1.36 g (64%); mp 80.1–80.9 °C; IR (ATR) ν_{max}/cm^{-1}: 3337, 2865, 1647, 1603, 1577, 1508, 1368, 1330, 1206, 1066, 876, 799, 764; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.40 (d, J = 5.3 Hz, 1H, H-2a), 7.85 (d, J = 2.3 Hz, 1H, H-8a), 7.74–7.66 (m, 4H, H-1, H-2″ and H-5a), 7.50 (d, J = 8.0 Hz, 2H, H-3″), 7.32 (d, J = 15.7 Hz, 1H, H-2), 7.26 (d, J = 3.5 Hz, 1H, H-3′), 7.24–7.21 (m, 1H, H-6a), 6.97 (t, J = 5.6 Hz, 1H, amide), 6.27 (d, J = 5.4 Hz, 1H, H-3a), 6.20 (d, J = 3.4 Hz, 1H, H-4′), 5.74 (t, J = 5.1 Hz, 1H, N-H), 3.78 (t, J = 5.1 Hz, 2H, H-2b), 3.68 (m, 6H, H-3b, H-4b and H-5b), 3.65 (q, J = 5.2 Hz, 2H, H-6b), 3.39 (q, J = 4.9 Hz, 2H, H-1b), 2.41 (s, 3H, H-6′); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.76 (C-3), 166.80 (C-5″), 158.54 (C-2′), 152.26 (C-4a), 151.50 (C-2a), 149.86 (c-9a), 148.61 (C-5′), 141.60 (C-1), 137.59 (C-1″), 135.54 (C-4″), 134.95 (C-7a), 128.27 (C-3″), 128.19 (C-8a), 127.44 (C-2″), 125.28 (C-6a), 122.82 (C-2), 121.34 (C-5a), 120.12 (C-3′), 117.16 (C-10a), 109.51 (C-4′), 99.01 (C-3a), 70.20 (C-3b), 70.07 (C-4b), 69.78 (C-5b), 68.60 (C-2b), 42.62 (C-1b), 39.73 (C-6b), 14.19 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 548.1942 (Calcd for $C_{30}H_{31}ClN_3O_5$: 548.1952).

2.4.3.8. *N*-[3-({3-[(7-Chloroquinolin-4-yl)amino]propyl}(methyl)amino)propyl]-4-[(1*E*)-3-(5-methylfurfan-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 18.

Dark yellow platelets; yield 1.1 g (52%); mp: 83.4-85.7 °C; IR (ATR) ν_{max}/cm^{-1}: 3250, 2943, 2847, 2798, 1647, 1601, 1577, 1508, 1367, 1328, 1205, 1066, 843, 801, 766; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.39 (d, J = 5.4 Hz, 1H, H-2a), 7.83 (d, J = 2.0 Hz, 1H, H-8a), 7.77–7.70 (m, 3H, H-1 and H-2″), 7.57 (dd, J = 11.2, 8.6 Hz, 3H, H-3″ and H-5a), 7.40 (t, J = 5.7 Hz, 1H, amide), 7.33 (d, J = 15.8 Hz, 1H, H-2), 7.24

(d, J = 4.0 Hz, 1H, H-3′), 7.19 (dd, J = 8.9, 2.2 Hz, 1H, H-6a), 6.97 (t, J = 4.3 Hz, 1H, N-H), 6.21 (d, J = 5.6 Hz, 1H, H-3a), 6.20 (d, J = 3.6 Hz, 1H, H-4′), 3.50 (q, J = 6.3 Hz, 2H, H-6b), 3.31 (q, J = 5.7 Hz, 2H, H-1b), 2.56 (t, J = 6.0 Hz, 2H, H-3b), 2.52 (t, J = 6.8 Hz, 2H, H-4b), 2.41 (s, 3H, H-6′), 2.32 (s, 3H, H-7b), 1.84 (m, 4H, H-2b and H-5b); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.76 (C-3), 166.77 (C-5″), 158.53 (C-2′), 152.26 (C-4a), 151.85 (C-2a), 150.20 (C-9a), 148.91 (C-5′), 141.63 (C-1), 137.55 (C-1″), 135.82 (C-4″), 134.61 (C-7a), 128.36 (C-3″), 128.32 (C-5a), 127.41 (C-2″), 124.90 (C-6a), 122.78 (C-2), 121.58 (C-8a), 120.08 (C-3′), 117.36 (C-10a), 109.50 (C-4′), 98.50 (C-3a), 56.90 (C-3b), 56.42 (C-4b), 43.01 (C-1b), 42.09 (C-7b), 39.09 (C-6b), 26.86 (C-5b), 24.95 (C-2b), 14.19 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 545.2303 (Calcd for $C_{31}H_{34}ClN_4O_3$: 545.2319).

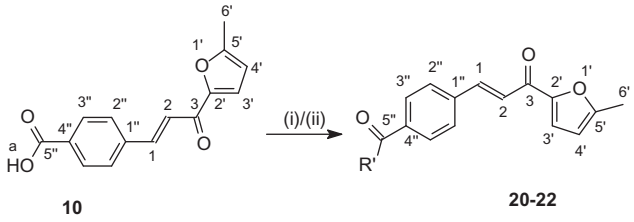
2.4.3.9. (2*E*)-3-(4-{[4-(7-Chloroquinolin-4-yl)piperazin-1-yl]carbonyl}phenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one, 19. Light brown powder; yield 0.65 g (34%); mp: 192.1–195.4 °C; IR (ATR) ν_{max}/cm^{-1}: 1656, 1631, 1601, 1574, 1511, 1426, 1380, 1247, 1207, 1125, 1061, 1008, 930, 812, 776; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.72 (d, J = 4.9 Hz, 1H, H-2a), 8.03 (d, J = 2.2 Hz, 1H, H-8a), 7.92 (d, J = 8.9 Hz, 1H, H-5a), 7.81 (d, J = 15.7 Hz, 1H, H-1), 7.68 (d, J = 8.1 Hz, 2H, H-2″), 7.48 (d, J = 8.1 Hz, 2H, H-3″), 7.43 (d, J = 2.2 Hz, 1H, H-6a), 7.40 (d, J = 15.7 Hz, 1H, H-2), 7.25 (d, J = 3.6 Hz, 1H, H-3′), 6.82 (d, J = 5.0 Hz, 1H, H-3a), 6.21 (d, J = 3.3 Hz, 1H, H-4′), 3.38–3.03 (m, 4H, H-1b), 2.42 (s, 3H, H-6′); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.77 (C-3), 169.78 (C-5″), 158.43 (C-2′), 156.22 (C-4a), 152.32 (C-9a), 151.89 (C-2a), 150.08 (C-5′), 141.74 (C-1), 136.74 (C-1″), 136.49 (C-4″), 135.15 (C-7a), 129.02 (C-8a), 128.51 (C-3″), 127.76 (C-2″), 126.61 (C-6a), 124.62 (C-5a), 122.64 (C-2), 121.73 (C-10a), 119.90 (C-3′), 109.48 (C-4′), 109.34 (C-3a), 52.13 (C-1b), 14.19 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 486.1545 (Calcd for $C_{28}H_{25}ClN_3O_3$: 486.1584).

2.4.4. Chalconyl amides, 20–22

2.4.4.1. 4-[(1*E*)-3-(5-Methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzamide, 20. The amide **20** was synthesized using a method by Fisher et al.[33] and described as follows: To the chalcone **10** (3.9 mmol, 1 g, 1 equiv) in DCM (50 ml) and stirring at 0 °C, was added oxalyl chloride (7.8 mmol, 0.99 g, 2 equiv) and 2 drops of DMF. The solution was allowed to warm to room temperature (rt) over a period of 1 h, and the solvent was subsequently removed. The residue was dissolved in DCM (50 ml), cooled to 0 °C and an excess NH_4OH (33%) was added. The solution was allowed to heat to rt and stirred for an additional 4 h (Scheme 2). The solvent was removed and the residue was washed with water (3 × 100 ml), then with cold 20% MeOH (2 × 10 ml) and dried to yield 0.92 g (92%) of the desired pure compound as a brown powder; mp: charcoal at 215 °C; IR (ATR) ν_{max}/cm^{-1}: 3347, 3180, 1649, 1595, 1507, 1067, 767; ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.07 (d, J = 4.4 Hz, 1H, 6″-α), 7.94–7.90 (m, 4H, H-2″ and H-3″), 7.80 (d, J = 3.5 Hz, 1H, H-3′), 7.73 (d, J = 5.5 Hz, 2H, H-1 and H-2), 7.47 (d, J = 5.9 Hz, 1H, 6″-β), 6.45 (d, J = 3.7 Hz, 1H, H-4′), 2.40 (s, 3H, H-6′); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 175.53 (C-3), 167.21 (C-5″), 158.86 (C-2′), 151.84 (C-5′), 141.02 (C-1), 137.13 (C-1″), 135.50 (C-4″), 128.50 (C-2″), 127.96 (C-3″), 123.45 (C-2), 121.90 (C-3′), 109.58 (C-4′), 13.77 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 256.0969 (Calcd for $C_{15}H_{13}NO_3$: 256.0973).

2.4.4.2. *N*-Butyl-4-[(1*E*)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]-benzamide, 21. Amide **21** was brought about as light yellow platelets in 55% yield (0.75 g) from chalcone **10** and *n*-butylamine (Scheme 2), similarly to compounds **11**–**19**; mp: 169.2-171.5 °C; IR (ATR) ν_{max}/cm^{-1}: 3359, 2866, 1643, 1593, 1536, 1069, 796; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.81–7.73 (m, 3H, H-2″ and H-1), 7.65–7.59 (m, 2H, H-3″), 7.38 (d, J = 15.8 Hz, 1H, H-2), 7.24 (d, J = 3.7 Hz, 1H, H-3′), 6.36 (t, J = 5.8 Hz, 1H, H-6″), 6.20 (d, J = 3.3 Hz, 1H, H-4′), 3.42 (td, J = 7.2, 5.6 Hz, 2H, H-7″), 2.41 (s, 3H, H-6′), 1.57 (tt, J = 7.7, 6.6 Hz, 2H, H-8″), 1.38 (h, J = 7.4 Hz, 2H, H-9″), 0.92 (t, J = 7.3 Hz, 3H, H-10″); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.81 (C-3), 166.70 (C-5″), 158.48 (C-2′), 152.30 (C-5′), 141.78 (C-1), 137.49 (C-1″), 136.12 (C-4″), 128.39 (C-2″), 127.43 (C-3″), 122.74 (C-2), 119.98 (C-3′), 109.47 (C-4′), 39.87 (C-7″), 31.64 (C-8″), 20.12 (C-9″), 14.17 (C-6′), 13.75 (C-10″); HRMS (APCI) m/z [M+H]$^+$ 312.1589 (Calcd for $C_{19}H_{21}NO_3$: 312.1599).

2.4.4.3. (2*E*)-1-(5-Methylfuran-2-yl)-3-{4-[(morpholin-4-yl)carbonyl]-phenyl}prop-2-en-1-one, 22. Amide **22** was synthesized as light yellow crystals in 71% (0.9), using chalcone **10** and morpholine (Scheme 2), as for **21** above; mp:195.5–197.2 °C; IR (ATR) ν_{max}/cm^{-1}: 1656, 1634, 1603, 1507, 064, 834, 797; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.66 (d, J = 15.7 Hz, 1H, H-1), 7.52 (d, J = 8.0 Hz, 2H, H-2″), 7.32–7.20 (m, 3H, H-3″ and H-2), 7.11 (d, J = 3.2 Hz, 1H, H-3′), 6.07 (d, J = 3.4 Hz, 1H, H-4′), 3.73–3.21 (m, 8H, H-6″ and H-7″), 2.28 (s, 3H, H-6′); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.76 (C-3), 169.55 (C-5″), 158.38 (C-2′), 152.29 (C-5′), 141.77 (C-1), 136.72 (C-1″), 136.29 (C-4″), 128.42 (C-2″), 127.68 (C-3″), 122.50 (C-2), 119.84 (C-3′), 109.42 (C-4′), 66.76 (C-7″), 48.11 (C-6″α), 42.49 (C-6″β), 14.15 (C-6′); HRMS (APCI) m/z [M+H]$^+$ 326.1381 (Calcd for $C_{19}H_{19}NO_4$: 326.1392).



Amide	R_2	Amide	R_2
20	6″ H_2N---	22	7″ 6″ O N---
21	9″ 7″ 10″ 6″ NH--- 8″		

Scheme 2. Synthesis of chalconyl amides **20**–**22**. Reagents and conditions: (i) oxalyl chloride, DCM/DMF, 0 °C to rt, 1 h then 33% NH_4OH (excess); (ii) CDI, DCM, 3 h, rt then *n*-butylamine or morpholine, 24 h.

3. Results

3.1. Chemistry

The aminoquinoline intermediates (**1**–**9**) were easily prepared in high yields through nucleophillic aromatic substitution on the 4th position of the quinoline ring by various diamines. In all instances the presence of one chloride was confirmed by means of the mass spectra having an M+2 ion peak being in a 4:1 height ratio to the M$^+$ peak. The chalcone intermediate **10** was attained by Claisen-Schmidt condensation of 2-acetyl-5-methylfuran and 4-formylbenzoic acid in basic methanolic water in high yields. Contrary, compounds **11**–**19** were synthesized in low to moderate yields through amide bond formation between the carboxylic acid of the chalcone and the free amine of the aminoquinolines, using CDI as coupling agent. The addition of different solvents (acetone and acetonitrile) to the reaction mixture, to facilitate solubility did not impact on the yields. The addition of DMF, however,

substantially increased the yields of the final reaction. With shortened reaction times, a significant amount of chalcone was extracted during workup, with very low yields (<5%) of the desired compound. Only after 24 h, the yields of the various hybrids seemed to reach a plateau. Increasing the reaction times further did not seem to impact on these yields.

3.2. Physicochemical properties

Three different techniques were adopted to determine the physicochemical properties of the prepared quinoline-chalcone amides. Differential scanning calorimetry (DSC) was used to determine both the physical states of the compounds and their different phase transitions. Thermogravimetric analysis (TGA) was employed to determine thermal stability. All the DSC and TGA thermograms are presented as Supporting Information. The ADMET properties, such as log*P*, solubility and absorption levels were determined using ACD/Chemsketch and Discovery studio. Preliminary ADMET calculations were performed in order to determine the drug-likeness properties of the synthesized compounds and of CQ and are summarized in Table 1.

The results show that the synthesized quinoline-chalcone amides were all had the same level of calculated poor water solubility, just like CQ at neutral pH value, which was consistent with their high log*P* values recorded in the 4–6 range. They demonstrated various absorption levels, for example good (**11**, **12**, **16** and **19**), moderate (**13**, **14**, **17** and **18**) and poor (**15**). None of them showed better calculated solubility and intestinal absorption levels than the intermediate chalcone **10** and the chalconyl amides **20**–**22**. Amide **15**, which showed low solubility, was the most lipophilic (log*P* = 6) and the poorest absorbed of all of the amides, while **19** was the least lipophilic, least soluble, but one of the best absorbed compounds. Overall, the three chalconyl amides **20**, **21** and **22** exhibited the best predicted drug-like properties.

Table 1
Calculated physicochemical properties of compounds **10**–**22** and CQ

Compound	Log *P*[a]	ADMET solubility level[b]	ADMET absorption level[c]
10	2.9	3	0
11	4.4	2	0
12	4.7	2	0
13	5.1	2	1
14	4.8	2	1
15	6.0	2	2
16	4.3	2	0
17	4.4	2	1
18	5.1	2	1
19	3.8	1	0
20	1.8	3	0
21	3.5	2	0
22	0.9	3	0
CQ	3.1	2	0

[a] Calculated with ACD/ChemSketch (2000), version 4.54.
[b] ADMET aqueous solubility level (Log S_w) at 25 °C and pH 7, values = 1, 2 and 3 indicate very low, low and good solubility, respectively, as determined using Accelrys Discovery Studio 3.1.
[c] ADMET human intestinal absorption, values = 0, 1 and 2 indicate good, moderate and poor absorption, respectively, as determined using Accelrys Discovery Studio 3.1.

3.3. In vitro antimalarial activity and cytotoxicity

The aminoquinoline-chalcone amides **11**–**19** and chalcone **10** were screened in vitro, alongside CQ and the equimolar **CQ-10** combination, **M**, against the 3D7 and W2 strains of *P. falciparum*. Their IC_{50} values are presented in Table 2. Compounds **11**–**19** were found active, with IC_{50} values ranging between 0.04–0.53 μM and 0.06–1.7 μM against the 3D7 and W2 strains, respectively. Amide **15** showed the highest activity against 3D7 and W2 strains, with an IC_{50} of 0.04 μM and 0.06 μM, respectively. Amides **11**–**15** and **18** showed activities comparable to that of CQ against the 3D7 strain, while **16**, **17** and **19** had much lower activities of ten-, seven- and five-fold less potent than CQ, respectively.

Amide **15** was found to be almost twice as potent against the W2 strain as CQ. Amides **13** and **14** displayed equipotency to CQ, while **12**, **17** and **18** were two-fold less potent, whereas **16** was four times less potent than CQ. Amides **11** and **19** were the least active, being fifteen- and eleven-fold less potent than CQ, respectively. The tertiary amides, **16, 19** and **22** displayed the lowest activity against both the CQS and CQR strains.

All synthesized compounds had resistance index (RI) values above 1, with amide **11** possessed the highest value of 17. They

Table 2
Antimalarial activity and cytotoxicity of screened amide compounds

	Activity IC_{50}[a] (μM)		Resistance Index	Cytotoxicity, IC_{50}[c] (μM)	Selectivity Index
Compound	3D7	W2	RI[b]	WI-38 HFLF[d]	SI[e]
10	18.4	14.2	0.8	>100	nd
11	0.11	1.78	16.7	47.3	446
12	0.07	0.23	3.4	74.5	1123
13	0.05	0.10	2.1	39.0	819
14	0.08	0.11	1.4	10.0	126.0
15	0.05	0.07	1.5	19.5	435
16	0.37	0.46	1.2	18.6	50
17	0.26	0.27	1.1	10.2	40
18	0.09	0.22	2.4	5.30	58
19	0.54	1.26	2.3	17.3	32
20	10–30	nd	nd	nd	nd
21	10–30	nd	nd	nd	nd
22	>30	nd	nd	nd	nd
M	0.01*	0.04*	3.1	20.9	1838.6
CQ	0.05 (0.02*)	0.12 (0.04*)	2.2	nd	nd
PTD	nd	nd	nd	16.4	nd

nd = not determined, M = equimolar combination of CQ and **10**, parthenolide (PTD).
* Expressed in μg/ml.
[a] Minimum concentration of compound inducing 50% parasitic cells inhibition.
[b] Resistance Index (RI) = IC_{50} W2/IC_{50} 3D7.
[c] Minimum concentration of compound inducing 50% of WI-38 HFLF cells inhibition.
[d] WI-38 cell line of normal human fetal lung fibroblast.
[e] Selectivity index (SI) = IC_{50} WI-38-HFLF/IC_{50} 3D7.

were also found either moderately or highly cytotoxic to the mammalian cells, with selectivity index values varying between 30 and 1000.

4. Discussion

4.1. Chemistry

The IR spectra of intermediates **1**–**9** commonly showed the presence of absorption frequencies in the 3350–3200 cm^{-1} region, which is associated with N–H stretching. The quinoline moiety was clearly identified from the presence of four doublets and one doublet of doublet, assigned to signals of the heterocyclic proton H-2a, H-3a, H-5a, H-8a and H-6a, respectively, in the ^1H spectra. Proton H-8a, which in normal circumstances would be a singlet, coupled weakly to H-6a to which the doublet of doublet (H-6a) could have been ascribed to. The DEPT90 of each intermediate showed five different tertiary aryl carbons, which further supported the presence of the quinoline moiety. Carbons of the various methylene linkers were identified on ^{13}C/DEPT135 between ca. 25–75 ppm, while the protons were identified on ^1H NMR at ca. 3.8–1.7 ppm.

The presence of the carboxylic acid of the chalcone **10** was confirmed by the signals of a highly deshielded proton at 13.07 ppm and a carbon at 166 ppm. The conjugated ketone's carbon was shielded and was allocated the signal at 175 ppm. The IR spectra showed a strong stretching band at 1654 cm^{-1}, which is associated with an unsaturated ketone. Additional evidence, such as six conjugated tertiary carbons (as determined with DEPT90) and six doublets (^1H NMR) were further indicative of the presence of the chalcone. The integration of the two doublets at 7.9 ppm accounted for four protons, which were assigned to H-2″ and H-3″, indicating that the *ortho*- and *meta* portions were non-equivalent. Due to the electronic effect of both the ketone and heterocyclic oxygen in the furan ring, the quaternary carbon C-2′ was deshielded and was assigned the peak at 158 ppm. The presence of the methyl group was indisputably assigned to the only aliphatic signal at 13 ppm.

The IR spectra of all target compounds **11**–**18** had a weak absorption frequency near 3300 cm^{-1}, assignable to N–H stretching. Further confirmation of this group was obtained by the presence of triplets/singlets in the ^1H-spectra between 6.9–8.8 ppm, as well as the coupling to the CH_2 of the linker displayed in COSY spectra. Additionally, ^1H NMR displayed ten doublets and one doublet of doublet between 6–8.5 ppm, which could have been credited to the chalcone together with the quinoline-H in compounds **11**–**19**, except for **12**. An interesting observation from the ^1H NMR of **12** was the number of singlet peaks. So long as the chemical shift difference in hertz ($\delta\ \nu$) is higher than the coupling constant (J), a simple splitting pattern appears, but within some compounds $\delta\ \nu/J$ is very small, resulting in multiplets to be displayed as singlets,[34] such as the case with amide **12**. The signals at 176 and 166 ppm in ^{13}C NMR were attributed to the ketone and amide of the hybrids, respectively. Supplementary information, such as signals at 99, 109 and 141 ppm, corresponding to C-3a, C-4′ and C-1, respectively, further indicated the presence of both chalcone and quinoline moiety in the structures of these amides. In all cases (except **19**), the theoretical number of CH_2 groups in the linker was in accordance with the one from DEPT135 spectra. In the case of **19**, only C-1b was displayed on ^{13}C NMR, although the HRMS confirmed the correct molecular ion.

4.2. Physicochemical properties

All compounds **11**–**19** displayed small endothermic phase transitions, which were consistent with glass transitions, with onset temperatures ranging between 50–75 °C in most cases. Amides **13** and **17** had onset temperatures of 121 °C and 37 °C, respectively, making them the extremities of the glass transition profile of the series. The glass transitions were not evidently expressed for amides **11**, **12** and **15** and were referred to as 'obscured' glass transitions, which could be readily elucidated by means of annealing.[35] However, since a thorough physicochemical analysis of the solid-state properties of the synthesized compounds, including strength and fragility parameters,[36] was not the main objective of this study, this aspect was not further investigated. The DSC thermograms suggested that all of the compounds **11**–**19** possessed amorphous structures (see Supporting information).

Thermal stability plays a vital role in the storing of compounds, especially when pharmaceuticals must withstand high storage temperatures as in Sub-Saharan Africa, where cooling facilities are limited. The TGA thermograms revealed weight losses of between 2–8.5% for all these compounds when heated to 300 °C. These endothermic events were characterized as glass transitions during the DSC analyses, rather than desolvation endotherms, due to the nature of the weight loss. Indeed, in all cases, the thermograms showed weight losses from the start of the heating runs, which was consistent with the loss of solvent from the surface of the solid, possibly due to adsorption. Although the presence of solvent trapped within crystalline regions of the solid could not be ruled out, the absence of a clear baseline before and after the thermal events made it difficult to explain the exact cause of the weight loss. Overall, all compounds showed a minor weight loss, which indicated minimal to no thermal degradation, therefore indicative of the ability to favourably withstand ordinary drug storage conditions, typical of hot, malaria endemic countries.

Drug development is largely hampered by poor pharmacokinetics and toxicity.[37] It is therefore important to determine drug-like properties during the early stages of the development of a drug. The aqueous solubility of any compound largely influences its ability to be absorbed when taken orally. Poor water solubility is generally associated with high lipophilicity, while hydrophilic compounds generally show poor permeability and hence low absorption, which to a large extent influence the drug's bioavailability. These two properties are thus of high importance when designing new drugs.

In order for drug uptake to occur through biological membranes, the drug must possess a limiting aqueous solubility and be neither too lipophilic, nor too hydrophilic. Lipophilic drugs show poor aqueous solubility and tend to be taken up in fatty globules in the intestine. Once they reach the blood stream, they may be absorbed into tissue. Their slow release may exacerbate toxicity, such as neurotoxicity. Contrary, hydrophilic drugs may be excreted directly by the kidneys, or should they be able to penetrate a cell membrane, become entrapped in intracellular aqueous media. An ideal drug must therefore possess balanced lipophilic/hydrophilic properties to both permeate biological membranes and be taken up in the systemic circulation. The *n*-octanol/water partition coefficient (Log$P$) offers a reliable measure of this balance, with values between 1 and 5 being targeted, and values between 1 and 3 being ideal.[38,39]

A combined analysis of the predicted solubility, absorption levels and log$P$ values revealed amides **11**, **12** and **16** to be the most drug-likeable compounds, similarly to CQ and they were thus expected to display the best biological profile. Compound **15**, however, appeared as the least drug-likeable, based upon its high lipophilicity, low solubility and poor absorption. Amides **20**, **21** and **22**, which also possessed favourable drug-like properties, were expected to demonstrate good antimalarial activity. Compound **19**, a tertiary amide, like **16**, had a predicted log$P$ value in the target

range, poor solubility, but good absorption and were they both therefore expected to show moderate activity.

4.3. In vitro antimalarial activity and cytotoxicity

The *P. falciparum* 3D7 strain, a line cloned from NF54, is of African origin and is CQ susceptible. It has frequently been used to evaluate in vitro antigametocytes activity of trial antimalarial compounds.[40] In contrast, the Indochina/Laos clone W2 is a multi-drug resistant strain. It is known to be the most resistant of all *P. falciparum* strains and has shown resistance against all traditional antimalarial drugs, including chloroquine, cycloguanil, pyrimethamine and sulfadoxine.[41] Both strains were used to determine the in vitro antiplasmodial activity of the synthesized target compounds.

Since the amides prepared during this study were tested in vitro and not metabolized by metabolic enzymes, they would act as new entities and not as prodrugs. The observed IC_{50} values were therefore most likely to be those of the hybrids, rather than that of any active metabolite.

Compared to the IC_{50} of 6.5 μM of Licochalcone A against 3D7 clone,[13] chalcone **10** was found inactive against both the 3D7 and W2 strains. Chalcones, containing electron withdrawing groups on ring A and a heterocyclic ring B, had been reported to show overall better antimalarial activities,[42] which was contradicted by the performance of chalcone **10** against the 3D7 strain in this study.

Due to the limited number of compounds prepared during this study, an accurate structure activity relationship (SAR) could not be drawn. However, the observations made with regards to SAR from these results may be useful in the design of future studies, which included:

Amides **11**–**18** had the same solubility levels and a comparison of their lipophilicity properties could therefore be made based on their log*P* values. In this Letter, the more lipophilic (higher log*P* value of 5–6) compounds, that is **13**, **15** and **18** happened to be more active, while the more hydrophilic (lower log*P* values of 3–4), that is **11**, **14**, **16**, **17** and **19** displayed lower activity against both strains. ADMET predicted amides **11**, **12** and **16** would be drug-likeable, whereas their activities showed the opposite. Contrary, amide **15**, predicted as the least drug-likeable, was found the most active. These outcomes indicated that the predicted absorption levels did not corroborate their antimalarial activity, resulting from their real absorption. These findings suggested that other properties, or other properties in combination with the predicted ones, may have been involved in the real absorption of these compounds. This was in accordance with a previous report that the properties, such as hydrogen bonding, molecular size and shape, polarity, flexibility and the charge/ionization of a compound/drug molecule as a whole (rather than a single property), affect absorption through membranes.[43]

Compounds **11**–**15** were furthermore found in the following increasing order of linker chain length: **11** < **12** = **14** < **13** < **15**, which was congruent with their lipophilicity and activity against both strains. In this sub-series of amides, featuring methylene chain linkers, the activity was found to be directly proportional to the chain length, hence as the length increased, so did the activity against the CQS and CQR strains. Compound **11** showed the highest RI value of 16, which might have been a result of the decreased flexibility of this short chain amide. As the chain increased the RI index decreased, indicating the importance of flexible linkers to overcome resistance.

The combination **M** was as potent as CQ against 3D7 and W2, suggesting that the activity of **M** was in fact equal to that of CQ, since the inactive chalcone **10** had no contribution to this combination's activity. The most active quinoline-chalcone amide, that is **15** was found to be as potent as CQ against both strains. No synthesized compound in this study therefore possessed overwhelmingly higher activity than CQ. This suggested that the chalcone moiety antagonized the antimalarial action of quinoline pharmacophore in most cases. The need for a better partner should thus be identified if one wishes to achieve synergism, using this quinoline based hybrid type.

The tertiary amides, **16** and **19**, despite their predicted favourable drug-like properties, demonstrated the lowest activity against both the CQS and CQR strains, which suggested that the tertiary amide bond was not conducive to increased antimalarial activity for this compound type. Since the 7-chloro-4-aminoquinoline moiety is a well-established antimalarial pharmacophore, this lower activity observed among these amides could only have been as a result of the presence of the chalcone portion in their structures. In order to validate this statement, basic chalconyl amides **20**–**22** were synthesized and screened against the 3D7 clones at three different concentrations that is 3, 10 and 30 μM. Compounds **20** and **21**, bearing a primary and secondary amide, respectively, showed activities between 10–30 μM, while amide **22** with a tertiary amide showed no activity, even at the highest concentration of 30 μM. Compounds **16**, **19** and **22** as tertiary amides, were depleted of hydrogen bonds, while all active amides had one hydrogen bond, involving N–H of the amide functional group. This emphasised the significance of hydrogen bonds as part of the parameters that influence the absorption of drugs or molecules and thus their antimalarial activity in this study.

Compared to the quinoline-chalcone ketones of Sharma et al.[22] (Fig. 2), the incorporation of methylene spacer between both moieties in this study, imparted the resulting conjugates with increased activity, as was observed throughout the series of amide compounds investigated. The amides had RI values above 1, an indication of a loss of activity against the CQR, and no compound were thus able to overcome the resistance of the W2 strain against CQ.

With the exception of **11** and **12**, all other amides showed moderate to high cytotoxicity against human fetal lung fibroblast WI-38 cells, compared to parthenolide. However, analysis of the selectivity index (SI), which is indicative of the effectiveness of a drug to clear only parasitic cells and not healthy cells, enabled the reaching of the following conclusions: (i) amides **16**–**19** had relatively low SI values, suggesting that their activities were generally that of toxicity rather than any other mechanism; (ii) compounds **11**–**15**, however, showed high SI values, demonstrative of their activities being intrinsic, hence not exerted by cytotoxicity, but rather through other mechanisms.

5. Conclusion

In this Letter, a series of novel 4-aminoquinolinyl-chalcone amides, **11**–**19**, were synthesized in a three-step process involving amino-functionalizing quinolines, a carboxylic acid-functionalized chalcone and 1,1′-carbonyldiimidazole as coupling reagent. Routinely used techniques, such as NMR, HRMS and IR served to confirm their structures. DSC and TGA analyses were used to reveal their amorphous structures and thermal stabilities, respectively, while ADMET served to predict their drug-like properties. Antimalarial screens, alongside CQ, showed that all of these amides proved to be active with IC_{50} values ranging between 0.05–0.53 μM and 0.07–1.8 μM against the 3D7 and W2 strains of *P. falciparum*, respectively. The amides displayed a loss of activity against the CQR strain, compared to that of the CQS, which resulted in resistance index values exceeding the unit thus no resistance to CQ was overcome in this study. They also demonstrated moderate to

high selective toxicity towards the mammalian cells in the presence of the parasitic ones. During this study, the antimalarial activity of the amides was found to increase as the lipophilicity and the linker chain length increased. The tertiary amides were the least active, which emphasized the significance of hydrogen bonding on the activity of this compound type. Amide **15**, featuring 1,6-diaminohexane linker was found the most active of all, being as potent as and two-fold more potent than CQ against the 3D7 and W2 strains, respectively, despite its predicted unfavourable high lipophilicity, low solubility and poor absorption properties. The above results generated necessitate the need for the inclusion of the intermediate amino-functionalized quinolines, as well as the aminoquinolines in a 1:1 molar ratio with the chalcone and to evaluate the interaction of these combinations against various *P. falciparum* cell lines. These results would have provided a more definitive conclusion about the role of the chalcone. Additionally the in vivo evaluation of amide **15** must also be investigated.

6. Disclaimer

Any opinion, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the NRF does not accept any liability in regard thereto.

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Supplementary data

Supplementary data (data of compounds **10**–**19**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.032.

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