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Application of multi-component reactions to antimalarial drug discovery. Part 1: Parallel synthesis and antiplasmodial activity of new 4-aminoquinoline Ugi adducts

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Abstract—The synthesis of a new class of Ugi adducts incorporating the 4-aminoquinoline moiety is described. The novel compounds are active against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* with the best compound showing an IC_{50} value of 73 nM against a resistant K1 strain.

At present malaria is considered to be the world's most important tropical parasitic disease, afflicting 300–500 million people and killing 1–2 million annually.[1] It is estimated that nearly 40% of the world's population lives in malaria endemic regions. Of the four species of the disease-causing parasites, *Plasmodium falciparum* (*P. falciparum*) is the most dangerous form, accounting for up to 95% of malaria-related deaths. The emergence of widespread resistance in the parasite to contemporary antimalarial drugs such as chloroquine, CQ (**1**), has given a new dimension to the malaria problem. This resistance has prompted the quest for new active compounds against the parasite.

The advent of combinatorial chemistry has in the recent past emerged as a powerful tool for the rapid generation, identification and optimization of lead compounds in the drug discovery process.[2–4] Multi-component reactions (MCRs),[5,6] reactions in which more than two starting materials all present together in one reaction vessel combine to form a final product, have in this regard been used to efficiently generate chemical diversity in the final products in a few reaction steps. Compared with conventional organic reactions, MCRs are advantageous in being highly convergent and in requiring minimum effort to achieve. Several MCRs are known to date among which the Ugi 4 component condensation (U-4CC)[7] of amine, oxo compound, carboxylic acid and isocyanide, and its variants remains, by far, the most documented and most versatile. Coupled with combinatorial chemistry, MCRs have been used to generate chemical diversity in a few reaction steps and usually in a one-pot operation.[8] Our interest in applying MCRs to antimalarial drug discovery stems from this attractive feature and the realization that the rational choice of inputs based on known antimalarial pharmacophores could lead to the generation of diverse biologically rich libraries designed to explore rapid structure–activity relationships (SARs). We report in this communication the synthesis and biological evaluation of a new class of Ugi adducts based on the aminoquinoline antimalarial pharmacophore. To the best of our knowledge this is the first report in which the Ugi isocyanide MCR (IMCR) chemistry has been used to synthesize antimalarial aminoquinoline analogues.

In the context of our on-going research, we wished to synthesize novel compounds for screening against malaria parasites and to this end we initially targeted α-acylamino amides accessible by way of the Ugi 4CC

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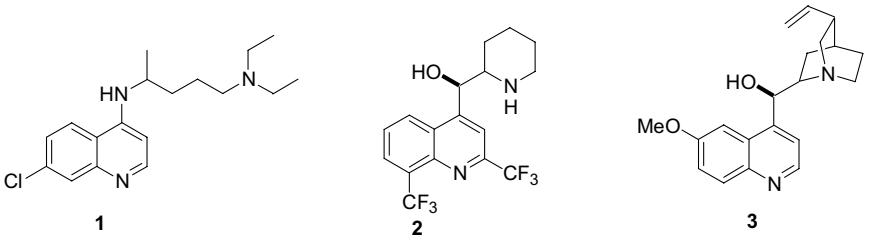


Figure 1. Chemical structures of chloroquine (**1**), mefloquine (**2**) and quinine (**3**).

reaction. We initially designed our exploratory library in such a way as to incorporate the quinoline substructure as part of our amine input in the U-4CC reactions. The basis of the design was that the quinoline moiety is present as the pharmacophore in a number of known antimalarial drugs including chloroquine, CQ, (**1**), mefloquine (**2**) and quinine (**3**) (Fig. 1), where it is believed to inhibit haemozoin formation in the malaria parasite.[9–11] The initial choice of hydroxyl-containing carboxylic acids was made on the basis of the ability of the hydroxyl functionality to chelate iron,[12] while it was reasoned that the presence of a nitrogen atom in the carboxylic acid would present a second protonatable site, which would aid in compound accumulation within the acidic parasite food vacuole via pH trapping,[13] as well as to improve the aqueous solubility of the compounds via salt formation. The initial use formaldehyde as the aldehyde input was governed by our desire to obtain low molecular weight compounds[14] while the choice of isocyanides was limited by their commercial availability.

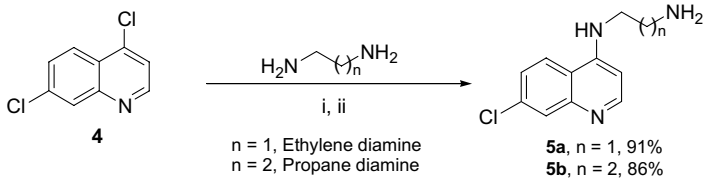
The quinoline-containing amines that were required for the U-4CC were synthesized by way of Scheme 1. Thus commercially available 4,7-dichloroquinoline **4** was reacted with excess ethylene or propane diamine in the neat at 80 °C for 1 h, then at 135–140 °C for 3 h to afford **5a** and **5b** in excellent yields.

Synthesis of the target compounds was then achieved in methanol at room temperature in parallel array format (Scheme 2).

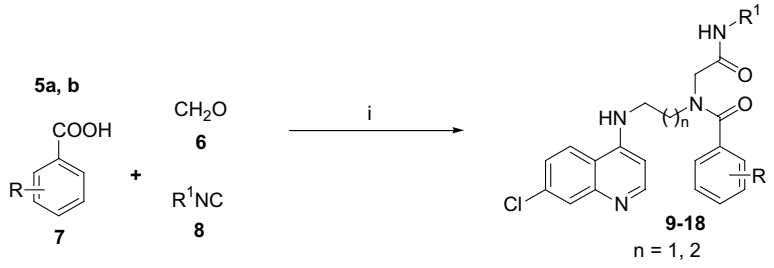
Condensation of stoichiometric amounts of amine, aldehyde, carboxylic acid and isocyanide at room temperature in anhydrous methanol afforded mixtures of the Ugi adducts. All the reactions with *tert*-butyl isocyanide went to completion within 36 h, while those with cyclohexylisocyanide took longer reaction times (up to 48 h). Figure 2 provides a summary of the target compounds synthesized.

The use of aldehydes besides formaldehyde in the U-4CC reaction would give rise to either enantiomers or diastereomers depending on the absence or presence of chirality in the other inputs. The use of formaldehyde in combination with achiral substrates thus eliminated the possibility of obtaining diastereomeric mixtures so that purification was not made a tedious effort. Purification was thus achieved simply by means of preparative thin layer chromatography following solvent removal under reduced pressure to afford the products in modest to good yields (Table 1).

Initial characterization of compounds **9**–**18** by ^{1}H NMR was complicated by duplication of each of the individual peaks. Rotation about the tertiary amide bond in the



Scheme 1. Synthesis of amines. Reagents and conditions: (i) 80 °C, 1 h; (ii) 135–140 °C, 3 h.



Scheme 2. Synthesis of target compounds. Reagents and conditions: (i) MeOH, rt, 36–48 h.

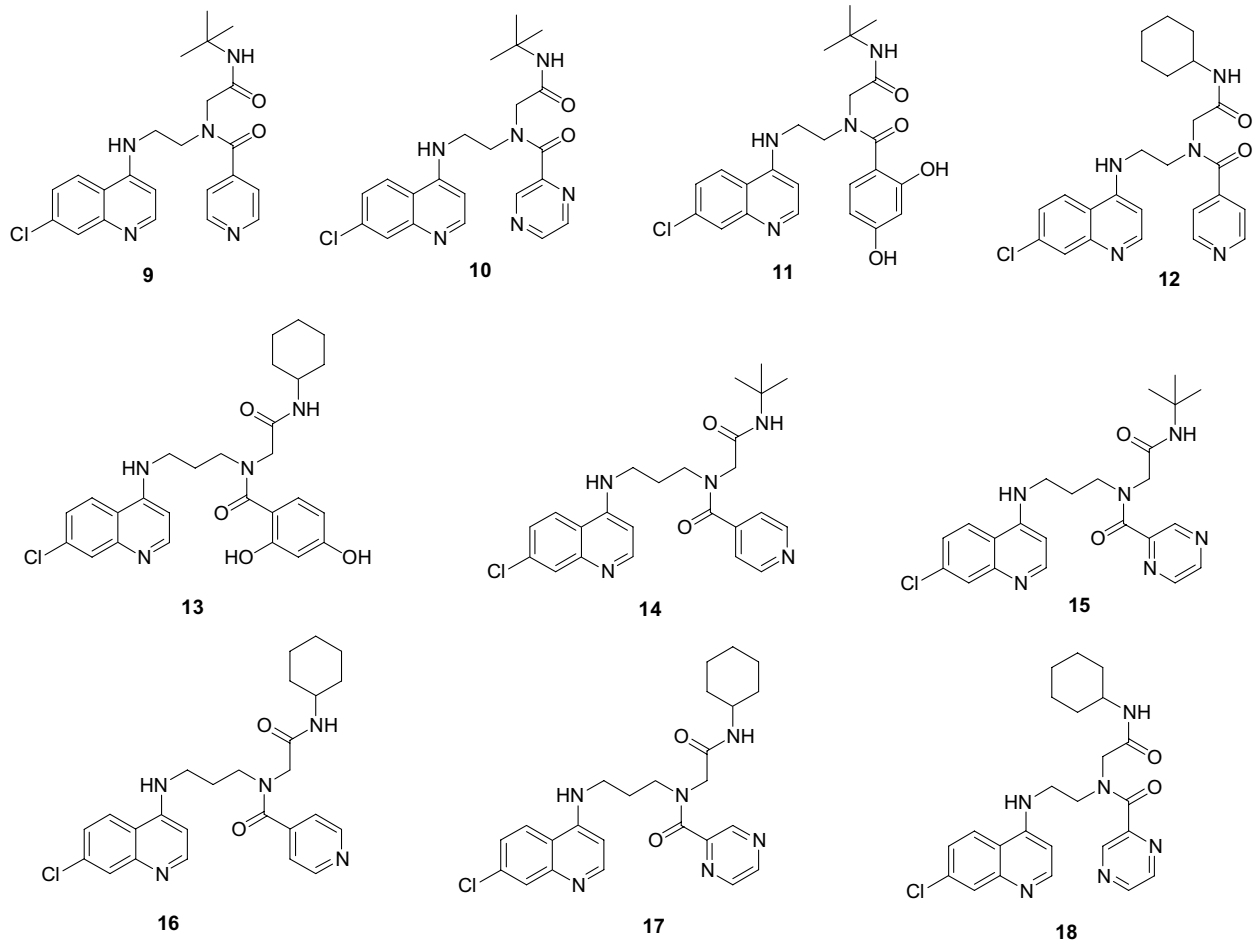


Figure 2. Structures of U-4CC reaction adducts synthesized.

Table 1. Isolated yields of compounds **9**–**18**

Compound	Yield (%)
9	65
10	69
11	68
12	76
13	70
14	65
15	63
16	60
17	74
18	72

Ugi adducts gave rise to two rotamers (major and minor) whose chemical shift values differed markedly. This phenomenon of conformational rotation was confirmed by the fact that duplicate peaks possessed the same coupling constants and also by running variable temperature (VT) NMR between 60 and 90 °C in deuterated DMSO, in which case coalescence of the duplicating peaks was observed at the higher temperature.

Three culture-adapted isolates of *P. falciparum*, K1 (CQ resistant; IC_{50} 568 nM), W2 (CQ resistant; IC_{50} 240 nM) and D10 (CQ sensitive; IC_{50} 37 nM) were used in this study. For K1 and D10 cultures were maintained according to the methods of Trager and Jensen[15] in O+ human erythrocytes. Growth medium was supplemented with gentamicin (1 mg/mL) and 0.5% (w/v) Albumax II. Parasites were synchronized at the trophozoite stage using the protocol of Lambros and Vandenberg[16] with 5% (w/v) D-sorbitol. Stock solutions of chloroquine diphosphate were made in Milli-Q water. Constituents for growth medium, CQ and D-sorbitol, were obtained from Sigma. Albumax II was purchased from GIBCO/Invitrogen. The parasite lactate dehydrogenase (pLDH) assay was used to measure drug activity in vitro.[17] The CQ IC_{50} was determined via regression analysis using Prism v4.0 (GraphPad Software, Inc. 5755 Oberlin Drive, #110 San Diego, CA 92121 USA). W2-strain *P. falciparum* parasites were cultured in RPMI-1640 medium with 10% human serum, following standard methods, and parasites were synchronized with 5% D-sorbitol.[18] Beginning at the ring stage, microwell cultures were incubated with different concentrations of compounds for 48 h. The compounds were added from DMSO stocks; the maximum concentration of DMSO used was 0.1%. Controls without inhibitors included 0.1% DMSO. After 48 h, when controls had progressed to new rings, the culture medium was removed, and cultures were incubated for 48 h with 1% formaldehyde in PBS, pH 7.4, at room temperature. Fixed parasites were then transferred into 0.1% Triton X-100 in PBS

containing 1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter vs fluorescence) acquired on a FACSort flow cytometer using CellQuest software (Beckton Dickinson). IC_{50} values for growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration using the Prism 3.0 program (GraphPad Software), with data from duplicate experiments fitted by nonlinear regression, as previously described.[19]

In order to ascertain their intrinsic antiplasmodial activity, compounds **9**–**18** were screened against three strains of *P. falciparum*, viz CQ-sensitive D10, and CQ-resistant K1 and W2. The antiplasmodial activities were determined as inhibitory concentrations at 50% parasite survival (IC_{50}) in the three strains and are tabulated in Table 2. For comparative purposes the antimalarial activity of CQ (**1**) in all three strains is also reported.

The compounds displayed moderate to significant antimalarial activity in all three strains of the malaria parasite with activity averaging in the single micromolar range. Compound **17** was generally the most active against all three strains. On the other hand **16** showed very high activity in the D10 and K1 strains (0.237 and 0.073 μM, respectively) but was not as active in the W2 strain ($IC_{50} = 1.566$ μM). Compound **12**, the most active in W2 ($IC_{50} = 0.619$ μM) was nearly as active in the resistant strains as in the CQ-sensitive strain. An increase in the size of the chain length in moving from compound **12** to **16** resulted in a marked increase in activity, whereas the reverse was observed with a similar change in moving from compounds **9** to **14** and **10** to **15**. A more general trend was however more observable with the change in the carboxylic acid input used. In this case the activity decreased for compounds based on isonicotinic acid (**9** and **16**), 2-pyrazinoic acid (**10** and **17**) and 2,4-dihydroxy benzoic acid (**11** and **13**) in this order for both sizes of methylene spacers in D10 and K1 but less so in W2. Although some general trends were observable from the compound activities, it must be emphasized that the effects of increasing the chain lengths beyond those presented herein need to be explored further before definite conclusions could be made. The varying results across different strains underscores the importance of testing compounds against multiple parasites with varying degrees of sensitivity and resistance as indeed the phenomenon of antimalarial drug resistance is often compound specific.

Table 2. Antimalarial activity of compounds **9**–**18**

Compound	IC_{50} D10 μM	IC_{50} K1 μM	IC_{50} W2 μM
CQ	0.037	0.568	0.24
9	1.064	1.105	0.991
10	0.712	1.133	1.190
11	5.655	5.786	4.145
12	0.540	0.531	0.619
13	1.672	3.826	5.936
14	>20	18.15	3.806
15	1.379	2.294	1.652
16	0.237	0.073	1.566
17	0.242	0.521	0.877
18	0.793	1.350	>10

Table 3. Comparison of resistance indexes of CQ with compounds **9**–**18**

Compound	RI[a]	RI[b]
CQ	15.5	6.49
9	1.04	0.93
10	1.60	1.67
11	1.02	0.73
12	0.98	1.15
13	2.29	3.55
14	nd[c]	nd
15	1.66	1.20
16	0.31	6.60
17	2.15	3.62
18	nd	nd

[a] K1 resistance index = K1 IC_{50}/D10 IC_{50}.
[b] W2 resistance index = W2 IC_{50}/D10 IC_{50}.
[c] Not determined.

Despite their low antiplasmodial activities in comparison to CQ, compounds **9**–**18** possess favourable resistance indexes (Table 3). Whereas CQ has resistance indexes of 15.5 and 6.49 in K1 and W2, respectively, compounds **9**–**18** generally have values far below that of the former, implying reduced cross-resistance between the CQ-sensitive and CQ-resistance strains. The only exception to this observation is compound **16** in W2.

It can be seen from the Table 3 that compounds **9**, **11** and **12** with RI values close to 1 were as active in the CQ-sensitive strains as in the CQ-resistant strains.

In summary we have synthesized new aminoquinoline-containing α-acylamino amides that are active against CQ-sensitive and CQ-resistant strains of *P. falciparum* Although the in vitro antiplasmodial activities of the aminoquinoline Ugi adducts described in this communication are not in the desirable low IC_{50} nanomolar range, we have demonstrated a simple new approach to novel analogues of existing aminoquinoline antimalarial drugs. We believe this approach will greatly assist in rapid SAR studies in this and other classes of antimalarial agents. Since the SAR libraries are already enriched in antimalarial pharmacophores, they need not be large in this approach. Thus the need for large libraries is precluded and the high cost often associated with such libraries significantly reduced. Combining antimalarial pharmacophores with other inputs, which may or may not be antimalarial pharmacophores themselves, offers many attractive features for accelerating antimalarial drug discovery in a rational manner. For example, MCR inputs can be designed in such a way that the final product is aimed at multiple targets within the malaria parasite. This approach could be useful in potentially slowing down or even overcoming the emergence of drug resistance and is analogous to conventional combination therapy wherein two or more antimalarial drugs, with different mechanisms of action, are co-administered.

Although the U-4CC reaction leads to the highly flexible aminoquinoline amides, which are not very attractive chemotherapeutic targets owing to the low bioavail-

ability associated with such compounds, the versatility of this reaction allows for the synthesis of more constrained, biologically relevant structures with improved stability and bioavailability. Efforts to synthesize antimalarial heterocycles based on the U-4CC[20] and other MCRs, incorporation of other antimalarial pharmacophores (and/or combinations thereof) as well as work to rotate the functionalities of the inputs of the Ugi 4CC in this context is currently underway in our laboratories.

Typical procedure: Aliquots of stock solution containing 0.226 mmol of amine were reacted with formaldehyde (6.78 mg, 0.226 mmol) in separate vials in methanol for 30 min at room temperature. A 1.2 excess-fold of isocyanide (0.27 mmol) was added, followed by acid (0.226 mmol). After consumption of starting materials (TLC, 36–48 h) the reaction mixtures were evaporated in vacuo, purified by preparative TLC (eluent MeOH–DCM 1:9) and concentrated under reduced pressure to afford the products in good to modest yields.

Data for compound **9**: Yield 65%, mp 106–108 °C; R_f (CH_2Cl_2–MeOH 9:1) 0.11; IR ν_{max} (KBr)/cm^{-1} 3420 (NH), 1674 (C=O), 1567 (C=C); δ_H (300 MHz, DMSO-d_6) 8.28 (d, 2H, *J* 6.0), 8.44 (d, 1H, *J* 5.7), 8.25 (d, 1H, *J* 9.3), 7.80 (d, 1H, *J* 2.4), 7.48 (dd, 1H, *J* 2.4, 9.3), 7.27 (d, 2H, *J* 6.0), 6.68 (d, 1H, *J* 5.7), 4.22 (s, 2H), 3.88 (t, 2H, *J* 6.0), 3.60 (t, 2H, *J* 6.0), 1.42 (s, 9H, $3 \times CH_3$); δ_C (75 MHz, DMSO-d_6) 170, 169, 151.9, 150 (2C), 149, 148.4, 148.3, 136.8, 133.2, 127.5, 123.9 (2C), 120.5, 117.5, 54.0, 52.3, 51.4, 29.3, 22.6; HRFABMS 440.18569 $C_{23}H_{26}ClN_5O_2$ + H, [M^++H requires 440.18533].

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