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# Application of multicomponent reactions to antimalarial drug discovery. Part 3: Discovery of aminoxazole 4-aminoquinolines with potent antiplasmodial activity in vitro

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**Abstract**—The synthesis and antimalarial activity of a novel series of first generation 4-aminoquinoline-containing 2,4,5-trisubstituted aminoxazoles against two strains of the *Plasmodium falciparum* parasite in vitro is described. A number of compounds significantly more potent than the standard drug chloroquine were identified.


The problem of endemic malaria continues unabated globally. Malaria affects 40% of the global population, causing an estimated annual mortality of 1.5–2.7 million people.[1,2] The World Health Organisation (WHO) estimates that 90% of these deaths occur in sub-Saharan Africa among infants under the age of five. There are four species of *Plasmodium* that cause malaria in humans, namely *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. Of these *P. falciparum* is the most virulent and causes the majority of deaths. While a vaccine against malaria continues to be elusive, chemotherapy remains the most viable alternative towards treatment of the disease. With the malaria parasite having developed multiple drug resistance to clinically established drugs, there is a compelling need to introduce new chemical entities that can overcome the resistance (Fig. 1).

In the context of our ongoing research towards the discovery of new antimalarial compounds, we have been successfully employing the isocyanide-based multicomponent reaction (MCR) strategy to synthesize new compounds in which diversity is introduced in a single step. To this end, we have synthesized varying classes of compounds based on the quinoline substructure, a pharmacophore found present in a number of antimalarial drugs including chloroquine (CQ) **1** and quinine **2**.[3,4]

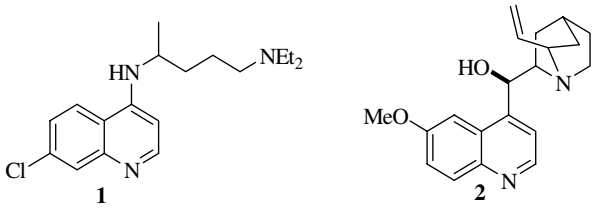


**Figure 1.** Chemical structures of chloroquine (**1**) and quinine (**2**).

In this communication, we wish to report on the synthesis and antiplasmodial activity of a novel class of 4-aminoquinolines that contain a heterocyclic 2,4,5-trisubstituted aminoxazole unit in the lateral side chain. The synthesis was based on the 3-component condensation domino reaction reported by Zhu et al.,[5,6] wherein an amine, aldehyde and isocyanoacetamide react to afford 2,4,5-trisubstituted aminoxazoles. We were attracted to this reaction owing to its simplicity and amenability to parallel synthesis and rapid library generation.

*Chemistry.* We intended to incorporate the 4-aminoquinoline substructural motif into the amine inputs required for the MCR. As such, the requisite amines **9**–**12** were synthesized according to Scheme 1,[7] whereby

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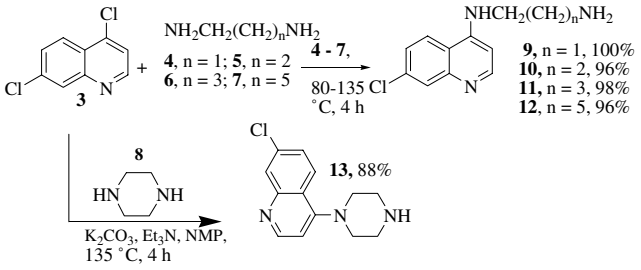
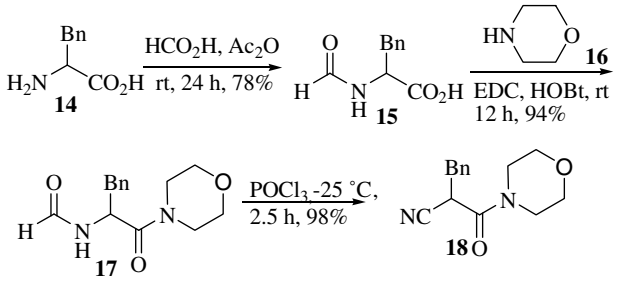



**Scheme 1.** Synthesis of 4-aminoquinoline amines **9**–**13**.




**Scheme 2.** Synthesis of isocyanoacetamide **18**.

commercially available 4,7-dichloroquinoline **3** was treated with an excess of the dialkyl amines **4**–**7**, initially at 80 °C and then at 135 °C for 3 h. On the other hand, treatment of 4,7-dichloroquinoline with excess piperazine[7] afforded the monosubstituted amine **13**, Scheme 1.[8] The five amines **9**–**13** were obtained in excellent yields (88–100%), the results of which are shown in Scheme 1.

The next step was to generate the isocyanoacetamide required for the multicomponent reaction, and this was realised from L-phenylalanine **14** according to Scheme 2 under standard conditions.[9]

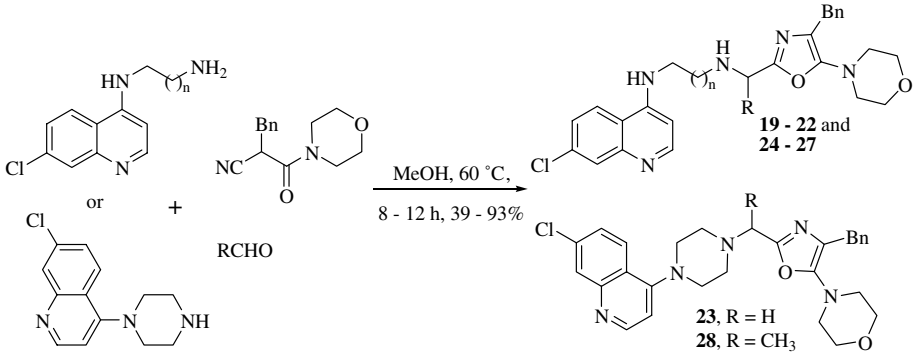
Treatment of L-phenylalanine with a mixture of formic acid (HCOOH) and $Ac_2O$ at sub-zero temperature for 20 min, followed by stirring at room temperature, afforded the formamide **15** in 78% yield.

Subsequent coupling of **15** with morpholine **16**, mediated by EDC and HOBt in DCM at room temperature, gave the formamide **17** in 94% yield. Finally, dehydration of **17** to the isocyanoacetamide **18** was achieved in 98% yield by treatment with $POCl_3$ in DCM at −25 °C.[10] Finally, the multicomponent reactions were conducted in parallel array format in MeOH at 60 °C according to Scheme 3.

Following pre-treatment of the amines **9**–**13** with either *para*-formaldehyde or acetaldehyde for 30 min, isocyanoacetamide **18** was added to each of the reaction mixtures. The initial choice of *para*-formaldehyde and acetaldehyde as the aldehyde inputs was governed by our desire to obtain low molecular weight compounds.[11] Isolation of the compounds was achieved by means of chromatography on silica ($SiO_2$) gel. Table 1 shows the isolated yields and purities of the compounds obtained.

*Biological results.* Compounds **19**–**28** were evaluated against two different strains of *P. falciparum*, namely a chloroquine-sensitive (CQS) 3D7 strain and a chloroquine-resistant

(CQR) K1 strain. Results of these assays are shown in Table 2. From the results in Table 2, compound **26** was the most active in the chloroquine-sensitive 3D7 strain which, with an $IC_{50}$ = 0.0038 μM, was 5 times more efficacious than chloroquine ($IC_{50}$ = 0.02 μM). Also of note was **19** ($IC_{50}$ = 0.0084 μM) which was also active in the low nanomolar range. On the other hand, **20** exhibited slightly weaker potency at $IC_{50}$ = 0.041 μM than chloroquine although not significantly so, while **25** ($IC_{50}$ = 0.079 μM), **22** ($IC_{50}$ = 0.15 μM), **23** ($IC_{50}$ = 0.52 μM) and **28** ($IC_{50}$ = 0.14 μM) exhibited weakened antiplasmodial activities in comparison to chloroquine. Interestingly, the introduction of a methyl substituent into the side chains of compounds **19** and **20** caused reductions in the activities of the corresponding derivatives **24** and **25**, respectively. In contrast, the introduction of a methyl substituent into the side chains of **21** and **22** resulted in significant improvements in the efficacies of the resulting compounds **26** and **27**. With $IC_{50}$s of 0.0038 and 0.018 μM, respectively, **26** and **27** exhibited the best potencies, showing 21-fold and eightfold improvements in efficacy against the chloroquine-sensitive strain.




**Scheme 3.** Multicomponent synthesis of compounds **19**–**28**.

**Table 1.** Isolated yields and purities of aminoxazoles **19**–**28**

| Entry | R | Product | *n* | %Yield | %Purity[a] |
|---|---|---|---|---|---|
| **1** | H | 19 | 1 | 70 | 93 |
| **2** | H | 20 | 2 | 68 | 95 |
| **3** | H | 21 | 3 | 62 | 97 |
| **4** | H | 22 | 5 | 39 | 98 |
| **5** | H | 23 | — | 93 | 99 |
| **6** | $CH_3$ | 24 | 1 | 68 | 96 |
| **7** | $CH_3$ | 22 | 2 | 51 | 97 |
| **8** | $CH_3$ | 26 | 3 | 67 | 96 |
| **9** | $CH_3$ | 27 | 5 | 43 | 95 |
| **10** | $CH_3$ | 28 | — | 87 | 98 |

[a] HPLC purity, conditions: flow rate: 1.0 ml/min, UV 220 and 254 nm, 30% $CH_3CN$: 70% 25 mM phosphate buffer.

**Table 2.** Antiplasmodial activity of **19**–**28** against CQS[a] 3D7 and CQR[b] K1

| Compound | *n* | R | $IC_{50}$ (μM) | |
|---|---|---|---|---|
| | | | 3D7 | K1 |
| CQ | — | — | 0.02 | 0.25[c]/1.03[d] |
| **19** | 1 | H | 0.0084 | 0.23[c] |
| **20** | 2 | H | 0.041 | 0.14[c] |
| **21** | 3 | H | 0.079 | 0.099[c] |
| **22** | 5 | H | 0.15 | 0.11[d] |
| **23** | — | H | 0.52 | 0.36[d] |
| **24** | 1 | $CH_3$ | 0.12 | 0.25[d] |
| **25** | 2 | $CH_3$ | 0.079 | 0.16[d] |
| **26** | 3 | $CH_3$ | 0.0038 | 0.019[d] |
| **27** | 5 | $CH_3$ | 0.018 | 0.037[d] |
| **28** | — | $CH_3$ | 0.14 | 0.97[d] |

[a] CQS, chloroquine-sensitive.
[b] CQR, chloroquine-resistant.
[c] Average CQ $IC_{50}$ was determined to be 0.25 μM.
[d] In this experiment the average CQ $IC_{50}$ was determined to be 1.03 μM.

The results also reveal that all compounds were more active than chloroquine in the resistant (K1) strain. In the first experiment, chloroquine had an $IC_{50}$ of 0.25 μM, whereas the 2 out of the 3 compounds tested along with the control drug, namely **20** and **21**, were both more potent than chloroquine with respective $IC_{50}$ values of 0.14 and 0.099 μM. In the second experiment, compounds **22**, **23** and **24**–**27** were several orders of magnitude more active than chloroquine. In fact, **25**, the least active among the three ($IC_{50}$ = 0.16 μM), was 6 times more potent than chloroquine ($IC_{50}$ = 1.03 μM), while **26** ($IC_{50}$ = 0.019 μM) was 54 times more efficacious than chloroquine and exhibited the greatest potency.

The results suggest that the introduction of a methyl substituent in **20** ($IC_{50}$ = 0.14 μM) to yield **25** ($IC_{50}$ = 0.16 μM) had no effect on the antiplasmodial activity, whereas a similar change for **21** ($IC_{50}$ = 0.099 μM) resulted in significantly improved efficacy for compound **26** ($IC_{50}$ = 0.019 μM). A similar increase in potency was seen in the analogous compounds **22** ($IC_{50}$ = 0.11 μM) and **27** ($IC_{50}$ = 0.037 μM).

The consequence of altering the length of the carbon spacer in the lateral side chain was not uniform in either the unsubstituted or methyl-substituted derivatives. In the unsubstituted compounds **19**–**22**, the two compounds with the 2- and 3-carbon spacers were the more active, while this activity was lost in the 4- and 6-carbon-spaced compounds. On the contrary, the methyl-substituted compound **24** with the 2-carbon spacer was not as active as the 3-carbon spaced compound **25**. The lowest activity was observed with the piperazinyl derivative **28**.

The observed improvement in the activities of these novel 4-aminoquinoline 2,4,5-trisubstituted aminoxazoles may be speculated to result from several factors. The 4-amino-7-chloroquinoline subunit is an antimalarial pharmacophore that inhibits haem dimerization into nontoxic haemozoin.[12] Therefore, the primary antiplasmodial activities associated with these compounds may be arising from inhibition of haem dimerization causing a build up of the toxic haem, resulting in parasite death. It is also possible that the increase in basicity caused by the side chain NH on N and the oxazole N may be improving the accumulation of the compounds in the acidic food vacuole of the parasite via pH trapping.[13] The presence of the heterocycle in the lateral chain may also be resulting in secondary interactions with other targets apart from haem, resulting in the observed antiplasmodial activities.

In conclusion, we have shown the potential of 4-aminoquinoline-containing 2,4,5-trisubstituted aminoxazoles as antiplasmodial agents in vitro and thereby demonstrated a simple approach to the synthesis of analogues of existing antimalarial drugs. The efficiency of this approach is manifested in the preclusion of large-sized libraries as the SAR libraries would already be enriched in antimalarial pharmacophores. Rationally, such a combination of antimalarial pharmacophores and other functionalities offers many attractive features for accelerating antimalarial drug discovery.

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## Supplementary data

Experimental details for the synthesis of all compounds and biological evaluation. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.070.

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