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The Synthesis and Testing of Arenearylpyrimidylmethanes as Antimalarial Agents

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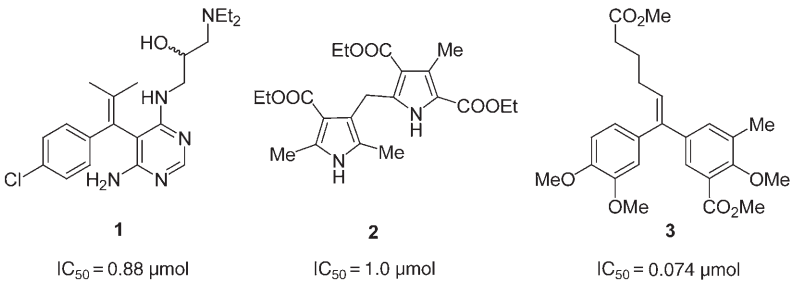
Malaria is a serious endemic disease that has resisted all efforts of eradication and control for over a century. It is a major threat to public health in more than 100 countries[1] and affects more than 500 million people per year, with an associated 2.7 million deaths.[2] The economic toll of malaria is tremendous,[2] and with the increasing globalization of commerce, the number of travellers to areas of high risk is increasing each year.[3] Thus, the need for a continual supply of new antimalarial therapeutics is still as relevant as ever. Malaria is caused by a protozoan parasite,[4] with *P. falciparum* being responsible for most malaria-related deaths.

We recently reported a serendipitous result in which a series of new lead compounds as antimalarial agents were unearthed from a study probing potential anti-HIV agents.[5] In summary, a series of pharmacophores were generated with different compound classes of non-nucleoside reverse transcriptase inhibitors of HIV-1 and were used as filters in database searching. Although none of the samples obtained showed HIV-1 reverse transcriptase inhibition, they were also subjected to antimalarial testing in a random screening study. Of the 15 compounds tested, nine showed significant activity, indicating that our pharmacophores and screening strategy are excellent for revealing new antimalarial leads. Most of these structures were previously reported by our research groups,[5] however, we publish herein the two most active antimalarial compounds that emerged from this study (**1** and **2**, Figure 1). Of particular interest was the arenearylpyrimidylmethane (AAPM) **1**; not only was this our most active antimalarial lead, it also has a scaffold that is similar to the known arenediarylmethanes (ADAM, such as **3**,[6] Figure 1), which have been extensively reported as anti-HIV agents that target reverse transcriptase.[6] Given the significant activity, the synthesis and retesting of **1** as a lead compound for potential antimalarial therapeutic development was our initial goal.

The development of a convergent synthetic strategy to the AAPM structural scaffold is important for the development of structure–activity relationships (SAR) through the synthesis and testing of analogues. The key intermediate is the AAPM **6**, as subsequent chemistry to produce the final target is routine. However, the published synthesis of **6** is a five-step linear sequence with an overall yield of 24% and contains little convergence.[8,9] We have developed a two-step sequence (Scheme 1) for the synthesis of **6** starting from materials analogous to those of the published synthesis, with an overall yield of 34%, thus more than halving the number of synthetic steps required to produce the key intermediate. This allows efficient access to the AAPM structural scaffold.



1 IC_{50} = 0.88 μmol **2** IC_{50} = 1.0 μmol **3** IC_{50} = 0.074 μmol

Figure 1. The two most active antimalarial leads are compounds **1** and **2**, which serendipitously emerged from an anti-HIV drug-discovery program.[5] The arenediarylmethane (ADAM) **3** is an example of a known anti-HIV reverse transcriptase inhibitor.[6] The antimalarial activities indicated were measured by using the WHO-approved Desjardins method[7] against multidrug-resistant *P. falciparum*.

The nucleophilic addition of 5-lithiated 4,6-dichloropyrimidine **4** to *p*-chloroisobutyrophenone yielded the alcohol **5** in modest yield (43–45%), which upon dehydration gave **6** in 74–78% yield. The steric hindrance associated with the initial nucleophilic addition is significant but is necessary for the required substitutions in subsequent derivatisation. The conversion of **6** into the final lead AAPM compound **1** was carried out under standard conditions[8] of monoamination at 140 °C with aqueous ammonia to yield **7**, followed by amination at 200 °C with 1-amino-3-diethylamino-2-propanol. In our hands, the reverse sequence of amination to initially produce **8** (see Figure 2) failed in the subsequent addition of ammonia, presumably due to the ever-increasing steric interactions associated with the bulky adjacent substituents.

The straightforward synthesis of **1** is complete in four steps (cf. seven steps previously reported[8,9]), with the AAPM scaffold being produced in two steps. It is convergent and rapid, using cheap and readily available starting materials.

The AAPM **1** has been reported as being negative in antimalarial testing.[9] However, a second sample from the NCI and a

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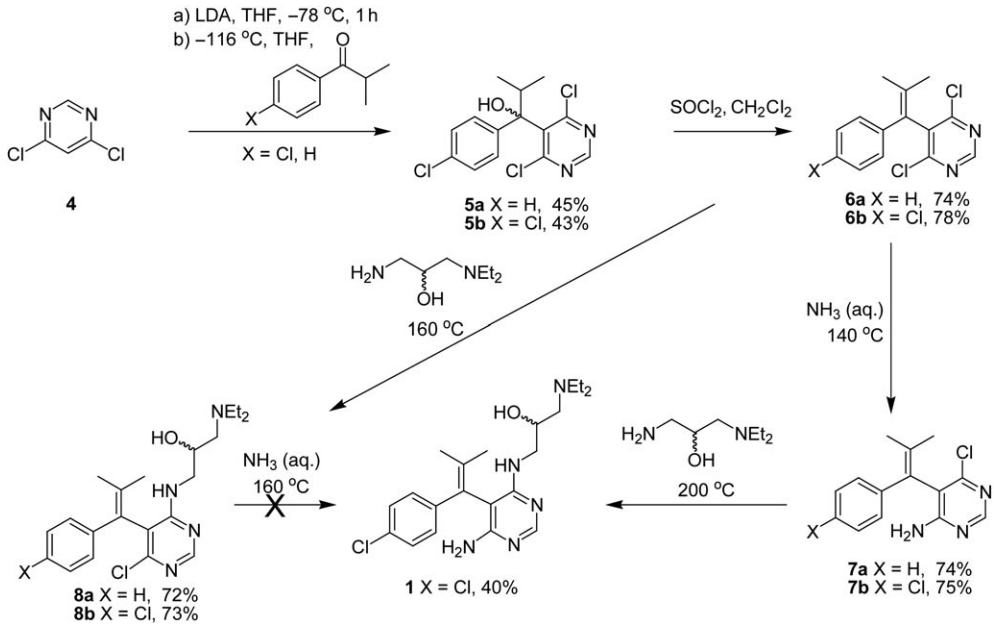
Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author: Synthetic procedures and spectroscopic data for compounds **1**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**, **8a**, and **8b**. A sample ^{13}C NMR spectrum for **1** is supplied to illustrate the doubling of peaks indicating atropisomerism.



Scheme 1. The short, convergent synthesis of the arenearylpyrimidylmethane (AAPM) skeleton **6** and the subsequent derivatisation in the total synthesis of the lead antimalarial compound **1**.

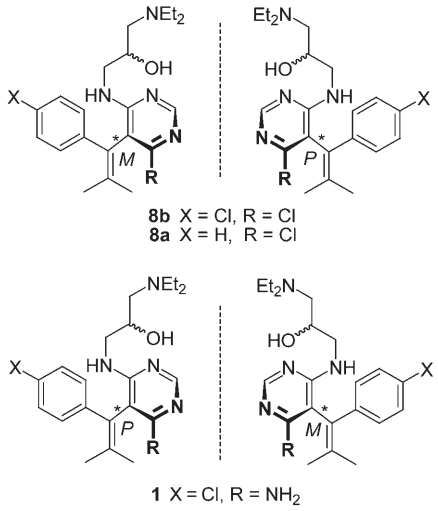


Figure 2. Atropisomerism due to a barrier of bond rotation in compounds **1**, **8a**, and **8b**. An asterisk indicates restricted rotation around the single bond.

sample prepared from our synthetic route were retested, and the results confirmed the antimalarial activity of **1**.

Analysis of the ^{13}C NMR and ^{1}H NMR spectra of **1**, **8a**, and **8b** (Figure 2) revealed a doubling of peaks of approximately equal intensity, indicating the presence of atropisomers. Atropisomerism is a phenomenon that results from slow rotation about a single bond[10] and can be observed here owing to the presence of diastereomers that arise from the atropisomeric bond and the presence of the racemic alcohol. Further confirmation of the likelihood of atropisomers is the absence of such doubling of peaks in the NMR spectra in systems in which atropisomerism would not be expected, for example, if the substituents adjacent to the relevant bond are identical (as in **6**) or if the relevant substituents are not sufficiently large to restrict bond rotation (as in **7**). The presence of atropisomerism in these compounds has not been previously reported.

In conclusion, a short two-step synthesis to the AAPM heterocyclic scaffold has been developed, and subsequent derivatisation to produce the lead antimalarial compound **1** was successfully completed. The biological activity of **1** was confirmed by retesting the synthesised sample. The presence of atropisomerism was observed in suitably substituted examples.

Experimental Section

Antimalarial testing: The parasite *P. falciparum* (K1, multidrug-resistant strain) was cultured continuously according to the method of Trager and Jensen.[11] Quantitative assessment of antiplasmodial activity in vitro was undertaken by means of the microculture radioisotope technique based on the method described by Desjardins et al.[7] Inhibition concentration (IC_{50}) represents the concentration that causes 50% decrease in parasite growth as indicated by the uptake of [^{3}H]hypoxanthine by *P. falciparum*.

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Keywords: antimalarial activity · atropisomerism · database searching · pharmacophore · synthesis design

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