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Disciplines
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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The Synthesis and Testing of Arenearylpyrimidylmethanes (AAPM) as Anti-Malarial Agents

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Keywords

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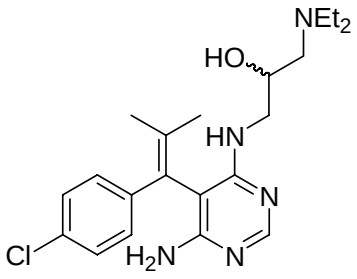
Abstract

The anti-malarial activity of the arenearylpyrimidylmethane (AAPM) class of compounds emerged from database searching of a pharmacophore. A new 2 step synthesis of the AAPM scaffold is reported and subsequent substitution yielded a short synthesis of the lead anti-malarial compound. The presence of atropisomerism in this class of compounds is also reported for the first time.

Introduction

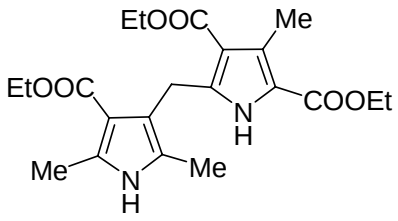
Malaria is a serious endemic disease that has resisted all efforts of eradication, and control for over a century. It is a major threat to public health in more than 100 countries[1] affecting more than 500 million people per year, with an associated 2.7 million deaths.[2] The economic toll of malaria 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 anti-malarial therapeutics is still as relevant as ever. Malaria is caused by the protozoan parasite,[4] with *P. falciparum* being responsible for most malaria related deaths.

We recently reported a serendipitous result where a series of new lead compounds as anti-malarial agents were unearthed from a study probing potential anti-HIV agents.[5] In summary, a series of pharmacophores were generated using 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 anti-malarial testing in a random screening study. Of the 15 compounds tested, 9 showed significant activity, indicating that our pharmacophores and screening strategy are excellent for revealing new anti-malarial leads. Most of these structures were previously reported by us,[5] however, we publish here the two most active anti-malarial compounds that emerged from this study (**1** and **2**, Figure 1). Of particular interest was the arenearylpyrimidylmethanes (AAPM) **1** – not only was this our most active anti-malarial lead, it also has a scaffold that is comparable to the known arenediarylmethanes (ADAM)(*e.g.* **3**,[6] Figure 1), which have been extensively reported as anti-HIV agents targeting the reverse transcriptase enzyme.[6] Given the significant activity, the synthesis and retesting of **1** as a lead compound for potential anti-malarial therapeutic development was our initial target.



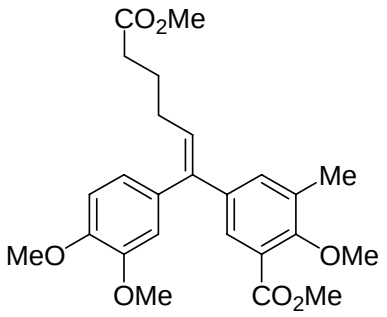
1

anti-malarial activity
0.88 μmole



2

anti-malarial activity
1.0 μmole



3

ADAM anti-HIV-1 compound
reverse transcriptase inhibition
0.074 μmol

Figure 1: The two most active anti-malarial leads that serendipitously emerged from an anti-HIV drug discovery program.[5] The anti-malarial activity was measured using the WHO approved Desjardins method[7] against multi-drug resistant *P. falciparum* and is reported as the IC_{50} in μmol. The arenediarylmethane (ADAM) **3** is an example of a known anti-HIV reverse transcriptase inhibitor.[6]

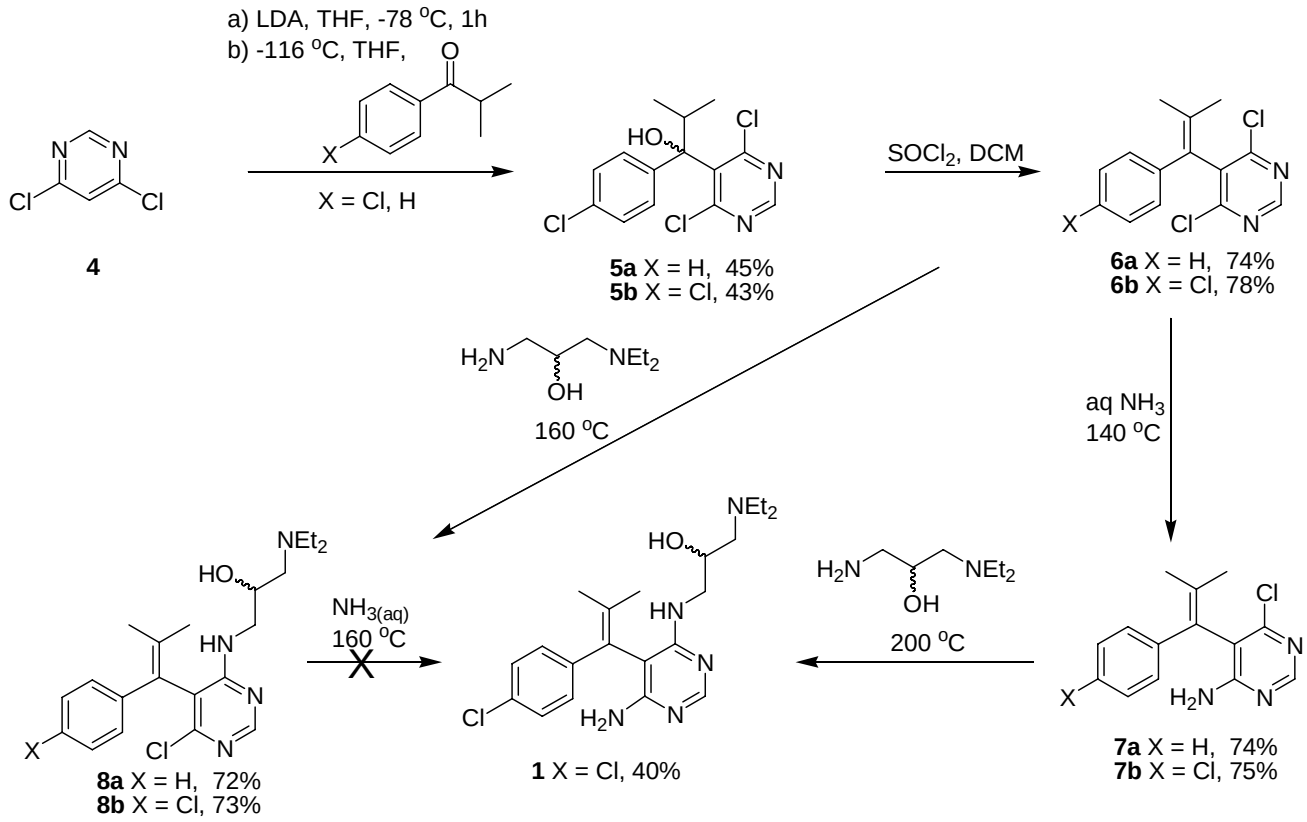
Results and Discussion

The development of a convergent synthetic strategy to the AAPM structural scaffold is important for the development of structure-activity relationships (SAR) via the synthesis and testing of analogues. The key intermediate is the AAPM **6** – subsequent chemistry to produce the final target is routine. However, the one published synthesis of **6** is a 5 step linear sequence with an overall yield of 24% and contains little convergence.[8,9] We have developed a 2 step sequence for the synthesis of **6** starting from analogous materials to the published synthesis (Scheme 1) with an overall yield of 34% – this more than halving of the synthetic steps required to produce the key intermediate allows for quick access to the AAPM structural scaffold.

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 in order to allow for the required substitutions for subsequent derivatisation. The conversion of **6** to the final lead AAPM compound **1** was achieved using standard conditions[8] of monoamination at 140 °C using aqueous ammonia yielding **6**, 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 4 steps (*c.f.* 7 steps previously reported[8,9]) with the AAPM scaffold being produced in 2 steps, and is convergent and quick, utilising cheap readily available starting materials.



Scheme 1: The short, convergent synthesis of the arenearylpyrimidylmethanes (AAPM) skeleton **6**, and the subsequent derivatisation yielding the total synthesis of the lead anti-malarial compound **1**.

The AAPM **1** has been reported as being negative in anti-malarial testing.[9] However, a second sample from the NCI and a sample prepared from our synthetic route were retested and confirmed the anti-malarial 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 which results from slow rotation about a single bond[10] and can be observed here due to the presence of diastereoisomers arising 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 where atropisomerism would not be expected, *e.g.* when the substituents adjacent to the relevant bond are identical (*e.g.* **6**) or if the relevant substituents are not sufficiently large to restrict the bond rotation (*e.g.* **7**). The presence of atropisomerism in these compounds has not been previously reported.

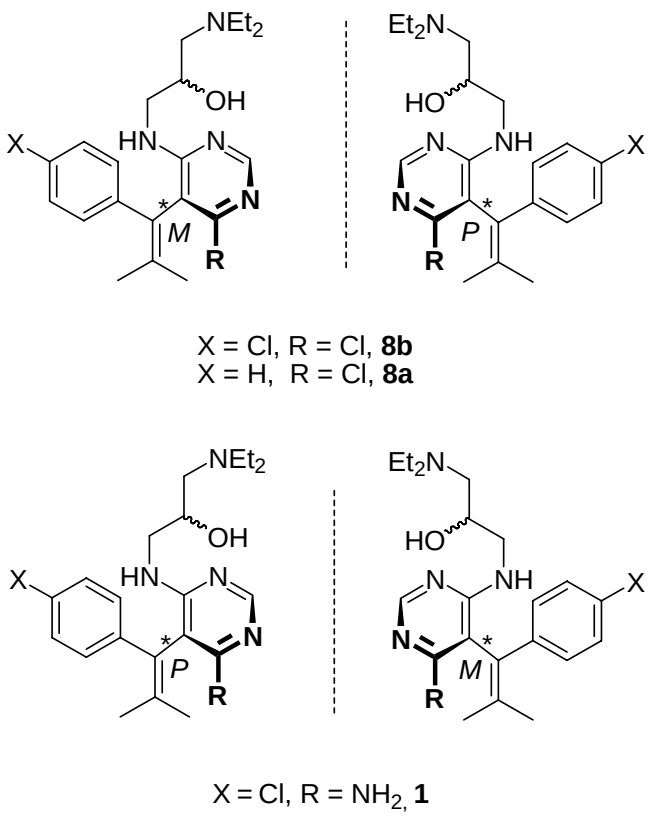


Figure 2: Atropisomerism due to a barrier of bond rotation in compounds **1**, **8a** and **8b**. An asterisk indicates restricted rotation around the single bond.

Conclusions

A short 2 step synthesis to the arenearylpyrimidylmethanes (AAPM) heterocyclic scaffold has been developed and subsequent derivatisation to produce the lead anti-malarial compound **1** was successfully completed. The biological activity of **1** was confirmed by retesting of the synthesised sample. The presence of atropisomerism was observed in suitably substituted examples.

Supporting Information

The synthetic procedures and spectroscopic data for compounds **1**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**, **8a**, and **8b**. A sample ^{13}C NMR spectrum (**1**) is supplied illustrating doubling of peaks indicating atropisomeriem.

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Anti-malarial testing

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

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