Synthesis of a small molecule used for selection of transfected *Plasmodium falciparum*

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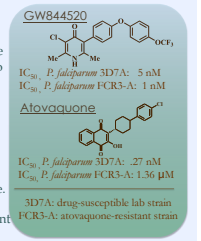
Introduction

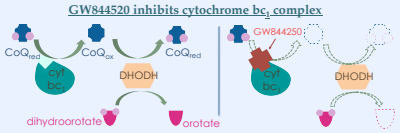
Humans have suffered from malaria for thousands of years, and it is still a major contributor to morbidity and mortality around the world. Malaria infects up to 500 million people every year, and the majority of the two million deaths it causes are of children under the age of five. The *Plasmodium* spp. parasites, which cause the disease, display a remarkable ability to develop resistance to antimalarial drugs.

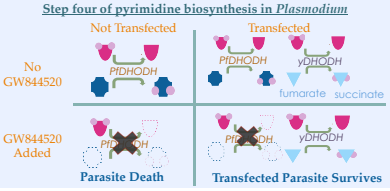
Many current studies of *Plasmodium* genetics aim to better understand specific biological mechanisms, some of which could be potential drug targets. The Broad malaria program is collaborating with other groups to discover small molecules that could serve as leads in the development of novel antimalarials. Small molecules can also serve as tools for genetic research. One example for their use is to select for parasites that have been transfected with plasmids possessing resistance markers, just as antibiotics are used to select for transformed bacteria.

The goal of our project was to synthesize GW844520 for use in *Plasmodium* genetic studies. The Duraisingh Laboratory at the Harvard School of Public Health wants to use this small molecule as a positive selector for *Plasmodium* that have been transfected with a plasmid containing a gene of interest. This small molecule can be used as a better selector than the laboratory's current one, atovaquone, as it shows less susceptibility to resistance and less additional toxicity to the parasite. GW844520 was recently developed by GlaxoSmithKline and demonstrated potent *in vitro* antiparasitic activity. However, because it was not available from their laboratory, it had to be synthesized.

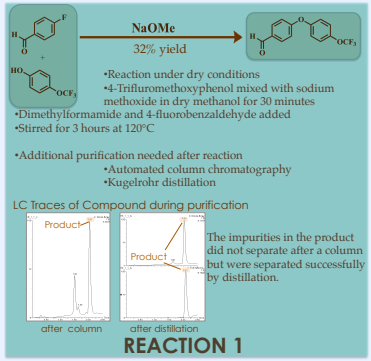


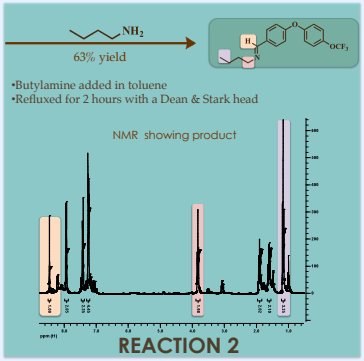
The mechanism of GW844520 is shown below. GW844520 can select for parasites that have been transfected with a plasmid harboring the yeast DHODH gene. This gene allows for pyrimidine biosynthesis in a Coenzyme Q-independent fashion, unlike *Plasmodium* DHODH.

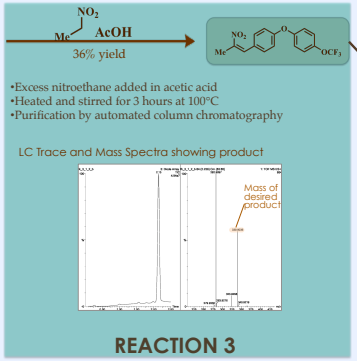


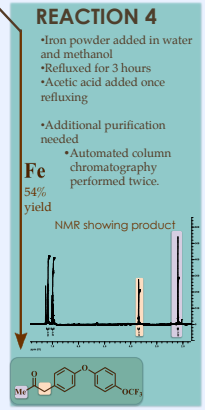


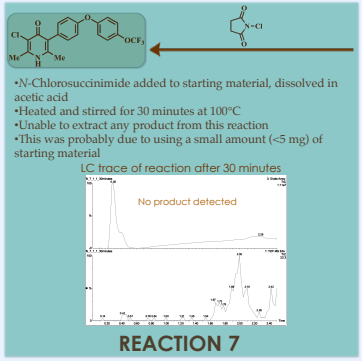
Methods & Results

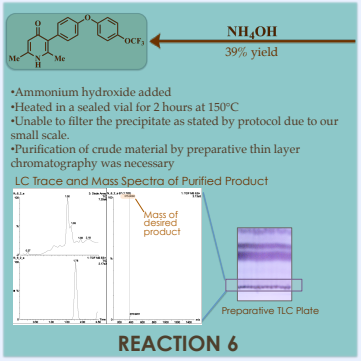


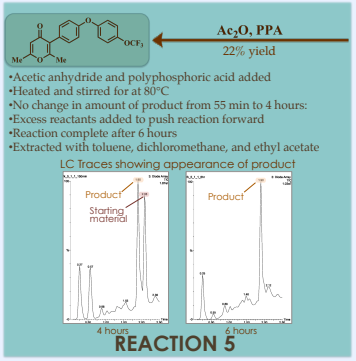












Reactions one through four were adapted from a patent containing minimal experimental detail.[1] Reactions five through seven were adapted from the J. Med Chem Journal.[2] Experiments in both sources were performed on a substantially larger scale than our experiment.

Conclusions

We were able to successfully complete the first six reactions in the synthetic route of GW844520. On the last reaction, we were unable to produce the final product due to low yields in previous steps and, consequentially, insufficient starting material for this step.

Through our experiments, we were able to determine specifics in the experimental design of each reaction which were not present in the brief patent and paper, both of which were also written for a much larger scale than what our synthesis was. We discovered the following improvements to the protocol:

We found that in reaction 1, distillation is required to purify the product. In reaction 5, reactants are needed in excess to what was stated,[2] and the resulting product needs to be extracted in dichloromethane and ethyl acetate in addition to toluene. Finally, reaction 6 can be completed in far less time than the 18 hours stated.

Literature Cited

1. Batchelor, John F. and Yeates, Clive L., EP0447164, 1991.
2. Yeates, Clive L. *et al. J. Med. Chem.* **2008,** *51,* 2845

Acknowledgements

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