

Development of a simple salen-based assay for comparing antimalarial activity of novel trioxanes.

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Research Proposal:

Malaria is one of the most common causes of illness in the world, infecting an estimated 300-500 million people per year and resulting in over one and a half million deaths. While very effective antimalarial agents drugs have been developed and used for years, malaria has continued to spread, in part due to *Plasmodium falciparum*, chloroquine resistant malaria. ¹

New drugs to combat chloroquine-resistance are constantly being sought and Mefloquine, Fansidar and Malarone are three that are successfully used today.² However, these drugs, and others currently on the market, are all nitrogen-based and therefore have similar mechanisms of toxicity to *P. falciparum*.³ Not surprisingly then, resistance to these drugs alone has already been reported.¹

Artemisinin and related compounds are drugs that have been used in combination with, and sometimes instead of, the nitrogen-based drugs. Artemisinin is a trioxane (a peroxide with an extra carbon and oxygen) that has been observed to alkylate hemes, suggesting that its mechanism of action is to damage the parasite's hemes, leading to death of the organism.⁴

While Artemisinin is an effective antimalarial, it is prohibitively expensive for most of the world infected by malaria. It is a natural product, extracted from *Artemisia annua* (Wormwood) with a maximum yield of 0.1% and even the most carefully cultivated plants may not contain any Artemisinin.⁵ Because of the expense, there is a need for simpler synthetic alternatives.

1 Bloland, P.B. Drug resistance in malaria. *WHO*, 2001.

2 http://www.cdc.gov/ncidod/dpd/parasites/malaria/hcp_malaria_drugs.htm accessed April 15, 2005

3 Jefford, C.W. Why artemisinin and certain synthetic peroxides are potent antimalarials, implications for the mode of action. *Curr. Med. Chem.* **2001**, *8*,1803-1826.

4 Robert, A.; Dechy-Cabaret, O.; Cazelles, J.; Meunier, B. From mechanistic studies on artemisin derivatives to new modular antimalarial drugs. *Acc. Chem. Res.* **2002**, *35*, 167-174.

5 Bez, G.; Kalita, B.; Sarmah, P.; Barua, N.C.; Dutta, D.K. Recent developments with 1,2,4-trioxane-type artemisinin analogues. *Curr. Org. Chem.* **2003**, *7*, 1231-1255.

In developing new synthetic trioxanes, it is important to have a simple, inexpensive and quick screening assay. Currently, common initial screening assays use *P. falciparum* infected mice.⁶ This methodology, although straightforward, is not one that our undergraduate institution can conceivably carry out, nor is it appropriate for an initial screening of antimalarial activity. A simpler, molecular-level assay would be preferable. The one that currently exists involves quantitating the ability of a trioxane to alkylate a manganese porphyrin, a process that mimics the biological mechanism of action.⁴ While this assay is possible for us to do, the required water soluble porphyrins are difficult for students to prepare and isolate. Currently, a senior research student, Joe Donnellon is working on designing a similar assay using a manganese salen, rather than a porphyrin, because the salens also form Mn(V)-oxo species but are more easily prepared, derivatized and purified. With UV/vis spectrometry, he has found that artemisinin does interact with manganese salen, but he has not yet determined if an alkylation occurs. As part of a course release, I would be able to continue with this part of the project so that, in spring of 2006, Laura Alamillo will be able to use the assay to quantitate the activity of a variety of simple trioxanes.

Many simple trioxanes have already been prepared and some have been designed specifically as antimalarials.⁷ Within each type of molecular framework, it is evident that effectiveness varies with the electron donating and withdrawing abilities of substituents, size of the molecule and steric hindrance to reaction of the heme.⁵ With a simple screening assay such as the one described above, it would be possible to make correlations between variables and activity, so that the simplest possible effective antimalarial can be designed. So, as a second part of this course release, I would like to explore the current research on trioxanes with the goal of identifying simple molecules that can be easily functionalized so that the differences in activity can be measured.

This project is an ideal one for students. It involves the development of basic spectroscopic and synthetic skills while it also connects with current events and ethical issues in drug development

Research Summary:

During Fall semester 2005, I developed a student research project on the investigation of mechanism of action of liminoids as anti-malarials. Malaria is one of the most common causes of illness in the world, infecting an estimated 300-500 million people per year and resulting in over one and a half million deaths. While very effective antimalarial agents have been developed and used for years, malaria has continued to spread, in part due to drug resistance. Interestingly, the mechanisms of most of the drugs on the market (the quinolines and artemisinin) are thought to involve the same pharmacological target:

6 Posner, G.H.; Jeon, H.B.; Parker, M.H.; Krasavin, M.; Paik, I-K.; Shapiro, T.A. Antimalarial simplified 3-aryltrioxanes: Synthesis and preclinical efficacy/toxicity testing in rodents. *J.Med.Chem.* **2001**, *44*, 3054-3058.

7 Posner, G.H.; O'Neill, P.M. Knowledge of the proposed chemical mechanism of action and cytochrome P450 metabolism of antimalarial trioxanes like artemisinin allows rational design of new antimalarial peroxides. *Acc.Chem.Res.* **2004**, *37*, 397-404.

heme. In light of problems with drug resistance, turning attention to drugs that react in different ways may lead new effective drugs or drug combinations. Leaves and bark from the Neem tree have been used as a treatment for Malaria for centuries and liminoid components of the Neem, such as Gedunin and Nimbolide, have been found to have anti-Malarial activity in vitro. These molecules are electrophiles, unlike the quinolines and artemisinin, which are nucleophiles. This suggests a decidedly different mechanism of action. Instead of reacting with heme, the liminoids could react with proteins or other nucleophilic biomolecules. During Fall semester, I gathered background resources and developed a research plan for students to extract the liminoids from Neem bark and leaves. During Spring semester (after the course release), two students worked on this project and I presented my literature research to the Division of Natural Science and Mathematics and Lewis-Clark State College. In the future, this project will be extended to classroom work as well as independent research