Evaluation of Triarylmethyl Dyes for Xenobiotic Transport Assay in the Mosquito *A. aegypti*

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M. ROUHIER LAB

Abstract

As a transmitter of disease-causing pathogens, the mosquito is responsible for millions of deaths every year. Pesticides can control the mosquito population, however with emerging resistance, pesticide development is imperative. A promising new pesticide target is the organic anion transporter (OAT) of the mosquito renal system. Previous work in our lab has shown that the presence of certain chemical moieties cause toxic molecules to be less lethal to a mosquito. This could be because the compound is preferentially recognized for transport by the OAT . In order to further study this trend, more compounds must be tested. Our study focused on the characterization of organic dye solutions with moieties of interest. Four dyes (two pairs) were evaluated for their solubility, stability, and spectroscopic properties in solutions of physiological relevance. Two dyes, Brilliant Green and Patent Blue VF, were selected as possible candidates for later experiments.

Methods

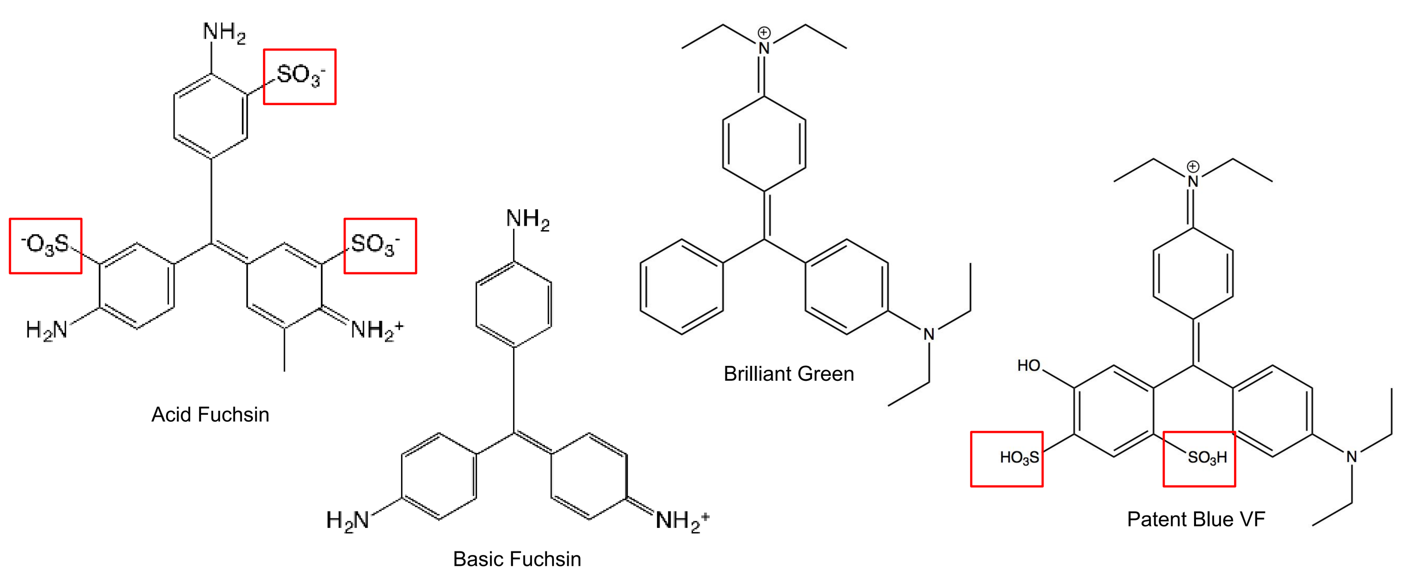

Figure 2. Structural diagrams of dyes used to characterize the OAT transport of *Aedes aegypti.* Red Box indicates sulfonate group.

- pH/light sensitivity
  - Prepare dye solutions in various conditions and track changes in absorption over time
- Dye characterization
  - Prepare dye stock solutions and dilutions
  - Quantify dye absorbance using Nanodrop
  - Characterize dye solutions by molar absorptivity

Background

Previous studies in the Rouhier Laboratory have shown that the sulfonate-containing dye, sulforhodamine b is several orders of magnitude less toxic than the non-sulfonate-containing rhodamine b, see Figure 1. Moreover, the sulforhodamine b treated mosquitoes were observed excreting large amount of dye, while the rhodamine b treated mosquitoes were not, suggesting that the transporters of mosquitoes have key chemical moieties they use to recognize xenobiotics for transport. To assign the role that the sulfonate group plays in xenobiotic recognition and excretion, similar studies must be performed using different dye pairs. This project aimed to evaluate dyes for use in future studies.

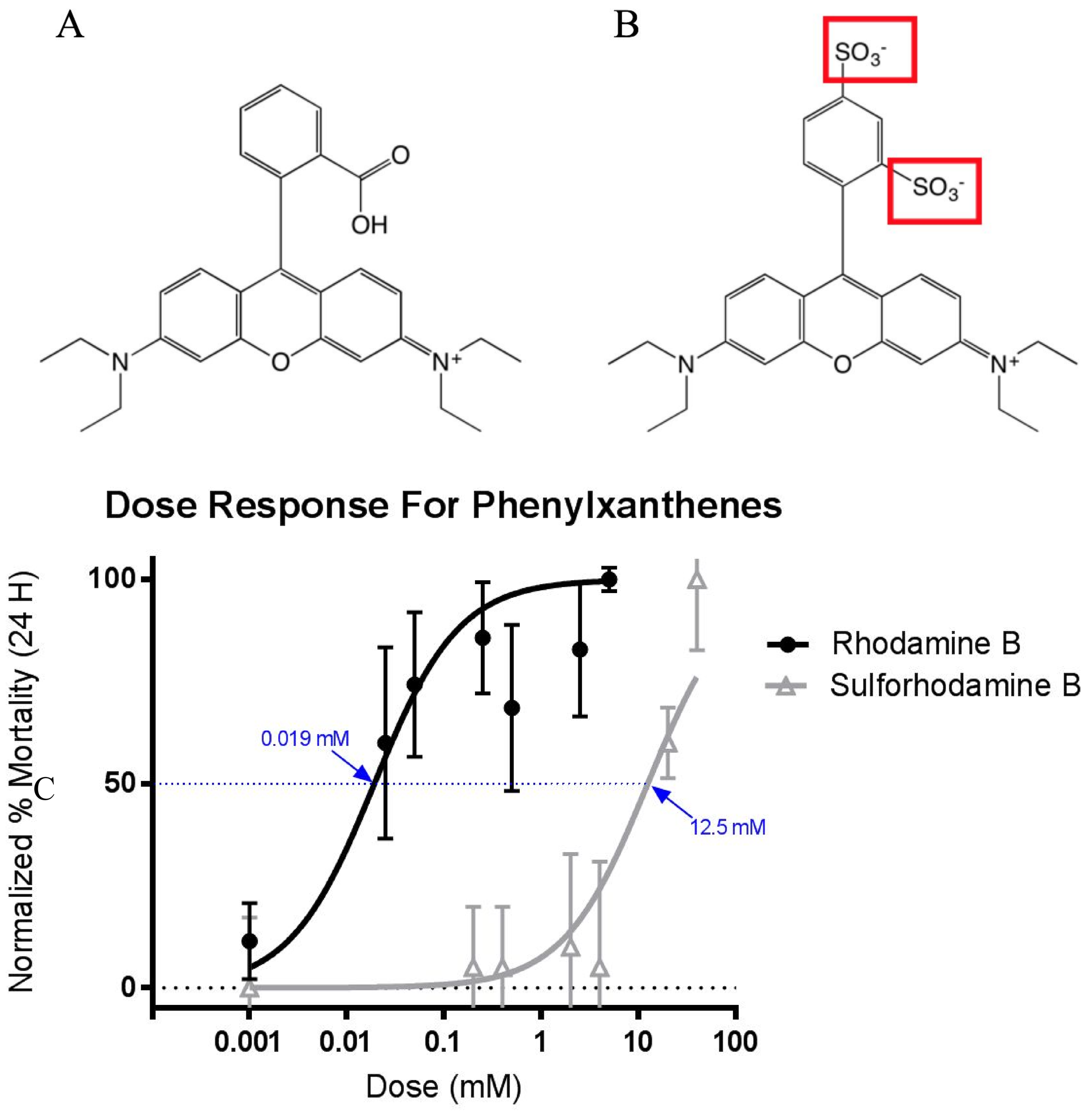

Figure 1. Structures of rhodamine b (A) and sulforhodamine b (B) Red box indicated the sulfonate functional groups. Dose-Response curve (C) for mosquitoes after injection with Sulforhodamine B and Rhodamine B.

Goals

- Evaluate solubility and stability of dyes in aqueous solutions and physiological conditions
- Characterize dyes using UV-Vis spectroscopy in aqueous solutions and physiological conditions

Results

		Patent Blue		Brilliant Green	
		Light	No Light	Light	No Light
DMSO	1 hr	2.4 ± 0.3	1.4 ± 0.7	1.6 ± 2.9	2.2 ± 0.2
	2 hr	2.4 ± 0.3	0.90 ± 1.49	1.2 ± 5.2	0.6 ± 1.0
	24 hr	-4.3 ± 4.9	-0.26 ± 1.36	2.8 ± 1.9	1.5 ± 0.2
PBS/ DMSO	1 hr	-0.1 ± 0.6	0.0 ± 0.5	20.2 ± 0.1	19.3 ± 3.5
	2 hr	0.6 ± 3.8	1.0 ± 1.4	33.1 ± 0.6	33.1 ± 3.9
	24 hr	0.2 ± 0.7	0.1 ± 3.8	96.1 ± 1.4	95.8 ± 0.9
pH=2/ DMSO	1 hr	-0.6 ± 1.3	0.0 ± 1.6	95.1 ± 0.6	93.3 ± 0.5
	2 hr	-0.1 ± 0.9	0.6 ± 1.1	97.5 ± 3.1	94.2 ± 3.3
	24 hr	1.6 ± 0.7	2.1 ± 1.1	97.0 ± 0.7	94.8 ± 0.1
pH=12/ DMSO	1 hr	-0.7 ± 1.9	4.3 ± 3.5		
	2 hr	-1.9 ± 4.0	-4.0 ± 4.3		
	24 hr	31.4 ± 0.6	27.5 ± 2.9		

Figure 3. Percent change in absorbance over time in solutions of Patent Blue and Brilliant Green. All solutions were initially 2.5 mM. Gray indicates insignificant changes based on standard deviation. Red indicates loss of absorption and black indicates absorbance gain.

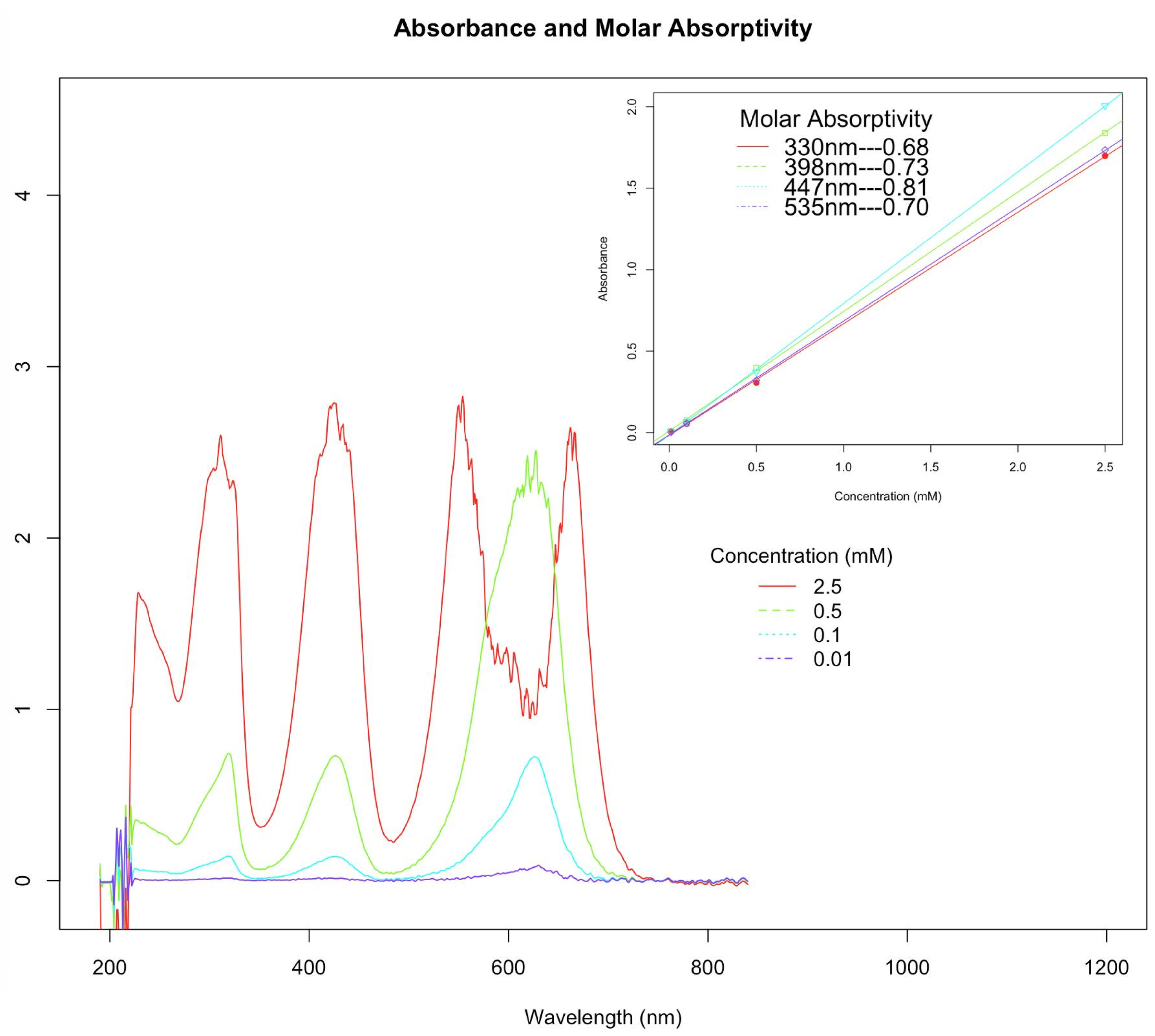

Figure 4. UV-vis absorbance of brilliant green in solutions of varying concentrations. Upper right is a Beer's law plot of absorbance vs. concentration at four wavelengths. Molar absorptivities of brilliant green at four wavelengths are recorded within the Beer's law plot.

Conclusions and Future Aims

- Brilliant green loses absorbance in aqueous solution, especially in solutions of high and low pH
- Molar Absorptivities of brilliant green at four wavelengths were calculated allowing the determination of the concentration of brilliant green in a given solution
- Future Aims
  - More precisely quantifying the absorbance loss in various brilliant green solutions
  - Perform mortality and excretion trials on mosquitos using the results of this study to accurately determine brilliant green concentration upon injection into the mosquito

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