

Proposed Half-Site Reactivity Towards NADPH Binding for *Plasmodium falciparum* Glutathione Reductase *in vivo*

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The aim of our interdisciplinary project is to substantiate glutathione reductase inhibitors as antimalarial agents.¹ Our strategy is based on the synthesis of suicide-substrates and of catalytic inhibitors of the selected targets, namely the glutathione reductases from human erythrocytes and from the malarial parasite *Plasmodium falciparum*.² Both enzymes are essential proteins for the survival of the malarial parasite infecting red blood cells. They maintain the redox equilibrium in the cytosol by catalyzing the physiological reaction: $\text{NADPH} + \text{H}^+ + \text{GSSG} \rightarrow \text{NADP}^+ + 2 \text{GSH}$. Different types of inhibitors (reversible, irreversible) were designed in the 1,4-naphthoquinone series to evaluate the impact of each inhibition mode on the growth of the parasites: uncompetitive³ or catalytic inhibitors, subversive substrates, fluorine-based suicide-substrates.⁴ The modifications of the enzyme structure and function exerted by the novel lead compounds were analyzed by mass spectrometry analyses and X-ray diffraction.⁴ In particular, a series of 2-benzoyl menadione derivatives was found to be photoreactive and potentially useful as photoaffinity labeling reagents upon irradiation.⁵ Based both on literature data and enzyme kinetics using naphthoquinones as substrates of *P. falciparum* GR showing a non-Michaelis behaviour, a putative half-site reactivity mechanism towards NADPH binding is suggested. The half-site reactivity towards NADPH binding, an extreme case of negative cooperativity, is proposed to be involved in *P. falciparum* GR catalysis and to render the enzyme insensitive to environmental changes, thus allowing a constant enzymatic activity despite large fluctuations in the metabolite concentration *in vivo*. Work is presently focused on the photoaffinity labeling of both glutathione reductases under oxygen-free conditions to identify the site where naphthoquinone reduction takes place.

¹ (a) Davioud-Charvet *et al.*, *J. Med. Chem* **2001**, 44, 4268 – 4276; (b) Friebolin, *et al.*, *J. Med. Chem.* **2008** 51, 1260 – 1277; (c) Müller *et al.*, European patent, EP 08290278.4, March 26, 2008.

² Krauth-Siegel *et al.*, *Angew. Chem. Int. Ed. Engl.* **2005**, 44, 690 – 715. Review.

³ Biot *et al.*, *J. Med. Chem.* **2004**, 47, 5972 – 5983.

⁴ Bauer *et al.*, *J. Am. Chem. Soc.* **2006**, 128, 10784 – 10794.

⁵ Müller *et al.*, In *Flavins and Flavoproteins 2008*, **2008** in press.