Hemolytic and antimalarial effects of tight-binding glyoxalase 1 inhibitors on the host-parasite unit of erythrocytes infected with Plasmodium falciparum

Cletus A. Wezena a,1, Miriam Urschera,1, Robert Vince b, Swati S. More b, Marcel Deponte a,*

a Department of Parasitology, Ruprecht-Karls University, D-69120 Heidelberg, Germany
b Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, MN 55455, USA

ARTICLE INFO

Article history:
Received 8 February 2016
Accepted 22 February 2016
Available online 2 March 2016

Keywords:
Glyoxalase
Inhibitor
Redox
Pharmacokinetics
Erythrocyte
Malaria

ABSTRACT

Glyoxalases prevent the formation of advanced glycation end products by converting glycolysis-derived methylglyoxal to D-lactate with the help of glutathione. Vander Jagt and colleagues previously showed that erythrocytes release about thirty times more D-lactate after infection with the human malaria parasite Plasmodium falciparum. Functional glyoxalases in the host-parasite unit might therefore be crucial for parasite survival. Here, we determined the antimalarial and hemolytic activity of two tight-binding glyoxalase inhibitors using infected and uninfected erythrocytes. In addition, we synthesized and analyzed a set of diester derivates of both tight-binding inhibitors resulting in up to threefold lower IC50 values and an altered methemoglobin formation and hemolytic activity depending on the type of ester. Inhibitor treatments of uninfected erythrocytes revealed an extremely slow inactivation of the host cell glyoxalase, irrespective of inhibitor modifications, and a potential dispensability of the host cell enzyme for parasite survival. Our study highlights the benefits and drawbacks of different esterifications of glutathione-derived inhibitors and demonstrates the suitability of glyoxalase inhibitors as a tool for deciphering the relevance and mode of action of different glyoxalase systems in a host-parasite unit.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The glyoxalase system is considered to be an ancient metabolic adaptation for the efficient conversion of reactive electrophilic 2-oxoaldehydes to 2-hydroxycarboxylic acids [1]. The system consists of reduced glutathione (GSH) and two enzymes, the isomerase glyoxalase 1 (Glo1) and the thioesterase glyoxalase 2 (Glo2). Even though there are organelles and organisms with in-sulcar glyoxalases pointing to alternative functions [1–3], Glo1 and Glo2 usually act together, for example, to catalyze the formation of D-lactate from glucose-derived methylglyoxal (MG) [1–5]. The glyoxalase system in humans prevents the accumulation of MG and of MG-derived advanced glycation end products (AGEs) with implications for numerous pathophysiological conditions including diabetes and cancer [6–9]. Inhibition of Glo1 is therefore supposed to be an elegant strategy to target cancer cells with high glycolytic fluxes that necessitate a potent MG removal system [7,10,11]. An inhibition of pathogen glyoxalases might likewise result in the accumulation of harmful MG and AGEs in bacteria and parasites with high glycolytic fluxes [2,3,12–15]. Moreover, the diverse glyoxalase systems and MG removal strategies of parasitic protists are not only potential drug targets but are also suited for the identification of alternative glyoxalase functions and mechanisms [1–3,15–19].

Two D-lactate producing glyoxalase systems are found in the host-parasite unit of erythrocytes that are infected with the human malaria parasite Plasmodium falciparum, one in the erythrocyte and one in the parasite cytosol [2,12,19]. The apicoplast of P. falciparum furthermore harbors a functional Glo2-isoform and a highly mutated Glo1-like protein that is inactive in standard enzymatic assays [2,19,20]. A methylglyoxal reductase activity was found to be negligible in the host-parasite unit and infected erythrocytes were shown to release about thirty times more D-lactate [12]. Cytosolic PfGlo1 from P. falciparum has been thoroughly analyzed in vitro. In contrast to its homodimeric human homologue, monomeric PfGlo1 has two different, allosterically coupled active sites [2,16,15,21]. Both active sites have deviating substrate
affinities [16] and can be targeted by non-glutathione [19] and glutathione-derived inhibitors [21]. Glutathione-derived Glo1 transition-state analogues, termed compound 1 and 2 (Fig. 1), were previously shown to act as glyoxalase-specific, non-competitive tight-binding inhibitors with nanomolar $K_c^{app}$ values for recombinant PfGlo1 [21]. Here, we tested the antimalarial activity and toxicity of these inhibitors as well as a set of novel ester derivatives in P. falciparum cell culture and on uninfected human erythrocytes. In addition, we used the inhibitors as a chemical tool to address the relevance of functional human Glo1 for parasite survival.

2. Materials and methods

2.1. Chemicals

Compounds 1 and 2 were synthesized as previously described [22]. The synthesis and validation of the ester derivatives 3–7 is described in the Supplementary materials and methods including Scheme S1. GSH, S-α-lactoylglutathione, MG, and DMSO were obtained from Sigma-Aldrich (Taufkirchen, Germany).

2.2. Growth inhibition assays and hemolysis

Direct growth inhibition of P. falciparum blood stage parasites was analyzed for strain 3D7 that was cultured according to standard protocols [23] at 37 °C, 5% CO$_2$, 5% O$_2$, 90% N$_2$ and 80% humidity in RPMI medium containing 0.45% (w/v) Albumax II, 0.2 mM hypoxanthine, 2.7 μg/mL gentamicin and human A erythrocytes at a hematocrit of 1.5–3.5%. Synchronization was carried out using the sorbitol method [24]. Inhibition of parasite growth was determined from three independent experiments by counting Giemsa-stained blood smears. Compounds 1–7 (50 or 25 mM stock solutions in DMSO) were diluted stepwise in culture medium in 48-well plates. Afterwards, either asynchronous parasite cultures or synchronized ring stage parasite cultures were added to the medium at a final hematocrit of 1.5% and an initial parasitemia of 0.25%. The highest final concentration of DMSO in the cultures was 0.8%. Parasites were grown for 48 h before preparation of blood smears. About 750–1500 erythrocytes were counted per Giemsa-stained blood smear and data were analyzed following the recommendations of the National Institutes of Health Chemical Genomics Center using the four parameter logistic model for the determination of IC$_{50}$ values. As a control, hemolytic effects of the tight-binding inhibitors on unparasitized erythrocytes were analyzed in parallel. After 48 h, erythrocytes were counted in a Neubauer chamber and the release of hemoglobin into the medium was determined spectrophotometrically at 405 nm.

2.3. Inhibition of the host cell Glo1 activity

Erythrocytes from five different donors were incubated in complete RPMI medium in the presence of 10 μM compounds 1–3 and 7 or DMSO as a control. The activities of human Glo1 and Glo2 were measured before the addition of each compound and monitored after the addition for 96 h. Every 24 h, erythrocytes were centrifuged (5 min, 300 g, room temperature) and a 40 μL aliquot was removed from the cell pellet. The remaining erythrocytes were resuspended in the original medium for further incubation, whereas the 40 μL aliquot was resuspended in 500 μL of cold phosphate-buffered saline (PBS containing 1.84 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$, 137 mM NaCl, 2.7 mM KCl, pH 7.4 at 24 °C) and centrifuged again (5 min, 2000 g, 4 °C). The supernatant was discarded and the erythrocyte aliquot was washed two more times with PBS to remove traces of extracellular inhibitors. Washed erythrocytes were subsequently lysed by resuspension in 200 μL of ice-cold water until the solution became clear. After centrifugation (10 min, 20,000 g, 4 °C), the supernatant was stored on ice pending immediate enzyme assays and hemoglobin measurements. The hemoglobin content in erythrocyte lysates was determined by adding 20 μL of lysate to 980 μL of 50 mM Tris/HCl, pH 7.4 at 37 °C and measuring the absorbance at the isosbestic point at 523 nm. The concentration was calculated using the formula [Hb] = Abs$_{523nm} - 50.712$ [mM] or [Hb] = Abs$_{523nm} - 16.1 - 50.712$ [g/L] [25]. Activities of human Glo1 and Glo2 were determined at 240 nm and 37 °C using a thermostatted Jasco V-550 UV–vis spectrophotometer according to standard protocols [28,29] with slight modifications. Briefly, stock solutions of 100 mM GSH and 100 mM MG were freshly prepared in cold Glo1 assay buffer (50 mM Na$_2$HPO$_4$, pH 6.6 at 37 °C) before each experiment and stored on ice. The hemithioacetal substrate was formed after mixing 955 μL of pre-warmed Glo1 assay buffer with 20 μL of each of the GSH and MG stock solutions followed by an incubation at 37 °C for 9.5 min. A baseline was subsequently recorded for 30 s and the Glo1 assay was started by the addition of 5 μL of erythrocyte lysate. The absorbance was monitored for 1.5 min and the activity was calculated using $E_{240nm} = 2.86 \text{mM}^{-1} \text{cm}^{-1}$. For human Glo2 measurements, a stock solution of 3 mM S-α-lactoylglutathione was freshly prepared in cold Glo2 assay buffer (50 mMTris/HCl, pH 7.4 at 37 °C) before each experiment and stored on ice. A baseline was recorded for 30 s after mixing 890 μL of pre-warmed Glo2 assay buffer with 100 μL of 3 mM S-α-lactoylglutathione. The Glo2 assay was started by the addition of 10 μL of cell lysate, and the absorbance was monitored for 1.5 min. Human Glo2 activities were calculated using $E_{240nm} = 3.1 \text{mM}^{-1} \text{cm}^{-1}$. All human Glo1 and Glo2 activity measurements were performed in triplicates and were normalized to the corresponding hemoglobin concentration of the lysates. Statistical analyses were performed in SigmaPlot 12.5 using the one way ANOVA on ranks method.

2.4. Effect of the host cell Glo1 activity on P. falciparum blood stage cultures

A potential indirect growth inhibition of P. falciparum blood stage cultures was determined with erythrocytes that were pretreated with compounds 1, 3 and 7 as described above. After 96 h inhibitor treatment, erythrocytes were washed three times with complete RPMI medium before adding synchronized schizont parasites (purity ≥ 98%) that were enriched by magnetic cell separation [28,29] using a VarioMACS™ Separator with CS columns.
3. Results

3.1. Direct growth inhibition of P. falciparum blood stage cultures

Our previous enzymatic studies showed that compounds 1 and 2 inhibit recombinant PfGlo1 at low nanomolar concentrations [21]. We therefore also examined the effect of these substances on the growth of P. falciparum blood stage cultures using Giemsa-stained blood smears. Compounds 1 and 2 inhibited parasite growth after 48 h with IC50 values around 70 or 90 μM (Table 1). Because esterification of the two carboxyl groups of glutathione-derived Glo1 inhibitors previously led to more potent agents, presumably because of an improved cellular uptake [11,13,30–32], we synthesized the diester derivatives 3–7 depicted in Fig. 1 and subsequently tested these compounds in cell culture. Methyl or ethyl esterifications of compound 1 had no influence on the IC50 values after 48 h drug treatment (data for compounds 4 and 5 not shown). In contrast, the cyclopentyl diester of 1 (compound 6) and the tert-butyl diesters of 1 and 2 (compounds 3 and 7) yielded two- to threefold decreased IC50 values for blood stage parasites (Fig. 2, Table 1). The IC50 curves displayed a steep slope with a well-defined toxic threshold resulting in complete parasite death at higher inhibitor concentrations. In summary, selected esterifications of compounds 1 and 2 improved their efficacy resulting in IC50 values for P. falciparum blood stage cultures around 30 μM.

3.2. Evaluation of hemolytic side effects

Next, we assessed the toxicity of the compounds on the host cell as a control. At high micromolar inhibitor concentrations, the colour of infected and uninfected erythrocytes turned brownish (Fig. 3A) which is indicative for methemoglobin formation.

Table 1

| Inhibitor | IC50 (μM) |
|-----------|-----------|
| Compound 1 | 70** |
| Compound 3 | 35** |
| Compound 6 | 30** |
| Compound 2 | 90/70 |
| Compound 7 | 30** |

Each compound was tested in at least two different experiments. Except for compound 2, no difference was observed between asynchronous (*) and synchronous (**) cultures (Fig. 2).

Furthermore, a loss of erythrocytes, as determined by cell counting, and the release of hemoglobin, as determined by spectrophotometry, were observed in a compound- and concentration-dependent manner (Fig. 3B, C). The hemolytic effect was most pronounced for 200 μM of compound 6 resulting in cell lysis of more than 40% of erythrocytes and a 30-fold increase of hemoglobin release as compared to the other compounds. However, except for the more toxic cyclopentyl diester compound 6, the inhibitors had rather moderate toxic side effects on uninfected erythrocytes at IC50 concentrations for P. falciparum around 30 μM. In summary, the tight-binding Glo1 inhibitors led to methemoglobin formation and hemolysis at high micromolar concentrations whereas concentrations around the IC50 values appeared to be safe for the host cell except for compound 6.

3.3. Effect of the host cell Glo1 activity on P. falciparum blood stage cultures

To date there are no glyoxalase knockout erythrocytes available. We therefore used our inhibitors as a molecular tool to analyze the relevance of functional human Glo1 on parasite growth (Fig. 4A). We therefore also examined the effect of these substances on the growth of P. falciparum blood stage cultures using Giemsa-stained blood smears. The inhibitors had a nearly identical influence on synchronous or asynchronous cultures (closed and open circles, respectively) yielding IC50 values of about 30 μM. Data points were averaged from at least two independent experiments. No difference was observed between asynchronous (•) and synchronous (○) cultures (Fig. 2).

Table 1

| Inhibitor | IC50 (μM) |
|-----------|-----------|
| Compound 1 | 70** |
| Compound 3 | 35** |
| Compound 6 | 30** |
| Compound 2 | 90/70 |
| Compound 7 | 30** |

Each compound was tested in at least two different experiments. Except for compound 2, no difference was observed between asynchronous (*) and synchronous (**) cultures (Fig. 2).

4. Discussion

What can we learn from our studies on glutathione-derived inhibitors? Regarding the activity of compounds 1 and 2 on P. falciparum blood stage parasites, the IC50 values around 70 μM were three to four orders of magnitude higher than the IC50 values with recombinant enzyme [21]. A plausible cause might be an inefficient cellular uptake of the inhibitors. According to previous studies [11,13,30–32], efficient delivery of glutathione-derived...
drugs to the site of action highly depends on the polarity of the compound. Esterification of the glutathione moiety reduces its polarity and is supposed to facilitate the uptake of the drugs. We were therefore surprised that, under the chosen assay conditions in Fig. 4, esters of compounds 1 and 2 did not result in a faster or more potent inhibition of human Glo1 in uninfected erythrocytes. Furthermore, in contrast to the reported effect of the ethyl diester of S-p-bromobenzylglutathione on parasite growth [13], we observed no improvement of the IC50 values when P. falciparum cultures were treated with ethyl (or methyl) diesters of compound 1. However, modification of compound 1 by cyclopentyl esters led to a more than twofold decrease of the IC50 value in accordance with previous studies on the inhibition of human leukemia 60 cells by the cyclopentyl diester of S-p-bromobenzylglutathione [11]. Another interesting observation was that the tert-butyl diesters of compounds 1 and 2 yielded similar IC50 values as the cyclopentyl diester but were far less toxic for the host cell. Hence, the type of ester can actually impact the growth inhibition of malaria parasites and the toxicity for the host cell in different ways. The slightly lower IC50 value for compound 7 in comparison to compound 3 might be explained by an absent turnover of compound 7 by γ-glutamyltranspeptidase [22]. In summary, the polarity and type of ester of glutathione-derived Glo1 inhibitors are both important. Bulky esters yielded lower IC50 values accompanied by an increased hemolytic toxicity, which was much higher for the cyclopentyl than for the tert-butyl diester of compound 1. Other factors, such as the drastically increased host cell permeability upon infection, as reviewed in [34] and [35], can also have significant effects on the potency of Glo1 inhibitors and are difficult to predict.

What is the mode of action of the tested inhibitors and how can they lead to methemoglobin formation? A physiologically relevant link between Glo1 activity and redox imbalances in vivo was recently suggested for diabetic rats that produced less reactive oxygen species upon overexpression of GLO1 [36]. Using a set of recombinant proteins, we previously showed that compounds 1 and 2 specifically act on the glyoxalase system and not on other glutathione-utilizing enzymes such as glutathione reductase or glutaredoxin [21]. An interesting question is therefore how methemoglobin formation upon inhibitor treatment of uninfected erythrocytes could be linked to glyoxalase inhibition. Two models appear to be plausible (Fig. 5). (i) While 2-oxoaldehyde formation continues upon glyoxalase inhibition, more and more GSH reacts with these electrophiles, and the pool of available GSH might get depleted. As a consequence, the ratio [GSH][GSSG] (as derived from the Nernst equation) decreases and oxidizing metabolites accumulate over time resulting in an increased methemoglobin concentration (Fig. 5A). (ii) Alternatively, MG and other 2-oxoaldehydes might inactivate selected antioxidant enzymes by modifying their essential active site (cysteine) residues (Fig. 5B). Since erythrocytes lack de novo protein biosynthesis, they should be particularly susceptible to enzyme inactivation resulting in redox imbalances. Such considerations are not only important for deciphering mechanistic links between glyoxalase and redox metabolic pathways but also regarding the potential necessity to generate non-toxic, parasite-specific glyoxalase inhibitors. For example, in analogy to the malaria protection hypothesis for people with glucose-6-phosphate dehydrogenase deficiency [37], a moderate effect of inhibitors on the erythrocyte glyoxalase system might even be beneficial because the parasite has to face a more hostile environment [2] and drug resistances are less likely to arise. Furthermore, if P. falciparum imports human Glo1 to cope with elevated MG concentrations (similar to peroxide detoxification by imported human peroxiredoxin 2 [38]), inhibition of the host cell enzyme might be a prerequisite for killing the parasite. Our data on the [ir]relevance of human Glo1 for parasite survival (Fig. 4) supports neither of both hypotheses, although we cannot fully exclude that the residual host cell Glo1 activity was sufficient to compensate a growth phenotype.

In conclusion, derivatives of compounds 1 and 2 have moderate antimalarial activities. They can also alter the redox milieu of human erythrocytes and could serve as an interesting tool for deciphering the molecular mechanism of how the glyoxalase system exerts its physiological protective function. The activity of
human Glo1 does not seem to play a major role for the development of \textit{P. falciparum} in cell culture. Regarding the esterification and optimization of Glo1 inhibitors, a balance between inhibitor activity, specificity, selectivity and toxicity has to be determined empirically. A future strategy could comprise the synthesis of conjugates between compounds 1 or 2 and existing antimalarial drugs. This might further facilitate the uptake into parasitized erythrocytes and may also exhibit a synergistic effect, as has been described for a double-drug glutathione reductase inhibitor [39].

**Acknowledgements**

The position of C.A.W. is funded by the German Academic Exchange Service (DAAD) and the Ministry of Education, Ghana. The work of S.S.M. and R.V. was supported by the Center for Drug Design research endowment funds. M.U. was funded by the Deutsche Forschungsgemeinschaft (DFG), Grant DE 1431/1. M.D. is grateful to the DFG for funding of his position in the frame of the Heisenberg Program, Grant DE 1431/9-1.

**References**

[1] M. Deponte, Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes, Biochim. Biophys. Acta 1830 (2013) 3217–3266.
[2] M. Urscher, R. Alisch, M. Deponte, The glyoxalase system of malaria parasites—implications for cell biology and general glyoxalase research, Semin. Cell Dev. Biol. 22 (2011) 262–270.
[3] M. Deponte, Glyoxalase diversity in parasitic protists, Biochem. Soc. Trans. 42 (2014) 473–478.
A. Wendler, T. Irsch, N. Rabbani, P.J. Thornalley, R.L. Krauth-Siegel, Glyoxalase II

M. Deponte, N. Sturm, S. Mittler, M. Harner, H. Mack, K. Becker, Allosteric

P.J. Thornalley, M. Strath, R.J. Wilson, Antimalarial activity

U. Suttisansanee, J.F. Honek, Bacterial glyoxalase enzymes, Semin. Cell Dev.

R. Vince, W.B. Wadd, Glyoxalase inhibitors as potential anticancer agents,

P.J. Thornalley, L.G. Edwards, Y. Kang, C. Wyatt, N. Davies, M.J. Ladan, J. Double,

M. Akoachere, R. Iozef, S. Rahlfs, M. Deponte, B. Mannervik, D.J. Creighton,

M. Urscher, M. Deponte, Distinct subcellular localization in the cytosol and apicoplast, unexpected dimerization and inhibition of Plasmodium falciparum glyoxalases, Mol. Microbiol. 76 (2010) 92–103.

M. Akouche, R. Iozef, S. Rahlf, M. Deponte, B. Mannervik, D.J. Creighton, H. Schirmer, K. Becker, Characterization of the glyoxalases of the malarial parasite Plasmodium falciparum and comparison with their human counterparts, Biol. Chem. 386 (2005) 41–52.

M. Urscher, S.S. More, R. Aliisch, R. Vince, M. Deponte, Tight-binding inhibitors efficiently inactivate both reaction centers of monomeric Plasmodium falciparum glyoxalase 1, FEBS J. 279 (2012) 2568–2578.

S.S. More, R. Vince, Inhibition of glyoxalase I: the first low-nanomolar tight-binding inhibitors, J. Med. Chem. 52 (2009) 4650–4656.

W. Trager, J.B. Jensen, Human malaria parasites in continuous culture, Science 193 (1976) 673–675.

C. Lambros, J.P. Vanderberg, Synchronization of Plasmodium falciparum erythrocytic stages in culture, J. Parasitol. 65 (1979) 418–420.

S.M. Snell, M.A. Marini, A convenient spectroscopic method for the estimation of hemoglobin concentrations in cell-free systems, J. Biochem. Biophys. Methods 17 (1988) 25–33.

P.J. Thornalley, Modification of the glyoxalase system in human red blood cells by glucose in vitro, Biochem. J. 254 (1988) 751–755.

M. Arai, N. Nihonmatsu-Kikuchi, M. Iokawa, N. Rabbani, P.J. Thornalley, Measurement of glyoxalase activities, Biochem. Soc. Trans. 42 (2014) 491–494.

T. Stalholm, H.A. Gia, D. Dodoo, T.G. Theander, L. Hvid, Detection of antibodies to variant antigens on Plasmodium falciparum-infected erythrocytes by flow cytometry, Cytometry 35 (1999) 328–336.

A. Vogt, Selection of Trophozoites by using Magnetic Cell Sorting (MACS), 5th ed., Manassas, Virginia, 2005.

R.E. Allen, T.W. Lo, P.J. Thornalley, Inhibitors of glyoxalase I: design, synthesis, inhibitory characteristics and biological evaluation, Biochem. Soc. Trans. 21 (1993) 535–540.

M.J. Kawarana, E.G. Kovalava, D.J. Creighton, M.B. Wollman, J.L. Eisenman, Mechanism-based competitive inhibitors of glyoxalase I: intracellular delivery, in vitro antitumor activities, and stabilities in human serum and mouse serum, J. Med. Chem. 42 (2009) 221–228.

T.W. Lo, P.J. Thornalley, Inhibition of proliferation of human leukaemia 60 cells by diethyl esters of glyoxalase inhibitors in vitro, Biochem. Pharmacol. 44 (1992) 2357–2363.

A.C. McLellan, P.J. Thornalley, J. Benn, P.H. Sonksen, Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications, Clin. Sci. 87 (1994) 21–29.

K. Kirk, A.M. Lehane, Membrane transport in the malaria parasite and its host erythrocyte, Biochem. J. 457 (2014) 1–18.

S. Baumeister, P. Gangapadhyay, U. Repnik, K. Lingelbach, Novel insights into red blood cell physiology using parasites as tools, Eur. J. Cell Biol. 94 (2015) 332–339.

G. Brouwers, P.M. Niesen, I. Ferreira, T. Myata, P.G. Scheffer, T. Teerlink, P. Schrauwen, M. Brownlee, C.D. Stehouwer, C.G. Schalkwijk, Novel insights into glyoxalase-I reduces hyperglycemia-induced levels of advanced glycation end products and oxidative stress in diabetic rats, J. Biol. Chem. 286 (2011) 1374–1380.

M.D. Cappellini, G. Fiorelli, Glucose-6-phosphate dehydrogenase deficiency, Lancet 371 (2008) 64–74.

S. Konarevic, P. Rohrbach, M. Deponte, G. Krohne, J.H. Prieto, J. Yates 3rd, S. Rahlf, K. Becker, The malarial parasite Plasmodium falciparum imports the human protein peroxiredoxin 2 for peroxide detoxification, Proc. Natl. Acad. Sci. USA 106 (2009) 13323–13328.

E. Davioud-Charvet, S. Delarue, C. Biot, B. Schwobel, C.C. Boehme, A. Mussigbrodt, L. Maes, C. Sержареерт, P. Grelle, R.H. Schirmer, K. Becker, A prodrug form of a Plasmodium falciparum glutathione reductase inhibitor conjugated with a 4-aminoquinoline, J. Med. Chem. 44 (2001) 4268–4276.