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# Antimalarial activity of thioacridone compounds related to the acronycine alkaloid

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**Abstract**—A series of thioacridone compounds that were previously shown to have DNA binding interaction, were screened for antimalarial activity. The new compounds were assessed for in vitro antimalarial activity against a chloroquine sensitive (D10) strain of the malaria parasite *Plasmodium falciparum*, using a lactate dehydrogenase (PfLDH) assay. In the series, the $IC_{50}$ values ranged from 0.4 to 27 μg/ml. 1-(2-Dimethylaminoethylamino)-9(10*H*)-thioacridone was found to be the most potent against *P. falciparum* (D10) with an $IC_{50}$ value of 0.4 μg/ml. This compound was also evaluated against a South African chloroquine resistant (RSA 11) *P. falciparum* strain and was found to have an $IC_{50}$ value of 1 μg/ml, compared with 0.16 μg/ml for chloroquine. Quantitative structure–activity relationships of this series were also investigated and a multiple linear regression $r^2$ of 0.58 was found for the best fit equation. The most potent compound, 1-(2-dimethylaminoethylamino)-9(10*H*)-thioacridone, was docked into the chloroquine binding site of PfLDH and it was found that the slightly lower activity of this compound, compared with chloroquine, is likely due to steric interference within a restricted binding pocket.


## 1. Introduction

Malaria remains a threat to almost half of the world's population.[1] The disease is a major health problem in tropical counties, where approximately 300 million malaria cases are reported annually.[1] In Africa, about 2 million children die of the disease every year.[2] This morbidity is largely due to the widespread emergence of strains of the malaria parasite that are resistant to presently available antimalarials, including frontline drugs such as chloroquine, primaquine, pyrimethamine, and mefloquine.[3,4] Therefore, an urgent need exists for the development of new simple, safe, and more effective antimalarial drugs. In this quest several groups, most notably those of Singh and Puri, and Jain have contributed elegant lead compounds. These include novel compounds such as substituted trioxanes,[5,6] trioxaquines,[7] and isonitriles of marine origin[8] by the groups of Singh and Puri, and ring-substituted-L-histidines and histamines,[9] multisubstituted quinolinamines and quinolinamine–amino acid conjugates,[10] and modified primaquine analogs that are resistant to metabolism of the quinoline ring[11] by that of Jain. The causative pathogen of human malaria in most affected areas is *P. falciparum*. In South Africa, about 90–95% of the locally contracted cases are due to *P. falciparum* and in most areas this strain of the parasite is resistant to the antimalarial drug chloroquine.[12]

Recently, we reported the in vitro cytotoxic activity of a series of thioacridone analogs related to the acronycine alkaloid.[13] The compounds investigated showed cytotoxic activity in HL-60 cells and exhibited DNA binding at $10^2\ M^{-1}$. One compound, (**4** in the current study) showed DNA binding at less than $10^4\ M^{-1}$. Furthermore, intercalation into the DNA helix was also suggested to be one of the molecular mechanisms characteristic of these compounds.[13] Structurally similar

*Keywords*: Thioacridones; Malaria; QSAR; DNA intercalation; *Plasmodium*; FLEXIDOCK; SYBYL; MOLCAD.
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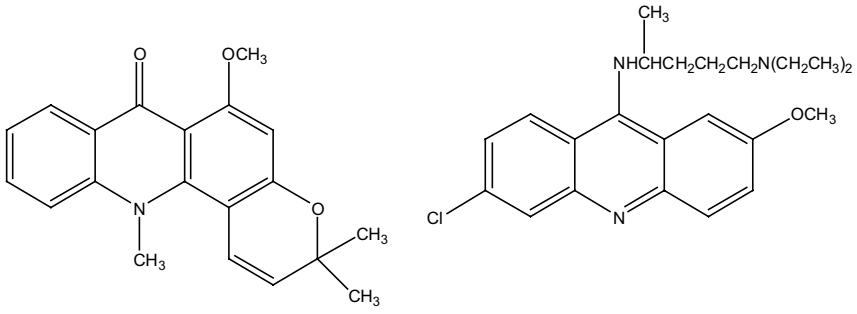



**Figure 1.** Structures of acridine compounds discussed in the text.

compounds to these novel thioacridones, including furo[2,3*b*]quinoline and acridone alkaloids,[14] and mepacrine (see below) are also known to be efficacious in *P. falciparum* malaria treatment. We therefore proceeded to also investigate the same series of compounds in an antimalaria paradigm.

Mepacrine, or quinacrine (Fig. 1) was one of the first synthetic antimalarials to be introduced clinically.[15] Due to the fact that both structural types have a fused planar tricyclic ring system and a basic side chain, mepacrine, and related drugs are structurally similar to the thioacridone compounds described in the current study. There is now evidence that the acridine ring structure of mepacrine intercalates with DNA in much the same way as proflavine,[15] a mechanism also believed to be operative with the novel thioacridones.[13]

The new compounds were assessed for in vitro antimalarial activity against a chloroquine sensitive (D10) strain of *P. falciparum*. After initial screening, the most active compound was also evaluated against a highly chloroquine resistant South African strain of *P. falciparum* (RSA 11). RSA 11 is the South African strain of *P. falciparum* that has been isolated from patients who contracted malaria in the Kwazulu-Natal province.[16]

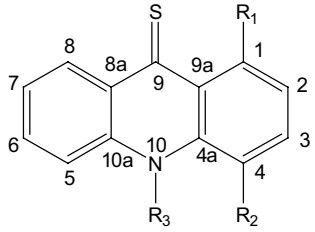
## 2. Materials and methods

The compounds (Table 1) used in this study were synthesized as described in detail in our earlier report.[13] The antimalarial properties of the compounds were evaluated against two strains of *P. falciparum*, a chloroquine sensitive strain (D10) and a chloroquine resistant strain (RSA 11).

Antimalarial tests were carried out as described earlier.[17] Briefly, sensitivity assays were initiated by adjusting the initial parasitemia to 1–2% with normal type A human red blood cells (2% hematocrit) suspended in complete tissue culture medium (RPMI 1640, containing 25 mM HEPES, 20 μg/ml gentamycin, 27 mM $NaHCO_3$, and 10% normal type A human serum). The suspensions were dispensed in triplicate plates. The test compounds and chloroquine (positive control) were prepared to contain concentrations varying from 0.1 to 100 μg/ml in RPMI 1640, before the addition of 10 μl volumes in triplicate to appropriate wells.

The cultures were incubated at 37 °C for 48 h in 3% $O_2$, 6% $CO_2$ and 91% $N_2$. At the end of the incubation period, the cultures were carefully resuspended and the aliquots removed for the analysis of *P. falciparum* lactate dehydrogenase (PfLDH) activity. Ten microliters from each well of suspended culture was transferred into a 96 well plate containing 100 μl of Malastad™ reagent, and the reduction of 3-acetyl pyridine adenine dinucleotide (APAD) followed for 10 min at 650 nm. At the end of this period, each well received 20 μl of a 20:1 mixture of nitroblue tetrazolium and phenazine ethosulfate (1 mg and 0.05 mg/ml respectively). The reduction of tetrazolium to blue formazan salts was followed for 10 min at 650 nm. Finally, the blue formazan product was evaluated after the addition of 30 μl of 2 N $H_2SO_4$ by end-point analysis at 650 nm using a 7520 Microplate

**Table 1.** Structures of thioacridone compounds used in this study




| Compound | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **1** | $NH(CH_2)_2(CH_3)_2$ | H | $CH_3$ |
| **2** | $NH(CH_2)_2(CH_3)_2$ | Cl | $CH_3$ |
| **3** | $NH(CH_2)_2(CH_3)_2$ | $CH_3$ | $CH_3$ |
| **4** | $NH(CH_2)_2(CH_3)_2$ | H | H |
| **5** | $NH(CH_2)_2(CH_3)_2$ | Cl | H |
| **6** | $NH(CH_2)_2(CH_3)_2$ | $CH_3$ | H |
| **7** | Cl | H | H |
| **8** | Cl | Cl | H |
| **9** | Cl | $CH_3$ | H |
| **10** | Cl | H | $CH_3$ |
| **11** | Cl | Cl | $CH_3$ |
| **12** | Cl | $CH_3$ | $CH_3$ |

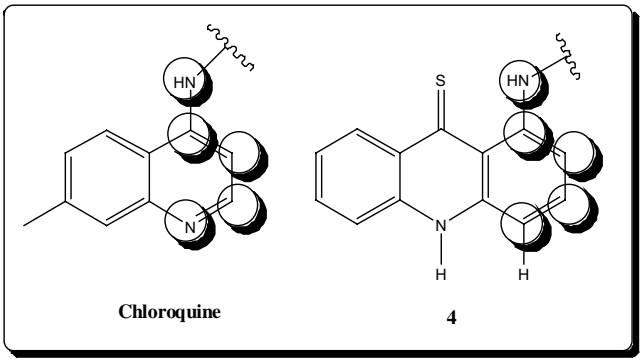



**Figure 2.** Alignment of **4** on chloroquine for the preparation of the docking studies. Atoms used for alignment are circled.

Reader spectrophotometer (Cambridge Technology Inc).

### 2.1. Statistical analysis

From the absorbance readings, the averages were calculated with their standard deviations (S.D.) for all the tests, blank, and control counts. The parasite viability was calculated using the relationship:

$$\%parasite\ survival = \frac{\bar{x}_{test} \pm \text{S.D.} - \bar{x}_{blank} \pm \text{S.D.}}{Control \pm \text{S.D.} - \bar{x}_{blank} \pm \text{S.D.}} \times 100\% \quad (1)$$

Concentration-curve data were analyzed by nonlinear least squares fit to a four parameter logistic equation, with resulting $IC_{50}$ values and goodness of fit statistics calculated using Prism™ (GraphPad Software, San Diego, CA).

### 2.2. QSAR analysis

The molecules were drawn in both SYBYL® 6.91[18] and HyperChem® 7.[19] In SYBYL®, the compounds were drawn using the SKETCH® module and energy minimized with the Tripos® Force Field engine (100 iterations) and Gasteiger-Hückel charges added. The compounds were then imported into a molecular spreadsheet, and the polar surface area (PSA) calculated. In HyperChem, energy minimization was done using the AMBER force field, with a conjugate gradient until the root mean square reached 0.01 kcal/mol Å. The physicochemical properties were then calculated for each compound.

### 2.3. Docking studies

Docking studies were carried out using the FLEXIDOCK® module in SYBYL®. Coordinates for *P. falciparum* lactate dehydrogenase (PfLDH)[3,20] were downloaded from the Protein Data Bank, Brookhaven National Laboratory, Upton, NY, USA (1CET.pdb). For the docking studies, chloroquine was extracted from the enzyme, and compound **4** was manually aligned (fitted) within the chloroquine occupied space using a root mean square (RMS) best fit algorithm (Fig. 2) to obtain the same orientation with **4** as that measured for chloroquine (which had been co-crystallized with PfLDH in the PDB file). MOLCAD surfaces were calculated for both chloroquine and compound **4**, to gain insight into possible steric or electronic interactions.

## 3. Results

The results of the antimalarial activity of the compounds tested in this study are given in Table 2 and the effects of concentration of chloroquine and compounds **4**–**6** on parasite survival (D10) are given in Figures 3 and 4. Figure 5 shows the effect of concentration of **4** on survival of the resistant (RSA 11) strain of *P. falciparum*. Biological data for the 1-chloroacridone precursors (**7**–**9** and **10**–**12**) were acquired in order to determine the effect of the side chain (present in the target compounds **1**–**3** and **4**–**6**, respectively) on antimalarial activity. Chloroquine, doxycycline, and proguanil were used as positive controls in these comparisons. Chloroquine is a cheap, commonly used drug for the treatment of chloroquine-sensitive *P. falciparum* malaria. Both doxycycline and proguanil have been widely used for malaria chemoprophylaxis[21] while doxycycline is also an effective drug in the treatment of chloroquine-resistant malaria.[22]

Our results show that all the compounds tested exhibited significant antimalarial activity against the chloroquine sensitive D10 *P. falciparum* strain with their $IC_{50}$ values ranging from 0.4 μg/ml to 27 μg/ml. Compound **4** was the most potent ($IC_{50}$ = 0.4 μg/ml), while **5** was the least effective with an $IC_{50}$ value of 27 μg/ml. Compound **4**, however, had lower activity than

**Table 2.** Antimalarial activity, cytotoxicity, and selective toxicity index of the new thioacridone derivatives

| Compound | Antimalarial activity $IC_{50}$ (μg/ml) (*P. falciparum*) | | Cytotoxicity $IC_{50}$ (μg/ml)[a] | Selectivity toxicity index[b] (Cytotoxicity/D10) |
|---|---|---|---|---|
| | D10 | RSA11 | | |
| **1** | 2.0 | — | 4.5 | 2.3 |
| **2** | 12 | — | 5.6 | 0.5 |
| **3** | 5.0 | — | 4.0 | 0.8 |
| **4** | 0.4 | 1 | 2.3 | 5.8 |
| **5** | 27 | — | 8.5 | 0.3 |
| **6** | 4.0 | — | 15 | 3.8 |
| **7** | 3.5 | — | 22.6 | 6.5 |
| **8** | 5.0 | — | >26 | [c] |
| **9** | 5.0 | — | >26 | [c] |
| **10** | 5.0 | — | 8.9 | 1.8 |
| **11** | 2.5 | — | 6.7 | 2.7 |
| **12** | 1.0 | — | 6.0 | 6 |
| **Chloroquine** | 0.01 | 0.16 | — | — |
| **Doxycycline** | 6.9 | — | — | — |
| **Proguanil** | 5.1 | — | — | — |

[a] HL-60 cells.[13]
[b] $IC_{50}$ for HL-60 cells/$IC_{50}$ for *P. falciparum* (D10).
[c] Underterminable.

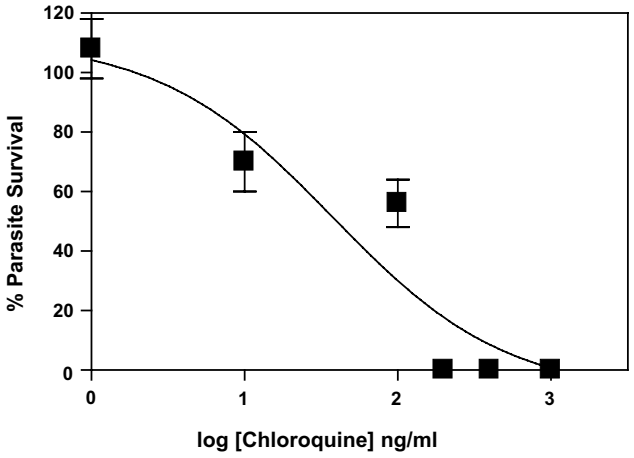



**Figure 3.** Effect of concentration of chloroquine (positive control) on the survival of a chloroquine-sensitive strain (D10) of *P. falciparum*.

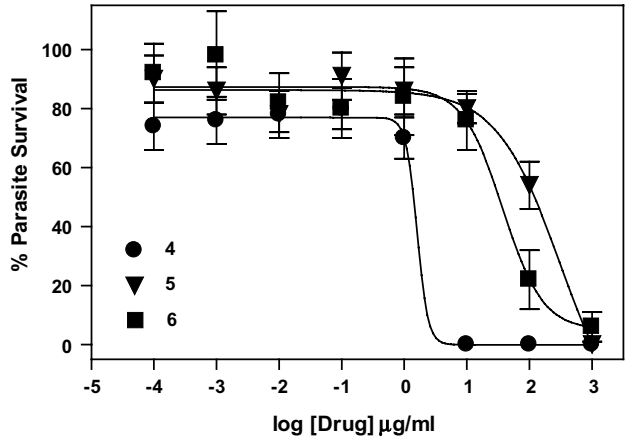



**Figure 4.** Effect of concentration of compounds **4**, **5**, and **6** on the survival of a chloroquine-sensitive strain (D10) of *P. falciparum*.

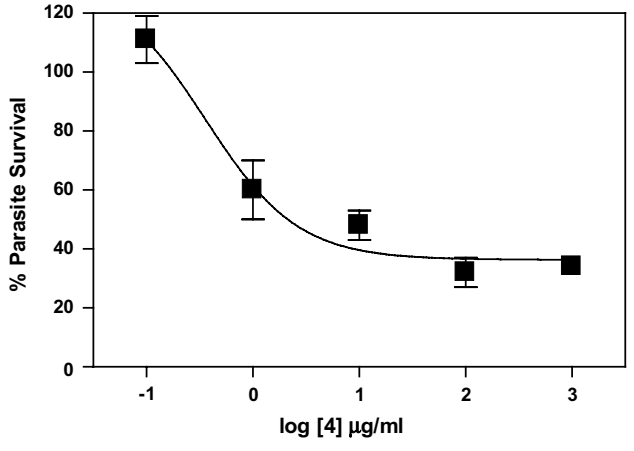



**Figure 5.** Effect of concentration of compound **4** on the survival of a chloroquine-resistant (RSA 11) strain of *P. falciparum*.

chloroquine ($IC_{50}$ = 0.01 µg/ml), but was significantly more potent than both doxycycline ($IC_{50}$ = 6.9 µg/ml) and proguanil ($IC_{50}$ = 5.1 µg/ml). In addition to **4**, compounds **1** ($IC_{50}$ = 2 µg/ml), **7** ($IC_{50}$ = 3.5 µg/ml), **11** ($IC_{50}$ = 2.5 µg/ml), and **12** ($IC_{50}$ = 1 µg/ml) all displayed better antimalarial activity than both doxycycline and proguanil. The antimalarial potency of compounds **3**, **6**, **8**, **9**, and **10** were found to be comparable to those of doxycycline and proguanil. When tested on the chloroquine resistant strain (RSA 11), **4** again showed lower potency than chloroquine with an $IC_{50}$ of 1 µg/ml as opposed to 0.16 µg/ml for chloroquine.

$$IC_{50} = -15.64\mathrm{Log}P + 395.91P + 0.72\mathrm{PSA} - 1550.13\mathrm{CMR} + 0.54\mathrm{MW} + 411.78$$
$$r^2 = 0.580 \quad \text{Standard Error} = 6.586 \quad n = 12 \quad (2)$$

The results of the quantitative structure–activity relationship (QSAR) are given in Figure 6 and Eq. 2. The descriptors used in the QSAR analysis are given in Table 3, where abbreviations are: polarizability (*P*), polar surface area (PSA), calculated molecular refractivity (CMR) and molecular weight (MW). A five descriptor

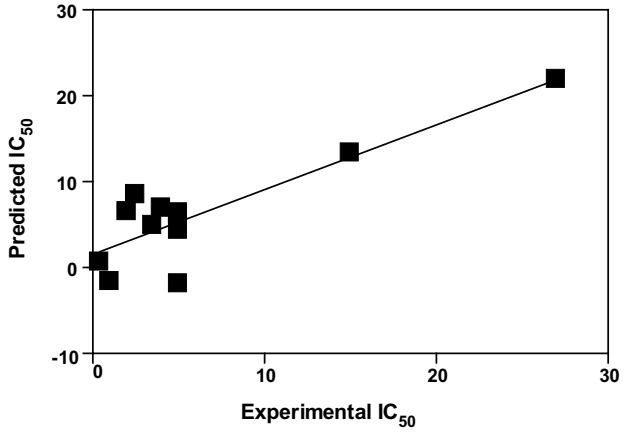



**Figure 6.** Correlation between the experimental $IC_{50}$ values and the predicted $IC_{50}$ values of the activity of the thioacridones against *P. falciparum* (D10). The resulting $r^2$ was found to be 0.58.

**Table 3.** Physicochemical properties of the thioacridone compounds tested in this study, calculated with SYBYL® 6.91 and HyperChem® 7

| Compound | $P$[a] | Log $P$[b] | PSA[c] | CMR[d] | MW[e] |
|---|---|---|---|---|---|
| **1** | 37.63 | 2.92 | 56.865 | 9.9792 | 311.447 |
| **2** | 39.56 | 3.44 | 55.242 | 10.4706 | 345.892 |
| **3** | 39.46 | 3.62 | 55.028 | 10.443 | 325.474 |
| **4** | 35.79 | 2.67 | 79.739 | 9.5154 | 297.42 |
| **5** | 37.72 | 3.19 | 75.659 | 10.0068 | 331.865 |
| **6** | 37.63 | 3.14 | 64.261 | 9.9792 | 311.447 |
| **7** | 27.68 | 3.64 | 66.547 | 7.4142 | 245.727 |
| **8** | 29.61 | 4.16 | 65.349 | 7.9059 | 280.172 |
| **9** | 29.51 | 4.11 | 55.924 | 7.878 | 259.754 |
| **10** | 29.51 | 3.29 | 43.371 | 7.878 | 259.754 |
| **11** | 31.44 | 4.4 | 41.347 | 8.3694 | 294.199 |
| **12** | 31.35 | 4.35 | 41.347 | 8.3418 | 273.781 |

[a] Polarizability.
[b] Octanol–water partition coefficient.
[c] Polar surface area.
[d] Calculated molecular refractivity.
[e] Molecular weight.

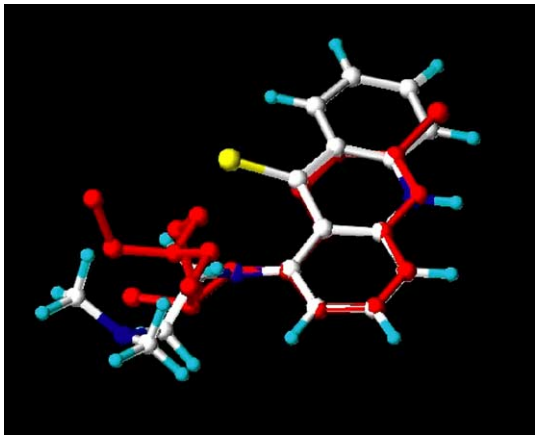


**Figure 7.** Alignment of compound **4** on chloroquine (red).

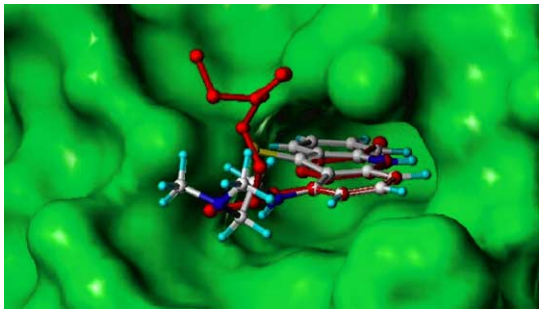


**Figure 8.** Compound **4** docked into the chloroquine binding site in PfLDH. The third aromatic ring of compound **4** extends deeper into the pocket than chloroquine.

multiple linear regression equation was generated to give a statistically favorable $r^2$ and keep the number of descriptors to a minimum to avoid over-fitting. Eq. (2) suggests that at a more positive $P$, PSA, and MW will lead to an increase in antimalarial activity, and that more negative Log $P$ and CMR will lead to an increase in antimalarial activity.

The most potent compound of the thioacridones, **4**, was docked into the chloroquine binding site of PfLDH. The results of the docking studies are shown in Figures 7–9. Compound **4** was docked with the same orientation found for chloroquine co-crystallized inside PfLDH. MOLCAD surface areas were generated for both compound **4** and chloroquine to gain perspective of interactions with amino acid side chains within the PfLDH catalytic site. The results indicate that the MOLCAD surface area for **4** extends closer to TYR85 and GLU122, with the oxygen of GLU122 breaching the MOLCAD surface area of the compound. This breach of the surface area was not seen for chloroquine.

## 4. Discussion

The cytotoxic properties of a novel series of thioacridones[13] led us to evaluate these compounds for possible antimalarial activity. Another lead for this study was the acridine derivative, mepacrine (quinacrine) that has been shown to have antimalarial as well as anticancer properties.[23]

The 1-aminothioacridones (**1**–**6**) and their 1-chlorothioacridone precursors (**7**–**12**) exhibited significant antimalarial activity against chloroquine sensitive *P. falciparum* (D10) in vitro, with compound **4** displaying similar activity to chloroquine. Some SAR could be seen in this group of compounds. The most active compound **4** lacks any substituent in the 4-position of the thioacridone nucleus. This compound also exhibited the highest DNA binding affinity of all the new compounds tested.[13] These results suggest that substitution at the 4-position with either a chloro or methyl group inhibits binding and that DNA binding might be an additional contributing factor in the antimalarial activity of these compounds.

In the case of the *des*-*N*-methyl-1-chlorothioacridones (**7**–**9**), it is seen that whereas substitution at the 4-position lowers activity, the antimalarial activity appears to be insensitive to the nature of the substituent since

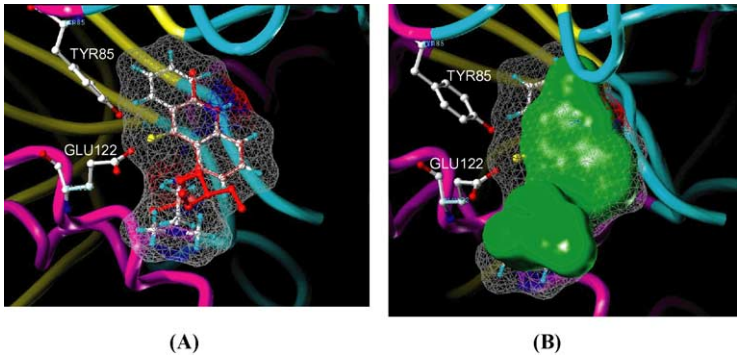



**Figure 9.** (A) MOLCAD surface area calculated for compound **4**. The chloroquine binding area of PfLDH is shown as ribbons and tube rendering in SYBYL. Red and blue areas indicate hydrogen bonding potential. Compound **4** is sterically closely located to glutamine 122 (GLU122) and tyrosine 85 (TYR85) in the chloroquine binding pocket of PfLDH. Chloroquine is shown in red. (B) MOLCAD surface area calculated for chloroquine is shown in green. The chloroquine surface area is not located in as close proximity to TYR85 and GLU122 as found for compound **4** (in A, above) and the MOLCAD area of chloroquine fits comfortably inside the MOLCAD area of compound **4**.

both **8** and **9** had a same $IC_{50}$ of 5 μg/ml. The *N*-methyl derivatives (**10**–**12**) showed an increase in antimalarial activity with substitution at the 4-position. The methyl group offers a higher potency than the chloro group, when compared to compounds **7**–**9**. The presence of a side chain on the thioacridone nucleus does not always result in improved activity, when comparing compound **3** with **12** and **5** with **8**. However, no clear relationship could be found between DNA binding and antimalarial activity, suggesting that different mechanisms of action might be at play with regards to antimalarial activity.

An important concept in identifying potential antimalarial compounds is the selectivity index (chemotherapeutic index). This index is the ratio of the $IC_{50}$ value of a toxin for mammalian cells, to the $IC_{50}$ value of the same toxin for the mammalian parasite.[24] The selectivity index values therefore provide a form of in vitro therapeutic index and are also a means of comparing SAR within this series. Drugs such as chloroquine and pyrimethamine have selectivity indices > 1000 whereas generally cytotoxic compounds produce selectivity indices of <10.[24] The indices for this series of compounds ranged from 0.3 to 6.5, indicating that these compounds might be too cytotoxic as antimalarial agents *in vivo*. Experiments evaluating their in vivo antimalarial activity are needed before a conclusion can be reached as to whether these compounds have very selective antimalarial activity or not.

QSAR analysis led to the development of a five descriptor multiple linear regression equation. This was the minimum number of descriptors required to give a statistically significant $r^2$. The equation shows that the relationship between the physicochemical properties of the compounds tested and their antimalarial activity is a complex one, but no easy generalizations could be made regarding SAR. Expanding the series for further QSAR investigation will probably give rise to a more generalized SAR for these compounds. The positive sign of PSA and negative Log *P* indicate that these compounds might undergo interactions more reliant on bonding, than merely a lipophilic interaction with the molecular target (e.g. DNA intercalation).

The DNA binding data obtained previously[13] on the compounds tested in this study, suggested that these compounds interact with DNA by means of intercalation between the base pairs of the DNA double helix. The antimalarial activity seen in this study might in part be due to this intercalative activity of the compounds.

Another possible mechanism of action is interaction of these compounds with *P. falciparum* lactate dehydrogenase (PfLDH). PfLDH has distinctive kinetic and structural properties that distinguish it from its human LDH homologues, thereby making it a very attractive potential drug target for inhibitors that may give rise to novel antimalarial therapy.[20] Even more encouraging are recent findings that unique structural features in PfLDH are shared among lactate dehydrogenase from other *Plasmodium* species such as *P. berghei*[25] and *P. vivax*.[26] This sequence similarity between PfLDH, PbLDH, and PvLDH (90% residue identity and no insertions or deletions) indicate that the same or, at the very least, a similar approach can be applied in the design of drugs targeting the *Plasmodium* genus. Chloroquine interacts with PfLDH,[27] and weakly binds ($K_i$ = 1.3 mM) to the NADH binding pocket of this malarial enzyme, thereby competing with NADH for binding to the enzyme.[3] We therefore investigated, in a drug design paradigm, how compound **4** would fit into the same chloroquine binding site. The results indicate that compound **4** possibly encounters steric hindrance, as seen by the close proximity of TYR85 and GLU122 to the compound (Fig. 9). This may account for the lower activity of compound **4** ($IC_{50}$ = 0.4), as compared to chloroquine ($IC_{50}$ = 0.01 for the D10 strain). These studies suggest that if compound **4** could be converted from a 3-membered ring system to a 2-membered ring system, its activity could possibly match or exceed that of chloroquine. We are currently investigating this hypothesis. It was interesting to observe from the crystal structure of PfLDH, that the amino side chain of chloroquine extends to the exterior of the binding pocket, which suggests that the side-chain itself does not play a major role in the binding interaction. Because the thioacridones that lacked the basic side chain fared worse than **4** against *P. falciparum*, DNA binding might provide a better explanation for the thioacridones' molecular mechanism than PfLDH inhibition.

In conclusion, the thioacridones evaluated in this study showed significant antimalarial activity against *P. falciparum* (D10) in vitro. Further design is required to improve the selectivity index, as seen from these and DNA binding experiments. The discovery of the antimalarial activity of compound **4** suggests that more studies should be undertaken to further test related thioacridones as potential antimalarial agents.

## Acknowledgements

The antimalarial screening tests were carried out in the Department of Pharmacology, Faculty of Medicine, University of Cape Town, South Africa.

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