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Aminothiol Multidentate Chelators as Antimalarials

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ABSTRACT. The antimalarial effects of two compounds from an aminothiol family of multidentate chelators, ethane-1,2-bis(*N*-1-amino-3-ethylbutyl-3-thiol) (BAT) and *N′,N′,N′*-tris(2-methyl-2-mercaptopropyl)-1,4,7-triazacyclononane (TAT), were studied in *Plasmodium falciparum* cultured in erythrocytes. Both drugs inhibited parasite growth, as was judged from [^{3}H]hypoxanthine incorporation into the nucleic acids of parasites, with 50% inhibitory concentrations (IC_{50} values: 7.6 ± 1.2 μM for BAT and 3.3 ± 0.3 μM for TAT) that exceeded the antimalarial action of desferrioxamine B by 5–10 times. The inhibitory effects of both agents on *P. falciparum* cultures were fully reversed by pre-complexation with iron, suggesting that this action was related mainly to the withholding of iron. Spectrofluorometric studies with the fluorescent iron-sensing probe calcein showed that both compounds withheld iron from calcein at pH 8.2. The trophozoite and schizont stages of parasite development were the stages most susceptible to inhibition. The IC_{50} values of BAT and TAT for mammalian cells, which were estimated by [^{3}H]thymidine incorporation into the nucleic acids of cells, were 10–20 times higher than those required to inhibit plasmodial growth. This indicates that multidentate aminothiols may prove to have a clinical margin of safety that makes them appropriate candidates for future clinical development. BIOCHEM PHARMACOL **54**;4:451–458, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. multidentate aminothiols; iron; iron chelators; malaria; antimalarial drugs

The spread of strains of *Plasmodium falciparum* that are resistant to chloroquine therapy necessitates a search for new therapeutic agents to combat malaria [1]. DFO§, an iron chelator, has clinical antimalarial activity both as a single agent [2] and in combination with quinine [3]. Other iron chelators have antimalarial activity *in vitro* [4, 5] and *in vivo* [6], but have not been tested in humans. Iron chelators may exert their inhibitory effects through two mechanisms: (i) arresting cell growth and replication by withholding iron from critical enzymes [7]; and/or (ii) damaging cells by toxic intracellular metal–chelator complexes [8]. Parasites seem to have a limited capacity to recover growth after temporary iron deprivation caused by an iron chelator [9]. In contrast, mammalian cells are able to tolerate a temporary iron deficit well and to rapidly replenish their iron stores after withdrawal of an iron chelator [10]. This difference appears to provide the biochemical basis for the selective cytotoxicity of iron chelators against *Plasmodia* [11].

The naturally occurring siderophore, DFO, is the only drug approved for iron chelation therapy in most countries. This agent has drawbacks as an antimalarial, including slow permeation across membranes, relatively high IC_{50} values, and fast clearance from the circulation of patients, necessitating continuous parenteral infusion [12]. Other iron-chelating compounds, including some derivatives of DFO, exert greater inhibitory action against malaria parasites both *in vitro* and *in vivo*, than DFO does [6, 13]. These findings suggest that a search for new iron-chelating antimalarial drugs that can outperform DFO is appropriate. Besides a therapeutic potential, the study of the unique properties of new iron-chelating drugs may enhance our knowledge of how malaria parasites handle iron.

Hemoglobin in the food vacuole has been proposed as the source of iron used by the parasite [14]. One way that iron chelators might influence malaria parasites is by accessing the food vacuole and/or parasite cytosol and complexing the metal. Because an iron chelator would have to reach the highly compartmentalized parasite within the red blood cell, the determinants of the efficacy of an iron chelator may include: (i) a hydrophilic/hydrophobic balance; (ii) an affinity for iron [8]; (iii) a selectivity for iron versus other cations [15]; (iv) a selectivity for iron(III) versus iron(II) [10]; and (v) permeability properties of the chelator with respect to the parasite and host membranes [16].

In the present study, we investigated the antimalarial effects of two multidentate aminothiol chelators that were developed initially as possible contrasting agents for radiographic procedures. These compounds form strong complexes with tin [17] and technecium-99m [18, 19] and were predicted to bind iron as well. The inhibitory concentra-

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§ *Abbreviations:* DFO, desferrioxamine B; BAT, ethane-1,2-bis(*N*-1-amino-3-ethylbutyl-3-thiol); and TAT, *N′,N′,N′*-tris(2-methyl-2-mercaptopropyl)-1,4,7-triazacyclononane.

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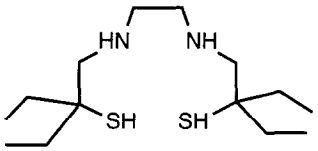
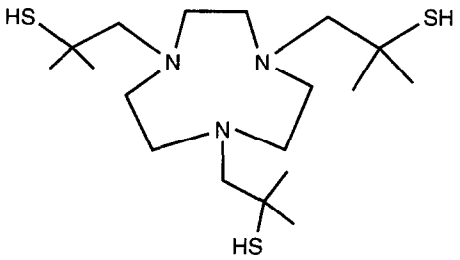


BAT



TAT

FIG. 1. Structure of the multidentate aminothiols used in the study.

tions of the chelators, their speed of action, their persistence of effects, the stages in the parasite cycle of development most sensitive to drug action, and the selective cytotoxicity of the chelators for parasites versus mammalian cells are described.

MATERIALS AND METHODS

Chemicals

Calcein was obtained from Molecular Probes (Eugene, OR). DFO in the form of the mesylate salt was purchased from Ciba–Geigy (Basel, Switzerland). RPMI-1640 medium and ferrous ammonium sulfate were obtained from the Sigma Chemical Co. (St. Louis, MO). [^{3}H]Hypoxanthine was obtained from the Amersham Corp. (Arlington Heights, IL). All other reagents were obtained from the Fisher Scientific Co. (Norcross, GA).

Iron Chelators Used in the Study

A tetradentate aminothiol ligand, BAT, and a hexadentate aminothiol ligand, TAT (Fig. 1), were synthesized and characterized as described elsewhere [17, 18]. These two chelators differ in the number of coordination sites that are available for binding of a transition metal such as iron (four in BAT and six in TAT). They also differ in lipophilicity, with TAT being more lipophilic, based upon molecular masses of two ligands and their HPLC retention times (John *et al.*, unpublished data) (Fig. 1). The more lipophilic the compound, the greater its ability to cross membranes [20]. The rationale for the selection of BAT and TAT was their predicted ability to bind iron; both compounds had been synthesized by one of the authors (C.J.) for potential use as radiographic imaging agents. These chelators probably bind metals other than iron, but this capability has not been investigated except for tin [17] and technecium-99m [18, 19]. The potential toxicity of these agents to humans is not known. Because BAT has only four coordination sites for iron rather than six, the possibility cannot be excluded that iron bound to this chelator may participate in the Fenton reaction. In a model system developed to study the generation of hydroxyl radicals in the presence of D-deoxyribose and Fe(II) [21], we found that in the concentration range of 10–100 μmol/L both BAT and TAT prevented the formation of hydroxyl radicals (data not shown).

Fluorescence Measurements

Calcein is a fluorescent compound, and the fluorescence is quenched when it binds iron [15]. Measurements of the fluorescence of calcein were performed on an LS50B Perkin–Elmer luminescence spectrometer (Perkin–Elmer Ltd., Beaconsfield, England). The fluorescence of calcein was recorded at 496 nm excitation wavelength and at 517 nm emission wavelength in a 1-cm-path plastic cuvette containing 1 mL of double-distilled and deionized water and adjusted to a pH of 8.2 with 0.5 N KOH. To assess the iron-binding ability of the iron chelators, the baseline fluorescence signal of 400 nmol/L of calcein was recorded after stabilization, the fluorescence was then quenched with 50 μmol/L of freshly prepared ferrous ammonium sulfate, and the recovery pattern of fluorescence elicited by the iron chelators was recorded by adding 300 μmol/L of either drug. All compounds were introduced into 1 mL of water in volumes of 5 μL from stock solutions.

Parasite Cultures

P. falciparum (strain 3D7; provided by D. Keister and O. Muratova of the National Institutes of Health, Bethesda, MD) was grown in culture flasks containing RPMI-1640 supplemented with 25 mM HEPES, 23 mM sodium bicarbonate, 10 mM glucose, 10% (v/v) heat-inactivated human plasma (O^+ or A^+), and washed human erythrocytes (A^+) at 2 to 2.5% hematocrit. The growth medium was replaced daily, and the cultures were gassed with a mixture of 90% N_2, 5% CO_2 and 5% O_2 [22]. Synchronization to the ring stage was achieved by the lysis of cells containing mature parasites with iso-osmotic sorbitol or alanine [23]. The morphological characteristics of the parasites and the degree of parasitemia were assessed by microscopic inspection of thin blood smears stained by Giemsa. For each smear, the number of rings, trophozoites, and schizont stages were counted per approximately 1000 red cells.

Effect of Drugs on Parasite Growth

Ring-stage synchronized cultures of parasites were adjusted to 2% parasitemia and 2% hematocrit, and 600-μL aliquots

were distributed into 24-well microtiter plates. The drugs used in the study were added either in a free form or in a form that had been complexed to iron. BAT was complexed with iron by adding ferrous ammonium sulfate in excess of the chelator in ratios ranging from 1:1 to 10:1 at pH 8.5. TAT was complexed with iron in a methanolic solution of ferric chloride with iron to chelator ratios ranging from 1:1 to 1:3 at pH 8.5. In water solutions, both BAT and TAT form stable complexes with iron(II) and iron(III) at a pH higher than 7.5, whereas no complexation occurs at a pH below 6.0. The higher than stoichiometric ratios of iron to chelator were used to compensate for rapidly precipitating iron salts at this pH, presumably iron hydroxide. The chelators were added at various concentrations in duplicate wells, and the plates were transferred to a candle jar and incubated at 37° for 24 hr, at which time [^{3}H]hypoxanthine was added to a final activity of 5 μCi/mL. After an additional 24-hr incubation period, the cells were transferred in triplicate to 96-well plates and harvested with a cell harvester (Cambridge Technology Inc., Watertown, MA). The filters were dried in the oven, transferred to vials containing the scintillation fluid Fluosafe (Fisher Scientific Co.), and counted on a Beckman LS5000CE scintillation counter (Beckman Instruments, Inc., Fullerton, CA). The viability of the parasites was assessed in terms of relative activity, calculated as the ratio of the incorporation of [^{3}H]hypoxanthine into parasitized red cells at a given concentration of the potential inhibitor to the incorporation in control red cells. Data are given as means ± SD.

Reversibility of the Effects of Drugs on Parasite Growth

The cells were prepared and processed as above, except that after the first 24 hr of incubation the drug was washed out of one-half of the samples with fresh RPMI-1640 medium by transferring into 15-mL sterile tubes and centrifugation. Thereafter, the samples were transferred into fresh 24-well plates and the wells were supplemented with RPMI-1640 medium lacking the drug. Then [^{3}H]hypoxanthine with a final activity of 5 μCi/mL was added to all of the samples, and the incorporation of radiolabel into parasitized cells was assessed at 48 hr.

Simultaneous Addition of [^{3}H]Hypoxanthine and Chelators to Synchronized Parasite Cultures

The simultaneous addition of iron chelators and [^{3}H]hypoxanthine may provide an indirect measure of a speed with which iron chelators cross membranes to access the parasite compartment and elicit their inhibitory effects on *P. falciparum* cultures [13]. Ring-stage synchronized parasites were distributed in duplicate into 24-well plates. The chelators and [^{3}H]hypoxanthine (5 μCi/mL) were added at the same time, and the plates were transferred to a candle jar and incubated at 37°. Duplicate aliquots were withdrawn at different intervals of time and frozen to −70°. They were thawed before processing and processed as described above. When the effect of drugs on [^{3}H]hypoxanthine incorporation by parasites was studied at a lower temperature, the procedure was the same except that the samples were incubated at 17° instead of 37°.

Mammalian Cell Cultures

Two lines of mammalian cells were used in this study: the human hepatoma cell line HepG2, and the human T-lymphocyte cell line SUP-1 (American Type Culture Collection, Rockville, MD). They were maintained in Falcon culture flasks (Becton Dickinson Labware, Franklin Lakes, NJ) and grown at 37° in a CO_2 incubator (5% CO_2) in 24-well plates. The human T-lymphocyte cell line, SUP-1, was grown in suspension in RPMI-1640 medium containing 10% (v/v) fetal bovine serum, 1% (v/v) glutamine and buffered with $NaHCO_3$. The human hepatoma cells, HepG2, were grown as monolayers in Earl's MEM (EMEM) medium containing 10% (v/v) fetal bovine serum, 1% (v/v) glutamine, 1% (v/v) non-essential amino acids, and $NaHCO_3$ buffer.

Effect of Drugs on the Incorporation of [^{3}H]Thymidine into Nucleic Acids of Cultured Mammalian Cells

Non-confluent HepG2 cells and exponentially growing SUP-1 cells in suspension were exposed to iron chelators for 24 hr and assayed in duplicate for incorporation of [^{3}H]thymidine into nucleic acids over 5 hr. Radiolabeled HepG2 cells were washed with isotonic buffer and fixed with ice-cold 5% (w/v) trichloroacetic acid. The precipitates that formed as a result of the addition of trichloroacetic acid were solubilized with 0.1 N KOH and counted for radioactivity. SUP-1 cells were transferred from the 24-well dishes to centrifuge tubes following 5 hr of labeling with tritiated thymidine and were washed with an isotonic phosphate-saline buffer (pH 7.4) twice by centrifugation (1000 *g* for 5 min); then the radioactivity was counted. Total cell protein was estimated on 96-well microplates with the bicinchoninic acid (BCA) method (Sigma); the plates were read at 490 nm wavelength in a V_{max} microtiter plate reader (Molecular Devices, Palo Alto, CA). Radioactivity recovered from the samples was normalized to the amount of protein, and the results were expressed as a ratio of the incorporation of [^{3}H]thymidine at a given concentration of the chelator to the incorporation in control cells.

Statistical Analysis of the Data

The concentrations of drugs that produced 50% inhibition of parasite growth (IC_{50} values) were calculated from concentration–response curves, using the best sigmoidal fit obtained with the program Prizm (GraphPad Software Corp., San Diego, CA).

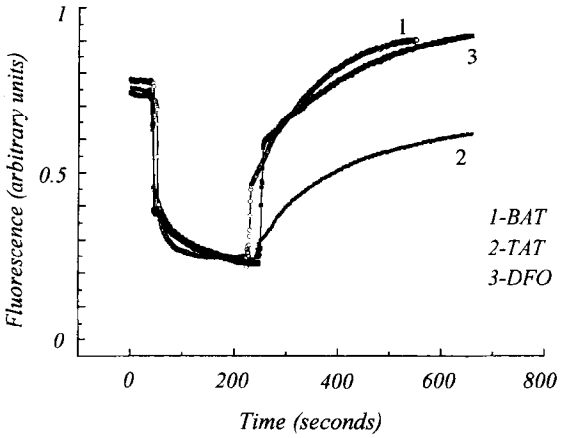


FIG. 2. Quenching of calcein fluorescence by ferrous ammonium citrate [Fe(II)] and its recovery with BAT, TAT, and DFO. Spectra: 496 nm excitation, 517 nm emission wavelengths; concentrations: 400 nM calcein, 50 μM ferrous ammonium sulfate, 300 μM BAT, TAT, or DFO; pH 8.2.

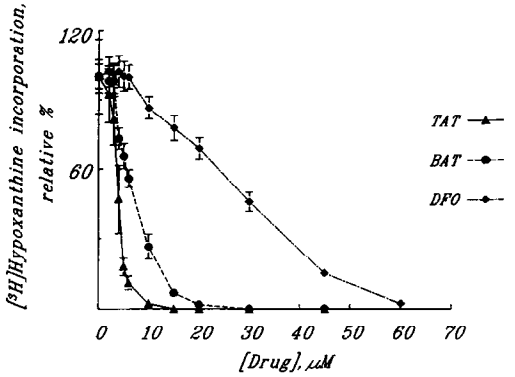


FIG. 3. Inhibition of parasite growth by BAT, TAT, and DFO. Ring-stage synchronized cultures were supplemented with the chelators at different concentrations. After 24 hr of incubation with drugs, [^{3}H]hypoxanthine was added for another 24 hr for assessment of nucleic acid synthesis. Each point represents inhibition relative to control. The results are means ± SD of six parallel samples of one representative experiment out of a series of 6 similar experiments performed. Incorporation of [^{3}H]hypoxanthine in control was 36.9 ± 2.3 × 10^3 dpm/sample.

RESULTS

Assessment of the Iron-Binding Properties of Iron Chelators

The iron-withholding capacity of the two chelators was estimated with the iron-sensing probe calcein (Fig. 2). DFO, which has demonstrated ability to withhold iron in the calcein system, was used as a reference iron chelator [15]. Both BAT and TAT displaced iron from its complex with calcein. BAT and DFO displayed a similar pattern of recovery of calcein fluorescence, whereas TAT displayed a shorter initial fast phase of recovery of calcein fluorescence followed by a slow phase that was similar to DFO or BAT (Fig. 2). On a larger time scale, the recovery of calcein fluorescence by TAT was complete (data not shown).

Effect of Chelators on Parasite Growth

Both BAT and TAT inhibited parasite growth (Fig. 3). The IC_{50} values, obtained from the concentration–response curves of one representative experiment out of a series of six, were 3.29 ± 0.33 μM for TAT and 7.64 ± 1.19 μM for BAT. The variations in calculated IC_{50} from experiment to experiment did not exceed 20%. Both drugs displayed greater inhibition than DFO.

Reversibility of Drug Effects on Parasite Growth

As shown in Fig. 4, TAT exerted a greater inhibitory effect than BAT, and the effect was more persistent after the drugs were washed out of the cultures. The removal of the chelator after 24 hr resulted in a slight increase in the IC_{50} values from 7.64 ± 1.19 to 9.69 ± 2.25 μM for BAT and from 3.29 ± 0.33 to 4.22 ± 0.16 μM for TAT.

Effect of Pre-complexation with Iron on the Inhibitory Potency of the Drugs

Figure 5 (panels A and B) shows that the inhibitory effects of BAT and TAT on parasite growth were fully reversible by pre-complexation with iron.

Time of Onset of the Inhibitory Effects of the Iron Chelators

When [^{3}H]hypoxanthine and the chelators were added simultaneously to the ring-stage synchronized cultures of *P. falciparum*, the uptake of the radioactive label in drug-treated cultures became significantly different from controls sometime between 6 and 18 hr of exposure (Fig. 6). The hexadentate and relatively more lipophilic TAT had a greater effect than the tetradentate BAT in equimolar concentrations. When the cultures were treated for the first 24 hr with a 15 μM concentration of either BAT or TAT followed by the addition of [^{3}H]hypoxanthine for the second 24 hr, only a residual incorporation of the label occurred (Fig. 3).

Effects of TAT and BAT on Parasite Growth at Lower Temperature

Incubation of parasite cultures at lower than optimal temperatures (15–17°) reversibly arrests parasite development at the ring stage [24]. To determine whether longer incubation times of ring-stage parasites with iron chelators could result in a higher degree of inhibition of [^{3}H]hypoxanthine incorporation into the nucleic acids of the parasites, the cultures were incubated at 17° in the presence of 15 μM TAT or BAT (Fig. 7). With the extended exposure of ring-stage parasites to both drugs, an antiparasitic effect was not observed at 24 hr but did appear at 30 hr. At 24 hr

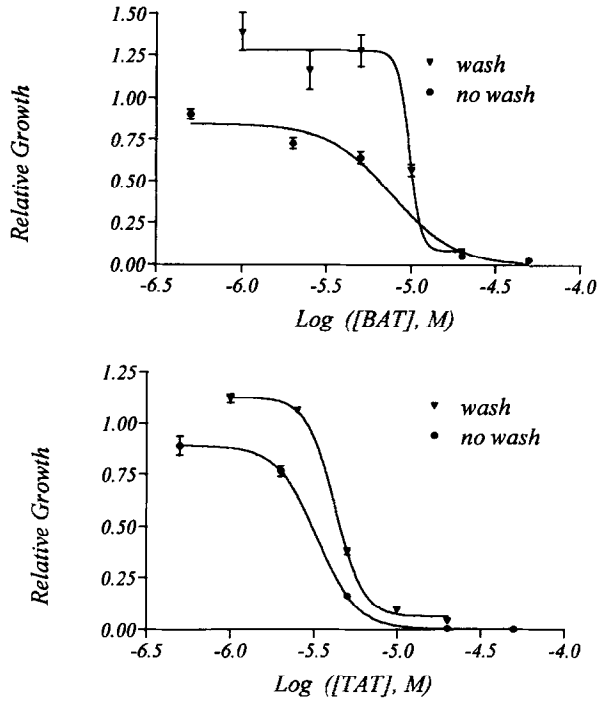


FIG. 4. (Top panel) Concentration–response curves of inhibition of parasite growth by BAT. Ring-stage synchronized cultures were supplemented with the chelator in different concentrations. After 24 hr of incubation, one-half of the samples were washed free of the chelator, and tritiated hypoxanthine was added for another 24 hr for assessment of nucleic acid synthesis. Each point represents inhibition relative to control. The lines are the best sigmoidal fits obtained with a Prizm program. The computed IC_{50} values were (in μmol/L): 7.64 ± 1.19 for washed cultures and 9.69 ± 2.25 for non-washed cultures. X-axis: log of concentration of the chelator in mol/L; Y-axis: relative growth. (Bottom panel) Concentration–response curves of inhibition of parasite growth by TAT. The calculated IC_{50} values were (in μmol/L): 3.29 ± 0.33 for washed cultures and 4.22 ± 0.16 for non-washed cultures. The results are means ± SD of six parallel samples of one representative experiment out of a series of 4 similar experiments performed. Incorporation of [^{3}H]hypoxanthine in control was 27.1 ± 1.8 × 10^3 dpm/sample.

the parasites were still in the ring stage of development as indicated by light microscopy, but at 30 hr the parasites had advanced to the early trophozoite stage.

Effects of TAT and BAT on the Stage Distribution of P. Falciparum

The stage distribution of parasite development was estimated microscopically (Fig. 8) in parallel with the uptake of [^{3}H]hypoxanthine by the ring-stage synchronized parasite cultures shown in Fig. 6. At 48 hr, the number of schizonts and the number of rings in the brood of the second generation in the control samples exceeded these numbers in the specimens treated with 7.5 and 15 μM BAT or TAT. Approximately 60% of trophozoites in the cultures treated with 15 μM BAT and 90% in the cultures exposed to 15 μM TAT looked abnormal morphologically at 48 hr, being more intensely stained with Giemsa and more dense.

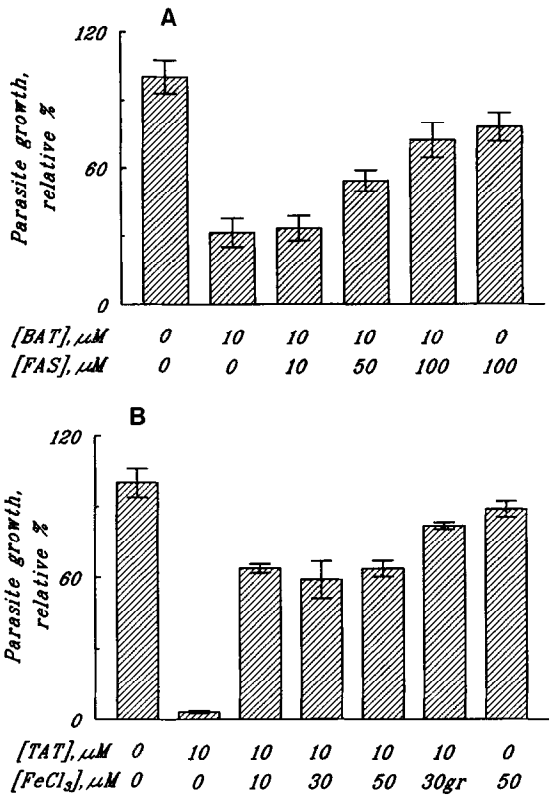


FIG. 5. Effect of pre-complexation with iron on parasite growth. BAT was preincubated with ferrous ammonium sulfate (Fig. 5A) and TAT with ferric chloride (Fig. 5B). In panel B, 30 gr = 30 μmol/L of ferric chloride added gradually in three portions of 10 μmol/L each to 10 μmol/L of TAT. The results are means ± SD of six parallel samples of one representative experiment out of a series of 3 similar experiments performed. Incorporation of [^{3}H]hypoxanthine in control was 24.8 ± 2.3 × 10^3 dpm/sample.

Effect of Chelators on Mammalian Cells

Both BAT and TAT exerted similar inhibitory effects on the proliferation of human hepatoma cells (grown in

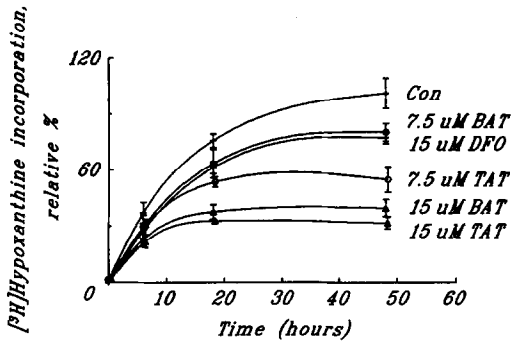


FIG. 6. Effects of BAT, TAT, and DFO on [^{3}H]hypoxanthine uptake by *P. falciparum*. Ring-stage synchronized cultures of parasites were supplemented simultaneously with 7.5 and 15 μM concentrations of either chelator and incorporation of radiolabel was followed over 48 hr. The results are means ± SD of six parallel samples of one representative experiment out of a series of 3 similar experiments performed. Incorporation of [^{3}H]hypoxanthine in control was 36.8 ± 2.7 × 10^3 dpm/sample.

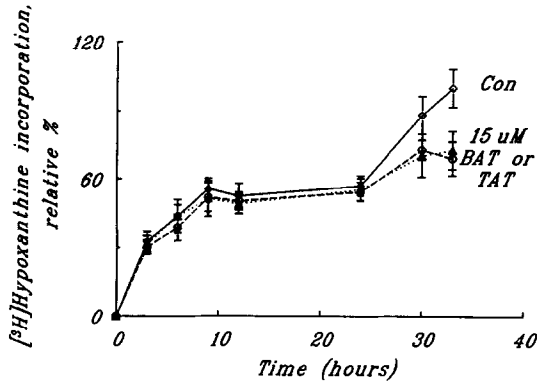


FIG. 7. Effects of BAT and TAT on [^{3}H]hypoxanthine uptake by P. *falciparum* at lower temperature (17°) over 33 hr. The results are means ± SD of six parallel samples of one representative experiment out of a series of 3 similar experiments performed. Incorporation of [^{3}H]hypoxanthine in control was 6.1 ± 0.5 × 10^3 dpm/sample.

monolayers) and human T-lymphocytes (grown in suspension). The IC_{50} values were higher than 30 and lower than 100 μM, which were 10–20 times higher than the IC_{50} values against *P. falciparum* (Fig. 9).

DISCUSSION

We found that two compounds from a family of multidentate aminothiol chelators inhibited the growth of *P. falciparum* cultured in erythrocytes and that they outperformed the inhibitory action of DFO by more than 5-fold (Fig. 3). Both BAT and TAT appeared to affect the trophozoite and schizont stages of parasite development (Figs. 6–8), and both drugs displayed selective cytotoxicity to malaria parasites versus mammalian cells (Fig. 9). The inhibitory effects of the aminothiols, BAT and TAT, like the antimalarial effects of structurally unrelated compounds such as hydroxamates and ferrichromes [4] or hydrazones [5], seem to be related mainly to their iron-withholding action, because pre-complexation with iron fully reversed the

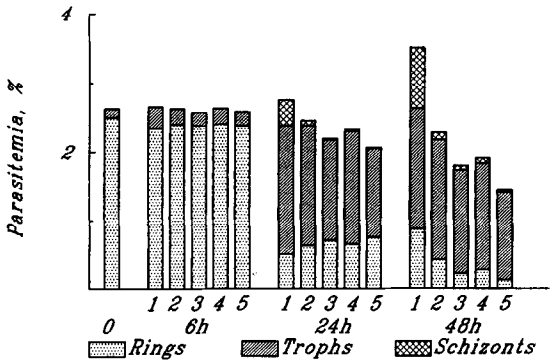


FIG. 8. Effects of BAT and TAT on parasitemia and stage distribution of ring-synchronized cultures of P. *falciparum* as assessed by light microscopy of thin smears. Key: (1) control; (2) 7.5 μM BAT; (3) 15 μM BAT; (4) 7.5 μM TAT; and (5) 15 μM TAT. Data are from one experiment of a series of 3.

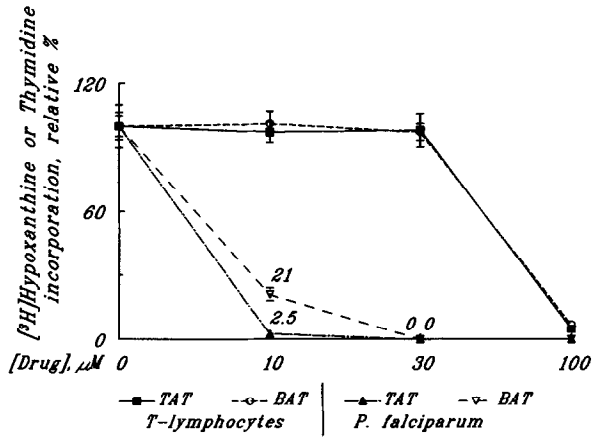


FIG. 9. Effects of BAT and TAT on malaria parasites versus mammalian cells, SUP-1 T-lymphocytes. Different concentrations of the drugs shown were applied to ring-stage synchronized cultures of P. *falciparum* and exponentially grown SUP-1 cells, and their viability was assessed. Malaria cultures were exposed to iron chelators for 24 hr and assayed for incorporation of [^{3}H]hypoxanthine into nucleic acids for another 24 hr. SUP-1 cells in suspension were exposed to iron chelators for 24 hr and assayed for incorporation of [^{3}H]thymidine into nucleic acids over 5 hr. The results are means ± SD of six parallel samples of one representative experiment out of a series of 3 similar experiments performed. Incorporation in control was 25.7 ± 2.1 × 10^3 dpm/sample for [^{3}H]hypoxanthine and 194.4 ± 22.6 × 10^3 dpm/sample for [^{3}H]thymidine. HepG2 cells displayed a quantitatively similar response to T-lymphocytes.

anti-parasitic effect (Fig. 5). The possibility that this effect is due to the generation of toxic intracellular iron–chelator complexes [8], or to the binding of metals other than iron, cannot be excluded.

Based on the partition in *n*-octanol and water, both of the aminothiols we studied were more lipophilic than DFO (Loyevsky *et al.*, unpublished observations). According to the molecular masses and retention times in a C-18 reverse-phase HPLC column in which the retention time of molecules depends on their polarity, TAT was more lipophilic than BAT (John *et al.*, unpublished observations). Also, TAT has six coordination sites for iron versus four coordination sites on the BAT molecule [17, 19]. These features predict that TAT will enter cells better and bind iron more tightly than BAT. In keeping with the relative lipophilic properties of the molecules and the number of binding sites, TAT was ten times more efficient than DFO and twice as efficient as BAT in antimalarial effect (Figs. 3 and 4). Furthermore, the inhibitory effect of TAT seemed to be more persistent than that of BAT (Fig. 4). These results are consistent with the perspective that compounds having lipid/water partition coefficients favoring better penetration of cells and having higher binding constants for iron will exert greater anti-parasitic effects [8, 11]. The fact that TAT displayed higher inhibitory potency than BAT in malaria cultures (Figs. 3 and 6–9) but seemingly had lower efficiency in displacing iron from calcein (Fig. 2) may be related to the relatively poor solubility of TAT in the conditions of the reaction with calcein.

Both compounds resembled DFO in time of onset of parasite growth inhibition [13], requiring more than 6 hr to elicit an inhibitory effect on the incorporation of [^{3}H]hypoxanthine into the nucleic acids of parasites (Figs. 6 and 7). These findings suggest either that ring-infected red cells are poorly permeable to BAT and TAT (Fig. 7) or that the ring-stage parasites are not sensitive to the action of the iron chelator [11].

Neither BAT nor TAT inhibited progression from the ring to the trophozoite stage (Figs. 6–8), but the apparent reduction in the number of rings of a second generation at 48 hr (Fig. 8) suggests an overall decline in the number of trophozoites that progressed to the schizont stage and underwent schizogony. Furthermore, trophozoites exposed to the aminothiols were morphologically abnormal by light microscopy. Since the effect of the chelators on merozoite invasion was not studied specifically, we could not rule out the possibility that these agents inhibited merozoite invasion. Thus, both drugs seemed to target the trophozoite and schizont stages that are marked by DNA replication and cellular division. The iron-dependent enzyme ribonucleotide reductase may be especially important during these stages [16].

BAT and TAT displayed selectivity in inhibiting the growth of malaria parasites and mammalian cells, inhibiting the growth of *Plasmodia* at substantially lower concentrations (Fig. 9). This selectivity may reflect differences in the handling of iron between malaria parasites and mammalian cells [10, 25]. These aminothiols seem to have an appropriate profile for possible clinical development. Our plans for future evaluation of these compounds include: (i) determination of the affinity constants for iron versus the other biologically essential metals, such as zinc, calcium, and magnesium; (ii) performing toxicity studies in animals; and (iii) examining antimalarial effect in a rodent model.

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