Inhibition of *Trypanosoma cruzi* Trypanothione Reductase by Acridines: Kinetic Studies and Structure−Activity Relationships

Susanne Bonse,[†] Christiane Santelli-Rouvier,[‡] Jacques Barbe,[‡] and R. Luise Krauth-Siegel*,[†]

Biochemie-Zentrum Heidelberg, Heidelberg University, Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany, and GERCTOP-UPRESA CNRS 6009, Faculté de Pharmacie, Université de la Mediterrannée, 27 Bd Jean-Moulin, F-13385 Marseille, France

Received July 27, 1999

Series of 9-amino and 9-thioacridines have been synthesized and studied as inhibitors of trypanothione reductase (TR) from *Trypanosoma cruzi*, the causative agent of Chagas' disease. The compounds are structural analogues of the acridine drug mepacrine (quinacrine), which is a competitive inhibitor of the parasite enzyme, but not of human glutathione reductase, the closest related host enzyme. The 9-aminoacridines yielded apparent K_i values for competitive inhibition between 5 and 43 μM. The most effective inhibitors were those with the methoxy and chlorine substituents of mepacrine and NH_2 or $NHCH(CH_3)(CH_2)_4N(Et)_2$ at C9. Detailed kinetic analyses revealed that in the case of 9-aminoacridines more than one inhibitor molecule can bind to the enzyme. In contrast, the 9-thioacridine derivatives inhibit TR with mixed-type kinetics. The kinetic data are discussed in light of the three-dimensional structure of the TR−mepacrine complex. The conclusion that structurally very similar acridine compounds can give rise to completely different inhibition patterns renders modelling studies and quantitative structure−activity relationships difficult.

Introduction

Trypanothione reductase is a flavoenzyme which has been found so far exclusively in trypanosomatid parasites[1] and recently—together with glutathione reductase—in *Euglena gracilis*.[2] Trypanosomes and leishmania are the causative agents of African sleeping sickness (*Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*), Chagas' disease (*Trypanosoma cruzi*), Nagana cattle disease (*Trypanosoma congolense*, *Trypanosoma brucei brucei*), Kala-azar (*Leishmania donovani*), and oriental sore (*Leishmania tropica*). All these parasitic protozoa have in common that they lack the nearly ubiquitous enzyme glutathione reductase (GR). Their main low molecular mass thiols are conjugates between glutathione and spermidine, namely monoglutathionyl spermidine and bis(glutathionyl)spermidine [trypanothione, $T(SH)_2$].[3] These compounds are kept reduced by the flavoenzyme trypanothione reductase (TR):

$$TS_2 + NADPH + H^+ \rightarrow T(SH)_2 + NADP^+$$

Mechanistically and structurally TR and human GR are closely related. The most important difference between parasite and host enzyme is their mutual exclusive substrate specificity which is based on the respective charge distributions of their active sites. The binding site for trypanothione disulfide in TR is hydrophobic and negatively charged, but the GSSG binding site in GR has an overall positive charge.[4−7] This finding has led to the elucidation of charge being a major discriminating factor for specific inhibitors of TR versus GR which should allow the design of inhibitors specific for the parasite enzyme.[8]

The known sensitivity of trypanosomatids toward oxidative stress and the absence of the enzyme from the mammalian host render TR an attractive target molecule for the development of new antiparasitic drugs. Several genetic approaches have unambiguously shown that the enzyme is essential for growth and virulence of the parasites.[9−11]

A prerequisite for a rational drug development is the knowledge of the three-dimensional structure of the target protein. The structures of TR[4−6] and of complexes with its substrates[6,12,13] as well as with the reversible inhibitor mepacrine have been elucidated.[14] A variety of lead compounds were revealed within the past few years (for reviews, see refs 15−17). The large group of reversible inhibitors include acridines, phenothiazines, benzoazepines, and pyridoquinolines.[18−22] None of these tricyclic derivatives interfere with human glutathione reductase, the closest related host enzyme.

Phenothiazines and acridines show trypanocidal activities.[21,23,24] Acridine derivatives are also well-known antibacterial and antitumor drugs. In addition, they have been considered for the treatment of several protozoan infections.[24] 9-Amino- and 9-thioacridines were highly active against African trypanosomes in culture but many of them were also toxic to human adenoma carcinoma cells.[24] In a serial screening of compounds licensed for human treatment, mepacrine was one of the most active drugs against *T. cruzi* in vitro.[23]

So far the TR−mepacrine structure is the only crystal structure of trypanothione reductase in complex with an inhibitor. Mepacrine is fixed in the active site close to the hydrophobic wall formed by Trp21 and Met113.

* To whom correspondence should be addressed. Phone: +49 6221 54 41 87. Fax: +49 6221 54 55 86. E-mail: krauth-siegel@urz.uni-heidelberg.de.
[†] Biochemie-Zentrum Heidelberg, Heidelberg University.
[‡] Faculté de Pharmacie, Université de la Mediterrannée, Marseille.

10.1021/jm990386s CCC: $18.00
Published on Web 12/04/1999

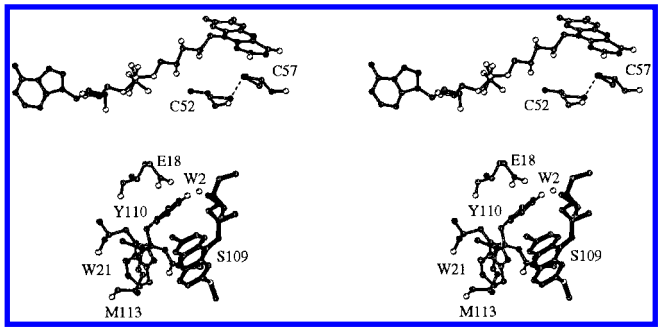
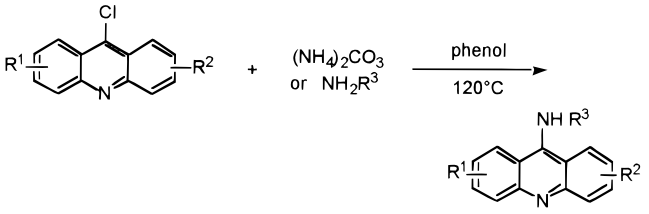


Figure 1. Stereoview of the active site of *T. cruzi* TR with bound mepacrine (after ref 14). The isoalloxazine ring of FAD forms the center of the active site. The dashed line represents the redox active disulfide. Mepacrine (bold lines) binds with the acridine ring close to the hydrophobic region formed by Trp21 and Met113. Specific pairwise interactions between functional groups of the drug and side chains of the protein include the ring nitrogen and Met113, the chlorine atom and Trp21, and the methoxy group and Ser109. The alkylamino chain is fixed to Glu18 via a solvent molecule (W2).

Scheme 1[a]



1-**7**

[a] **1** R^1 = 3-Cl, R^2 = H, R^3 = H; **2** R^1 = 6-Cl, R^2 = 2-OCH_3, R^3 = H; **3** R^1 = H, R^2 = 2-OCH_3, R^3 = $CH_3CH(CH_2)_3N(C_2H_5)_2$; **4** R^1 = 3-Cl, R^2 = H, R^3 = $CH_3CH(CH_2)_3N(C_2H_5)_2$; **5** R^1 = 6-Cl, R^2= 2-OCH_3, R^3 = $CH_3CH(CH_2)_3N(C_2H_5)_2$; **6** R^1 = 3-Cl, R^2 = H, R^3 = $CH_3CH(CH_2)_4N(C_2H_5)_2$; **7** R^1 = 6-Cl, R^2 = 2-OCH_3, R^3 = $CH_3CH(CH_2)_4N(C_2H_5)_2$.

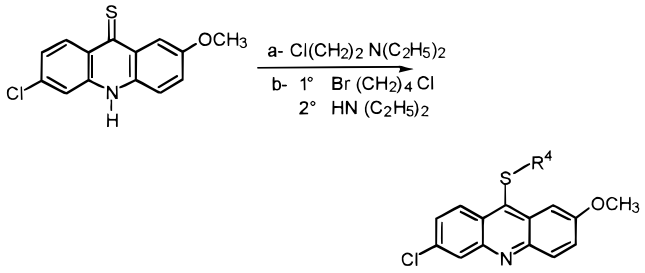
The acridine ring and its substituents are in direct contact with side chains of the protein. The interactions include the ring nitrogen and Met113, the chlorine atom and Trp21, and the methoxy group and Ser109. The alkylamino chain of mepacrine points into the inner region of the active site and may undergo a water-mediated hydrogen bond to Glu18.[14] The residues in contact with the bound mepacrine are four out of five residues in the active site which are not conserved when comparing *T. cruzi* TR and human GR. To get a deeper insight in the relative contribution of each substituent upon binding to the enzyme, several analogues of mepacrine have been synthesized and studied as inhibitors of *T. cruzi* TR. The kinetics will be discussed in light of the three-dimensional structure of the TR−mepacrine complex (Figure 1).

Results

Synthesis of Acridine Derivatives. The 9-aminoacridines **1**−**7** were prepared by heating the corresponding 9-chloroacridines (Scheme 1) in phenol (a) with ammonium carbonate (**1**, **2**);[25] (b) with 5-diethylamino-2-aminopentane (**3**, **4**, **5**);[26] and (c) with 6-diethylamino-2-aminohexane (**6**, **7**). These diamines were obtained by reductive amination with $NaBH_3CN$[27] of the corresponding diethylaminoalkane-2-one.[28]

The 9-thioacridine derivatives were obtained by alkylation of the corresponding 9-thioacridinone either with

Scheme 2[a]



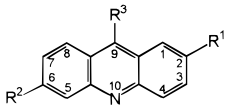
[a] a: **8** R^4 = $(CH_2)_2N(C_2H_5)_2$. b: **9** R^4 = $(CH_2)_4N(C_2H_5)_2$.

2-diethylaminoethyl chloride (**8**)[29] or with 1,4-bromochlorobutane followed by amination with diethylamine (Scheme 2) (**9**).

9-Aminoacridines Are Competitive Inhibitors of TR with More Than One Binding Site. Acridine derivatives with an amino substituent at position C9 of the aromatic ring inhibited the parasite enzyme competitively (Table 1). The most effective compounds were **2** and **7** which have in common the chlorine and the methoxy substituents of mepacrine but differ in the nature of their side chain at C9. The weakest inhibitor was tacrine which represents 9-aminoacridine with one of the outer rings saturated. The inhibitor constants given in Table 1 were derived from Lineweaver Burk plots conducted in the absence of inhibitor and in the presence of at least two different inhibitor concentrations. The double reciprocal plots were linear and intersected in a common point on the $1/v$ axis in accordance with a competitive type of inhibition. A striking observation was that the calculated inhibitor constants were not independent of the inhibitor concentration in the assay. In each case the value obtained at the higher inhibitor concentration was (slightly) lower than that calculated at the lower [I]. The inhibitor constant determined from the slope of the reciprocal plot is actually $K_{i,slope}$, which is not the K_i for the dissociation of an EI complex but rather a more complex function of K_i varying with [I].[30] The $K_{i,apparent}$ values given in Table 1 are the mean of these $K_{i,slope}$ values at the respective inhibitor concentrations (see below). Subsequent variation of the concentration of 9-aminoacridine as well as of compounds **1** and **5**, respectively, at fixed concentrations of trypanothione disulfide resulted in Dixon plots which curved upward parabolically (Figure 2a). These forms of inhibition occur when more than one molecule of inhibitor can bind to the same form of the enzyme.[30,31] It should be emphasized that mechanisms in which more than one inhibitor molecule can bind to different enzyme intermediates do not give rise to curved inhibitor plots.[31]

Such two- or more than two-site competitive inhibition is not distinguishable from one-site pure competitive or partial competitive inhibition by the usual v versus S or $1/v$ versus $1/S$ plots. The best indication of the nature of inhibition is obtained from the replots of the slopes of the reciprocal plot versus the corresponding [I].[30] For two-site competitive inhibition, this replot yields a parabola. One-site pure competitive and one-site partial competitive systems would give linear and hyperbolic replots, respectively. Figure 2b shows the replot of $slope_{1/S}$ versus [I] of the Lineweaver Burk plot (not shown) for compound **1**. In the case of two inhibitor

Table 1. Inhibition of *T. Cruzi* TR by Acridine Derivatives[a]



9-Aminoacridines

compd	substituent R1	substituent R2	substituent R3	$K_{i,\ apparent}$ (μM)[b] (competitive)
9-aminoacridine			NH_2	38 ± 10 [100; 200]
1		Cl	NH_2	43 ± 5 [29; 58]
2	OCH_3	Cl	NH_2	7 ± 2 [26; 58]
3	OCH_3		$NHCH(CH_3)(CH_2)_3N(Et)_2$	27 ± 2 [56; 92]
4		Cl	$NHCH(CH_3)(CH_2)_3N(Et)_2$	10 ± 1 [23; 60]
5 (mepacrine)	OCH_3	Cl	$NHCH(CH_3)(CH_2)_3N(Et)_2$	19 ± 5 [20; 46]
6		Cl	$NHCH(CH_3)(CH_2)_4N(Et)_2$	11 ± 1 [50; 100]
7	OCH_3	Cl	$NHCH(CH_3)(CH_2)_4N(Et)_2$	5.5 ± 1 [26; 60]
tacrine[c]			NH_2	140 ± 18 [100; 200]
6-chlorotacrine[d]		Cl	NH_2	80 ± 20 [50; 100]

9-Thioacridines[e]

compd	substituent R1	substituent R2	substituent R3	K_i (μM) (mixed-type)	K_i' (μM)
8[29]	OCH_3	Cl	$S(CH_2)_2N(Et)_2$	37 ± 1	83 ± 2 [46; 91]
9	OCH_3	Cl	$S(CH_2)_4N(Et)_2$	21 ± 3	67 ± 3 [21; 53]

[a] The kinetics were measured at 25 °C in TR assay buffer (40 mM Hepes, 1 mM EDTA, pH 7.5) at 100 μM NADPH. The TS_2 concentration was varied in the presence of two different inhibitor concentrations as well as in the absence of inhibitor. Each data point has been measured at least two times and differed by ≤5%. [b] The inhibitor constants for competitive inhibition were derived from Lineweaver Burk plots. They do not represent true dissociation constants for the EI complex since they were dependent on the respective inhibitor concentration. In any case, the lower value resulted at the higher [I]. The $K_{i,apparent}$ values given in the table are the mean of the kinetic constants (actually $K_{i,slope}$ values) calculated at the different inhibitor concentrations (given in brackets). For a detailed discussion, see text. [c] 9-Amino-1,2,3,4-tetrahydroacridine. [d] 9-Amino-6-chloro-1,2,3,4-tetrahydroacridine. [e] The inhibitor constants for mixed-type inhibition were derived from Lineweaver Burk and Cornish-Bowden plots.

molecules binding to the enzyme, a replot of $1/K_{i,slope}$ versus [I] yields a straight line from which K_i can be derived. For compound **1** this plot was not perfectly linear. In addition, for most other 9-aminoacridines the kinetics were measured in the presence of only two inhibitor concentrations. Therefore the $K_{i,apparent}$ values given in Table 1 are used for comparing the inhibitory potency of the different derivatives.

The Role of the Substituents for Inhibitor Binding. In the three-dimensional structure of the TR−mepacrine complex[14] the chlorine, the methoxy, and the alkylamino group have been shown to be in contact with specific side chains of the protein (Figure 1). The newly synthesized derivatives contain one or two of the substituents of mepacrine or have slightly modified ligands (Table 1). As outlined in the previous section more than one molecule of the 9-aminoacridine derivatives may bind to the same form of the enzyme. Since the crystallographic analysis revealed the binding mode of only one mepacrine molecule in the active site of TR, quantitative structure−activity relationships cannot be established. In addition, the contributions of the substituents to the binding strength of the inhibitor was not additive, an observation also made for other tricyclic inhibitors of TR.[21,32] Subtle structural changes can obviously cause significantly different binding modes of the compounds. In the following section we will therefore compare groups of two or three derivatives and discuss their kinetics in light of the TR−mepacrine structure.

The Alkylamino Chain. The derivatives **1**, **4**, and **6** are 3-chloroacridines with a different substituent at C9 (R^3 in Table 1). The latter two compounds with long alkylamino chains were more efficient inhibitors than **1** with only an amino group at C9. On the other hand, in the series of compounds **2**, **5** (mepacrine), and **7** which have a chlorine and a methoxy group, elongation of the alkylamino chain by one methylene group (**7**) but also replacement of this substituent by a single amino group (**2**) improved inhibition relative to mepacrine. In the TR−mepacrine complex the terminal diethylamino group of the side chain may undergo a water-mediated hydrogen bond with the carboxylate of Glu18. The introduction of an additional methylene group could thus make possible a direct contact with the protein. The efficient interaction between **2** and the enzyme may indicate that in the absence of the bulky alkylamino chain the acridine derivative binds in the active site in a completely different orientation.

The Methoxy Group. The crystal structure of the TR−mepacrine complex revealed that the methoxy group of the inhibitor is in hydrogen bonding distance to the side chain oxygen of Ser109. The kinetic studies presented here also indicate that the methoxy group contributes to the interaction with the enzyme. Three pairs of derivatives differing only by the presence/absence of the methoxy group have been measured. Comparison of compounds **6** and **7** as well as **1** and **2** shows that the derivatives with the methoxy group have a significantly lower K_i value than those lacking the substituent. Only in the case of **4** and **5** did introduction of the methoxy group not improve the interaction with the enzyme.

The Chlorine Atom. In the TR−mepacrine structure, the chlorine at C6 of the acridine ring is fixed in the vicinity of the ring nitrogen of Trp21. The kinetic results are in agreement with a positive effect of the

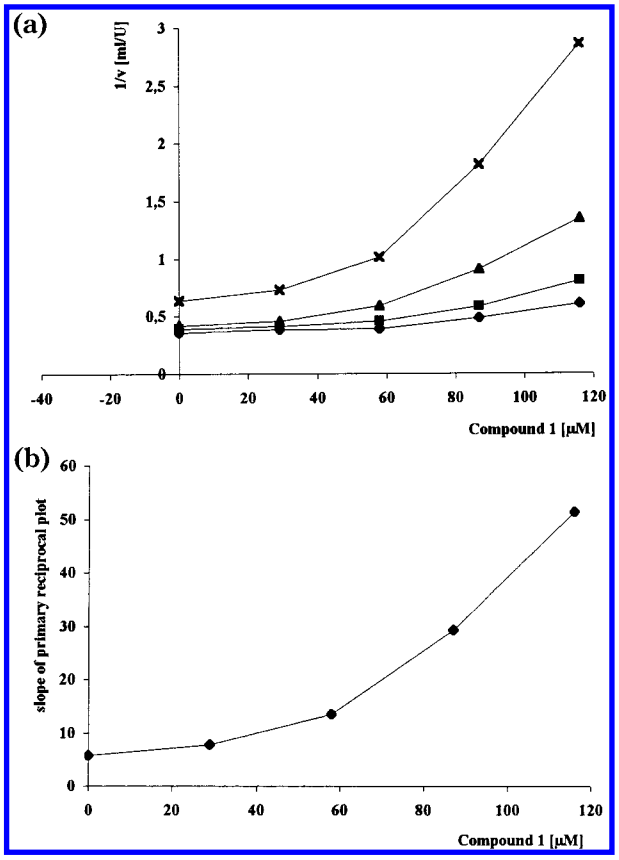


Figure 2. Inhibition of *T. cruzi* trypanothione reductase by compound **1**. The kinetics were measured at 25 °C as described under Experimental Procedures at a constant concentration of 100 μM NADPH. (a) Dixon plot. The inhibitor concentration was varied in the presence of fixed concentrations of (◆) 200 μM, (■) 100 μM, (▲) 50 μM, and (×) 20 μM TS_2. The values are the mean of two measurements which differed by less than 5%. The complete data set was measured at least in dublicate. (b) Replot of the slopes of the reciprocal plot (a) versus [I].

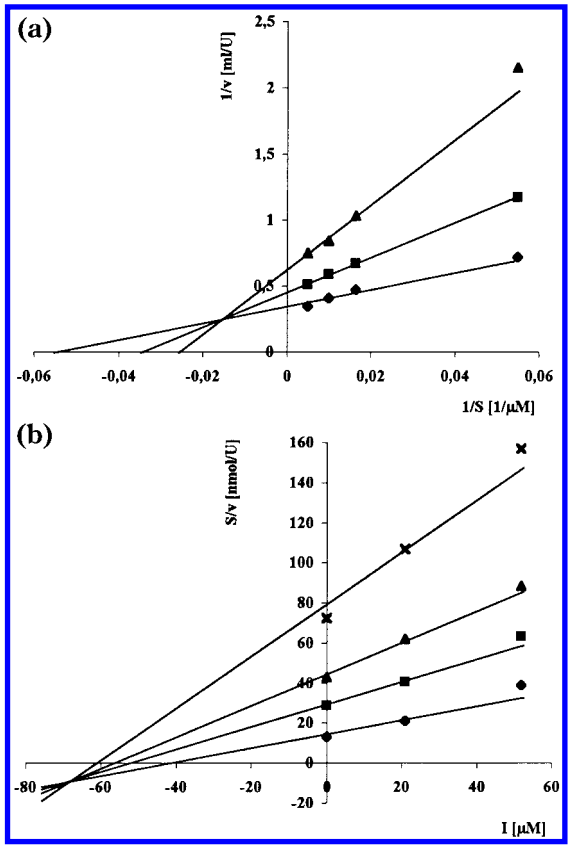


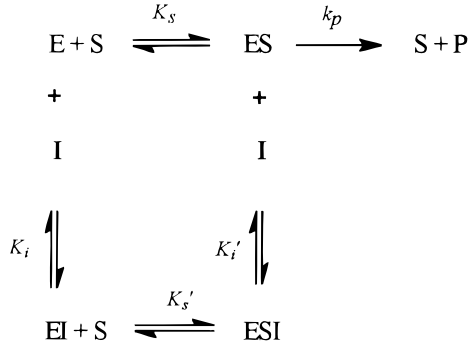
Figure 3. Inhibition of TR by compound **9**. The kinetics were performed as described in the legend of Figure 2. (a) Lineweaver Burk plot. The TS_2 concentration was varied in the presence of (◆) none, (■) 21 μM, and (▲) 53 μM inhibitor. (b) Cornish-Bowden plot. The inhibitor concentration was varied in the presence of (◆) 18 μM, (■) 61 μM, (▲) 105 μM, and (×) 210 μM TS_2. The plot directly gives the inhibitor constants: The lines intersect at a point where $[I] = -K_i'$; K_i is calculated from $S/v = K_m[1 - (K_i'/K_i)]/V$.

halogen upon inhibitor binding. Derivatives with the chlorine at C6 of the acridine ring, namely mepacrine (**5**) and compound **1**, show improved binding in comparison to compound **3** and 9-aminoacridine, respectively. It should be kept in mind that the apparent K_i values (Table 1) depend on the inhibitor concentration, resulting in lower K_i values at higher concentrations of inhibitor. For instance, at 58−116 μM the $K_{i,apparent}$ value for compound **1** is only 24 μM and thus significantly lower than that for 9-aminoacridine (for details see Figure 2 and the text below).

The Aromatic Ring System. In the mepacrine−TR structure, the aromatic ring is fixed close to the hydrophobic wall formed by Trp21, Met113, Leu17, and Phe114. Tacrine (9-amino-1,2,3,4-tetrahydroacridine) and 6-chlorotacrine are inhibitors of acetylcholine esterase where they bind in the vicinity of aromatic residues.[33] As shown in Table 1, tacrine and 6-chlorotacrine bind to TR much weaker than do the respective acridines, whereby the presence of a chlorine atom again improves binding. This finding indicates that the planar acridine ring system is favorable for TR binding.

9-Thioacridines Inhibit TR with Mixed-Type Kinetics. Compounds **8** and **9** (Table 1) are mepacrine analogues which have an S-alkylamino chain at position C9 of the acridine ring. In contrast to the 9-aminoacridines, they show mixed-type kinetics when studied as inhibitors of *T. cruzi* TR. K_i and K_i' values (αK_i) have been obtained from the Lineweaver Burk plot (Figure 3a) as well as graphically from the Cornish-Bowden plots (Figure 3b).[34] In addition, replots of the primary reciprocal plot data, namely slope versus [I] and intercept on the vertical axis versus [I], were linear, showing that the ESI complex is catalytically inactive. The kinetics are in accordance with linear mixed-type inhibition (Scheme 3). Originally, compound **8** had been reported to inhibit TR competitively with a K_i value of 70 μM.[20] Since the inhibitor constant was derived from a Dixon plot, which does not allow to distinguish between competitive and mixed-type kinetics, the true inhibition type was overlooked.

Scheme 3



Discussion

All acridine derivatives studied here as inhibitors of *T. cruzi* trypanothione reductase are positively charged under physiological conditions. The crucial role of a positive charge for binding in the active site of TR has been outlined by Faerman et al.[8] As shown previously, acridine itself and anionic derivatives do not bind to the enzyme.[14] The 9-aminoacridine derivatives inhibit TR competitively, consistent with a localization at the disulfide substrate binding site.[13] This region is mainly formed by Glu18, Trp21, Ser109, and Met113 and is the binding site for mepacrine (Figure 1).[14] As indicated by the parabolic Dixon plots observed with three different 9-aminoacridine derivatives, more than one inhibitor molecule can bind to the enzyme. The crystal structure of the TR−mepacrine complex has visualized a single inhibitor molecule in the active site. No other localization was observed, but this may be due to the low occupancy for mepacrine and the relatively low resolution of 2.8 Å.[14] Therefore, we can only speculate about probable additional binding sites. One site may be the cavity at the 2-fold axis of the homodimeric protein. The respective pocket in human GR has been shown to accommodate several ligands such as menadione[35] as well as the tricyclic compounds safranin,[35,36] xanthene,[36] and isoalloxazine derivatives.[37] In the complex of GR with dinitrophenylglutathione (DNPG), two binding sites for the inhibitor have been revealed.[38] The main localization was in the active site coinciding with the binding site for glutathione disulfide; an additional minor binding was observed in the cavity at the 2-fold axis. Interestingly, the kinetics of GR inhibition by DNPG are very similar to those found here for the 9-aminoacridines and TR. In the former case, the reciprocal plot also showed competitive inhibition and the Dixon plot was parabolic.[38]

Another possibility is that two inhibitor molecules bind in the active site. Modeling approaches on TR have suggested a second hydrophobic pocket in the outer region of the active site formed by Phe396, Pro398, and Leu399.[21,39]

The finding that the 9-aminoacridine derivatives can bind at more than one site in the enzyme makes quantitative structure−activity relationships based on the TR−mepacrine structure difficult. In addition, the calculated inhibitor constants for competitive inhibition depend on the inhibitor concentration in the assay and are therefore not directly comparable. All 9-aminoacridines are inhibitors of TR with apparent K_i values ranging from 5 to 43 μM. The most potent inhibitors are compounds **2** and **7** which have a methoxy group and a chlorine atom at C2 and C6, respectively, of the acridine ring. A general statement about the contribution of each individual substituent upon binding to TR cannot be made since the effects were not additive (Table 1). An important result of the studies presented here is that the often-used reciprocal plot at one inhibitor concentration is not sufficient to elucidate the real type of inhibition.

The 9-thioacridine derivatives **8** and **9** inhibit TR with mixed-type kinetics, the K_i values being comparable to those for the 9-aminoacridines. K_i' values of about 70 μM show that binding of the inhibitor to the enzyme substrate complex is weaker than that to the free enzyme. The linearity of the Dixon plots as well as of the replots of slopes and intercepts, respectively, of the Lineweaver Burk plot versus [I] show that the ESI complex is not active. Such linear mixed-type (intersecting linear noncompetitive) inhibition pattern has also been observed for human GR complexed with xanthene and safranin.[36] Isoalloxazines are another class of GR inhibitors with mixed-type kinetics as revealed by plotting the published Dixon data[40] into a Cornish-Bowden diagram. The crystal structures of GR in complex with xanthene, safranin, as well as two isoalloxazine derivatives showed, in any case, one inhibitor molecule fixed in the cavity at the 2-fold axis of the homodimeric protein.[36,37] No binding was observed in the active site. One may therefore speculate that the respective cavity in TR is the binding site for the 9-thioacridines.

Conclusions

Inhibition of *T. cruzi* TR by 9-aminoacridines and 9-thioacridines, respectively, follows different types of kinetics. 9-Aminoacridines are competitive inhibitors with more than one binding site in the enzyme whereas the 9-thioacridines give a mixed-type inhibition pattern. These findings were surprising and showed that the binding mode of even structurally very similar compounds can be unpredictible. From the structural analyses of several inhibitor complexes of TR and GR and the kinetic results presented here for TR, one may conclude that competitive inhibitors with multiple binding sites are fixed at the disulfide binding site with additional localizations in the cavity at the 2-fold axis and/or the outer region of the active site. On the other hand, compounds with mixed-type inhibition pattern preferably bind at the 2-fold axis.[36] For drug development approaches based on inhibitors of TR it would be very helpful if the binding locus in the enzyme could be deduced from the kinetic data. Clearly, some more kinetic and crystallographic data of TR inhibitors have to be available before a general correlation can be established.

Experimental Procedures

Materials. *T. cruzi* trypanothione reductase was purified from recombinant *Escherichia coli* SG5 cells as described.[41] Trypanothione disulfide (TS_2) was purchased from Bachem, Heidelberg, Germany. Tacrine was obtained from Sigma, Deisenhofen. 6-Chlorotacrine was kindly provided by Parke-Davis, Ann Arbor, MI. 9-Aminoacridine was purchased from Aldrich, Steinheim, Germany.

TR Assay. TR activity was measured at 25 °C in TR assay buffer (40 mM Hepes, 1 mM EDTA, pH 7.5) as described.[42] The standard assay mixture (1 mL) contained 100 μM NADPH and 7−10 mU TR. The reaction was started by adding 105 μM TS_2, and the absorption decrease at 340 nm was followed. V_{max} is calculated using a K_m value of 18 μM for TS_2.

Determination of Inhibitor Constants. The structural formula of the compounds tested are given in Table 1. Stock solutions (3−5 mM) of 9-aminoacridine and tacrine were prepared in TR assay buffer. Stock solutions of the other acridine derivatives and 6-chlorotacrine were made in DMSO. Control assays containing the respective amount of DMSO were carried out where appropriate. Inhibitor constants were derived from Lineweaver Burk, Dixon, and Cornish-Bowden plots as described.

Chemistry. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX 200 spectrometer with TMS as internal reference; chemical shifts are given on the δ (ppm) scale with J values in hertz. The compounds were purified by liquid chromatography on silica gel 60 (70−230 mesh).

Synthesis of Aminoacridines 1 and 2. Ammonium carbonate (1.5 g, 17 mmol) was added to the corresponding

9-chloroacridine[25] (10 mmol) and mixed with phenol (8 g). The mixture was stirred and heated to 120 °C for 45 min and then poured into an excess of 5 N NaOH to give pH 12. The solid was filtered, washed with 1 N NaOH and water, and dried (yield: 70%).

9-Amino-3-chloroacridine (1).[43] Mp: 267 °C. ^{1}H NMR (DMSO-d_6): 8.44 (d, J = 9.3, 1 H); 8.39 (d, J = 8.8, 1 H); 7.97 (b s, 2 H); 7.82 (d, J = 2.1, 1 H); 7.81 (d, J = 7.8, 1 H); 7.67 (dd, J = 7.8, J = 6.6, 1 H); 7.32 (m, 2 H). ^{13}C NMR (DMSO-d_6): 150.6 (s); 149.4 (s); 149.2 (s); 134.8 (s); 130.6 (d); 128.7 (d); 126.8 (d); 125.8 (d); 123.4 (d);122.1 (d); 121.9 (d); 113.1 (s); 111.4 (s). Anal. ($C_{13}H_9N_2Cl$) C, H, N.

9-Amino-6-chloro-2-methoxyacridine (2).[44] Mp: 274 °C. ^{1}H NMR (DMSO-d_6): 8.40 (d, J = 9.2, 1 H); 7.81 (d, J = 2.2, 1 H); 7.76 (d, J = 9.3, 1 H); 7.68 (sh, 2 H); 7.67 (d, J = 2.5, 1 H); 7.38 (dd, J = 9.3, J = 2.5, 1 H); 7.30 (dd, J = 9.2, J = 2.2, 1 H); 3.91 (s, 3 H). ^{13}C NMR (DMSO-d_6): 154.8 (s); 148.9 (s); 147.8 (s); 146 (s); 133.7 (s); 130.5 (d); 126.9 (d); 125.4 (d); 124.3 (d); 122.2 (d); 113.3 (s); 111.3 (s); 100.4 (d); 55.8(q). Anal. ($C_{14}H_{11}N_2OCl$) C, H, N.

Preparation of Substituted Aminoacridines 3, 4, 5, 6, 7. The corresponding 9-chloroacridine (10 mmol) was reacted with 5-diethylamino-2-aminopentane (1.58 g, 10 mmol) (**3**, **4**, **5**) or 6-diethylamino-2-aminohexane (1.72 g, 10 mmol,) (**6**, **7**) in phenol (15 g), heated at 110 °C for 2 h. The reaction mixture was poured into 5 N NaOH solution, and the product was extracted with chloroform. The solvent was removed under reduced pressure and the amine purified by chromatography on SiO_2 in ether−methanol (90/10 to 80/20) (yield: 45−55%).

2-Methoxy-9-(4′-diethylamino-1′-methylbutylamino)-acridine (3).[45] ^{1}H NMR ($CDCl_3$): 8.11 (d, J = 9.3, 1 H); 8.05 (b d, J = 9.4, 2 H); 7.64 (td, J = 6.7, J = 1.3, 1 H); 7.42 (m, 2 H); 7.26 (b s, 1 H); 4.41 (d, J = 10.5, 1 H); 4.03 (m, 1 H); 3.96 (s, 3 H); 2.6−2.3 (m, 6 H); 1.66 (m, 4 H); 1.27 (d, J = 6.3, 3 H); 0.95 (t, J = 7.1, 6 H). ^{13}C NMR ($CDCl_3$): 155.9 (s); 148.9 (s); 148 (s); 146.5 (s); 131.6 (d); 130 (d); 128.8 (d); 124.2 (d); 124.1 (d); 122 (d); 119.3 (s); 119.3 (s); 99.4 (d); 55.7 (d); 55.4 (q); 52.8 (t); 46.8 (t); 37 (t); 24 (t); 22.2 (q); 11.5 (q). Anal. ($C_{23}H_{31}N_3O$) C, H, N.

3-Chloro-9-(4′-diethylamino-1′-methylbutylamino)-acridine (4).[26] ^{1}H NMR ($CDCl_3$): 8.05−7.96 (m, 4 H); 7.65 (dd, J = 7.1, J = 7.6, 1 H); 7.35 (dd, J = 7.5, J = 7.7, 1 H); 7.23 (m, 1 H); 4.90 (m, 1 H); 4.11 (m, 1 H); 2.40 (q, J = 7.1), 2.31 (t, J = 6.2) (6 H); 1.7−1.5 (m, 4 H); 1.26 (d, J = 6.3, 3 H); 0.91 (t, J = 7.1, 6 H). ^{13}C NMR ($CDCl_3$): 151.2 (s); 149.9 (s); 149.7 (s); 135.7 (s); 130.3 (d); 128.9 (d); 128.1 (d); 124.5 (d); 124.2 (d); 123.7 (d); 122.4 (d); 117.9 (s); 116 (s); 56.1 (d); 52.5 (t); 46.6 (t); 36.8 (t); 23.8 (t); 22.1 (q); 11.4 (q). Anal. ($C_{22}H_{28}N_3Cl$) $(H_2O)_{1.5}$ C, H, N.

6-Chloro-2-methoxy-9-(4′-diethylamino-1′-methylbutylamino)-acridine (5).[46] Mp: 248−250 °C. ^{1}H NMR (D_2O) (hydrochloride): 7.63 (d, J = 9.3, 1 H); 7.24−7.19 (m, 3 H); 7.11 (dd, J = 9.3, J = 1.6, 1 H); 6.92 (b s, 1 H); 4.03 (q, J = 6.3, 1 H); 3.78 (s, 3 H); 2.75 (q, J = 7.3, 4 H + 2 H); 1.62 (m, 2 H); 1.40 (d, J = 6.2, 3 H); 1.28 (m, 2 H); 0.88 (t, J = 7.3, 6 H). ^{13}C NMR (D_2O): 156.2 (s); 154.9 (s); 141.8 (s); 139.7 (s); 127 (d); 126.7 (d); 125.1 (d); 123.6 (d); 120.3 (d); 114.9 (s); 111.6 (s); 102.2 (d); 56.7 (d or q); 56 (q or d); 51.5 (t); 47.9 (t); 34.9 (t); 21.4 (q); 21 (t); 8.7 (q). Anal. ($C_{23}H_{30}N_3OCl$) $(HCl)_2(H_2O)_{1.5}$ C, H, N.

3-Chloro-9-(5′-diethylamino-1′-methylpentylamino)-acridine (6). ^{1}H NMR ($CDCl_3$): 8.09−7.99 (m, 4 H); 7.69 (m, 1 H); 7.40 (m, 1 H); 7.30 (dd, J = 9.2, J = 2.0, 1 H); 4.72 (b s, 1 H); 4.11 (m, 1 H); 2.46 (q, J = 7.2, 4 H); 2.33 (m, 2 H); 1.67 (m, 2 H); 1.41 (m, 4 H); 1.32 (d, J = 6.3, 3 H); 0.97 (t, J = 7.2, 6 H). ^{13}C NMR ($CDCl_3$): 151.3 (s); 149.7 (s); 149.5 (s); 135.8 (s); 130.4 (d); 129.5 (d); 127.9 (d); 124.4 (d); 124.3 (d); 123.7 (d); 122.3 (d); 117.8 (s); 115.9 (s); 56.2 (d); 52.4 (t); 46.6 (t); 38.8 (t); 26.6 (t) 24.2 (t); 22.1 (q); 11.2 (q). Anal. ($C_{23}H_{30}N_3Cl$) C, H, N.

6-Chloro-2-methoxy-9-(5′-diethylamino-1′-methylpentylamino)-acridine (7).[25] ^{1}H NMR ($CDCl_3$): 8.0 (d, J = 2.0, 1 H); 7.92 (d, J = 9.4, 1 H); 7.88 (d, J = 9.5, 1 H); 7.34 (dd, J = 9.4, J = 2.6, 1 H); 7.23 (dd, J = 9.5, J = 1.7, 1 H); 7.11 (d, J = 2.6, 1 H); 4.22 (d, J = 10.5, 1 H); 3.9 (m, 1 H); 3.87 (s, 3 H); 2.35 (q, J = 7.1, 4 H); 2.25 (m, 2 H); 1.58 (m, 2 H); 1.33 (m, 4 H); 1.17 (d, J = 6.3, 3 H); 0.88 (t, J = 7.1, 6 H). ^{13}C NMR ($CDCl_3$): 156.2 (s); 149.3 (s); 148.2 (s); 147 (s); 134.7 (s); 131.7 (d); 128.5 (d); 125.1 (d); 124.7 (d); 123.8 (d); 119.4 (s); 117.4 (s); 99.3 (d); 56.1 (d); 55.5 (q); 52.7 (t); 46.9 (t); 39.1 (t); 27.2 (t); 24.5 (t); 22.2 (q); 11.6 (q). Anal. ($C_{24}H_{32}N_3OCl$) C, H, N.

Preparation of Aminothioacridine 8 and 9. 6-Chloro-2-methoxy-9-(4′-diethylaminoethyl)-thioacridine (8).[29] A mixture of 6-chloro-2-methoxy-9-thioacridinone (2.7 g, 10 mmol), 2-diethylaminoethyl chloride hydrochloride (1.9 g, 11 mmol), toluene (150 mL), and 5 N KOH solution (50 mL) was refluxed 4 h. After cooling, the organic phase was separated, the aqueous phase was extracted with chloroform, and the organic phases were dried over drierite. The solvent was removed under reduced pressure, and the product was purified by chromatography on SiO_2 and eluted with ether and ether−methanol (70/30) (yield: 75%) as an oil. By addition of HCl saturated ether, the hydrochloride was obtained. Mp: 188−190 °C. ^{1}H NMR (D_2O) (hydrochloride): 8.40 (d, J = 9.4, 1 H); 7.82 (d, J = 2.0) and 7.80 (d, J = 8.7) (2 H); 7.66−7.57 (m, 2 H); 7.46 (d, J = 2.5, 1 H); 3.92 (s, 3 H); 3.27−3.19 (m, 2 H); 3.24 (m) and 2.91 (q, J = 7.3) (2 H + 4 H); 0.92 (t, J = 7.3, 6 H). ^{13}C NMR (D_2O): 159.9 (s); 150.2 (s); 142.2 (s); 138.3 (s); 137.4 (s); 132.3 (d); 131 (d); 130.5 (s); 129 (d); 127.4 (s); 123.8 (d); 120.7 (d); 103.6 (d); 57 (q); 51.5 (t); 48.2 (t); 31.6 (t); 8.7 (q). Anal. ($C_{20}H_{23}N_2OSCl$) $(HCl)_2$ (H_2O) C, H, N.

6-Chloro-2-methoxy-9-(4′-chlorobutyl)-thioacridine. This intermediate compound was synthesized by mixing 6-chloro-2-methoxy-9-thioacridinone (2.7 g, 10 mmol), excess 1-bromo-4-chlorobutane (2.6 g, 15 mmol), TEBAC (triethylbenzylammonium chloride, 0.5 g), toluene (100 mL), and NaOH (8 g) in H_2O (20 mL) and refluxing for 4 h.[47] After cooling, the organic phase was washed to pH 7 and dried over $MgSO_4$. The product was purified by chromatography on SiO_2 using ether−petroleum ether (50/50) as eluent. The yield of pure product was 60%. ^{1}H NMR ($CDCl_3$): 8.67 (d, J = 9.3, 1 H); 8.19 (d, J = 2.0, 1 H); 8.09 (d, J = 9.4, 1 H); 7.51 (m, 2 H); 4.04 (s, 3 H); 3.43 (t, J = 6.4, 2 H); 2.94 (t, J = 7.0, 2 H); 1.90 (m, 2 H); 1.64 (m, 2 H).

6-Chloro-2-methoxy-9-(4′-diethylaminobutyl)-thioacridine (9). 6-Chloro-2-methoxy-9-(4′-chlorobutyl)-thioacridine (3.6 g, 5 mmol), excess diethylamine (2 g, 27 mmol), and Na_2CO_3 (1 g, 10 mmol) were refluxed in acetone (40 mL) for 4 days. The solvent was removed under vacuum, and NaOH was added to give pH 12. The solution was extracted with chloroform, and the organic phase was dried over drierite. Chromatography on SiO_2 and elution with ether and ether−methanol (70/30) yielded pure compound **9** as an oil. The hydrochloride was obtained as described for compound **8**. Mp: 185−187 °C. ^{1}H NMR (D_2O) (hydrochloride): 8.38 (d, J = 9.3, 1 H); 7.81 (m, 2 H); 7.65 (d, J = 9.3, 1 H); 7.58 (d, J = 9.7, 1 H); 7.44 (b s, 1 H); 3.91 (s, 3 H); 3.03 (t, J = 6.7) and 2.94 (q, J = 7.3) (6 H); 2.79 (m, 2 H); 1.49 (m, 2 H); 1.31 (m, 2 H); 1.03 (t, J = 7.3, 6 H). ^{13}C NMR (D_2O): 159.6 (s); 155.4 (s); 142.7 (s); 137.4 (s); 135.9 (s); 132.5 (d); 130.6 (d); 130.2 (s); 129.5 (d); 127.2 (s); 123 (d); 120 (d); 104.2 (d); 57.1 (q); 51.5 (t); 48 (t); 38.3 (t); 27.5 (t); 23 (t); 8.9 (q). Anal. ($C_{22}H_{27}N_2OSCl$) $(HCl)_2$ (H_2O) C, H, N.

Acknowledgment. Heike Lüdemann and Edith Röckel are acknowledged for excellent technical assistance. We thank Dr. Heiner Schirmer for many helpful discussions. Our work is supported by the Deutsche Forschungsgemeinschaft (DFG Kr 1242/1-3 and SFB 544) and the Bundesministerium für Bildung und Forschung (BMBF Schwerpunkt Tropical Medicine Heidelberg, TMH).

References

(1) Fairlamb, A. H.; Cerami, A. Metabolism and functions of trypanothione in the Kinetoplastida. *Annu. Rev. Microbiol.* **1992**, *46*, 695−729.

(2) Montrichard, F.; Le Guen, F.; Laval-Martin, D. L.; Davioud-Charvet, E. Evidence for the coexistence of glutathione reductase and trypanothione reductase in the nontrypanosomatid Euglenozoa: *Euglena gracilis* Z. *FEBS Lett.* **1999**, *442*, 29−33.

(3) Fairlamb, A. H.; Blackburn, P.; Ulrich, P.; Chait, B. T.; Cerami, A. Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. *Science* **1985**, *227*, 1485−1487.
(4) Kuriyan, J.; Kong, X. P.; Krishna, T. S.; Sweet, R. M.; Murgolo, N. J.; Field, H.; Cerami, A.; Henderson, G. B. X-ray structure of trypanothione reductase from *Crithidia fasciculata* at 2.4 Å resolution. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8764−8768.
(5) Hunter, W. N.; Bailey, S.; Habash, J.; Harrop, S. J.; Helliwell, J. R.; Aboagye-Kwarteng, T.; Smith, K.; Fairlamb, A. H. Active site of trypanothione reductase. A target for rational drug design. *J. Mol. Biol.* **1992**, *227*, 322−333.
(6) Lantwin, C. B.; Schlichting, I.; Kabsch, W.; Pai, E. F.; Krauth-Siegel, R. L. The structure of *Trypanosoma cruzi* trypanothione reductase in the oxidized and NADPH reduced state. *Proteins* **1994**, *18*, 161−173.
(7) Stoll, V. S.; Simpson, S. J.; Krauth-Siegel, R. L.; Walsh, C. T.; Pai, E. F. Glutathione reductase turned into trypanothione reductase: structural analysis of an engineered change in substrate specificity. *Biochemistry* **1997**, *36*, 6437−6447.
(8) Faerman, C. H.; Savvides, S. N.; Strickland, C.; Breidenbach, M. A.; Ponasik, J. A.; Ganem, B.; Ripoll, D.; Krauth-Siegel, R. L.; Karplus, P. A. Charge is the major discriminating factor for glutathione reductase versus trypanothione reductase inhibitors. *Bioorg. Med. Chem.* **1996**, *4*, 1247−1253.
(9) Dumas, C.; Ouellette, M.; Tovar, J.; Cunningham, M. L.; Fairlamb, A. H.; Tamar, S.; Olivier, M.; Papadopoulou, B. Disruption of the trypanothione reductase gene of *Leishmania* decreases its ability to survive oxidative stress in macrophages. *EMBO J.* **1997**, *16*, 2590−2598.
(10) Tovar, J.; Cunningham, M. L.; Smith, A. C.; Croft, S. L.; Fairlamb, A. H. Down-regulation of *Leishmania donovani* trypanothione reductase by heterologous expression of a trans-dominant mutant homologue: effect on parasite intracellular survival. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5311−5316.
(11) Krieger, S.; Schwarz, W.; Ariyanayagam, M. R.; Fairlamb, A. H.; Krauth-Siegel, R. L.; Clayton, C. H. Trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidative stress. *Mol. Microbiol.*, in press.
(12) Bailey, S.; Smith, K.; Fairlamb, A. H.; Hunter, W. N. Substrate interactions between trypanothione reductase and N_1-glutathionylspermidine disulphide at 0.28-nm resolution. *Eur. J. Biochem.* **1993**, *213*, 67−75.
(13) Bond, C. S.; Zhang, Y.; Berriman, M.; Cunningham, M. L.; Fairlamb, A. H.; Hunter, W. N. Crystal structure of *Trypanosoma cruzi* trypanothione reductase in complex with trypanothione, and the structure-based discovery of new natural product inhibitors. *Structure* **1999**, *7*, 81−89.
(14) Jacoby, E. M.; Schlichting, I.; Lantwin, C. B.; Kabsch, W.; Krauth-Siegel, R. L. Crystal structure of the *Trypanosoma cruzi* trypanothione reductase mepacrine complex. *Proteins* **1996**, *24*, 73−80.
(15) Schirmer, R. H.; Müller, J. G.; Krauth-Siegel, R. L. Disulfide-reductase inhibitors as chemotherapeutic agents: The design of drugs for trypanosomiasis and malaria. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 141−154.
(16) Krauth-Siegel, R. L.; Schöneck, R. Flavoprotein structure and mechanism. 5. Trypanothione reductase and lipoamide dehydrogenase as targets for a structure-based drug design. *FASEB J.* **1995**, *9*, 1138−1146.
(17) Krauth-Siegel, R. L.; Coombs, G. H. Enzymes of parasite thiol metabolism as drug targets. *Parasitol. Today* **1999**, *15*, 404−409.
(18) Krauth-Siegel, R. L.; Lohrer, H.; Bücheler, U. S.; Schirmer, R. H. The antioxidant enzymes glutathione reductase and trypanothione reductase as drug targets. In *Biochemical Protozoology*; Coombs, G., North, M., Eds; Taylor & Francis: London, 1991; pp 493−506.
(19) Benson, T. J.; McKie, J. H.; Garforth, J.; Borges, A.; Fairlamb, A. H.; Douglas, K. T. Rationally designed selective inhibitors of trypanothione reductase. Phenothiazines and related tricyclics as lead structures. *Biochem. J.* **1992**, *286*, 9−11.
(20) Krauth-Siegel, R. L.; Jacoby, E. M.; Jockers-Scherübl, M. C.; Schlichting, I.; Barbe, J. *T. cruzi* trypanothione reductase: structure−function relationships of enzyme−inhibitor complexes. In *Flavins and Flavoproteins*; Stevenson, K. J., Massey, V., Williams, C. H., Jr., Eds.; University of Calgary Press: Canada, 1996; pp 35−44.
(21) Chan, C.; Yin, H.; Garforth, J.; McKie, J. H.; Jaouhari, R.; Speers, P.; Douglas, K. T.; Rock, P. J.; Yardley, V.; Croft, S. L.; Fairlamb, A. H. Phenothiazine inhibitors of trypanothione reductase as potential antitrypanosomal and antileishmanial drugs. *J. Med. Chem.* **1998**, *41*, 148−156.
(22) Girault, S.; Davioud-Charvet, E.; Salmon, L.; Berecibar, A.; Debreu, M. A.; Sergheraert, C. Structure−activity relationships in 2-aminodiphenylsulfides against trypanothione reductase from *Trypanosoma cruzi*. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1175−1180.
(23) Hammond, D. J.; Cover, B.; Gutteridge, E. W. A novel series of chemical structures active in vitro against the trypomastigote form of *Trypanosoma cruzi*. *Trans. R. Soc. Trop. Med. Hyg.* **1984**, *78*, 91−95.
(24) Obexer, W.; Schmid, C.; Barbe, J.; Galy, J. P.; Brun, R. Activity and structure relationship of acridine derivatives against African trypanosomes. *Trop. Med. Parasitol.* **1995**, *46*, 49−53.
(25) Albert, A. *The Acridines*; Edward Arnold: London, 1966.
(26) Shepard, E. R.; Shonle, H. A. Nuclear substituted 9-(4′-diethylamino-1′-methylbutylamino)-acridines. *J. Am. Chem. Soc.* **1948**, *70*, 1979−1980.
(27) Borch, R. F.; Bernstein, M. D.; Durst, H. D. The cyanohydridoborate anion as a selective reducing agent. *J. Am. Chem. Soc.* **1971**, *93*, 2897−2904.
(28) Breslow, D. S.; Yost, R. S.; Walker, H. G.; Hauser, C. R. Synthesis of atabrine analogues having various aliphatic a-substituents in the side chain. *J. Am. Chem. Soc.* **1944**, *66*, 1−1924.
(29) Bsiri, N.; Johnson, C.; Kayirere, M.; Galy, A. M.; Galy, J. P.; Barbe, J.; Osuna, A.; Mesa-Valle, M. C.; Castilla Calvente, J. J.; Rodriguez-Cabezas, M. N. Trypanocidal structure- activity relationship in 9-thioalkylacridines. *Ann. Pharm. Fr.* **1996**, *54*, 27−33.
(30) Segel, I. H. *Enzyme kinetics*; John Wiley & Sons: New York, 1993.
(31) Dixon, M.; Webb, E. C. *Enzymes*; Academic Press: London, 1979.
(32) Garforth, J.; Yin, H.; McKie, J. H.; Douglas, K. T.; Fairlamb, A. H. Rational design of selective ligands for trypanothione reductase from *Trypanosoma cruzi*. Structural effects on the inhibition by dibenzazepines based on imipramine. *J. Enzyme Inhib.* **1997**, *12*, 161−173.
(33) Wlodek, S. T.; Antosiewicz, J.; McCammon, J. A.; Straatsma, T. P.; Gilson, M. K.; Briggs, J. M.; Humblet, C.; Sussman, J. L. Binding of tacrine and 6-chlorotacrine by acetylcholinesterase. *Biopolymers* **1996**, *38*, 109−117.
(34) Cornish-Bowden, A. A simple graphical method for determining the inhibition constants of mixed, uncompetitive and noncompetitive inhibitors. *Biochem. J.* **1974**, *137*, 143−144.
(35) Karplus, P. A.; Pai, E. F.; Schulz, G. E. A crystallographic study of the glutathione binding site of glutathione reductase at 0.3 nm resolution. *Eur. J. Biochem.* **1989**, *178*, 693−703.
(36) Savvides, S. N.; Karplus, P. A. Kinetics and crystallographic analysis of human glutathione reductase in complex with a xanthene inhibitor. *J. Biol. Chem.* **1996**, *271*, 8101−8107.
(37) Schönleben-Janas, A.; Kirsch, P.; Mittl, P. R.; Schirmer, R. H.; Krauth-Siegel, R. L. Inhibition of human glutathione reductase by 10-arylisoalloxazines: crystalline, kinetic, and electrochemical studies. *J. Med. Chem.* **1996**, *39*, 1549−1554.
(38) Bilzer, M.; Krauth-Siegel, R. L.; Schirmer, R. H.; Akerboom, T. P.; Sies, H.; Schulz, G. E. Interaction of a glutathione S-conjugate with glutathione reductase. Kinetic and X-ray crystallographic studies. *Eur. J. Biochem.* **1984**, *138*, 373−378.
(39) Horvath, D. A virtual screening approach applied to the search for trypanothione reductase inhibitors. *J. Med. Chem.* **1997**, *40*, 2412−2423.
(40) Becker, K.; Christopherson, R. I.; Cowden, W. B.; Hunt, N. H.; Schirmer, R. H. Flavin analogues with antimalarial activity as glutathione reductase inhibitors. *Biochem. Pharmacol.* **1990**, *39*, 59−65.
(41) Sullivan, F. X.; Walsh, C. T. Cloning, sequencing, overproduction and purification of trypanothione reductase from *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* **1991**, *44*, 145−147.
(42) Jockers-Scherübl, M. C.; Schirmer, R. H.; Krauth-Siegel, R. L. Trypanothione reductase from *Trypanosoma cruzi*. Catalytic properties of the enzyme and inhibition studies with trypanocidal compounds. *Eur. J. Biochem.* **1989**, *180*, 267−272.
(43) Albert, A.; Dyer, F. J.; Linnel, W. H. Chemotherapeutic studies in the acridine series. IV Relation between structure and toxicity. *Q. J. Pharm. Pharmacol.* **1937**, *10*, 649−658.
(44) Holliman, F. G.; Mann, F. G. Synthetic application of o-β-bromoethylbenzyl bromide. *J. Chem. Soc.* **1945**, 34−37.
(45) Cherntsov, O. M.; Drozdov, N. S. The preparation and antimalarial action of substituted 9-aminoacridine. *Zh. Obshchei. Khim.* **1939**, *9*, 1435−1440.
(46) Mietzsch, F.; Mauss, H. I. I. G. Farben, Deutsches Patent, 553,072. *Chem. Abstr.* **1932**, 4683.
(47) Galy, J. P.; Vincent, E. J.; Galy, A. M.; Barbe, J.; Elguero, J. A comparative study of reactivity of acridanones, aminoacridines and thioacridanones toward alkylating agents using phase transfer catalysis. *Bull. Soc. Chim. Belg.* **1981**, *90*, 947−954.

JM990386S