Coupling of a Competitive and an Irreversible Ligand Generates Mixed Type Inhibitors of *Trypanosoma cruzi* Trypanothione Reductase

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9-Aminoacridines and (terpyridine)platinum(II) complexes are competitive and irreversible inhibitors, respectively, of trypanothione reductase from *Trypanosoma cruzi*, the causative agent of Chagas' disease. Four chimeric compounds in which 2-methoxy-6-chloro-9-aminoacridine was covalently linked to the (2-hydroxyethanethiolate)(2,2′:6′,2″-terpyridine)platinum(II) complex were synthesized and studied as inhibitors of the parasite enzyme. The derivatives differed by the nature and/or the length of the spacer connecting the two aromatic systems. All four compounds were effective mixed type inhibitors of trypanothione reductase with K_i and K_i' values of 0.3−4 and 2−11 μM, respectively. The most potent inhibitor had an ethylthioether linkage between the two aromatic ring systems, and the other compounds contained an alkyl ether group with 4−6 methylene groups. In contrast to the parasite enzyme, human glutathione reductase, the closest related host enzyme was not inhibited by these compounds. The finding that the conjugation of a competitive and an irreversible inhibitor can give rise to reversible mixed type inhibitors underlines the difficulties associated with inhibitor design based on the three-dimensional structure of trypanothione reductase.

Introduction

Trypanosomes and Leishmania are the causative agents of African sleeping sickness (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*), Chagas' disease (*Trypanosoma cruzi*), Nagana cattle disease (*Trypanosoma congolense* and *Trypanosoma brucei brucei*), Kala-azar (*Leishmania donovani*), and Oriental sore (*Leishmania tropica*). All of these parasitic protozoa lack the ubiquitous enzyme glutathione reductase. For maintaining a reducing intracellular redox equilibrium, Trypanosomes and Leishmania depend on the flavoenzyme trypanothione reductase (TR),[1] which keeps their main thiols, bisglutathionylspermidine (trypanothione, $T(SH)_2$)[2] and monoglutathionylspermidine (Gsp), in the thiol state:

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

TR shares many physical and chemical properties with human GR, the closest related host enzyme. The most important difference between parasite and host enzyme is the mutually exclusive specificity toward their disulfide substrates. Genetic approaches revealed that trypanosomes lacking TR are avirulent and show increased sensitivity to oxidative stress.[3] The absence of TR from mammalian cells and the essential role of TR in the defense of oxidative stress render the enzyme well-suited as a target molecule for rational drug development (for reviews, see refs 6−8). 9-Aminoacridines such as mepacrine are competitive inhibitors.[4] The crystal structure of the TR−mepacrine complex revealed that the drug is fixed in the active site close to the hydrophobic wall formed by Trp21 and Met113. The residues in contact with the bound mepacrine are four out of five residues in the active site, which are not conserved between *T. cruzi* TR and human GR.[5] The fact that the activity of TR in bloodstream *T. brucei* has to be lowered to 10% to cause an increased sensitivity toward oxidative stress[3] indicates that purely competitive inhibitors may not become suitable drug candidates, unless a ligand with a nanomolar inhibition constant and/or slow binding behavior was discovered.[6] However, most competitive inhibitors of TR described so far have inhibitor constants that are of the same order of magnitude as the K_m of the enzyme for trypanothione disulfide.[6−8] In contrast to competitive ligands, irreversible inhibitors may be effective at much lower concentrations and in addition, accumulation of substrate due to blockage of the pathway cannot overcome inhibition. This renders irreversible inhibitors promising drug candidates. (2,2′:6′,2″-Terpyridine)platinum(II) complexes have been shown to be irreversible inhibitors of *T. cruzi* TR but not of human GR. Most probably, Cys52 in the active site of TR is specifically modified.[9] Acridines[10,11] and (2,2′:6′,2″-terpyridine)platinum(II) complexes[12] have trypanocidal activities.

The selective inhibition of TR by both 9-aminoacridines and (2,2′:6′,2″-terpyridine)platinum(II) complexes caused us to synthesize chimeric ligands composed of (2-hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)platinum(II) complexes and 2-methoxy-6-chloro-9-aminoacridines. The two aromatic moieties were connected by either an alkyl ether or an alkylthioether group. It was assumed that the acridine moiety may exert specificity for the parasite enzyme at the trypanothione binding site of TR (following mepacrine, see above) whereas the (2,2′:6′,2″-terpyridine)platinum(II)

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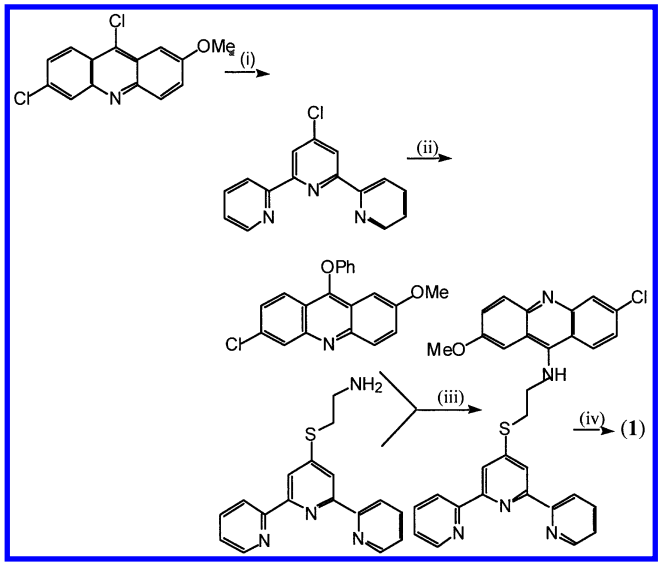
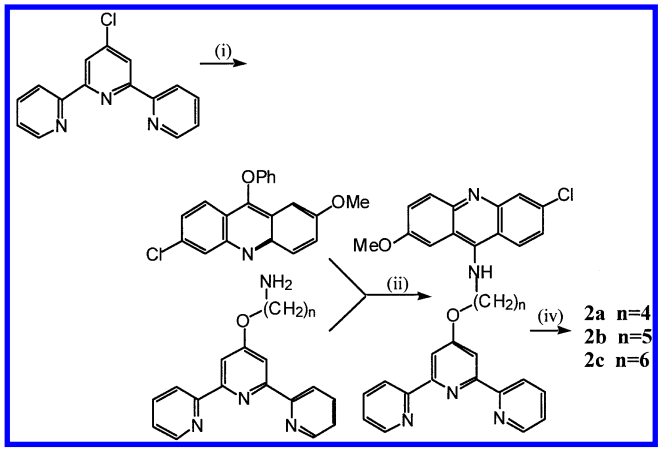
Scheme 1. Synthesis of (2,2′:6′,2″-Terpyridine)platinum(II) Acridine Conjugate (**1**)[a]



[a] Reagents: (i) PhOH, NaOH. (ii) 2-Mercapto-ethylamine. (iii) Heat in weakly acidic solution. (iv) (1,5-Cyclooctadiene)diiodo Platinum(II).

Scheme 2. Synthesis of (2,2′:6′,2″-Terpyridine)platinum (II) Acridine Conjugate (**2a**−**c**)[a]



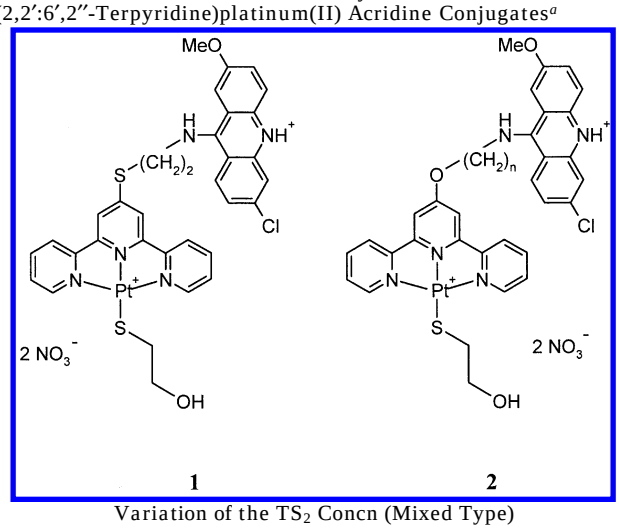
[a] Reagents: (i) α,ω-Hydroxyalkylamines, NaH. (ii) Heat in weakly acidic solution. (iii) (1,5-Cyclooctadiene)diiodo Platinum(II).

moiety may lead to an irreversible modification of the enzyme. Four (2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates were synthesized and studied as irreversible and reversible inhibitors of *T. cruzi* TR and as reversible inhibitors of human GR.

Results

Synthesis of the Compounds. 2-Methoxy-6,9-dichloro-acridine can be converted to the 9-phenoxy derivative, which is more reactive toward nucleophilic substitution at the 9-position.[13] Acridine−terpyridine conjugates can be made by reacting 2-methoxy-6-chloro-9-phenoxy-acridine and 4′-chloro-2,2′;6′,2″-terpyridine with a suitable linker. Thus, 2-mercaptoethylamine reacts with 4′-chloro-2,2′:6′,2″-terpyridine to give 4′-(2-aminoethylthio)-2,2′:6′,2″-terpyridine, which reacts with 2-methoxy-6-chloro-9-phenoxyacridine to give the acridine−terpyridine conjugate, which is then converted to the 2-hydroxyethane-thiolate platinum(II) complex (**1**) by established procedures (Scheme 1).[14]

Table 1. Inhibition of *T. cruzi* TR by (2,2′:6′,2″-Terpyridine)platinum(II) Acridine Conjugates[a]



Variation of the TS_2 Concn (Mixed Type)

compd	*n*	inhibitor concn in the assay (μM)	$K_{i,slope}$ (μM)[b]	$K_{i,int}$ (μM)
1		0.4	0.59	2.7
		1	0.32	2.5
		2	0.27	2
2a	4	0.9	1.8	5.7
		1.5	1.2	2.5
		3	0.7	2.1
2b	5	2	4	11.4
		4	2.5	7.6
		6	2.6	7.1
2c	6	3	0.53	4
		7.1	0.42	4.4

Variation of the NADPH Concn (Mixed Type)

compd	inhibitor concn (μM)	$K_{i,slope}$ (μM)[b]	$K_{i,int}$ (μM)
1	1	2	2.8
	2	0.8	2

[a] The kinetics were measured at 25 °C in TR assay buffer (40 mM Hepes, 1 mM EDTA, pH 7.5). The TS_2 and NADPH concentration was varied in the presence of 100 μM NADPH and 110 μM TS_2, respectively, and two or three inhibitor concentrations as well as in the absence of inhibitor. Each set of kinetics was measured twice whereby the single data points differed by ≤15%. [b] The inhibitor constants $K_{i,slope}$ and $K_{i,int}$ were calculated for each inhibitor concentration from the slope and intersection values derived from Lineweaver−Burk plots as described under the Experimental Procedures. See text for details.

Alkoxide ions are known to displace chloride ion from 4′-chloro-2,2′:6′,2″-terpyridine,[12] and α,ω-hydroxyalkylamines were found to react preferentially through the alkoxide ion rather than the amine. By using a variety of α,ω-hydroxyalkylamines of increasing chain length under alkaline conditions, it was possible to make three further acridine−terpyridine conjugates, which were then converted into their 2-hydroxyethane-thiolate platinum(II) complexes (Scheme 2).

(2-Hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)platinum(II)−Acridine Conjugates Are Mixed Type Inhibitors of *T. cruzi* TR. Four compounds (**1** and **2a**−**c**) in which a 9-aminoacridine derivative was covalently linked to a (terpyridine)-platinum(II) complex were studied as inhibitors of *T. cruzi* TR (Table 1). The derivatives differed by the nature and/or the length of the spacer connecting the two aromatic systems. In compound **1**, the spacer was an alkylthioether, whereas the other compounds (**2a**−**c**) contained alkyl ether groups with 4−6 methylene

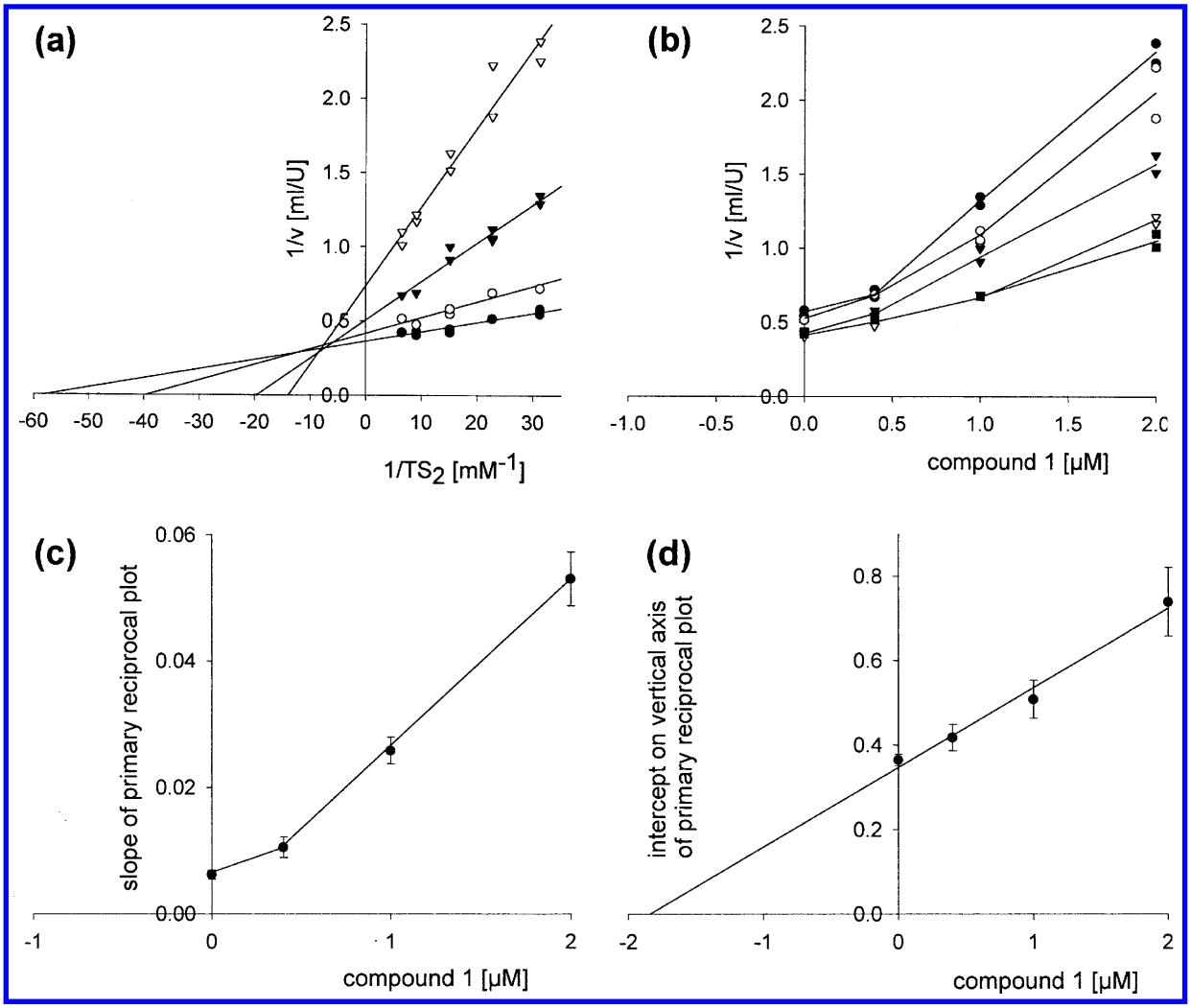


Figure 1. Inhibition of *T. cruzi* TR by compound **1**. The kinetics were measured at 25 °C as described under Experimental Procedures at a constant concentration of 100 μM NADPH. Each set of kinetics was measured twice whereby the single data points differed by ≤10%. (a) Lineweaver−Burk plot; the TS_2 concentration was varied in the presence of (●) none, (○) 0.4, (▲) 1, and (△) 2 μM inhibitor. The data points were fitted to a straight line by linear regression analysis. (b) Dixon plot; 1/*v* is plotted against the inhibitor concentration in the presence of fixed concentrations of (■) 154, (△) 110, (▲) 66, (○) 44, and (●) 32 μM TS_2. (c) Replot of the slopes of the reciprocal plot a vs the inhibitor concentration. (d) Replot of the points of intersection of the reciprocal plot a vs the inhibitor concentration. In panels c and d, standard errors based on the linear regression analysis of a are given.

groups. All four derivatives were effective inhibitors of TR. The type of inhibition was derived from Lineweaver−Burk plots conducted in the absence and presence of at least two different inhibitor concentrations. The double reciprocal plots were linear and intersected to the left of the 1/*v* axis and above the baseline in accordance with mixed type[15,16] or noncompetitive[17] inhibition (Figure 1a). The inhibitor constants were calculated from the slope and intersection values of the Lineweaver−Burk plots as described under the Experimental Procedures. Table 1 gives the $K_{i,slope}$ and $K_{i,int}$ values[16] obtained at each inhibitor concentration.

Plots of 1/*v* against [I] (Dixon) and replots of the slopes and intercepts on the vertical axis of the reciprocal plots vs the corresponding inhibitor concentration yield a parabola when more than one inhibitor molecule can bind to the same form of the enzyme. Linear mechanisms in which more than one inhibitor molecule can bind to different enzyme intermediates do not give rise to curved inhibitor plots.[15] With partially mixed inhibitors, reciprocal plots of velocity against substrate concentration in the presence of a series of inhibitor concentrations will also be linear, but plots against [I] will curve downward to level off at a finite velocity.[15] As shown for compound **1**, the Dixon plot did not yield straight lines but curved upward parabolically in accordance with the inhibitor constants not being independent of the inhibitor concentration in the assay (Figure 1b). The replot of the slopes of the reciprocal plot against [I] also curved upward parabolically whereas the replot of the intercepts against [I] is probably linear (Figure 1c,d). This may indicate that two inhibitor molecules can bind to the free enzyme but only one molecule can bind to the ES complex.

To study a probable binding of the compounds at the reduced nicotinamide adenine dinucleotide phosphate (NADPH) binding site, kinetics were conducted at a fixed concentration of 110 μM TS_2 and varying concentrations of 8−102 μM NADPH in the absence and presence of compound **1**. Also in this case, strong mixed type inhibition was observed (Table 1).

(2-Hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)platinum(II)−Acridine Conjugates Do Not Inhibit TR Irreversibly. TR was

incubated with the chimeric compounds in the presence and absence of NADPH as described under the Experimental Procedures. At different time intervals, aliquots of the incubation mixture were removed and assayed for remaining activity in a standard TR assay. The compounds inhibited neither the oxidized- nor the NADPH-reduced enzyme in a time-dependent manner. The partial inhibition observed remained constant over time and corresponded to the reversible inhibition of TR by the (terpyridine)platinum(II)−acridine conjugates.

(2-Hydroxyethanethiolate)(4′-substituted-2,2′:6′,2′-terpyridine)platinum(II)−Acridine Conjugates Do Not Inhibit Human GR. Assays containing GR, 0.1−1 mM glutathione disulfide (GSSG), and 100 μM NADPH were carried out in the presence and absence of the respective chimeric compound as described under the Experimental Procedures. Control assays contained all components except that the inhibitor was replaced by dimethyl sulfoxide (DMSO). After an initial lag phase, the reaction rate slowly increased to give a final activity nearly identical with that of the control. Obviously, the (terpyridine)platinum(II)−acridine conjugates undergo a reversible interaction with the mammalian enzyme, which is abolished under assay conditions. To get an insight in the underlying mechanism, three assays containing 81 μM **1**, 100 μM NADPH, and 100 μM GSSG were conducted starting the reaction with GSSG, GR, and NADPH, respectively. The lag phase was most pronounced when starting the reaction with GSSG. Independently of the order of additions, an initial lag phase was observed that abolished during the course of the assay resulting in a steady state velocity identical with that in the absence of inhibitor.

Discussion

(2-Hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates are effective mixed type inhibitors of *T. cruzi* TR but do not inhibit human GR. The complexes studied here are bulky hydrophobic aromatic compounds, which are positively charged under physiological conditions. They contain a positive charge on the platinum ion, and N10 of the acridine ring is probably protonated.[18] In addition to the crucial hydrophobic nature of the compounds,[19] the contribution of positive charges for ligands binding in the active site of TR has been outlined by Faerman et al.[20] As shown previously, acridine itself and anionic derivatives do not bind to the enzyme.[5] (2-Hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)-platinum(II) complexes are irreversible inhibitors of TR. The irreversible inhibition implies the coordinative replacement of 2-mercaptoethanol by Cys52 in the active site of TR.[9] The surprising finding that combination of an irreversible with a competitive ligand did not conserve the irreversible type of inhibition indicates that the acridine moiety determines the binding mode. Obviously, conjugation with the acridine moiety prevents the platinum complex from binding in the vicinity of Cys52 thus not allowing the replacement of the fourth ligand by the protein thiol group. Increase in size and loss of flexibility cannot be responsible for the lack of irreversible inhibition, since the most bulky (2,2′:6′,2″-terpyridine)platinum(II) complexes, comparable with the (2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates, inhibit the enzyme irreversibly.[9] The crystal structure of the TR−mepacrine complex has shown a single inhibitor molecule at the disulfide substrate binding site close to a hydrophobic wall formed by Trp21 and Met113.[5] Consistent with the X-ray crystal structure, mepacrine is a competitive inhibitor vs trypanothione disulfide with an apparent K_i value of about 19 μM. The detailed kinetic analysis of a series of 9-aminoacridines revealed the probable presence of more than one binding site in the enzyme.[4] The conjugation of 2-methoxy-6-chloro-9-aminoacridine with a (2,2′:6′,2″-terpyridine)platinum(II) complex lowers the inhibitor constants of 9-aminoacridines by an order of magnitude and leads to a mixed type inhibition pattern. In addition, similar to the 9-aminoacridines, the chimeric conjugates have probably more than one binding site in the enzyme. One binding locus 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 mixed type inhibitors such as xanthene,[21] safranin,[21,22] and isoalloxazines.[23] No binding was observed in the active site. Thus, it is tempting to speculate that the respective cavity in TR, which is comparable in size and in contrast to hGR, shows an overall negative charge[24] can accommodate the compounds tested here. The disulfide substrate binding site of TR is approximately 20 Å long, 15 Å wide, and 15 Å deep[25] and thus much more spacious than that of GR.[26] Especially the outer region of the active site of TR is much wider due to the different orientation of two helices in the flavin adenine dinucleotide (FAD) domain.[25] In this region, a second hydrophobic area formed by Phe396, Pro398, and Leu399, called the Z-site, has been described.[27] Modeling studies on TR have suggested that tricyclics can assume different binding modes at the hydrophobic pockets in the active site.[19] Therefore, in addition to a possible binding site at the 2-fold axis, the acridine moiety may interact with one of the hydrophobic regions at the disulfide binding site of TR with the (2,2′:6′,2″-terpyridine)-platinum(II) moiety pointing away from the active site so that it is not in a position to interact with Cys52 directly. For compound **1**, kinetics at varying concentrations of NADPH also showed a mixed type inhibition pattern. This finding supports the assumption that the compounds do not exclusively bind at the trypanothione disulfide binding site but probably have more than one binding site.

The (2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates do not inhibit human GR. An initial lag phase is completely abolished within the first minutes of the assay. A fast recovery of GR activity after an initial strong "inhibition" has also been observed with (2,2′:6′,2″-terpyridine)platinum(II) complexes.[9] A possible explanation is that the disulfide substrate displaces the inhibitor from the active site and that the establishment of the equilibrium (EI ↔ E ↔ ES) is a relatively slow process.[9] In accordance with this assumption is the observation that starting the reaction with GSSG showed a slightly longer initial lag phase than the assays started with GR or NADPH. In the assays started with substrate, EI can be formed before GSSG is added, whereas in assays started with enzyme or NADPH, no preformed EI complex is present. Another explanation is that the DMSO present as solvent in the

assay is responsible for a slow conformational change as reported earlier for yeast GR.[28]

Conclusions

The (2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates studied here are mixed type inhibitors of *T. cruzi* TR but do not interact with human GR. From the structural analyses of several complexes of GR with linear mixed type inhibitors and the kinetic results presented here for TR, one may conclude that the (2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates can bind in the cavity at the 2-fold axis of the homodimeric enzyme with a probable additional binding site in the active site. Unexpectedly, the combination of an irreversible inhibitor [(2-hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)platinum(II) complexes] and a competitive ligand (9-aminoacridine) created reversible mixed type inhibitors. These findings underline the difficulty to set up structure−activity relationships based on known kinetic and crystallographic data on inhibitors of TR.

Experimental Procedures

Materials. Recombinant *T. cruzi* TR[29] and human GR[30] were prepared according to published procedures. Trypanothione disulfide was purchased from Bachem, Heidelberg, Germany. Stock solutions (1−4 mM) of the (2,2′:6′,2″-terpyridine)platinum(II)−acridine conjugates were made in DMSO and stored at −20 °C.

TR Inhibition Studies Assay. TR activity was measured spectrophotometrically at 25 °C in TR assay buffer (40 mM Hepes, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5) as described.[31] In the assay mixture (1 mL) containing 7−10 mU of *T. cruzi* TR, the enzyme activity was followed in the absence and presence of different fixed concentrations of inhibitor varying the trypanothione disulfide (TS_2) concentration (24−186 μM). The reaction was started by adding 100 μM NADPH, and the absorption decrease at 340 nm due to NADPH consumption was followed. In the presence of compound **1**, starting the reaction with TS_2 led to a slight initial lag phase. Therefore, all assays were started with NADPH where no lag phase was observed. In addition, for compound **1**, *T. cruzi* TR activity was followed in the presence of a fixed concentration of 110 μM TS_2 and varying concentrations of 8−102 μM NADPH in the absence and presence of inhibitor. The assays lacking inhibitor contained the respective amount of DMSO.

GR Inhibition Studies Assay. GR activity was measured in GR assay buffer (20.5 mM KH_2PO_4, 26.5 mM K_2HPO_4, 200 mM KCl, 1 mM EDTA, pH 6.9) at 25 °C as described.[30] The assay mixture (1 mL) contained 100 μM NADPH, 5−10 mU GR, 1 mM or 0.1 mM GSSG and 54−81 μM **1**, 19.2 μM **2a**, 23 μM **2b**, and 14.2 μM **2c**, respectively. The reaction was started by adding either GSSG, GR, and NADPH, respectively, and the absorption decrease at 340 nm was followed. Control samples contained all components except that the inhibitor was replaced by DMSO.

Test of Irreversible Inhibition of TR by (2-Hydroxyethanethiolate)(4′-substituted-2,2′:6′,2″-terpyridine)platinum(II)-2-methoxy-6-chloro-acridine Conjugates. In a total volume of 0.4 mL of TR assay buffer, pH 7.5, 0.55 μM TR was incubated at 25 °C with 54 μM **1**, 19 μM **2a**, 5 μM **2b**, and 50 μM **2c**, respectively, in the presence and absence of 100 μM NADPH and varying concentrations of inhibitor. Control samples contained all components except that the inhibitor was replaced by DMSO. At different time intervals (0−120 min), aliquots of 4−20 μL were removed and assayed for remaining activity in a standard TR assay.[31]

Determination of Inhibitor Constants. The activity of *T. cruzi* TR was followed in the absence and presence of different fixed concentrations of inhibitor varying the substrate concentration. The type of inhibition was derived from Lineweaver−Burk plots, and the inhibitor constants were calculated from the expressions for the slopes and the intercepts on the vertical axis. For mixed type inhibition, the equations are[15]

$$\text{slope} = K_s\frac{(1 + [I]/K_i)}{V} \qquad \text{and}$$

$$\text{intercept on vertical axis} = \frac{(1 + [I]/K_i')}{V}$$

where K_s is equated with K_m assuming equilibrium conditions.

Chemical Synthesis. Chemicals were obtained from Aldrich, Lancaster, and Sigma and were used without further purification. NMR spectra were taken on a Bruker DPX 250 spectrometer with a 5 mm multinuclear probe. The temperature was kept constant (298 K) by a variable unit. Mass spectra were recorded by Dr. Robin Aplin either by electrospray (complexes) or chemical ionization techniques (ligands and organic compounds).

6-Chloro-2-methoxy-9-phenoxyacridine. 2-Methoxy-6,9-dichloroacridine (2 g, 7 mmol) was added to a solution of NaOH (400 mg, 10 mmol) in phenol (10 g, 106 mmol), and the mixture was stirred at 100 °C for 2 h and then poured into NaOH solution (2 M, 50 mL). The solution was extracted with $CHCl_3$ (3 × 50 mL), and the organic phase was dried ($MgSO_4$) and concentrated in vacuo. Recrystallization of the residue from hot MeOH afforded the product as yellow needles (2.02 g, 84%). mp: measured 154 °C; literature value 155 °C.[13] ^{1}H NMR (CD_2Cl_2): δ = 3.79 [s, 3 H, OCH_3], 6.86 [d, 2 H, J = 10 Hz, phenoxy H 2], 7.06 [t, 1 H, J = 7 Hz, phenoxy H 4], 7.16 [d, 1 H, J = 2.75 Hz, H1″ or H5″], 7.26 [d, 2 H, J = 2.80 Hz, phenoxy H 3], 7.37 [dd, 1 H, J = 9.25, 2 Hz, H3″ or H7″], 7.45 [dd, 1 H, J = 2.75, 9.5 Hz, H3″ or H7″], 7.97 [d, 1 H, J = 9.25 Hz, H4″ or H8″], 8.08 [d, 1 H, J = 9.5 Hz, H4″ or H8″], and 8.17 [d, 1 H, J = 2 Hz, H1″ or H5″]. DCI-MS m/z (%): 336 (100) [MH^+] $C_{20}H_{14}ClNO_2$ (335.5).

4′-(2-Amino-ethanethio)-2,2′:6′,2″-terpyridine. 4′-Chloro-2,2′:6′,2″-terpyridine (1.34 g, 5 mmol) and 2-mercaptoethylamine hydrochloride (6.82 g, 60 mmol) were suspended in 50 mL of 2-methylpropan-1-ol, and the mixture was stirred at reflux for 54 h. The crude product was isolated by filtration of the hot suspension and redissolved in 20 mL of water, and the acidic solution (pH 2.5) was washed with 2 × 20 mL dichloromethane. The aqueous phase was heated to 90 °C, and 1 M aqueous sodium hydroxide solution was added dropwise until no further solid was seen to precipitate. The suspension was cooled slowly to 5 °C, and the precipitated solid was isolated by filtration, washed with aqueous base, and dried under vacuum to yield the product as a white solid (821.8 mg, 53.3%); mp 93.5−94.5 °C. $\delta 1_H$ (250 MHz, $CDCl_3$)/ppm: 3.12 (2H, t, J = 5.8 Hz, CH_2N), 3.31 (2H, t, J = 6.1 Hz, CH_2S), 7.36 (2H, dd, J = 6.6, 4.7 Hz, H5,5″), 7.87 (2H, t, J = 7.0 Hz, H4,4″), 8.39 (2H, s, H3′,5′), 8.63 (2H, d, J = 7.9 Hz, H3,3″), 8.71 (2H, d, J = 3.2 Hz, H6,6″); $\delta 13_C$ (62.9 MHz, $CDCl_3$)/ppm: 35.4 (*C*N), 41.0 (*C*S), 118.5 (C3′,5′), 121.8 (C3,3″), 124.3 (C5,5″), 137.3 (C4,4″), 149.5 (C6,6″), 151.1 (C4′), 155.5 (C2′,6′), 156.2 (C2,2″) (assigned from HMQC and HMBC spectra); m/z ($APCI^+$) 309 (80%, $[MH]^+$), 331 (100%, $[MNa]^+$). HR−CI: 309.1174 ($C_{17}H_{16}N_4S$ requires 309.1174).

4′[*N*-(6-Chloro-2-methoxyacridine-9-aminoethanethio)]-2,2′:6′,2″-terpyridine. 4′-(2-Amino-ethanethio)-2,2′:6′,2″-terpyridine (500 mg, 1.62 mmol) and 6-chloro-2-methoxy-9-phenoxyacridine (1.0 g, 2.98 mmol) were suspended in 30 mL of acetonitrile, and 1 mL of glacial acetic acid was added. The resulting suspension was heated at reflux, and the progress of the reaction was followed by TLC (normal phase silica, eluting with 10% MeOH in $CHCl_3$). After 2.5 h, no terpyridine starting material could be detected. The solvent was removed under vacuum, and the residual gum was resuspended in 25 mL of chloroform and 25 mL of aqueous sodium hydroxide solution. The solid material was removed by filtration, the phases were separated, and the aqueous layer was extracted with chloroform (2 × 10 mL). The combined organic fractions

were dried over K_2CO_3, and the solvent was removed in vacuo. The residual gum was redissolved in chloroform and purified by column chromatography (eluting with a gradient of 1−4% MeOH in $CHCl_3$). Removal of solvent in vacuo left a gum, which was triturated with chloroform and hexane. The resulting solid was dried under vacuum to leave the product as a yellow powder (370.6 mg, 41.6%); mp 68−71 °C. δ^1_H (400 MHz, CD_2Cl_2)/ppm: 3.50 (2H, t, J = 6.2 Hz, CH_2S), 3.95 (3H, s, CH_3), 4.06 (2H, t, J = 6.2 Hz, CH_2N), 7.3−7.4 (5H, m, H5,5″ + HA3 + HA1 + HA7), 7.9−8.0 (3H, m, H4,4″ + HA4), 8.02 (1H, d, J = 2.0 Hz, HA5), 8.17 (1H, d, J = 9.2 Hz, HA8), 8.42 (2H, s, H3′,5′), 8.64 (2H, d, J = 8.0 Hz, H3,3″), 8.69 (2H, d, J = 4.8 Hz, H6,6″). δ^{13}_C (100.6 MHz, CD_2Cl_2)/ppm: 33.1 (*C*S), 49.0 (*C*N), 56.2 (*C*O), 99.3 (CA3 or CA1), 117.9 (CA13), 118.8 (C3′,5′), 120.0 (CA10), 121.8 (C3,3″), 124.7 (C5,5″), 124.9 (CA8), 125.4 (CA1 or CA3), 125.6 (CA7), 128.8 (CA5), 132.1 (CA4), 135.0 (CA12), 137.5 (C4,4″), 147.4 (CA11), 148.7 (CA6), 149.2 (CA9), 149.7 (C6,6″ + C4′), 156.0 (C2,2″ + C2′,6′), 157.2 (CA2) (assigned from HMQC and HMBC spectra); *m*/*z* (APCI+): 551 (100%, $[M.H]^+$), 573 (25%, $[M.Na]^+$). HR−CI: 550.1456, 552.1434 ($C_{31}H_{25}N_5OSCl$ requires 550.1468, 552.1439).

4′[*N*-(6-Chloro-2-methoxyacridine-9-aminoethanethio)]-2,2′:6′,2″-terpyridine Platinum(II)-2-hydroxyethanethiolate (1). The title compound was prepared by the published method[14] from 4′[*N*-(6-chloro-2-methoxyacridine-9-aminoethanethio)]-2,2′:6′,2″-terpyridine (0.2 mmol) and mercaptoethanol (32 μL 36.5 mg, 0.47 mmol). The title compound was a brown solid (27.8 mg, 14.7%); mp >230 °C. δ^1_H (400 MHz, d_6-DMSO)/ppm: 2.66 (2H, t, J = 7.5 Hz, CH_2SPt), 3.69 (2H, t, J = 7.5 Hz, CH_2O), 3.73 (3H, s, CH_3), 3.87 (2H, br (t), CH_2-Sterpy), 4.65 (2H, br (t), CH_2N), 7.36 (2H, s, HA3, HA4), 7.41 (1H, d, J = 9.0 Hz, HA7), 7.47 (1H, d, J = 2.0 Hz, HA5), 7.61 (1H, s, HA1), 8.03 (2H, m, H5,5″), 8.19 (2H, s, H3′,5′), 8.30 (2H, d, J = 8.0 Hz, H3,3″), 8.5 (3H, m, HA8, H4,4″), 9.40 (2H, br d, J = 5.0 Hz, H6,6″), 9.56 (1H, br (t), N*H*). δ^{13}_C (100.6 MHz, d_6-DMSO)/ppm: 33.5 (CH_2Sterpy), 34.0 (*C*H_2SPt), 49.0 (*C*H_2N), 57.0 (*C*H_2O), 66.0 (*C*H_3), 103.5 (CA1), 110.5 (CA13), 118.0 (CA7), 122.0 (CA3), 123.0 (C3′,5′), 125.0 (CA12), 126.5 (C3,3″), 128.0 (CA8, CA10), 129.5 (CA4), 130.5 (C5,5″), 134.5 (CA11), 140.5 (CA6), 143.5 (C4,4″), 152.0 (C2′,6′), 153.5 (C6,6″), 155.5 (C4′), 157.0 (CA2), 159.0 (C2,2″) (assigned from HMQC and HMBC spectra; no cross-peaks for CA9 or CA5). *m*/*z* (ESI+): 411.6 (100%, $[MH]^{2+}$), 822.2 (60%, $[M]^+$). HR-ESI of $[M]^{2+}$: 411.62 ($C_{33}H_{31}N_5S_2O_2ClPt$ requires 411.56).

4′-(6-Aminohexyloxy)-2,2′:6′,2″-terpyridine. A solution of NaH (120 mg, 3 mmol) and 6-amino-1-hexanol (585 mg, 5 mmol) in 10 mL of dimethyl formamide (DMF) was stirred for 30 min at room temperature. 4′-Chloro-2,2′:6′,2″-terpyridine (267 mg, 1 mmol) was added, and the mixture was stirred overnight at 80 °C under argon. A few drops of water were added, and DMF was evaporated. The product was taken up in dichloromethane and washed with basic NaOH/H_2O solution for several times, and the organic layer was dried ($MgSO_4$) and concentrated in vacuo to give the product (0.301 g, 87%). ^{1}H NMR ($CDCl_3$): δ 1.38 [m, 6 H, (CH_2)$_n$], 1.91 [t, 2 H, J = 7 Hz, CH_2CH$_2$O], 2.75 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.27 [t, 2 H, J = 7 Hz, CH_2O], 7.37 [ddd, 2 H, J = 7.5, 5.0, 1.0 Hz, H5,5″], 7.89 [dt, 2 H, J = 8.0, 7.6, 1.8 Hz, H4,4″], 8.04 [s, 2 H, H3′,5′], 8.65 [ddd, 2 H, J = 8.0, 2.0, 1.0 Hz, H3,3″], 8.73 [ddd, 2 H, J = 5.0, 2.0, 1.0 Hz, H6,6″] DCI-MS; *m*/*z* (%): 348 (100) $[MH^+]$ $C_{21}H_{24}N_4O$ (348.0).

6-Chloro-2-methoxy-9-[4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine]acridine. A solution of the phenoxyacridine (220 mg, 0.655 mmol) and 4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine (152 mg, 0.437 mmol) in 10 mL of acetonitrile and 1 mL of acetic acid was refluxed for 3 h. The MeCN was evaporated, and the product was taken up in CH_2Cl_2 and washed with basic NaOH/H_2O solution. The organic phase was dried ($MgSO_4$) and flash-chromatographed over silica with dichloromethane−methanol−ammonia 9:1:0.1. The fractions containing acridone and product were combined and flash-chromatographed over silica again with dichloromethane−ammonia 90:1 yielding the product as a yellow solid (170 mg, 66%) (found: C, 68.9; H, 5.5; N,11.6. Calcd for $C_{35}H_{32}ClN_5O_2\cdot H_2O$: C, 69.1; H, 5.6; N, 11.5%). ^{1}H NMR (CD_2Cl_2): δ 1.60 [m, 4 H, $CH_2CH_2CH_2CH_2$], 1.91 [m, 4 H, $CH_2CH_2CH_2CH_2$], 3.82 [t, 2 H, J = 7 Hz, CH_2-NH$_2$], 4.01 [s, 3H, OCH$_3$], 4.27 [t, 2 H, J = 7 Hz, CH_2O], 7.37 [ddd, 2 H, J = 7.5, 5.0, 1.0 Hz, H5,5″], 7,40 [m, 3 H, acridine], 7.92 [dt, 2 H, J = 8.0, 7.6, 1.8 Hz, H4,4″], 8.04 [s, 2 H, H3′,5′], 8,08 [m, 3 H, acridine], 8.67 [ddd, 2 H, J = 8.0, 2.0, 1.0 Hz, H3,3″], 8.73 [ddd, 2 H, J = 5.0, 2.0, 1.0 Hz, H6,6″] DCI-MS; *m*/*z* (%): 590 (100) $[MH^+]$ $C_{35}H_{33}ClN_5O_2$ (590.0).

[6-Chloro-2-methoxy-9-[4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine Platinum(II) Hydroxyethanethiolate]acridine Bisnitrate (2c). Silver nitrate (35.7 mg, 0.21 mmol) in aqueous acetone (80% acetone, 25 mL) was added to a suspension of Pt(COD)I_2 (55.7 mg, 0.1 mmol) in aqueous acetone (0.75 mL). Silver iodide was removed by centrifugation. The supernatant was added to a suspension of ligand (53 mg, 0.09 mmol) in MeCN (5 mL). After 10 min, the solid was isolated, and the supernatant was discarded. The solid was washed with ether:acetone 3:1 and taken up in DMF, and mercaptoethanol (20 μL, 0.20 mmol) was added. The solid was precipitated with ether, isolated by centrifugation, washed with ether several times, and dried in vacuo; yield (23 mg, 30%) (found: C, 41.5; H, 4.7; N, 8.5. Calcd for $C_{37}H_{38}ClSN_7O_9Pt$: C, 41.3; H, 4.4; N, 9.1%). ^{1}H NMR (DMSO): δ 1.58 [m, 4 H, $CH_2CH_2CH_2CH_2$], 1.92 [m, 4 H, $CH_2CH_2CH_2CH_2$], 2.50 [t, 2 H, J = 7 Hz, SCH_2], 3.64 [t, 2 H, J = 7 Hz, HOCH_2], 3.92 [s, 3H OCH$_3$], 4.10 [t, 2 H, J = 7 Hz, CH_2NH], 4.35 [t, 2 H, J = 7 Hz, CH_2O], 7.73 [m, 7 H, acridine + terpy], 8.21 [s, 2 H, H3′,5′], 8,58 [m, 5 H, acridine + terpy], 9.31 [s, 2 H, H6,6″], 9.58 [s, 1 H, N*H* (acridine)]. ^{13}C NMR (DMSO): δ 32.4, 33.3 [$CH_2CH_2CH_2CH_2$], 35.5, 36.5 [$CH_2CH_2CH_2CH_2$], 40.4 [CH$_2$-NHAcr], 72.8 [CH_2OTerpy], 77.9 [CH_3O], 56.3, 63.7, [mercaptoethanol]. ESI-MS; *m*/*z* (%): 862 (100) $[MH^+]$ $C_{37}H_{38}ClSN_5O_3$-Pt (862.0).

4′-(5-Aminopentyloxy)-2,2′:6′,2″-terpyridine. The compound was prepared as for 4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine; yield (0.916 g, 70%). ^{1}H NMR ($CDCl_3$): δ 1.38 [m, 2 H, $CH_2CH_2CH_2$], 1.98 [t, 4 H, J = 7 Hz, CH_2CH$_2$CH_2], 2.75 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.31 [t, 2 H, J = 7 Hz, CH_2O], 7.41 [ddd, 2 H, J = 7.5, 5.0, 1.0 Hz, H5,5″], 7.92 [dt, 2 H, J = 8.0, 7.6, 1.8 Hz, H4,4″], 8.09 [s, 2 H, H3′,5′], 8.70 [ddd, 2 H, J = 8.0, 2.0, 1.0 Hz, H3,3″], 8.73 [ddd, 2 H, J = 5.0, 2.0, 1.0 Hz, H6,6″] DCI-MS; *m*/*z* (%): 334 (100) $[MH^+]$ $C_{21}H_{24}N_4O$ (334.0).

6-Chloro-2-methoxy-9-[4′-(5-aminopentyloxy)-2,2′:6′,2″-terpyridine]acridine. The compound was prepared as 6-chloro-2-methoxy-9-[4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine]-acridine; yield (0.792 g, 58%) (found: C, 69.9; H, 5.4; N,12.3. Calcd for $C_{34}H_{31}ClN_5O_2$: C, 70.8; H, 5.2; N, 12.2%). ^{1}H NMR (CD_2Cl_2): δ 1.69 [m, 2 H, $CH_2CH_2CH_2$], 1.95 [m, 4 H, CH_2-CH$_2$CH_2], 3.82 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.01 [s, 3H, OCH$_3$], 4.29 [t, 2 H, J = 7 Hz, CH_2O], 7.37 [ddd, 2 H, J = 7.5, 5.0, 1.0 Hz, H5,5″], 7,40 [m, 3 H, acridine], 7.92 [dt, 2 H, J = 8.0, 7.6, 1.8 Hz, H4,4″], 8.04 [s, 2 H, H3′,5′], 8,08 [m, 3 H, acridine], 8.67 [ddd, 2 H, J = 8.0, 2.0, 1.0 Hz, H3,3″], 8.73 [ddd, 2 H, J = 5.0, 2.0, 1.0 Hz, H6,6″] DCI-MS; *m*/*z* (%): 576 (100) $[MH^+]$ $C_{34}H_{31}ClN_5O_2$ (576.0).

6-Chloro-2-methoxy-9-[4′-(5-aminopentyloxy)-2,2′:6′,2″-terpyridine Platinum(II)hydroxyethanethiolate]acridine Bisnitrate (2b). This was prepared as [6-chloro-2-methoxy-9-[4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine platinum(II)hydroxyethanethiolate]acridine bisnitrate; yield (22 mg, 30%) (found: C, 42.3; H, 4.1; N, 9.5. Calcd for $C_{36}H_{36}ClSN_7O_9Pt\cdot 3H_2O$: C, 42.1; H, 4.1; N, 9.5%). ^{1}H NMR (DMSO): δ 1.68 [m, 2 H, ($CH_2CH_2CH_2$)], 2.00 [m, 4 H, ($CH_2CH_2CH_2$)], 2.50 [t, 2 H, J = 7 Hz, SCH_2], 3.64 [t, 2 H, J = 7 Hz, HOCH_2], 3.92 [s, 3H OCH$_3$], 4.10 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.35 [t, 2 H, J = 7 Hz, CH_2O], 7,73 [m, 7 H, acridine + terpy], 8.21 [s, 2 H, H3′,5′], 8.58 [m, 5 H, acridine + terpy], 9.31 [s, 2 H, H6,6″], 9.58 [s, 1 H, N*H* (acridine)] ESI-MS; *m*/*z* (%): 848 (100) $[MH^+]$ $C_{36}H_{36}$-$ClSN_5O_3Pt$ (848.0).

4′-(4-Aminobutyloxy)-2,2′:6′,2″-terpyridine. This was prepared as for 4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine; yield (1.082 g, 85%). ^{1}H NMR ($CDCl_3$): δ 1.38 [m, 2 H, (CH_2)], 1.98 [t, 2 H, J = 7 Hz, CH_2], 2.81 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.30 [t, 2 H, J = 7 Hz, CH_2O], 7.41 [ddd, 2 H, J = 7.5, 5.0, 1.0

Hz, H5,5″], 7.92 [dt, 2 H, J = 8.0, 7.6, 1.8 Hz, H4,4″], 8.09 [s, 2 H, H3′,5′], 8.70 [ddd, 2 H, J = 8.0, 2.0, 1.0 Hz, H3,3″], 8.73 [ddd, 2 H, J = 5.0, 2.0, 1.0 Hz, H6,6″] DCI-MS; *m/z* (%): 320 (100) [MH^+] $C_{20}H_{22}N_4O$ (320.0).

6-Chloro-2-methoxy-9-[4′-(4-aminobutyloxy)-2,2′:6′,2″-terpyridine]acridine. This was prepared as 6-chloro-2-methoxy-9-[4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine]acridine; yield (1.230 g, 65%) (found: C, 69.2; H, 6.3; N,12.5. Calcd for $C_{33}H_{28}ClN_5O_2 \cdot H_2O$: C, 68.4; H, 5.3; N, 12.1%). ^{1}H NMR (CD_2Cl_2): δ 1.70 [m, 2 H, (CH_2)], 2.11 [m, 2 H, (CH_2)], 3.89 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.01 [s, 3H OCH$_3$], 4.32 [t, 2 H, J = 7 Hz, CH_2O], 7.37 [ddd, 2 H, J = 7.5, 5.0, 1.0 Hz, H5,5″], 7,40 [m, 3 H, acridine], 7.92 [dt, 2 H, J = 8.0, 7.6, 1.8 Hz, H4,4″], 8.04 [s, 2 H, H3′,5′], 8,08 [m, 3 H, acridine], 8.67 [ddd, 2 H, J = 8.0, 2.0, 1.0 Hz, H3,3″], 8.73 [ddd, 2 H, J = 5.0, 2.0, 1.0 Hz, H6,6″] DCI-MS; *m/z* (%): 562 (100) [MH^+] $C_{33}H_{29}ClN_5O_2$ (562.0).

6-Chloro-2-methoxy-9-[4′-(4-aminobutyloxy)-2,2′:6′,2″-terpyridine Platinum(II)hydroxyethanethiolate]acridine Bisnitrate (2a). This was prepared as [6-chloro-2-methoxy-9-[4′-(6-aminohexyloxy)-2,2′:6′,2″-terpyridine platinum(II)hydroxyethanethiolate]acridine bisnitrate; yield (25 mg, 36%) (found: C, 42.1; H, 4.1; N, 9.7. Calcd for $C_{35}H_{34}ClSN_7O_9Pt \cdot 3H_2O$: C, 41.5; H, 4.0; N, 9.7%). ^{1}H NMR (DMSO): δ 2.05[m, 2 H, (CH_2)], 2.15 [m, 2 H, (CH_2)], 2.50 [t, 2 H, J = 7 Hz, SCH_2], 3.64 [t, 2 H, J = 7 Hz, HOCH_2], 3.92 [s, 3H OCH$_3$], 4.10 [t, 2 H, J = 7 Hz, CH_2NH$_2$], 4.35 [t, 2 H, J = 7 Hz, CH_2O], 4.81 [s, 1 H, N*H* (acridine)], 7,73 [m, 7 H, acridine + terpy], 8.21 [s, 2 H, H3′,5′], 8,58 [m, 5 H, acridine + terpy], 9.31 [s, 2 H, H6,6″]. ESI-MS; *m/z* (%): 834 (100) [MH^+] $C_{35}H_{33}ClSN_5O_3Pt$ (834.0).

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References

(1) Fairlamb, A. H.; Cerami, A. Metabolism and functions of trypanothione in the Kinetoplastida. *Annu. Rev. Microbiol.* **1992**, *46*, 695−729.
(2) 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.
(3) Krieger, S.; Schwarz, W.; Ariyanayagam, M. R.; Fairlamb, A. H.; Krauth-Siegel, R. L.; Clayton, C. Trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidative stress. *Mol. Microbiol.* **2000**, *35*, 542−552.
(4) Bonse, S.; Santelli-Rouvier, C.; Barbe, J.; Krauth-Siegel, R. L. Inhibition of *Trypanosoma cruzi* trypanothione reductase by acridines: kinetic studies and structure−activity relationships. *J. Med. Chem.* **1999**, *42*, 5448−5454.
(5) 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.
(6) Krauth-Siegel, R. L.; Coombs, G. H. Enzymes of parasite thiol metabolism as drug targets. *Parasitol. Today* **1999**, *15*, 404−409.
(7) Austin, S. E.; Khan, M. O.; Douglas, K. T. Rational drug design using trypanothione reductase as a target for anti- trypanosomal and anti-leishmanial drug leads. *Drug Des. Discovery* **1999**, *16*, 5−23.
(8) Werbovetz, K. A. Target-based drug discovery for malaria, leishmaniasis, and trypanosomiasis. *Curr. Med. Chem.* **2000**, *7*, 835−860.
(9) Bonse, S.; Richards, J. M.; Ross, S. A.; Lowe, G.; Krauth-Siegel, R. L. (2,2′:6′,2″-Terpyridine)platinum(II) complexes are irreversible inhibitors of *Trypanosoma cruzi* trypanothione reductase but not of human glutathione reductase. *J. Med. Chem.* **2000**, *43*, 4812−4821.
(10) Hammond, D. J.; Cover, B.; Gutteridge, W. E. 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.
(11) 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.
(12) Lowe, G.; Droz, A. S.; Vilaivan, T.; Weaver, G. W.; Tweedale, L.; Pratt, J. M.; Rock, P.; Yardley, V.; Croft, S. L. Cytotoxicity of (2,2′:6′,2″-terpyridine)platinum(II) complexes to *Leishmania donovani*, *Trypanosoma cruzi*, and *Trypanosoma brucei*. *J. Med. Chem.* **1999**, *42*, 999−1006.
(13) Ross, S. A.; Pitié, M.; Meunier, B. Synthesis of two acridine conjugates of the bis(phenanthroline) ligand "Clip-Phen" and evaluation of the nuclease activity of the corresponding copper complexes. *Eur. J. Inorg. Chem.* **1999**, 557−563.
(14) Lowe, G.; Vilaivan, T. An improved method for the synthesis of (2,2′:6′,2″-terpyridine)platinum(II) complexes. *J. Chem. Res.* **1996**, 386−387.
(15) Dixon, M.; Webb, E. C. *Enzymes*; Academic Press: London, 1979.
(16) Segel, I. H. *Enzyme Kinetics*; John Wiley & Sons: New York, 1993.
(17) Bisswanger, H. *Enzyme Kinetics—Principles and Methods*; Wiley-VCH: Weinheim, 2002.
(18) Courseille, C.; Busetta, B.; Hospital, M. Structure cristalline et moléculaire de la quinacrine. *Acta Crystallogr.* **1973**, *B29*, 2349−2355.
(19) 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.
(20) 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.
(21) 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.
(22) 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.
(23) 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.
(24) Salmon-Chemin, L.; Buisine, E.; Yardley, V.; Kohler, S.; Debreu, M.-A.; Landry, V.; Sergheraert, C.; Croft, S. L.; Krauth-Siegel, R. L.; Davioud-Charvet, E. 2- and 3-Substituted 1,4-naphthoquinone derivatives as subversive substrates of trypanothione reductase and lipoamide dehydrogenase from *Trypanosoma cruzi*: Synthesis and correlation between redox cycling activities and in vitro cytotoxicity. *J. Med. Chem.* **2001**, *44*, 548−565.
(25) 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-A resolution. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8764−8768.
(26) 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.
(27) 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.
(28) Lüönd, R. M.; McKie, J. H.; Douglas, K. T.; Dascombe, M. J.; Vale, J. Inhibitors of glutathione reductase as potential antimalarial drugs. Kinetic cooperativity and effect of dimethyl sulphoxide on inhibition kinetics. *J. Enzyme Inhib.* **1998**, *13*, 327−345.
(29) Sullivan, F. X.; Walsh, C. T. Cloning, sequencing, overproduction and purification of trypanothione reductase from *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* **1991**, *44*, 145−147.
(30) Nordhoff, A.; Bücheler, U. S.; Werner, D.; Schirmer, R. H. Folding of the four domains and dimerization are impaired by the Gly446→Glu exchange in human glutathione reductase. Implications for the design of antiparasitic drugs. *Biochemistry* **1993**, *32*, 4060−4066.
(31) 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.

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