Trypanosoma cruzi: Inhibition of Parasite Growth and Respiration by Oxazolo(thiazolo)pyridine Derivatives and Its Relationship to Redox Potential and Lipophilicity

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*Program of Clinical and Molecular Pharmacology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, P.O. Box 70086 Santiago 7, Chile; †Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Salamanca, Spain; and ‡Laboratory of Bioelectrochemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, P.O. Box 233, Santiago, Chile

Maya, J. D., Morello, A., Repetto, Y., Rodriguez, A., Puebla, P. Caballero, E., Medarde, M., Núñez-Vergara, I. J., Squella, J. A., Ortiz, M. E., Fuentealba, J., and San Feliciano, A. 2001. Trypanosoma cruzi: inhibition of parasite growth and respiration by oxazolo(thiazolo)pyridine derivatives and its relationship to redox potential and lipophilicity. Experimental Parasitology 99, 1–6. Chagas’ disease constitutes a therapeutic challenge because presently available drugs have wide toxicity to the host and are generally ineffective in the chronic stages of the disease. A series of oxazolo(thiazolo)pyridine derivatives were studied on Trypanosoma cruzi epimastigote growth and oxygen consumption and their electrochemical (redox) potentials and lipophilicity. The derivatives produced different degrees of parasite growth and respiration inhibition on CL Brener, I, Q, and Tulahuen strains of T. cruzi epimastigotes. Respiratory chain inhibition appears to be a determinant of the trypanosomicidal activity of these compounds, since a significant correlation between respiration and culture growth inhibition was found. A similar correlation was found, within the different structural subfamilies, between toxic effects and the ability of the compounds to be oxidized in aqueous media. The inhibition of respiration and of parasite growth in culture increases with the lipophilicity of the substituents on the oxazolopyridine nucleus. No difference in the action of these derivatives was found among the different parasite strains. It is concluded that these compounds may have a potential usefulness in the treatment of Chagas’ disease. © 2001 Academic Press

Index Descriptors and Abbreviations: oxazolopyridines; thiazolopyridine; Trypanosoma cruzi; epimastigotes; oxygen consumption; apparent partition coefficient; oxidation peak potential; respiratory chain.

INTRODUCTION

There are over 20 million people infected with Trypanosoma cruzi in Latin America, and mortality indices range from 8 to 12%, depending on patient’s age and physiological state (WHO, 1998). This makes Chagas’ disease (American trypanosomiasis) a serious health problem. The available antichagasic therapies are inadequate; in the first place, nifurtimox and benznidazole, classical nitroheterocyclic antichagasic agents, have serious side effects that force half of the patients to stop treatment. Because of nifurtimox’s toxicity and its ineffectiveness in chronic stages of Chagas’ disease, it is no longer used in some countries. Additionally, the many different parasite strains so far isolated show important differences in susceptibility (Filardi and Brener 1987; Gustafsson et al. 1987; Maya et al. 1997; Morello et al. 1994; WHO 1998). Due to these problems, hundreds of chemical compounds, both natural and synthetic, have been tested as

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antichagasic agents, but the potential of actual toxicity and the low water solubility of many of these have curtailed their use (Chiari et al. 1991; Rivas et al. 1999; Sepulveda-Bosa and Cassels 1996).

Among the tested compounds, a series of nitroaryl-1,4-dihydropyridine and 3-chlorophenyl-1,4-dihydropyridine derivatives proved to be active against several strains of *T. cruzi* epimastigotes (Maya et al. 2000; Nuñez-Vergara et al. 1997). The results demonstrated that all compounds have an inhibitory effect on the growth and respiration of parasites. Due to the positive results previously found and with the aim of predicting the antichagasic activity of new compounds structurally related to the dihydropyridines, we have now extended the studies to a series of fused heterocyclic analogues, with the basic skeleton of oxazol(othiazolo)pyridines.

**MATERIALS AND METHODS**

*Chemicals and drugs.* Tryptose, fetal calf serum, yeast extract, and tryptone were obtained from Difco. Hemin and all other chemicals were purchased from Sigma Chemical Co. for oxazol(othiazolo)pyridines, synthesis of compounds 1 to 8 (Fig. I) was reported previously (Caballero et al. 1996; San Feliciano et al. 1991).

*Apparent partition coefficient determination (Papp).* Studies were carried out in a system containing octanol and 50 mM phosphate buffer, pH 7.4. Both phases were mutually saturated at 23°C prior to use. Drugs were dissolved in octanol to obtain final concentrations about 0.5 mM. After the equilibrium was achieved, absorbance in both phases were determined by UV-Vis spectroscopy (Unicam UV 160 spectrophotometer) between 345 and 360 nm, depending on the derivative. Final drug concentrations were calculated from the corresponding calibration curves. All the experiments were performed in triplicate.

*Retention time (Rt) determination of oxazol(othiazolo) derivatives by HPLC.* HPLC measurements were carried out by using a Waters assembly equipped with a Model 600 Controller pump and a Model 996 Photodiode Array Detector. The acquisition and treatment of data were made by means of Millenium version 2.1 software. As a chromatographic column, a Bondapak/Porasil C-18 column of 3.9 × 150 mm was used. As a column guard, a C18 Bondapak (30 × 4.6 mm) was employed. The injector was a 20-μl Rheodyne valve.

Chromatograms were obtained through the photodiode array detector at 340 nm. An isotropic method was employed, using a mobile phase composed of acetonitrile/water (60/40) at 1 ml/min and applying helium spurring (30 ml/min) to remove dissolved gases. The temperature was kept constant at 35°C.

*Electrochemical measurements.* All the studies were carried out in aprotic media (dimethylformamide containing 0.1 M tetrabutylammonium perchlorate) or in aqueous media (ethanol/Britton Robinson buffer, pH 7.4, KCl) using an electrochemical BAS equipment Model 50W. A glassy carbon electrode, an Ag/AgCl-electrode, and a platinum wire electrode were used as the working electrode, reference electrode, and auxiliary electrode, respectively. Consequently, oxidation peak potential values were measured against an Ag/AgCl reference electrode (Nuñez-Vergara et al. 1997, 1999). Drugs were used at a 0.5 mM concentration.

*Parasites.* *T. cruzi* epimastigotes (CL Brener, Tulahuen, and LQ strains), from our collection, were grown at 28°C in Diamond's monophase medium as reported earlier (Aldunate et al. 1986), with blood replaced by 4 μM hemin. Fetal calf serum was added to a final concentration of 4%. For parasites, 8 × 10⁶ cells correspond to 1 mg of protein or 12 mg of fresh weight.

*Oxygen uptake.* The parasites were harvested at the fourth or fifth day of growth by centrifugation at 500g for 10 min and then washed and resuspended with 0.05 M potassium phosphate buffer, pH 7.4, containing 0.107 M sodium chloride. Respiration measurements were carried out polarographically with a Clark No. 5331 electrode (Yellow Spring Instruments) in a Gilson 5/6 oxygen meter (Letelier et al. 1990). The chamber volume was 2 ml and the temperature was 28°C. The number of parasites used for the assays was equivalent to 2 mg of protein. Drugs were added at a 100 μM final concentration in DMSO. Control respiration was 35 ± 5 n-at. oxygen/min/mg of protein. Values are expressed as the mean ±SD of three or more independent experiments.

*Epimastigote growth inhibition.* Four to five different concentrations of each drug dissolved in dimethylsulfoxide were added to a suspension of 3 × 10⁶ *T. cruzi* epimastigotes/ml (Tulahuen and LQ strains and CL Brener). Final concentrations in the culture growth were between 10 and 250 μM for each drug. Parasite growth was followed by nephelometry for 10 days (Ferreira et al. 1988; Aldunate et al. 1992). No toxic effect of dimethylsulfoxide (DMSO) alone was observed.

The growth constant (Kc) for each drug concentration employed and the control was calculated using the epimastigote exponential growth curve (regression coefficient >0.97, P < 0.05). The slope resulting from plotting the Ln of nephelometry lecture versus time corresponds to the Kc (hours⁻¹). The IC₅₀ is defined as the drug concentration needed to diminish the control growth Kc in 50%, calculated by linear regression analysis from the Kc values at the employed concentrations.

*Drug toxicity.* To perform toxicity determinations, parasite suspensions (3 × 10⁶ cells/ml of CL Brener epimastigotes suspended in Diamond media) were incubated for 2 and 24 h at 28°C, with drugs at a 100 μM concentration in DMSO. Changes in parasite motility and shape were microscopically (40× magnification) observed (Letelier et al. 1990).

Toxicity grades expressed as 0, 1, 2, 3, and 4 represent the sequential changes in motility, shape, and lysis of parasites (Letelier et al. 1990).

No effect on cell growth, oxygen consumption, or drug toxicity attributable to DMSO was observed at the maximum concentration used of 2%.

Statistical analysis. Pearson's correlation and linear regression analysis were performed using Prism Graphpad software from Graphpad Software Inc.

**RESULTS AND DISCUSSION**

Nifurtimox, benznidazole, and many 1,4-dihydropyridines, such as nicardipine and felodipine, have inhibitory effects upon epimastigote growth and oxygen uptake (Maya
The oxazolo(thiazolo)pyridine derivatives evaluated in this work share some structural similarity with dihydropyridines (Fig. 1). To determine the inhibitory ability of these compounds, we investigated their effect on epimastigote growth in culture and respiration.

Table I shows oxazolo(thiazolo)pyridines effect on the growth of *T. cruzi*, CL Brener, epimastigotes. The inhibition is expressed as the IC₅₀ (see culture growth inhibition under Materials and Methods for experiments and calculations). A global dose-response dependence trend was observed. The 3-chlorophenyl oxazolopyridines 2, 3, and 4 and the thio analogue 8 are more potent inhibitors than the nitro 5 and 6 and the unsubstituted furyl 7 derivatives. These potency values are comparable to that of nifurtimox and benznidazole, which have IC₅₀ of 10 and 20 μM, respectively, under the same experimental conditions.

With these results, some general structure–activity relationships observations can be made. The presence of the chlorophenyl moiety seems to be important for antitrypanosomal activity. Nevertheless, if the comparison is restricted to the 3-chlorophenyl-oxazolopyridines 1 to 4, an influence on activity is observed for the size (lipophilicity) of the R² substituent, attached to the ester group at position C-8. The presence of a carbonyl function at position C-3 also induces enhancement of the antiparasitic potency (compound 4 vs compound 1).

Values for the inhibition of parasite respiration by the same derivatives, at 100 μM concentration, are also included in Table I. When parasite growth and respiration inhibitions are studied together for all the compounds, a reasonably good positive correlation is found (Pearson's correlation coefficient, 0.8883, *P* < 0.005), indicating that the respiratory chain is an important target for this type of compound (Fig. 2A, Table I). When the lipophilicity of the substituents is considered (Table I, Fig. 2B), it becomes clear that an increase in lipophilicity increases the inhibitory action of these

<table>
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<tr>
<th>Compound</th>
<th>Ar</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>R¹</th>
<th>R¹</th>
<th>Type</th>
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<td>3-Cl</td>
<td>O</td>
<td>H₂</td>
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<td>Methyl</td>
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<td>H₂</td>
<td>H</td>
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<td>H₂</td>
<td>H</td>
<td>Benzyl</td>
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<td>O</td>
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<td>5</td>
<td>phenyl</td>
<td>3-</td>
<td>O</td>
<td>H₂</td>
<td>H</td>
<td>Methyl</td>
<td>nitrophenyl-oxazolopyridines</td>
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<tr>
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<td>3-Cl</td>
<td>S</td>
<td>H₂</td>
<td>H</td>
<td>Methyl</td>
<td>chlorophenyl-thiazolopyridine</td>
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**FIG. 1.** Chemical structures of oxazolo(thiazolo)pyridines.
TABLE I
Effect of Oxazolo(thiazolo)pyridine Derivatives on Culture Growth, Oxygen Uptake, and Toxicity upon Trypanosoma cruzi Epimastigotes (CL Brener) and Oxidation Potentials and the Lipophilicity of Drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Inhibition of epimastigote growth ( [IC_{50} \mu M] )</th>
<th>Inhibition of respiration (%)</th>
<th>Toxicity (h)</th>
<th>Peak potential( ^c )</th>
<th>Lipophilicity( ^d )</th>
</tr>
</thead>
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<tr>
<td>3</td>
<td>8.3 ± 1.0</td>
<td>70.6 ± 6</td>
<td>0</td>
<td>938</td>
<td>10.9 ± 0.9</td>
</tr>
<tr>
<td>8</td>
<td>9.6 ± 0.6</td>
<td>90.0 ± 4</td>
<td>3</td>
<td>928</td>
<td>8.7 ± 1.1</td>
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<tr>
<td>4</td>
<td>14.6 ± 2.5</td>
<td>68.5 ± 7</td>
<td>2</td>
<td>1126</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>14.0 ± 1.2</td>
<td>24.5 ± 4</td>
<td>1</td>
<td>948</td>
<td>20.0 ± 1.3</td>
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<tr>
<td>6</td>
<td>287.4 ± 65.5</td>
<td>10.0 ± 3</td>
<td>1</td>
<td>950</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>5</td>
<td>214.6 ± 43.7</td>
<td>10.0 ± 3</td>
<td>1</td>
<td>978</td>
<td>6.8 ± 1.1</td>
</tr>
<tr>
<td>7</td>
<td>147.8 ± 10.7</td>
<td>8.0 ± 3</td>
<td>1</td>
<td>1158</td>
<td>17.0 ± 2.3</td>
</tr>
<tr>
<td>1</td>
<td>147.8 ± 13.1</td>
<td>5.0 ± 2</td>
<td>1</td>
<td>1068</td>
<td>14.3 ± 1.5</td>
</tr>
</tbody>
</table>

\( ^a \)See Fig. 1 for chemical structures.
\( ^b \)The \( IC_{50} \) corresponds to the concentration of drug needed to inhibit 50% of the control culture growth.
\( ^c \)Calculated with respect to control (35 ± 5 n-at, oxygen/min/mg protein) at a 100 \( \mu M \) drug concentration at 28°C.
\( ^d \)Toxicity grades expressed as 0 (Control); 1, 2, 3, and 4 represent the sequential changes in motility, shape, and lysis of parasites at a 100 \( \mu M \) drug concentration.
\( ^e \)\( E_p \) is the anodic peak potential value measured in aqueous media or in dimethylformamide expressed in millivolts.
\( ^f \)\( K_p \) is the apparent partition coefficient octanol-buffer, pH 7.4.

Note. All values are expressed as the mean ± SD of three or more independent experiments. For further details see Materials and Methods.

Compounds. Thus, the chlorophenyl group increases inhibition compared with the nitrophenyl substituent. It also should be noted that the values of respiratory inhibition induced by compounds 3 and 8 are inverted with respect to those found for growth inhibition. The greater potency of 8 with respect to its parental compound 3 can be explained by: (i) the higher lipophilicity of the sulfur atom of the thiazolo derivative 8, with respect to that of the oxygen atom of the oxazolo derivative 3, (ii) the possible oxidation of 8, withdrawing some oxygen of the medium during the experiment, or (iii) the incipient formation of the sulfone of 8, which could display a higher potency than the parent compound as a respiratory inhibitor.

Table I also shows the results of toxicity measured as

FIG. 2. (A) Correlation between oxygen uptake inhibition and culture growth inhibition at 10 \( \mu M \) oxazolo(thiazolo)pyridines on T. cruzi epimastigotes. Pearson's correlation and regression coefficients are 0.8883 and 0.7891, respectively (\( P = 0.0032 \)). (B) Relationship between apparent partition coefficient and percentage of inhibition of cultures at 10 \( \mu M \) concentration of oxazolo(thiazolo)pyridines. Oxygen uptake inhibition and apparent partition coefficient obtained from Table I.
changes in motility, shape, and lysis induced by the oxazo-
lo(thiazolo)pyridine derivatives at 2 and 24 h. The most
toxic compounds at 24 h were compound 3, 8, 4, and 2.
These results agree reasonably well with those for growth
inhibition, indicating that the changes observed are mainly
due to the inhibition of the respiratory chain of the parasites
and their energy production.

To compare strain susceptibilities, studies with Tulahuen
and LQ strains of T. cruzi were conducted. Results similar
to those reported in Table I were obtained (data not shown).
These studies are consistent with previous work where drugs
showing inhibition of the respiratory chain also showed no
important differences among different strains of T. cruzi
(Aldunate et al. 1986, 1992; Ferreira et al. 1988).

In order to substantiate the biological results we have
conducted some additional studies. Thus, the apparent parti-
tion coefficients (Papp = octanol/phosphate buffer, pH 7.4)
and retention times of all the derivatives were determined
(Table I). A good correlation between the above-mentioned
parameters was found. These results are consistent with the
fact that there is a close parallel between the retention of
drugs on reverse-phase high-pressure liquid chromatography
columns and the octanol–water partition coefficients. All
compounds exhibited Papp's higher than unity, evidencing
the lipophilic character of the studied derivatives.

In Fig. 2B, the correlation between the percentage of
inhibition cultures and the apparent partition coefficients
of octanol–phosphate buffer, pH 7.4, is shown. Two types
of linear correlation were found: a first group of derivatives
represented by the chlorophenylloxazolopyridines 2 and 3,
furyloxazolopyridine 7, and chlorophenylthiazolopyridine 8,
with \( r = 0.98 \); a second group \( r = 0.95 \) including the
nitrophenyloxazolopyridines 5 and 6; and the other chloro-
phenyloxazolopyridines 1 and 4, compound 4 being the most
lipophilic derivative, with an apparent coefficient of 20
(Table I). At present, we have no explanation for the two
types of linear correlations. Nevertheless, lipophilicity,
which indicates penetration of the drugs into the parasite,
is important for respiration inhibition, but other factors,
such as calcium homeostasis, might also be involved. However,
two well-known channel calcium antagonists, verapamil and
diltiazem, which can inhibit epimastigote calcium uptake,
do not have trypanocidal activity. Other dihydroxypyridine
derivatives with trypanocidal activity, such as nifedipine and
nicardipine, do not have inhibitory activity on parasite cal-
cium homeostasis, supporting the idea that inhibition of
respiration or other factors, but not calcium homeostasis, is
involved (Nuñez-Vergara et al. 1997).

Also, we have correlated the pharmacological effects and
the ease of the derivatives to becoming oxidized. As can be
seen from Table I, when all the compounds are globally
considered, they showed no strict correlation \( r^2 = 0.62 \)
between oxidation peak potential and corresponding growth
inhibitory effects on T. cruzi epimastigotes. Nevertheless,
if only aqueous \( E_p \) values are taken into account and the
comparisons are made excluding those with high lipophilicity
(Table I, compounds 1, 4, 5, and 6), a good correlation
between parasite growth or respiration inhibition \( r^2 = 0.87 \)
and the corresponding oxidation ease for each compound
can be observed. This could support the recent results that
we reported for several 3-chlorophenyl-1,4-dihydropyr-
dines, which showed a good correlation between toxic effects
upon T. cruzi and ease of oxidation (Maya et al. 2000).

Much extensive chemical and pharmacological work must
be done in order to design, synthesize, and evaluate new
molecules, to explain thoroughly the structure–activity rela-
tionships and the mechanistic aspects mentioned in this pa-
per. Nevertheless, it can be concluded that within the family
of oxazolo(thiazolo)pyridines related to those evaluated in
this work, compounds can be found displaying significant
inhibitory effects on culture growth and oxygen uptake, as
well as toxic effects inducing changes in shape and motility
or lysis on the parasites. They constitute a new structural
category of potentially useful anti-Chagas agents.

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REFERENCES

Aldunate, J., Ferreira, J., Letelier, M. E., Repetto, Y., and Morello, A.
1986. 3-Butyl-4-hydroxy anisole, a novel respiratory chain inhibitor:
Effects on Trypanosoma cruzi epimastigotes. *FEBS Lett.* 195,
295–297.

Effects of hydroquinones on intact Trypanosoma cruzi epimastigotes.
*Comp. Biochem. Physiol.* 103C, 97–100.

Caballero, E., Puebla, P., Sánchez, M., Medarde, M., Morán del Prado,
L., and San Feliciano, A. 1996. Enantiospecific synthesis of dihydro-

Chiari, E., Oliveira, A., Raslan, D., Mesquita, A., and Tavares, K.


