BIOCHEMICAL PHARMACOLOGY 73 (2007) 1939–1946

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Activity of "reversed" diamidines against *Trypanosoma cruzi* "in vitro"

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ARTICLE INFO

Article history:
Received 15 January 2007
Accepted 20 March 2007

Keywords:
Chagas disease
Trypanosoma cruzi
Reversed diamidines
Chemotherapy

ABSTRACT

Chagas' disease is an important parasitic illness caused by the flagellated protozoan *Trypanosoma cruzi*. The disease affects nearly 17 million individuals in endemic areas of Latin America and the current chemotherapy is quite unsatisfactory based on nitroheterocyclic agents (nifurtimox and benznidazol). The need for new compounds with different modes of action is clear. Due to the broad-spectrum antimicrobial activity of the aromatic dicationic compounds, this study focused on the activity of four such diamidines (DB811, DB889, DB786, DB702) and a closely related diguanidine (DB711) against bloodstream trypomastigotes as well as intracellular amastigotes of *T. cruzi in vitro*. Additional studies were also conducted to access the toxicity of the compounds against mammalian cells *in vitro*. Our data show that the four diamidines compounds presented early and high anti-parasitic activity (IC50 in low-micromolecular range) exhibiting trypanocidal dose-dependent effects against both trypomastigote and amastigote forms of *T. cruzi* 2 h after drug treatment. Most of the diamidines compounds (except the DB702) exerted high anti-parasitic activity and low toxicity to the mammalian cells. Our results show the activity of reversed diamidines against *T. cruzi* and suggested that the compounds merit *in vivo* studies.

1. Introduction

Aromatic diamidines such as pentamidine isethionate, diminazene aceturate and furamidine are DNA minor groove-binding ligands, which present broad-spectrum antimicrobial activity [1]. Pentamidine (Pentacarinat®-Rhodia) first synthesized as a synthetic analog of insulin, represents the only compound from the aromatic diamidine class that is extensively used in the clinic. Pentamidine is used to treat early stage of African trypanosomosis, antimony-resistant leishmaniasis and *Pneumocystis jiroveci* infection, mostly in AIDS patients [2,3]. Although possessing high activity *in vitro* and *in vivo* against fungi, amoeba, bacteria and protozoan parasites, these compounds lack oral bioviability, which leads to important limitations to their use [1,4]. To overcome these limitations, prodrugs have been developed and one such compound, the methamidoxime prodrug of furamidine (DB289), is currently undergoing phase III clinical trials against human African trypanosomiasis [3]. Parasites from the Tripanosomatidae family such as *Trypanosoma brucei*, *Trypanosoma cruzi* and various species of *Leishmania* cause a variety of important diseases in humans and other mammals, being responsible for considerable human mortality and morbidity in developing countries [5].

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.03.020

T. cruzi is the etiological agent of Chagas' disease, a zoonosis, which is considered a major public health problem in the developing countries of Central and South America [6]. The most important transmission mechanism of this parasite among its hosts is by hematophagous reduviid vectors and the overall prevalence of human infection is about 17 million cases. Furthermore, approximately 120 million people are at risk of contracting the infection [7]. Myocardial damage is one of the most important pathological features responsible for the high morbidity and mortality rates. The current chemotherapy is mostly based on nitrofurans and nitroimidazoles, such as nifurtimox and benznidazole. These type compounds were empirically developed over three decades ago and are not very effective for the treatment of chronic disease [8,9].

Although much attention has being devoted to the trypanocidal effect of aromatic diamidines against African trypanosomosis, few studies have evaluated their effect against *T. cruzi*. Furamidine and its *N*-phenyl substituted analogue (DB569) display trypanocidal activity against *T. cruzi* and although both compounds have equivalent DNA binding properties; DB569 exhibits higher activity against a variety of strains and stages of *T. cruzi in vitro*, with inhibitory values in the low-micromolar range [10]. The *N*-phenyl substituted analog reduced the cardiac parasitism of *T. cruzi*-infected mice and also displayed increased mouse survival rates [11], encouraging additional studies with aromatic diamidines against this parasite. In this context, the present study aims to investigate the microbicidal efficacy of four different reversed diamidines and one diguanidine against the relevant parasite forms of *T. cruzi* found in the mammalian hosts the intracellular amastigotes and bloodstream trypomastigotes.

2. Materials and methods

2.1. Drugs

The synthesis of DB702 and the diguanidine DB711 have seen reported [12] and the synthesis of DB786, DB811 and DB889 was achieved by the same approach (Fig. 1). Stock solutions (5 mM) of the drugs were prepared in DMSO (dimethyl sulfoxide) and fresh dilutions were prepared extemporaneously.

2.2. Cell cultures

For both infection and cytotoxic assays, Vero cell lineage cells (from green monkey kidney) were seeded at a density of 10^5 or 5×10^4 cells/well into 24- and 96-well culture plates, respectively, and sustained in RPMI 1640 medium (Roswell Park Memorial Institute, Sigma–Aldrich, USA) supplemented with 5% fetal bovine serum and 1 mM L-glutamine. Primary cultures of peritoneal mouse macrophages, also assayed for drug toxicity, were obtained as described elsewhere [13], seeded at a density of 5×10^4 cells/well into 96-well culture plates and sustained in Dulbecco's modified medium supplemented with 5% fetal bovine serum and 4 mM L-glutamine (DMES). All the cell cultures were maintained at 37 °C in an atmosphere of 5% CO_2 and air and the assays were run three times at least in duplicates.

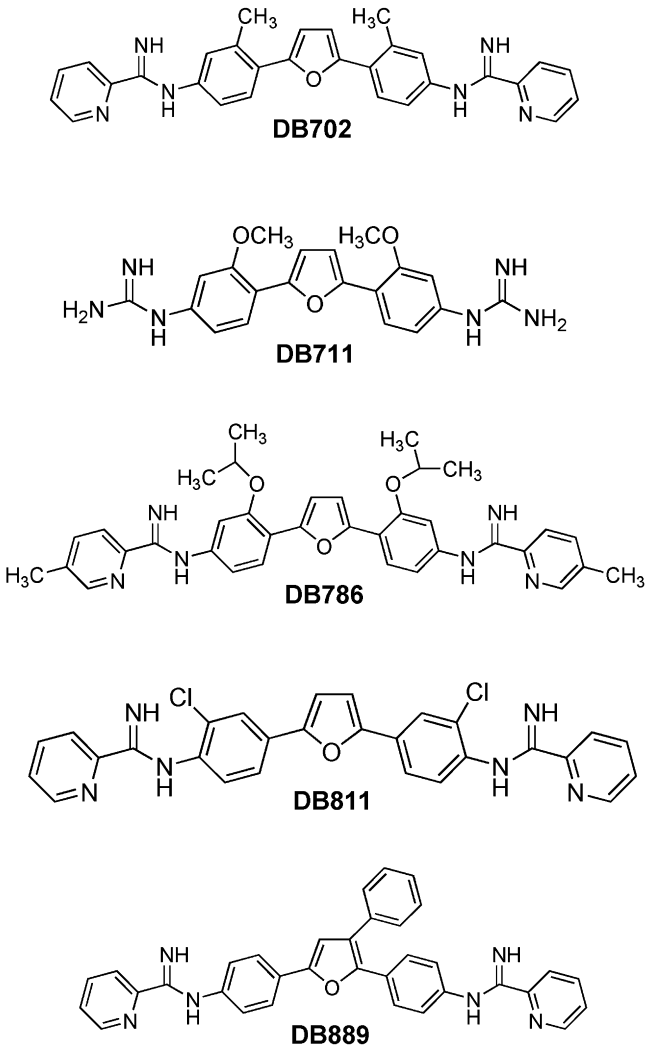

Fig. 1 – Structures of the five drugs used in this study.

2.3. Parasites

Y (moderately resistant to benznidazole and nifurtimox) [14] and Dm28c stocks of *T. cruzi*, representatives of biodemes II and I, respectively; were used throughout the experiments. Cell culture-derived trypomastigotes (Dm28c clone and Y strain) were isolated from the supernatant of Vero cells, which have been previously infected with trypomastigote forms [10]. Bloodstream trypomastigotes from Y strain were harvested by heart puncture from *T. cruzi*-infected Swiss mice at the parasitaemia peak day [15]. All procedures were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (CEUA 0099/01), resolution 242/99.

2.4. Trypanocidal assays

For the analysis of the effect of the drugs upon the bloodstream trypomastigote forms, isolated parasites were incubated at 4 °C for 2 and 24 h in the presence of increasing doses (0.043–32 μM) of each compound diluted in DMES or in whole blood collected from *T. cruzi*-infected mice. After drug