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Evaluation of proline analogs as trypanocidal agents through the inhibition of a *Trypanosoma cruzi* proline transporter

Melisa Sayé[a], Lucía Fargnoli[b], Chantal Reigada[a], Guillermo R. Labadie[b,c], Claudio A. Pereira[a,*]

[a] Laboratorio de Parasitología Molecular, Instituto de Investigaciones Médicas "A. Lanari", IDIM-CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Instituto de Química Rosario, UNR, CONICET, Suipacha 531, S2002LRK Rosario, Argentina
[c] Departamento de Química Orgánica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina

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ABSTRACT

Background: *Trypanosoma cruzi*, the etiological agent of Chagas disease, uses proline as its main carbon source, essential for parasite growth and stage differentiation in epimastigotes and amastigotes. Since proline is involved in many essential biological processes in *T. cruzi*, its transport and metabolism are interesting drug targets.
Methods: Four synthetic proline analogues (ITP-1B/1C/1D/1G) were evaluated as inhibitors of proline transport mediated through the *T. cruzi* proline permease TcAAAP069. The trypanocidal activity of the compounds was also assessed.
Results: The compounds ITP-1B and ITP-1G inhibited proline transport mediated through TcAAAP069 permease in a dose-dependent manner. The analogues ITP-1B, -1D and -1G had trypanocidal effect on *T. cruzi* epimastigotes with IC_{50} values between 30 and 40 μM. However, only ITP-1G trypanocidal activity was related with its inhibitory effect on TcAAAP069 proline transporter. Furthermore, this analogue strongly inhibited the parasite stage differentiation from epimastigote to metacyclic trypomastigote. Finally, compounds ITP-1B and ITP-1G were also able to inhibit the transport mediated by other permeases from the same amino acid permeases family, TcAAAP.
Conclusions: It is possible to design synthetic amino acid analogues with trypanocidal activity. The compound ITP-1G is an interesting starting point for new trypanocidal drug design which is also an inhibitor of transport of amino acids and polyamines mediated by permeases from the TcAAAP family, such as proline transporter TcAAAP069 among others.
General significance: The *Trypanosoma cruzi* amino acid transporter family TcAAAP constitutes a multiple and promising therapeutic target for the development of new treatments against Chagas disease.

1. Introduction

Trypanosoma cruzi is the protozoan parasite that causes Chagas disease, an illness that affects approximately 6 million people in Latin America [1]. Nowadays there are only two drugs approved for Chagas treatment, the nitroimidazole benznidazole and the nitrofuran nifurtimox. Both drugs were discovered over 50 years ago, and despite their high efficacy during the acute phase of the disease, they have limited antiparasitic activity in the chronic phase. Moreover, both drugs have many side effects, such as anorexia, nauseas and dermopathies, highlighting the urgent need for develop new therapies and find new alternative drug targets [2]. Since proline is involved in many essential biological processes in *T. cruzi*, its transport and metabolism are interesting drug targets. Besides its role as carbon source, proline sustains cell invasion and differentiation from intracellular epimastigotes to trypomastigotes [3,4]. There is also evidence of its participation in resistance to nutritional and oxidative stress as well as drug resistance [5,6].

Proline can be obtained from glutamate or it can be acquired from the extracellular medium *via* membrane transporters. Two proline transport systems with different affinities have been biochemically characterized in *T. cruzi*, and one proline transporter has been recently identified [6,7]. The proline transporter, named TcAAAP069, belongs to the first multigenic family of amino acid transporters identified in *T. cruzi*, the TcAAAP family (*Trypanosoma cruzi* Amino Acid/Auxin Permeases) [8]. This protein family is absent in mammals and its members are responsible for *T. cruzi* ability for acquiring essential metabolites like amino acids and polyamines, thus the TcAAAP family constitutes not only an interesting drug target *per se* but also a novel way of entering drugs like toxic analogues or metabolites conjugated with

* Corresponding author: IDIM, Av. Combatientes de Malvinas 3150, 1427 Buenos Aires, Argentina.
E-mail address: cpereira@retina.ar (C.A. Pereira).

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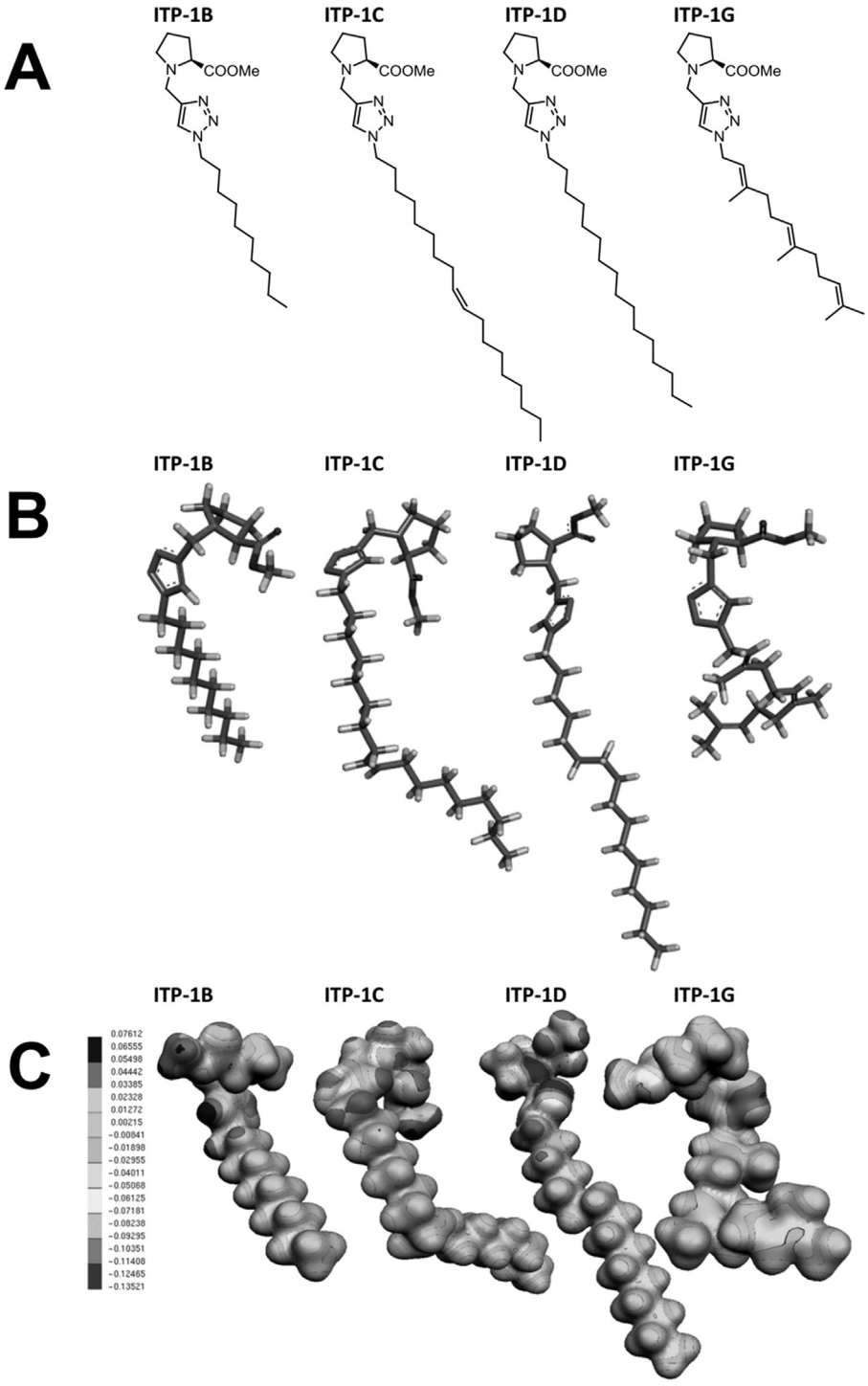

Fig. 1. Proline analogues used in this work. **A**) **Structure of the four synthetic analogues**. ITP-1B: (*S*)-methyl 2-(methyl((1-undecyl-1H-1,2,3-triazol-4-yl)methyl)amino) propanoate; ITP-1C: (S,Z)-methyl 2-(methyl((1-(nonadec-10-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)propanoate; ITP-1D: (*S*)-methyl 2-(((1-heptadecyl-1H-1,2,3-triazol-4-yl) methyl)(methyl)amino)propanoate; ITP-1G: (*S*)-methyl 2-methyl((1-(3E,7E)-4,8,12-trimethyltrideca-3,7,11-trien-1-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)propanoate. **B**) **Global minimum energy conformers of the four synthetic analogues**. The conformation of the compounds is highly dependent on the number of double bounds and substituents of the triazol chain, going from linear for ITP-1B to a twisted conformation for ITP-1G. **C**) **Molecular electronic potential maps**. Molecular electronic potential maps onto a van der Waals surface (isodensity 0.001 e/au^3) for compounds ITP-1B/1C/1D/1G. The grayscale-code comprises the range from − 0.1352 au to + 0.0761 au.

inhibitors into the parasite.

The use of metabolite analogues has been largely exploited, mainly as metabolic pathway inhibitors. One of the most known metabolic inhibitors used in therapy is the eflornithine (α-difluoromethylornithine or DFMO), an ornithine analogue which is one of the four drugs currently used as treatment for Human African Trypanosomiasis (HAT), a disease caused by *Trypanosoma brucei* [9]. The proline analogue L-thiazolidine-4-carboxylic acid (T4C) has been proved to diminish *T. cruzi* viability and also decreased the resistance to nutritional and oxidative stress [5]. Related to the use of chimeric molecules, some uracil amino acid conjugates have been tested as *T. cruzi* dUTPase inhibitors [10].

Several efforts have been also made to design site-directed drugs. The melamine moiety present in melarsoprol, another drug used to treat HAT, directs its entry into the parasite through the TbAT1 (P2) aminopurine transporter [11,12]. Many compounds have been synthesized using this moiety in combination with different trypanocidal agents, such as polyamine analogues, nitroheterocycles, fluoroquinolones, artesunate and eflornithine [13–16]. In addition, many quinone conjugates have been designed in combination with amino acids or polyamines and successfully tested for leishmanicidal activity [17,18].

The aim of this study was to evaluate new proline analogues that may target the *Trypanosoma cruzi* proline transporter TcAAAP069 and