Parasitology International 61 (2012) 604–613

Contents lists available at SciVerse ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

# Nuclease activity and ultrastructural effects of new sulfonamides with anti-leishmanial and trypanocidal activities

Pablo Bilbao-Ramos [a], Cristina Galiana-Roselló [b], M. Auxiliadora Dea-Ayuela [a,b], Marta González-Alvarez [c], Celeste Vega [d], Miriam Rolón [d], Jorge Pérez-Serrano [e], Francisco Bolás-Fernández [a], M. Eugenia González-Rosende [b,*]

[a] Department of Parasitology, Faculty of Pharmacy, University Complutense of Madrid, Plaza Ramón y Cajal s/n, 28040-Madrid, Spain
[b] Department of Chemistry, Biochemistry and Molecular Biology, University CEU-Cardenal Herrera, Avda. Seminario s/n, 46113-Moncada, Spain
[c] Faculty of Pharmacy, University Miguel Hernández of Elche, Ctra. Nacional 332 s/n, 03550-Sant Joan D'Alacant, Spain
[d] Centro para el Desarrollo de la Investigación Científica (CEDIC), Pai Perez 165 c/ Mariscal Estigarribia, Asunción, Paraguay
[e] Department of Microbiology and Parasitology, Faculty of Pharmacy, University Alcalá de Henares, Ctra. Madrid-Barcelona Km. 33.600, 28871-Alcalá de Henares, Spain

ARTICLE INFO

*Article history:*
Received 10 January 2012
Received in revised form 9 May 2012
Accepted 10 May 2012
Available online 2 June 2012

*Keywords:*
*Leishmania spp.*
*Trypanosoma cruzi*
Electron microscopy
Nuclease activity
Sulfonamides

ABSTRACT

Our aim was to evaluate the *in vitro* efficacy of a series of *N*-benzenesulfonamides of amine substituted aromatic rings, sulfonamides **1**–**6**, against *Trypanosoma cruzi* and *Leishmania spp.* and to compare their trypanocidal and leishmanicidal profile. In order to elucidate the probable mechanism of action, the interaction of selected sulfonamides with pUC18 plasmid DNA was investigated by nuclease activity assays. In addition, the cellular targets of these sulfonamides in treated parasites were also searched by transmission and scanning electron microscopy. The most active compounds 4-nitro-*N*-pyrimidin-2-ylbenzenesulfonamide **1a** and 4-chloro-*N*-5-methyl-thiazol-2-yl-benzenesulfonamide **2d** displayed significant *in vitro* activity against *Leishmania spp.* promastigotes, without toxicity to J774 macrophages. Selected sulfonamides **1a**, 4-nitro-*N*-pyrazin-2-yl-benzenesulfonamide **1n** and **2d** were also active against *Leishmania infantum* intracellular amastigotes. Compounds **1n** and **2d** showed nuclease activity in the presence of copper salt analogous to our previous results with sulfonamide **1a**. Mechanistic data reveal the involvement of a redox process. Evidence for the formation of reactive oxygen species (ROS) responsible for DNA strand scission is provided for sulfonamides **1a**, **1n** and **2d**. Transmission electron microscopic (TEM) analysis of *L. infantum* promastigotes treated with compounds **1a**, **1n** and **2d** shows an overall cellular disorganization effects which are mainly addressed to DNA bearing structures such as the nucleus, mitochondria and kinetoplast. Disruption of double nuclear membrane and loss of cellular integrity along with accumulation of cytoplasmic electrodense bodies were also frequently observed.



## 1. Introduction

Protozoa of the order Kinetoplastida are the causative agents of a number of human and animal diseases including Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania spp.*) [1]. These infections have a large disease burden [2,3]. However, few therapeutic agents are currently available. Moreover, many of them produce adverse side effects, in certain cases with high toxicity, require inconvenient routes of administration, long-term treatments and show low activity in immunosuppressed patients [4–6]. In addition, the widespread development of resistance by some parasite strains such as *T. cruzi* resistant to benznidazole and nifurtimox and *Leishmania* to antimonial compounds constitutes an important health problem [7,8]. Therefore there is an urgent need for the discovery of new therapeutics displaying antitrypanosomal and leishmanicidal activities [9,10].

It is well-known that the sulfonamide pharmacophore is an important structural core in medicinal chemistry that shows a broad spectrum of pharmacological activities. Several compounds containing the sulfonamide scaffold have been used as antimicrobial drugs [11,12], diuretics [13,14], hypoglycemics [15,16], antithyroid agents [17], antitumor [18–21], antiviral drugs [22–24] and a number of other biological activities. In addition, the antiparasitic activity of several benzenesulfonamides has been reported [25–33]. Thus *in vitro* anti-leishmanial and trypanocidal effects of compounds containing the sulfonamido moiety have been shown [34–44]. However, a limited number have been tested in a murine animal model and neither of them displayed significant *in vivo* activity. In this context, we have previously demonstrated the anti-leishmanial *in vivo* efficacy of 4-nitro-*N*-pyrimidin-2-ylbenzenesulfonamide **1a** and 4-nitro-*N*-pyrazin-2-yl-benzenesulfonamide **1n** against *L. infantum* [45].

Herein our initial work on a series of *N*-substituted benzenesulfonamides **1**–**6** with antiprotozoal activity against *L. infantum*

* Corresponding author. Tel.: +34 961 369 000; fax: +34 961 395 272.
*E-mail address:* eugenia@uch.ceu.es (M.E. González-Rosende).

1383-5769/$ – see front matter 
doi:10.1016/j.parint.2012.05.015

has been extended to four parasites: *Leishmania guayanensis*, *Leishmania amazonensis* and *Leishmania braziliensis*, ethiological agents responsible for most of the cases of cutaneous and mucocutaneous leishmaniasis (CL, MCL) [46], and *T. cruzi*, the causative agent of Chagas disease, the largest parasitic disease burden on the American continent [47] (Table 1). In this study, we report on the *in vitro* activity spectrum of sulfonamides **1**–**6** against both *Leishmania spp.* promastigote forms and *T. cruzi* epimastigotes. The most interesting compounds were also investigated on intracellular amastigote forms of *L. infantum*.

Nuclease activity studies of selected **s**ulfonamides were carried out to elucidate the probable mechanism of action. In addition, the cellular targets in parasites of these sulfonamides were sought by transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) analysis.

## 2. Materials and methods

### 2.1. Drugs and reagents

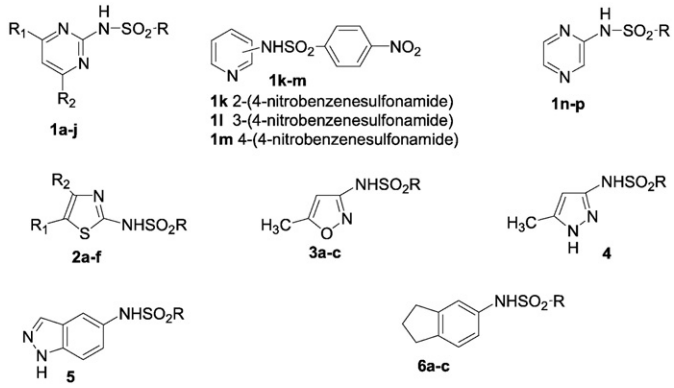
Sulfonamides **1**–**6** were prepared as previously reported by our group [45]. Resazurin sodium salt was obtained from Sigma-Aldrich, St. Louis, USA and stored at 4 °C protected from light. The solution of resazurin was prepared at 2.5 mM in phosphate buffered saline solution (PBS), pH 7.4, and filtered through 0.22 μm prior use. Chlorophenol red-β-D-galactopyranoside (CPRG; Roche, Indianapolis, IN) was dissolved in 0.9% Triton X-100 (pH 7.4). Reference drugs (miltefosine and benznidazole) were purchased from Sigma-Aldrich.

### 2.2. Anti-leishmanial and anti-trypanosomal assays

#### 2.2.1. In vitro leishmanicidal assays

*2.2.1.1. Parasites and culture procedure.* The following species of *Leishmania* were used: an autochthonous isolate of *Leishmania infantum* (MCAN/ES/92/BCN83) obtained from an asymptomatic dog from the Priorat region (Catalonia, Spain), kindly given by Prof. Montserrat Portús (Universidad de Barcelona), *L. braziliensis* 2903, *L. amazonensis* (MHOM/Br/79/Maria) and *Leishmania guyanensis* 141/93, kindly provided by Prof. Alfredo Toraño (Instituto del Salud Carlos III, Madrid). Promastigotes were cultured in Schneider's Insect Medium (Sigma, St. Louis, MO) at 26 °C supplemented with 20% heat-inactivated foetal bovine serum (FBS) (Sigma, St. Louis, MO) and 100 U/mL of penicillin plus 100 μg/mL of streptomycin (Sigma, St. Louis, MO) in 25 mL culture flasks.

**Table 1**
Sulfonamides evaluated against *Leishmania spp.* and *T. cruzi*.




| Compound | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **1a** | 4-$NO_2$Ph | H | H |
| **1b** | 2-$NO_2$Ph | H | H |
| **1c** | $CH_3$ | H | H |
| **1d** | 3-$NO_2$Ph | H | H |
| **1e** | Ph | H | H |
| **1f** | 4-FPh | H | H |
| **1g** | 4-ClPh | H | H |
| **1h** | 4-$CH_3$OPh | H | H |
| **1i** | 4-$NO_2$Ph | $CH_3$ | $CH_3$ |
| **1j** | 4-ClPh | $CH_3$ | $CH_3$ |
| **1k** | – | – | – |
| **1l** | – | – | – |
| **1m** | – | – | – |
| **1n** | 4-$NO_2$Ph | – | – |
| **1o** | 4-ClPh | – | – |
| **1p** | Ph | – | – |
| **2a** | 4-$NO_2$Ph | H | H |
| **2b** | 4-ClPh | H | H |
| **2c** | 4-$NO_2$Ph | $CH_3$ | H |
| **2d** | 4-ClPh | $CH_3$ | H |
| **2e** | Ph | $CH_3$ | H |
| **2f** | 4-$NO_2$Ph | H | $CH_3$ |
| **3a** | 4-$NO_2$Ph | – | – |
| **3b** | 4-ClPh | – | – |
| **3c** | Ph | – | – |
| **4** | 4-$NO_2$Ph | – | – |
| **5** | 4-$NO_2$Ph | – | – |
| **6a** | 4-$NO_2$Ph | – | – |
| **6b** | 4-ClPh | – | – |
| **6c** | Ph | – | – |