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Identification and preliminary structure-activity relationship studies of novel pyridyl sulfonamides as potential Chagas disease therapeutic agents

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ABSTRACT

Chagas disease is a neglected pathology responsible for about 12,000 deaths every year across Latin America. Although six million people are infected by the *Trypanosoma cruzi*, current therapeutic options are limited, highlighting the need for new drugs. Here we report the preliminary structure activity relationships of a small library of 17 novel pyridyl sulfonamide derivatives. Analogues **4** and **15** displayed significant potency against intracellular amastigotes with EC_{50} of 5.4 μM and 8.6 μM. In cytotoxicity assays using mice fibroblast L929 cell lines, both compounds indicated low toxicity with decent selectivity indices (SI) >36 and >23 respectively. Hence these compounds represent good starting points for further lead optimization.

Chagas disease (CD) is a parasitic infection caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*). This neglected disease is endemic to 21 countries across Latin America with more than six million people infected worldwide. A further 70 million people are at risk of infection and approximately 12,000 deaths annually are associated with CD.[1] CD has become an important public health concern in other non-endemic countries across North America, Europe and Asia because of globalization mainly due to the migration of infected individuals to these areas.[2,3]

CD treatment is based on two nitroheterocyclic drugs: nifurtimox (Nf) and benznidazole (Bz), both introduced in clinics >50 years ago.[4] These nitroderivatives require long administration periods and produce adverse side effects, which may result in the discontinuation of the treatment by 20–30% of the patients. Furthermore, there are several naturally resistant strains and very low cure rates (≤20%) once the disease reaches chronic phase.[5,6]

Two inhibitors of the ergosterol biosynthetic route Posaconazole and E1224 (a pro-drug of ravuconazole) were recently evaluated in clinical trials on chronic chagasic patients. Unfortunately, both failed to sustain high therapeutic cure rates after one year of follow up despite promising results in experimental murine and canine models of *T. cruzi* infection.[7,8] Also, the recently concluded clinical trial BENEFIT, revealed that Bz is not able to impair or reduce the progression of the chagasic cardiomyopathy when administered to chronic patients, despite the remarkable reduction of the parasite load assessed through qPCR.[9] Those findings emphasize the importance of pre-clinical studies to investigate the biological activity of novel molecules against *T. cruzi*, aiming to find new alternative treatments that are more tolerable, potent, orally adequate, with broader efficacy, lower costs and reduced administration periods.[10]

Presently, we have identified a 3-pyridyl sulfonamide derivative (compound **1**) active against *T. cruzi* from a high throughput screening of a small set of 100 diverse compounds based on our previous study.[11] Compound **1** showed an EC_{50} (minimal concentration able to reduce the infection index by 50%) of 5.5 μM against intracellular forms of *T. cruzi* (Silvio X10/7 strain), with minimal toxicity to the mammalian hosts (Vero cell line), and comparable activity to nifurtimox, with EC_{50} of 0.9 μM (Fig. 1).

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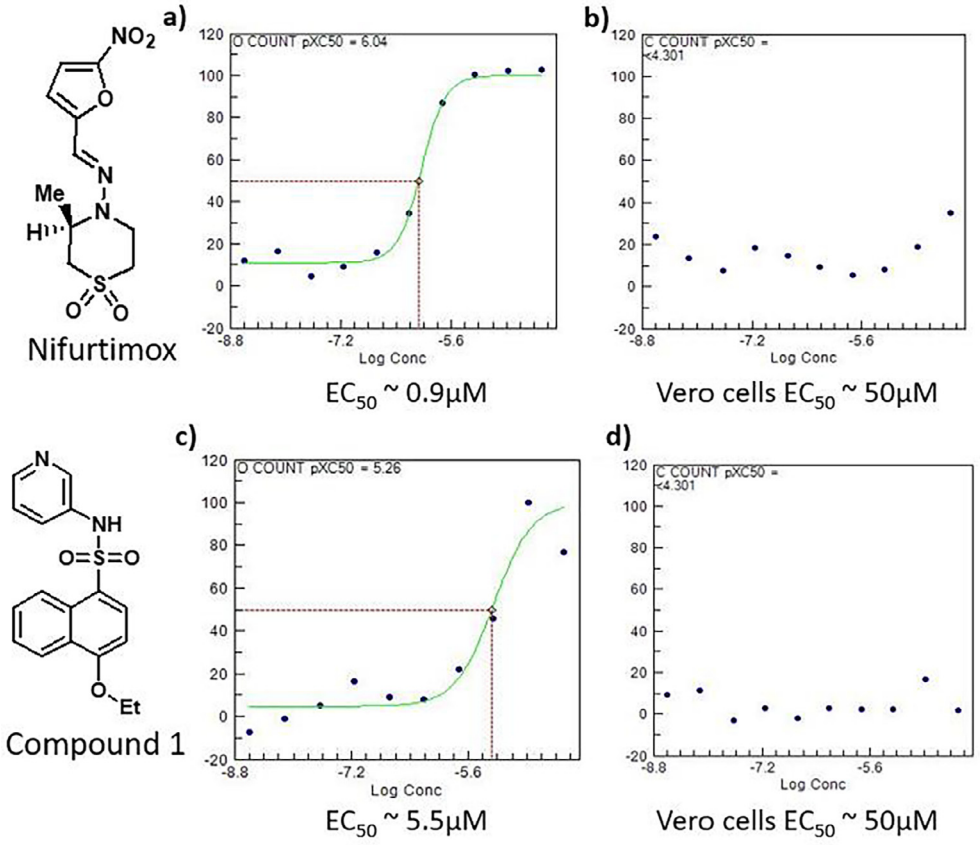

Fig. 1. Silvio strain of *T. cruzi*; Ten-point dose response curves and Vero cells toxicity of Nifurtimox (**a** and **b**) and compound **1** (**c** and **d**) with calculated potency values. The X-axis shows log of compound molar concentrations (M) and Y-axis shows the normalized activity based on the measurement of number of amastigotes per host cell.

As a part of the project related to Chagas disease drug discovery that recommends testing against different parasite strains and forms relevant for mammalian infection (intracellular and bloodstream forms), compound **1** was selected as a lead compound due to its low toxic profile, drug likeness (Lipinski's rule of five) and synthetic accessibility. A small set of 16 analogues (**2**–**17**) were purchased (Table 1) to establish the initial structure activity relationships (SARs). As a part of SAR development, compounds (Supporting information) were selected based on various modifications that differ in steric properties (naphthyl *Vs* phenyl), location of nitrogen on the pyridyl ring (2nd, 3rd and 4th position), alkyl chain length (methoxy, ethoxy and propoxy substituents) and extent of branching (methyl, ethyl, isopropyl and tertiary butyl). Compounds with methylene linker were also selected and parameters such as the EC_{50} values against the intracellular parasites were taken into consideration while establishing the SARs.

Next, regarding the phenotypic biological analysis, the novel analogues were initially assayed using a fixed concentration (10 μM) that corresponds to the EC_{90} of Bz. The non-infected cells (controls) were treated with DMSO. Analogues that reached similar or higher activity than Bz were further evaluated in assays with increasing concentrations (serially diluted) for the determination of the EC_{50}. In both assays, the cultures were maintained at 37 °C for 96 h.[12]

When compound **1** was further screened against intracellular forms of *T. cruzi* but now against Tulahuen strain using Bz as reference drug, the lead compound with 3-pyridyl moiety and 4-ethoxy naphthyl ring **A** displayed lower activity than Bz (Table 1), but sustained a low toxicity profile against the other cell line (L929 cells reaching $LC_{50} > 400$). When a small set of analogues (**2**–**4**) was screened to investigate the contribution of pyridyl moiety towards the anti-*T. cruzi* activity we found that the replacement of 3-pyridyl moiety from compound **1** ($EC_{50} > 25$ μM) either with 4-pyridyl (**2**) or 2-substituted pyridyl (**3**) moieties did not improve the potency of the compounds towards the intracellular forms (see Table 1 regarding the % of reduction levels using a fixed concentration of 10 μM). These results suggest that the position of the nitrogen on the pyridyl ring is not relevant for the anti-parasitic activity.

Replacement of 4-ethoxy naphthyl ring **A** in compound **1** with 6-methoxy naphthyl ring **C** (**4** in Table 1) resulted in over 5-fold increase in potency (EC_{50} of ~5.44 μM). This suggests that the substitution pattern of the naphthyl ring is important for activity.

Then, we have examined the effect of combination of 3-pyridyl moiety with substituted phenyl rings (**D** to **J**) towards the activity. 3-pyridyl moiety in combination with 2,3-dimethyl-4-ethoxy substituted phenyl ring **D** (**5**) and 2,5-dimethyl-4-propoxy substituted phenyl ring **E** (**7**) displayed better trypanocidal activity than the lead compound **1**, with EC_{50} of ~14 μM and ~13 μM, respectively. The compound **6** with 4-pyridyl moiety resulted in complete loss of activity.

Interestingly combination of 3-pyridyl moiety with bulky ethyl (compound **8** with **F**), trimethyl substitutions (compound **9** with **H**), branched isopropyl (i-Pr; compound **10** with **I**) and tertiary butyl (*t*-butyl; compound **11** with **J**) moieties were not tolerated suggesting that dimethyl substitutions (**D** and **E**) along with ethoxy or propoxy or methoxy groups on the phenyl ring are optimal. The substitution pattern on the aromatic ring attached to the sulfonyl group also had a large impact on activity as the methyl substituted phenyl derivatives **5** and **7** showed activity with an EC_{50} of ~14 μM and ~13 μM compared to inactive branched i-Pr and *t*-butyl analogues **10** and **11**. Extension of the aromatic amine with methylene-4-pyridyl ring (**12**) did not result in anti-parasitic effect