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In Vitro Evaluation of Arylsulfonamide Derivatives against *Trypanosoma cruzi*

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Abstract: Chagas disease is caused by the parasite protozoan *Trypanosoma cruzi* (*T. cruzi*) and affects millions of people in over 21 countries in around the world. The main forms of treatment of this disease, benznidazole and nifurtimox, present low cure rates in the chronic phase and often have serious side effects. Herein, we describe the evaluation of the trypanocidal activity of arylsulfonamides. The arylsulfonamides were evaluated in vitro against the amastigote and trypomastigote forms of the parasite. An enantiomerically pure example of arylsulfonamide was also tested. The initial results suggest that the arylsulfonamides evaluated act as DNA binding agents. A moderate activity was monitored against the intracellular forms of *T. cruzi*, with the best compound exhibiting an IC_{50} value at 22 μM and a selectivity index of 120. However, the level of activity was not favorable for progressing towards in vivo studies for Chagas disease.

Keywords: chagas disease; sulfonamides; *Trypanosoma cruzi*; in vitro

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1. Introduction

Parasitic infections are capable of causing serious and life-threating health problems, especially in developing countries, which are affected most by neglected tropical diseases that mainly afflict the poorest communities. The increase in protozoan infections is exacerbated by the deficiency of efficient and safe medicines or vaccines. Chagas disease is an infectious disease caused by the protozoan parasite *Trypanossoma cruzi* (*T. cruzi*) through direct contact with contaminated feces of insects known as Triatominae [1,2]. When contaminated areas are brought into contact with the nose or eyes or licked, infiltration can occur through the mucous membranes, or even through the orifice caused by the bite of triatomine bugs [1,2]. There is an ever growing concern that oral transmission caused from eating contaminated food containing triatomine bugs is becoming the principal mode of transmission [2–4]. Moreover, there have been reports of vertical transmission involving the passing of the parasitic infection to babies during pregnancy from the infected mothers [3,4]. Approximately 7 million people are infected with Chagas disease worldwide, most of which reside in Latin America [3–5]. The acute phase is characteristically silent and most symptoms that do present themselves at this stage are similar to those experienced with common viral infections. Symptoms that do typically manifest may include prolonged fever (more than 5 days), severe weakness, headache and swelling of the legs and face. In the chronic phase, these symptoms are seldom observed; however, many patients frequently develop very serious problems such as digestive disorders and heart failure or cardiomegaly. Approximately 20% of patients suffering from Chagas disease develop a digestive tract disease known as megaesophagus and/or megacolon. Cardiac complications are the most life-threating consequence of the chronic phase, affecting up to 40% of infected patients. These types of cardiac lesions can usually take decades to manifest themselves in the chronic phase of Chagas disease; for this reason, cardiomyopathy is the principal cause of cardiovascular mortality for patients with Chagas disease

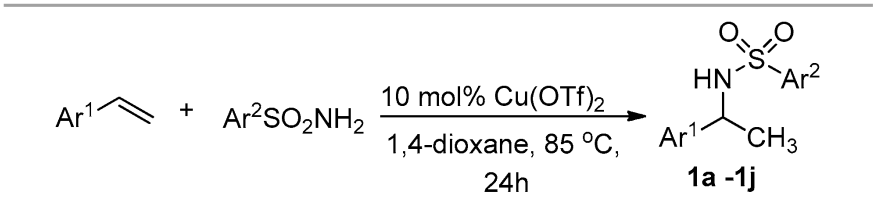
between the ages of 30 and 50 years. At the present moment, only two approved medicines are prescribed for the treatment of Chagas disease: nifurtimox (NFX) and benznidazole (BZ) [6]. Both benznidazole and nifurtimox are nitroheterocyclic compounds that have been on the market since the 1960s. Positive outcomes achieved by these two medicines vary according to and are dependent on the time of treatment, the age of the infected patients, geographic location and at what stage the disease has been diagnosed. Both benznidazole and nifurtimox can achieve high cure rates in the acute phase (up to 80% of cases); however, in the chronic phase, the cure rate drop drastically to between 10 and 20%. Given the negative economic and social impact caused by Chagas disease, the search for new drugs has become increasingly necessary to treat this disease. Arylsulfonamide compounds have demonstrated promising activity against *T. cruzi* [7–17]. The arylsulfonamide motif was an important structural component of piperazines with anti *T. cruzi* activity and improved metabolic stability [7]. Interestingly, arylsulfonamide compounds demonstrated promising activity against *T. cruzi* by targeting the *T. cruzi* proteasome, binding at the interface key subunits important for catalyzing chymotrypsin-like peptidase activity [8]. Furthermore, the *T. cruzi* cytosolic malic enzyme (TcMEc) has been targeted by an arylsulfonamide TcMEc inhibitor, TCMDC-143108, and 14 analogues of this same compound [9]. Impressive results have been described with the sulfonamide series of 4-aminopyridyl-based inhibitors, which were reported to have achieved high picomolar and low nanomolar inhibition of TcCYP51, important for its role in the biosynthesis of ergosterol [10]. Hit-to-lead optimization of a series of benzenesulfonylpiperazine derivatives against *T. cruzi* uncovered arylsulfonamides with <10.0 μM anti-trypanosomal activity and with good predicted ADMET profiles [11]. The 3-nitrotriazole-based sulfonamides displayed significant in vitro antichagasic at low to intermediate nanomolar concentrations and selectivity >200, with some even being 50 times more potent than the reference compound benznidazole [12]. Protozoan carbonic anhydrases are metalloenzymes that are involved in many different critical physiologic and pathologic processes. For this reason, the DNA cloning and purification of α-CA isolated from *T. cruzi* (TcCA) was reported, and subsequent inhibition studies were carried out [13]. Unfortunately, on this occasion, the aromatic sulfonamides were generally weak inhibitors (K_I 192 nM to 84 μM); nevertheless, TcCA remains an interesting target for developing antitrypanosomal drugs with an alternative mechanism of action. The limited number sulfonamides that have been tested in murine animal models motivated a study encompassing the in vivo evaluation of four aromatic sulfonamide derivatives. The study concluded that an isoquinolyl sulfonamide derivative exhibited the highest antiparasitic activity with 72% and 60% reduction in parasitemia [14]. Examples of sulfonamides displaying in vitro biological activities against specific strains of *T. cruzi*, NINOA and INC-5, which are both endemic in Mexico, have also been reported [16]. On this occasion, the 2-methyl-4-quinolinamine ring scaffold was chosen and derivatized with arylsulfonamide groups. The importance of the sulfonamide group for the anti-*T. cruzi* activities of these quinolone derivatives was demonstrated and the lead compound also exhibited better lytic activity on NINOA and INC-5 than the reference drugs, nifurtimox and beznidazole.

Although some promising drug candidates have made their way to the early phases of clinical trials, no new drugs have been approved for Chagas' disease for more than 30 years. In order to meet the demand for new alternative therapies to treat Chagas disease, we have prepared and tested arylsulfonamide derivatives against *T. cruzi*, evaluated their cytotoxic effects against L929 cells, and determined their selectivity index.

2. Results and Discussion

Employing copper(II) triflate in a hidden Brønsted acid catalyzed hydroamidation reaction (Figure 1), the Markovnikov addition of arylsulfonamides to vinylarenes and norbornene was carried according to the previously described methodology [18]. Brønsted acid catalysis has been shown to be effective for the hydroamidation reactions of alkenes. In this case, triflic acid generated in situ using a metal triflate is a very convenient method for this task given that it allows for easier handling and control of the catalytic loading

needed to avoid competitive side reactions. In certain cases, metal catalysts may afford cleaner reactions. The yields of the target compounds **1a**–**m** range from modest to excellent and depend on the electron and steric nature of the substituents. Arylsulfonamides **1a**–**m** were purified via flash column chromatography using silica gel 200–400 mesh and ethyl acetate/hexane as eluent. Both ^{1}H and ^{13}C NMR analysis were performed in order to confirm the formation of the desired products. The proton NMR spectra of compounds **1k**–**m** confirmed that the addition of arylsulfonamides to norbornene favored the exclusive formation of the exo-isomer. High-resolution mass spectrometry was also used to confirm the identity of the final compounds. The rationale for compounds **1k**–**m** allows for the comparison of arylsulfonamides with more aliphatic character to arylsulfonamides **1a**–**j** with an extra aromatic moiety and a higher proportion of Sp2 carbons.



Entry	Ar^1	Ar^2	Yield[b] [%]	Product
1	C_6H_5	4-Me-C_6H_4	92	**1a**
2	4-F-C_6H_4	4-Me-C_6H_4	90	**1b**
3	4-Cl-C_6H_4	4-Me-C_6H_4	78	**1c**
4	4-Me-C_6H_4	4-Me-C_6H_4	62	**1d**
5	2,5-Me_2-C_6H_3	4-Me-C_6H_4	49	**1e**
6	2-napthyl	4-Me-C_6H_4	65	**1f**
7	C_6H_5	4-NO_2-C_6H_4	90	**1g**
8	4-Cl-C_6H_4	4-NO_2-C_6H_4	53	**1h**
9	4-Me-C_6H_4	4-NO_2-C_6H_4	58	**1i**
10	2,5-Me_2-C_6H_3	4-NO_2-C_6H_4	43	**1j**

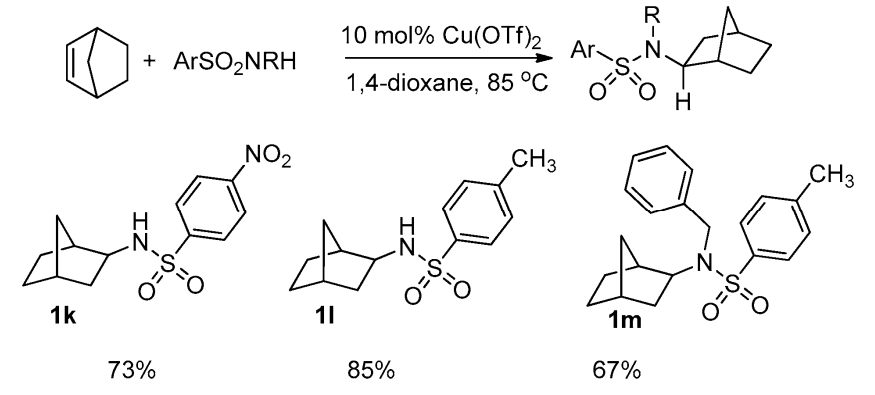
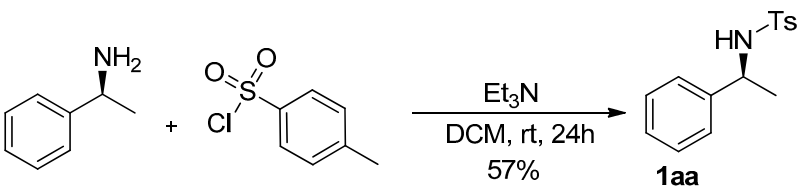


Figure 1. Synthesis of arylsulfonamide derivatives.

The (*S*)-enantiomer of compound **1a** was synthesized by tosylation of commercially available (*S*)-1-phenylethylamine in the presence of Et_3N as a base to provide enantiomerically pure **1aa** (Scheme 1) [19].



Scheme 1. Tosylation of (*S*)-1-phenylethlamine.

Thus, with the target compounds in hand, in vitro bioassays using trypomastigote and amastigote forms of Y-strain *T. cruzi* of β-galactosidase transfected the Tulahuen strain of *T. cruzi*. The whole cell-based approach is an ideal screening methodology given that it enables the evaluation of bioactive compounds against anti *T. cruzi* activity in infected cells whilst simultaneously monitoring their effects on both amastigotes and trypomastigotes in the same system [20–23]. Benznidazole was used as a positive control against *T. cruzi* (IC_{50} = 3.81 μM, CC_{50} = 2381 μM, and SI = 625) and cytotoxicity was determined in mammalian L929 cells (Table 1). Amongst the 14 compounds tested, none were more active than the reference drug benznidazole or presented selectivity indexes above 50, which is the minimum SI for advancing lead compounds to in vivo assays according to DNDi guidelines [24].

Table 1. In vitro trypanocidal activity, cytotoxicity, selectivity index, and physicochemical properties of bioactive sulfonamides.

	In Vitro Activity			Lipinski's Rule of Five							
Compound	**Trypanocide IC_{50} (μM)**	**Cytotoxicity CC_{50} (μM)**	**SI**	**HBA**	**HBD**	**MW (g·mol^{-1})**	**log P**	**Violations**	**TPSA (A^2)**	**Volume A^3**	**NRB**
1a	109 ± 2	145 ± 2	1.3	3	1	276.11	3.41	0	46.17	249.24	4
1aa	116 ± 1	290 ± 3	2.5	3	1	276.11	3.41	0	46.17	249.24	4
1b	48 ± 1	273 ± 2	5.7	3	1	293.36	3.58	0	46.17	254.17	4
1c	39 ± 1	129 ± 2	3.3	3	1	309.82	4.09	0	46.17	262.77	4
1d	45 ± 1	138 ± 2	3.1	3	1	289.40	3.86	0	46.17	265.80	4
1e	26 ± 1	138 ± 2	5.3	3	1	303.43	4.24	0	46.17	282.36	4
1f	28 ± 1	123 ± 2	4.4	3	1	325.43	4.60	0	46.17	293.23	4
1g	101 ± 1	254 ± 3	2.5	6	1	306.34	2.92	0	91.99	256.01	5
1h	22 ± 1	118 ± 2	5.4	6	1	340.79	3.60	0	91.99	269.55	5
1i	34 ± 1	91 ± 1	2.6	6	1	320.37	3.37	0	91.99	272.57	5
1j	22 ± 1	120 ± 2	5.4	6	1	334.40	3.75	0	91.99	289.13	5
1k	30 ± 1	159 ± 2	5.3	6	1	296.35	2.50	0	91.99	247.22	4
1l	116 ± 3	301 ± 5	2.6	3	1	265.38	2.99	0	46.17	240.44	3
1m	27 ± 1	169 ± 2	6.4	3	0	355.50	4.63	0	37.38	329.04	5
Bnz	3.81	2381	625	-	-	260.25	0.78	0	92.75	224.99	5

IC_{50}: 50% inhibitory concentration. CC_{50}: 50% cytotoxic concentration determined using mammalian L929 cells. SI: selectivity index calculated from CC_{50}/IC_{50}. LogP: octanol/water partition coefficient. TPSA: total polar surface area. HBA = hydrogen bond acceptors. HBD = hydrogen bond donors. NRB = number of rotatable bonds.

Regarding the structure activity relationships, it is worth noting that the enantiomerically pure compound **1aa** displayed almost equal potency to **1a** and was only slightly less cytotoxic which suggests that no specific protein target is responsible for the mechanism of action. This observation may also suggest that the evaluated sulfonamides act as DNA binding agents. The most potent sulfonamide **1h** contains a nitro group and chlorine atom and is almost four times more potent than compound **1g**, which differs from **1h** only by the absence of the chlorine atom. Moreover, a similar improvement in trypanocidal activity caused by the presence of chlorine can be observed between compounds **1a** and **1c**. The introduction of either a halogen, methyl, or naphtyl group to the benzyl portion of **1a–j** improved potency and all exhibited sub <100 μM activity, whereas in comparison, unsubstituted **1a** or **1g** displayed activities >100 μM. In the case of norbornyl derivatives **1k–1m**, the nosyl bearing compound **1k** was three times more potent and slightly more selective than its tosyl counterpart **1l**. The addition of a benzyl moiety to **1l** afforded the most potent and selective norbornyl derivative **1m** and this result supports the notion that these compounds are exhibiting mostly DNA binding properties given than the benzyl moiety is a known DNA intercalation switch [25]. Planar and linear derivatives are expected to be DNA intercalators, but compounds with cyclic and aromatic moieties may also bind to DNA in a more complex way. Further investigations are needed to clearly discern the non-covalent binding modes of arylsulfonamide derivatives given that they may bind to the minor or major groove of DNA and these binding properties are strongly dependent on structure. The pharmacokinetic profile of the arylsulfonamide compounds were calculated using the online Molinspiration software. The partition coefficient (log P), topological polar surface

area (TPSA), molecular mass, number of hydrogen bond donors and acceptors and volume are the main physicochemical parameters utilized to provide a reasonable prediction of the potential oral bioavailability of biologically active substances. Good absorption is expected for compounds that have fewer than 10 hydrogen bond acceptors (sum of O and N atoms), have fewer than 5 hydrogen bond donors (OH and/or NH), have a molecular weight less than or equal to 500 $gmol^{-1}$ and a calculated log P of less than 5. The results presented in Table 1 showed that all sulfonamides **1a**–**1m** satisfied the Lipinski rules without any violations. More than two violations that are outside these ranges tend to result in poorly bioavailable drugs. Some extensions to these parameters were later proposed to include TPSA values, which should be less than 140 Å2, log P ranging between −0.4 and +5.6, number of atoms between 20 and 70, and less than 10 rotating bonds. Nosyl derivatives **1g**–**k** have double the hydrogen bond acceptors and TPSA than their tosyl counterparts **1a**–**f**. Although the sulfonamides do not violate the Lipinski parameters, their "druglike" character is compromised by the high proportion of sp2 carbons [26].

3. Conclusions

According to the WHO, 6–7 million people worldwide are infected with *T. cruzi*. The current chemotherapy relies on mainly two drugs, benznidazole or nifurtimox, both of which suffer from toxicity issues and low efficacy for the chronic stage of the disease. It is clear that new anti-Chagas initiatives and campaigns should prioritize lead compounds that are effective against the intracellular forms of the parasite whilst maintaining their efficacy in the chronic stage of Chagas disease. To this end, we describe the evaluation of the trypanocidal activity of arylsulfonamides. In conclusion, the trypanocidal properties of 14 arylsulfonamides were assayed in vitro against trypomastigote and amastigote forms of the parasite. Utilizing the intracellular forms of *T. cruzi*, we found that most of the evaluated substances displayed some promising activity; unfortunately, however, considering the selectivity index, a criterion used for the screening of possible candidates for subsequent in vivo tests, none were deemed suitable candidates for in vivo assays. Therefore, further work is required to improve the potency and selectivity of this series. The inclusion of the arylsulfonamide moiety into trypanocidal compounds has been a common strategy in prior studies and our results suggest that this subunit possibly introduces DNA intercalating characteristics to bioactive compounds. Since DNA binding is usually linked to cytotoxicity, it should be noted that the arylsulfonamide compounds did exhibit significant cytotoxicity to the host cell because of their possible DNA-binding characteristics. This will ultimately present a challenge for developing highly selective arylsulfonamide derivatives with the minimum selectivity index required for in vivo assays. Although the pharmacological target in *T. cruzi* on which arylsulfonamide derivative compounds act has not been conclusively elucidated, given their ability to enhance trypanocidal properties of bioactive compounds, it is interesting to continue the development of these types of compounds.

4. Materials and Methods

All commercial reagents were used as received. Anhydrous solvents were purchased from Sigma Aldrich. Flash column chromatography was performed using silica gel 200–400 Mesh. TLC analyses were performed using silica gel plates using ultraviolet light (254 nm), phosphomolybdic acid, or vanillin solution for visualization. The melting points were uncorrected and were recorded on a Buchi B-540 apparatus [23]. ^{1}H and ^{13}C NMR spectra were acquired using a Bruker DRX 400 MHz instrument (^{1}H at 400 MHz, ^{13}C at 100 MHz). For NMR data, the chemical shifts are reported in δ (ppm) referenced to residual solvent protons and ^{13}C signals in deuterated chloroform. Coupling constants (J) are expressed in Hertz (*Hz*). Full assignment of resonance signals was aided by relevant 2D NMR experiments: COSY, NOESY and HMQC. High resolution mass spectra were obtained on a Shimadzu HPLC-ESI-IT-TOF. Compounds **1aa** and **1a**–**m** were prepared according to the literature procedures [18]. In brief, for a typical catalytic experiment, round-bottom flasks were loaded with copper trifluoromethanesulfonate (0.05 mmol), a Teflon-coated stirrer

bar was used, styrene or norbornene (1.5 mmol) and the requisite sulfonamide (2.0 mmol) were dissolved in 1,4-dioxane (5 mL) and refluxed at 75 °C (18 h). Upon completion, the reaction mixture was concentrated under vacuum, and purified via column chromatography (4:1 hexanes/ethyl acetate). SMILES notations of the arylsulfonamide derivatives were inputted into an online software and subjected to molecular properties prediction using Molinspiration software (software version v2015.01, https://www.molinspiration.com/ accessed on 29 March 2023).

4.1. Charcterization Data

4-Methyl-*N*-(1-phenyl-ethyl)-benzenesulfonamide, **1a**. White crystalline solid. mp. 77–79 °C (Lit.[27] mp. 78–80 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (2H, d, *J* 8.0), 7.18 (5H, m), 7.10 (2H, d, *J* 8.0), 4.84 (1H, d, *J* 6.8), 4.46 (1H, quintet, *J* 6.8), 2.39 (3H, s, Ar-CH_3), 1.43 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.2, 142.0, 137.7, 129.5, 128.6, 127.5, 127.1, 126.1, 53.6, 23.6, 21.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{15}H_{18}NO_2S$: 276.1053; Found: 276.1049.

N-[1-(4-flurophenyl)ethyl]-4-methylbenzenesulfonamide, **1b**. White crystalline solid. mp. 106–107 °C (Lit.[28] mp. 106–108 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.61 (2H, d, *J* 8.4), 7.18 (2H, d, *J* 8.4), 7.10 (2H, m), 6.89 (2H, m) 4.89 (1H, d, *J* 6.8), 4.48 (1H, quintet, *J* 6.8), 2.39 (3H, s), 1.40 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 163.2, 160.9 (d, J_{CF} 245, C-4), 143.3 (C_{ipso}), 137.8 (d, J_{CF} 4, C-1), 137.6, 129.4, 127.8 (d, J_{CF} 8, C-3), 127.1, 115.4 (d, J_{CF} 21, C-2), 52.9, 23.5, 21.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{15}H_{17}FNO_2S$: 294.0959; Found: 294.0951.

N-[1-(4-chlorophenyl)ethyl]-4-methylbenzene-sulfonamide, **1c**. White crystalline solid. mp. 128–129 °C (Lit.[29] mp. 128–129 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.59 (2H, d, *J* 8.4), 7.20 (2H, d, *J* 8.4), 7.10 (2H, d, *J* 8.4), 6.89 (2H, d, *J* 8.4) 5.19 (1H, d, *J* 6.8), 4.44 (1H, quintet, *J* 6.8), 2.39 (3H, s), 1.39 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.3, 140.5, 137.4, 133.2, 129.4, 128.5, 127.6, 127.0, 53.1, 23.5, 21.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{15}H_{17}ClNO_2S$: 310.0663; Found: 310.0666.

N-[1-(4-methylphenyl)ethyl]-4-methylbenzenesulfonamide, **1d**. White crystalline solid. mp. 117–118 °C (Lit.[30] mp. 114–116 °C); R_f = 0.4 (Hexanes/EtOAc, 4:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (2H, d, *J* 8.4), 7.20 (2H, d, *J* 8.4), 7.00 (4H, m), 4.81 (1H, d, *J* 6.8), 4.41 (1H, quintet, *J* 6.8), 2.39 (3H, s), 2.28 (3H, s), 1.40 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.0, 139.0, 137.7, 137.2, 129.4, 129.2, 127.1, 126.0, 53.4, 23.4, 21.5, 21.0; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{16}H_{20}NO_2S$: 290.1209; Found: 290.1212.

N-[1-(2,4-dimethylphenyl)ethyl]-4-methyl-benzenesulfonamide, **1e**. White crystalline solid. mp. 124–126 °C; R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.61 (2H, d, *J* 8.4), 7.17 (2H, d, *J* 8.4), 7.03 (1H, s), 6.85 (2H, m), 5.05 (1H, d, *J* 6.8), 4.67 (1H, quintet, *J* 6.8), 2.37 (3H, s), 2.23 (3H, s), 2.14 (3H, s) 1.36 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.0, 137.8, 137.3, 136.9, 134.3, 131.3, 129.4, 127.1, 127.0, 125.4, 49.6, 23.1, 21.5, 20.9, 18.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{17}H_{22}NO_2S$: 304.1366; Found: 304.1359.

4-Methyl-N-(1-naphthalene-2-yl-ethyl)-benzene-sulfonamide, **1f**. White crystalline solid. mp. 147–149 °C (Lit.[31] mp. 148–149 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (2H, d, *J* 8.0), 7.56–7.43 (7H, m), 7.01 (2H, d, *J* 8.0), 5.35 (1H, d, *J* 6.8), 4.63 (1H, quintet, *J* 6.8), 2.18 (3H, s), 1.32 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.1, 139.1, 137.6, 133.1, 132.7, 129.3, 128.4, 127.9, 127.5, 127.0, 126.1, 125.9, 125.1, 124.1, 53.9, 23.5, 21.3; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{19}H_{20}NO_2S$: 326.1209; Found: 326.1211.

4-Nitro-*N*-(1-phenylethyl)benzenesulfonamide, **1g**. White crystalline solid. 125–126 °C (Lit.[32] mp. 118–120 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.13 (2H, d, *J* 8.8), 7.79 (2H, d, *J* 8.8), 7.13 (3H, m), 7.05 (2H, m), 5.28 (1H, d, *J* 6.8), 4.60 (1H, quintet, *J* 6.8), 1.49 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.7, 146.6, 141.0, 128.6, 128.2, 127.9, 126.2, 123.8, 54.2, 23.6; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{14}H_{15}N_2O_4S$: 307.0747; Found: 307.0741.

4-Nitro-*N*-(1-(4-chlorophenyl)ethyl)benzenesulfonamide, **1h**. White crystalline solid. 122–123 °C; R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.22 (2H, d, *J* 8.8), 7.83 (2H, d, *J* 8.8), 7.16 (2H, d, *J* 8.4), 7.03 (2H, d, *J* 8.4), 5.21 (1H, d, *J* 6.8), 4.57 (1H, quintet, *J* 6.8), 1.46 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.8, 146.4, 139.6, 133.8, 128.8, 128.2, 127.6, 127.4, 53.5, 23.4; *m*/*z* (EI): 340 (M^+, 2%), 327 (45%), 325 (100%), 186 (55%), 154 (73%), 153 (56%), 139 (36%), 138 (56%), 122 (68%), 103 (40%), 77 (25%). HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{14}H_{14}ClN_2O_4S$: 341.0357; Found: 341.0359.

4-Nitro-*N*-(1-(*p*-tolyl-ethyl)benzenesulfonamide, **1i**. White crystalline solid. 94–95 °C; R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.13 (2H, d, *J* 8.8), 7.78 (2H, d, *J* 8.8), 6.92 (4H, m), 5.32 (1H, d, *J* 6.8), 4.55 (1H, quintet, *J* 6.8), 2.21 (3H, s), 1.44 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.6, 146.5, 138.0, 137.8, 129.2, 128.3, 126.1, 123.8, 54.0, 23.5, 20.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{15}H_{17}N_2O_4S$: 321.0904; Found: 321.0899.

4-Nitro-*N*-(1-(2,4-dimethylphenyl)ethyl)benzenesulfonamide, **1j**. White crystalline solid. 125–126 °C; R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.10 (2H, d, *J* 8.8), 7.73 (2H, d, *J* 8.8), 6.80–6.71 (3H, m), 5.22 (1H, d, *J* 6.8), 4.82 (1H, quintet, *J* 6.8), 2.22 (3H, s), 2.18 (3H, s), 1.44 (3H, d, *J* 6.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.6, 146.5, 138.0, 136.1, 129.2, 128.3, 128.1, 125.3, 123.7, 50.0, 23.1, 20.8, 18.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{16}H_{19}N_2O_4S$: 335.1060; Found: 335.1050.

exo-*N*-Bicyclo[2.2.1]hept-2-yl-4-methylbenzene-sulfonamide, **1k**. Crystalline white solid. mp. 129–131 °C (Lit.[33] mp. 138–139 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, *J* 9.0), 7.31 (2H, d, *J* 9.0), 4.67 (1H, d, *J* 7.4), 3.11 (1H, br s), 2.43 (3H, s) 2.18 (1H, br s), 2.09 (1H, br s), 1.77 (1H, ddd, *J* 2.0, 7.0, 13), 1.10–1.60 (7H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.1, 137.9, 129.6, 127.1, 55.6, 42.4, 40.7, 35.5, 35.1, 28.0, 26.3, 21.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{14}H_{20}NO_2S$: 266.1209; Found: 266.1210.

exo-*N*-Bicyclo[2.2.1]hept-2-yl-4-nitrobenzene-sulfonamide, **1l**. Crystalline pale yellow solid. mp. 125–127 °C (Lit.[34] mp. 127–129 °C); R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.37 (2H, d, *J* 12.0), 8.07 (2H, d, *J* 12.0), 4.76 (1H, d, *J* 6.8), 3.22 (1H, br s), 2.23 (1H, br s), 2.21 (1H, br s), 1.68 (1H, ddd, *J* 2.0, 7.0, 13), 1.1–1.6 (7H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 150.1, 146.9, 129.6, 127.1, 56.9, 42.6, 40.8, 35.6, 35.1, 27.8, 26.2; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{13}H_{17}N_2O_4S$: 297.0904; Found: 297.0909.

exo-*N*-Benzyl-*N*-bicyclo[2.2.1]hept-2-yl-4-methylbenzenesulfonamide, **1m**. Crystalline white solid. mp. 84–86 °C; R_f = 0.4 (Hexanes/EtOAc, 4/1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.73 (2H, d, *J* 6.8), 7.40 (2H, d, *J* 6.8), 7.20–7.35 (5H, m), 4.58 (1H, closeAB), 4.38 (1H, closeAB) 3.91 (1H, m), 2.44 (3H, s) 2.10 (1H, br s), 1.94 (1H, br s), 1.77 (1H, ddd, *J* 2.0, 7.0, 13), 1.1–1.6 (7H, m); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.4, 137.9, 129.6, 128.6–127.1, 127.0, 61.2, 47.6, 47.3, 40.5, 37.8, 35.8, 29.5, 27.5, 21.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ Calculated for $C_{21}H_{26}NO_2S$: 356.1679; Found: 356.1671.

4.2. Anti-Trypanosoma cruzi Activity Assay (Amastigotes and Trypomastigotes)

The in vitro anti-*T. cruzi* activity was evaluated on L929 cells (mouse fibroblasts) infected with the Tulahuen strain of the parasite expressing the *Escherichia coli β*-galactosidase as a reporter gene [23]. Briefly, for the bioassay, 4000 L929 cells were added to each well of a 96-well microtiter plate. After an overnight incubation, 40,000 trypomastigotes were added to the cells and incubated for 2 h. Then, the medium containing extracellular parasites was replaced with 200 µL of fresh medium and the plate was incubated for an additional 48 h to establish the infection. For IC_{50} determination, the cells were exposed to each synthesized compound at serially decreasing dilutions and the plate was incubated for 96 h. After this period, 50 µL of 500 µM chlorophenol red beta-D-galactopyranoside (CPRG) in 0.5% Nonidet P40 was added to each well, and the plate was incubated for 16–20 h, after which the absorbance at 570 nm was measured. Controls with uninfected cells, untreated infected cells, infected cells treated with benznidazole at 3.8 µM (positive control), or DMSO 1% were used. The results are expressed as the percentage of *T. cruzi* growth inhibition

in compound-tested cells as compared to the infected cells and untreated cells. The IC_{50} values were calculated by linear interpolation. Quadruplicates were run on the same plate, and the experiments were repeated at least once.

4.3. In Vitro Cytotoxic Test of Trypanocidal Compounds

The active compounds were tested in vitro for the determination of cellular toxicity against uninfected L-929 cells using the alamarBlue® dye [20]. The cells were exposed to compounds at increasing concentrations starting at IC_{50} value for *T. cruzi*. After 96 h of incubation with the tested compounds, the alamarBlue® was added and the absorbance at 570 and 600 nm was measured after 4–6 h. The cell viability was expressed as the percentage of difference in the reduction between the treated and untreated cells. CC_{50} values were calculated via linear interpolation, and the selectivity index (SI) was determined based on the ratio of the CC_{50} value in the host cell divided by the IC_{50} value of the parasite. Quadruplicates were run on the same plate, and the experiments were repeated at least once.

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