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Synthesis, *in vitro* and *in vivo* anti-*Trypanosoma cruzi* and toxicological activities of nitroaromatic Schiff bases

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ABSTRACT

Chagas disease is a major health problem not only in Latin America but also in Europe and North America due to the spread of this disease into nonendemic areas. In terms of global burden, this major tropical infection is considered to be one of the most neglected diseases, and there are currently only two available chemotherapies: benznidazole and nifurtimox. Unfortunately, although these chemotherapies are beneficial in the acute phase of the disease, benznidazole and nifurtimox lead to significant side effects, including hepatitis and neurotoxicity. Therefore, the search for and development of more effective, safe and inexpensive anti-*Trypanosoma cruzi* drugs are required. In this work, a series of 10 nitroaromatic Schiff bases bearing different (nitro) aromatic rings-was synthesized. Subsequently, the *in vitro* and *in vivo* anti-*T. cruzi* activities of the Schiff bases were investigated, as well as the *in vivo* toxicity and the biological effects. The basic structure of the most promising *in vivo* Schiff base, **10** would be useful in the synthesis of new compounds for Chagas disease treatment.

1. Introduction

Chagas disease, which is caused by the protozoan parasite *Trypanosoma cruzi*, is a serious health problem in Latin America and is an emerging disease in nonendemic countries [1]. According to estimates from the World Health Organization updated in March 2017, approximately 6–7 million people are infected worldwide. More than 10,000 people die annually from clinical manifestations of Chagas disease, and 25 million are at risk of acquiring the infection [2].

Although historically, Chagas disease is a poverty-associated disease because transmission of *T. cruzi* occurs mostly in rural areas where humans live in poor-quality houses and in close contact with potential vectors [3], the epidemiological profile of Chagas disease has changed over the last several decades. Factors such as immigration, transmission *via* blood transfusion and pregnancy, and consumption of contaminated food have contributed to the spread of this disease into nonendemic areas, and Chagas disease is becoming a major health threat in Europe and North America [4].

In addition to being a prevalent disease with clinical and epidemiological relevance, Chagas disease is important in terms of its economic impact. The early mortality and incapacity caused by the disease, which often occurs in the most productive populations, result in great economic losses. Further, up to 30% of chronically infected patients develop cardiac alterations, and 10% develop digestive, neurological or mixed alterations approximately 20 years after the initial infection. In this sense, there is a need for specific and long-term treatment, which increases the costs related to the disease [2,5].

Despite being one of the major tropical infections in terms of global burden, Chagas disease is considered one of the most neglected diseases. The currently available chemotherapy is based on two agents introduced to the market in the 1970s: the nitroimidazole compound benznidazole (Benzonidazol, LAFEPE®) and the nitrofuran compound nifurtimox (Lampit®, Bayer Healthcare) (Fig. 1) [6,7]. Both act as prodrugs and exert their activities through the bioreduction of the nitro group *via* nitroreductases-mediated reactions. Oxidative stress, DNA damage and thiol depletion are the major mechanisms responsible for the trypanocidal activity of these substances [8,9]. Unfortunately, although they are beneficial in the acute phase of the disease,

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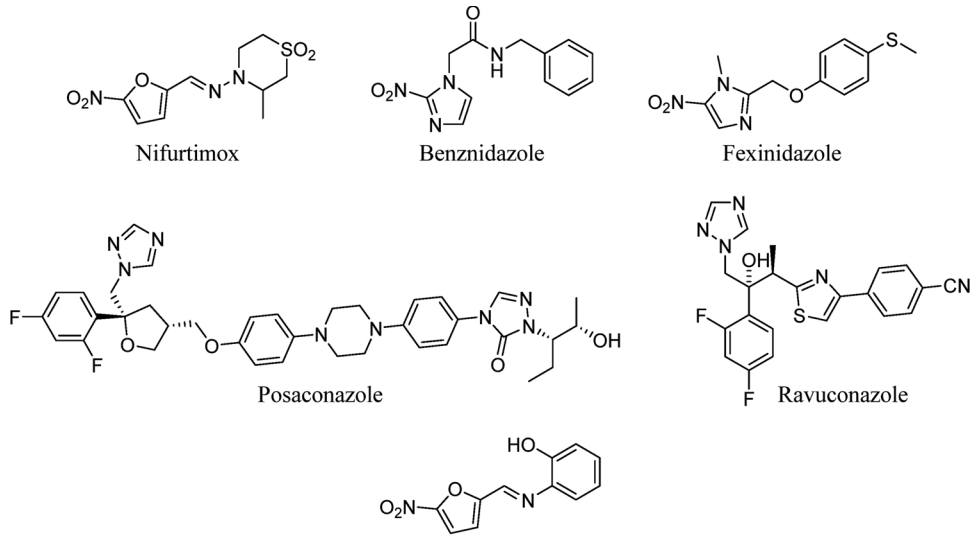


Fig. 1. Chemical structures of nifurtimox, benznidazole, fexinidazole, posaconazole, ravuconazole and 2-(((5-nitrofuran-2-yl)methylene)amino)phenol.

benznidazole and nifurtimox are not uniformly efficacious in the chronic stage, and both lead to significant side effects, including hepatitis and neurotoxicity, which result in a sizable number of individuals not completing a full course of treatment [10,11]. This scenario clearly indicates that research on new anti-*T. cruzi* agents is required.

Over the last decade, some efforts have been dedicated to developing a more efficient treatment for Chagas disease. The BENEFIT trial, carried out in 2004 and 2011, was the largest multicenter, multinational, randomized study ever conducted in patients infected with *T. cruzi*. This study aimed at evaluating the efficacy and safety of benznidazole in reducing clinical outcomes among patients with chronic Chagas cardiomyopathy. However, only modest and unsustained antiparasitic activity was observed [12]. The STOP-CHAGAS, CHAGASAZOL and DNDi-CH-E1224-001 trials evaluated the trypanocidal activity of the ergosterol biosynthesis inhibitors posaconazole and ravuconazole (Fig. 1) in chronic patients. However, these compounds were ineffective and resulted in a higher percentage of treatment failures, thus offering no potential as monotherapies for Chagas disease [13–15]. Finally, fexinidazole (Fig. 1), another nitroheterocyclic compound, has shown promising results and is currently undergoing phase II trials [16]. Although some advances have been made, an effective, safe and inexpensive anti-*T. cruzi* drug is still not available.

Schiff bases are compounds containing an azomethine group, usually obtained by the condensation of primary amines and aldehydes or ketones [17]. These compounds have attracted considerable attention from organic and medicinal chemists due to their broad range of antimicrobial activities, including antifungal, antibacterial, antileishmanial and antiplasmodial activities [18–21]. However, to date, there have been few reports on the trypanocidal activity of this class of compounds. Recently, Martins et al. showed that the Schiff base 2-(((5-nitrofuran-2-yl)methylene)amino)phenol (**1**, Fig. 1) had a promising activity against *T. cruzi*. This compound was as active as benznidazole in inhibiting *T. cruzi* bloodstream trypomastigotes (Y strain) (EC_{50} 14 μmol L^{-1}) [22]. Encouraged by this result, we decided to synthesize ten Schiff bases and determine their *in vitro* and *in vivo* activity against *T. cruzi*.

2. Methods

2.1. Synthesis of Schiff bases

Schiff bases **1–10** were prepared by microwave-assisted condensation between corresponding aldehydes and aromatic amines [23]. The chemicals were solubilized in ethanol and the solutions were irradiated in a CEM Discover® reactor for 2 min. After this period, the reaction mixtures were stored in a refrigerator until the crystallization of the products. The resulting solids were filtered and washed with ice-cold ethanol. Compounds **1**, **2**, and **6–10** were previously synthesized by our research group and their spectroscopic data are in accordance with those published elsewhere [23]. The structures of Schiff bases **3, 4** and **5** were confirmed by IR, ^{1}H and ^{13}C NMR.

2-(((5-Nitrofuran-2-yl)methylene)amino)phenol (1): Mp: 160 °C (dec) (lit.: 158 °C (dec) [24]). IR (neat, cm^{-1}) $\bar{\nu}$: 3357, 3154, 1618, 1585, 1568, 1527, 1487, 1471, 1455, 1397, 1351, 1242, 1168, 1150, 1110, 1018, 971, 956, 941, 884, 821, 804, 760, 749, 739. ^{1}H NMR (200 MHz, DMSO-d_6): 6.84 (t, 1H, J = 7.8 Hz), 6.93 (d, 1H, J = 7.8 Hz), 7.13 (t, 1H, J = 7.8 Hz), 7.28 (d, 1H, J = 7.8 Hz), 7.49 (d, 1H, J = 3.9 Hz), 7.80 (d, 1H, J = 3.9 Hz), 8.74 (s, 1 H), 9.50 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 114.4, 116.6, 116.8, 119.6, 120.9, 129.0, 136.4, 146.4, 151.7, 152.2, 153.7.

4-(((5-Nitrofuran-2-yl)methylene)amino)phenol (2): Mp: 170 °C (dec) (lit.: 183.6 °C (dec) [25]). IR (neat, cm^{-1}) $\bar{\nu}$: 3118, 1621, 1579, 1561, 1528, 1505, 1481, 1455, 1399, 1384, 1348, 1337, 1260, 1169, 1017, 971, 963, 943, 818, 753, 736, 658. ^{1}H NMR (200 MHz, DMSO-d_6): 6.82 (d, 2H, J = 8.7 H_z), 7.27–7.39 (m, 3 H), 7.77 (d, 1H, J = 3.9 H_z), 8.58 (s, 1 H), 9.79 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 114.4, 115.9, 116.4, 123.4, 141.0, 143.6, 152.1, 153.6, 157.9.

***N*-(4-Methoxyphenyl)-1-(5-nitrofuran-2-yl)methanimine (3):** Mp: 122–123 °C (dec) (lit.: 130 °C [26]). IR (neat, cm^{-1}) $\bar{\nu}$: 3143, 2835, 1617, 1575, 1529, 1495, 1456, 1393, 1352, 1239, 1180, 1112, 1024, 952, 899, 867, 837, 813, 792, 738, 672. ^{1}H NMR (200 MHz, DMSO-d_6): 3.78 (s, 3 H), 6.99 (d, 2H, J = 8.8 Hz), 7.34 (d, 1H, J = 3.9 Hz), 7.41 (d, 2H, J = 8.8 Hz), 7.79 (d, J = 3.9 Hz), 8.62 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 55.3, 114.3, 114.6, 116.9, 123.2, 142.4, 144.9, 152.1,

153.4, 159.2.

***N*-(4-Clorophenyl)-1-(5-nitrofuran-2-yl)methanimine (4):** Mp: 157–158 °C (lit.: 158 °C [26]). IR (neat, cm^{-1}) $\bar{\nu}$: 3145, 2958, 1617, 1603, 1574, 1530, 1505, 1487, 1391, 1354, 1321, 1253, 1179, 1091, 1023, 981, 951, 922, 899, 862, 818, 773, 758, 739, 692, 665. ^{1}H NMR (200 MHz, DMSO-d_6): 7.30–7.60 (m, 5 H), 7.81 (d, J = 3.9 Hz), 8.62 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 114.1, 118.4, 123.2, 129.3, 131.8, 148.6, 152.4, 152.5.

2-(((5-Nitrofuran-2-yl)methylene)amino)benzonitrile (5): Mp: 135–136 °C (dec). IR (neat, cm^{-1}) $\bar{\nu}$: 3153, 2228, 1620, 1585, 1567, 1519, 1494, 1468, 1442, 1399, 1351, 1341, 1253, 1162, 1097, 1024, 962, 861, 815, 761, 741, 736, 722, 660. ^{1}H NMR (200 MHz, DMSO-d_6): 7.41–7.58 (m, 3 H), 7.70–7.95 (m, 3 H), 8.71 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 107.4, 113.9, 117.0, 119.1, 119.9, 127.6, 133.5, 134.5, 151.5, 151.7, 152.4, 152.8.

1-(5-Nitrofuran-2-yl)-*N*-phenylmethanimine (6): Mp: 131 °C (lit.: 127.5 °C [27]). IR (neat, cm^{-1}) $\bar{\nu}$: 3143, 1618, 1574, 1526, 1489, 1448, 1390, 1351, 1339, 1319, 1253, 1179, 1161, 1077, 1024, 965, 954, 856, 815, 772, 755, 738, 692. ^{1}H NMR (200 MHz, DMSO-d_6): 7.24–7.53 (m, 6 H), 7.90 (d, 1H, J = 3.8 H_z), 8.61 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 114.1, 117.9, 121.3, 127.5, 129.3, 147.8, 149.8, 152.3, 152.7.

4-((Furan-2-ylmethylene)amino)phenol (7): Mp: 188 °C (lit.: 187–188 °C [28]). IR (neat, cm^{-1}) $\bar{\nu}$: 1627, 1584, 1559, 1505, 1478, 1443, 1367, 1277, 1232, 1198, 1168, 1152, 1102, 1072, 1017, 960, 928, 882, 834, 818, 744, 655. ^{1}H NMR (200 MHz, DMSO-d_6): 6.66 (dd, 1H, J = 3.3 Hz, J = 1.7 H_z), 6.80 (d, 2H, J = 8.7 H_z), 7.04 (d, 1H, J = 3.3 H_z), 7.18 (d, 2H, J = 8.7 H_z), 7.88 (br, 1 H), 8.41 (s, 1 H), 9.54 (br, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 112.4, 115.4, 115.8, 122.4, 142.3, 145.1, 145.7, 152.4, 156.4.

2-((4-Nitrobenzylidene)amino)phenol (8): Mp: 161 °C (lit.: 159–160 °C [29]). IR (neat, cm^{-1}) $\bar{\nu}$: 3321, 1625, 1587, 1514, 1478, 1374, 1341, 1316, 1284, 1231, 1190, 1169, 1142, 1101, 1028, 1009, 965, 940, 849, 836, 787, 757, 730, 683, 671. ^{1}H NMR (200 MHz, DMSO-d_6): 6.85 (t, 1H, J = 7.5 H_z), 6.94 (d, 1H, J = 8.1 H_z), 7.14 (t, 1H, J = 7.5 H_z), 7.29 (d, 1H, J = 7.5 H_z), 8.21–8.41 (m, 4 H), 8.88 (s, 1 H), 9.26 (br, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 116.4, 119.3, 119.5, 123.8, 128.6, 129.8, 137.1, 142.0, 148.6, 151.8, 156.9.

4-((4-Nitrobenzylidene)amino)phenol (9): Mp: 173 °C (lit.: 176 °C [30]). IR (neat, cm^{-1}) $\bar{\nu}$: 3436, 1625, 1597, 1578, 1505, 1443, 1336, 1262, 1206, 1160, 1105, 1009, 973, 952, 890, 849, 831, 817, 783, 748, 719, 688, 673. ^{1}H NMR (200 MHz, DMSO-d_6): 6.83 (d, 2H, J = 8.7 Hz), 7.29 (d, 2H, J = 8.7 H_z), 8.09 (d, 2H, J = 8.8 H_z), 8.30 (d, 2H, J = 8.8 H_z), 8.75 (s, 1 H), 9.71 (br, 1H, OH). ^{13}C NMR (50 MHz, DMSO-d_6): 115.8, 123.1, 123.9, 129.0, 141.6, 142.1, 148.3, 154.6, 157.3.

2-((3-(2-Nitrophenyl)allylidene)amino)phenol (10): Mp: 127 °C. IR (neat, cm^{-1}) $\bar{\nu}$: 3075, 2924, 2854, 1627, 1607, 1584, 1518, 1454, 1341, 1293, 1261, 1235, 1158, 1103, 1041, 991, 959, 802, 786, 756, 741, 696, 673. ^{1}H NMR (200 MHz, DMSO-d_6): 6.81 (t, 1H, J = 7.5 H_z), 6.90 (d, 1H, J = 7.8 H_z), 7.00–7.29 (m, 3 H), 7.53–7.68 (m, 2 H), 7.75 (t, 1H, J = 7.4 H_z), 7.92–8.08 (m, 2 H), 8.55 (d, 1H, J = 8.7 H_z), 9.13 (s, 1 H). ^{13}C NMR (50 MHz, DMSO-d_6): 116.1, 119.5, 124.6, 127.7, 128.4, 130.1, 130.2, 133.2, 133.7, 137.0, 137.9, 148.0, 151.2, 160.4.

2.2. Anti-T. cruzi activity

2.2.1. In vitro assays

2.2.1.1. Cell culture. *T. cruzi* epimastigotes (Y strain) were grown at 28 °C in LIT medium (liver infusion tryptose (LIT) medium containing 20 mg L^{-1} hemin, 10% fetal calf serum and antibiotics (penicillin/streptomycin)) as described previously [31]. After 4 days of growth (*log* phase), the cells were harvested by centrifugation (1000 × *g* at 4 °C) and washed in phosphate buffered saline (PBS) at pH 7.3, and the number of cells was determined in a Neubauer chamber.

2.2.1.2. Drug screening. Cells (10^6 mL^{-1}) were incubated in the presence of serial dilutions of each compound in LIT medium. Since the Schiff bases were diluted in DMSO, an equal volume of this solvent was added to the control. After 4 days of incubation, the number of cells was determined, and the EC_{50} was calculated using the program Excel (Microsoft Office®).

2.2.1.3. Statistical analysis. The experimental data represent the averages ± standard deviations of at least three independent experiments performed in triplicate. Comparisons were conducted using Student's *t*-test in Origin 6.0 software, and data with $p < 0.05$ were considered significant.

2.2.2. In vivo assays

2.2.2.1. Chemicals and solvents used for in vivo studies. Benznidazole: 2-nitro-imidazole-(*N*-benzyl-2-nitro-1-imidazoleacetamide was purchased from LAFEPE, – Laboratório Farmacêutico de Pernambuco, Brazil). Nifurtimox: *N*-(3-methyl-1,1-dioxido-4-thiomorpholinyl)-1-(5-nitro-2-furyl)methanimine was donated by the Drugs for Neglected Diseases initiative. Tween® 80 was purchased from Sigma Aldrich (St. Louis, MO, USA). Polyethylene glycol 300 (PEG 300), ethanol (EtOH) and propylene glycol (PG) were all of analytical grade and were purchased from Vetec (Rio de Janeiro, Brazil). Methylcellulose was purchased from Dow Chemical (São Paulo, Brazil).

2.2.2.2. Preparation of Schiff base and reference drugs suspensions for in vivo administration. Schiff base suspensions for oral administration were prepared by dissolving Schiff bases in a mixture of surfactants and cosolvents containing polyethylene glycol 300 (60%), propylene glycol (32%), Tween® 80 (5%) and ethanol (3%) [32]. For toxicity and rapid test, 125 mg of Schiff base was dissolved in 1 mL of the solution previously described, obtaining a suspension with the concentration of 12.5%. Subsequently, a 1:5 (v/v) dilution in distilled water was performed to reach the correct dose (500 mg kg^{-1}) at a final volume of 400 µL for oral administration. For classical tests, 25 mg of Schiff base was dissolved in 1 mL of the solution previously described, obtaining a suspension with the concentration of 2.5%. Subsequently, a 1:5 (v/v) dilution in distilled water was performed to reach the correct dose (100 mg kg^{-1}) at a final volume of 400 µL for oral administration. Benznidazole and nifurtimox were administered orally in a 4% methylcellulose aqueous suspension.

2.2.2.3. The formatation of this paragraphy is different from the others. Should be justified. Animals. Female Swiss mice, aged 28–30 days and weighing 25–30 g, obtained from the Animal Science Center of the Universidade Federal de Ouro Preto (UFOP) were used in the experiments. The mice were maintained in a room at 20–24 °C under a 12/12-h light/dark cycle and were provided with sterilized water and food *ad libitum*. The experiments were approved by the Ethical Committee on Animal Experimentation of the UFOP (protocol 2011/60).

2.2.2.4. The formatation of this paragraphy is different from the others. Should be justified. Treatment of healthy animals with doses to evaluate acute toxic effects. To determine the no-observed-adverse-effect-level (NOAEL), groups of six healthy animals were treated with Schiff bases orally with a single dose (500 mg kg^{-1}). Postapplication animals were weighed and observed daily (*i.e.*, general movement and behavior, skin appearance and lesion developments), and their microenvironment was examined (*i.e.*, presence of blood in urine and snout; general characteristics of fecal material). The animals were evaluated for 48 h for the occurrence of toxic and subtoxic symptoms according to OECD guidelines [33]. At day 3, the animals were weighed, anesthetized with ketamine (7.5 mg kg^{-1}) and xylazine (60 mg kg^{-1}), sacrificed and

necropsied. The liver and blood were submitted to further studies [34–36].

2.2.2.5. The formatation of this paragraphy is different from the others. Should be justified. Hematological investigations and clinical chemistry. Hematological and clinical chemistry examinations were performed on all animals after three days of treatment with the Schiff bases (at a dose of 500 mg kg^{-1}) following the method described by Boiani et al. and Cabrera et al. [34,35]. The mice were anesthetized with a mixture of xylazine (7.5 mg kg^{-1}) and ketamine (60 mg kg^{-1}), and blood samples were collected in tubes containing 0.18% w/v EDTA (used for hematological exams) and in tubes without anticoagulants (used for clinical chemistry exams). The evaluated hematology parameters included hemoglobin, hematocrit, and total leukocyte count. All analyses were performed using an automated analyzer (Mindray BC-2800Vet). The liver function was evaluated by measuring the alanine aminotransferase (ALT) and by aspartate aminotransferase (AST) activities, which were determined by spectrophotometric analysis in an autoanalyzer (Wiener Lab model CM200, Kinetic Analysis) and diagnostic kits for the kinetic analysis of the Cristal line, donated by the Bioclin-Quibasa laboratory (Belo Horizonte, Minas Gerais, Brazil).

2.2.2.6. Histopathological examination. The liver was removed, washed with 0.9% w/v NaCl solution and fixed in 10% v/v buffered formalin. The tissue was embedded in a paraffin block, cut into 5-μm-thick sections and placed on glass slides. After hematoxylin-eosin staining, photos were taken using an optical microscope. The parameters observed were inflammation, congestion and degeneration. This organ was chosen because the liver is related to drug biotransformation. A pathologist who was blinded to the treatment performed the qualitative analysis of the slides for pathology.

2.2.2.7. Rapid susceptibility test of T. cruzi to treatment. The evaluation of the effect of the Schiff bases on *T. cruzi* was made through a rapid susceptibility test, according to Filardi and Brener [37]. Infection was performed *via* an intraperitoneal injection of 10^4 blood trypomastigotes (Y strain). Groups of six animals were treated with the Schiff bases or with the reference drugs, nifurtimox and benznidazole. The treatment was performed on the day of the parasitemia peak (7th day of infection), orally and using a single dose (500 mg kg^{-1}). The evaluation of the compounds was performed by comparing the curves of parasitemia and the survival rate of the animals treated with Schiff bases and the animals treated with the reference drugs.

Parasitemia: The evaluation of parasitemia was performed using blood collected from the caudal veins of the mice (fresh blood test), and quantification of the parasites was performed as described by Brener [38]. The number of parasites was established prior to treatment, at two and six hours, and at one, two and five days after treatment. The parasitemia curves represent the mean number of parasites observed in the peripheral blood of the mice of each experimental group. *Rate of survival:* The survival of the animals was evaluated on the 14th day after infection.

2.2.2.8. Evaluation of the therapeutic action in a long test. The evaluation of therapeutic action in a long test was based on the protocol of Filardi and Brener [39]. Groups of eight animals were infected with 5×10^3 blood trypomastigotes (Y strain) and treated with each of the Schiff bases. Treatment was started on the 4th day of infection, was given orally at 100 mg kg^{-1} day^{-1}, and continued for 20 consecutive days. The evaluation of the therapeutic action of the Schiff bases was performed by comparing the parasitemia curves and survival rates of these animals with those of the animals treated with nifurtimox (120 mg kg^{-1} day^{-1}) and benznidazole (100 mg kg^{-1} day^{-1}) and those of the animals in the untreated group.

Parasitemia: The evaluation of parasitemia was performed in the blood collected from the caudal veins of the mice (fresh blood test), and the quantification of parasites was determined as described by Brener [38]. The parasite number was determined on the 4th day after inoculation and was evaluated on alternate days until no more parasites were observed in the fresh blood test for five consecutive days or until the death of the mice. The parasitemia curves represent the mean number of parasites observed in the peripheral blood of the mice of each experimental group. *Survival:* The survival of the animals was evaluated daily until the 60th day after infection.

2.2.2.9. The formatation of this paragraphy is different from the others. Should be justified. Statistical analysis. GraphPad Prism 5 software was used for statistical analysis of *in vivo* tests. The Kolmogorov-Sminorv test was used to evaluate the normality of the data. For data with a non-normal distribution, the *t*-test was used. For data with a normal distribution, the ANOVA test was used, followed by the Newman-Keuls (parasitemia data) or Tukey (body weight, biochemical and hematological data) test. The results were considered statistically significant when $p < 0.05$. All values are expressed as the means ± SD.

3. Results and discussion

3.1. Synthesis of Schiff bases

Compounds containing a nitroaromatic group are known for their clinical applications. This class of substances is used to treat a wide variety of diseases, including Parkinson's disease and hypertension [40,41]. Additionally, several nitroaromatic compounds are also used as anti-infective agents, including drugs to treat parasitic infections such as giardiasis, cryptosporidiosis, trichomoniasis, amoebiasis, sleeping sickness and Chagas disease [42]. In this work, we synthesized a series of nitroaromatic and nitroheteroaromatic Schiff bases with different substitution patterns (Fig. 2).

Compounds **1–10** were obtained by microwave-assisted condensation between the corresponding aldehydes and aromatic amines. Schiff bases were isolated by recrystallization at good to excellent yields (73–92%) after only 2 min of reaction. Following synthesis, the compounds were characterized using infrared (IR), ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopies. All of the synthesized compounds presented similar spectra in the infrared region, with the main absorption bands being observed at ~1620 cm^{-1}, corresponding to C═N bond stretching. The ^{1}HNMR spectra obtained for the Schiff bases showed signals characteristic of azomethine protons in $\delta \sim 8.50$ (Table 1).

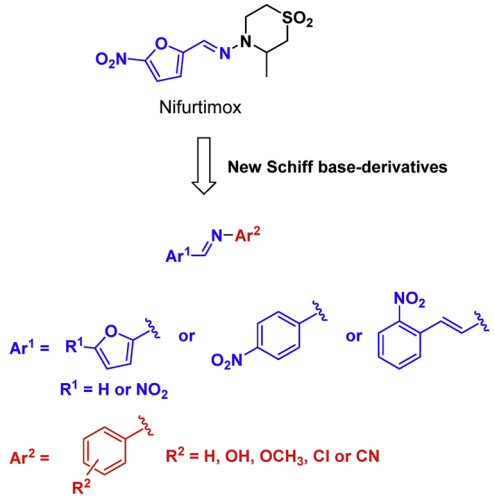
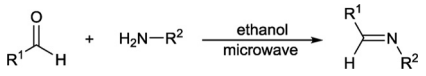


Fig. 2. Chemical structures of synthesized Schiff bases.

Table 1
Yields obtained in the synthesis of Schiff bases **1–10** employing microwave irradiation.



Compound	R^1	R^2	Yield[a](%)	HC=N (cm^{-1})	δHC = N (ppm)
1	5-NO_2-2-furanyl	2-OH-phenyl	92	1618	8.74 (singlet)
2	5-NO_2-2-furanyl	4-OH-phenyl	73	1621	8.58 (singlet)
3	5-NO_2-2-furanyl	4-OCH_3-phenyl	75	1617	8.62 (singlet)
4	5-NO_2-2-furanyl	4-Cl-phenyl	80	1617	8.62 (singlet)
5	5-NO_2-2-furanyl	2-CN-phenyl	90	1620	8.71 (singlet)
6	5-NO_2-2-furanyl	phenyl	75	1618	8.61 (singlet)
7	2-furanyl	4-OH-phenyl	78	1627	8.41 (singlet)
8	4-NO_2-phenyl	2-OH-phenyl	84	1625	8.88 (singlet)
9	4-NO_2-phenyl	4-OH-phenyl	90	1625	8.75 (singlet)
10	(*E*)-2-NO_2-cinnamyl	2-OH-phenyl	84	1627	8.55(doublet)

[a] Yields of isolated and purified products.

Table 2
Trypanosoma cruzi susceptibility to Schiff bases **1–10**.

Compound	EC_{50}* (μM)	CI (95%)
1	13.67 ± 0.33[a]	13.21–14.13
2	15.12 ± 0.97[b]	13.82–16.42
3	9.87 ± 0.52[a,b,c]	9.15–10.59
4	14.04 ± 0.23[d]	13.72–14.36
5	4.04 ± 0.05	3.97–4.11
6	9.67 ± 0.07[a,d,e]	9.57–9.77
7	81.95 ± 1.51	79.85–84.05
8	15.63 ± 0.38 [d,e]	15.10–16.16
9	104.45 ± 1.75	102.05–106.85
10	14.09 ± 0.11[c,e]	13.94–14.24
Benznidazole	2.23 ± 0.08	2.0–2.0

* Values are expressed as the mean ± standard deviation. Statistical analysis: *t*-test ($p < 0.05$ were considered significant) among the groups marked with the same letters. In relation to compounds **5**, **7**, **9** and benznidazole, all differences were statistically significant from all. CI (95%) 95% confidence interval values.

3.2. In vitro anti-T. cruzi activity

Initially, we investigated the *in vitro* effects of Schiff bases **1-10** against *T. cruzi* epimastigotes (Y strain) proliferation. The EC_{50} values obtained for each compound are presented in Table 2. Among the synthesized compounds, Schiff bases **3**, **5** and **6** were the most active, with EC_{50} values of 9.87, 4.04 and 9.67 μM, respectively. Compound **7**, which is the non-nitrated analogue of **2**, was one of the least active compounds, (EC_{50} of 81.95 μM). Since the 2-nitrofuranyl moiety is the pharmacophoric group of nifurtimox, the Schiff bases containing this nucleus were expected to be more active. However, the substituents present on the other aromatic portions of the synthesized compounds interfered in the anti-*T. cruzi* activity. While unsubstituted compound **6** and 2-hydroxy-substituted compound **1** showed EC_{50} values of 9.67 and 13.67 μM, respectively, the cyano derivative **5** was twice and three times more potent, respectively, than these compounds, with an EC_{50} of 4.04 μM (Table 2).

Schiff base **8**, an *ortho*-hydroxylated derivative, (EC_{50} = 15.63 μM) was almost seven times more active than the *para*-hydroxylated analogue **9**. Compound **10**, a nitrocinnamaldehyde derivative, was found to be as potent as compound **8**, indicating that the additional C=C double bond does not have a strong influence on the trypanocidal activity of these compounds. It is known that compounds containing a nitro-furanyl moiety have a greater reduction potential than nitrophenyl derivatives do [43]. Thus, these results suggest that the bioreduction of nitro groups is correlated with the observed trypanocidal activities. Interestingly, the position of the hydroxyl group in the aromatic ring also had exerted a strong influence on the anti *T. cruzi* activity of the compound. In addition to promising *anti-T. cruzi* activity showed by the Schiff bases described here, it is important to highlight that the derivatives **1**,**2** and **6–10** have shown only moderate cytotoxicity activity (GI_{50} = 27.1–117.6 μM) against African green monkey kidney cells (nontumorigenic VERO cell line) [23]. Derivatives **3**, **4** and **5** also presented only moderate antiproliferative activities against the VERO cell line (GI_{50} = 115.7, 93.7 and 25.3 μM, respectively) (data not published). Based on these results, the selectivity indexes (determined as the ratio of the GI_{50} value for VERO to the EC_{50} value obtained for *T. cruzi*) for the Schiff bases are: 5 (**1**), 11 (**2**), 6 (**4**), 6 (**5**), 3 (**6**), 8 (**7**) and 5 (**10**). Derivatives **3**, **8** and **9** were more cytotoxic for the VERO cell line than for *T. cruzi*.

3.3. Toxic and biological effects

Our initial goal was to develop formulations in solution with Schiff bases. Several solvents, surfactants and cosurfactants have been previously assayed. Solutions are preferred for preclinical evaluation because the new molecules are present in an absorption-ready state. Therefore, several cosolvents and nonionic surfactants were used to obtain a homogeneous formulation [32]. Using the mixture of TWEEN 80:PEG 300: EtOH: PG, a suspension was obtained. However, the use of a suspension is limited by the nonuniformity of the dose due to time-dependent settling and particle agglomeration. Thus, a good absorption profile could not be obtained. The inability new substance to reach the desired therapeutic concentrations provides strong motivation to develop new distribution systems that will help the therapeutic efficacy [32].

To determine the possible toxic effects, the animals were treated orally with a single dose of each Schiff base (500 mg kg^{-1}), and individual parameters were analyzed. No changes, such as diarrhea,

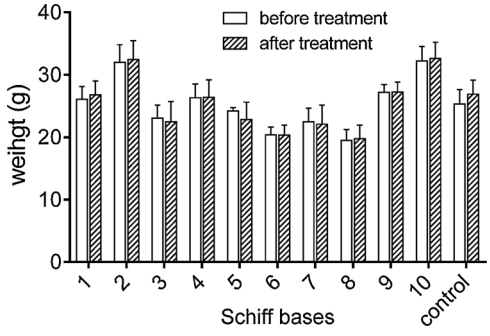


Fig. 3. Weight of animals before and after treatment with Schiff bases (single dose, 500 mg kg^{-1}). Control group: animals treated with the suspension excipients (60% PEG 300, 32% PG, 5% Tween 80, 3% EtOH).

Table 3
Mean values of the biochemical and the hematological finding in heathy animals treated orally with single dose of Schiff bases (500 mg kg^{-1}).

Animal treated	Leucocyte (10^9 l^{-1})	Hemoglobin (g dl^{-1})	Hematocrite (%)	ALT (U l^{-1})	AST (U l^{-1})
1	8.7	13.4	24.6	83.5	291.7
2	8.0	13.0	27.0	85.4	275.6
3	11.6	13.1	23.9	159.6	319.5
4	9.2	13.9	22.2	126.8	339.9
5	8.6	12.5	22.2	107.3	332.3
6	7.9	13.5	26.7	159.1	378.3
7	11.0	12.1	23.1	76.1	317.8
8	9.4	12.4	23.0	131.8	287.9
9	9.5	12.3	22.4	76.5	270.2
10	9.0	12.8	24.8	101.3	315.8
Control	8.2	12.5	22.8	104.0	263.8

ALT = alanine aminotransferase, AST = aspartate aminotranferase. Control group: animals treated with the suspension excipients (60% PEG 300, 32% PG, 5% Tween 80, 3% EtOH).

ataxia or weakness, were observed in the behavioral and functional parameters of any of the animals treated with the Schiff bases, compared to the vehicle-treated mice. Furthermore, there were no cutaneous symptoms, lesions or appearance of blood in the fecal material.

Monitoring of body weight is an important indicator for evaluating the toxicity of a substance. There was no significant difference ($p > 0.05$) in the weights of the animals before and after treatment with the Schiff bases, as shown in Fig. 3. Biochemical and hematological analysis showed that there were no significant differences ($p > 0.05$) between the animals treated with the Schiff bases and the control groups. No differences suggestive of the absence of liver damage were observed in the AST and ALT levels in mice exposed to the Schiff bases (Table 3) [44].

Macroscopic observation in the dissection process and histological studies of liver (Table 4) showed that there were no differences in the analyzed parameters between the treated and healthy untreated animals, suggesting the absence of hepatic damage in the animals treated with the Schiff bases. The survival rates were 100% for all of the groups.

Our results showed a good safety profile for the Schiff bases, certainly indicating that studies of acute and chronic toxicity should be conducted to obtain a better understanding of the toxicity; additionally, the screening performed in our work was very useful, and we can even compare our results with studies by Cabrera et al. (2009) that

Table 4
Histological results for liver in healthy animals treated orally with single dose of Schiff bases.

Animal treated	Inflammation	Congestion	Degeneration
1	+	+	–
2	–	–	–
3	–	–	–
4	+	–	–
5	–	++	+
6	–	+	–
7	–	–	–
8	–	–	–
9	++	–	+
10	+	–	+
Control	–	–	–

Histological results – without changes respect to normal tissue; + the organ not present relevant changes respect the normal tissue; ++ the organ present moderate kind of change respect to normal tissue; +++ the organ present a great number of changes respect to normal tissue. Control group: animals treated with the suspension excipients (60% PEG 300, 32% PG, 5% Tween 80, 3% EtOH).

demonstrated that some 5-nitro-2-furyl derivatives were toxic. The literature shows that animals treated with the nifurtimox - the reference drug used in our study - showed a reduction of 6% in the weight of treated animals and a large number of alterations in liver histology [35]. However, no change in biochemical and hematological parameters were observed for benznidazole and nifurtimox in the studies reported in the literature [33–35,45].

3.4. *In vivo anti-T. cruzi activity*

3.4.1. *T. cruzi susceptibility to treatment in vivo (rapid test)*

The parasitemia curves of the animals infected with the *T. cruzi* strain Y, partially sensitive to benznidazole, and treated with a single dose (500 mg kg^{-1}) of the Schiff bases, benznidazole or nifurtimox by oral route are represented in Fig. 4(A–B) (here, only the Schiff base derivatives with a nitro group in their structure were evaluated). The results showed that only animals treated with compound **2** showed a reduction in parasitemia ($p < 0.05$), which was similar to the results observed in the animals treated with benznidazole or nifurtimox and different from the results observed in the groups of animals treated with the other synthesized compounds. Interestingly, this compound was among the least active of the 2-nitrofuranyl derivatives in the *in vitro* assays. In particular, this result was observed for the vast majority of the compounds or substances tested for anti-*T. cruzi* activity. However, these results only mean that the parasite is susceptible to this compound, and no prediction can be made regarding the effectiveness of compound **2** *in vivo*.

The results for the survival of the animals infected with the *T. cruzi* Y strain and treated with a single oral dose of Schiff bases, benznidazole or nifurtimox is shown in Table 5. The animals treated with Schiff bases **5** and **10** showed 100% survival, identical to the animals treated with benznidazole or nifurtimox. Animals treated with compounds **2**, **4**, **6** and **8** had survival rates higher than 80% and were then chosen for the standard *in vivo* treatment test (treatment for 20 days). Compounds **1**, **3** and **9** had lower survival rates (25%, 62% and 16%, respectively) and were not evaluated in the classical test of *T. cruzi* susceptibility to treatment.

Rapid tests were performed to screen the Schiff bases that had an impact on parasitemia and showed survival that was better or equal to those of the reference drugs (benznidazole and nifurtimox) in a short period of time (three days). Thus, compounds **2**, **4**, **5**, **6**, **8**, and **10** were chosen for evaluation by the classical test *in vivo* (treatment for 20 days).

3.4.2. *Classical test of T. cruzi susceptibility to treatment in vivo*

The parasitemia curves of the animals infected with the *T. cruzi* Y strain and treated for 20 days *via* an oral route, with each Schiff base (100 mg kg^{-1} day^{-1}), benznidazole (100 mg kg^{-1} day^{-1}) or nifurtimox (120 mg kg^{-1} day^{-1}) are shown in Fig. 5. The results showed that all animals (100%) treated with benznidazole or nifurtimox had subpatent parasitemia during and after treatment. Animals treated with compounds **5** and **6** showed a significant reduction in parasitemia ($p > 0.05$) relative to the control group (infected and not treated) but with a lower intensity than observed in the animals treated with reference drugs ($p > 0.05$). It should be noted that Schiff base **5** was also the most active in the *in vitro* assays. Unlike for the rapid test, the Schiff bases that were more active for significantly reducing of the parasitemia were **5** and **6**.

Although these compounds were less active than the benznidazole and nifurtimox reference drugs which maintained the animals with subpatent parasitemia throughout the experiment, it is important to keep in mind that the Colombian strain is a typical example of a *T. cruzi* strain that is resistant to the reference drugs [37,39]. Thus, these active Schiff bases should be assayed with partially resistant and susceptible parasites, which represent 64.27% of the 104 strains evaluated for resistance/susceptibility to the classical compounds nifurtimox and

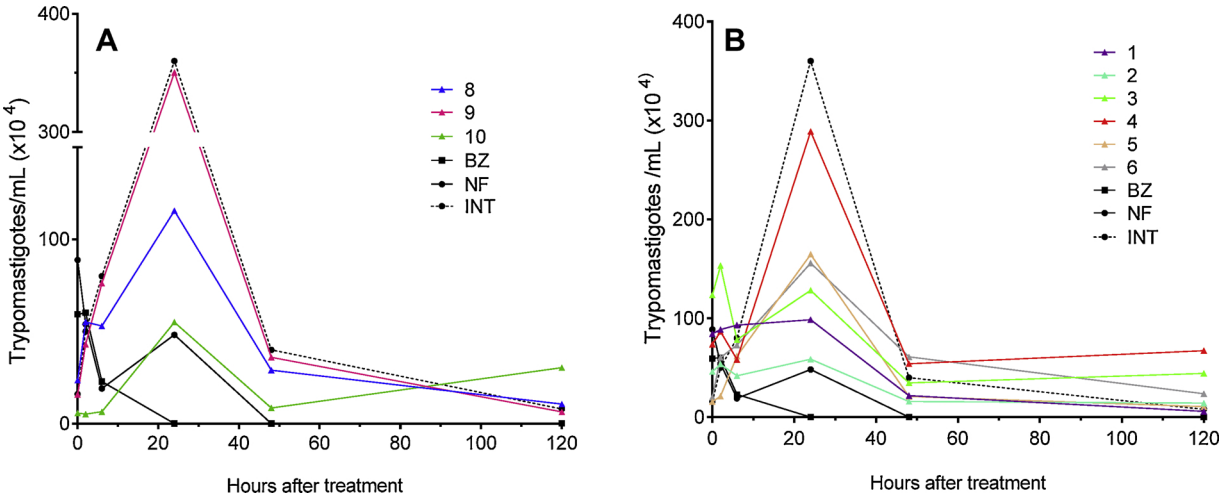


Fig. 4. Rapid test: Parasitemia curve of mice infected with 10^4 blood trypomastigotes of *T. cruzi* Y strain and treated by oral route. (A) Compounds **8–10**, benznidazole (BZ) and nifurtimox (NF). (B) Compounds **1–6**, benznidazole (BZ) and nifurtimox (NF). Control groups: INT (infected and not treated). All animals were treated with a single dose (500 mg kg^{-1}). Treatment started in the patent period on the 7^{th} day after infection. Schiff base **7** was not evaluated in the *in vivo* tests due to its low activity in the *in vitro* assays.

Table 5
Rapid test: Survival of animals infected with *T. cruzi* Y strain and treated by oral route with a single dose (500 mg kg^{-1}) of each Schiff base, nifurtimox and benznidazole.

Compound	Survival (%)
1	25
2	83
3	62
4	87,5
5	100
6	83
8	83
9	16
10	100
Nifurtimox	100
Benznidazole	100
INT	0

INT (infected and not treated (control)). Treatment started on the 7^{th} day after infection when the animals were in the peak of parasitemia.

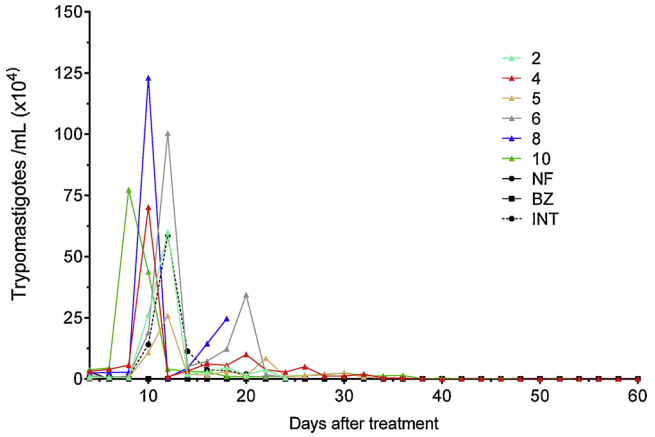


Fig. 5. Classical test: Parasitemia curve of mice infected with 5×10^3 blood trypomastigotes of *T. cruzi* Y strain and treated by oral route. Schiff bases **2**, **4**, **5**, **6**, **8** and **10** at 100 mg kg^{-1} day^{-1}; benznidazole (BZ) at 100 mg kg^{-1} day^{-1}; nifurtimox (NF) at 120 mg kg^{-1} day^{-1}. Control groups: INT (infected and not treated). All animals were treated daily for 20 consecutive days (treatment started in the patent period on the 4^{th} day after infection).

Table 6
Classical test: Survival of animals infected with *T. cruzi* Y strain and treated by oral route with each Schiff base (100 mg kg^{-1} day^{-1}), benznidazole (100 mg kg^{-1} day^{-1}) or nifurtimox (120 mg kg^{-1} day^{-1}).

Compound	Survival (%)
2	0
4	12.5
5	25
6	0
8	0
10	62.5
Nifurtimox	100
Benznidazole	100
INT	0

INT (infected and not treated (control)). All animals were treated daily for 20 consecutive days. Treatment started on the 4th day after infection when the infection was confirmed.

benznidazole in mice [46].

The survival rates of the animals infected with the *T. cruzi* Y strain and treated for 20 days *via* an oral route with each Schiff base (100 mg kg^{-1} day^{-1}), benznidazole (100 mg kg^{-1} day^{-1}) or nifurtimox (120 mg kg^{-1} day^{-1}) are shown in Table 6. The animals treated with benznidazole and nifurtimox showed 100% survival. The group treated with Schiff base **10** presented 62.5% survival, followed by the group treated with **5** (25%) and the groups treated with **4** and **3** (12.5%).

The results obtained for Schiff base **10** correlate with the results obtained in the rapid susceptibility test, in which the increase in parasitemia was suppressed. There was also a correlation between the tests in relation to survival. This compound presented the best results among all the synthesized compounds. Interestingly, Schiff base **10** was also the second most active compound in the rapid test. Although the animals treated with this compound in the classical test presented a relatively high level of parasitemia on the 7th day after infection, **10** was the compound that subsequently maintained the best control of the parasitemia. Furthermore, in the rapid test, the animals treated with compound **10** showed subclinical parasitemia on the 12th day of infection, that is, 8 days after treatment.

Using a partially sensitive strain of *T. cruzi* (strain Y) a good

correlation was not observed between the results obtained with the rapid test and those obtained with the classical regimens involving the administration of Schiff bases for 20 consecutive days and a prolonged evaluation period, as performed by Filardi and Brener [37]. However, the rapid test is recommended and applied for the screening of compounds or chemicals active against *T. cruzi* because this test rapidly evaluated the impact of the active substances on parasitemia and mortality caused by this strain in the acute phase of the infection in the murine model. The classical studies in the literature demonstrate that the Y strain is considered to be partially resistant to BZ and nifurtimox in mice [39] when submitted to the classical treatment leading to an approximately 50% cure rate in mice and 100% of reduction in blood trypomastigotes forms (parasitemia). This strain is ideal for this type of test due to its high virulence (parasitemia and mortality during the acute phase). Therefore, the effect of the active substances against *T. cruzi* may be easily observed by using these parameters in both tests.

When the rapid test was performed, a parasitemia reduction of approximately 77% was verified, as observed by Filardi and Brener [37]. Although with an exception of compound 2, the Schiff bases did not show a reduction in parasitemia in the rapid test, we used the results of the animals' survival to justify the subsequent evaluation by the classical test.

Regarding the survival of the animals, the reference drugs achieved 100%, and the only Schiff base that effectively increased survival in 62.5% of the mice was **10**; compound **10** was also the second most active compound in the rapid test. Thus, this Schiff base offers some potential for the development of a treatment for Chagas disease and should be explored.

4. Conclusion

Nitro-derivative Schiff bases have been shown to have promising anti-*T. cruzi* activity. Although Schiff base **10** was not the most active in the *in vitro* assays, this compound was the most promising in the *in vivo* tests, showing a significant reduction in parasitemia and the highest survival rate under control conditions. For any new compound, extensive pharmacological and safety studies are required for the possible development of a pharmaceutical formulation to improve the therapeutic efficacy of the compound. The synthesis and evaluation of new nitro derivative Schiff bases will hopefully lead to the identification of improved candidates for treating Chagas disease.

Conflict of interest

The authors report no conflict of interest.

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Appendix A. Supplementary data0

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2018.09.176.

References

[1] M.C.P. Nunes, W. Dones, C.A. Morillo, J.J. Encina, A.L. Ribeiro, Chagas disease: an overview of clinical and epidemiological aspects, J. Am. Coll. Cardiol. 62 (9) (2013) 767–776, https://doi.org/10.1016/j.jacc.2013.05.046.

[2] World Health Organization, Chagas Disease (American Trypanosomiasis), World Health Organization, Geneva, Switzerland, 2016 2017 (Accessed December 2017) http://www.who.int/mediacentre/factsheets/fs340/en/.

[3] A. Hidron, N. Vogenthaler, J.I. Santos-Preciado, A.J. Rodriguez-Morales, C. Franco-Paredes, A. Rassi Jr., Cardiac involvement with parasitic infections, Clin. Microbiol. Rev. 23 (2) (2010) 324–349, https://doi.org/10.1128/CMR.00054-09.

[4] M. Sánchez-Moreno, F. Gómez-Contreras, P. Navarro, C. Marín, F. Olmo, M.J.R. Yunta, A.M. Sanz, M.J. Rosales, C. Cano, L. Campayo, Phthalazine derivatives containing imidazole rings behave as Fe-SOD inhibitors and show remarkable anti-*T. cruzi*activity in immunodeficient-mouse mode of infection, J. Med. Chem. 55 (22) (2012) 9900–9913, https://doi.org/10.1021/jm3011004.

[5] C. Franco-Paredes, A. Von, A. Hidron, A.J. Rodríguez-Morales, I. Tellez, M. Barragán, D. Jones, C.G. Náquira, J. Mendez, Chagas disease: an impediment in achieving the millennium development goals in Latin America, BMC Int. Health Hum. Rights 7 (2007) 7, https://doi.org/10.1186/1472-698X-7-7.

[6] A. Cavalli, M.L. Bolognesi, Neglected tropical diseases: multi-target-directed ligands in the search for novel lead candidates against *Trypanosoma* and *Leishmania*, J. Med. Chem. 52 (2009) 7339–7359, https://doi.org/10.1021/jm9004835.

[7] P. Trouiller, P. Olliaro, E. Torreele, J. Orbinski, R. Laing, N. Ford, N. Drug development for neglected diseases: a deficient market and a public-health policy failure, Lancet 359 (2002) 2188–2194, https://doi.org/10.1016/S0140-6736(02)09096-7.

[8] B.S. Hall, C. Bot, S.R. Wilkinson, Nifurtimox activation by trypanosomal type nitroreductases generates cytotoxic nitrile metabolites, J. Biol. Chem. 286 (15) (2011) 13088–13095, https://doi.org/10.1074/jbc.M111.230847.

[9] B.S. Hall, S.R. Wilkinson, Activation of benznidazole by trypanosomal type I nitroreductases results in glyoxal formation, Antimicrob. Agents Chemother. 56 (1) (2012) 115–123, https://doi.org/10.1128/AAC.05135-11.

[10] R.L. Tarleton, J.W. Curran. is Chagas Disease really the "new HIV/AIDS of the Americas?", PLoS Negl. Trop. Dis. 6 (10) (2012) 1861, https://doi.org/10.1371/journal.pntd.0001861.

[11] E. Fischer, C.N.L. Macpherson, Update on neglected tropical diseases of the Western Hemisphere, Infect. Dis. Res. Treat. 4 (2011) 17–25, https://doi.org/10.4137/IDRT.S7401.

[12] C.A. Morillo, J.A. Marin-Neto, A. Avezum, S. Sosa-Estani, A. Rassi Jr, F. Rosas, E. Villena, R. Quiroz, R. Bonilla, C. Britto, F. Guhl, E. Velazquez, L. Bonilla, B. Meeks, P. Rao-Melacini, J. Pogue, A. Mattos, J. Lazdins, A. Rassi, S.J. Connolly, S. Yusuf, Randomized trial of benznidazole for chronic chagas' cardiomyopathy, N. Engl. J. Med. 373 (14) (2015) 1295–1306, https://doi.org/10.1056/NEJMoa1507574.

[13] I. Molina, J.G. Prat, F. Salvador, B. Treviño, E. Sulleiro, N. Serre, D. Pou, S. Roure, J. Cabezos, L. Valerio, A. Blanco-Grau, A. Sánchez-Montalvá, X. Vidal, A. Pahissa, Randomized trial of posaconazole and benznidazole for chronic Chagas' Disease, N. Engl. J. Med. 370 (20) (2014) 1899–1908, https://doi.org/10.1056/NEJMoa1313122.

[14] E. Chatelain, Chagas disease drug discovery: toward a new era, J. Biomol. Screen. 20 (1) (2015) 22–35, https://doi.org/10.1177/1087057114550585.

[15] B. Zingales, M.A. Miles, C.B. Moraes, A. Luquetti, F. Guhl, A.G. Schijman, I. Ribeiro, Drug discovery for Chagas disease should consider *Trypanosoma cruzi* strain diversity, Mem. Inst. Oswaldo Cruz 109 (6) (2014) 828–833, https://doi.org/10.1590/0074-0276140156.

[16] M.A. Miles, fourth edition, 124 - Chagas Disease (American Trypanosomiasis). Infectious Diseases Vol. 2 (2017), pp. 1065–1072, https://doi.org/10.1016/B978-0-7020-6285-8.00124-6.

[17] C.M. da Silva, D.L. da Silva, L.V. Modolo, R.B. Alves, M.A. de Resende, C.V.B. Martins, Â. de Fátima, Schiff bases: a short review of their antimicrobial activities, J. Adv. Res. 2 (2011) 1–8, https://doi.org/10.1016/j.jare.2010.05.004.

[18] C.M. da Silva, D.L. da Silva, C.V.B. Martins, M.A. de Resende, E.S. Dias, T.F.F. Magalhães, L.P. Rodrigues, A.A. Sabino, R.B. Alves, Â. de Fátima, Synthesis of aryl aldimines and their activity against fungi of clinical interest, Chem. Biol. Drug Des. 78 (2011) 810–815, https://doi.org/10.1111/j.1747-0285.2011.01185.x.

[19] Â. de Fátima, C.P. Pereira, C.R.S.D.G. Olímpio, B.G.F. Oliveira, L.L. Franco, P.H.C. da Silva, Schiff bases and their metal complex as urease inhibitors – a brief review, J. Adv. Res. 13 (2018) 113–126, https://doi.org/10.1016/j.jare.2018.03.007.

[20] Y.M.S.A. Al-Kahraman, H.M.F. Madkour, D. Ali, M. Yasinzai, Antimicrobial and antifungal activities of some new aryl azomethines, Molecules 15 (2010) 660–671, https://doi.org/10.3390/molecules15020660.

[21] V. Razakantoanina, N.K.P. Phung, G. Jaureguiberry, Antimalarial activity of new gossypol derivatives, Parasitol. Res. 86 (2000) 665–668, https://doi.org/10.1007/PL00008549.

[22] D.A. Martins, L.F.B. Filho, C.M. da Silva, Â. de Fátima, S.R.W. Louro, D.G.J. Batista, M.N.C. Soeiro, J.E. de Carvalho, L.R. Teixeira, Copper(II) nitroaromatic Schiff base complexes: synthesis, biological activity and their interaction with DNA and albumins, J. Braz. Chem. Soc. 28 (1) (2017) 87–97, https://doi.org/10.5935/0103-5053.20160150.

[23] C.M. da Silva, M.M. Silva, F.S. Reis, A.L.T.G. Ruiz, J.E. de Carvalho, J.C.C. Santos, I.M. Figueiredo, R.B. Alves, L.V. Modolo, Â. de Fátima, Studies on free radical scavenging, cancer cell antiproliferation and calf thymus DNA interaction of Schiff bases, J. Photochem. Photobiol. B 172 (2017) 129–138, https://doi.org/10.1016/j.jphotobiol.2017.05.020.

[24] T. Toyonori, I. Nobuharu, M. Teruaki, Synthetic control by internal interaction. The intramolecular Diels-Alder reactions of furan derivatives and α, β-unsaturated amides, Bull. Chem. Soc. Jpn. 56 (1983) 1107–1112, https://doi.org/10.1246/bcsj.56.1107.

[25] C.E.A. de Melo, C.R. Nicoleti, M.C. Rezende, A.J. Bortoluzzi, R.S. Heying, R.S. Oliboni, G.F. Caramori, V.G. Machado, Reverse solvatochromism of imine dyes comprised of 5-nitrofuran-2-yl or 5-nitrothiophen-2-yl as electron acceptor and phenolate as electron donor, Chem. Eur. J. 24 (9364) (2018) -9376, https://doi.org/10.1002/chem.201800613.

[26] T. Takahashi, H. Saikachi, S. Yoshina, C. Mizuno, Syntheses of furan derivatives. III, Yakugaku Zasshi 69 (1949) 284–286, https://doi.org/10.1248/yakushi1947.69.6-10_284.
[27] R.W. Drisko, H. McKennis Jr., The reaction of certain substituted furfurals with aniline and aniline hydrochloride, J. Am. Chem. Soc. 74 (1951) 2626–2628, https://doi.org/10.1021/ja01130a048.
[28] J. Rombaut, G. Smets, Azométhines III dérivés du furfuraldéhyde, Bull. Soc. Chim. Belg. 58 (1949) 421–431, https://doi.org/10.1002/bscb.19490581002.
[29] A.W. Baker, A.T. Shulgin, Intramolecular hydrogen bonding. II. The determination of Hammett sgma constants by intramolecular hydrogen bonding in Schiff's bases, J. Am. Chem. Soc. 81 (1959) 1523–1529, https://doi.org/10.1021/ja01516a001.
[30] A. Iqbal, H.L. Siddiqui, C.M. Ashraf, M.H. Bukhari, C.M. Akram, Synthesis, spectroscopic and cytotoxic studies of biologically active new Schiff bases derived from *p*-nitrobenzaldehyde, Chem. Pharm. Bull. 55 (2007) 1070–1072, https://doi.org/10.1248/cpb.55.1070.
[31] O. Castellani, L.V. Ribeiro, J.F. Fernandes, Differentiation of *Trypanosoma cruzi* in culture, J. Protozool. 14 (1967) 447–451, https://doi.org/10.1111/j.1550-7408.1967.tb02024.x.
[32] S.M. Shah, A.S. Jain, R. Kaushik, M.S. Nagarsenker, M.J. Nerurkar, Preclinical formulations: insight, strategies, and practical considerations, AAPS PharmSciTech 15 (2014) 1307–1323, https://doi.org/10.1208/s12249-014-0156-1.
[33] D.D.G.J. Batista, M.M. Batista, G.M. de Oliveira, P.B. do Amaral, J. Lannes-Vieira, C.C. Britto, A. Junqueira, M.M. Lima, A.J. Romanha, P.A. Sales Junior, C.E. Stephens, D.W. Boykin, Mde N. Soeiro, Arylimidamide DB766, a potential chemotherapeutic candidate for Chagas' disease treatment, Antimicrob. Agents Chemother. 54 (2010) 2940–2952, https://doi.org/10.1128/AAC.01617-09.
[34] L. Boiani, C. Davies, C. Arredondo, W. Porcal, A. Merlino, A. Gerpe, M. Boiani, J.P. Pacheco, M.A. Basombrio, H. Cerecetto, M. Gonzalez, *In vivo* studies of 5-arylethenylbenzofuroxans in acute murine models of Chagas' disease, Eur. J. Med. Chem. 43 (2229-) (2008) 2237, https://doi.org/10.1016/j.ejmech.2007.12.016.
[35] E. Cabrera, M.G. Murguiondo, M.G. Arias, C. Arredondo, C. Pintos, G. Aguirre, M. Fernandez, Y. Basmadjian, R. Rosa, J.P. Pacheco, S. Raymondo, R. Di Maio, M. Gonzalez, H. Cerecetto, 5-Nitro-2-furyl derivative actives against *Trypanosoma cruzi*: preliminary *in vivo* studies, Eur. J. Med. Chem. 44 (2009) 3909–3914, https://doi.org/10.1016/j.ejmech.2009.04.015.
[36] B.L. Timm, P.B. da Silva, M.M. Batista, F.H. da Silva, C.F. da Silva, R.R. Tidwell, D.A. Patrick, S.K. Jones, S.A. Bakunov, S.M. Bakunova, Mde N. Soeiro, *In vitro* and *in vivo* biological effects of novel arylimidamide derivatives against *Trypanosoma cruzi*, Antimicrob. Agents Chemother. 58 (2014) 3720–3726, https://doi.org/10.1128/AAC.02353-14.
[37] L.S. Filardi, Z. Brener, A rapid method for testing *in vivo* the susceptibility of different strains of *Trypanosoma cruzi* to active chemotherapeutic agents, Mem. Inst. Oswaldo Cruz 79 (2) (1984) 221–225, https://doi.org/10.1590/S0074-02761984000200008.
[38] Z. Brener, Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi*, Rev. Inst. Med. Trop. São Paulo 4 (6) (1962) 389–396.
[39] L.S. Filardi, Z. Brener, Susceptibility and natural resistance of *Trypanosoma cruzi* strains to drugs used clinically in Chagas disease, Trans. R. Soc. Trop. Med. Hyg. 81 (1987) 755–759, https://doi.org/10.1016/0035-9203(87)90020-4.
[40] D.D. Truong, Tolcapone: review of its pharmacology and use as adjunctive therapy in patients with Parkinson's disease, Clin. Interv. Aging 4 (2009) 109–113, https://doi.org/10.2147/CIA.S3787 PMC2685232.
[41] J. Varon, P.E. Marik, Clinical review: The management of hypertensive crises, Crit. Care 7 (5) (2003) 374–384, https://doi.org/10.1186/cc2351.
[42] S. Patterson, S. Wyllie, Nitro drugs for the treatment of trypanosomatid diseases: past, present, and future prospects, Trends Parasitol. 30 (6) (2014) 289–298, https://doi.org/10.1016/j.pt.2014.04.003.
[43] J. Rodríguez, V.J. Arán, L. Boiani, C. Olea-Azar, M.L. Lavaggi, M. González, H. Cerecetto, J.D. Maya, C. Carrasco-Pozo, H.S. Cosoy, New potent 5- nitroindazole derivatives as inhibitors of *Trypanosoma cruzi* growth: synthesis, biological evaluation, and mechanism of action studies, Bioorg. Med. Chem. 17 (24) (2009) 8186–8196, https://doi.org/10.1016/j.bmc.2009.10.030.
[44] I.M. Washington, G. van Hoosier, Chapter 3 – Clinical Biochemistry and Hematology, The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents, Academic Press, Boston, 2012, pp. 57–116.
[45] J.A. Castro, M.M. de Mecca, L.C. Bartel, Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis), Hum. Exp. Toxicol. 25 (2006) 471–479, https://doi.org/10.1191/0960327106het653oa.
[46] M.J. Toledo, M.T. Bahia, C.M. Carneiro, O.A. Martins-Filho, M. Tibayrenc, C. Barnabe, W.L. Tafuri, M. de Lana, Chemotherapy with benznidazole and itraconazole for mice infected with different*Trypanosoma cruzi* clonal genotypes, Antimicrob. Agents Chemother. 47 (2003) 223–230, https://doi.org/10.1128/AAC.47.1.223-230.2003.