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Synthesis and Antitrypanosomal Profile of Novel Hydrazonoyl Derivatives

Natália N. Santiago [a,b], Giulianna P. de Alcântara[a,b], Juliana Silva da Costa[a,c], Samir A. Carvalho[a], Juliana M. C. Barbosa[d], Kelly Salomão[d], Solange L. de Castro[d], Henrique M. G. Pereira[b,e] and Edson F. da Silva[a,c]*

[a]*Instituto de Tecnologia em Fármacos, Farmanguinhos, Laboratório de síntese 1, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil;* [b]*Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal do Rio de Janeiro;* [c]*Escola de Ciência e Tecnologia, ECT, Universidade do Grande Rio, Duque de Caxias, RJ, Brazil;* [d]*Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil;* [e]*LBCD - LADETEC / IQ-UFRJ, 21949-900 Rio de Janeiro, RJ, Brazil;*

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Abstract: This work describes the construction of a new family of hidrazonoyl substituted derivatives, structurally designed exploring the molecular hybridization between megazol and nitrofurazone. The compounds were evaluated for their *in vitro* activity against bloodstream trypomastigotes of *Trypanosoma cruzi*, etiological agent of Chagas disease, and for their potential toxicity to mammalian cells. Derivative (*Z*)-2-(2-(4-fluorophenyl) hydrazono)-2-(1-methyl-5-nitro-1H-imidazol-2-yl)-1-phenylethanone (4) (IC_{50}/24h = 15.0 ± 2.7 µM) showed an activity similar to that of benznidazole, used for the clinical treatment of chagasic patients (IC_{50}/24h = 10.8 ± 0.4 µM).

Keywords: Hydrazonoyl, Megazol, Nitrofurazone, Chagas' disease, *Trypanosoma cruzi*, Chemotherapy.

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1. INTRODUCTION

Chagas disease is a neglected tropical disease promoted by the protozoan hemoflagellate *Trypanosoma cruzi* that is primarily transmitted to humans by the faeces of haemophagic insects, from the family Reduviidae, subfamily Triatominae. Also, blood transfusion, oral and congenital transmission are important routes of infection [1]. This disease, classically associated with poor and rural populations, underwent an urbanization process in the 1970s and 1980s to Latin American cities and later beyond endemic countries creating new epidemiological, economic and social challenges [2]. Approximately 5-7 million people are infected with *T. cruzi* in the world, and approximately 10,000 people per year die of complications linked to this disease [3].

Benznidazole (Bz), the first-line treatment in most countries, together with nifurtimox are the only two drugs available for in the treatment of Chagas disease, both introduced in the 1970s. The results obtained with these two nitroheterocycles vary according to the phase of the disease, the period of treatment and dose, and the age and geographical origin of the patients, being their major limitations the limited and curative activity in the established chronic form and their toxic effects [4].

*Address correspondence to this author at the Instituto de Tecnologia em Fármacos, Farmanguinhos, Fundação Oswaldo Cruz, Rua Sizenando Nabuco 100, 21041, Rio de Janeiro, RJ, Brazil; Phone/Fax: +55 (21) 3977-2462; E-mail: edson.ferreira@far.fiocruz.br

Interest into nitroheterocycle drugs for the treatment of infectious diseases has undergone a resurgence in recent years [5], despite their potential adverse properties associated with DNA damage; metronidazole was included in the WHO list of Essential Medicines for the treatment of bacterial and protozoal infection [6]. In special, the 5-nitroimidazole core is a useful framework for medicinal chemistry investigation as exemplified by the performance of metronidazole and fexinidazole against pathogenic trypanosomatids, including a current clinical trial of fexinidazole for patients with chronic indeterminate Chagas disease (EudraCT 2016-004905-15) [7].

Megazol (**1**), a 5-nitroimidazole-thiadiazole derivative, have remarkable activity in preclinical *in vitro* and *in vivo* studies for Chagas disease, even against strains refractory to Bz [8]. This compound showed also great efficacy in experimental treatment of different animal models infected with *Trypanosoma brucei gambiense* [9]. Its trypanocidal activity is described as a scavenger of trypanothione, the cofactor for trypanothione reductase with interference with the oxygen metabolism of the parasite [10]. However, the development of megazol was discontinued due to the toxicity and mutagenicity induced by its use in animals [11]. Trying to avoid this unfavourable profile, there have been numerous efforts to use megazol as a core structure for the design of new compounds [12].

Nitrofurazone (**2**) a 5-nitro-2-furfurylidenesemicarbazone, has been known for a long time to exhibit antimicrobial activity and against *T. cruzi*. In mice experimentally infected nitrofurazone led to parasitological cure [13] and when used for the treatment of children at the acute phase, and adults, in both the acute and chronic phases, was effective, but it was abandoned due to its severe side effects [14]. The mechanism of action of nitrofurazone was associated with the inhibition of cruzain [15], a cysteine protease extensively investigated as a drug target in preclinical studies for Chagas disease [16].

In our attempt to develop new potent trypanocidal compounds, we constructed a new class of hydrazonoyl derivatives aiming two distinct molecular targets of *T. cruzi*. It was designed by molecular hybridization (functional retroisomerism) between two nitroheterocyclic compounds megazol (1) and nitrofurazone (2). This class planned in order to include inhibitory profile against cruzain (site B) to the 5-nitroimidazole subunit (site A), which has a recognized capacity to interfere in the redox metabolism of the parasite [10a,17].

The designed hydrazonoyl derivatives (**3-16**) were synthesized presenting substituents with different stereoelectronic properties attached to the phenyl subunit C (Figure 1). After that, they were tested against *T. cruzi* and the more active compounds had their activity against mammalians cells evaluated.

2. RESULTS AND DISCUSSION

2.1 Chemistry

The synthetic route used for the preparation of the hydrazonoyl compounds (**3-16**) is delineated in the Scheme 1. 1,2-Dimethyl-5-nitro-1H-imidazole (**17**) was converted into the corresponding phenylvinyl benzoate (**18**) in 92% yield through its base-catalysed condensation with benzoyl chloride [18]. Subsequently, the phenylvinyl benzoate (**18**) was hydrolysed in acid medium to provide the ketone intermediate (**19**) in 92% yield [19]. Finally, the hydrazonoyl derivatives were obtained through condensation reaction via the diazonium salt. The condensation of the ketonic intermediate with the corresponding anilines previously diazotized occurs in ethanolic sodium hydroxide solution [20]. The ^{1}H- and ^{13}C-NMR spectra, IR and mass spectra of the synthesised compounds (**3-16**) were consistent with the proposed structures.

2.2 Biological activity

The para fluoro substituted (**4**) was the most active

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compound against *T. cruzi* (IC_{50}/24h = 15.0 ± 2.7 µM), with activity similar to that of Bz (IC_{50}/24h = 10.8 ± 0.4 µM) (Table 1). Substitution of fluorine for hydrogen results in minor steric alterations, but electrostatic repulsive interaction or attraction of fluorine in para phenyl position can lead to significant interactions changes [21]. One of the major effects of substitution of fluorine for hydrogen in this series is the reduce of amine basicity [22], preventing the amine to be protonated resulting in higher bioavailability which directly affects the absorption process [23]. The lower activity of para trifluoromethyl (10) (IC_{50}/24h = 451.9 µM), when compared with (**4**) (IC_{50}/24h = 15.0 µM), could be explained by the drastic steric change as its van der Waals volume may impose [24].

The trypanocidal activity displayed by the electron donating substituents (**7**, **8**, **15** and **16**), mono and di-substituted derivatives, showed it was of great importance for the activity against trypomastigote forms of *T. cruzi*, probably due to its capacity to be identified through target bioreceptor by dipolar interactions or as hydrogen bond acceptor.

The *ortho*-bromo derivative (**13**) had important trypanocidal activity (IC_{50}/24h = 43.7 µM), superior to that of the *ortho*-chloro derivative (**11**) (IC_{50}/24h >500 µM) and para-*bromo* derivatives (**6**) (IC_{50}/24h = 263.3 µM) pointing out that this higher activity is relative to a very specific interaction with the target bioreceptor.

The cellular viability in the presence of most active derivatives against bloodstream forms of *T. cruzi* (IC_{50}/24 h) (**4**, **7**, **13**, **15** and **16**) was determined by mammalian cells (LC_{50}/24 h), providing resolve the selectivity index (SI) calculated from the ratio of LC_{50}/IC_{50} (Table 1). The most active compound against the parasite (**4**) also presented the lowest cytotoxicity profile, leading to a SI of 18.7.

3. CONCLUSIONS

We have described herein a different structural hydrazonoyl derivatives profile able to display an important antitrypanosomal profile *in vitro*. Among these hydrazonoyl derivatives, we identified the derivative (**4**) that showed trypanocidal activity (IC_{50}/24 h = 15.0 µM) similar to Bz, the standard drug, and low toxicity to mammalian cells, reaching a SI value of 18.7.

4. EXPERIMENTAL PROTOCOLS

4.1 General Procedures for preparing 3-16

Melting points were determined on a Buchi apparatus (B-545) and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer in potassium bromide pellets. Bond position are presented as wavenumbers (V) whose unit is cm^{-1}. ^{1}H-NMR spectra were recorded at room temperature on Bruker Avance 500 and Bruker Avance 400 spectrometers operating at 500/125 and 400/100 MHz (^{1}H/^{13}C), respectively. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane, which was used as an internal standard. Low resolution mass spectra (MS) were obtained by electron-spray ionisation in a LC/MS Amazon SL. Microanalysis data were obtained using a Perkin–Elmer 240 analyser, using a Perkin–Elmer AD-4 balance. The progress of all reactions was monitored by TLC, which was performed on 2.0 X 6.0 cm aluminium sheets that were precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light (254 and 265 nm).

(*E*)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-phenylhydrazono)ethanone (**3**)

Yellow solid, 54% yield, mp. 170-171 °C, FTIR (KBr, cm^{-1}) υmax: 3218 (N-H); 1635 (C=O stretch); 1537 and 1369 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.75 (3H, s, N-CH_3); 7.06 (1H, t, *J*=7.3Hz, H-17); 7.22 (2H, d, *J*=7.8Hz, H-15 and H-19); 7.34 (2H, t, *J*=7,8Hz, H-16 and H-18); 7.57 (2H, t, *J*=7.4Hz, H-10 and H-12); 7.66 (1H, t, *J*=7.3Hz, H-11); 7.95 (2H, d, *J*=7.2Hz, H-9 and H-13); 8.37 (1H, s, H-4); 11.16 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.38 (N-CH_3); 115.02 (C-15 and C-19), 123.47 (C-1); 127.88 (C-10 and C-12); 128.36 (C-6), 129.36 (C-16 and C-18); 129.90 (C-9 and C-13); 131.90 (C-11); 133.11 (C-4); 137.34 (C-8); 139.67 (C-2); 142.42 (C-5); 143.13 (C-14); 189.45 (C-7).; MS/ESI (*m/z* $[M-1]^+$): 348.1. Anal. Calcd. for $C_{18}H_{15}N_5O_3$: C, 61.89; H, 4.33; N, 20.05; O, 13.74 Found: C, 61.81; H, 4.55; N, 19.81; O, 13.83

(*E*)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-fluorophenyl)-hydrazono)ethanone (**4**)

Yellow solid, 80% yield, mp. 163-164°C, FTIR (KBr, cm^{-1}) υmax: 3201 (N-H), 1624 (C=O stretch), 1538 and 1362 (NO_2 stretch), 1206 (C-F); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.75 (3H, s, N-CH_3); 7.20 (4H, m, H-15, H-16, H-18 and H-19); 7.56 (2H, d, *J*=7.46Hz, H-10 and H-12); 7.66 (1H, tt, *J*=3,15Hz, H-11); 7.94 (2H, d, H-9 and H-13); 8.37 (1H, s, H-4); 11.17 (1H, s N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.37 (N-CH_3); 116.10 (d, *J*=22.78Hz, C-16 and C-18); 116.57 (d, *J*= 8.05Hz, C-15 and C-19); 127.91 (C-10 and C-12); 128.36 (C-6); 128.36 (C-6); 129.87 (C-9 and C-13); 131.90 (C-11); 133.10 (C-4); 137.33 (C-8); 139.01 (d, *J*=2,37Hz C-14); 139.66 (C-2); 143.07 (C-5); 157.22 (C-17); 189.45 (C-7); MS/ESI (*m/z* $[M-1]^+$): 366.1. Anal. Calcd. for $C_{18}H_{14}FN_5O_3$: C, 58.85; H, 3,84; F, 5.17; N, 19.07; O, 13,07 Found: C, 58.81; H, 4.55; F, 4.14; N, 18.99; O, 13.91

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-chlorophenyl)hydrazono) ethanone (**5**)

Yellow solid, 64% yield, mp. 192-193°C, FTIR (KBr, cm^{-1}) υmax: 3221 (N-H), 1634 (C=O stretch),

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1540 and 1361 (NO_2 stretch), 740 (C-Cl); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.75 (1H, s, N-CH_3); 7.20 (2H, d, *J*= 9.92 Hz, H-16 and H-18); 7.39 (2H, d, *J*= 8.88 Hz, H-15 and H-19); 7.57 (2H, t, *J*= 7.50 Hz, H-10 and H-12); 7.67 (1H, t, *J*= 7.38 Hz, H-11); 7.95 (2H,d. H-9 and H-13); 8.37 (1H, s, H-4); 11.19 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.39 (N-CH_3); 116.58 (C16 and C18); 127.11 (C-17); 127.95 (C-10 and C-12); 128.99 (C-6); 129.27 (C-15 and C-19); 129.92 (C-9, C-13); 132.04 (C-11); 133.10 (C-4); 137.15 (C-8); 139.68 (C-2); 141.43 (C-5); 142.92 (C-14); 189.42 (C-7); MS/ESI (*m/z* [M-1]$^+$): 382.1. Anal. Calcd. for $C_{18}H_{14}ClN_5O_3$: C, 56.33; H, 3.68; Cl, 9.24; N, 18.25; O, 12.81 Found: C, 56.31; H, 3.65; Cl, 9.12; N, 18.19; O, 12.75

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-bromophenyl)hydrazono) ethanone (**6**)

Yellow solid, 74% yield, mp. 280-281°C, FTIR (KBr, cm^{-1}) υmax: 3140 (N-H), 1674 (C=O stretch), 1540 and 1359 (NO_2 stretch), 1021 (C-Br); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.75 (3H, s, N-CH_3); 7.15 (2H, *J*= 8.88 Hz, H-16 and H-18); 7.55 (4H, m, H-10, H-12, H-15 and H-19); 7.67 (1H, t, *J*= 7.38 Hz, H-11); 7.96 (2H, d, *J*= 7.20 Hz, H-9 and H-13); 8.37 (1H, s, H-4); 11.18 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.38 (N-CH_3); 116.97 (C-16 and C-18); 127.95 (C-10 and C-12); 129.06 (C-6); 129.93 (C-9 and C-13); 132.06 (C-11); 132.13 (C-15 and C-19); 133.09 (C-4); 137.12 (C-8); 139.67 (C-2); 141.83 (C-5); 142.89 (C-14); 189.40 (C-7); MS/ESI (*m/z* [M-1]$^+$): 428.0. Anal. Calcd. for $C_{18}H_{14}BrN_5O_3$: C, 50.48; H, 3.30; Br, 18.66; N, 16.35; O, 11.21 Found: C, 50.71; H, 3.27; Br, 18.78; N, 16.37; O, 11.01

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-hydroxyphenyl)-hydrazono)ethanone (**7**)

Yellow solid, 73% yield, mp. 245-246°C, FTIR (KBr, cm^{-1}) υmax: 3266 (N-H), 3188 (O-H) 1594 (C=O stretch), 1543 and 1336 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.72 (3H, s, N-CH_3); 6.73 (2H, d, *J*=8.88 Hz, H-16 and H-18); 7.05 (2H, d, *J*= 8.8 Hz, H-15 and H-19); 7.55 (2H, t, *J*=7.46 Hz, H-10 and H-12); 7.63 (1H, m, H-11); 7.91 (2H, d, H-9 and H-13); 8.35 (1H, s, H-4); 9.08 (1H, s, O-H); 11.08 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.37 (N-CH_3); 115.84 (C-16 and C-18); 116.61 (C-5 and C-19); 126.66 (C-6); 127.79 (C-10 and C-12); 129.80 (C-9 and C-13); 131.57 (C-11); 133.10 (C-5); 134.60 (C-4); 137.71 (C-8); 13.58 (C-2); 143.60 (C-14); 154.10 (C-17); 189.09 (C-7); MS/ESI (*m/z* [M-1]$^+$): 364.1. Anal. Calcd. for $C_{18}H_{15}N_5O_4$: C, 59.18; H, 4.14; N, 19.17; O, 17.52 Found: C, 59.08; H, 4.14; N, 19.25; O, 17.42

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-methoxyphenyl)-hydrazono)ethanone (**8**)

Yellow solid, 87% yield, mp. 250 – 251°C, FTIR (KBr, cm^{-1}) υmax: 3222 (N-H), 2948 (O-CH_3), 1623 (C=O stretch), 1525 and 1355 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.71 (3H, s, O-CH_3); 3.75 (1H, s, N-CH_3); 6.92 (2H, d, *J*=9.04 Hz, H-16 and H-18); 7.15 (2H, d, *J*= 9.04 Hz, H-15 and H-19); 7.55 (2H, t, *J*=7.46 Hz, H-10 and H-12); 7.64 (1H, t, *J*=7.43 Hz, H-11); 7.93 (2H, d, H-9 and H-13); 8.36 (1H, s, H-4); 11.14 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.38 (N-CH_3); 55.20 (O-CH_3); 114.60 (C-16 and C-18); 116.41 (C-15 and C-19); 127.20 (C-6); 127.82 (C-10 and C-12); 129.81 (C-9 and C-13); 131.66 (C-5); 135.98 (C-4); 137.63 (C-8); 139,61 (C-2); 143.43 (C-14); 155.83 (C-17); 189.24 (C-7); MS/ESI (*m/z* [M-1]$^+$): 378.1. Anal. Calcd. for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46; O, 16.87 Found: C, 59.51; H, 4.15; N, 19.23; O, 17.12

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-nitrophenyl)-hydrazono)ethanone (**9**)

Yellow solid, 65% yield, mp. 241–243°C, FTIR (KBr, cm^{-1}) υmax: 3130 (N-H), 1646 (C=O stretch), 1538 and 1329 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.88 (3H, s, N-CH_3); 7.42 (2H, *J*= 9.16 Hz, H-15 and H19); 7.63 (2H, t, *J*= 7.38 Hz, H-10 and H-12); 7.71 (1H, m, H-11); 8.14 (2H, m, H-16 and H-18); 8.23 (3H, m, H-4, H-9 and H-13); 11.93 (1H, s, N-H).^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 36.19 (N-CH_3); 115.90 (C-16 and C-18); 126.56 (C-10 and C-12); 129.21 (C-16, C-18); 129.76 (C-17); 132.06 (C-11); 130.02 (C-6); 131.43 (C-9 and C-13); 132.69 (C-11); 133.71 (C-4); 137.85 (C-8); 143.60 (C-2); 143.98 (C-5); 148.86 (C-14); 190.53 (C-7); MS/ESI (*m/z* [M-1]$^+$): 393.2. Anal. Calcd. for $C_{18}H_{14}N_6O_5$: C, 54.82; H, 3.58; N, 21.31; O, 20.29 Found: C, 55.01; H, 3.51; N, 21.30; O, 20.30

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(4-(trifluoromethyl)phenyl)hydrazono)ethanone (**10**)

Yellow solid, 54% yield, mp. 170-171 °C, FTIR (KBr, cm^{-1}) υmax: 3223 (N-H); 1633 (C=O stretch); 1537 and 1369 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.77 (3H, s, N-CH_3); 7.36 (2H, d, *J*= 8.4Hz, H-15 and H-19); 7.69 (2H, t, *J*= 8.7Hz, H-16 and H-18); 7.59 (2H, t, *J*= 7.8Hz, H-10 and H-12); 7.68 (1H, t, *J*= 7.3Hz, H-11); 7.98 (2H, d, *J*= 7.3Hz, H-9 and H-13); 8.38 (1H, s, H-4); 11.31 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.42 (N-CH_3); 115.11 (C-15 and C-19), 122.96 (C-1); 123.28 (C-10 and C-12); 125.66 (C-6); 126.70 (C-20), 128.05 (C-16 and C-18); 130.05 (C-9 and C-13); 130.31 (C-11); 132.29 (C-4); 133.12 (C-8); 136.90 (C-2); 139.76 (C-5); 142.65 (C-14); 145.75 (C-17); 189.52 (C-7); MS/ESI (*m/z* [M-1]$^+$): 348.1. Anal. Calcd. for $C_{19}H_{14}F_3N_5O_3$: C, 54.60; H, 4.46; F, 13.62; N, 15.79; O, 11.53 Found: C, 54.68; H, 3,38; F, 13.66; N, 16.78; O, 11,50

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-

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phenyl-2-(2-(2-chlorophenyl)hydrazono) ethanone (**11**)

Yellow solid , 92% yield, mp. 280-281°C, FTIR (KBr, cm^{-1}) υmax: 3152 (N-H), 1669 (C=O stretch), 1540 and 1359 (NO_2 stretch), 1023 (C-Cl); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.71 (3H, s, N-CH_3); 7.02 (1H, t, *J*= 8.0 Hz, H-17); 7.27 (1H, d, *J*= 8.2 Hz, H-19); 7.38 (1H, t, *J*= 8.0 Hz, H-18); 7.61 (2H, t, *J*= 8.0 Hz, H-10 and H-12); 7.66 (1H, d, *J*= 8.0 Hz, H-16); 7.71 (1H, t, *J*= 8.0 Hz, H-11); 8.05 (2H, t, *J*= 8.4 Hz, H-9 and H-13); 8.44 (1H, s, H-4); 12.40 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 35.99 (N-CH_3); 109.25 (C-18); 115.77 (C-4); 124.95 (C-9 and C-13); 128.23 (C-10 and C-12); 129.09 (C-19); 129.19 (C-11); 130.32 (C-16); 131.53 (C-8); 132.83 (C-17); 132.93 (C-15) 136.52 (C-5); 139.30 (C-14); 139.71 (C-6); 142.86 (C-2); 189.49 (C-7); MS/ESI (*m/z* [M-1]$^+$): 383.0. Anal. Calcd. for $C_{18}H_{14}ClN_5O_3$: C, 50.48; H, 3.30; Cl, 18.66; N, 16.35; O, 11.21 Found: C, 50.71; H, 3.27; Cl, 18.78; N, 16.37; O, 11.01

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1-phenyl-2-(2-(3-chlorophenyl)hydrazono) ethanone (**12**)

Yellow solid, 92% yield, mp. 280-281°C, FTIR (KBr, cm^{-1}) υmax : 3149 (N-H), 1666 (C=O stretch), 1540 and 1359 (NO_2 stretch), 1019 (C-Cl); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.75 (3H, s, N-CH_3); 7.09 (1H, d, *J*= 7.92 Hz, H-17); 7.15 (1H, d, *J*= 6.96 Hz, H-18); 7.23 (1H, t, *J*= 2.0 Hz, H-15); 7.36 (1H, t, *J*= 8.1 Hz, H-19); 7.57 (2H, t, *J*= 7.28 Hz, H-10 and H-12); 7.68 (1H, m, H-11); 7.97 (2H, d, H-9 and H-13); 8.38 (1H, s, H-4); 11.18 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.37 (N-CH_3); 113.59 (C-15); 114.72 (C-17); 122.83 (C-16); 127.90 (C-10 and C-12); 129.43 (C6); 129.95 (C-9 and C-13); 131.08 (C-11); 132.11 (C-18); 133.09 (C-4); 133.80 (C-19); 137.08 (C-8); 139.71 (C-2); 142.68 (C-5); 143.97 (C-14); 189.52 (C-7). MS/ESI (*m/z* [M-1]$^+$): 428.0. Anal. Calcd. for $C_{18}H_{14}ClN_5O_3$: C, 50.48; H, 3.30; Cl, 18.66; N, 16.35; O, 11.21 Found: C, 50.71; H, 3.27; Cl, 18.78; N, 16.37; O, 11.01

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(2-bromophenyl)hydrazono) ethanone (**13**)

Yellow solid, 92% yield, mp. 280-281°C, FTIR (KBr, cm^{-1}) υmax: 3140 (N-H), 1674 (C=O stretch), 1540 and 1359 (NO_2 stretch), 1025 (C-Br); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.71 (3H, s, N-CH_3); 7.02 (1H, t, *J*= 8.0 Hz, H-17); 7.27 (1H, d, *J*= 8.2 Hz, H-19); 7.38 (1H, t, *J*= 8.0 Hz, H-18); 7.61 (2H, t, *J*= 8.0 Hz, H-10 and H-12); 7.66 (1H, d, *J*= 8.0 Hz, H-16); 7.71 (1H, t, *J*= 8.0 Hz, H-11); 8.05 (2H, t, *J*= 8.4 Hz, H-9 and H-13); 8.44 (1H, s, H-4); 12.40 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 35.99 (N-CH_3); 109.25 (C-18); 115.77 (C-4); 124.95 (C-9 and C-13); 128.23 (C-10 and C-12); 129.09 (C-19); 129.19 (C-11); 130.32 (C-16); 131.53 (C-8); 132.83 (C-17); 132.93 (C-15) 136.52 (C-5); 139.30 (C-14); 139.71 (C-6); 142.86 (C-2); 189.49 (C-7); MS/ESI (*m/z* [M-1]$^+$): 428.0. Anal. Calcd. for $C_{18}H_{14}BrN_5O_3$: C, 50.48; H, 3.30; Br, 18.66; N, 16.35; O, 11.21 Found: C, 50.71; H, 3.27; Br, 18.78; N, 16.37; O, 11.01

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(3,5-dichlorophenyl)hydrazono)ethanone (**14**)

Yellow solid, 92% yield, mp. 280-281°C, FTIR (KBr, cm^{-1}) υmax: 3152 (N-H), 1669 (C=O stretch), 1540 and 1359 (NO_2 stretch), 1023 (C-Cl); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.76 (3H, s, N-CH_3); 7.18 (1H, t, H-15 and H-18); 7.23 (1H, t, H-19); 7.57 (2H, t, *J*= 7.5 Hz, H-10 and H-12); 7.69 (1H, t, *J*= 7.38 Hz, H-11); 7.97 (2H, d, H-9 and H-13); 8.38 (1H, s, H-4); 11.21 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.91 (N-CH_3); 114.14 (C-18); 128.22 (C-10 and C-12); 131.00 (C-6); 129.58 (C-15 and C-19); 130.57 (C-9 and C-13); 132.88 (C-11); 133.20 (C-4); 137.44 (C-8); 140.31 (C-2); 140.40 (C-5) 142.83 (C-14); 190.11 (C-7). MS/ESI (*m/z* [M-1]$^+$): 428.0. Anal. Calcd. for $C_{18}H_{13}Cl_2N_5O_3$: C, 50.48; H, 3.30; Cl, 18.66; N, 16.35; O, 11.21 Found: C, 50.71; H, 3.27; Cl, 18.78; N, 16.37; O, 11.01

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(3,5-dimethoxyphenyl)hydrazono)ethanone (**15**)

Yellow solid, 82% yield, mp. 163–165°C, FTIR (KBr, cm^{-1}) υmax: 3132 (N-H), 1649 (C=O stretch), 1540 and 1332 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.67 (6H; s; O-CH_3); 3.75 (3H; s; N-CH_3); 6.19 (1H, s, H-17); 6.41 (2H, s, H-15 and H-19); 7.56 (2H, t, *J*=7,56Hz, H-10 and H-12); 7.64 (1H, t, *J*=7,28Hz, H-11); 7.96 (2H, d, *J*=7,32Hz, H-9 and H-13); 8.37 (1H, s, H-4); 11.08 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.47 (N-CH_3); 55.12 (O-CH_3); 93.42 (C-16 and C-18); 96.01 (C-15 and C-19); 127.85 (C-10 and C-12); 131.91 (C-5); 133.23 (C-4); 137.47 (C-8); 139.78 (C-2); 142.99 (C-14); 189.59 (C-7).; MS/ESI (*m/z* [M-1]$^+$): 392.2. Anal. Calcd. for $C_{19}H_{15}N_5O_5$: C, 58.01; H, 3.84; N, 17.80; O, 20.34 Found: C, 58.09; H, 3.43; N, 17.65; O, 20.46

(E)-2-(1-methyl-5-nitro-1*H*-imidazol-2-*yl*)-1-phenyl-2-(2-(benzo[d][1,3]dioxophenyl)-hydrazono)ethanone (**16**)

Yellow solid, 82% yield, mp. 163–165°C, FTIR (KBr, cm^{-1}) υmax: 3130 (N-H), 1646 (C=O stretch), 1538 and 1329 (NO_2 stretch); ^{1}H-NMR (DMSO-$_{d6}$, 400 MHz) δ (ppm): 3.74 (3H; s; N-CH_3); 5.99 (2H; s; H-20); 6.68 (1H, dd, H-18); 6.75 (1H, d, *J*=2.12Hz, H-19); 6.88 (1H, d, *J*=8.4Hz, H-15); 7.55 (2H, t, *J*=7.40Hz, H-10 and H-12); 7.65 (1H, m, H-11); 7.91 (2H, m, H-9 and H-13); 8.36 (1H, s, H-4); 11.12 (1H, s, N-H). ^{13}C-NMR (DMSO-$_{d6}$, 100 MHz) δ (ppm): 34.39 (N-CH3); 96.67 (C-19); 101.22 (C-20); 108.18 (C-15); 108.51 (C-18); 127.34 (C-6); 127.81 (C-10 and C-12); 129.77 (C-9 and C-13); 131.71 (C-11); 133.09 (C-4); 137.56 (C-8); 139.61 (C-2); 143.23 (C-5); 143.61 (C-14); 148.03 (C-16 and

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C-17); 189.35 (C-7).; MS/ESI (*m/z* $[M-1]^+$): 392.2. Anal. Calcd. for $C_{19}H_{15}N_5O_5$: C, 58.01; H, 3.84; N, 17.80; O, 20.34 Found: C, 58.09; H, 3.43; N, 17.65; O, 20.46.

4.2 Trypanocidal activity

The trypanocidal profile of novel hydrazonoyl derivatives (**3-16**) was carried out using the Y strain of *T. cruzi*. Bloodstream trypomastigotes were obtained from infected mice at peak parasitemia [25]. Stock solutions of the compounds were prepared in DMSO, and the assays were performed in Dulbecco's modified Eagle medium. The final concentration of the solvent never exceeded 0.5%, which has no deleterious effect on the parasite. All tests were performed by mixing 100 µL of cell suspension with an equal volume of the desired test-compound solution to make a final drug concentration ranging from 1.5 to 500 µM, and incubating at 37° C for 24 h. Untreated and Bz-treated parasites were used as controls. The results were analysed by plotting % lysis of *T. cruzi* against the concentration of the test compound.

4.5 Cytotoxicity to mammalian cells

The cytotoxicity assays were performed using primary cultures of peritoneal macrophages obtained from Albino Swiss mice. For the experiments, 2.5 x 10^4 cells in 200 µL of RPMI-1640 medium (pH 7.2 plus 10% fetal bovine serum and 2 mM glutamine) were added to each well of a 96-well microtiter plate and incubated for 24 h at 37° C. The treatment of the cultures was performed in fresh supplemented medium (200 µL/well) for 24 h at 37° C. After this period, 110 µL of the medium was discarded and 10 µL PrestoBlue (Invitrogen) was added to complete the final volume of 100 µL. Thus, the plate was incubated for 2 h and the measurement was performed at 560 and 590 nm, as recommended by the manufacturer. The results were expressed as the difference in the percentage of reduction between treated and untreated cells being the LC_{50} value, corresponding to the concentration that leads to lysis of 50% of the mammalian cells. The selectivity index (SI) were calculated by the ratio between LC_{50} and IC_{50} [26].

CONFLICT OF INTEREST

None

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Figure 1. Design concept of new hydrazonoyl derivatives (**3-16**). See Table 1 for the nature of substituents R_1-R_4.

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Table 1. *In vitro* trypanocidal activity against trypomastigotes, cytotoxicity to mammalian cells and Selectivity Index (SI) of new hydrazonoyl compounds (**3**-**16**).

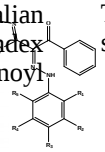


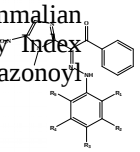
Figure 1. Design concept of new hydrazonoyl derivatives (**3**-**16**). See Table 1 for the nature of substituents R_1-R_4.

Cpd	R_1	R_2	R_3	R_4	IC_{50}/24h (μM)	LC_{50}/24h (μM)	SI
3	H	H	H	H	>500	-	-
4	H	H	F	H	15.0±2.7	280.5±29.4	18.
5	H	H	Cl	H	312.4±59.0	-	-
6	H	H	Br	H	263.3±44.1	-	-
7	H	H	OH	H	36.6±2.8	26.1±4.7	0.7
8	H	H	OCH_3	H	92.6±10.3	-	-
9	H	H	NO_2	H	183.8±28.2	-	-
10	H	H	CF_3	H	451.9±72.4	-	-
11	Cl	H	H	H	>500	-	-
12	H	Cl	H	H	>500	-	-
13	Br	H	H	H	41.2±5.5	62.5	1.5
14	H	Cl	H	Cl	331.4±25.5	-	-
15	H	OCH_3	H	OCH_3	43.7±5.7	66.7±4.4	1.5
16	H	O-CH_2-O		H	22.0±3.5	41.7±9.8	1.9

Scheme 1. Synthetic route for the preparation of the new hydrazonoyl compounds (**3**-**16**).

Review Version

Table 1. *In vitro* trypanocidal activity against trypomastigotes, cytotoxicity to mammalian cells and Selectivity Index (SI) of new hydrazonoyl compounds (**3**-**16**).



Cpd	R_1	R_2	R_3	R_4	IC_{50}/24h (μM)	LC_{50}/24h (μM)	SI
3	H	H	H	H	>500	-	-
4	H	H	F	H	15.0±2.7	280.5±29.4	18.
5	H	H	Cl	H	312.4±59.0	-	-
6	H	H	Br	H	263.3±44.1	-	-
7	H	H	OH	H	36.6±2.8	26.1±4.7	0.7
8	H	H	OCH_3	H	92.6±10.3	-	-
9	H	H	NO_2	H	183.8±28.2	-	-
10	H	H	CF_3	H	451.9±72.4	-	-
11	Cl	H	H	H	>500	-	-
12	H	Cl	H	H	>500	-	-
13	Br	H	H	H	41.2±5.5	62.5	1.5
14	H	Cl	H	Cl	331.4±25.5	-	-
15	H	OCH_3	H	OCH_3	43.7±5.7	66.7±4.4	1.5
16	H	O-CH_2-O		H	22.0±3.5	41.7±9.8	1.9

Scheme 1. Synthetic route for the preparation of the new hydrazonoyl compounds (**3**-**16**).

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Figure 1. Design concept of new hydrazonoyl derivatives (**3-16**). See Table 1 for the nature of substituents R_1-R_4.

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Scheme 1. Synthetic route for the preparation of the new hydrazonoyl compounds (**3-16**).

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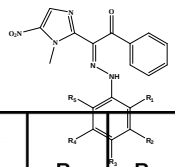
Table 1. *In vitro* trypanocidal activity against trypomastigotes, cytotoxicity to mammalian cells and Selectivity Index (SI) of new hydrazonoyl compounds (**3-16**).



Cpd	R_1	R_2	R_3	R_4	IC_{50}/24h (μM)	LC_{50}/24h (μM)	SI
3	H	H	H	H	>500	-	-
4	H	H	F	H	15.0±2.7	280.5±29.4	18.7
5	H	H	Cl	H	312.4±59.0	-	-
6	H	H	Br	H	263.3±44.1	-	-
7	H	H	OH	H	36.6±2.8	26.1±4.7	0.71
8	H	H	OCH_3	H	92.6±10.3	-	-
9	H	H	NO_2	H	183.8±28.2	-	-
10	H	H	CF_3	H	451.9±72.4	-	-
11	Cl	H	H	H	>500	-	-
12	H	Cl	H	H	>500	-	-
13	Br	H	H	H	41.2±5.5	62.5	1.52
14	H	Cl	H	Cl	331.4±25.5	-	-
15	H	OCH_3	H	OCH_3	43.7±5.7	66.7±4.4	1.53
16	H	O-CH_2-O		H	22.0±3.5	41.7±9.8	1.90

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