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# Synthesis, anti-*Trypanosoma cruzi* activity and quantitative structure relationships of some fluorinated thiosemicarbazones

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ABSTRACT

Synthesis and spectroscopic characterization of ten fluorinated thiosemicarbazones are reported. All synthesized compounds were evaluated for their anti-*Trypanosoma cruzi* activity, and the $IC_{50}$ values were obtained in the range of 5.64–29.19 $\mu g\,mL^{-1}$ in 24 h of cultures. Among all assayed thiosemicarbazones the 2,3,4-trifluoro-substituted compound showed the higher activity with $IC_{50} = 5.64\ \mu g\,mL^{-1}$. QSAR studies involving electronic and hydrophobic parameters, as well as the $^{13}C$ NMR chemical shifts of iminic carbon indicated that the deshielding effect caused by the fluorine atoms and their hydrophobicity are significant features for the anti-*T. cruzi* activity.



## 1. Introduction

The protozoan diseases such as Chagas disease and leishmaniasis are responsible for significant mortality of tropical and subtropical regions of the world. Additionally, the prevalence of the disease has been increasing in other areas such as North America and Europe due to human migration [1,2]. The protozoan parasites infect billion of humans, world-wide and are associated with large morbidity and economic impacts [3]. The trypanosomatids are parasitic protozoa of Kinetoplastida class that cause among others the Chagas disease by *Trypanosoma cruzi*. The Chagas disease affects around 25 million people which several effects such as cardiac, gastrointestinal or neurological commitment [4,5]. Chagas disease presents an initial acute phase followed by a chronic phase. The acute phase is generally asymptomatic, while the progression of disease into its chronic phase often results in cardiomyopathy, myocardium damage and other heart diseases [6].

For treatment of Chagas disease only two drugs, nifurtimox (Lampit™) and benzinidazole (Radanil™, Rochagan™) are decades old used and, curing at least 50% after long time of use with severe side-effects [7,8], further, are not uncommon the failure of treatment. In addition, the parasite naturally resistance to these compounds has been observed [9], including the induction of maintaining the parasite under prolonged drug exposition [10]. The search of new, safe and efficient anti-parasitic agents for the treatment of Chagas disease is an urgent need for health programs in all world.

Thiosemicarbazones are now well established class of nitrogen and sulphur donor ligands very attractive because of their structural, and diversified biological activities, such as, anticancer [11,12], antimicrobial [13,14], antiviral [15], antimalarial [16], anti-leishmanial [17] and anti-trypanosomal [16,18]. There are few years ago we reported the anti-*Trypanosoma cruzi* activity of some thiosemicarbazones, and the most efficient compound, 2-methoxy-styryl-thiosemicarbazone, demonstrated a significant decrease in nitric oxide synthase enzyme activity. Further, also was observed the absence of macrophage toxicity for any of the assayed compounds [19].

The fluorine moiety when present in biological active molecules led to very important increase of their biological effects [20,21]. In several times, the drug-receptor interactions are improved in the presence of fluorine moiety and the transport of drug is facilitated by the high lipophilicity of organofluorine compounds [22].

Thus, the early results of anti-*T. cruzi* effects by styrylaldehyde-thiosemicarbazones motivated us to prepare a series of thiosemicarbazones containing fluorine moieties in the benzaldehyde portion. Furthermore, the literature reports that among the thiosemicarbazones, assayed for anti-parasitic activities, fluorinated benzaldehydes-thiosemicarbazones have been rarely tested. So, in this paper we report the synthesis and full characterization

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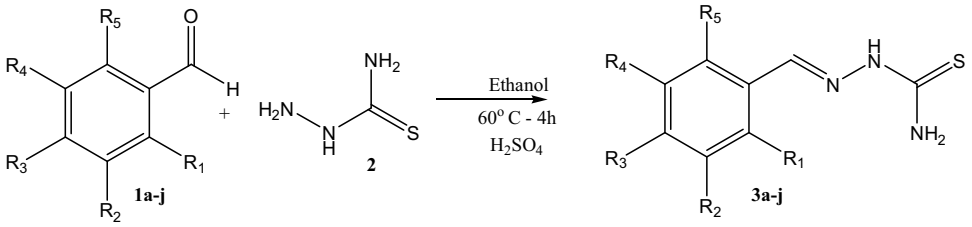



**a:** $R_1$=$R_2$=F, $R_3$=$R_4$=$R_5$=H
**b:** $R_1$=$R_4$=F, $R_2$=$R_3$=$R_5$=H
**c:** $R_1$=$R_5$=F, $R_2$=$R_3$=$R_4$=H
**d:** $R_1$=$R_2$=$R_3$=F, $R_4$=$R_5$=H
**e:** $R_1$=$R_2$=$R_4$=$R_5$=F, $R_3$=H
**f:** $R_1$=$R_2$=$R_3$=$R_4$=$R_5$=F
**g:** $R_2$=$R_4$=F, $R_1$=$R_3$=$R_5$=H
**h:** $R_3$=F, $R_1$=$R_2$=$R_4$=$R_5$=H
**i:** $R_2$=$R_3$=F, $R_1$=$R_4$=$R_5$=H
**j:** $R_3$=$CF_3$, $R_1$=$R_2$=$R_4$=$R_5$=H

**Scheme 1.** Synthesis of the compounds 3a–j.

by $^1$H, $^{13}$C and $^{19}$F NMR, of ten fluoro-benzaldehyde-thiosemicarbazones (**3a–j**), the anti-*T. cruzi* epimastigotes effects and the preliminary quantitative structure activity relationship (QSAR).

## 2. Results and discussion

### 2.1. Chemistry

A series of ten fluoro-benzaldehyde-thiosemicarbazones (**3a–j**), in which the compounds **3a, 3d**, **3e** and **3f** are described here for the first time, was prepared from the fluorinated benzaldehydes (**1a–j**) with thiosemicarbazide (**2**) in ethanol, as solvent, and few drops of $H_2SO_4$ at 60 °C within 4 h, according to literature, [17] as shown in the Scheme 1. The products were recrystallized from methanol in 92–96% yield. The infrared spectra show the band at 1500–1530 $cm^{-1}$ and 1030–1097 $cm^{-1}$ related to $\nu$ C=S and $\nu$ (C=N), respectively, as reported in the literature [23]. $^1$H, $^{13}$C and $^{19}$F NMR spectra (Supplementary data) permitted the full characterisation of all thiosemicarbazones.

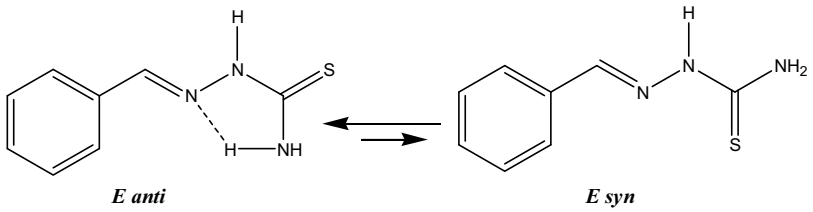
The $^{13}$C NMR spectra showed that all the chemical shift values of C-1′ to the fluorinated-benzaldehyde thiosemicarbazones (**3a–j**) have upfield shift ($\Delta\delta < 0$) when compared to benzaldehyde thiosemicarbazone (**TH**), as can be observed in Table 1. In addition, when there is at least one fluorine atom is present in *ortho* position, the shielding is most pronounced ($\Delta\delta \geq |8{\cdot}9|$). Moreover, other increase in shielding is observed of $\Delta\delta = -11{\cdot}3$ and $\Delta\delta = -12{\cdot}0$ to compounds with 4 (**3e**) and 5 (**3f**) fluorine atoms, respectively. The C-1 (*ipso* carbon) also stay shielding ($\Delta\delta < 0$), with exception of the compound **3g** (two fluorine atoms in *meta* position) and **3j** (4-$CF_3$). The difference of chemical shifts ($\Delta\delta$) these carbon atoms are higher than C-1′ atoms, and the polyfluorinated compounds, **3e** and **3f**, present values of $\Delta\delta = -19{\cdot}9$ and $\Delta\delta = -24{\cdot}6$, respectively, when compared to benzaldehyde thiosemicarbazone. These results can be attributed to mesomeric and polar effects caused by fluorine atom in the *ortho* position. A dependence linear was obtained ($r^2 = 0.89$) between Δδ C-1′ and Δδ C-1, with two outliers, **3b** and **3h**.

The $^1$H NMR shifts for azomethine hydrogen (H-1′) present two tendencies, when there is one or more fluorine atom are present in *orto* position; the chemical shift of H-1′ atom is downfield ($\Delta\delta > 0$), and the opposite effect is observed to C-1′ atom. It can be due to anisotropic effect caused by N atom, since another compounds (**3g–j**) are upfield ($\Delta\delta < 0$) with exception of compound **3j**. This same tendency was observed for chemical shifts of the Schiff bases [24]. The hydrogens of the $NH_2$ group present different chemical shifts, indicating that these not is homotopic, probably due to establishing of an intramolecular hydrogen bond (Scheme 2) with the N-5′ of azomethine group. However, as can be observed in Table 1, at least one H-5′ is more deshielding than the corresponding hydrogen of the benzaldehyde-thiosemicarbazone ($\delta$ 8.22). The presence of an intramolecular strong hydrogen bond can be the cause of this hydrogen chemical shift in downfield, highlight the compounds **3e** and **3f** that present the chemical shifts in $\delta$ 8.54 and $\delta$ 8.52, respectively. The $^{19}$F NMR chemical shifts present δ −162.7 to −109.4 according with the contribution of electronic effect due the fluorine atoms at *ortho*, *meta*, and *para* position, with exception the compound **3j** ($R_3$ = $CF_3$, δ = −65.0). Further, were observed values of F–F coupling in the range of 5 Hz to 25 Hz due to distance between the fluorine atoms. The $^{19}$F NMR chemical shifts as F–F coupling were in concordance with the literature [25].

**Table 1**
Some $^1$H and $^{13}$C chemical shifts for compounds **3a–j** in DMSO-$d_6$.

| Entry | Comp. | Substituent | | | | | δ C-1′ | Δδ C-1′-C-1′[a] | δ C-1 | Δδ C-1-C-1[a] | δ H-1′ | Δδ H-1′-H-1′[a] | H-5′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | | | | | | | |
| 1 | **TH**[a] | H | H | H | H | H | 142.8 | 0 | 134.6 | 0 | 8.05 | 0 | 8.22/8.01 |
| 2 | **3a** | F | F | H | H | H | 133.9 | −8.9 | 124.6 | −10.0 | 8.26 | 0.21 | 8.38/8.15 |
| 3 | **3b** | F | H | H | F | H | 134.3 | −8.4 | 123.5 | −11.1 | 8.21 | 0.16 | 8.36/8.16 |
| 4 | **3c** | F | H | H | H | F | 133.8 | −9.7 | 111.1 | −23.4 | 8.21 | 0.16 | 8.43/7.36 |
| 5 | **3d** | F | F | F | H | H | 133.2 | −9.6 | 120.6 | −14.0 | 8.18 | 0.13 | 8.36 |
| 6 | **3e** | F | F | H | F | F | 131.5 | −11.3 | 114.7 | −19.9 | 8.16 | 0.11 | 8.54/7.42 |
| 7 | **3f** | F | F | F | F | F | 130.8 | −12.0 | 110.0 | −24.6 | 8.12 | 0.07 | 8.52/7.43 |
| 8 | **3g** | H | F | H | F | H | 139.8 | −3.0 | 138.6 | +4.0 | 7.98 | −00.7 | 8.33/8.28 |
| 9 | **3h** | H | H | F | H | H | 141.6 | −1.2 | 131.1 | −3.5 | 8.04 | −001 | 8.24 |
| 10 | **3i** | H | F | F | H | H | 140.2 | −2.6 | 132.6 | −2.0 | 8.00 | −0.05 | 8.27/8.21 |
| 11 | **3j** | H | H | $CF_3$ | H | H | 140.7 | −2.1 | 138.7 | +4.1 | 8.10 | 0.05 | 8.36/8.20 |

[a] Phenyl-thiosemicarbazone.




**Scheme 2.** *Anti* and *syn* conformation for the *E* configuration of benzaldehyde thiosemicarbazone.

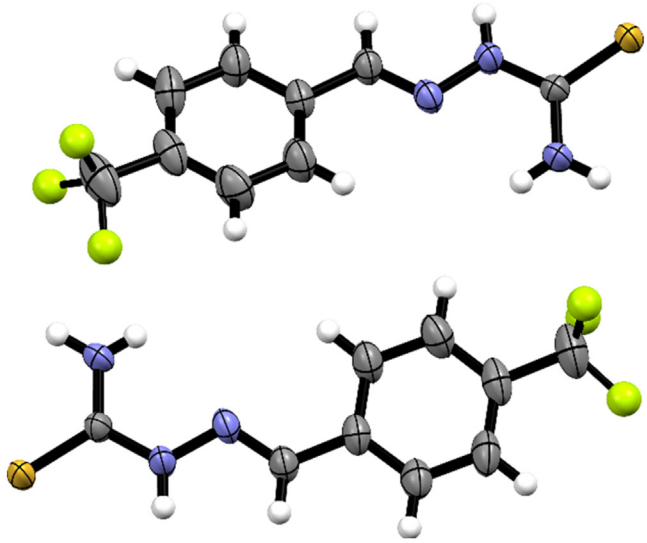


**Fig. 1.** Crystal structure of **3j** showing two co-crystallized molecules in the asymmetry unit. Ellipsoids at 40% of probability. For sake of clarity, fluorine atoms are shown as spheres. Color codes: gray – carbon; white – hydrogen; blue – nitrogen; yellow – sulphur; green – fluorine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A single crystal X-ray diffraction enabled the determination of the structure of compound **3j**; the same crystal structure showed two co-crystallized molecules. The *anti*-configuration (*E*) was observed as seen in Fig. 1, this conformation favours an intramolecular hydrogen bond, as previously mentioned.

### 2.2. *Anti*-Trypanosoma cruzi *assays and 2D-QSAR model*

The benzaldehyde fluoro-substituted thiosemicarbazones **3a–j** were evaluated *in vitro* against culture epimastigotes of *T. cruzi*. The assays were performed with concentrations in the range of 100–3.125 mgmL$^{-1}$ in 24 h of cultures, and showed values of $IC_{50}$ = 5.64–29.19 μg mL$^{-1}$ (Table 2). The most active compounds were di and tri-fluoro-substituted and, the best effect was showed by 2,3,4-trifluorobenzaldehyde thiosemicarbazone with $IC_{50}$ = 5.64 μg mL$^{-1}$. The assays using the benzaldehyde-thiosemicarbazone (unsubstituted thiosemicarbazone), performed in same conditions against epimastigotes of *T. cruzi*, showed $IC_{50}$ value of 78.05 μM (13.98 μg mL$^{-1}$), reinforcing the importance of the presence of fluorine atoms in aromatic ring, as shown in Table 2.

In order to investigated the 2D-QSAR model of relationships of anti-*T. cruzi* effects and the chemical structures of the benzaldehyde fluoro-substituted thiosemicarbazones the physical-chemistry descriptors, molar refractivity (MR), molar volume (MV), log *P* and $pK_a$ were calculated from ACDLabs software package, version 12.0 [26]. Further, the electronic parameters, Hammett constant (σ) [27] and the chemical shift of iminic carbon atoms (C-1′), and the Hansh hydrophobicity constant (π) [28] were also considered, as showed in Table 2.

After analysing the several generated linear and parabolic 2D-QSAR models, utilizing BuildQSAR software [29], only two models presented satisfactory statistical data as shown in the Eqs. (1) and (2). As can be seen in the Eqs. (1) and (2) the lipophilicity parameter (log *P*) and the electronic parameter expressed by the chemical shift of iminic carbon (δ C-1′) proved be important for inhibition of *T. cruzi*.

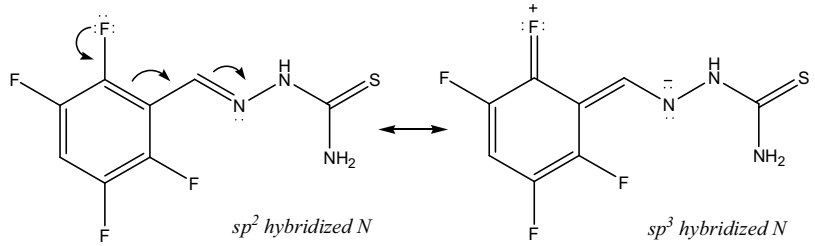
The Eq. (1), parabolic model, with two outliers: **3b** and **3g**, demonstrate the lipophilic parameter is crucial for inhibition of *T. cruzi*. Each fluorine atom added in the aromatic ring increases the lipophilicity, and it is benefits of the trypanocidal activity, however, when four fluorine atoms are added in the aromatic ring (**3e** and **3f**), there was a decreasing of the trypanocidal effect.

$$\log (1/C) = 27.5228\ (\pm 10.4241)\ \log P - 5.6353\ (\pm 2.1092)\ \log P^2 - 34.8845\ (\pm 12.7096)\quad (n = 8;\ r^2 = 0.91;\ s = 0{,}098;\ F = 26.996;\ p = 0.0021;\ q^2 = 0.605;\ \text{outliers: } \mathbf{3b} \text{ and } \mathbf{3g}) \tag{1}$$

$$\log (1/C) = 6.2573\ (\pm 3.3990)\ \text{C-}1' - 0.0230\ (\pm 0.0125)\ \text{C-}1'^2 - 426.0708\ (\pm 231.6093)\quad (n = 8;\ r^2 = 0.86;\ s = 0.119;\ F = 15.657;\ p = 0.0070;\ q^2 = 0.659;\ \text{outliers: } \mathbf{3b} \text{ and } \mathbf{3i}) \tag{2}$$

**Table 2**
$IC_{50}$ values for compounds **3a–j**, and **TH** (phenyl-thiosemicarbazone), assayed against *Trypanosoma cruzi* epimastigotes and, the descriptors log *P*, molar refractivity (MR), molecular volume (MV), Hansch (π) and sigma (σ) constants, *pK*a and chemical shifts (ppm) of C-1′ carbon.

| Entry | Comp. | $IC_{50}$ (ug/mL) | $IC_{50}$ (μM) | log $1/IC_{50}$ | log*P* | RM | VM | π | σ | $pK_a$ | δC-1′ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **3a** | 8.69 | 40.56 | −1.60 | 2.17 | 51.95 | 153.5 | 0.31 | 0.34 | 4.542 | 133.99 |
| **2** | **3b** | 19.14 | 89.35 | −1.95 | 2.33 | 51.95 | 153.5 | 0.47 | 0.34 | 4.542 | 133.38 |
| **3** | **3c** | 8.21 | 38.32 | −1.58 | 2.19 | 51.95 | 153.5 | 0.33 | 0 | 4.202 | 133.18 |
| **4** | **3d** | 5.64 | 24.28 | −1.38 | 2.33 | 51.82 | 156.4 | 0.47 | 0.49 | 4.692 | 133.19 |
| **5** | **3e** | 12.96 | 51.80 | −1.71 | 2.69 | 51.69 | 159.4 | 0.83 | 0.34 | 4.542 | 131.52 |
| **6** | **3f** | 28.52 | 106.34 | −2.02 | 2.79 | 51.56 | 162.3 | 0.93 | 0.83 | 5.032 | 130.79 |
| **7** | **3g** | 10.52 | 49.11 | −1.69 | 2.40 | 51.95 | 153.5 | 0.54 | 0.68 | 4.882 | 139.84 |
| **8** | **3h** | 29.19 | 148.76 | −2.17 | 2.07 | 52.08 | 150.6 | 0.21 | 0.15 | 4.352 | 141.61 |
| **9** | **3i** | 6.95 | 32.44 | −1.51 | 2.23 | 51.95 | 153.5 | 0.37 | 0.49 | 4.692 | 140.21 |
| **10** | **3j** | 25.84 | 105.04 | −2.02 | 2.83 | 56.96 | 177.9 | 0.97 | 0.87 | 5.072 | 140.7 |
| **11** | **TH** | 13.98 | 78.05 | – | – | – | – | – | – | – | – |




**Scheme 3.** Mesomeric effect caused by fluorine atom in the *ortho* position in 2,3,5,6-tetrafluoro-benzaldehyde thiosemicarbazones.

Considering the Eq. (2), also parabolic model, when there are four and five fluorine atoms linked to the aromatic ring, and with two fluorine atoms are linked to *ortho* position, caused a stronger shielding in the C-1′, decreasing the trypanocidal activity. This model indicates that the trypanocidal effect is increasing due to deshielding of the C-1′ atom. It can be observed that the addition of the fluorine atoms showed an opposite effect, because while the trypanocidal activity is favored by increase of lipophilicity, these does not benefit the electronic effect, because the increasing the shielding in the C-1′ carbon is observed. Moreover, the mesomeric effect caused by fluorine atom in the *ortho* or *para* position can take the nitrogen atom (N-1) to $sp^3$ hybridization (Scheme 3), therefore, changing the geometry of compound, and consequently the bio-receptor interaction.

## 3. Conclusion

All fluorinated thiosemicarbazones were synthesized in good yields, and fully characterized, including by $^{19}F$ NMR. These compounds were evaluated against epimastigotes of *T. cruzi*, and the results showing the 2,3,4-trifluoro-benzaldehyde derivative is a potential candidate to Chagas disease treatment. The QSAR model obtained was satisfactory indicating that the hydrophobicity is an important property for anti-*T. cruzi* activity.

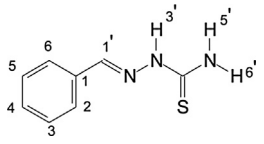
## 4. Experimental

### 4.1. Instruments and materials

The melting points were recorded on a Gehaka (PF1500 Farma) capillary melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker Vertex 70 spectrophotometer using potassium bromide tablets. $^1H$ and $^{13}C$ NMR spectra were obtained in Bruker NMR Ultrashield 400 MHz and 500 MHz spectrometers, with tetramethysilane as the internal reference and DMSO-$d_6$ as the solvent; the chemical shifts are reported in ppm. Reactions were monitored by TLC on Merck silica gel 60 F254 aluminium sheets. TLC spots were visualised by inspection of the plates under UV light (254 and 365 mm). All commercial reagents were obtained from Aldrich or Merck Co. and used without any further purification.

### 4.2. General synthetic procedure for the benzaldehyde fluoro-substituted thiosemicarbazones (**3a–j**)

To a solution of benzaldehyde fluoro-substituted (**1a–j**) (0.01 mol), in hot ethanol (30 mL) was added two drops of concentrated sulphuric acid and thiosemicarbazide (**2**) (0.01 mol). The reaction mixture was stirred at 40 °C for 4 h, and monitored by TLC. The precipitate was filtered off and recrystallized from ethanol to afford the target compounds **3a–j**.



#### 4.2.1. 2,3-Difluoro-benzaldehyde thiosemicarbazone (**3a**)

Yield: 94%; m.p. 210 °C. IR (KBr) $\nu_{max}$ in $cm^{-1}$: 3433, 3253 ($NH_2$), 3151 (NH), 1519 (C=N) 1288 (C—F), 1053 (C=S). $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.66 (s, 1H, H-3′); 8.26 (s, 1H, H-1′); 8.38 (bs, 1H, H-5′); 8.15 (bs, 1H, H-6′); 8.05 (t, $^3J_{5,6}$ = 8 Hz, 1H, H-6); 7.43 (q, $^3J_{5,6}$ = 8 Hz, $^3J_{4,5}$ = 8 Hz, 1H, H-5); 7.21 (q, $^4J_{2,4}$ = 8 Hz, $^3J_{3,4}$ = 12 Hz, 1H, H-4). $^{13}C$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 178.8 (C-4′); 150.3 (dd, $J_{C\text{-}F}$ = 245.5 Hz, $^2J_{2,3}$ = 12 Hz, 1C, C-2); 149.0 (dd, $J_{C\text{-}F}$ = 251.5 Hz, $^2J_{2,3}$ = 13 Hz, 1C, C-3); 134.0 (m, 1C, C-1′); 125.3 (dd, $^3J_{6,2}$ = 7 Hz, $^4J_{6,3}$ = 4 Hz, 1C, C-6); 124.7 (d, $^2J_{1,2}$ = 6 Hz, 1C, C-1); 122.4 (d, $^4J_{5,2}$ = 2 Hz, 1C, C-5); 118.6 (d, $^2J_{4,3}$ = 17 Hz, 1C, C-4). $^{19}F$ NMR (500 MHz, DMSO-$d_6$): δ −139.3 (d, $^3J_{2,3}$ = 20 Hz, 1F, $F_{C\text{-}3}$); −147.3 (d, $^3J_{2,3}$ = 20 Hz, 1F, $F_{C\text{-}2}$). Anal. Calcd for $C_9H_9F_2N_3S$: C, 44.65; H, 3.28; N, 19.52; S, 14.90; Found: C, 44.53; H, 3.47; N,19.66; S, 14.72.

#### 4.2.2. 2,5-Difluoro-benzaldehyde thiosemicarbazone (**3b**)

Yield: 92%; m.p. 222 °C. IR (KBr) $\nu_{max}$ in $cm^{-1}$: 3427, 3255 ($NH_2$); 3155 (NH); 1521 (C=N); 1272 (C—F); 1082 (C=S). $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.64 (s, 1H, H-3′); 8.36 (bs, 1H, H-5′); 8.21 (s, 1H, H-1′); 8.16 (bs, 1H, H-6′); 8,17 (m, 1H, H-2); 7,30 (m, 1H, H-5); 7,28 (m, 1H, H-4). $^{13}C$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 178.6 (C-4′); 158.5 (dd, $J_{C\text{-}F}$ = 237.4 Hz, 1C, C-2); 156.9 (d, $J_{C\text{-}F}$ = 248.5 Hz, 1C, C-5); 133.4 (C-1′); 123.5 (q, $^2J_{1,2}$ = 12.5 Hz, $^3J_{1,5}$ = 8 Hz, 1C, C-1); 117.7 (ddd, $^2J_{4,5}$ = 25 Hz, $^3J_{2,4}$ = 9 Hz, 2C, C-4, C-6); 112.4 (dd, $^2J_{2,3}$ = 25 Hz, $^3J_{3,5}$ = 3 Hz, 1C, C-3). $^{19}F$ NMR (500 MHz, DMSO-$d_6$): δ −118.1 (d, $^5J_{2,5}$ = 15 Hz, 1F, C-2); −127.0 (d, $^5J_{2,5}$ = 15 Hz, 1F, C-5). Anal. Calcd for $C_9H_9F_2N_3S$: C, 44.65; H, 3.28; N, 19.52; S, 14.90; Found: C, 44.47; H, 3.42; N, 9.71; S, 14.83.

#### 4.2.3. 2,6-Difluoro-benzaldehyde thiosemicarbazone (**3c**)

Yield: 93%; m.p. 209 °C. IR (KBr) $\nu_{max}$ in $cm^{-1}$: 3422, 3244 ($NH_2$); 3145 (NH); 1541 (C=N); 1290 (C—F); 1072 (C=S). $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.69 (s, 1H, H-3′); 8.43 (bs, 1H, H-5′); 8.21 (s, 1H, H-1′); 7.47 (m, 1H, H-4); 7.36 (bs, 1H, H-6′); 7.16 (m, 2H, H-3, H-5). $^{13}C$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 178.8 (C-4′); 160.0 (dd, $J_{C\text{-}F}$ = 254.5 Hz, $^3J_{2,6}$ = 7 Hz, 2C, C-2, C-6); 132.0 (t, $^3J_{2,4}$ = 10–11 Hz, 1C, C-4); 133.2 (C-1′); 112.8 (dd, $^2J_{2,3}$ = 22 Hz, $^3J_{3,6}$ = 5 Hz, 2C, C-3, C-5); 111.8 (t, $^2J_{1,2}$ = 13–14 Hz, 1C, C-1). $^{19}F$ NMR (500 MHz, DMSO-$d_6$): δ −112.5. Anal. Calcd for $C_9H_9F_2N_3S$: C, 44.65; H, 3.28; N, 19.52; S, 14.90; Found: C, 44.48; H, 3.38; N, 19.72; S, 4.82.

#### 4.2.4. 2,3,4-Trifluoro-benzaldehyde thiosemicarbazone (**3d**)

Yield: 96%; m.p. 216 °C. IR (KBr) $\nu_{max}$ in $cm^{-1}$: 3408, 3255 ($NH_2$); 3163 (NH); 1506 (C=N); 1311 (C—F); 1070 (C=S). $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.64 (s, 1H, H-3′); 8.36 (bs, 1H, H-5′); 8.18 (s, 1H, H-1′); 8.10 (bs, 1H, H-6′); 7.35 (q, $^5J_{2,5}$ = 4 Hz, $^4J_{3,5}$ = 8 Hz,

$^3J_{4,5}$ = 9.5 Hz, $^3J_{5,6}$ = 10 Hz, 1H, H-5); 8.11 (q, $^4J_{2,6}$ = 8 Hz, $^5J_{3,6}$ = 4 Hz, $^4J_{4,6}$ = 6 Hz, 1H, H-6). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 178.8 (s, C-4′); 151.2 (dddd, $J_{C\text{-}F}$ = 249.5 Hz, $^2J_{3,4}$ = 9 Hz, $^3J_{2,4}$ = 3 Hz, 1C, C-4); 148.5 (dddd, $J_{C\text{-}F}$ = 253.0 Hz, $^2J_{2,3}$ = 11 Hz, $^3J_{2,4}$ = 3 Hz, 1C, C-2); 139.4 (dt, $J_{C\text{-}F}$ = 247.5 Hz, $^2J_{2,3}$ = 15–16 Hz, 1C, C-3); 133.2 (s, 1C, C-1′); 121.8 (t, $^3J_{2,6}$ = 3–4 Hz, 1C, C-6); 120.6 (q, $^2J_{1,2}$ = 6.5 Hz, $^3J_{1,3}$ = 3 Hz, 1C, C-1); 113.6 (dd, $^2J_{4,5}$ = 18 Hz, $^3J_{3,5}$ = 3 Hz, 1C, C-5). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −133.3 (1F, $F_{C\text{-}4}$); −142.9 (1F, $F_{C\text{-}2}$); −161.7 (1F, $F_{C\text{-}3}$). Anal. Calcd for $C_8H_5F_3N_3S$: C, 41.20; H, 2.59; N; 18.02; S, 13.75; Found: C, 41.08; H, 2.67; N, 18.32; S, 13.64.

*4.2.5. 2,3,5,6-Tetrafluoro-benzaldehyde thiosemicarbazone (**3e**)*

Yield: 93%; m.p. 227 °C. IR (KBr) $\nu_{max}$ in cm$^{-1}$: 3410, 3238 ($NH_2$); 3140 (NH); 1523 (C=N); 1296 (C—F); 1076 (C=S). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 11.83 (s, 1H, H-3′); 8.54 (bs, 1H, H-5′); 8.16 (s, 1H, H-1′); 7.89 (m, 1H, H-4); 7.42 (bs, 1H, H-6′). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 178.9 (s, C-4′); 146.1 (ddd, $J_{C\text{-}F}$ = 244.5 Hz, $^2J_{2,3}$ = 13 Hz, $^3J_{2,4}$ = 4 Hz, 2C, C-2, C-6); 144.4 (ddd, $J_{C\text{-}F}$ = 253.0 Hz, $^2J_{2,3}$ = 14 Hz, $^3J_{3,5}$ = 5 Hz, 2C, C-3, C-5); 131.5 (t, $^3J_{1',2}$ = 3 Hz, 1C, C-1′); 114.7 (t, $^2J_{1,2}$ = 11–12 Hz, 1C, C-1); 107.6 (t, $^2J_{3,4}$ = 23 Hz, 1C, C-4). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −139.6 (q, $^3J_{5,6}$ = 20 Hz, $^4J_{3,5}$ = 15 Hz, 2F, $F_{C\text{-}3}$, $F_{C\text{-}5}$); −142.5 (q, $^3J_{2,3}$ = 25 Hz, $^4J_{2,6}$ = 15 Hz, 2F, C-2, C-6). Anal. Calcd for $C_8H_4F_4N_3S$: C, 38.25; H 2.01; N, 16.73; S,12.76; Found: C, 38.07; H, 2.32; N, 16.86; S, 12.65.

*4.2.6. 2,3,4,5,6-Pentafluoro-benzaldehyde thiosemicarbazone (**3f**)*

Yield: 95%; m.p. 245 °C. IR (KBr) $\nu_{max}$ in cm$^{-1}$: 3417, 3244 ($NH_2$); 3143 (NH); 1517 (C=N); 1292 (C—F); 1072 (C=S). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 11.79 (s, 1H, H-3′); 8.52 (bs, 1H, H-5′); 8.12 (s, 1H, H-1′); 7.43 (bs, 1H, H-6′). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 179.0 (s, C-4′); 146.3 (m, $J_{C\text{-}F}$ = 253.5 Hz, 2C, C-2, C-6); 141.5 (m, $J_{C\text{-}F}$ = 257.5 Hz, 1C, C-4); 137.9 (m, $J_{C\text{-}F}$ = 246.5 Hz, 2C, C-3, C-5); 130.8 (d, $^3J_{1',2}$ = 2 Hz, 1C, C-1′); 110.1 (dt, $^2J_{1,2}$ = 12.3 Hz, $^3J_{1,3}$ = 4 Hz, $^4J_{1,4}$ = 3 Hz, 1C, C-1). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −162.7 (td, $^3J_{3,4}$ = 25 Hz, $^5J_{3,6}$ = 10 Hz, 2F, $F_{C\text{-}3}$, $F_{C\text{-}5}$); −153.4 (t, $^3J_{4,5}$ = 25 Hz, $^4J_{4,6}$ = 22.5 Hz, 2F, $F_{C\text{-}3}$, $F_{C\text{-}5}$); −142.1 (dd, $^3J_{2,3}$ = 25 Hz, $^4J_{2,4}$ = 22.5 Hz, $^5J_{2,5}$ = 10 Hz, $^4J_{2,6}$ = 15 Hz, 2F, C-2, C-6). Anal. Calcd for $C_8H_3F_5N_3S$: C, 35.69; H, 1.50; N, 15.61; S, 11.91; Found: C, 35.48; H, 1.72; N, 5.79; S, 11.76.

*4.2.7. 3,5-Difluoro-benzaldehyde thiosemicarbazone (**3g**)*

Yield: 92%; m.p. 205 °C. IR (KBr) $\nu_{max}$ in cm$^{-1}$: 3446, 3394 ($NH_2$); 3159 (NH); 1508 (C=N); 1315 (C—F); 1097 (C=S). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 11.63 (s, 1H, H-3′); 8.33 (bs, 1H, H-5′); 8.28 (bs, 1H, H-6′); 7.98 (s, 1H, H-1′); 7.62 (dd, $^3J_{2,3}$ = 8 Hz, $^4J_{2,4}$ = 4 Hz, $^5J_{2,5}$ = 1.6 Hz, $^4J_{2,6}$ = 4 Hz, 2H, H-2 e H-6); 7.22 (tt, $^3J_{3,4}$ = 8 Hz, 1H, H-4). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 178.8 (s, C-4′); 162.0 (dd, $J_{C\text{-}F}$ = 245.0 Hz, $^3J_{3,5}$ = 13–14 Hz, 2C, C-3 e C-5); 139.8 (m, 1C, C-1′); 138.7 (t, $^3J_{1,3}$ = 9–11 Hz, 1C, C-1); 110.6 (dd, $^2J_{2,3}$ = 22 Hz, 2C, C-2, C-6); 104.7 (t, $^2J_{3,4}$ = 26 Hz, 1C, C-4). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −109.7 (2F, C-3, C-5). Anal. Calcd for $C_9H_9F_2N_3S$: 44.65; H, 3.28; N, 19.52; S, 14.90; Found: C, 44.59; H, 3.52; N, 19.74; S, 14.98.

*4.2.8. 4-Fluoro-benzaldehyde thiosemicarbazone (**3h**)*

Yield: 96%; m.p. 193 °C. IR (KBr) $\nu_{max}$ in cm$^{-1}$: 3433, 3253 ($NH_2$); 3151 (NH);1521 (C=N); 1286 (C—F); 1085 (C=S). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 11.47 (s, 1H, H-3′); 8.31 (bs, 1H, H-5′); 8.10 (bs, 1H, H-6′); 8.04 (s, 1H, H-1′); 7.84 (t, $^4J_{2,6}$ = 6 Hz, 2H, H-2, H-6); 7.19 (t, $^3J_{3,4}$ = 8 Hz, $^4J_{3,5}$ = 4 Hz, 2H, H-3, H-5). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 178.4 (s, C-4′); 164.4 (d, $J_{C\text{-}F}$ = 247.5 Hz, 1C, C-4); 141.6 (s, 1C, C-1′); 131.2 (d, $^4J_{1,4}$ = 2 Hz, 1C, C-1); 129.9 (d, $^3J_{2,4}$ = 8 Hz, 2C, C-2, C-6); 116.1 (d, $^2J_{3C\text{-}F}$ = 22 Hz, 2C, C-3, C-5). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −110.9 (s, 1F, $F_{C\text{-}4}$). Anal. Calcd for $C_8H_7FN_3S$: C, 48.72; H, 4.09; N, 21.31; S, 16.26; Found: C, 48,68; H, 4.17; N, 21.46; S, 16.05.

*4.2.9. 3,4-Difluoro-benzaldehyde thiosemicarbazone (**3i**)*

Yield: 95%; m.p. 198 °C. IR (KBr) $\nu_{max}$ in cm$^{-1}$: 3421, 3390 ($NH_2$); 3159 (NH); 1508 (C=N); 1280 (C—F); 1093 (C=S). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 11.53 (s, 1H, H-3′); and 8.21 (bs, 1H, H-6′); 8.00 (s, 1H, H-1′); 7.27 (bs, 1H, H-5′); 7.55 (m, 1H, H-2); 7.45 (q, $^4J_{3,5}$ = 8 Hz, $^3J_{4,5}$ = 12 Hz, $^3J_{5,6}$ = 10 Hz, 1H, H-5); 8.12 (dddd, $^5J_{3,6}$ = 2 Hz, $^4J_{4,6}$ = 4 Hz, 1H, H-6). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 178.7 (s, C-4′); 151.9 (dd, $J_{C\text{-}F}$ = 248.5 Hz, $^2J_{3,4}$ = 13 Hz, 1C, C-3); 149.1 (d, $J_{C\text{-}F}$ = 245.5 Hz, $^2J_{3,4}$ = 13 Hz, 1C, C-4); 140.2 (s, 1C, C-1′); 132.7 (d, $^3J_{1,3}$ = 6 Hz, $^4J_{1,4}$ = 3 Hz, 1C, C-1); 125.6 (t, $^4J_{6,3}$ = 3 Hz, 1C, C-6); 118.1 (d, $^2J_{4,5}$ = 18 Hz, 1C, C-5); 115.5 (d, $^2J_{2,3}$ = 18 Hz, 1C, C-2). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −136.5 (d, $^3J_{3,4}$ = 25 Hz, 1F, $F_{C\text{-}3}$); −138.2 (d, $^3J_{3,4}$ = 25 Hz, 1F, $F_{C\text{-}4}$). Anal. Calcd for $C_9H_9F_2N_3S$: C, 44.65; H, 3.28; N, 19.52; S, 14.90; Found: C, 44.57; H, 3.36; N, 19.71; S, 14.83.

*4.2.10. 4-Trifluoromethyl-benzaldehyde thiosemicarbazone (**3j**)*

Yield: 95%; m.p. 170 °C. IR (KBr) $\nu_{max}$ in cm$^{-1}$: 3417, 3244 ($NH_2$); 3143 (NH); 1517 (C=N); 1294 (C—F); 1089 (C=S). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 11.63 (s, 1H, H-3′); 8.36 (bs, 1H, H-5′); 8.20 (bs, 1H, H-6′); 8.11 (s, 1H, H-1′); 8.03 (d, $^4J_{2,6}$ = 2 Hz, 2H, H-2, H-6); 7.74 (d, $^4J_{3,5}$ = 2 Hz, 2H, H-3, H-5). $^{13}$C NMR (500 MHz, DMSO-$d_6$): δ 178.8 (s, C-4′); 140.7 (s, 1C, C-1′); 138.7 (s, 1C, C-1); 129.9 (d, $^2J_{4C\text{-}F}$ = 32 Hz, 1C, C-4); 128.3 (s, 2C, C-2, C-6); 125.9 (d, $^3J_{3C\text{-}F}$ = 3 Hz, 2C, C-3, C-5); 123.9 (d, $J_{C\text{-}F}$ = 544.3 Hz, 1C, $F_{CF3}$). $^{19}$F NMR (500 MHz, DMSO-$d_6$): δ −61.0 (3F, $CF_3$). Anal. Calcd for $C_9H_7F_3N_3S$: C, 43.72; H, 3.26; N, 17.00; S, 12.97; Found: C, 43.61; H, 3.41; N, 17.23; S, 12.73.

### 4.3. Biological activity assays

#### 4.3.1. Parasites

*T. cruzi* epimastigotes from Y strain [30], were cultivates at 26 °C in liver infusion LIT (Liver Infusion Triptose) medium supplemented with 10% heat inactivated fetal calf serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. The epimastigotes were collected in the log phase of cell culture growth. The parasites were centrifuged (2500 rpm) by 15 min at 4 °C and, resuspended with PBS (Phosphate Buffered Saline) buffer and the pellet were counted in Neubauer chamber to final concentration of $4 \times 10^6$ parasites/mL of the cell suspension.

#### 4.3.2. Assays

*T. cruzi* epimastigotes at $5.10^6$/mL were incubated in LIT medium with fluorinated thiosemicarbazones (**3a–j**), and in the absence (negative control), and in the presence of benzinidazol as positive control. The compounds were solubilized in DMSO (1.5% v/v) in the range concentration of 100–3.125 μg/mL. The parasite cultures were incubated at 26 °C by 24 h in 96-well plates. The living epimastigotes were counted using a Neubauer chamber, and the $EC_{50}$ values, corresponding to effective dose that kills 50% of the parasites were calculated using a perceptual of live parasites *versus* log dose and, after applied linear regression to resulting curve. The untreated parasites were used as controls. Pentamidine isothionate was used as reference drug ($IC_{50}$ = 10 ug/mL, 16.90 μM). All tests were carried out in triplicate.

### 4.4. Descriptors calculation and 2D-QSAR

The molecular structures were drawn by ACD/ChemSketch software (ACDLabs software package, version 12.0), and the values of physical-chemistry descriptors, such as, log P, molar refractivity (MR) and, molar volume (MV) were calculated for each compound.

The 2d-QSAR models were derived by multiple regression analysis that were performed using the BuildQSAR program [29] to determine the equations and the statistic parameters. In equations the numbers in parentheses represent the 95% of confidence intervals of the coefficients, *n* is number of data points, *r* is the

correlation coefficient, $s$ is the standard deviation, $q^2$ is the cross-validated, and $F$ is the Fisher value, measures for the statistical significance.

### 4.5. X-Ray diffraction

Single crystal X-ray data were collected on Bruker D8 Venture diffractometer using graphite monochromated Mo Ka radiation (λ = 0.71073 Å) at room temperature. Data collection and cell refinement were performed using Bruker Instrument Service v6.2.1 software and APEX3 v2016.1-0, respectively. Data reduction was carried out using SAINT. Empirical multiscan absorption correction using equivalent reflections was performed with SHELXS-97 [31,32] and SHELXL-2014 softwares [33]. All atoms, except the hydrogen, were anisotropically refined. Hydrogen atoms were treated by a mixture of independent and constrained refinement.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfluchem.2017.01.013.

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