



# Organometallic Schiff bases derived from 5-nitrothiophene and 5-nitrofurane: Synthesis, crystallographic, electrochemical, ESR and anti*Trypanosoma cruzi* studies

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## ABSTRACT

In the search for new therapeutic tools for the treatment of American trypanosomiasis, a series of novel ferrocene and cyrrhretrene imine compounds, derived from 5-nitro-heterocycles, were designed, synthesized and characterized. The <sup>1</sup>H and <sup>13</sup>C NMR spectra indicated that these compounds adopted an anti-(*E*) conformation in solution, and this was confirmed by X-ray crystallography for one of the complexes (**NT2**). To study the relationship between the physical–chemical properties of N-iminyl substituents of nitrofurfuryl and nitrothienyl groups and their antitrypanosomal activity, we have carried out cyclic voltammetry and electron spin resonance studies of a series of organometallic imine compounds. The results demonstrated that the electronic properties of the side chain of the 5-nitroheterocyclic compound could be correlated to its trypanocidal effect.

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## 1. Introduction

According to recent data from the World Health Organization (WHO), Chagas' disease (American trypanosomiasis) is one of the most common endemic parasitic diseases in Central and South America [1]. The disease is caused by the protozoa *Trypanosoma cruzi* (*T. cruzi*), and current pharmacological treatments have been based on dated and nonspecific drugs, such as Nifurtimox (Nfx, a nitrofurane derivative) and Benznidazole (Bnz, a nitroimidazole derivative) [2–4]. Although both of these drugs produce significant toxic effects, their mechanism of action is not fully understood and a large number of new antitrypanosomal agents have been developed using derivatives that are structurally related to these drugs. It has been shown that the bioreduction of the nitro group during metabolism in these pharmacological recommended drugs caused antitrypanosomal activity [5–7].

Electron spin resonance (ESR) and electrochemical methods, such as cyclic voltammetry (CV), have been used as powerful tools in the generation and identification of paramagnetic intermediates found in compounds with potential antichagasic activities [8]. It is important to note that electrochemical parameters do not absolutely correlate with biological activity data, but they do provide important information about the potential activities of compounds [9]. In this regard, Olea and coworkers have explored a series of potential antiprotozoal agents containing 5-nitrofurane and 5-nitrothiophene groups and have reported the electrochemical generation of nitro radical species, which were characterized by ESR spectroscopy [10,11].

The search for new antitrypanosomal agents has been focused on several different strategies. Organic and inorganic compounds containing nitro-aromatic systems, have been extensively studied as anti-*T. cruzi* agents [12,13]. Recently we have reported the synthesis of the first organometallic-organic hybrids containing a 5-nitrofurane group and have evaluated their anti-*T. cruzi* activity (Fig. 1) [14]. Although all of the reported compounds were less active than Nfx, we observed that the antichagasic activity was dependent on the electronic nature of the N-iminyl substituents. Therefore, we established

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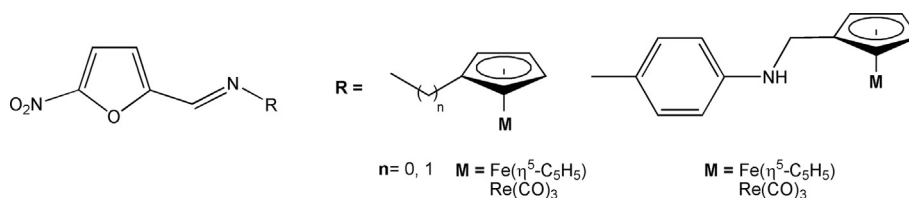


Fig. 1. Organometallic-organic hybrids derived from 5-nitrofurane.

that electronic effects should be considered when designing new molecules with potential trypanocidal activity.

In this study, we aim to gather insight on organometallic complexes bearing antitrypanosomal groups, and we report the synthesis, characterization (including X-ray crystallography) and biological evaluation of new bioorganometallics based on the 5-nitrothiophene fragment. We also include a complete CV and ESR study of these new compounds and their analogues containing 5-nitrofurane, which were previously reported.

## 2. Results and discussion

### 2.1. Design and synthesis

All nitro compounds were rationally designed based on the Nifurtimox structure backbone (Fig. 2). This core structure allowed for different substitutions on the iminyl moiety such as a 5-nitrofurane or 5-nitrothiophene group bound to C-iminyl and organometallic fragments attached to the N-iminyl bridge, with and without electronic communication. The organometallic groups were chosen based on their electronic effects (electron-donating or electron-withdrawing substituents) (Fig. 2).

The imine compounds derived from 5-nitrothiophene were easily synthesized following the same procedure described for the analogs of 5-nitrofurane [14]. This was accomplished by the condensation of equimolar amounts of the appropriate organometallic amine with 5-nitro-2-thiophenecarboxaldehyde (Scheme 1). In all cases, the products were obtained in high yields and isolated as microcrystalline or crystalline solids after recrystallization from a  $\text{CH}_2\text{Cl}_2$ –hexane mixture. The resulting compounds were air-stable and soluble in most organic solvents.

The IR spectra of these compounds showed the value of the  $\nu\text{C}=\text{N}$  stretch in the expected range of  $1615$ – $1637\text{ cm}^{-1}$  in either a  $\text{CH}_2\text{Cl}_2$  solution or KBr disc. Similar  $\nu\text{C}=\text{N}$  stretching frequencies have been reported for Schiff bases derived from aminoferrocene [15] and aminocyrhrene [14]. Each of the imine complexes showed a strong molecular ion peak in their mass spectra and the elemental analyses were in good agreement with their proposed formula.

For all complexes, in addition to the resonance characteristics of the ferrocenyl and cyrhetrenyl fragments, the  $^1\text{H}$  NMR spectra of the heterocyclic and imine portions of the molecules showed patterns similar to that previously reported for the 5-nitrothiophene and 5-nitrofurane analogues [16]. Specifically, the presence of a sharp singlet at approximately  $8.30$ – $8.59\text{ ppm}$  that were assigned

to the iminic proton and two doublets at  $\delta 7.18$ – $7.91$ , which were attributed to the hydrogen atoms of the 5-nitrothiophene ring.

The  $^{13}\text{C}$  NMR data were also in agreement with the existence of the  $\text{CH}=\text{N}$  entity because the resonance of the iminyl carbon was observed at a low field ( $\delta 151$ – $155$ ) and was confirmed by  $^1\text{H}$ – $^{13}\text{C}$  NMR COSY. This resonance occurred at nearly the same  $\delta$  as those reported for the other Schiff bases mentioned above. The resonances for the other carbon nuclei present in the molecules showed no unusual features. Despite the fact that these types of compounds could adopt one of two different forms (*E*- or *Z*-), their  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were consistent with the presence of only one isomer (*E*-form) in solution and were in good agreement with previously reported ferrocenyl and cyrhetrenyl Schiff bases [17]. Further proof was provided by the X-ray crystal structure determination of **NT2** (see below).

It is important to note that the  $^{13}\text{C}$  shifts of the iminyl carbons of the 5-nitrothiophene derivatives (**NT1** and **NT2**) did not show any dependence on the electronic properties of the organometallic substituents in the side chain, as we had previously observed in their 5-nitrofurane analogues (**NF1** and **NF2**) [14]. In these cases, we believe that the lower electronegativity of the S atom could better stabilize any charge generated in the heterocyclic ring; therefore, no difference in the iminyl carbon resonance would be evidenced. Similar results have been reported by Rando for a series of 5-nitrofurane and 5-nitrothiophene-2-benzylidene hydrazides [18].

### 2.2. X-ray crystal structure

With the aim of comparing the structural parameters of 5-nitrothiophenyl imines with the crystallographic data reported for their analogues containing 5-nitrofurane and related compounds, we undertook a crystallographic study of **NT2**. Fig. 3 shows an ORTEP representation of **NT2** and the most relevant bond lengths and angles. The structure confirms the *E* configuration tentatively assigned by NMR.

The crystallographic data obtained for **NT2** (Supplementary material) allowed us to make a comparison with the previously reported 5-nitrofurane analogue (**NF2**) [14]: i) in both cases the imine moiety and the Cp ring were nearly coplanar and the dihedral angle between the  $\text{C}_5$  ring and the iminyl group plane was  $6.4^\circ$  in **NT2** vs  $6.3^\circ$  in **NF2**; ii) the iminic double bond  $\text{N}(1)$ – $\text{C}(9)$  in **NT2** ( $1.284\text{ \AA}$ ) was slightly longer than the one measured for **NF2** ( $1.266\text{ \AA}$ ), whereas the same trend was observed for the bond lengths of  $\text{C}(9)$ – $\text{C}(10)$  ( $1.443$  vs  $1.439$  for **NT2** and **NF2**, respectively); iii) in contrast, the bond distance  $\text{C}(4)$ – $\text{N}(1)$  in **NT2** ( $1.403\text{ \AA}$ ) was much shorter than in

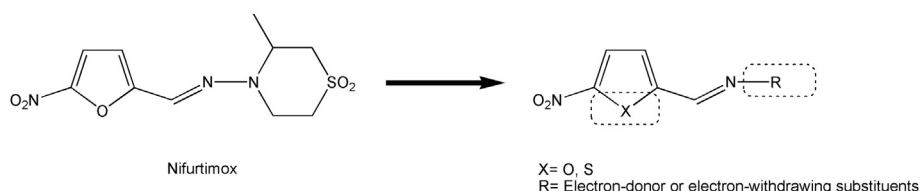
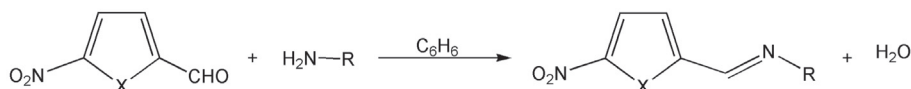


Fig. 2. Nifurtimox: model compound and structural substitutions for synthetic analogues.



Compound	X	R <sup>a</sup>	Compound	X	R <sup>a</sup>
NF1	O	Fc	NT1	S	Fc
NF2	O	Cy	NT2	S	Cy
NF3	O	CH <sub>2</sub> Fc	NT3	S	CH <sub>2</sub> Fc
NF4	O	CH <sub>2</sub> Cy	NT4	S	CH <sub>2</sub> Cy

<sup>a</sup> Fc = (η<sup>5</sup>-C<sub>5</sub>H<sub>4</sub>)Fe(η<sup>5</sup>-C<sub>5</sub>H<sub>5</sub>); Cy = (η<sup>5</sup>-C<sub>5</sub>H<sub>4</sub>)Re(CO)<sub>3</sub>

**Scheme 1.** Synthesis of imine compounds derived from 5-nitrothiophene (**NT**) and 5-nitrofuran (**NF**).

**NF2** (1.449 Å); iv) within the cyrhetrenyl group the average C–C (Cp), Re–C(O), Cp (centroid)–Re and Re–C–O angles were concordant with **NF2** [14] and other related tricarbonyl cyclopentadienyl rhenium (I) complexes [19]. With respect to the 5-nitrothiophene group, the internal C–C, C–S and C–N(NO<sub>2</sub>) bond distances were within the range found in aromatic compounds containing the 5-nitro-2-thiophenylideneamino group: O<sub>2</sub>N–C<sub>4</sub>H<sub>2</sub>S–CH=N–C<sub>6</sub>H<sub>5</sub>-o-Et [20] and (O<sub>2</sub>N–C<sub>4</sub>H<sub>2</sub>S–CH=N–)<sub>2</sub> [21].

### 2.3. Cyclic voltammetry

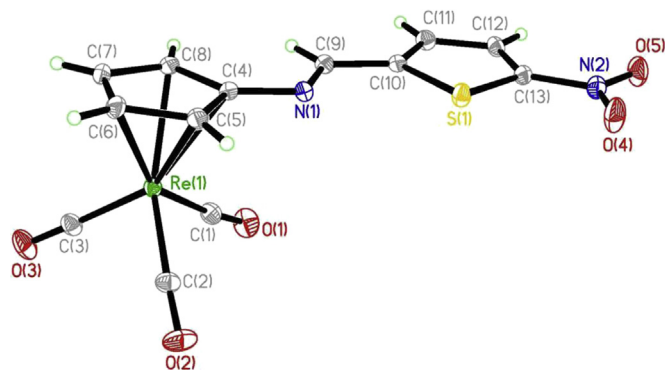
To study the correlation between electronic effects of the N-iminyl substituents with their  $E_{1/2}$  and trypanocidal activity, we performed cyclic voltammetric experiments. Under the recommended experimental conditions [7], all nitroheterocyclic compounds displayed similar electrochemical behaviours; for example, Fig. 4 shows the cyclic voltammogram of **NF2**.

The first wave for all compounds studied corresponded to a reversible one-electron transfer. The reverse scan showed the anodic counterparts to the reduction waves. The width of the cathodic wave at half intensity had a relatively constant value of 60 mV. According to the standard reversibility criteria, this couple corresponded to a reversible diffusion-controlled single electron transfer, and it was attributed to the reduction of Ar–NO<sub>2</sub> to the stable anion radical Ar–NO<sub>2</sub><sup>•−</sup> at room temperature [7,10]. These imine derivatives exhibited lower cathodic peak potentials (E<sub>pc</sub>) than Nifurtimox, (Table 1). The second cathodic peak was

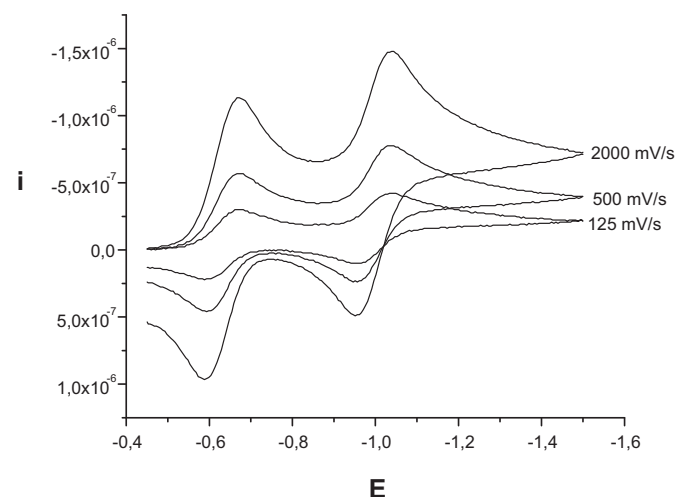
irreversible in the range of sweep rates used (125–2000 mV/s), and it was attributed to the production of the hydroxylamine derivative. Similar behaviors have been observed for a large number of 5-nitrofuran and 5-nitrothiophene containing compounds [7,11,22].

Similar to many other antichagasic compounds studied by CV, with the only exception being **NT1**, the 5-nitrothiophene derivatives (**NT**) possessed lower  $E_{1/2}$  values than their nitrofuran (**NF**) counterparts, as shown in Table 1 [16a].

Taking into account electronic effects, some general conclusions could be reached: i) in the two series of compounds (**NT** and **NF**), the  $E_{1/2}$  values correlated with the electronic nature of the substituent attached to the iminyl nitrogen; the more electron-withdrawing group, the lower the  $E_{1/2}$ ; ii) the  $E_{1/2}$  values showed a clear dependence on the presence of the ferrocenyl or cyrhetrenyl group bound to the nitrogen atom; in all cases the  $E_{1/2}$  ferrocenyl >  $E_{1/2}$  cyrhetrenyl. This phenomenon was not observed in compounds **NT3–4** and **NF3–4**, for which the electronic communication between the organometallic fragment and the nitroheterocyclic ring was impeded by a methylene group (see Table 1). These results confirmed our previous observations concerning the close relationship between the electronic nature of the substituent and the ability of the nitro group to be reduced in these types of compounds.



**Fig. 3.** ORTEP of the asymmetrical unit of **NF2**. Relevant bond lengths (Å): C(4)–N(1) 1.403(4); N(1)–C(9) 1.284(4); C(9)–C(10) 1.443(4); Cp(centroid)–Re(1) 1.963(6) and angles (°): N(1)–C(9)–C(10) 119.2(3); C(9)–N(1)–C(4) 119.1(11).



**Fig. 4.** Cyclic voltammogram of **NF2** in DMSO obtained at the following sweep rates: 2000, 500, and 125 mV/s.

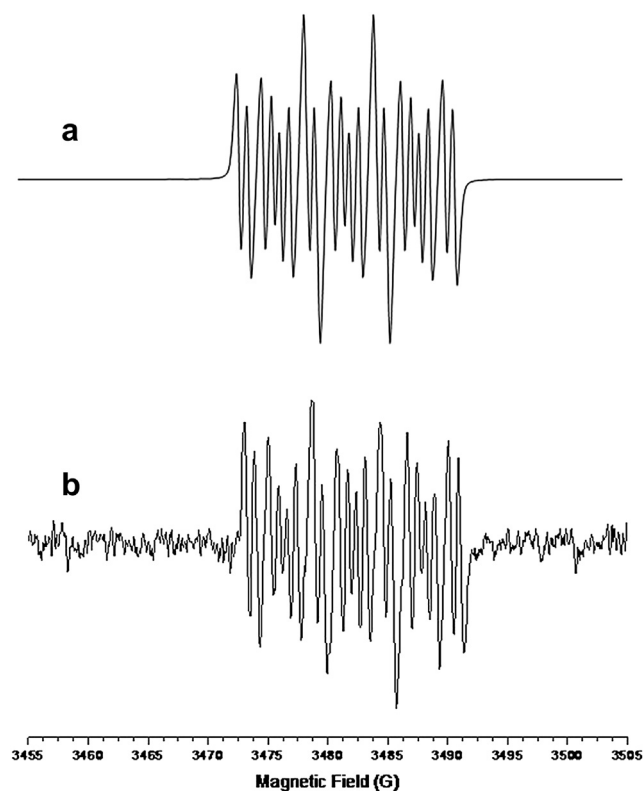
**Table 1**  
Comparison of activities of the 5-nitrothiophene (NT) and 5-nitrofuran (NF) derivatives on *T. cruzi* (epimastigotes and trypomastigote) and their reduction potentials.

Compound	IC <sub>50</sub> (μM) epimastigote	IC <sub>50</sub> (μM) trypomastigote	E <sub>1/2</sub> (NO <sub>2</sub> group)
<b>NT1</b>	17.9 ± 1.1	18.3 ± 0.7	-0.780
<b>NT2</b>	15.9 ± 0.9	3.7 ± 0.7	-0.560
<b>NT3</b>	35.9 ± 0.5		-0.680
<b>NT4</b>	43.1 ± 0.8		-0.650
<b>NF1</b>	48.8 ± 0.8	12.3 ± 1.7	-0.735
<b>NF2</b>	12.7 ± 3.1	0.4 ± 0.1	-0.630
<b>NF3</b>	83.1 ± 1.3		-0.750
<b>NF4</b>	87.7 ± 2.1		-0.740
<b>Nifurtimox</b>	17.2 ± 3.3	19.8 ± 2.5	-0.880

#### 2.4. ESR spectroscopy

The free radicals generated *in situ* by the electrochemical reduction of all NF and NT derivatives were characterized by ESR spectroscopy, in DMSO (see Supplementary material). This was accomplished by applying a potential corresponding to the first mono-electronic wave [11]. Two hyperfine patterns were found for all derivatives and were independent of the N-iminyl substituent (Fig. 5a). However, as expected, the nature of the heteroatom on the ring affected the hyperfine splitting pattern of the compounds. Thus, the lower electronegativity and the presence of empty d orbitals on the sulphur atom can more efficiently stabilize the generated radical in comparison to its furane analogues.

The ESR spectra of the generated radicals could be simulated using the experimentally obtained hyperfine coupling constants and by changing different parameters to find the major coincidences between the simulated spectra and the experimental ones [8].



**Fig. 5.** (a) ESR experimental spectrum of NF2 nitro radical produced by electrochemical generation in DMSO and (b) computer simulation of the same spectrum.

Accordingly, the ESR spectra of imine complexes were simulated in terms of i) one triplet that was due to the nitrogen of the nitro group or ii) three doublets that were due to nonequivalent hydrogen corresponding to the furane or thiophene ring and iminic group (Fig. 5b). In all cases, the calculated hyperfine constants were in good agreement with those obtained experimentally (see Supplementary material), and the values were similar to those previously reported for compounds containing 5-nitrofuran and 5-nitrothiophene fragments [7,11,23]. According to these results, the electronic nature of the N-iminyl substituent did not affect the stabilization of the generated nitro radical species.

#### 2.5. *In vitro* anti *T. cruzi* activity

To confirm the relationship between the electronic effects of the substituents on the trypanocidal activities of 5-nitrothiophene, compounds NT1–4 were assayed on epimastigote and trypomastigote forms of the Dm28c strain of *T. cruzi*. Table 1 shows the IC<sub>50</sub> values that were obtained for the new compounds. For comparison, we have also included the values of their 5-nitrofuran analogues, NF1–4 and Nifurtimox, which were also obtained with the Dm28c strain.

The results found on the epimastigote form of *T. cruzi* (Table 1) indicated that there was not a linear correlation between nitro-reduction (E<sub>1/2</sub>) with the trypanocidal activity (IC<sub>50</sub>). This finding was in good agreement with other nitro-heterocyclic compounds with antiparasitic activity [24]. Nevertheless, a clear trend could be established between the electronic effects of the substituent bound directly to the imine nitrogen and the IC<sub>50</sub>. Imines containing the electron-withdrawing cyrhetrenyl group (NT2 and NF2) were more active than their ferrocenic analogues (NT1 and NF1) and were comparable to Nifurtimox. This was in contrast to the compounds without electronic communication (NT3–4 and NF3–4) where the IC<sub>50</sub> values appeared to be independent of the organometallic fragment because comparable antitrypanosomal activity was observed within each series.

However, a much more interesting result was found when the trypanocidal activity of the compounds that possessed the 5-nitroheterocyclic group and the organometallic fragment connected electronically through the imine bridge (NF1–2 and NT1–2) were assessed against trypomastigote, which is the infective form of *T. cruzi* found in the mammal host (Table 1). Even though the ferrocene derivatives possessed comparable trypanocidal activity to Nifurtimox, the cyrhetrenyl analogues showed a remarkably enhanced anti-*T. cruzi* activity, particularly the cyrhetrenyl that contained the nitrofuran group (NF2).

At present we do not have a plausible explanation for the substantial improvement in the cytotoxicity of the 5-nitrofurfuryl group in NF2, but it is most likely associated with the electron-withdrawing nature of the cyrhetrenyl moiety. This assumption is in good agreement with the lower E<sub>1/2</sub> that was measured for the compounds containing the cyrhetrenyl fragment bound to pharmacophore. However, we cannot discard other factors, such as the increase in the lipophilic character of the molecule by incorporating the organometallic entity [25,26].

### 3. Conclusions

Ferrocenyl and cyrhetrenyl Schiff bases containing the pharmacophore 5-nitrothiophene were successfully synthesized. Like their 5-nitrofuran analogues, these complexes adopted an *anti*-configuration for the iminyl moiety, which was confirmed by NMR and X-ray crystallography. The results of an *in vitro* antitrypanosomal assay of the compounds against strains of *T. cruzi* confirmed the relationship between the electronic effects of the substituents on the trypanocidal activities of these organics-

organometallics hybrids. Compounds that possessed electronic communication between the organometallic substituent and the 5-nitroheterocyclic groups (**NT1–2** and **NF1–2**) showed higher antichagasic activity than those without electronic communication (**NT3–4** and **NF3–4**). When we compared compounds with organometallic fragments connected electronically through an imine bridge, the cyrhetrenyl derivatives (**NT2** and **NF2**) showed a remarkable enhancement of anti-*T. cruzi* activity when compared to their ferrocenic analogues (**NT1** and **NF1**). This is in good agreement with the lower  $E_{1/2}$  that was measured for the compounds containing the cyrhetrenyl fragment bound to the pharmacophore. This result was likely due to electron-withdrawing effects of the cyrhetrenyl moiety that were efficiently transferred to the heterocyclic group that facilitated the reduction of  $\text{NO}_2$  to  $\text{NO}_2^{\cdot-}$ . Therefore, electronic effects should be considered an influencing factor in designing new molecules with potential antitrypanosomal activity.

## 4. Experimental

### 4.1. Materials

Ferrocene (98%) and 5-nitro-2-thiophenecarboxaldehyde were obtained from Aldrich. Tetrabutylammonium perchlorate (TBAP), used as supporting electrolyte, was obtained from Fluka. Solvents such as  $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_6\text{H}_6$ , MeOH, DMSO and THF were obtained commercially and purified using standard methods. Complexes ( $\eta^5\text{-C}_5\text{H}_4\text{NH}_2$ ) $\text{Re}(\text{CO})_3$  [27], ( $\eta^5\text{-C}_5\text{H}_4\text{NH}_2$ )Fc [28] (Fc =  $\text{Fe}(\eta^5\text{-C}_5\text{H}_5)$ ) and imine compounds derived from 5-nitro-2-furaldehyde [14] were synthesized according to procedures found in the literature. Infrared spectra were recorded in solution (NaCl cell) or solid (KBr) on a Perkin–Elmer FT-1605 spectrophotometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were obtained using a Bruker AVANCE 400 spectrometer.  $^1\text{H}$  NMR chemical shifts were referenced using the chemical shifts of the residual solvent resonances, and  $^{13}\text{C}$  chemical shifts to the solvent peaks. Mass spectra were obtained at the Laboratorio de Servicios Analíticos, P. Universidad Católica de Valparaíso.

### 4.2. Synthesis of imine compounds. General procedure

**Caution!** Some of the preparations described here required the use of benzene which should be handled with care.

The organometallic compounds derived from 5-nitrothiophene were prepared following the same procedure as of their 5-nitrofurane analogues [14]. Equimolar amounts of the amino compound and 5-nitro-2-thiophenecarboxaldehyde were dissolved in anhydrous benzene (20 mL) and refluxed for 1 h under a nitrogen atmosphere. After, the solvent was removed under vacuum and the colored solids obtained were purified by crystallization from  $\text{CH}_2\text{Cl}_2$ /hexane (1:5) at  $-18^\circ\text{C}$ .

#### 4.2.1. (5-Nitro-2-thiophenylideneamino)ferrocene (**NT1**)

Purple solid, Yield: 80% (272.0 mg, 0.8 mmol). IR (KBr): ( $\nu_{\text{C}=\text{N}}$   $\text{cm}^{-1}$ ): 1615 (w).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  4.21 (s, 5H,  $\text{C}_5\text{H}_5$ ); 4.41 (t,  $J = 2.2$  Hz,  $\text{C}_5\text{H}_4$ ); 4.64 (t, 2H,  $J = 2.2$  Hz,  $\text{C}_5\text{H}_4$ ); 7.22 (d, 1H,  $J = 4.1$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 7.86 (d, 1H,  $J = 4.1$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 8.59 (s, 1H,  $\text{CH}=\text{N}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  62.5 ( $\text{C}_5\text{H}_4$ ); 69.2 ( $\text{C}_5\text{H}_4$ ); 70.1 ( $\text{C}_5\text{H}_5$ ); 101.7 ( $\text{C}_5\text{H}_{4\text{ipso}}$ ); 126.3 ( $\text{C}_4\text{H}_2\text{S}$ ); 128.3 ( $\text{C}_4\text{H}_2\text{S}$ ); 143.5 ( $\text{C}_4\text{H}_2\text{S}_{\text{ipso}}$ ); 154.0 ( $\text{CH}=\text{N}$ ). Mass spectrum ( $m/z$ ): 340 [ $\text{M}^+$ ]. Anal. (%) Calc. for  $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_2\text{SFe}$ : C, 52.96; H, 3.56 and N, 8.23; found: C, 52.98; H, 3.60 and N, 8.21.

#### 4.2.2. (5-Nitro-2-thiophenylideneamino)cyrhetrene (**NT2**)

Yellow crystals, Yield: 85% (59.0 mg, 0.12 mmol). IR ( $\text{CH}_2\text{Cl}_2$ ,  $\text{cm}^{-1}$ ): 2028 (s) ( $\nu\text{CO}$ ); 1936 (vs) ( $\nu\text{CO}$ ); 1625 (w) ( $\nu_{\text{C}=\text{N}}$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.32 (t, 2H,  $J = 2.3$  Hz,  $\text{C}_5\text{H}_4$ ); 5.59 (t, 2H,  $J = 2.3$  Hz,  $\text{C}_5\text{H}_4$ ); 7.38 (d, 1H,  $J = 4.3$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 7.88 (d, 1H,  $J = 4.3$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 8.49 (s,

1H,  $\text{CH}=\text{N}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  78.6 ( $\text{C}_5\text{H}_4$ ); 82.5 ( $\text{C}_5\text{H}_4$ ); 112.8 ( $\text{C}_4\text{H}_2\text{S}_{\text{ipso}}$ ); 114.6 ( $\text{C}_5\text{H}_{4\text{ipso}}$ ); 118.4 ( $\text{C}_4\text{H}_2\text{S}$ ); 130.4 ( $\text{C}_4\text{H}_2\text{S}$ ); 146.1 ( $\text{C}_4\text{H}_2\text{S}_{\text{ipso}}$ ); 154.7 ( $\text{CH}=\text{N}$ ); 193.4 (CO). Mass spectrum (based on  $^{187}\text{Re}$ )  $m/z$ : 490 [ $\text{M}^+$ ], 462 [ $\text{M}^+ - \text{CO}$ ], 434 [ $\text{M}^+ - 2\text{CO}$ ], 406 [ $\text{M}^+ - 3\text{CO}$ ]. Anal. (%) Calc. for  $\text{C}_{13}\text{H}_7\text{N}_2\text{O}_5\text{SRe}$ : C, 31.90; H, 1.44 and N, 5.72; found: C, 31.93; H, 1.43 and N, 5.74.

#### 4.2.3. (5-Nitro-2-thiophenylideneaminomethyl)ferrocene (**NT3**)

The synthesis of complex **NT3** was carried out similarly to that described above (general procedure), however the reflux was prolonged for 6 h using a Dean–Stark apparatus. Compound **NT3** was obtained as a red solid, Yield: 90% (318.0 mg, 0.9 mmol). IR (KBr): ( $\nu_{\text{C}=\text{N}}$   $\text{cm}^{-1}$ ): 1637 (w). NMR  $^1\text{H}$  ( $\text{CDCl}_3$ ):  $\delta$  4.17 (s, 9H,  $\text{CH}_2$ ,  $\text{C}_5\text{H}_4$ ,  $\text{C}_5\text{H}_5$ ); 4.57 (s, 2H,  $\text{C}_5\text{H}_4$ ); 7.18 (d, 1H,  $J = 4.2$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 7.85 (d, 1H,  $J = 4.2$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 8.24 (s, 1H,  $\text{CH}=\text{N}$ ). NMR  $^{13}\text{C}$  ( $\text{CDCl}_3$ ):  $\delta$  59.2 ( $\text{CH}_2$ ); 68.1 ( $\text{C}_5\text{H}_4$ ); 68.5 ( $\text{C}_5\text{H}_4$ ); 68.6 ( $\text{C}_5\text{H}_5$ ); 84.5 ( $\text{C}_5\text{H}_{4\text{ipso}}$ ); 127.9 ( $\text{C}_4\text{H}_2\text{S}$ ); 128.3 ( $\text{C}_4\text{H}_2\text{S}$ ); 148.8 ( $\text{C}_4\text{H}_2\text{S}_{\text{ipso}}$ ); 153.0 ( $\text{CH}=\text{N}$ ). Mass spectrum  $m/z$ : 354 [ $\text{M}^+$ ]. Anal. (%) Calc. for  $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_2\text{SFe}$ : C, 54.25; H, 3.98 and N, 7.91; found: C, 54.26; H, 3.99 and N, 7.96.

#### 4.2.4. (5-Nitro-2-thiophenylideneaminomethyl)cyrhetrene (**NT4**)

The synthesis of complex **NT4** was carried out similarly to that described for **NT3**. Complex **NT4** was obtained as brown solid, Yield: 50% (69.0 mg, 0.14 mmol). IR ( $\text{CH}_2\text{Cl}_2$ ,  $\text{cm}^{-1}$ ): 2025 (s) ( $\nu\text{CO}$ ); 1933 (s) ( $\nu\text{CO}$ ); 1625 (w) ( $\nu_{\text{C}=\text{N}}$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  4.15 (s, 2H,  $\text{CH}_2$ ); 5.31 (t, 2H,  $J = 2.3$  Hz,  $\text{C}_5\text{H}_4$ ); 5.49 (t, 2H,  $J = 2.3$  Hz,  $\text{C}_5\text{H}_4$ ); 7.28 (d, 1H,  $J = 4.2$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 7.85 (d, 1H,  $J = 4.2$  Hz,  $\text{C}_4\text{H}_2\text{S}$ ); 8.31 (s, 1H,  $\text{CH}=\text{N}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  40.1 ( $\text{CH}_2$ ); 83.1 ( $\text{C}_5\text{H}_4$ ); 84.2 ( $\text{C}_5\text{H}_4$ ); 112.8 ( $\text{C}_4\text{H}_2\text{S}$ ); 114.3 ( $\text{C}_5\text{H}_{4\text{ipso}}$ ); 118.4 ( $\text{C}_4\text{H}_2\text{S}$ ); 130.4 ( $\text{C}_4\text{H}_2\text{S}$ ); 146.1 ( $\text{C}_4\text{H}_2\text{S}_{\text{ipso}}$ ); 153.2 ( $\text{CH}=\text{N}$ ); 193.4 (CO). Mass spectrum (based on  $^{187}\text{Re}$ )  $m/z$ : 504 [ $\text{M}^+$ ]; 476 [ $\text{M}^+ - \text{CO}$ ]; 448 [ $\text{M}^+ - 2\text{CO}$ ]; 420 [ $\text{M}^+ - 3\text{CO}$ ]. Anal. (%) Calc. for  $\text{C}_{14}\text{H}_9\text{N}_2\text{O}_5\text{SRe}$ : C, 33.40; H, 1.80 and N, 5.56; found: C, 33.41; H, 1.82 and N, 5.54.

### 4.3. X-ray crystal structure determinations

A suitable X-ray single crystal of the compound **NT2** was obtained as described above and was mounted on top of glass fibers in a random orientation. Crystal data, data collection, and refinement parameters are given in the [Supplementary materials](#). Compound **NT2** was studied at 150(2) K, on a Bruker Smart Apex diffractometer equipped with bidimensional CCD detector using graphite-monochromated Mo- $K\alpha$  radiation ( $\lambda = 0.71073$  Å). The diffraction frames were integrated using the SAINT package [29] and were corrected for absorption with SADABS [30]. The structures were solved using XS in SHELXTL-PC [31] by the Patterson method and completed (non-H atoms) by difference Fourier techniques. The complete structure was then refined by the full matrix least-squares procedures on reflection intensities ( $F_2$ ) [32]. All non-hydrogen atoms were refined with anisotropic displacement coefficients, and all hydrogen atoms were placed in idealized locations.

### 4.4. Cyclic voltammetry experiments

Cyclic voltammetry experiments were carried out using a Metrohm 693 VA instrument equipped with a 694 VA Stand convertor and a 693 VA Processor, and performed in DMSO (ca.  $1.0 \times 10^{-3}$  mol  $\text{L}^{-1}$ ) under a nitrogen atmosphere at room temperature using TBAP (ca. 0.1 mol  $\text{L}^{-1}$ ) as the electrolyte in a three-electrode cell. A hanging mercury drop electrode (HMDE) was used as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) was used as the reference electrode.

#### 4.5. ESR spectroscopy

ESR spectra were recorded in the X band region (9.85 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation. The hyperfine splitting constants were estimated to be accurate within 0.05 G and they are included in the Supplementary material. The nitro anion radicals were generated by electrolytic reduction *in situ* under identical conditions that were used for the CV experiments (temperature, atmosphere and concentration). The ESR spectra were simulated using the WINEPR Simfonia software package, version 1.25.

#### 4.6. Biological assays: antitrypanosomal activity

Trypanocidal activity of the 5-nitrothiophene and 5-nitrofurane derivatives were evaluated against the *T. cruzi* trypomastigote and epimastigote stages (clone Dm28c). The compounds were dissolved in DMSO, and added to suspensions of  $1 \times 10^7$  trypomastigotes/mL and  $3 \times 10^6$  epimastigotes/mL. Trypomastigotes were incubated in non-supplemented red phenol-free RPMI 1640. Epimastigotes were incubated in Diamond's monophasic medium supplemented with 4  $\mu$ M hemin and 4% inactivated bovine calf serum. Trypomastigotes and epimastigotes were incubated at 37 °C and 28 °C, respectively, for 24 h. Afterwards, the trypanocidal activity was measured through the MTT assay as described elsewhere [33]. Briefly, MTT was added at a final concentration of 0.5 mg/mL and incubated at 37 °C for 4 h. The parasites were solubilized with 10% sodium dodecyl sulfate–0.1 mM HCl and incubated overnight. Formazan formation was measured at 570 nm in a multiwell reader (Asys Expert Plus®, Austria). The DMSO final concentration was less than 0.1% v/v. The trypanocidal Nifurtimox was added as a drug control. We determined the IC<sub>50</sub> value of viable parasites (IC<sub>50</sub> was the drug concentration needed to reduce by 50% the parasite viability) at 24 h after compound addition by nonlinear regression analysis from the log of concentration vs. the percentage of viable cells curve.

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#### Appendix A. Supplementary material

CCDC 943805 contains the supplementary crystallographic data for NT2. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html>, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk).

#### Appendix B. Supplementary material

Supplementary material related to this article can be found at <http://dx.doi.org/10.1016/j.jorganchem.2013.06.014>.

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