Cyrhetrenylaniline and new organometallic phenylimines derived from 4- and 5-nitrothiophene: Synthesis, characterization, X-Ray structures, electrochemistry and in vitro anti-\textit{T. brucei} activity

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1. Introduction

Neglected tropical diseases (NTDs) represent a collection of infections prevalent in many regions of the developing world. They are responsible for substantial global morbidity, mortality, and economic adversity that together affect about 1 billion people worldwide [1]. One such infection is African trypanosomiasis, a debilitating condition caused by the tsetse fly-transmitted protozoan parasite \textit{Trypanosoma brucei} (\textit{T. brucei}) that is prevalent across sub-Saharan Africa [2]. In addition to affecting an estimated 70 million individuals living in endemic sites [2,3], this pathogen also is of veterinary and economic importance, being one of several trypanosomes responsible for a wasting disease known as Nagana in ungulate animals.

At present, pentamidine, suramin, melarsoprol and eflornithine are the only drugs available to treat the human form of the disease, although there are significant issues relating to toxicity, administration, the disease stage being treated and the \textit{T. brucei} subspecies being targeted [4]. Additionally, due to long periods of treatment, the subsequent lack of completion of the treatment course and strain variation, resistance is emerging as a major problem [5]. Recently, the WHO added nifurtimox (Nfx), a nitroheterocycle normally used against Chagas disease, to the Essential Medicines list (EML) as part of the nifurtimox-eflornithine combination therapy (NECT) for treatment of \textit{T. brucei gambiense} [2,6]. This recommendation, together with several emerging reports on new nitroheterocyclic compounds with potential or significant \textit{in vitro} activity against \textit{Trypanosoma cruzi} (\textit{T. cruzi}) and \textit{T. brucei}, has reinvigorated interest in the use of nitroheterocyclic compounds as...
antitrypanosomal agents [7–12].

With the aim to find new and more efficient antitrypanosomal agents, inorganic compounds containing nitroaromatic systems have also been extensively explored [13–15]. The focus has been oriented mainly toward the protozoa T. cruzi, the caustive agent of American trypanosomiasis (also known as Chagas Disease) and to a lesser extent to T. brucei. The strategy consists in the coordination of a transition metal of known pharmacological activity to the structure of a bioactive organic molecule. Based on this approach, the pioneering work of Sanchez-Delgado [16–18] and Gambino [19–21] can be cited as remarkable examples in the search for new antitrypanosomal agents. For example, some of the ruthenium [19], rhenium [18,19], and palladium [21] complexes with 5-nitrofuryl containing an electron-donor ferrocenyl and electron-withdrawing cyrhetrenyl groups; ii) an iminophenyl bridge and, iii) a thiophene ring containing thiosemicarbazones as ligands have proven to be more active against T. cruzi than the corresponding free ligands.

In the last decades, a large number of organometallic compounds have attracted great interest because of their broad spectra of biological and pharmacological properties [22–25]. In particular, metallocene-based chemotherapeutics are known to exhibit a wide diversity of biological activity [26,27]. Among them, ferrocenyl compounds have emerged as an important research field in the ongoing discovery of metallo-therapeutic agents (i.e., antibacterial, antitumor, antimarial, and antitrypanosomal activities) [28–31]. Promising results have been reported when targeting diseases such as cancer and malaria, indicating that the incorporation of a ferrocenyl fragment may enhance biological activities or generate new medicinal properties [32–36]. Additionally, the chemistry of cyrhetrene, [Re(C5H4-2a)3] (the typical example of a three-legged half-sandwich rhenium(I) complex), has undergone rapid development in the last decade [37]. Among its many other applications, this organometallic core has been recognized as a promising anticancer drug candidate [38]. For example, Re-Tamoxifen has been demonstrated to be slightly more active than Tamoxifen for the treatment of hormone-responsive breast tumors [39]. Recently, the cyrhetrenyl fragment was conjugated to sulfonamide moieties to target human carbonic anhydrides [40] and has also been incorporated into several pharmacophores for evaluation as potential antimalarial agents [41].

In recent years, our research group has been involved in the development of ferrocene and cyrhetrene derivatives bound covalently to 5-nitrofuran and 5-nitrothiophene groups as a new class of anticagastic compounds. So far, we have connected the organometallic and 5-nitro heterocyclic groups through conjugated and non-conjugated bridges. We have established the existence of a relationship between electronic effects of the organometallic fragments and the trypanocidal activities [42,43].

In view of the aforementioned potential applications of 5-nitro heterocyclic groups containing organometallic fragments, in this paper we would like to report the synthesis and characterization, including the X-ray crystallography, of an unreported cyrhetrenylamine and a new series of bioorganometallics possessing: i) electron-donor ferrocenyl and electron-withdrawing cyrhetrenyl groups; ii) an iminophenyl bridge and, iii) a thiophene ring substituted with a nitro group in the 4- and 5-position. In addition, in the present work, we included the cyclic voltammetry studies and the anti-T. brucei evaluation of the Schiff bases.

2. Experimental

2.1. Materials

All manipulations were conducted under a nitrogen atmosphere using Schlenk techniques. The complexes cyrhetrene [44], 4-nitrophenylferrocene [45] and 4-ferrocenylaniline [46] were synthesized as described in the literature. Ferrocene (98%), 2-thiophencarboxaldehyde (98%), 5-nitro-2-thiophencarboxaldehyde (98%), 4-nitroanilin (99%), sodium nitrite (99%), hexadecyltrimethylammonium bromide (99%), n-Buti 2.0 M, ZnCl2 anhydrous, PdCl2(PPh3)2 (99%), 1-bromo-4-nitrobenzene (99%), and KNO3 (99%) were purchased from Aldrich and used as such. Solvents were obtained commercially and purified using standard methods. FT-IR spectra were recorded in solution (CH2Cl2) or solid state (KBr disc) on a Thermo Scientific, model Nicolet FT-IR spectrophotometer in the range of 4000-500 cm−1. 1H and 13C NMR spectra were measured on a Bruker Advance 300 spectrometer using tetramethylsilane (TMS) as the internal standard and CDCl3 as a solvent. The following abbreviations were used to describe the peak patterns: s = singlet, d = doublet, t = triplet, and ps = pseudo-triplet. Mass spectra were obtained on a Shimadzu model QP5050A GC-MS at the Laboratorio de Servicios Analíticos, Pontificia Universidad Católica de Valparaíso.

2.2. Synthesis of organometallic precursors

2.2.1. Synthesis of the 4-cyrhetrenylaniline (2a)

The preparation of the unreported 4-cyrhetrenylaniline complex was performed in two synthetic steps (Scheme 1), which involved first, a Negishi coupling reaction between cyrhetrene and 1-bromo-4-nitrobenzene and then the reduction of 4-nitrophenylcyrhetrene (1a).

2.2.1.1. Synthesis of 4-nitrophenylcyrhetrene (1a). n-Buti (0.30 mL, 2.0 M in cyclohexane, 0.64 mmol) was added dropwise to a solution of cyrhetrene (100 mg, 0.30 mmol) in anhydrous THF (8.0 mL) at −78 °C. After that, the reaction mixture was stirred for 1.5 h at −78 °C and ZnCl2 (48.0 mg, 0.35 mmol) was added. Subsequently, the mixture was allowed to warm to room temperature and was stirred for an additional 1.5 h. Then, a suspension of PdCl2(PPh3)2 (10.0 mg, 0.014 mmol) in dry THF (2.0 mL) and a solution of 1-bromo-4-nitrobenzene (BrC6H4NO2) (61.0 mg, 0.30 mmol) in dry THF (2.0 mL) were added to the reaction mixture and the stirring was continued, at room temperature, for 12 h. After this time, the solution was poured into water (10 mL) and extracted with dichloromethane (3 × 10 mL). The organic layers were dried over Na2SO4, filtered and evaporated under a vacuum. The complex (1a) was isolated as a pale yellow solid. The yield was 39% (43.0 mg, 0.12 mmol). IR (CH2Cl2, cm−1): 2026, 1932 (νCO); 1523, 1349 (νNO2). 1H NMR (CDCl3): δ 5.48 (t, 2H, J = 2.3 Hz, C6H4); 5.88 (t, 2H, J = 2.3 Hz, C6H4); 7.54 (d, 2H, J = 8.9 Hz, C6H4); 8.21 (d, 2H, J = 8.9 Hz, C6H4); 13C NMR (CDCl3): δ 83.4 (C6H4); 85.3 (C6H4); 103.4 (C6H4); 124.4 (C6H4); 126.8 (C6H4); 139.3 (C6H4); 147.5 (C6H4); 193.2 (CO). Mass spectrum (based on 187Re) (m/z): 457 [M+]; 429 [M+ − CO]; 401 [M+ − 2CO]; 373 [M+ − 3CO].

2.2.1.2. Synthesis of 4-cyrhetrenylaniline (2a). First, 4-nitrophenylcyrhetrene (1a) (50.0 mg, 0.13 mmol) was added to a magnetically stirred solution of concentrated hydrochloric acid
(0.40 mL) and ethanol (6.0 mL). Then granulated tin (750.0 mg, 0.63 mmol) was added and the reaction mixture was refluxed in a nitrogen atmosphere for 4 h. After cooling to room temperature, the yellow mixture that formed was treated with 3.0 mL of water and 10 mL of 0.5 M aqueous NaOH. The solid crude product was extracted with CH2Cl2 (2 × 20 mL), dried over Na2SO4, filtered and the solvent was removed in a rotary evaporator. The solid thus obtained was crystallized in a mixture with CH2Cl2/hexane (1:5) at −18 °C. 2a was obtained as a dark orange solid in 47% yield (22.0 mg, 0.062 mmol). IR: (KBr, cm−1) 3440, 3339 (νNH2); (CDCl3, cm−1) 2020, 1923 (νCO). 1H NMR (CDCl3): δ 5.36 (t, 2H, J = 2.2 Hz, C4H2S); 5.35 (t, 2H, J = 2.2 Hz, C4H2S); 6.62 (d, 2H, J = 8.8 Hz, C6H4); 7.19 (d, 2H, J = 8.8 Hz, C6H4); 84.1 (C5H4); 111.0 (C6H4ipso); 121.5 (C6H4); 127.7 (C6H4); 147.0 (C6H4); 194.6 (CO). Mass spectrum (based on 187Re) (m/z): 427 [M]+; 399 [M − CO]+; 343 [M − 3CO].

2.3. Synthesis of cyrhetrenyl and ferrocenyl imines. General procedure

The Schiff bases (Scheme 2) were achieved following the procedure reported by Zaheer et al. for the preparation of 4- and 5-nitrothiophene complexes [51] and a re-extraction with CH2Cl2/hexane (1:5) at −18 °C. 4a was obtained similarly to that described above (general procedure); nevertheless, the reflux was prolonged for 5 h. Brown crystals, yield: 56% (27.0 mg, 0.060 mmol). IR (KBr, cm−1): 2025 (s) (νCO), 1912 (vs) (νCO): 1617 (w) (νC = N). 1H NMR (CDCl3): δ 5.43 (t, 2H, J = 2.1 Hz, C4H2S); 5.79 (t, 2H, J = 2.1 Hz, C4H2S); 7.21 (d, 2H, J = 8.5 Hz, C6H4); 7.44 (d, 2H, J = 8.5 Hz, C6H4); 7.96 (d, 1H, J = 1.4 Hz, C6H2S); 8.42 (d, 1H, J = 1.4 Hz, C6H2S); 8.55 (s, 1H, CH = N). 13C NMR (CDCl3): δ 81.9 (C6H4); 84.7 (C6H4); 107.8 (C6H4ipso); 121.8 (C6H4); 125.5 (C6H2S); 127.4 (C6H4); 130.9 (C4H5); 150.9 (CH = N); 194.1 (CO). Mass spectrum (based on 187Re) (m/z): 566 [M]+; 482 [M − 3CO].

2.3.2. N-(5-nitro-2-thiophenylidene)-4-phenylcyrhetrene (3a)

The synthesis of complex 4a was carried out similarly to that described above (general procedure); nevertheless, the reflux was prolonged for 5 h. Brown crystals, yield: 56% (27.0 mg, 0.060 mmol). IR (KBr, cm−1): 2025 (s) (νCO), 1912 (vs) (νCO): 1617 (w) (νC = N). 1H NMR (CDCl3): δ 5.43 (t, 2H, J = 2.2 Hz, C4H2S); 5.79 (t, 2H, J = 2.2 Hz, C4H2S); 7.24 (d, 2H, J = 8.7 Hz, C6H4); 7.39 (d, 1H, J = 4.3 Hz, C6H2S); 7.91 (t, 1H, J = 4.3 Hz, C6H2S); 8.55 (s, 1H, CH = N). 13C NMR (CDCl3): δ 82.0 (C6H4); 84.7 (C6H4); 107.5 (C6H4ipso); 121.9 (C6H4); 127.4 (C6H2S); 128.7 (C6H2S); 129.9 (C4H5); 151.5 (CH = N); 194.0 (CO). Mass spectrum (based on 187Re) (m/z): 566 [M]+; 482 [M − 3CO].

2.3.3. N-(4-nitro-2-thiophenylidene)-4-phenylferrocene (3b)

Red solid, yield: 53% (40.0 mg, 0.10 mmol). IR (KBr, cm−1): 1617 (w) (νC = N). 1H NMR (CDCl3): δ 4.05 (s, 5H, C5H5); 4.35 (ps, 2H, C6H4); 1.9 Hz); 4.67 (ps, 2H, C6H4); J = 1.8 Hz); 7.21 (d, 2H, J = 8.5 Hz, C6H4); 7.51 (2HJ = 8.5 Hz, C6H4); 7.94 (d, 1H, J = 1.5 Hz, C6H2S); 8.40 (t, 1H, J = 1.5 Hz, C6H2S); 8.61 (s, 1H, CH = N). 13C NMR (CDCl3): δ 66.6 (C6H4); 69.4 (C6H4); 69.8 (C6H3); 121.5 (C6H3); 124.8 (C6H3); 126.9 (C6H4); 130.6 (C6H2S); 149.3 (CH = N). Mass spectrum (m/z): 416 [M]+.

2.3.4. N-(5-nitro-2-thiophenylidene)-4-phenylferrocene (4b)

Green solid, yield: 25% (19.0 mg, 0.050 mmol). IR (KBr, cm−1): 1611 (w) (νC = N). 1H NMR (CDCl3): δ 4.07 (s, 5H, C5H5); 4.38 (ps, 2H, C6H4); J = 1.9 Hz); 4.70 (ps, 2H, C6H4); J = 1.9 Hz); 7.25 (d, 2H, J = 8.7 Hz, C6H4); 7.39 (d, 1H, J = 4.3 Hz, C6H2S); 7.54 (d, 2H, J = 8.7 Hz, C6H4); 7.94 (d, 1H, J = 4.3 Hz, C6H2S); 8.64 (s, 1H, CH = N). 13C NMR (CDCl3): δ 66.7 (C6H4); 69.5 (C6H3); 69.9 (C6H3); 121.8 (C6H3); 126.9 (C6H4); 128.7 (C6H2S); 129.1 (C6H2S); 149.6 (CH = N). Mass spectrum (m/z): 416 [M]+.

2.4. X-ray crystal structure determinations

Single crystal X-ray diffraction studies have been successfully implemented for 2a, 4a and 3b. Table 1 summarized the fundamental crystal and refinement data for the compounds. Crystals of the cyrhetrenylaniline (2a) and imines (4a and 3b) were mounted using MiTeGen MicroMounts and a random orientation for a single crystal X-ray diffraction experiment. The compounds were studied at room temperature on a Bruker D8 QUEST diffractometer equipped with a bidimensional CMOS Photon100 detector using graphite monochromated Mo-Kα radiation. The diffraction frames were integrated using the APEX2 package [48] and were corrected for absorptions with SADABS [49]. The unit cell dimensions were determined by a least-squares fit procedure collected with I > 2s(I). Data were integrated and scaled using the APEX2 package and the scale correction was based on the equivalent reflection carried out using SADABS. The solution and refinement for compounds 2a, 4a and 3b were determined using Olex2 [50]. The 2a and 3b structures were solved using direct methods, while the 4a structure was solved by the Patterson Method, using ShelXL software for all the complexes [51] and a refinement package using Least Squares minimization. Calculations were performed with SMART software for data collection, while data reduction used ShelXL. The complete structures were refined using the full matrix least squares procedure on the reflection intensities (I2) with anisotropic thermal parameters for all the nonhydrogen atoms and all the hydrogen atoms were placed in idealized locations.

2.5. Cyclic voltammetry (CV)

DMSO (spectroscopy grade) was used as a solvent and was obtained from Aldrich. Tetrabutylammonium perchlorate (TBAP), which was used as supporting electrolyte, was obtained from Fluka. CV measurements were obtained using a Metrohm 693 VA instrument with a 694 VA Stand converter and a 693 VA Processor, in DMSO (ca. 1.0 × 10−3 mol L−1), using TBAP (ca. 0.1 mol L−1), under a nitrogen atmosphere at room temperature, using a three electrode cell. A hanging mercury drop electrode was used as the working electrode with an area of 0.208 cm2. The reference electrode was a silver/silver chloride electrode and the counter electrodes were platinum mesh. The potential was scanned from −1.5 to 0.8 V vs Ag/AgCl with a scan rate of 100 mV s−1.
2.6.2. Anti-proliferative assays
well plate format. L6 rat skeletal myoblasts or
Software Inc.).
using the non-linear regression tool on GraphPad Prism (GraphPad
CO2 atmosphere in RPMI-1640 medium supplemented with 20 mM
brucei
Gemini Fluorescent Plate reader (Molecular Devices) set at
2.6. Biological evaluation

2.6.1. Cell culturing
L6 rat skeletal myoblasts were grown at 37 °C under a 5% (v/v) 
CO2 atmosphere in RPMI-1640 medium supplemented with 20 mM 
HEPES pH 7.4, 2 mM sodium glutamate, 2 mM sodium pyruvate, 2.5 
U mL−1 penicillin, 2.5 mg mL−1 streptomycin and 10% (v/v) foetal 
calf serum (Pan Biotech UK Ltd).
T. brucei brucei BSF trypomastigotes (MITat 427 strain; clone 
221a) were grown at 37 °C under a 5% (v/v) CO2 atmosphere and 
10% (v/v) heat-inactivated foetal calf serum (Pan Biotech UK Ltd).

2.6.2. Anti-proliferative assays

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<th>2a</th>
<th>4a</th>
<th>3b</th>
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<td>C21H16N2O2SFe</td>
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<td>296.15</td>
<td>296.15</td>
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<td>P21/c</td>
</tr>
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<td>a = 7.3225(15) Å; b = 86.074°; c = 76.288°</td>
<td>a = 22.0594(9) Å; b = 98.851°; c = 11.3482(17) Å</td>
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<td>920.3(3)</td>
<td>1829.3(5)</td>
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<td>4</td>
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<tr>
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<td>F(000)</td>
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<td>3825 [R int = 0.0351]</td>
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<td>87.6, 99%</td>
<td>87.6, 99%</td>
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3. Results and discussion

3.1. Design and synthesis

As part of our continued interest in the study on the electronic 
influence of the organometallic groups into hybrid organic-
organometallic imines compounds with potential antiparasitic ac-
tivity, we decided to form new Schiff bases containing a [-C6H4-
N–CH3] bridge between the organometallic entity and 4- and 5-
nitro-2-thiophenyl groups. To do that we inspired on the several 
reports dealing with imines derived from ferrocenylanilines, which 
have proved to be excellent and versatile building blocks with the 
ability to produce several compounds with interesting electro-
chemical [54], antimicrobial [55–58], antioxidant [59] and antitu-
moral properties [60,61]. On the other hand, we selected the nitro-
thiophenyl group substituted into the 4- and 5-position because 
they allowed us to compare the reduction potential of the nitro 
group and the anti-T. brucei activity. Since ferrocenylaniline is a 
known compound [46,62–64], our first goal was the synthesis of 
the analogous cyrhetrene derivative (2a), which was prepared 
following a modified procedure reported for ferrocenylaniline. The 
first step involved the preparation of 4-nitrophenylcyrhetrene (1a) 
by a Negishi cross-coupling reaction between cyrhetrene and 1-
bromo-4-nitrobenzene, following a strategy similar to that re-
ported for 2-pyridylcyrhetrene [65], followed by its reduction with 
tin in hydrochloric acid [46] (see Scheme 1). Compound 2a 
was isolated in low yield and characterized by spectroscopic and 
crystallographic techniques.

In the IR spectrum, 2a showed N-H stretching absorption bands 
at 3440 and 3339 cm⁻¹ (in KBr) which are similar to those reported 
for 4-ferrocenylaniline [46]. As expected, the ν(CO) bands observed 
at 2020 and 1923 cm⁻¹ (in CH2Cl2) are shifted to lower energy 
compared to the ones measured for 1a, due to the electron-donor 
capability of the NH2 group. The 1H NMR spectrum of 2a showed 
the resonances for the C3 ring at 5.35 and 5.64 ppm, whereas 
the NH2 and aryl hydrogen’s resonances deferred by about 0.1 ppm 
with those reported for ferrocenylaniline [64]. The 13C NMR spectra 
of the compound showed signals at 80.3, 84.1 and 111.0 ppm, which
is indicative of a monosubstituted cyrhetrene subunit [65]. The aromatic carbon resources were assigned by comparison of the experimental chemical shift with those measured for their ferrocenyl analogue [64]. The X-ray structure of 2a will be discussed in the crystallography section.

To prepare the Schiff bases described below, we synthesized the unreported 4-nitro-2-thiophencarboxaldehyde, which was prepared by nitration of thiophencarboxaldehyde according to the procedure described by Fabricynii et al. [66] (Supplementary Material).

The organometallic imines derived from 4- and 5-nitrothiophene were obtained as described in Scheme 2, following the same procedure reported for some N-(arylidene)-4-ferrocenylanilines [58–61,67,68], that is, by the reaction of the appropriate organometallic amine (2a or 2b) and the corresponding 4- or 5-nitrothiophencarboxaldehyde in anhydrous EtOH. All compounds were isolated in low to moderate yields as pure material (by NMR), after crystallization from the CH2Cl2/hexane mixture. They were air stable and soluble in most common polar organic solvents, but insoluble in hexane.

In all cases, the infrared spectral analysis of these compounds showed the characteristic absorption corresponding stretching vibration of the ν(C–N) bond in the range of 1611–1618 cm−1 in KBr disk. Similar ν(C–N) frequency values have been previously reported for other organometallic Schiff bases derived from 5-nitrothiophene [43]. The absence of the band assigned to the aldehyde carbonyl group of nitro-heterocycle as well as the (N–H) stretching absorption of the amine precursors confirmed the formation of the organometallic imines. In addition, the cyrhetrenylimines (3a) and (4a) exhibited the characteristic ν(CO) absorption bands, in the region of 2025 and 1912 cm−1.

For all complexes, the 1H NMR spectra showed only the presence of a single compound. As expected, the 1H NMR spectra of the ferrocenyl derivatives (3b) and (4b) exhibited a singlet at δ 4.0 and two resonances between 4.35 and 4.70 ppm, which were assigned to the protons of the ferrocenyl group. Similarly, the two resonances for the cyrhetrenyl group in compounds (3a) and (4a) were observed at identical chemical shifts (5.43 and 5.79 ppm). In all cases, the ary hydrogen’s were observed as doublets in the range 7.21–7.54 ppm. The most interesting feature of the 1H NMR spectra of these Schiff bases was (i) the imine proton of the cyrhetrenyl derivatives are slightly upfield when compared to the ferrocenyl analogues, and (ii) the heterocyclic hydrogen atoms were observed in a higher field (7.94–8.42 ppm) and showed smaller coupling constants (1.4–1.5 Hz) when the nitro group was at the 4-position (3a–b) compared to their 5-substituted analogues (7.39–7.94 ppm, J = 4.3 Hz), meaning that they follow the same trend observed in the nitro-thiophencarboxaldehyde precursors.

The 13C NMR data also indicated the existence of a single compound. Despite that, these types of compounds could adopt two different forms (E- or Z-) and their 1H and 13C NMR spectra agreed with those reported for the related ferrocenyl and cyrhetrenyl Schiff bases [42,43]. This finding indicates that only one isomer (E-form) was present in the solution. Further proof was provided by an X-ray crystal structure determination of (4a) and (3b) (see below).

The most important feature of these spectra is the presence of low field resonance (δ 149–151) assigned to the iminyl carbon. This resonance occurs at almost the same δ as those reported for other Schiff bases [59,58] and were corroborated by 1H–13C NMR HMOC and HSQC. It is important to note that the 13C shifts of the iminyl carbon of these nitrothiophene derivatives did not show a clear dependence on the electronic properties of the organometallic substituents in the side chain and the position of the nitro group in the thiophene ring. We previously observed similar results in other imines containing 5-nitrothiophene groups [43].

### 3.2. X-ray crystallography

A single crystal of cyrhetrenylaniline (2a) was grown by slow evaporation of the CH2Cl2/hexane solution containing the product. The ORTEP diagram of the molecule giving its numbering scheme is shown in Fig. 1. The summary of the structural refinement data is included in Table 1 and the bond lengths and bond angles are provided as supplementary materials (Table S1). Also, 2a was shown to crystallize in the orthorhombic crystal system containing one molecule per asymmetric unit.

The molecular structure of compound 2a [[[(η⁵-C₅H₄)-(C₅H₅)]Re(CO)₃] (Fig. 1) was confirmed by X-ray diffraction studies showing the expected three-legged piano-stool structure and the presence of an aniline unit attached to the C5H4 ring. Average values of the Re–CO (1.907 Å) and C–O (1.146 Å) bond lengths and the Re centroid distance (1.961(2) Å) are similar to those reported for [[[(η⁵-C₅H₄)-2-(C₅H₅)N]Re(CO)₃] [65]. The short C(1)-(C(6) (1.473(6) Å) bond length on 2a does suggest enhanced conjugation between the NH2 on the phenyl and the C5H4 ring. This was also supported by the torsion angles (C(2)-C(1)-C(6)-C(11), 0.5(6)°), which indicate that the C5H4 ring and the aromatic group are almost co-planar allowing for efficient overlap of the π-electrons for bonding. Similar findings have been reported by Coville for ferrocenylandaniline [62].

To compare the structural parameters of the organometallic Schiff bases with the crystallographic data reported for the related compounds [55,58,60,67], single-crystal X-ray diffraction studies were successfully carried out for [[[(η⁵-C₅H₄)-C₅H₄-N–CH-(C₅H₅S-5-NO₂)]Re(CO)₃]2 and [[[(η⁵-C₅H₄)-C₅H₄-N–CH-(C₅H₅S-4-NO₂)]Fe[(η⁵-C₅H₄)]3] (3b). The molecular structures of 4a and 3b are shown in Figs. 2 and 3, respectively, including the selected bond lengths and bond angles. A full description of the bond lengths and bond angles are listed in Tables S2 and S3 in the Supplementary Material.

The crystallographic structures of compounds 4a and 3b showed that the (C₅H₅S) moiety and [[[(η⁵-C₅H₄)-C₅H₄]]] unit are in a trans arrangement, thus confirming that these imines also adopt the E form in the solid state. In addition, for imine 3b, the presence of the nitro group at the 4-position of thiophene was confirmed.

The cyrhetrenyl group of structure 4a exhibited a typical three-legged piano-stool structure (Fig. 2), which is commonly observed for other half-sandwich rhenium(I) complexes studied by X-ray crystallography [43,69,70]. In 3b (Fig. 3), the ferrocenyl fragment adopted an eclipsed conformation, similar to that found in many

Fig. 1. Molecular structure of 2a drawn with 30% probability displacement ellipsoids. Selected bond lengths (Å) and bond angles (°): C₅H₄(centroid)–Re 1.961(2); Re(1)–C(12) 1.895(5); Re(1)–C(13) 1.911(5); Re(1)–C(14) 1.914(5); C(1)–C(6) 1.473(6); C(9)–N(1) 1.398(7).
Fig. 2. Molecular structure of 4a drawn with 30% probability displacement ellipsoids. Selected bond lengths (Å) and bond angles (°): C5H4(centroid)–Re 1.959(18); C(1)–C(6) 1.488(5); N(1)–C(9) 1.259(5); C(12)–C(13) 1.413(5); N(1)–C(12) 1.259(5); C(1)–C(6) 1.350(6); N(2)–N(2) 1.218(3). C(2)–C(1)–C(6) 125.9(4); C(1)–C(6)–C(11) 120.1(3); C(10)–C(9)–N(1) 124.9(3); C(9)–N(1)–C(12) 119.7(3); N(1)–C(12)–C(13) 121.7(4); C(12)–C(13)–S(1) 120.5(3).

Fig. 3. Molecular structure of 3b drawn with 30% probability displacement ellipsoids. Selected bond lengths (Å) and bond angles (°): C5H4(centroid)–Fe 1.6487(16); C(1)–C(6) 1.4699(4); N(1)–C(9) 1.412(3); N(1)–C(12) 1.258(3); C(12)–C(13) 1.440(4); C(13)–C(14) 1.359(3); C(14)–C(15) 1.406(4); C(15)–C(16) 1.350(3); N(2)–C(15) 1.463(3); O(1)–N(2) 1.214(3); O(2)–N(2) 1.218(3); C(2)–C(1)–C(6) 127.6(2); C(1)–C(6)–C(11) 122.0(2); C(10)–C(9)–N(1) 125.3(2); C(9)–N(1)–C(12) 122.3(2); N(1)–C(12)–C(13) 120.1(2); C(12)–C(13)–S(1) 118.3(4).

3.3. Electrochemical studies

To establish the possible correlation between the electronic effects of the phenylimine bridge with their $E_{1/2}$ and trypanocidal activity, we measured the reduction potentials of nitro compounds by cyclic voltammetry. The large amount of electrochemical information available in the literature for 5-nitroheterocycles (furane and thiophene) [72–74] contrasts with the limited studies dealing with nitrothiophenes and nitrofuranes nitrated in different ring positions [75]. For that reason, in this study we compared the reduction potential of 4-nitro (3a, 3b) and 5-nitro (4a, 4b) thiienyl derivatives.

Under the recommended experimental conditions [74], the 4-nitro derivatives exhibit a different cathodic performance to that measured for 4-nitro derivatives. For example, Fig. 4 shows the comparative cyclic voltammograms of compounds 3a and 4a. It is important to mention that the electrochemical parameters obtained for all compounds are detailed in the Supplementary Material (Table S4).

The voltammograms obtained for 4-nitro compounds (3a and 3b) show the presence of only one reduction peak with a potential near $-1.0$ V. This wave was correlated to an one-electron transfer process due to the reduction of the nitro group to the nitro radical anion [76a]. According to the Nicholson diagnostic criteria, a quasi-reversible reduction process should be involved [76b]. Both the shape of voltammograms and the displacement of the wave to cathodic potentials can be correlated to similar results observed by Sarragiotto and co-workers in nitro aromatic systems derived from tetrahydro-β-carbolines [76a]. On the other hand, 5-nitro derivatives (4a and 4b) exhibited two reduction cathodic peaks in their voltammograms, similar to those observed in several organometallic Schiff bases derived from 5-nitrofuran and 5-nitrothiophene [42,43]. The first one, observed at $-0.65$ V, was attributed to the nitro/nitro-radical anion couple [74,77], comparatively lower than those obtained for 4-nitro derivatives.
3.4. In vitro anti-\textit{T. brucei} activity

According to the standard reversibility criteria, this couple corresponded to a reversible diffusion-controlled single electron transfer. The second irreversible peak displayed a more negative reduction potential (\( \approx -1.1 \) V), which was assigned to the electro-reduction of the nitro-radical anion to form a hydroxylamine derivative (this reduction wave is not observed in the 4-nitro derivatives in the potential range used) \([77]\).

Taking into account the electrochemical behaviour for all the compounds, it is possible to make some general conclusions: (i) in the two series of compounds (4-nitro and 5-nitro) the \( E_{1/2} \) values \([E_{1/2} = (E_a + E_c)/2]\) \((\text{Table 2})\) did not correlate with the electronic nature of the organometallic fragment attached to the phenylimine bridge, suggesting that there is no electronic communication between the two substituents of the imine group, which is probably due to the lack of planarity of the phenylimine bridge with the organometallic and nitrothiophene groups \((\text{see crystallographic section})\) and, (ii) 5-nitro derivatives exhibited lower \( E_{1/2} \) values than nifurtimox and 4-nitroderivatives, under the same conditions, indicating that these compounds had a better ability to generate radical species \([74]\). These results are in agreement with the studies indicating that these compounds had a better ability to generate radical species \((\text{NO}_2^-)\) in the biological target \([5, 11, 78]\). These results are probably related to the generation (in agreement with the electrochemical results) and a better stabilization of the anion nitro derivative \((\text{NO}_2^-)\) formed from the nitro-reduction \((\text{NO}_2^-)\) in the biological target \([5, 11, 78]\).

From the data depicted in Table 2, some general observations can be achieved. Firstly, there was not a linear correlation between the nitro-reduction \((E_{1/2})\) with the anti-\textit{T. brucei} activity \((EC_{50})\). Similar results have been previously reported for related nitrothiophene compounds with antichagasic activity \([43]\). Second, 5-nitrothiophenes are more efficient anti-\textit{T. brucei} agents \((EC_{50} = 0.44)\) for \(3a\) and \(EC_{50} = 2.72 \mu M\) for \(4b\) than their 4-nitro analogues \((EC_{50} = 16.32)\) for \(3a\) and \(9.77 \mu M\) for \(3b\). These results are probably related to the generation (in agreement with the electrochemical results) and a better stabilization of the anion nitro radical \((\text{NO}_2^-)\) in the biological target \([5, 11, 78, 80]\). Third, the cyrhetrenyl derivative containing the 5-nitrothiophene \((4a)\) was a more potent trypanocidal agent than the ferrocenyl analogue \((4b)\) possibly due to the better lipophilicity of the cyrhetrenyl compared to ferrocenyl fragment \([41b]\). Four, independent of the substitution on the thiophene ring, the cyrhetrenyl derivatives \((3a, 4a\) and \(4a)\) showed comparable cytotoxicity, whereas the ferrocenyl containing the 5-nitrothiophenyl ring \((4b)\) is less cytotoxic than its 4-nitro analogue \((3b)\) and nifurtimox. Lastly, the Selectivity Index (SI)


\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{Structure} & \textbf{EC}_{50} \((\mu M)^a\) \pm SE\textsuperscript{b} for: & \multicolumn{2}{c|}{\textbf{Selectivity Index}} & \\textbf{EC}_{50} \((\mu M)^a\) \pm SE\textsuperscript{b} for: \\ & & \textit{T. brucei} & \textit{L}_6 & \textit{EC}_{50} \textit{T. b. brucei} & \textit{EC}_{50} \textit{L}_6/\textit{T. b. brucei} \\
\hline
\textit{a} & \text{4-NO}_2 & 16.32 \pm 1.72 & 20.77 \pm 3.71 & 1.27 & \\textit{3a} & \text{5-NO}_2 & 0.44 \pm 0.01 & 13.27 \pm 1.58 & 30.2 & \\textit{b} & \text{4-NO}_2 & 9.77 \pm 1.21 & 9.22 \pm 1.90 & 0.94 & \\textit{b} & \text{5-NO}_2 & 2.72 \pm 0.45 & >150 & >55.1 & \\textit{b} & \text{5-NO}_2 & 3.65 \pm 0.16 & 88.67 \pm 3.49 & 24.3 & \\textit{c} & \text{Nfx} & 3.71 \pm 0.98 & 0.560 & \textit{3.49} & 0.590 & \\textit{d} & \text{Nfx} & 0.560 & 0.980 & \\textit{e} & \text{Nfx} & 0.985 & -0.590 & \\textit{f} & \text{Nfx} & -0.590 & -0.880 & \\hline
\end{tabular}
\caption{In vitro anti-\textit{T. brucei} activity, cytotoxicity in \textit{L}_6 cells, selectivity index and reduction potentials of cyrhetrenyl \((3a, 4a)\) and ferrocenyl \((3b, 4b)\) imines.}

\textsuperscript{a} EC_{50}: concentration that inhibits 50% of growth. Values shown are the average of four or more experiments.

\textsuperscript{b} Standard error \((SE)\).

\textsuperscript{c} The selectivity index was calculated as a ratio of the \([EC_{50} \text{ L}_6 \text{ cells}: EC_{50} \text{ T. brucei}]\).

\textsuperscript{d} \(E_{1/2} = (E_a + E_c)/2\).

\textsuperscript{e} Data From Ref. \([74]\).}
\end{table}

\textbf{Fig. 4.} (i) Cyclic voltammogram of compounds \(3a\) and (ii) Cyclic voltammogram of compounds \(4a\) in DMSO of 0.1–2.5 V\(^1\).
calculated as the ratio between the EC$_{50}$ values of Ls cell and T. brucei (Table 2) demonstrated that the organometallic compounds derived from 5-nitrothiophene, 4a and 4b, (SI = 30.2 and >55, respectively) have improved selectivity against the parasite than nifurtimox (SI = 24.3). Therefore, these new organometallic compounds could represent a promising family for the design of novel anti-T. brucei agents.

4. Conclusion

New organometallic phenylimines derived from 4- and 5-nitrothiophene were synthesized and characterized. Like many other organometallic Schiff bases, these compounds adopt an anti-configuration for the iminyl fragment in solution and in the solid state. Cyclic voltammetry studies demonstrated that phenylimines derived from 5-nitrothiophene presented reduction potentials of the NO$_2$ group (E$_{1/2} = -0.575$ V) that were more anodic than those registered for their 4-nitro analogues (E$_{1/2} = -0.982$ V) and nifurtimox (E$_{1/2} = -0.880$ V). Evaluation of the in vitro activity against Trypanosoma brucei indicated that the most active complexes are those possessing the 5-nitrothiophene moiety, such as 4a, which is 8-fold more active than nifurtimox, whereas complex 4b was more active and less toxic to host Ls cells than nifurtimox. The non-coplanarity of the [[(n$_2$-C$_5$H$_4$)$_2$C$_6$H$_4$N$^-$(CH$_2$S)$_5$]] system impeded the fluid electronic communication between the cyrene-trenyl and ferrocenyl fragments with the nitrothiophene groups. Accordingly, the electronic effects of the organometallic units are not influential factors in E$_{1/2}$ and the anti-Trypanosoma brucei activity of these compounds.

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Appendix A. Supplementary data

CCDC 1586086-1586088 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-363-033; or by e-mail: deposit@ccdc.cam.ac.uk.

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jorganchem.2018.03.004.

References
