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Synthesis, characterization and *in vitro* anti-*Trypanosoma cruzi* and anti-*Mycobacterium tuberculosis* evaluations of cyrhetrenyl and ferrocenyl thiosemicarbazones

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ABSTRACT

To study the electronic influence of the organometallic moieties in thiosemicarbazones, two short libraries of cyrhetrenyl thiosemicarbazone and ferrocenyl thiosemicarbazone hybrids were synthesized and tested for their antichagasic and antitubercular activities. The unreported cyrhetrenyl thiosemicarbazone derivatives of the form $[(\eta^5\text{-}C_5H_4)\text{–}C(R_1) = NNHC(S)NHR_2]Re(CO)_3$ (R_1 = H, CH_3; R_2 = H, CH_3, CH_2CH_3, C_6H_5) were prepared from cyrhetrenylcarbaldehyde (**1a**) or acetylcyrhetrene (**1b**) and the corresponding thiosemicarbazide. The ^1H and ^{13}C NMR spectra indicate that these compounds have the anti-(*E*) conformation in solution, and the X-ray crystal structure of formylcyrhetrene 4-methyl-thiosemicarbazone (**2b**) confirms that this complex also adopts the anti-(*E*) form in the solid state. The new cyrhetrenyl thiosemicarbazones and their ferrocene analogues were screened *in vitro* against *Trypanosoma cruzi* and *Mycobacterium tuberculosis*. The anti-*T. cruzi* evaluation showed that the ferrocenyl derivatives were more efficient trypanocidal agents compared to their cyrhetrenyl counterparts. The incorporation of any organometallic fragment into thiosemicarbazone scaffold showed moderate antituberculosis activity against mc^{2}7000 strain.

1. Introduction

Thiosemicarbazones (TSCs) are a class of small molecules that have been extensively studied for several decades because they allow a great number of substitution patterns and because of their capability to act as a ligand for metal ions in several bonding modes [1–4].

In addition, the pharmacological properties of TSCs and their metal complexes have been of interest since 1946 [5]. At present, there is a large amount of literature that is entirely dedicated to their broad range of biological and therapeutic applications, such as their antiviral [6], antibacterial [7], antifungal [8] and antitumoral properties [9]. Recently, there has been greater interest in TSCs and their transition metal complexes as potential antichagasic and antitubercular agents, with the goal of finding new and more effective therapies to decrease toxic effects and the growing incidence of drug resistance against clinically established drugs [10–18].

Several organic TSCs as well as TSC–metal complexes have been studied with regard to their trypanocidal activity toward the parasites *Trypanosoma brucei* and *Trypanosoma cruzi* [19]. Although the mechanism of their activity remains unclear, the nonpeptidic nature of these compounds, coupled with their low cost of synthesis, makes this class of reversible covalent inhibitors very promising candidates for the development of new antitrypanosomal chemotherapy [19–21]. For this reason, considerable attention has been focused on aromatic and nitroheterocyclic thiosemicarbazones for designing new anti-*T. cruzi* prodrugs [22,23]. Coordination compounds based on TSC also appear to be a promising alternative in the search for a pharmaceutical solution to Chagas disease [24–28].

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Recently, Gambino and co-workers reported the synthesis and trypanocidal evaluation of the first organoruthenium compounds containing coordinated TSCs [28b,c]. To the best of our knowledge, organometallic TSCs have not been explored previously with regard to their antitrypanosomal activity.

Among a wide spectrum of bioactivities already mentioned, TSCs and related analogues have also been extensively used against *Mycobacterium tuberculosis* (MTB), the pathogenic agent of tuberculosis (TB) [29]. Since Domagk's first report on the antituberculosis activity of TSCs, a large number of organic compounds that contain thiosemicarbazones have been reported and evaluated against MTB, both *in vitro* and *in vivo* [30]. One of them, *p*-acetamidobenzaldehyde thiosemicarbazone, is currently being used for the treatment of TB in Africa and South America and is commercially available as thiacetazone. The rise of multidrug resistance in MTB has complicated and prolonged treatment, and for that reason, new strategies have emerged to develop therapeutics for TB, which can reduce the duration of treatment and provide a more effective therapy against active and latent TB. To this end, ferrocenyl thiosemicarbazones and their metal complexes have been demonstrated to have promising properties as anti-TB agents [31–33].

Taking into account the promising reports that involve organometallic groups being incorporated into various trypanocidal and antitubercular agents, we present a study on the synthesis and characterization of new cyrhetrenyl thiosemicarbazones (**2a**–**h**). We also include in this report the anti-*T. cruzi* and anti-*M. tuberculosis* evaluation of the new compounds and their ferrocene analogues (**2i**–**p**).

2. Experimental

2.1. Materials

All manipulations were conducted under an N_2 atmosphere using Schlenk techniques. The complexes (η^5-C_5H_4CHO)$Re(CO)_3$ (**1a**) [34], (η^5-$C_5H_4COCH_3$)$Re(CO)_3$ (**1b**) [35] and the ferrocenyl thiosemicarbazones (Fc-TSCs) (**2i**–**p**) [36] were prepared according to published procedures. Ferrocenecarboxaldehyde (98%), acetylferrocene (95%), thiosemicarbazide (98%), 4-methyl-thiosemicarbazide (98%), 4-ethyl-thiosemicarbazide (98%), and 4-phenyl-thiosemicarbazide (98%) were obtained from Aldrich. Solvents such as dichloromethane (CH_2Cl_2), hexane, acetone, ethanol (EtOH), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) were obtained commercially and purified using standard methods. Infrared spectra were recorded in solution (CH_2Cl_2) or solid state (KBr disc) on a Perkin–Elmer FT-1605 spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Bruker AVANCE 400 spectrometer. ^1H NMR chemical shifts were referenced using the chemical shifts of residual solvent resonances, and ^{13}C chemical shifts were referenced to solvent peaks. Elemental analyses were measured on a Perkin–Elmer CHN Analyzer 2400. Mass spectra were obtained at the Laboratorio de Servicios Analíticos, Universidad Católica de Valparaíso, and masses are quoted in reference to ^{187}Re.

2.2. Synthesis of cyrhetrenyl thiosemicarbazones. General procedure

The cyrhetrenyl–TSCs were prepared following the same procedure as for their ferrocenyl analogues [36]. Equimolar amounts of **1a** or **1b** and the corresponding thiosemicarbazide were dissolved in anhydrous ethanol (25 mL) and refluxed for 24 h, under nitrogen atmosphere. After this time, the solvent was removed under vacuum and the solid obtained was purified by crystallization from CH_2Cl_2/hexane (1:5) at −18 °C.

2.2.1. Formylcyrhetrene thiosemicarbazone (**2a**)

White solid, yield: 90% (54 mg, 0.12 mmol). IR (CH_2Cl_2, cm^{-1}): 2027 (s) (νCO), 1935 (s) (νCO), 1605 (w) (νC=N). IR (KBr, cm^{-1}): 3120 (m) (νNH), 2025 (s) (νCO), 1915 (s) (νCO), 1582 (w) (νC=N), 837 (m) (νC=S). ^1H NMR ($CDCl_3$): δ 5.38 (t, 2H, J = 2.2 Hz, C_5H_4); 5.80 (t, 2H, J = 2.2 Hz, C_5H_4); 6.40 (bs, 1H, NH_2); 7.03 (bs, 1H, NH_2); 7.58 (s, 1H, CH=N); 9.76 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 84.5 (C_5H_4); 84.6 (C_5H_4); 97.1 (C_5H_{4ipso}); 134.3 (CH=N); 178.2 (C=S); 192.7 (Re–CO). Mass spectrum m/z: 438 [M^+]; 409 [M^+ − CO]; 381 [M^+ − 2CO]; 353 [M^+ − 3CO]. Anal. (%) Calc. for $C_{10}H_8N_3O_3SRe$: C, 27.52; H, 1.85 and N, 9.63; found: C, 27.59; H, 1.86 and N, 9.62.

2.2.2. Formylcyrhetrene 4-methyl-thiosemicarbazone (**2b**)

Yellow crystal, yield: 90% (56 mg, 0.12 mmol). IR (CH_2Cl_2, cm^{-1}): 2027 (s) (νCO), 1933 (s) (νCO), 1604 (w) (νC=N). IR (KBr, cm^{-1}): 3128 (m) (νNH), 2025 (s) (νCO), 1913 (s) (νCO), 1581 (w) (νC=N), 828 (m) (νC=S). ^1H NMR ($CDCl_3$): δ 3.23 (d, 3H, J = 4.9 Hz, CH_3); 5.39 (t, 2H, J = 2.0 Hz, C_5H_4); 5.78 (t, 2H, J = 2.0 Hz, C_5H_4); 7.51 (s, 1H, CH=N); 9.29 (bs, 1H, $NHCH_3$). ^{13}C NMR ($CDCl_3$): δ 31.4 (CH_3); 84.4 (C_5H_4); 84.5 (C_5H_4); 97.1 (C_5H_{4ipso}); 134.5 (CH=N); 178.1 (C=S); 192.5 (Re–CO). Mass spectrum m/z: 451 [M^+]; 423 [M^+ − CO]; 367 [M^+ − 3CO]. Anal. (%) Calc. for $C_{11}H_{10}N_3O_3SRe$: C, 29.33; H, 2.24 and N, 9.33; found: C, 29.31; H, 2.25 and N, 9.32.

2.2.3. Formylcyrhetrene 4-ethyl-thiosemicarbazone (**2c**)

Yellow solid, yield: 85% (54 mg, 0.11 mmol). IR (CH_2Cl_2, cm^{-1}): 2027 (s) (νCO), 1933 (s) (νCO), 1604 (w) (νC=N). IR (KBr, cm^{-1}): 3130 (m) (νNH), 2025 (s) (νCO), 1913 (s) (νCO), 1590 (w) (νC=N), 828 (m) (νC=S). ^1H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 4.2 Hz, CH_3); 3.71 (m, 2H, CH_2); 5.38 (t, 2H, J = 2.0 Hz, C_5H_4); 5.80 (t, 2H, J = 2.0 Hz, C_5H_4); 7.20 (pst, 1H, NHC_2H_5); 7.54 (s, 1H, CH=N); 9.74 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.3 (CH_3); 39.4 (CH_2); 84.4 (C_5H_4); 84.7 (C_5H_4); 97.2 (C_5H_{4ipso}); 134.7 (CH=N); 176.8 (C=S); 192.7 (Re–CO). Mass spectrum m/z: 465 [M^+]; 437 [M^+ − CO]; 381 [M^+ − 3CO]. Anal. (%) Calc. for $C_{12}H_{12}N_3O_3SRe$: C, 31.03; H, 2.60 and N, 9.05; found: C, 31.10; H, 2.59 and N, 9.07.

2.2.4. Formylcyrhetrene 4-phenyl-thiosemicarbazone (**2d**)

Yellow solid, yield: 85% (60 mg, 0.11 mmol). IR (CH_2Cl_2, cm^{-1}): 2027 (s) (νCO), 1932 (s) (νCO), 1594 (w) (νC=N). IR (KBr, cm^{-1}): 3126 (m) (νNH), 2025 (s) (νCO), 1912 (s) (νCO), 1587 (w) (νC=N), 830 (m) (νC=S). ^1H NMR ($CDCl_3$): δ 5.38 (t, 2H, J = 2.2 Hz, C_5H_4); 5.83 (t, 2H, J = 2.2 Hz, C_5H_4); 7.29 (m, 1H, C_6H_5); 7.41 (t, 2H, J = 7.8 Hz, C_6H_5); 7.58 (d, 2H, J = 7.8 Hz, C_6H_5); 7.67 (s, 1H, CH=N); 8.93 (s, 1H, NHC_6H_5); 10.55 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 84.5 (C_5H_4); 85.1 (C_5H_4); 96.5 (C_5H_{4ipso}); 124.9 (C_6H_5); 126.6 (C_6H_5); 128.9 (C_6H_5); 135.8 (CH=N); 137.5 (C_6H_5); 175.8 (C=S); 192.9 (Re–CO). Mass spectrum m/z: 513 [M^+]; 485 [M^+ − CO]; 429 [M^+ − 3CO]. Anal. (%) Calc. for $C_{16}H_{12}N_3O_3SRe$: C, 37.49; H, 2.36 and N, 8.20; found: C, 37.48; H, 2.36 and N, 8.22.

2.2.5. Acetylcyrhetrene thiosemicarbazone (**2e**)

White solid, yield: 50% (30 mg, 0.1 mmol). IR (CH_2Cl_2, cm^{-1}): 2025 (s) (νCO), 1933 (s) (νCO), 1606 (w) (νC=N). IR (KBr, cm^{-1}): 3151 (m) (νNH), 2023 (s) (νCO), 1913 (s) (νCO), 1590 (w) (νC=N), 819 (m) (νC=S). ^1H NMR ($CDCl_3$): δ 2.03 (s, 3H, CH_3); 5.37 (t, 2H, J = 2.2 Hz, C_5H_4); 5.76 (t, 2H, J = 2.2 Hz, C_5H_4); 6.40 (bs, 1H, NH_2); 7.03 (bs, 1H, NH_2); 8.75 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 30,8 (CH_3); 84.3 (C_5H_4); 85.7 (C_5H_4); 96.9 (C_5H_{4ipso}); 138.3 (C=N); 178.5 (C=S); 192.9 (Re–CO). Mass spectrum m/z: 451 [M^+]; 423 [M^+ − CO]; 367 [M^+ − 3CO]. Anal. (%) Calc. for $C_{11}H_{10}N_3O_3SRe$: C, 29.33; H, 2.24 and N, 9.33; found: C, 29.31; H, 2.25 and N, 9.32.

*2.2.6. Acetylcyrhetrene 4-methyl-thiosemicarbazone (**2f**)*

Yellow solid, yield: 60% (37 mg, 0.1 mmol). IR (CH_2Cl_2, cm^{-1}): 2025 (s) (νCO), 1931 (s) (νCO), 1604 (w) (νC═N). IR (KBr, cm^{-1}): 3145 (m) (νNH), 2023 (s) (νCO), 1911 (s) (νCO), 1583 (w) (νC═N), 820 (m) (νC═S). ^{1}H NMR ($CDCl_3$): δ 2.02 (s, 3H, CH_3); 3.23 (d, 3H, $J = 4.9$ Hz, CH_3); 5.37 (t, 2H, $J = 2.0$ Hz, C_5H_4); 5.77 (t, 2H, $J = 2.0$ Hz, C_5H_4); 7.40 (pst, 1H, $NHCH_3$); 8.59 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.3 (CH_3); 31.4 (CH_3); 84.5 (C_5H_4); 85.6 (C_5H_4); 97.1 (C_5H_{4ipso}); 138.6 (C═N); 178.6 (C═S); 193.0 (Re−CO). Mass spectrum *m/z*: 465 [M^+]; 437 [$M^+ - CO$]; 381 [$M^+ - 3CO$]. Anal. (%) Calc. for $C_{12}H_{12}N_3O_3SRe$: C, 31.03; H, 2.60 and N, 9.05; found: C, 31.04; H, 2.62 and N, 9.07.

*2.2.7. Acetylcyrhetrene 4-ethyl-thiosemicarbazone (**2g**)*

Yellow pale solid, yield: 70% (44 mg, 0.1 mmol). IR (CH_2Cl_2, cm^{-1}): 2025 (s) (νCO), 1931 (s) (νCO), 1606 (w) (νC═N). IR (KBr, cm^{-1}): 3144 (m) (νNH), 2023 (s) (νCO), 1911 (s) (νCO), 1583 (w) (νC═N), 822 (m) (νC═S). ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, $J = 4.2$ Hz, CH_3); 2.01 (s, 3H, CH_3); 3.73 (m, 2H, CH_2); 5.38 (t, 2H, $J = 2.0$ Hz, C_5H_4); 5.76 (t, 2H, $J = 2.0$ Hz, C_5H_4); 7.33 (pst, 1H, NHC_2H_5); 8.48 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 31.3 (CH_3); 39.4 (CH_2); 84.4 (C_5H_4); 84.7 (C_5H_4); 97.1 (C_5H_{4ipso}); 138.7 (C═N); 178.7 (C═S); 193.1 (Re−CO). Mass spectrum *m/z*: 479 [M^+]; 451 [$M^+ - CO$]; 395 [$M^+ - 3CO$]. Anal. (%) Calc. for $C_{13}H_{14}N_3O_3SRe$: C, 32.63; H, 2.95 and N, 8.78; found: C, 32.62; H, 2.96 and N, 8.80.

*2.2.8. Acetylcyrhetrene 4-phenyl-thiosemicarbazone (**2h**)*

Brown solid, yield: 60% (42 mg, 0.1 mmol). IR (CH_2Cl_2, cm^{-1}): 2025 (s) (νCO), 1930 (s) (νCO), 1605 (w) (νC═N). IR (KBr, cm^{-1}): 3133 (m) (νNH), 2023 (s) (νCO), 1910 (s) (νCO), 1580 (w) (νC═N), 827 (m) (νC═S). ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3H, CH_3); 5.38 (t, 2H, $J = 2.2$ Hz, C_5H_4); 5.83 (t, 2H, $J = 2.2$ Hz, C_5H_4); 7.29 (m, 1H, C_6H_5); 7.41 (t, 2H, $J = 7.8$ Hz, C_6H_5); 7.58 (d, 2H, $J = 7.8$ Hz, C_6H_5); 8.73 (s, 1H, NHC_6H_5); 9.11 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 84.4 (C_5H_4); 85.5 (C_5H_4); 97.0 (C_5H_{4ipso}); 124.9 (C_6H_5); 126.6 (C_6H_5); 128.9 (C_6H_5); 138.5 (CH═N); 137.5 (C_6H_5); 178.6 (C═S); 193.0 (Re−CO). Mass spectrum *m/z*: 527 [M^+]; 499 [$M^+ - CO$]; 443 [$M^+ - 3CO$]. Anal. (%) Calc. for $C_{17}H_{14}N_3O_3SRe$: C, 38.77; H, 2.68 and N, 7.98; found: C, 38.80; H, 2.69 and N, 7.99.

2.3. In vitro antitrypanosomal activity

Trypanocidal activity of the organometallic TSC derivatives was evaluated against the *T. cruzi* epimastigote stages (Dm28c strain). The compounds were dissolved in DMSO and were added to 3×10^6 epimastigotes/mL suspensions. Epimastigotes were incubated (28 °C, 24 h) in Diamond's monophasic medium, supplemented with 4 μM hemin and 4% inactivated bovine calf serum. Afterwards, trypanocidal activity was measured through the MTT assay as described elsewhere [37]. Briefly, MTT was added at a final concentration of 0.5 mg/mL and incubated at 37 °C for 4 h. Parasites were solubilized with 10% sodium dodecyl sulphate and 0.1 mM HCl and incubated overnight. Formazan formation was measured at 570 nm in a multiwell reader (Asys Expert Plus©, Austria). The final concentration of DMSO was less than 0.1% v/v. The trypanocidal Nifurtimox was added as a drug control. We determined the IC_{50} value of viable parasites (IC_{50} is the drug concentration needed to reduce by 50% the parasite viability) at 24 h after compound addition by non-lineal regression analysis from the log of the concentration *vs* the percentage of viable cells curve.

2.4. In vitro anti-tubercular activity

Bacterial strains and growth conditions: *M. tuberculosis* $mc^2$7000, an unmarked version [38] of $mc^2$6030, was grown at 37 °C in Sauton's medium, supplemented with 20 μg ml^{-1} of pantothenic acid. The susceptibility of *M. tuberculosis* $mc^2$7000 to the various compounds was determined as reported previously [39]. In brief, the Middlebrook 7H10 solid medium that contains oleic-albumin-dextrose-catalase enrichment (OADC) and 20 μg ml^{-1} of pantothenic acid was supplemented with increasing concentrations of the chemical analogues. Serial 10-fold dilutions of each actively growing culture were plated and incubated at 37 °C for 2–3 weeks. The Minimum Inhibitory Concentration (MIC) was defined as the minimum concentration required to inhibit 99% of the growth.

2.5. X-ray crystal structure determinations

A suitable X-ray single crystal of the compound **2b** was obtained as described above and was mounted on top of glass fibres in a random orientation. Crystal data, data collection, and refinement parameters are given in the Supplementary material. Compound **2b** was studied at 150(2) K, on a Bruker Smart Apex diffractometer equipped with a bidimensional CCD detector, using graphite-monochromated Mo-Kα radiation ($l = 0.71073$ Å). The diffraction frames were integrated using the SAINT package [40] and were corrected for absorption with SADABS [41]. The structures were solved using XS in SHELXTL-PC [42] by the Patterson method and completed (non-H atoms) by use of difference Fourier techniques. The complete structure was then refined by the full matrix least-squares procedures on the reflection intensities (F^2) [43]. All non-hydrogen atoms were refined with anisotropic displacement coefficients, and all hydrogen atoms were placed in idealized locations.

3. Results and discussion

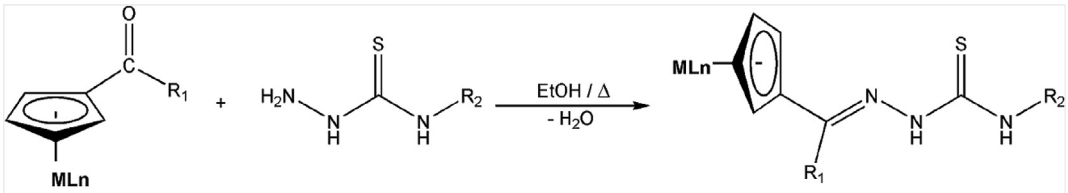
3.1. Design and synthesis

In view of the large amount of literature that addresses organic-TSCs and their applications as potential antitrypanosomal [24–28] and antituberculosis [29] agents, it is surprising that organometallic analogues with these biological targets have not been extensively studied. Recently, Chibale et al. reported the incorporation of ferrocenyl TSCs into the structure of metallodendrimers [32] and gold (III) complexes [31] and their biological evaluations against malaria and TB.

To continue our studies on the electronic influence of the organometallic moiety on bioconjugates [44–47], two short libraries of unreported cyrhetrenyl-thiosemicarbazones and their previously reported ferrocenyl-thiosemicarbazone hybrids were synthesized.

The cyrhetrenyl TSCs were prepared following the same procedure that was described for their ferrocenic analogues [36], that is, the reaction of cyrhetrenecarboxaldehyde (**1a**) or acetylcyrhetrene (**1b**) with the corresponding thiosemicarbazide in anhydrous EtOH (Scheme 1). In all cases, the products were isolated as solids after crystallization with the CH_2Cl_2–hexane mixture. These products are air-stable and are soluble in most organic solvents.

The IR spectra of all compounds showed the expected absorption band for the νC═N, νC═S and νN-H stretchs in the range of 1594–1606 cm^{-1}, 819-837 cm^{-1} and 3120-3151 cm^{-1}, respectively, in a CH_2Cl_2 solution or in solid state (KBr). Similar frequencies values have been reported for thiosemicarbazones derived from ferrocene [48,49]. In addition, the spectra show that the νCO bands of the carbonyls bound to rhenium are shifted to lower wavenumber than the bands for their cyrhetrene precursors [50]. The stoichiometry of the complexes was established by elemental analysis and mass spectrometry. The mass spectra for compounds



Compound	MLn	R_1	R_2
2a	$Re(CO)_3$	H	H
2b	$Re(CO)_3$	H	CH_3
2c	$Re(CO)_3$	H	CH_2CH_3
2d	$Re(CO)_3$	H	Ph
2e	$Re(CO)_3$	CH_3	H
2f	$Re(CO)_3$	CH_3	CH_3
2g	$Re(CO)_3$	CH_3	CH_2CH_3
2h	$Re(CO)_3$	CH_3	Ph
2i	$Fe(\eta^5\text{-}C_5H_5)$	H	H
2j	$Fe(\eta^5\text{-}C_5H_5)$	H	CH_3
2k	$Fe(\eta^5\text{-}C_5H_5)$	H	CH_2CH_3
2l	$Fe(\eta^5\text{-}C_5H_5)$	H	Ph
2m	$Fe(\eta^5\text{-}C_5H_5)$	CH_3	H
2n	$Fe(\eta^5\text{-}C_5H_5)$	CH_3	CH_3
2o	$Fe(\eta^5\text{-}C_5H_5)$	CH_3	CH_2CH_3
2p	$Fe(\eta^5\text{-}C_5H_5)$	CH_3	Ph

Scheme 1. Synthesis of cyrhetrenyl and ferrocenyl thiosemicarbazones.

2a–**h** all showed a strong molecular ion and fragments that correspond to the successive loss of three CO groups.

For all complexes, the ^{1}H NMR spectra showed the presence of a single compound. In the case of **2a**–**d**, a sharp singlet was observed near 7.5–7.7 ppm and was assigned to the iminic proton. In addition, a signal due to the methyl protons of the $-C(CH_3)=$N-fragment was also observed at approximately 2.0 ppm for compounds **2e**–**h**. These results are in agreement with the values reported for ferrocenyl TSCs [48,49]. For all complexes, proton NMR spectra showed two triplets in the region of 5.3–5.8 ppm, which are ascribed to the two types of protons of the cyrhetrenyl moiety [50,51]. It is important to note that the TSCs are capable of exhibiting the thione or the thiol tautomeric forms [3]. However, the ^{1}H NMR spectra of all cyrhetrenyl TSCs exhibit the –NH– resonance as a broad singlet in the range of 8.5–10.5 ppm, indicating that they remain solely as the thione form in solution, just like their ferrocene counterparts do [52].

^{13}C NMR data were also in accordance with the existence of a single compound. The most important feature of these spectra is the presence of a low field resonance (134–138 ppm), which was assigned to the iminyl carbon [C=N]. The carbon chemical shift of this group, compared with that of their ferrocene analogues (144–150 ppm), shows a clear dependence on the presence of an organometallic fragment. The upfield shift observed for the cyrhetrenyl TSC *vs* ferrocenyl TSC ($\Delta\delta = 10.0$ ppm) can be related to the opposite electronic effects of these organometallic fragments [53,54]. We previously reported similar results for Schiff bases and chalcones that contain ferrocenyl and cyrhetrenyl moieties [44,45].

Despite the fact that these types of compounds can adopt two different forms (*E*- or *Z*-) [55,56], their ^{1}H and ^{13}C NMR spectra, which agreed with those previously reported for the related ferrocenyl thiosemicarbazones, revealed that only one isomer (the *E*-form) was present in solution. Further proof was provided by the X-ray crystal structure determination of **2b** (see below).

3.2. X-ray crystallography

With the aim of comparing the structural parameters of cyrhetrenyl TSCs with the crystallographic data reported for their ferrocenic analogues, we undertook a crystallographic study of **2b**. Fig. 1 shows an ORTEP representation of **2b** and the most relevant bond lengths and angles. The structure confirms the *E* configuration tentatively assigned by NMR.

The crystallographic data obtained for **2b** did not show any remarkable differences from the structures previously reported for the ferrocenyl analogues [57–59]. However, the internal C–C bond distances of the cyclopentadienyl ring are slightly different than those measured by Fun for **2k** [58], so some degree of delocalization

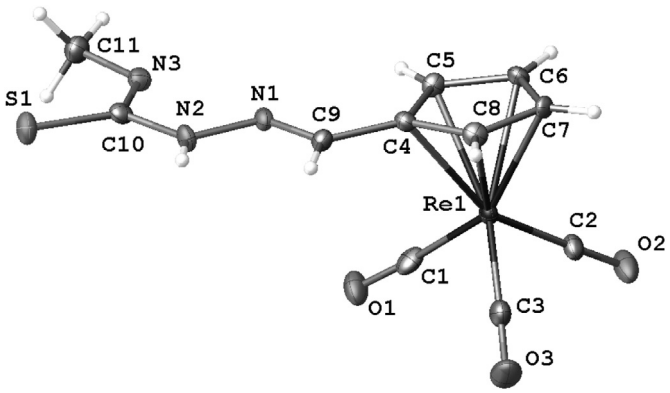


Fig. 1. ORTEP representation of the asymmetrical unit of **2b**. Relevant bond lengths (Å): C(4)–C(9) 1.455(8); C(9)–N(1) 1.287(7); N(1)–N(2) 1.391(6); N(2)–C(10) 1.377(7); C(10)–SC(1) 1.681(6); Cp(centroid)–Re(1) 2.297(6) and angles (°): C(4)–C(9)–N(1) 119.5(6); C(9)–N(1)–N(2) 114.6(5); N(1)–N(2)–C(10) 117.3(5); N(2)–C(10)–S(1) 118.8(5).

Table 1
In vitro anti-*T. cruzi* and antitubercular activity against the *T. cruzi* and *M. tuberculosis* strains.

Compound	*T. cruzi* (IC_{50}/μM)	*M. tuberculosis* (MIC/μM)
	Dm28c strain	$mc^2$7000 strain
2a	>100	45–115
2b	>100	111
2c	>100	>107
2d	>100	>98
2e	>100	–
2f	>100	–
2g	>100	>105
2h	>100	95
2i	17.9 ± 4.0	174
2j	15.8 ± 1.1	66–166
2k	9.1 ± 1.2	63–159
2l	20.8 ± 1.6	>138
2m	30.8 ± 2.6	166
2n	29.4 ± 1.1	>159
2o	27.5 ± 1.6	>152
2p	32.7 ± 2.1	>133
Nfx	17.4 ± 5.1	–
Rifampicin	–	0.06

of electron density between the CH=N group and the cyrhetrenyl fragment can be considered. On the other hand, within the cyrhetrenyl group, the average Re–C(O) distance and the Re–C–O angle are concordant with the distances and angles reported for the related tricarbonyl cyclopentadienyl rhenium (I) complexes [51]. In addition, the bond distances of the N(1)–N(2)–C(10)–S(1) fragment are slightly shorter than those reported for the ferrocenyl analogue [59] and may suggest some contribution of the thione form.

3.3. Biological evaluations

3.3.1. In vitro anti-T. cruzi activity

The anti-*T. cruzi* activities of the cyrhetrenyl TSCs (**2a**–**h**) and the ferrocenyl TSCs (**2i**–**p**) are shown in Table 1, along with IC_{50} values for the standard trypanocidal drug Nifurtimox (Nfx). Ferrocenyl TSCs (IC_{50} = 9.1–31.0 μM) were more active than their analogues that contained the cyrhetrenyl fragment (IC_{50} > 100 μM). At present, we have not a plausible explanation to this phenomenon which might be associated with the redox properties of the ferrocenyl fragment [60]. On the other hand, it is interesting to note that for the ferrocenyl TSCs series, when a hydrogen atom is replaced by a methyl group on the imine carbon, a two-fold decrease in trypanocidal activity was observed.

3.3.2. In vitro anti-tubercular activity

The Minimum Inhibitory Concentrations (MICs) are reported in Table 1. Taking into account the similar MIC values reported for the cyrhetrenyl (**2a**–**h**) and the ferrocenyl TSCs (**2i**–**p**) (MIC = 20–50 μg ml^{-1}), we observed that their opposite electronic effects are not an important factor in the antitubercular activities of TSCs. In addition, the moderate activity of metallo-TSCs could possibly be attributed to the presence of the lipophilic organometallic moiety, which allows these fragments to partially access the lipid-rich mycobacterial cell wall. In fact, Collins et al. [61] suggested that the antimycobacterial activity of thiosemicarbazones was as a result of their optimum hydrophobicity. It is noteworthy that the most active anti-*T. cruzi* ferrocenyl TSCs (**2i**–**k**) were significantly less active against *M. tuberculosis*, which suggests selective trypanocidal activity.

4. Conclusions

The cyrhetrenyl fragment was successfully incorporated into the thiosemicarbazone skeleton. Like many other organic and ferrocenic TSCs, these complexes adopt an *anti* configuration for the iminyl moiety and a thione tautomeric form, both in solution and in the solid state. The electron-donating (ferrocenyl) and electron-withdrawing (cyrhetrenyl) capability of the organometallic fragment on the imine moiety was correlated properly with the ^{13}C shift of the carbon nuclei of the C=N group. The results of an *in vitro* antitrypanosomal assay of the compounds against *T. cruzi* (Dm28c strain) indicate that the ferrocenyl TSCs were more active than their cyrhetrene analogues, most likely due to the redox properties of the ferrocenic entity. Within the ferrocenyl series, the replacement of a hydrogen atom by a methyl group on the imine carbon produced a two-fold decrease in the trypanocidal activity. The incorporation of any organometallic fragment into thiosemicarbazone scaffold showed moderate antituberculosis activity against $mc^2$7000 strain.

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Appendix A. Supplementary material

CCDC 963290 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jorganchem.2013.12.049.

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