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Short communication

Naphthoquinoidal [1,2,3]-triazole, a new structural moiety active against *Trypanosoma cruzi*

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Abstract

[1,2,3]-Triazole derivatives of nor-β-lapachone were synthesized and assayed against the infective bloodstream trypomastigote form of *Trypanosoma cruzi*, the etiological agent of Chagas disease. All the derivatives were more active than the original quinones, with IC_{50}/1 day values in the range of 17 to 359 μM, the apolar phenyl substituted triazole **6** being the most active compound. These triazole derivatives of nor-β-lapachone emerge as interesting new lead compounds in drug development for Chagas disease.

Keywords: Chagas disease; *Trypanosoma cruzi*; Chemotherapy; [1,2,3]-Triazoles; Naphthoquinones

1. Introduction

Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is endemic in Latin America [1]. The infection is transmitted by triatomine insects while blood feeding on a human host. The trypomastigote form ingested by the vector via the blood of an infected individual differentiates into epimastigote form, which, after proliferation, reaches the posterior intestine and differentiates into metacyclic trypomastigote. This latter infective form, following invasion of vertebrate cells, undergoes differentiation into amastigote, which, after several reproductive cycles, transforms into trypomastigote, responsible for the dissemination of the infection. Acute infections are usually asymptomatic, but the ensuing chronic *T. cruzi* infections have been associated with high ratios of morbidity and mortality [2]. Currently, treatment is unsatisfactory, being limited to two drugs nitroheterocycles, benznidazole and nifurtimox. Their use to treat the acute phase of the disease is widely accepted, while the treatment of the chronic phase is controversial [3]. The undesirable side effects of both drugs have a major drawback in their uses, frequently forcing the abandonment of the treatment [4].

In this context, an intensive research program has been focused upon the search for alternative natural, semi-synthetic and synthetic drugs. Among several naturally occurring quinones, emerge the naphthoquinones with a broad distribution in the plant kingdom and involved in oxidative processes such as photosynthesis and electron transfer reactions [5]. In folk medicine, plants containing naphthoquinones have been employed for the treatment of many diseases, especially among Indian populations [6]. The involvement of quinones in numerous biochemical processes has led to the study of a wide variety of their synthetic derivatives. The facile reduction–oxidation of the quinone moiety appears to be the basis for the participation of a number of different quinones in electron transport and oxidative-phosphorylation processes.

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The biological activities of the naphthoquinone lapachol extracted from the heartwood of trees of the genus *Tabebuia* (Bigoniaceae) and its cyclization product, β-lapachone, have been intensively studied [7]. Previous work of our group showed the activity of new heterocyclic naphthoimidazole derivatives obtained from the reaction of naphthoquinones with amino-compounds [7–13]. Recently, our group also reported the synthesis and trypanocidal activity of new naphthofuranquinones [14] and of heterocyclic oxyranes [15,16]. Continuing our studies on the chemical reactivity of quinones from the Brazilian flora, we now focus on the search of compounds with naphthofuranquinoidal endowed linked to a triazolic moiety. We synthesized and characterized five derivatives of nor-lapachol (**1**), and assayed their activity against infective bloodstream forms of *T. cruzi*.

The triazolic nucleus and the naphthoquinone ring are moieties with independent biological activities [5,17–19] and in this context the Huisgen cyclization under copper catalysis was employed in the present work for the obtention of naphthoquinones coupled to 1,4-triazolic nuclei and analysis and their potential trypanocidal activity was evaluated.

2. Chemistry

Lapachol was extracted in large scale from the heartwood of *Tabebuia* sp. and purified by a series of recrystalizations, as previously described [20]. Nor-lapachol (**1**, 2-hydroxy-3-(2′-methyl-1-propenyl)-1,4-naphthoquinone) was obtained from lapachol by Hooker oxidation, and bromo-β-nor-lapachone (**2**, 3-bromo-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione) was prepared through cyclization of **1** with bromine in chloroform. From **2**, through nucleophilic substitution with sodium azide in dichloromethane was obtained the azide (**3**, 3-azido-2,2-dimethyl-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione), the key intermediate for the synthesis of quinones coupled to the triazolic nucleus, employing 1,3-dipolar reaction between the azidoquinone and an alkyne, catalyzed by Cu(I), known as ''click chemistry''. Through this type of reaction, the naphthoquinoidal triazoles **4–8** were obtained and their physical and spectroscopic data are in agreement with the structures depicted in Scheme 1. The atoms of carbons present in molecules **3** and **4–8** were differentiated using the spectra of ^{13}C-APT (attached proton test), where the carbons CH and CH_3 are positive phases and C (quaternary) and CH_2 are negative phases. The regioselectivity of the reactions was fully ascertained through X-ray crystallography study of derivative **4**.

3. Results and discussion

[1,2,3]-Triazoles have gained increasing attention in drug discovery since the introduction of the concept of ''click'' chemistry by Sharpless [21,22]. This kind of substances can actively participate in hydrogen bonding and dipole–dipole interactions due to their strong dipole moments, being extremely stable to hydrolysis and oxidative/reductive conditions. Several different procedures have been described for their synthesis, but the most suitable method is the 1,3-dipolar cycloaddition reaction [23] between substituted acetylenes and an alkyl azide derivative. This reaction originates two regioisomers, 1,4- and 1,5-triazoles. However, recently it was demonstrated that copper(I) salt's regioselectivity promotes formation of the 1,4-triazole adduct. The improvement for this reaction was reported independently by the groups of Sharpless and Meldal [24] demonstrating that it can be carried out at room temperature in aqueous media under copper(I) catalysis.

[1,2,3]-Triazoles are an important class of heterocyclic compounds due to their wide range of activities such as antiplatelet agents [25], dopamine D2 receptor ligands related to schizophrenia [26], anticonvulsants [27], anti-inflammatory, anti-allergic [28], antiviral [29] and antimicrobial agents [30].

Structure of **4** was confirmed by X-ray diffraction (Fig. 1). All the interatomic distances and angles are within the expected values for similar chemical bondings [31].

The naphthoquinonic ring is planar with maximum deviation for C6 [0.022(2) Å]. The distances of atoms O1 and C12 of furan ring to the average reference plane are 0.012(3) Å and 0.006(2) Å, respectively. Atoms O2 and O3 are practically inside this plane with distances of 0.002(4) Å and 0.012(2) Å, respectively. The furan ring adopts a pure envelope conformation with Puckering parameters [$q_2 =$ 0.006(1) Å and $\varphi = 292(1)^\circ$]. Atoms C12, C15, O4 and atoms of the triazole ring are all inside of the same plane of least squares with maximum distances for O4 [0.1215(4) Å]. The dihedral angle between this least squares plane and that of the naphthoquinonic ring is 89.47°. In the crystal structure packing molecules interact through strong intermolecular O4–H11···N2 hydrogen bond [symmetry code: $(x-1,y,z)$ with O4···N2 = 2.890(4), H11···N2 = 1.899(3) Å and O4–H11···N2 = 156(2)°, forming a network (Fig. 2).

In order to analyze the role of the triazole substitution pattern on the furan ring moiety of **3**, it was planned to prepare compounds **4–8**, whose structures are dependent on the available alkynes: (a) triazoles containing polar groups (**4**, **5** and **7**); (b) a derivative containing an aromatic apolar group, such as (**6**) and (c) a triazole possessing a polar group (**8**).

The triazolic quinones **4**, **5** and **7** present a hydroxyl group (leaving group), susceptible to elimination as exemplified in the Scheme 2, by elements of the biological milieu, including blood components, leading to the formation of an ammonium salt, suggesting a detoxification mechanism reducing the concentration (bioavailability) of the original compound and consequently leading to lower trypanocidal activity. Compound **8** being a hemiacetal could be hydrolyzed in aqueous medium leading to the generation of an intermediate with chemical characteristics similar to **4**, **5** and **7**. On the other hand, the most active compound, **6**, does not present such a leaving group, not suffering similar detoxification reaction and, due to its higher lipophylic character when compared with the other triazoles, could display a better penetration through the parasite's plasma membrane.

The activity of these triazoles against trypomastigote forms of *T. cruzi* is shown in Table 1. All the derivatives were more active than the original quinone, nor-lapachol, being the IC_{50}/1

Scheme 1. Synthesis of the naphthoquinoidal triazoles obtained through 1,3-dipolar reaction with the azide intermediate **3**.

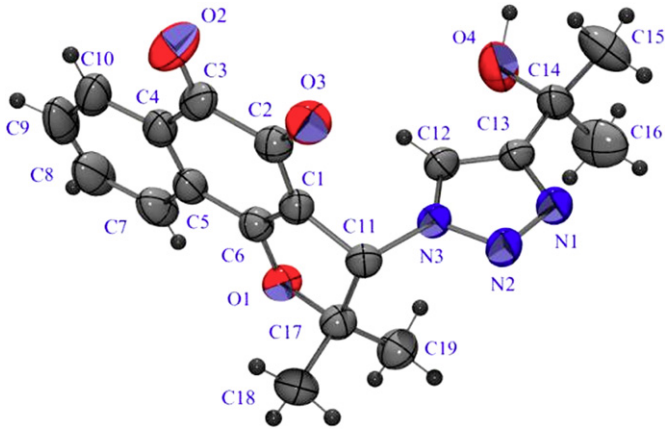


Fig. 1. Projection ORTEP-3 of triazole **4**.

day values in the range of 17–359 μM. Among them, the triazole **6** is the most active one. Statistical analysis showed the following order of decreasing activity ($p < 0.05$): **6** > **3** = **7** > **4** > **8** > **5**. In the same experimental condition, the IC_{50}/1 day value for crystal violet, the standard drug, is 536.0 ± 3.0 μM [8] and for benznidazole 103.6 ± 0.6.

Compounds **3** and **4**–**8** were synthesized and assayed against the infective bloodstream trypomastigote form of *T. cruzi*. All the derivatives were more active than the original quinone, with IC_{50}/1 day values in the range of 17–359 μM, showing that the triazolic nucleus acted in the increase of the biological activity, the apolar substituted triazole **6** being the most active. These triazole derivatives of nor-β-lapachone emerge as interesting new lead compounds in drug development for the treatment of Chagas disease.

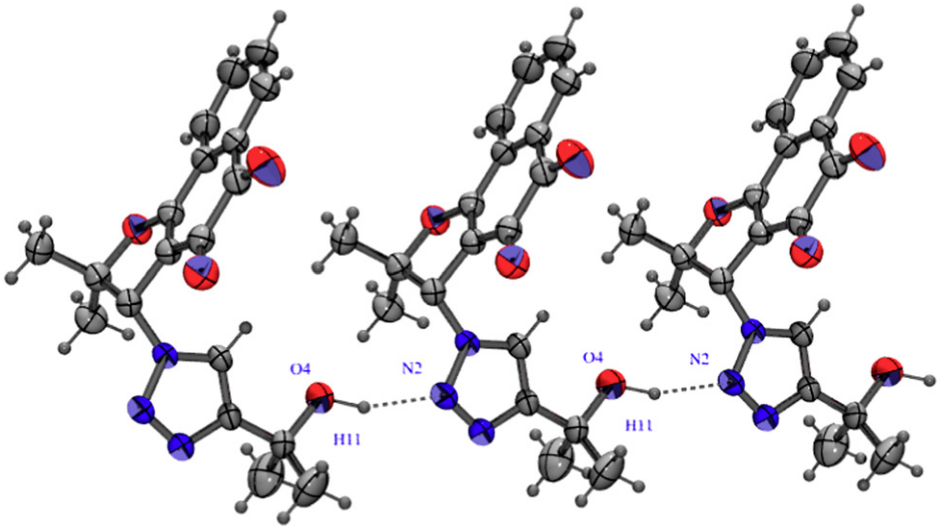


Fig. 2. Packing diagram showing the H-bonding.

4. Experimental protocols

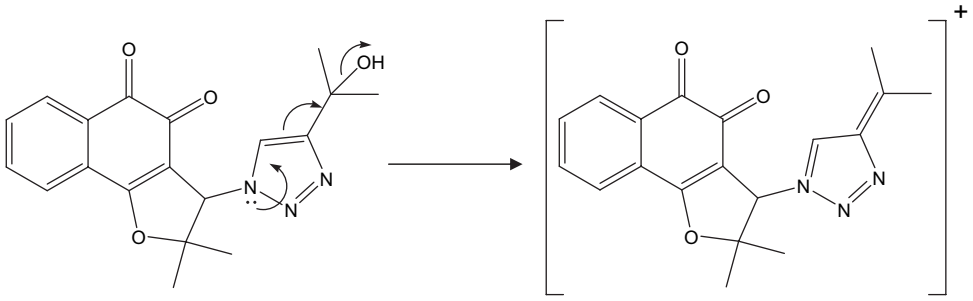
Melting points were determined on a Reichert micro hot stage and are uncorrected. Analytical grade solvents were used. The solvents were previously purified as described in the literature [32]. Column chromatography was performed on silica gel (Acros Organics 0.035–0.070 mm, pore diameter ca 6 nm). Infrared spectra were recorded on a Perkin–Elmer FT-IR Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature using a Varian Unity Plus 300 instrument, in the solvents indicated, with TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. High-resolution electron-impact mass spectra (70 eV) were obtained using a MAT8500 instrument. The main fragments were described as a relation between atomic mass units and the charge (m/z) and the relative abundance in percentage of the base peak intensity.

*4.1. Synthesis of 3-azido-2,2-dimethyl-2,3-dihydro-naphtho[1,2-b]furan-4,5-dione (**3**)*

A solution of **1** (228 mg, 1 mmol) in 25 ml of dichloromethane and 2 ml bromine (26 mg, 38 mmol) was stirred until an orange precipitate was formed. To this mixture, sodium azide (130 mg, 2 mmol) was added as a solid and stirred overnight. The azide-quinone was removed by filtration and dried at room temperature to produce **3** (269 mg, 1 mmol, 100% yield) as an orange solid (mp 200–202 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.14 (1H, ddd, $J = 6.9$, 2.1, 0.9), 7.72–7.65 (3H, m), 4.77 (1H, s), 1.67 (3H, s), 1.55 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 180.3 (C=O), 175.2 (C=O), 170.2 (C_0), 134.5 (CH), 132.7 (CH), 131.1 (C_0), 113.5 (C_0), 129.5 (CH), 125.1 (CH), 126.7 (C_0), 95.5 (C_0), 67.3 (CH), 27.1 (CH_3), 21.9 (CH_3). IR (film) ν_{max} 1655 (C=O), 1644 (C=O), 2109 (N_3) cm^{-1}. EI-HRMS (70 eV, m/z) 269.08000. Calcd for $C_{14}H_{11}O_3N_3$: 269.08004; (%) 227 (100), 199 (18), 104 (14), 173 (8), 76 (10), 42 (6), 50 (4), 269 (2), 181 (3), 157 (5), 128 (6).

*4.2. General procedure for the synthesis of **4** to **8***

Compound **3** (223.4 mg, 0.83 mmol) in 12 mL CH_2Cl_2/H_2O 1:1 is reacted with $CuSO_4 \cdot 5H_2O$ (9.3 mg, 0.04 mmol) and sodium ascorbate (22 mg, 0.11 mmol) and the desired substituted alkyne (see below). The mixture was maintained under agitation at room temperature till the total formation of the product, monitored by thin layer chromatography. The organic phase was extracted with dichloromethane, dried



Scheme 2. Mechanism of formation of cationic species in compound **4**.

Table 1
Activity of compounds **3**–**8** against trypomastigote forms of *T. cruzi*

Compound	IC_{50}/1 day (μM)
Nor-lapachol	1281.0 ± 167.0[a]
3	50.2 ± 3.8
4	151.9 ± 8.0
5	348.1 ± 44.2
6	17.3 ± 2.0
7	57.8 ± 5.6
8	256.7 ± 38.7
Crystal violet	536.0 ± 3.0[a]
Benznidazole	103.6 ± 0.6

[a] Ref. [8].

with $NaSO_4$ and concentrated under reduced pressure. The residue obtained was purified by column chromatography on silica gel using as eluent a gradient mixture of hexane/ethyl acetate with increasing polarity up to 100% ethyl acetate. The alkynes employed were the following: for **4**, 2-methyl-but-3-yn-2-ol; for **5**, prop-2-yn-1-ol; for **6**, ethynyl-benzene; for **7**, 1-ethynyl-cyclohexanol and for **8**, 2-prop-2-ynyloxy-tetrahydro-pyran.

4.2.1. Synthesis of 3-[4-(1-hydroxy-1-methyl-ethyl)-[1,2,3]triazol-1-yl]-2,2-dimethyl-2,3-dihydro-naphtho-[1,2-b]furan-4,5-dione (*4*)

Yield: 90%; orange solid; mp 220–222 °C. IR (KBr) ν_{max} 2926–2981 (CH_2, CH_3), 1656 (C=O), 1615 (C=O), 3423 (OH) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.95 (1H, s), 8.17 (1H, dd, $J = 6.6$, 2.1), 7.82–7.64 (3H, m), 1.75 (3H, s), 1.18 (3H, s), 7.4 (1H, s), 1.59 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 95.5 (C_0), 66.1 (CH), 111.3 (C_0), 174.2 (C=O), 180.0 (C=O), 132.1 (C_0), 134.7 (CH), 133.1 (CH), 128.8 (CH), 125.1 (CH), 126.8 (C_0), 170.0 (C_0), 27.1 (CH_3), 20.8 (CH_3), 121.0 (CH), 155.9 (C_0), 67.2 (C_0), 30.8 (CH_3), 30.7 (CH_3). EI-HRMS (70 eV, *m/z*) 353.13750. Calcd for $C_{19}H_{19}O_4N_3$: 353.13756; (%) 227 (100), 353 (12), 213 (6), 199 (18), 183 (2), 171 (7), 157 (5), 128 (7), 115 (4), 105 (3), 43 (5).

4.2.2. Synthesis of 3-(4-hydroxymethyl-[1,2,3]triazol-1-yl)-2,2-dimethyl-2,3-dihydro-naphtho[1,2-b]furan-4,5-dione (*5*)

Yield: 92%; yellow solid; mp 212–214 °C. IR (KBr) ν_{max} 2937–2987 (CH_2, CH_3), 1655 (C=O), 1614 (C=O), 3342 (OH) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.97 (1H, s), 8.19 (1H, ddd, $J = 6.4$, 2.4, 0.5), 7.81–7.73 (3H, m), 1.21 (3H, s), 1.76 (3H, s), 7.5 (1H, s), 4.77 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 95.3 (C_0), 66.1 (CH), 111.4 (C_0), 174.7 (C=O), 179.9 (C=O), 132.0 (C_0), 134.7 (CH), 133.1 (CH), 128.8 (CH), 125.1 (CH), 126.7 (C_0), 169.8 (C_0), 20.8 (CH_3), 27.1 (CH_3), 123.1 (CH), 148.1 (C_0), 55.2 (CH_2).

4.2.3. Synthesis of 2,2-dimethyl-3-(4-phenyl-[1,2,3]triazol-1-yl)-2,3-dihydro-naphtho[1,2-b]furan-4,5-dione (*6*)

Yield: 100%; yellow solid; mp 177–179 °C. IR (KBr) ν_{max} 2926–2980 (CH_2, CH_3), 1612 (C=O), 1650 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 6.02 (1H, s), 8.21 (1H, dd, $J = 6.8$, 2.4), 7.84–7.71 (3H, m), 1.25 (3H, s), 1.79 (3H, s), 7.30 (1H, s), 7.84–7.33 (5H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 95.3 (C_2), 66.3 (C_3), 111.1 (C_{3a}), 174.7 (C=O), 179.8 (C=O), 132.0 (C_{5a}), 130.6 ($C_{1'}$), 134.8 (CH), 133.2 (CH), 129.1 (CH), 125.2 (CH), 126.7 (C_{9a}), 170.0 (C_{9b}), 21.0 (C_{10} or C_{11}), 27.2 (C_{10} or C_{11}), 131.7 (C_5), 146.4 (C_4), 129.0 (CH), 128.8 (CH), 128.7 (CH), 128.1 (CH), 125.3 (CH).

4.2.4. Synthesis of 3-[4-(1-hydroxy-cyclohexyl)-[1,2,3]triazol-1-yl]-2,2-dimethyl-2,3-dihydro-naphtho-[1,2-b]furan-4,5-dione (*7*)

Yield: 90%; yellow solid; mp 186–188 °C. IR (KBr) ν_{max} 2854–2981 (CH_2, CH_3), 1662 (C=O), 1623 (C=O), 3372 (OH) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.95 (1H, s), 8.17 (1H, dd. $J = 6.6$, 2.2), 7.82–7.69 (3H, m), 1.17 (3H, s), 1.75 (3H, s), 7.40 (1H, s), 1.96–1.52 (10H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 95.5 (C_0), 66.0 (CH), 111.3 (C_0), 174.7 (C=O), 179.9 (C=O), 132.0 (C_0), 134.7 (CH), 133.1 (CH), 128.8 (CH), 125.1 (CH), 127.0 (C_0), 169.8 (C_0), 20.7 (CH_3), 27.1 (CH_3), 121.5 (CH), 155.5 (C_0), 68.0 (C_0), 37.9 (CH_2), 25.3 (CH_2), 21.9 (CH_2).

4.2.5. Synthesis of 2,2-dimethyl-3-[4-(tetrahydro-pyran-2-yloxymethyl)-[1,2,3]triazol-1-yl]-2,3-dihydro-naphtho-[1,2-b]furan-4,5-dione (*8*)

Yield: 90%; orange solid; mp 190–192 °C. IR (film) ν_{max} 2937–2985 (CH_2, CH_3), 1655 (C=O), 1698 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.97 (1H, s), 8.19 (1H, dd, $J = 6.1$, 1.9), 7.83–7.73 (3H, m), 1.20 (3H, s), 1.76 (3H, s), 7.48 (1H, s), 4.84 (1H, dd, $J = 12.7$, 5.4), 4.63 (1H, dd, $J = 12.7$, 3.6), 4.68 (1H, t, $J = 3.9$), 1.95–1.46 (8H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 95.1 (C_0), 66.1 (CH), 111.3 (C_0), 174.7 (C=O), 179.8 (C=O), 132.0 (C_0), 134.7 (CH), 133.0 (CH), 128.7 (CH), 125.0 (CH), 126.7 (C_0), 169.9 (C_0), 20.7 (CH_3), 27.0 (CH_3), 124.1 (CH), 97.2 (CH) 143.7 (C_0), 30.0 (CH_2), 24.9 (CH_2), 61.4 (CH_2), 61.3 (CH_2), 19.0 (CH_2).

4.3. X-Ray analysis

Crystallographic data for compound **4**: $C_{19}H_{18}N_3O_4$; $M = 704.74$; triclinic, space group *P*-1; $a = 7.1998(3)$ Å, $b = 9.5301(7)$ Å, $c = 13.5644(12)$ Å; $\alpha = 89.742(3)°$, $\beta = 89.350(5)°$, $\gamma = 71.586(4)°$; $V = 883.01(11)$ $Å^3$; $Z = 2$; $D_c = 1.325$ g cm^{-1}; λ (Mo K_α) = 0.71013 Å; $F(000) = 370$; $T = 293$ K; colorless sheet, size $0.15 \times 0.11 \times 0.09$ mm; 5185 measured reflections, refinement based on F^2 to give R_1 $[F^2 > 2\sigma(F^2)] = 0.070$ and $w_2 = 0.215$ for 3730 observed reflections, and 240 parameters. The Flack absolute structure parameter was determined to be 0.8(5) and the refinement of the opposite enantiomer resulted in a value of 1.32(2). Unfortunately, the weak distinguishing value of the Flack parameter (which should be 0.0 for the correct enantiomer) cannot be used to definitively assign the absolute stereochemistry. The positions of H atoms bonded to C were determined based on

stereochemical parameters and their displacement parameters calculated as $1.5U_{eq}$ (C-methyl) or $1.2U_{eq}$ (other).

X-ray data collection was accomplished on an Enraf-Nonius KappaCCD area-detector diffractometer. The programs used in the crystallographic study were: COLLECT [33] for data collection; HKL Denzo-Scalepack system [34] for integration and scaling of the reflections; SHELXS-97 [35] for solving the structure and SHELXL-97 [35] for refining by full-matrix least squares on F2. The programs SHELXL-97 [35] and ORTEP-3 [36] were used within WinGX [37] to prepare materials for publication. A complete set of data of compound **4** has been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 651356.

4.4. Assay for in vitro trypanocidal activity

Stock solutions of the compounds (**1**–**8**) were prepared in dimethylsulfoxide (DMSO), with the final concentration of the latter in the experiments never exceeding 0.1%. Preliminary experiments showed that at concentrations of up to 0.5%, DMSO has no deleterious effect on the parasites. Bloodstream trypomastigotes of the Y strain [38] were obtained at the peak of parasitaemia from infected albino mice, isolated by differential centrifugation and resuspended in Dulbecco's modified Eagle medium (DME) to a parasite concentration of 10^7 cells/ml in the presence of 10% of mouse blood. This suspension (100 μl) was added in the same volume of each compound previously prepared at twice the desired final concentrations. Cell counts were performed in Neubauer chamber and the trypanocidal activity was expressed as IC_{50}, corresponding to the concentration that leads to lysis of 50% of the parasites.

4.5. Statistical analysis

The comparison between the IC_{50} values for *T. cruzi* was performed by ANOVA followed by the Student–Newman–Keuls and Mann–Whitney tests ($p < 0.05$).

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Appendix. Supplementary data

The X-ray crystallographic data of compound **4** is available free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CH21EZ, UK (fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2007.10.015.

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