Bioorganic & Medicinal Chemistry 18 (2010) 3224–3230

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

The evaluation of quinonoid compounds against *Trypanosoma cruzi*: Synthesis of imidazolic anthraquinones, nor-β-lapachone derivatives and β-lapachone-based 1,2,3-triazoles

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ARTICLE INFO

Article history:
Received 7 January 2010
Revised 11 March 2010
Accepted 12 March 2010
Available online 16 March 2010

Keywords:
Naphthoquinones
Nor-β-lapachone
Chagas' disease
Trypanosoma cruzi
Experimental chemotherapy

ABSTRACT

In continuing our screening program of naphthoquinone activity against *Trypanosoma cruzi*, the aetiological agent of Chagas' disease, new β-lapachone-based 1,2,3-triazoles, 3-arylamino-nor-β-lapachones, 3-alkoxy-nor-β-lapachones and imidazole anthraquinones were synthesised and evaluated against bloodstream trypomastigote forms of the parasite. Compounds 2,2-dimethyl-3-(2,4-dibromophenylamino)-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione, IC_{50}/24 h 24.9 ± 7.4 and 4-azido-3-bromo-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione with 23.4 ± 3.8 μM showed a trypanosomicidal activity higher than benznidazole. These results demonstrate the potential of naphthoquinone derivatives as novel structures for the development of alternative drugs for Chagas' disease.

1. Introduction

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, remains a major health problem in Latin America. The transmission of this disease occurs mostly by vector, but it can also be transmitted by blood transfusion and through congenital pathways.[1] Rarer instances of transmission occur by laboratory accidents,[2] organ transplantations[3,4] and the ingestion of contaminated food with infected triatomines or their dejection.[5,6] Recently, Chagas' disease has also been recognised as an opportunistic disease in HIV-infected individuals.[7]

Introduced in the 1960s and 1970s, nifurtimox and benznidazole are the nitroderivatives currently available for the treatment of Chagas' disease. While these drugs are effective for acute infections, the data regarding their use and efficacy during the chronic phase are still controversial primarily due to the undesirable side effects and poor indices of apparent cure from the disease.[8] However, as also noted with other diseases, the development of new drugs for Chagas' disease is not of interest to pharmaceutical industries in developed countries due to a lack of potential profits.[9]

In *T. cruzi*, the presence of a single mitochondrion and a specialised region rich in DNA named the kinetoplast,[10] make this organelle an extraordinary drug target.[11] In addition, the *T. cruzi* mitochondrion is known to be deficient in reactive oxygen, making it especially sensitive to oxidative stress conditions.[12] In this context, since the microbicidal mechanism of quinones is mainly related to the generation of reactive oxygen species,[13] these molecules are interesting for medicinal chemistry studies.[14] Among the bioactive quinones, lapachol (**1**) and β-lapachone (**14**) originally isolated from the heartwood of trees of the Bignoniaceae family (*Tabebuia* sp.)[15] were selected as starting points for the screening of trypanosomicidal drugs[16] because they are easily accessible in Brazilian flora and facile synthetic routes have been previously developed when exploring the reactivity of 1,2-quinoidal carbonyls.[17]

Taking in account that molecular hybridisation[18] is a powerful approach for the design of new compounds, quinone derivatives

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doi:10.1016/j.bmc.2010.03.029

were prepared by reaction of naphthoquinones with [1,2,3]-triazoles or arylamines and evaluated as potential anti-*T. cruzi* compounds. Recently, several triazole derivatives (nor-β-lapachone-based 1,2,3-triazoles) were prepared from nor-lapachol (**2**), and the most active was 2,2-dimethyl-3-(4-phenyl-1,2,3-triazol-1-yl)-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione,[19] with a phenyl group attached to the triazolic ring, which, due to its higher lipophylic character, could allow better penetration through the parasite's plasma membrane as reported.[19] The screening of a series of *ortho*-naphthodihydrofuranquinones obtained from the reaction of 3-bromo-nor-β-lapachone (**3**) with substituted arylamines revealed two derivatives with a high activity on trypomastigote forms better than that of benznidazole.[20] Such hybrid molecules obtained from quinones and triazoles with redox properties are an interesting prospect for a medicinal chemistry program directed toward the chemotherapy of Chagas'.

Continuing our studies on the chemical reactivity of quinones from the Brazilian flora, we now focus on the search of new compounds with trypanosomicidal activity. We synthesised and characterised six novel derivatives (**16–21**) of lapachol (**1**) and two imidazolic anthraquinones (**25** and **27**), that together with 13 other compounds previously synthesised (**4–13**, **22**, **24** and **26**) were assayed for their activity against infective bloodstream forms of *T. cruzi* and cytotoxicity mammalian cells.

2. Results and discussion

2.1. Chemistry

Lapachol (**1**) (2-hydroxy-3-(3′-methyl-2′-butenyl)-1,4-naphthoquinone) was extracted from the heartwood of *Tabebuia* sp. (*Tecoma*) and purified by a series of recrystallisations. From this naphthoquinone, nor-lapachol (**2**) (2-hydroxy-3-(2′-methyl-propenyl)-1,4-naphthoquinone) was obtained by Hooker oxidation method,[21] in which the presence of molecular bromine undergoes cyclisation and produces in situ 3-bromo-nor-β-lapachone (**3**). This bromo derivative by reaction with arylamines led to the synthesis of arylamino-nor-β-lapachones **4–10**, as previously reported.[22,23] When compound **3** is submitted to nucleophilic substitution with alcohols, alkoxy-nor-β-lapachones **11–13** were formed[24] (Scheme 1). Only three 3-alkoxy nor-β-lapachones were obtained due to difficulty in removal of the solvent and reagents after the end of the reaction under our laboratory conditions. The reactions using alcohols with high boiling points failed due to the difficulty of product isolation.

The β-lapachone-based 1,2,3-triazoles **18–21**, described herein for the first time, were obtained from β-lapachone (**14**), synthesised by cyclisation of lapachol (**1**) in the presence of H_2SO_4. Substance **14** reacted with N-bromosuccinimide (NBS) in CCl_4 with benzoyl peroxide as initiator to produce 3,4-dibromo-β-lapachone (**15**).[24] Compound **15** was stirred overnight with sodium azide in dichloromethane and **16** (3-bromo-4-azide-α-lapachone) and **17** (3-bromo-4-azide-β-lapachone) were obtained. A hypothesis that can be considered for the formation of the compounds **16** and **17** is that after the formation of the compound **15** (3,4-dibromo-β-lapachone) may occur the exit of the bromine of the position 3, by anchimeric assistance of the oxygen in position 1, resulting in an unstable pyran based-oxygen carbenium ion that suffers nucleophilic attack through its less hindered side and the stereochemistry *trans* was observed for both compounds **16** and **17**. The formation of compound **16** occurs due to isomerisation of 3-bromo-4-azide-β-lapachone (**17**). After separating substances **16** and **17** by column chromatography, compound **17** was submitted to azide-alkyne Huisgen's cycloaddition, producing the new 1,2,3-triazolic compounds **18–21**. This reaction catalysed by Cu(I), which is known in the literature as 'click chemistry'.[25] The compounds **18–21** were obtained in racemic form, but the stereochemistry *trans* were defined by X-ray crystallography.

Other types of naphthoquinones were included for trypanosomicidal activity screening. The first substance was derived from the reaction of naphthoquinones with lead dioxide in acetic acid, a type of reaction first described by Hooker in 1936.[26] Using lapachol (**1**), we obtained the corresponding alkoxyquinone **22** (Scheme 1), which was purified by recrystallisation in ethanol, since the use of column chromatography on silica gel led to degradation of this compound.[27]

The second series are the anthraquinone imidazoles **24–27** described here for the first time, except for compound **24** and **26**.[28] All are easily obtained from the reaction of 1,2-diaminoanthraquinone (**23**) and substituted aldehydes (Scheme 2).

The structures of the compounds **16–21** and **25–27** were determined by ^1H and ^{13}C NMR and IR by comparing their spectroscopic data with those of similar compounds already reported[29] and together with high-resolution (electrospray ionisation) mass spectra. For the novel compounds, β-lapachone-based 1,2,3-triazoles, the chemical shift of the hydrogens of the pyranic ring appears in the same region (δ 5.00–6.00). In general, the signals of the hydrogens of the triazole ring appear in the aromatic region (δ 8.00) and the others signals corresponding to the substitution pattern observed for each substance.

The structures of compounds **16** and **17** were reconfirmed by X-ray crystallography data studies (Fig. 1). The ring *trans*-substitution is clearly observed in both cases.

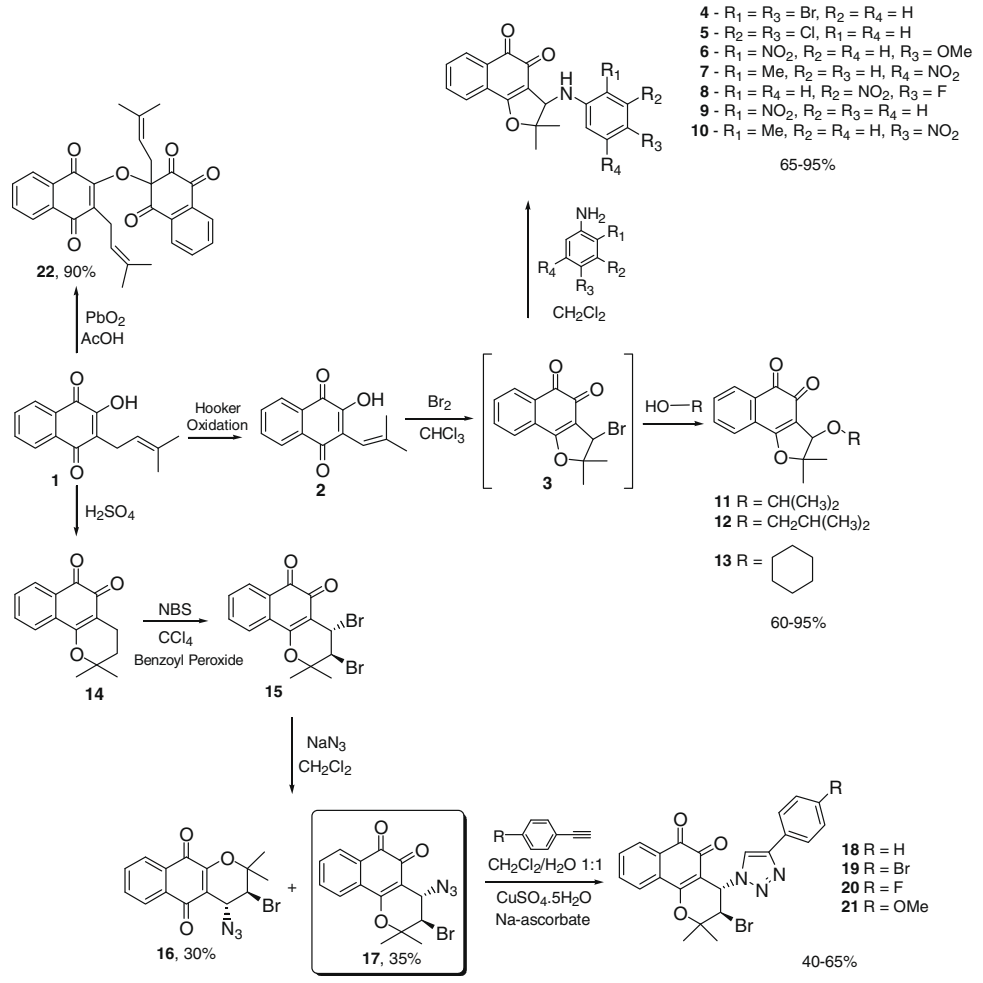
2.2. X-ray analysis

The molecular dimensions in each of the two structures are essentially in agreement with expected values, in particular, those found among the pyranic rings reported in the Cambridge Structural Database,[30] with exception of the compound **17** in which one of the methyl groups is disordered over two sets of sites C13 and C13′ with refined occupancies of 0.843(2) and 0.157(3). However hydrogen atoms of this methyl group do no participate in intermolecular hydrogen bond in the packing. The pyranic ring in the structure of **16** adopts a twisted boat conformation. The structure of **17** also adopts a half boat conformation, as assessed by the ring puckering $q2 = 0.769(1)$, $q3 = -0.037(1)$, $Q = 0.769(1)$ Å, $\theta = 92.72(9)$, $\varphi = 209.76°$ for **16** and $q2 = 1.10(3)$, $q3 = -0.47(4)$, $Q = 1.20(3)$ Å, $\theta = 113.0(2)$, $\varphi = 0.0(2)°$ for **17**.[31] The molecular packing involves weak intermolecular hydrogen contacts in both structures: Compound **16** presents one weak interaction between C6–H6···O1^i where C6–H6 = 0.930 Å; H6···O1^i = 2.58(3) Å and C6–H6···O1^i = 139° [$i = -x + 1, -y, -z$]. Compound **17** presents two weak interaction between C2–H2···O2^i where C2–H2 = 0.980 Å; H2···O2^i = 2.42(1) Å; C2–H2···O2^i = 143° [$i = -x - 1/2, -y + 1/2, z + 1/2$] and C2–H2···O3^i where C2–H2 = 0.980 Å; H2···O3^i = 2.55(3) Å; C2–H2···O3^i = 137° [$i = -x - 1/2, -y + 1/2, z + 1/2$].

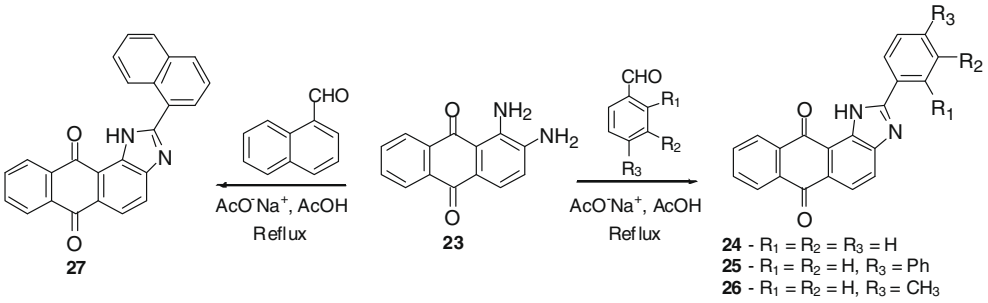
2.3. Biological assays

We recently reported the synthesis of a series of 3-arylamino-nor-β-lapachones substituted by arylamine moiety containing electron withdrawing and electron donating groups[20,22] and these compounds were evaluated against *T. cruzi* trypomastigotes. Other compounds we recently reported[19] were the nor-β-lapachone-based 1,2,3-triazoles with potent trypanosomicidal and antitumor activities.

In this paper, the anti-*T. cruzi* activity of the β-lapachone-based 1,2,3-triazoles (**18–21**), 3-alkoxy-nor-β-lapachones (**11–13**) and 3-arylamino-nor-β-lapachones (**4–10**) with other types of arylamino rings, obtained from modification of the C ring (Scheme 3, Table 1), are described.



Scheme 1. Synthetic route for the preparation of **4–10**, **11–13**, **16–21** and **22**.



Scheme 2. Synthetic route for the preparation of the imidazolic anthraquinones **24–27**.

The arylamino rings of **4–10** were substituted by electron withdrawing (–Br, –Cl, $-NO_2$ and –F) or electron donating groups (–OMe, –Me), as well as the aromatic triazoles (–Br, –F and –OMe).

Important trypanosomicidal compounds and structural features were defined from present studies with comparisons of previously reported data,[19a,20] which include: (i) the difference of the activity observed between lapachol (**1**) and nor-lapachol (**2**) is reproduced in the case of β-lapachone (**14**) and nor-β-lapachone (**28**); and (ii) the 3-substituted arylaminonaphthoquinones provided four derivatives in structural classes [**4** (24.9 ± 7.4 μM), **5** (43.8 ± 4.2 μM), **6** (59.6 ± 13.2 μM) and **9** (55.6 ± 4.6 μM)] with activity between about 1.7 and 4.2 higher than that of the standard drug, benznidazole (103.6 ± 0.6 μM).[19a] In this class of compounds, the position and nature of the substituents play important roles, particularly for halogens in the arylamino ring. In the bromo derivatives [*m*-Br (140.8 ± 11.9 μM); *p*-Br (952.5 ± 71.1 μM)],[20] the presence of the second bromine atom led to increase of the biological activity [2,4-Br, compound (**4**) (24.9 ± 7.4 μM)]; the same trend was observed for the chloro derivatives [*m*-Cl (332.8 ± 23.3 μM); *p*-Cl (384.4 ± 52.5 μM)][20] and 3,4-dichloro, compound (**5**) (43.8 ± 4.2 μM)]. The presence of the atom of fluor does not enhance the trypanosomicidal activity as previously shown [*m*-F (>4000 μM, *p*-F (2517.9 ± 169.8 μM)].[20] The substitution by nitro groups enhances the anti-*T. cruzi* effect, except in *p*-substitution [*o*-NO_2, compound **9**, (55.6 ± 4.6 μM); *m*-NO_2 (86.3 ± 4.6 μM),[20] *p*-NO_2 (857.3 ± 96.4 μM)[20]]. As a second substituent, in the case of

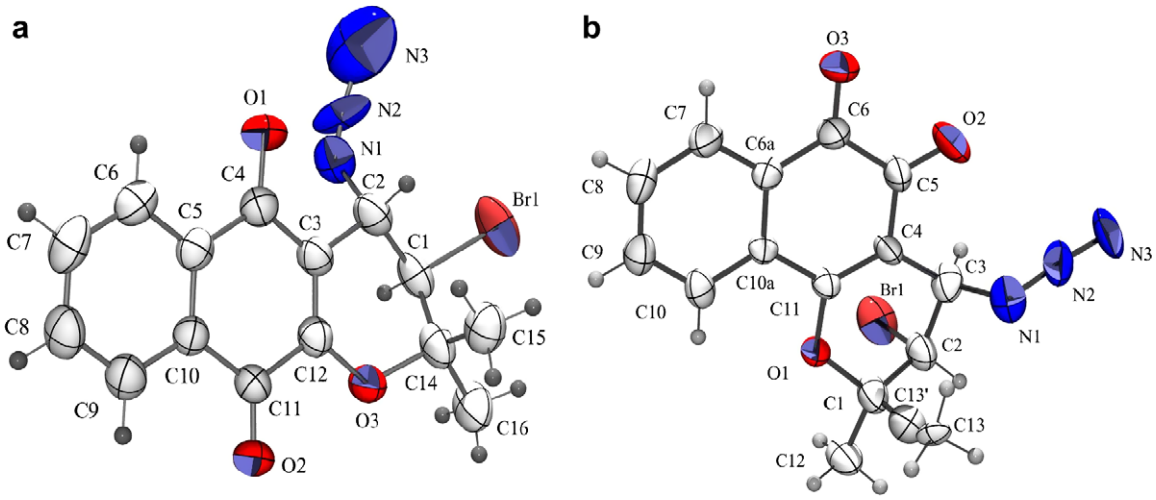
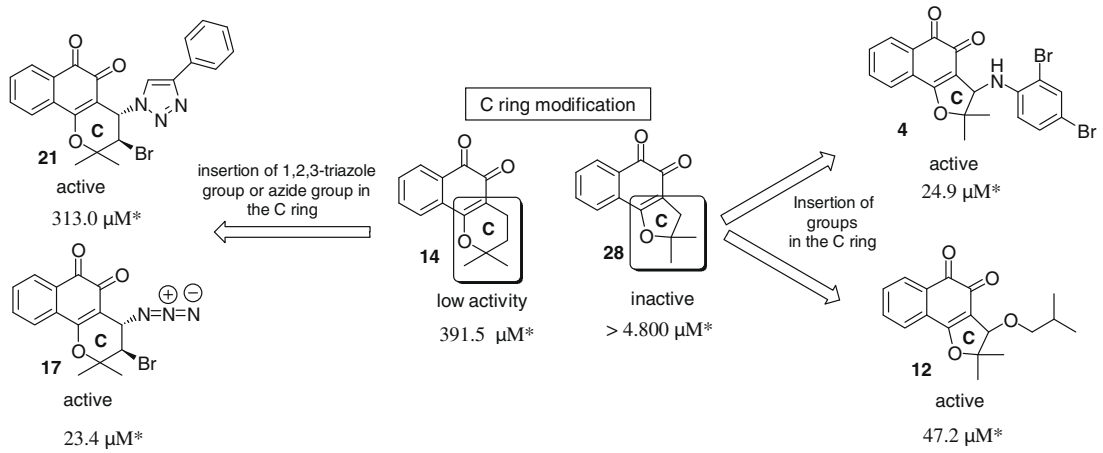


Figure 1. ORTEP3 projection of the compounds (a): **16** and (b): **17**, showing the atom numbering and 50% probability displacement ellipsoids.



Scheme 3. New compounds active against *T. cruzi*. *Mean values of IC_{50}/24 h (see Table 1).

methoxy derivative [*p*-OMe (88.2 ± 6.7 μM)],[20] the activity is considerably increased [compound **6** (59.6 ± 13.2 μM)]. The presence of a methyl group in position 2 of the arylamino ring stunts the activity in compound **7** (284.3 ± 60.9 μM); however, this methyl group is highly favourable in compound **10** (156.2 ± 9.1 μM). Despite the impossibility of rationalisation, it seems that the substitution in position 2 is favourable.

Compound **12** (47.2 ± 4.1 μM), from the 3-substituted alkoxyquinones, is two times more active than benznidazole,[19a] indicating that the insertion of alkoxy groups intensifies the activity of nor-β-lapachone (**28**).

The key intermediate azide **17** used for the synthesis of β-lapachone-based 1,2,3-triazoles **18–21** displays an important activity against the parasite, with an IC_{50}/24 h of 23.4 ± 3.8 μM, about four times more active than the standard drug and seventeen times more active than its precursor, β-lapachone (**14**). Nor-β-lapachone azide-substituted was recently reported[19a] to have important activity with IC_{50}/24 h of 50.2 ± 3.8 μM, and this result highlights the importance of the azide-substituted lapachones. In comparison with β-lapachone (**14**), the substitution by a triazole ring appears to be a promising approach. β-Lapachone-based 1,2,3-triazole **18** with an IC_{50}/24 h value of 313.0 ± 26.4 μM is slightly more active than compound **14**. However, compound **21,** with IC_{50}/24 h of 106.1 ± 19.0 μM, presented a high lytic activity against *T. cruzi* that was 3 times more responsive than β-lapachone (**14**).

The anthraquinone imidazoles **24–27** were not active against *T. cruzi* trypomastigote forms. Previous studies have shown that *p*-naphthoquinones are not active compounds against *T. cruzi*. Based on previous results,[17] we insert the imidazolic nucleus in attempt to increase activity for *p*-naphthoquinones, but the obtained results were not promising.

The cytotoxicity of the compounds against mammalian cells (human peripheral blood mononuclear cells—PBMC) was also investigated, and the IC_{50} values obtained for the majority of the compounds were in the range of 2.1–10 μM, while for **7**, **22**, **24–26** and benznidazole the values were higher than 12 μM.

3. Conclusions

To obtain trypanosomicidal compounds, we synthesised and evaluated the activity of nor-β-lapachones 3-arylamino and 3-alkoxy substituted, imidazolic anthraquinones and β-lapachone-based 1,2,3-triazoles. In comparison with previous studies, we concluded that in a nor-β-lapachones-3-arylamino-substituted compound, the insertion of chlorine, bromine, nitro and methoxy groups in the arylamino ring intensifies the activity, while fluorine is harmful for trypanosomicidal activity. By the Alamar blue test, it was observed that after 72 h of treatment, the IC_{50} values were lower than those obtained for the trypanosomicidal activity, monitored after 24 h. This result points to toxicity of the compounds for

Table 1
Activity of napththoquinones and precursors on trypomastigote forms of *T. cruzi*

Compounds	IC_{50}/24 h[a] (μM)
4	24.9 ± 7.4
5	43.8 ± 4.2
6	59.6 ± 13.2
7	284.3 ± 60.9
8	526.2 ± 80.5
9	55.6 ± 4.6
10	156.2 ± 9.1
11	268.3 ± 29.2
12	47.2 ± 4.1
13	558.5 ± 77.1
16	248.3 ± 29.1
17	23.4 ± 3.8
18	313.0 ± 26.4
19	439.6 ± 31.6
20	219.8 ± 27.2
21	106.1 ± 19.0
22	233.3 ± 29.2
24	>2000
25	>4000
26	>4000
27	>4000
Lapachol (**1**)[b]	410.8 ± 53.5
Nor-lapachol (**2**)[b]	1280.6 ± 167.2
β-Lapachone (**14**)[b]	391.5 ± 16.5
Nor-β-lapachone (**28**)[b]	>4800
Benznidazole[c]	103.6 ± 0.6

[a] Mean ± SD of at least three independent experiments.
[b] Ref. 16a.
[c] Ref. 19a.

PBMC, however since compounds **4** and **17** were about 4 times more active than benznidazole, they will be further investigated in relation to the in vivo toxicity monitoring hepatic, renal and cardiac muscle marker. The compounds, 3-bromo-4-azide-β-lapachone (**17**) and β-lapachone-based 1,2,3-triazoles were recognised as important prototypes and can aid in the development of new anti-*T. cruzi* agents.

4. Experimental protocols

4.1. General procedures

Melting points were determined in a capillary Thomas Hoover apparatus (Thomas Co., Philadelphia, PA, USA) and are uncorrected. Analytical grade solvents were used. 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 (Perkin–Elmer Inc., Wellesley, MA, USA). ^{1}H and ^{13}C NMR were recorded at room temperature using a Varian Mercury Plus 300 instrument (Varian, Palo Alto, CA, USA) in the solvents indicated with TMS as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) are reported in Hertz. High-resolution mass spectra (electrospray ionisation) were obtained using a MicroTOF Ic—Bruker Daltonics. (Bruker–Franzen Analytic, Bremen, Germany). Elemental analysis was performed in a Perkin–Elmer CHN 2400 analyser (Perkin–Elmer Inc., Wellesley, MA, USA).

The compounds **4–10**, **11–13**, **22**, **24** and **26** were synthesised as previously described.[22,27,28b]

4.2. Synthesis of 4-azido-3-bromo-2,2-dimethyl-3,4-dihydro-2*H*-benzo[g]chromene-5,10-dione (16) and 4-azido-3-bromo-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (17)

β-Lapachone (**14**) (242 mg, 1 mmol) in carbon tetrachloride (80 mL) was reacted with NBS (352 mg, 2 mmol) and catalytic amounts of benzoyl peroxide. The reaction mixture was gently heated to near vaporisation and the product formed, **15**, was observed by TLC. Compound **15** was treated with an excess of sodium azide (2 mmol, 130 mg) at room temperature. The reaction system was stirred for 24 h, filtered and the compounds **16** and **17** were isolated by column chromatography over silica gel, using an eluent as a gradient mixture of hexane/ethyl acetate with increasing polarity. Compound **16** was isolated as an orange solid (108 mg, 0.3 mmol, 30% yield, mp 163–164).

IR ν_{max} (cm^{-1}, KBr): 1682 (C=O), 1652 (C=O), 2105 (N_3); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.18–8.11 (2H, m), 7.82–7.70 (2H, m), 5.02 (1H, d, J = 5.1 Hz), 4.15 (1H, d, J = 5.1 Hz), 1.65 (3H, s), 1.64 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 183.3 (C=O), 178.9 (C=O), 153.4 (C_0), 134.4 (CH), 133.5 (CH), 131.6 (C_0), 130.7 (C_0), 126.5 (CH), 126.3 (CH), 116.1 (C_0), 80.7 (C_0), 58.8 (CH), 54.0 (CH), 25.4 (CH_3), 25.0 (CH_3). EI-HRMS (m/z) [M+Na]$^+$ 383.9953. Calcd for $[C_{15}H_{12}BrN_3O_3Na]^+$: 383.9959.

Compound **17** was isolated as an orange solid (126 mg, 0.3 mmol, 35% yield, mp 186–187).

IR ν_{max} (cm^{-1}, KBr): 1654 (C=O), 1702 (C=O), 2107 (N_3); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.14 (1H, dd, J = 7.5; 1.2 Hz), 7.88 (1H, dd, J = 7.8; 1.2 Hz), 7.72 (1H, td, J = 7.5; 1.4 Hz), 7.26 (1H, td, J = 7.5; 1.2 Hz), 4.96 (1H, d, J = 5.1 Hz), 4.17 (1H, d, J = 5.1 Hz), 1.71 (3H, s), 1.67 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.6 (C=O), 178.3 (C=O), 162.2, 135.4, 132.3, 131.0, 130.6, 129.3, 125.4, 110.3, 81.9, 59.2, 54.3. EI-HRMS (m/z) [M+Na]$^+$ 383.9946. Calcd for $[C_{15}H_{12}BrN_3O_3Na]^+$: 383.9959.

4.3. General procedure for the synthesis of the β-lapachone-based 1,2,3-triazoles 18–21

$CuSO_4{\cdot}5H_2O$ (9.3 mg, 0.04 mmol), sodium ascorbate (22 mg, 0.11 mmol) and the desired substituted phenylacetylene (see below) were added to a solution of **17** (299 mg, 0.83 mmol) in 12 mL CH_2Cl_2/H_2O (1:1). The mixture was stirred at room temperature for 12 h and was monitored by TLC. The organic phase was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel, using an eluent as a gradient mixture of hexane/ethyl acetate with increasing polarity. The alkynes used were the following: to synthesise **18,** phenylacetylene; for **19**, 1-bromo-4-ethynylbenzene; for **20**, 1-ethynyl-4-fluorobenzene and for **21**, 1-ethynyl-4-methoxybenzene.

4.3.1. 3-Bromo-2,2-dimethyl-4-(4-phenyl-1,2,3-triazol-1-yl)-3,4-dihydro-2*H*-benzo[*h*] chromene-5,6-dione (18)

Using phenylacetylene (84 mg, 0.83 mmol), 18 was obtained as an orange solid (300 mg, 0.6 mmol, 65% yield, mp 184–185 °C).

IR ν_{max} (cm^{-1}, KBr): 1700 (C=O), 1652 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.13–8.08 (2H, m), 8.09 (1H, s), 7.95–7.92 (1H, m), 7.87–7.82 (1H, m), 7.79–7.71 (1H, m), 7.68–7.59 (1H, m), 7.45–7.39 (2H, m), 7.35–7.29 (1H, m), 5.72 (1H, d, J = 8.8 Hz), 5.07 (1H, d, J = 8.8 Hz), 1.78 (3H, s), 1.69 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.8 (C=O), 176.5 (C=O), 162.7 (C_0), 146.7 (C_0), 135.1 (CH), 132.2 (CH), 130.6 (C_0), 130.4 (C_0), 129.1 (CH), 128.6 (CH), 128.0 (CH), 125.7 (CH), 125.2 (CH), 122.7 (CH), 110.3 (C_0), 83.4 (C_0), 58.9 (CH), 54.3 (CH), 24.4 (CH_3), 20.7 (CH_3). EI-HRMS (m/z) [M+Na]$^+$ 486.0404. Calcd for $[C_{23}H_{18}BrN_3O_3Na]^+$: 486.0429.

4.3.2. 3-Bromo-4-[4-(4-bromo-phenyl)-1,2,3-triazol-1-yl]-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (19)

Using 1-bromo-4-ethynylbenzene (148 mg, 0.83 mmol), **19** was obtained as an orange solid (280 mg, 0.5 mmol, 52% yield, mp 185–186 °C).

IR ν_{max} (cm^{-1}, KBr): 1701 (C=O), 1647 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.13–8.09 (1H, m), 8.09 (1H, s), 7.95–7.88

(1H, m), 7.78–7.76 (3H, m), 7.66–7.59 (1H, m), 7.56–7.50 (2H, m), 5.70 (1H, d, J = 9.0 Hz), 5.04 (1H, d, J = 9.0 Hz), 1.79 (3H, s), 1.68 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.8 (C=O), 176.5 (C=O), 162.7 (C_0), 145.7 (C_0), 135.2 (CH), 132.3 (CH), 131.8 (C_0), 130.6 (C_0), 130.4 (C_0), 129.3 (C_0), 129.2 (CH), 127.3 (CH), 125.3 (CH), 122.9 (CH), 121.9 (C_0), 110.2 (C_0), 83.5 (C_0), 59.1 (CH), 54.2 (CH), 27.4 (CH_3), 20.6 (CH_3). EI-HRMS (m/z) [M+Na]$^+$ 563.9538. Calcd for $[C_{23}H_{17}Br_2N_3O_3Na]^+$: 563.9534.

4.3.3. 3-Bromo-4-[4-(4-fluoro-phenyl)-1,2,3-triazol-1-yl]-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (20)

Using 1-ethynyl-4-fluorobenzene (99 mg, 0.83 mmol), **20** was obtained as an orange solid (192 mg, 0.4 mmol, 40% yield, mp 182–183 °C).

IR ν_{max} (cm^{-1}, KBr): 1701 (C=O), 1652 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.14–8.09 (1H, m), 8.09 (1H, s), 7.95–7.92 (1H, m), 7.84–7.71 (3H, m), 7.68–7.60 (1H, m), 7.15–7.06 (2H, m), 5.70 (1H, d, J = 9.0 Hz), 5.05 (1H, d, J = 9.0 Hz), 1.79 (3H, s), 1.69 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.8 (C=O), 176.5 (C=O), 162.8 (C_0), 145.9 (C_0), 133.2 (CH), 132.3 (CH), 130.6 (C_0), 130.4 (C_0), 129.1 (CH), 127.6 (CH), 127.5 (CH), 126.7 (C_0, J = 3.3 Hz), 125.3 (CH), 122.6 (CH), 115.8 (CH), 115.5 (CH), 110.3 (C_0), 83.5 (C_0), 59.0 (CH), 54.3 (CH), 27.4 (CH_3), 20.7 (CH_3). EI-HRMS (m/z) [M+Na]$^+$ 504.0324. Calcd for $[C_{23}H_{17}BrFN_3O_3Na]^+$: 504.0335.

4.3.4. 3-Bromo-4-[4-(4-methoxy-phenyl)-1,2,3-triazol-1-yl]-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (21)

Using 1-ethynyl-4-methoxybenzene (109 mg, 0.83 mmol), **21** was obtained as an orange solid (271 mg, 0.5 mmol, 55% yield, mp 187–188 °C).

IR ν_{max} (cm^{-1}, KBr): 1707 (C=O), 1654 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10–8.06 (1H, m), 8.01 (1H, s), 7.94–7.88 (1H, m), 7.79–7.69 (3H, m), 7.65–7.58 (1H, m), 6.97–6.90 (2H, m), 5.71 (1H, d, J = 8.8 Hz), 5.04 (1H, d, J = 8.8 Hz), 3.83, (3H, s), 1.76 (3H, s), 1.68 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 177.9 (C=O), 176.5 (C=O), 162.7 (C_0), 159.5 (C_0), 146.6 (C_0), 135.2 (CH), 132.3 (CH), 130.6 (C_0), 130.5 (C_0), 129.1 (CH), 127.1 (CH), 125.2 (CH), 125.1 (CH), 123.1 (C_0), 122.0 (CH), 114.1 (CH), 110.4 (C_0), 83.4 (C_0), 58.9 (CH), 55.2 (CH_3), 54.3 (CH), 27.4 (CH_3), 20.8 (CH_3). EI-HRMS (m/z) [M+Na]$^+$ 516.0553. Calcd for $[C_{24}H_{20}BrN_3O_4Na]^+$: 516.0535.

4.4. General procedure for the synthesis of the antraquinone imidazoles 25 and 27

A solution of 1,2-diamino-anthraquinone (**23**) (238 mg, 1 mmol) in 5 mL acetic acid, sodium acetate (106 mg, 1,3 mmol) and the desired aldehyde (see below) were stirred in reflux. TLC was used to monitor the end of the reaction. After the addition of water, the formed precipitate was filtered, and the product was purified by column chromatography using an eluent as a mixture of dichloromethane/ethyl acetate with the ratio 5:1.

4.4.1. 2-Biphenyl-4-yl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (25)

Using 4-phenylbenzaldehyde (200 mg, 1.1 mmol), **25** was obtained as a yellow solid (196 mg, 0.49 mmol, 49% yield, mp 269–270 °C).

IR ν_{max} (cm^{-1}, KBr): 3334, 1664, 1645, 1609, 1562, 1540, 1476, 1437, 1420, 1327, 1290, 1214, 1199, 1153, 1060, 1008, 843, 772, 738, 717, 702, 667, 651, 596, 509. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 13.10 (1H, s), 8.50 (2H, d), 8.16–8.22 (2H, m), 8.03–8.10 (2H, q), 7.75–7.92 (6H, m), 7.38–7.52 (3H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 183.9, 183.0, 158.1, 150.1, 143.1, 139.9, 135.1, 135.0, 133.9, 133.7, 133.7, 129.8, 129.5, 128.8, 128.6, 127.6, 127.5, 126.9, 125.6, 121.7, 119.4. Anal. Calcd for $C_{27}H_{16}N_2O_2$: C. 80,99; H. 4,03; N. 7,00. Found: C, 81.05; H, 4.09; N, 7.08.

4.4.2. 2-Naphthalen-1-yl-1H-anthra[1,2-d]imidazole-6,11-dione (27)

Using 1-naphthaldehyde (172 mg, 1,1 mmol), **27** was obtained as a yellow solid (190 mg, 0.51 mmol, 51% yield, mp 263–267 °C).

IR ν_{max} (cm^{-1}, KBr): 3600, 3487, 3443, 3420, 3060, 3010, 2923, 2853, 1670, 1606, 1583, 1519, 1504, 1488, 1475, 1438, 1329, 1294, 1214, 1182, 1154, 1063, 1003, 855, 804, 772, 715, 563, 488. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.86 (1H, d, J = 8.23 Hz), 8.37 (1H, dd, J = 7.17, 1.49 Hz), 8.30 (1H, d, J = 8.38 Hz), 8.29–8.22 (2H, m) 8.08–7.93 (3H, m), 7.87–7.75 (2H, m), 7.69–7.55 (3H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 182.9, 182.5, 157.7, 134.3, 134.1, 133.2, 133.0, 130.7, 129.6, 128.3, 128.1, 127.1, 126.6, 126.1, 125.7, 124.9. Anal. Calcd for $C_{25}H_{14}N_2O_2$: C, 80.20; H, 3.77; N, 7.48. Found: C, 80.25; H, 3.81; N, 7.52.

4.5. Trypanosomicidal activity

Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO) with the final concentrations in the experiments never exceeding 0.1%. Preliminary experiments showed that at concentrations up to 0.5% DMSO had no deleterious effect on the parasites. Bloodstream trypomastigotes from the Y strain[32] were obtained at the peak of parasitemia from infected albino mice, isolated by differential centrifugation and resuspended in Dulbecco's modified Eagle's medium (DMEM) to a parasite concentration of 10^7 cells mL^{-1} in the presence of 10% of mouse blood. This suspension (100 μL) was added in the same volume to each compound previously prepared at twice the desired final concentrations. Cell counts were performed in Neubauer chamber, and the trypanosomicidal activity was expressed as IC_{50}, corresponding to the concentration that leads to lysis of 50% of the parasites.

4.6. Inhibition of PBMC proliferation

To investigate the selectivity of the compounds toward a normal proliferating cell, the Alamar blue assay was performed using peripheral blood mononuclear cells (PBMC).[22] After 24 h of plating, the compounds were added to each well and incubated for 72 h. Doxorubicin was used as positive control. Twenty-four hours before the end of the incubation, 10 μL Alamar Blue (Resazurin, Sigma–Aldrich Co) (0.312 mg/mL) was added to each well. The absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter®) and the drug effect was quantified as the percentage of control absorbance at 570 nm and 595 nm. The percent of reduced Alamar Blue is expressed as ALW—(AHW × R0) × 100. R0 is the correction factor obtained by the ratio AOLW/AOHW, corresponding AOLW to the difference between the absorbance of Alamar Blue in culture medium and that of the medium alone at the lower wavelength and AOHW to the value obtained at the higher wavelength.

4.7. X-ray crystallographic analysis

X-ray data collection for compounds **16** and **17** was accomplished on a Smart APEXII diffractometer and Enraf–Nonius KappaCCD area-detector diffractometer, respectively. Data collection strategy and frames integration were conducted using SAINT PLUS and COLLECT program for the crystallographic study.[33] Integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs.[34] The structures of **16** and **17** were resolved by direct methods with SHELXS-97.[35] The models were refined by full-matrix least squares on F^2 with SHELXL-97.[35] The programs SHELXL-97 and ORTEP-3[36] were used within WinGX[37] to

prepare materials for publication. Crystallographic data for compound **16** and **17** have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 754510 and 754041, respectively. Copies of the data can be obtained free of charge by application to CCDC, 12 Union Road, Cambridge CH21EZ, UK (fax: +44 1223 336 033 or e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgments

This research was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), FIOCRUZ, Fundação Carlos Chagas Filho de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), CAPES, UFRJ, UFAL, USP, UFC and UnB. The authors are also indebted to the PRONEX-FAPERJ and to PRONEX-CNPq-FAPEAL. Dr. Eufrânio N. da Silva Júnior thanks the CNPq for post doctoral grant (No. 151558/2009-4).

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