European Journal of Medicinal Chemistry 60 (2013) 51–56

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Synthesis and biological activity against *Trypanosoma cruzi* of substituted 1,4-naphthoquinones

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ARTICLE INFO

*Article history:*
Received 2 July 2012
Received in revised form
16 November 2012
Accepted 22 November 2012
Available online 1 December 2012

*Keywords:*
*Trypanosoma cruzi*
Naphthoquinones
Chagas disease
Alkylation reaction
Lapachol
β-Lapachone
Lawsone

ABSTRACT

The discovery and development of essential drugs for Chagas disease is a major concern worldwide. New substituted 1,4-naphthoquinones were synthesized and tested against the infective bloodstream form of *Trypanosoma cruzi*, the etiological agent of Chagas disease. These products exhibited substantial activity against *T. cruzi*, especially 2-((8E,11Z)-heptadeca-8,11-dienyl)-3-hydroxynaphthalene-1,4-dione (**9**) with $IC_{50}$ of 7.8 μM.



## 1. Introduction

Chagas disease is a long-term debilitating disease caused by the flagellate protozoan *Trypanosoma cruzi*, which is mainly transmitted by triatomine insects or by blood *transfusion* [1]. The usual mode of transmission of *T. cruzi* to humans is through the bug bite on an exposed area of skin. Alternative modes of infection include blood transfusion, congenital transmission from infected mothers and the ingestion of contaminated foods [2].

The life cycle of *T. cruzi* involves a haematophagous triatomine insect, a vertebrate host, and different parasitic forms. Briefly, a bloodstream trypomastigote ingested by the insect differentiates into an epimastigote, which proliferates and, in the posterior intestine, differentiates into the metacyclic form. This infective form invades the vertebrate cell and undergoes differentiation into an intracellular amastigote, which proliferates and then transforms into a trypomastigote, the *T. cruzi* form that causes the infection [3].

Chagas disease has two successive phases: acute and chronic. The acute phase lasts six to eight weeks. Once the acute phase subsides, most infected patients recover an apparently healthy state, in which no organ damage can be detected using the current standard methods of clinical diagnosis [4].

An estimated 10 million people are infected worldwide, mostly in Latin America [5]. After one hundred years of discovering this chronic disease, there is still no effective drug treatment. The therapeutic agents available are benznidazole (Rochagan® or Radanil®) and Nifurtimox (Lampit®), which present severe side effects and they are not effective for chronic phase patients [6,7].

Naphthoquinones are aromatic cyclic α,β-dienones with a basic skeleton derived from naphthalene. They are found in various vegetative parts of higher plants, algae, fungi and, as a product of the metabolism of some bacteria, possessing broad biological activities such as antibacterial, anti-inflammatory, antitumor, anticancer; and trypanocidal [8–10]. Naphthoquinone derivatives are also widespread in nature and their biological and pharmacological properties are of great interest such as the alkyl-1,4-

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0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.11.034

naphthoquinones that comprise a class of compounds that show important biological activity [11,12].

Lapachol (**1**) and β-lapachone (**2**) [13], (Fig. 1) are very well known biologically active naphthoquinones. The pharmacological potential of **1** is explained by its varied biological activity such as antimicrobial and antifungal [14], cercaricida, molluscicide, anti-leishmanial [15,16], trypanocidal [17–19], antimalarial [20], anti-viral [21] and anti-inflammatory [22].

The potential use of β-lapachone and semi-synthetic derivatives has already been investigated as a chemotherapeutic agent against Chagas disease [9]. In the present article, we prepared ten substituted naphthoquinones from different starting materials (Table 1) and assayed their activity against *T. cruzi*.

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of the substituted 1,4-naphthoquinones was made by using as starting materials: 1,4-naphthoquinone (**3**), 2-methyl-1,4-naphthoquinone (menadione) (**4**) and, 2-hydroxy-1,4-naphthoquinone (lawsone) (**5**) (Table 1). Benli Liu et al. [23] reported the synthesis of vitamin-K derivatives with different side-chain lengths using radical alkylation reaction. A radical is generated by decarboxylation of carboxylic acids with silver ions and ammonium persulfate. However, the insertion of the long chain alkenyl functionality is a general synthetic problem [24].

The carboxylic functional group allows the introduction of different chains in order to obtain substituted 1,4-naphthoquinones. Following the mentioned methodology [23], compounds **3**, **4** and **5** were treated with carboxylic acids in the presence of $AgNO_3$ and $(NH_4)_2S_2O_8$ to give the naphthoquinones shown in Table 1. Eight new naphthoquinones were synthesized and assayed against the infective bloodstream form of *T. cruzi*. The structures of the synthesized compounds were unequivocally determined by spectroscopic techniques, such as $^1H$ and $^{13}C$ NMR, infrared spectroscopy, MS (EI), HRMS (ESI), and elemental analysis.

Naphthoquinones **8**–**11** and **12**–**15** are described for the first time and, although the synthesis of **6** and **7** has been reported [24], we found no results about their biological activity against *T. cruzi*.

The influence of the large carboxylic acid chains on the yield of quinones derivatives previously reported [23] is in accordance with our low yields results for compounds **6**–**11**, resulting from the reaction with oleic and linoleic acids (Table 1, Entries 1–3 and 4–6). It is also known that this type of reaction starting with lawsona (**5**) usually gives unsatisfactory yields [25] and sometimes, no product is formed (Table 1, Entries 7, 10 and 13). This was also the result for alkylation of menadione (**4**) with octanoic acid (Table 1, Entry 8).

Treatment of 1,4-naphthoquinone (**3**) with octanoic acid and 5-phenylpentanoic, under the same conditions, led to the formation of the dialkylated products **12** and **13** (Table 1, Entry 9 and 12). As for the reaction of 3-(4-methoxyphenyl) propanoic acid with **3**–**4**, compounds **14** and **15** were obtained as a racemic mixture (Table 1, Entry 14 and 15), probably via a reaction mechanism going through a secondary radical intermediate.

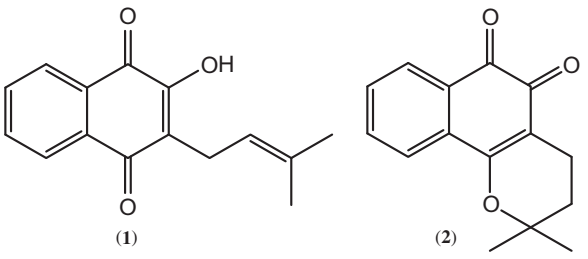



**Fig. 1.** Chemical structure of lapachol (**1**) e β-lapachone (**2**).

### 2.2. Biological activity

Compounds **6**-**15** were tested against bloodstream forms of *T. cruzi* and only three of them showed low activity, when compared with positive control crystal violet (Table 2). Compound **9**, demonstrated the best trypanocidal activity with $IC_{50}$ of 7.8 μM.

A comparison of the $IC_{50}$ values for compounds **9**–**11** indicates the importance of substitution the pattern at C-2 of the naphthoquinone. The insertion of electron-withdrawing groups possibly increases the redox potential of the naphthoquinoidal structure, leading to an important biological activity [26]. In most cases, this activity is related to the ability of quinones to accept one and/or two electrons to form the corresponding radical anion or dianion species as well as the acid–base properties of the compounds [27]. The exchange of hydrogen in the compound **11** by the methyl (**10**) or hydroxyl (**9**) group in the C-2 position increases the activity from $IC_{50}$ of 26.4 μM, 16.9 μM–7.8 μM, respectively. Similarly, the same effect can be observed with compounds **15** and **14**, where trypanocidal activity significantly increases from $IC_{50}$ of 48.5 μM–17.4 μM, respectively. Notably, in the case of compounds **6**–**8** this effect is reversed, since compound **8**, with no substitution at C-2, was the most active. On the other hand, compounds **6**–**8** only differ from compounds **9**–**11** on the number of double bonds at the carbon side chain; it is also observed that the greater unsaturation degree decreases the trypanocidal activity of compound **11** compared to the compound **8**. Oppositely, diunsaturated compounds **9** and **10** have better trypanocidal activity than monounsaturated **6** and **7**.

Considering compounds with attached hydroxyl group, we observed that the prepared compounds **6** ($IC_{50}$ 10.6 μM) and **9** ($IC_{50}$ 7.8 μM), are remarkably more active (Table 2) than known compounds such as lawsone (**5**) with $IC_{50} > 2500$ μM and lapachol (**1**) with $IC_{50}$ of 410.8 (Table 3), suggesting that the presence and the size of alkyl chain, increases the activity against *T. cruzi*.

Recently, it was reported that the attachment of a 1,2,3-triazole group in the 1,4-naphthoquinoidal structure (compound **16**, Table 3) led to an enhancement of its pharmacological activity showing an $IC_{50}$ of 10.9 while benznidazole presents $IC_{50}$ of 103.6 [26]. If compared to the synthesized compounds **8**, **11** and **15**, that are substituted in only one position, the triazole **16** is two and four times more active than **11** and **15** respectively, however, it is less active than **8**.

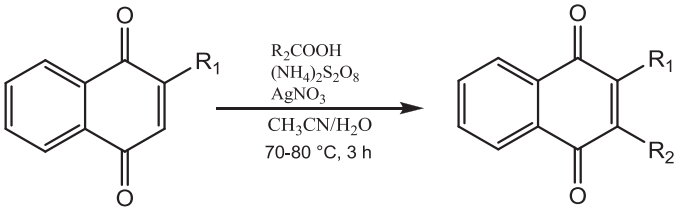
Another interesting result was related to the activity of compounds **12** and **13**, derived from dialkylation of 1,4-naphthoquinone, that showed $IC_{50}$ of 123.7 μM and 25.1 μM, respectively. Compound **12** is the least active of the entire series but **13** is more active than **12** because it bears aromatic rings in its structure which could be related with hydrophobic properties.

The obtained results can be considered very significant and can lead to develop potential Chagas disease drug candidates. Compound **9** could be considered a good prototype after investigation of its cytotoxicity in mammalian cells.

## 3. Conclusions

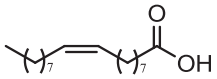
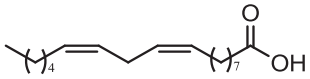
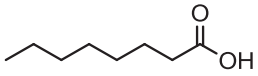
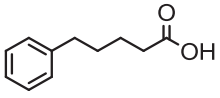
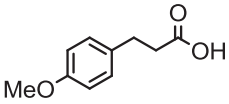
We prepared substituted 1,4-naphtoquinones and their trypanocidal activity was evaluated. Data of all activities revealed that compound **9** displayed the highest activity. Within the limits of the compounds synthesized and examined, the structure-activity might be related to the position of substitution, the functional groups and the degree of unsaturation of carbon side chain attached to 1,4-naphthoquinoidal structure. Therefore, the prepared compounds merit further research as potential drugs against Chagas disease.

**Table 1**
Synthesis of naphthoquinones.




$R_1$=H (**3**)
$R_1$=$CH_3$ (**4**)
$R_1$=OH (**5**)

**6**, $R_1$ = OH, $R_2$ = $CH_3$-$(CH_2)_7$-CH=CH-$(CH_2)_7$-
**7**, $R_1$ = $CH_3$, $R_2$ = $CH_3$-$(CH_2)_7$-CH=CH-$(CH_2)_7$-
**8**, $R_1$ = H, $R_2$ = $CH_3$-$(CH_2)_7$-CH=CH-$(CH_2)_7$-
**9**, $R_1$ = OH, $R_2$ = $CH_3$-$(CH_2)_4$-CH=CH-$CH_2$-CH=CH-$(CH_2)_7$-
**10**, $R_1$ = $CH_3$, $R_2$ = $CH_3$-$(CH_2)_4$-CH=CH-$CH_2$-CH=CH-$(CH_2)_7$-
**11**, $R_1$ = H, $R_2$ = $CH_3$-$(CH_2)_4$-CH=CH-$CH_2$-CH=CH-$(CH_2)_7$-
**12**, $R_1$ = $R_2$ = $CH_3$-$(CH_2)_6$-
**13**, $R_1$ = $R_2$ = $C_6H_5$-$(CH_2)_4$-
**14**, $R_1$ = $CH_3$, $R_2$ = (p-$CH_3OC_6H_4$)($CH_2CO_2H$)CH-
**15**, $R_1$ = H, $R_2$ = (p-$CH_3OC_6H_4$)($CH_2CO_2H$)CH-

| Entry | Naphthoquinone | Carboxylic acid | Product[a] | Yield. [%] |
|---|---|---|---|---|
| 1 | **5** ($R_1$ = OH) |  | 6 | 8 |
| 2 | **4** ($R_1$ = $CH_3$) | | 7 | 67 |
| 3 | **3** ($R_1$ = H) | | 8 | 40 |
| 4 | **5** ($R_1$ = OH) |  | 9 | 39 |
| 5 | **4** ($R_1$ = $CH_3$) | | 10 | 13 |
| 6 | **3** ($R_1$ = H) | | 11 | 20 |
| 7 | **5** ($R_1$ = OH) |  | n. r. | – |
| 8 | **4** ($R_1$ = $CH_3$) | | n. r. | – |
| 9 | **3** ($R_1$ = H) | | 12 | 40 |
| 10 | **5** ($R_1$ = OH) |  | n. r. | – |
| 11 | **4** ($R_1$ = $CH_3$) | | n. r. | – |
| 12 | **3** ($R_1$ = H) | | 13 | 41 |
| 13 | **5** ($R_1$ = OH) |  | n. r. | – |
| 14 | **4** ($R_1$ = $CH_3$) | | 14 | 52 |
| 15 | **3** ($R_1$ = H) | | 15 | 30 |

[a] n. r. = no reaction.

## 4. Experimental

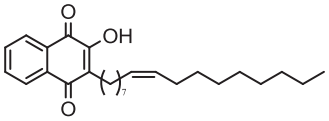
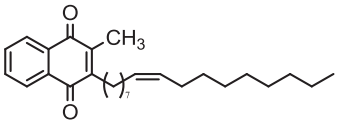
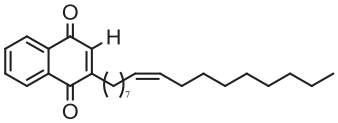
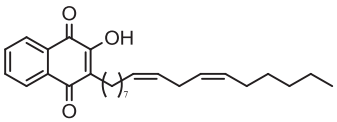
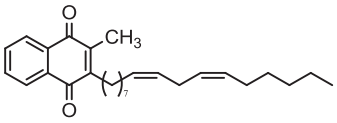
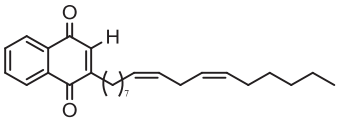
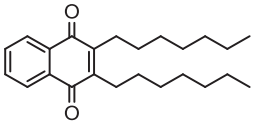
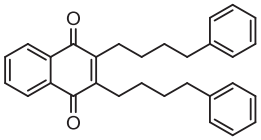
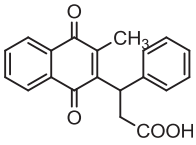
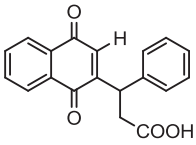
### 4.1. General

All the laboratory grade reagents were commercial. Purifications of compounds were made by column chromatography (CC) using Merck silica gel 60 (230–400 mesh) and a mixture of hexane and ethyl acetate was used for elution. All the reactions were monitored by TLC using Merck silica gel 60. $^1$H (300 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded with a Bruker DPX-300 spectrometer and, calibrated with residual non-deuterated solvent as an internal reference. Chemical shifts are reported in ppm and tetramethylsilane was used as the internal standard ($\delta$ = 0 ppm) and coupling constants ($J$) in Hertz. Mass spectra (EI, 70 eV) were run on a Shimadzu CGMS QP2010 Plus gas chromatography mass spectrometer. 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. Infrared spectra were recorded on a Bomen FT-IR-MB100 Spectrometer. HRMS and Elemental analysis results were obtained in the services of Central Analytical – Institute of Chemistry – USP.

### 4.2. General preparation of *6–15*

General procedure for the synthesis of substituted 1,4-naphthoquinones **6–15** 1,4-naphthoquinone (**3**), 2-hydroxy-1,4-naphthoquinone (**4**) and 2-methyl-1,4-naphthoquinone (**5**) was as described in literature [1–3]. A solution of $(NH4)_2S_2O_8$ (1,0 g) in $H_2O$ (10 mL) was added dropwise over 90–120 min to a stirred suspension of $H_2O$ (10 mL), $CH_3CN$ (20 mL), $AgNO_3$ (0.25 g), **2**, **4** and **5** (0.2 g)

**Table 2**
Activity of the compounds **6**–**15** against bloodstream trypomastigote forms of *T. cruzi*.

| Compound | | Concentration (μM) ×% lysis | | | | $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|
| | | 0.5 | 2.0 | 8.0 | 32.0 | |
| **6** |  | 13.1 ± 5.5 | 22.5 ± 7.6 | 51.3 ± 6.3 | 57.6 ± 3.9 | 10.6 |
| **7** |  | 27.2 ± 4.3 | 25.1 ± 1.9 | 35.1 ± 3.4 | 34.0 ± 4.2 | 32.6 |
| **8** |  | 37.2 ± 1.8 | 45.5 ± 4.6 | 46.1 ± 2.3 | 49.2 ± 4.1 | 8.1 |
| **9** |  | 44.5 ± 1.9 | 47.6 ± 2.3 | 42.9 ± 3.7 | 50.8 ± 1.0 | 7.8 |
| **10** |  | 15.2 ± 3.1 | 25.6 ± 1.0 | 47.1 ± 7.0 | 44.5 ± 1.9 | 16.9 |
| **11** |  | 0.0 | 7.8 ± 3.5 | 30.9 ± 5.4 | 49.2 ± 3.0 | 26.4 |
| **12** |  | 8.1 ± 0.8 | 8.9 ± 4.7 | 10.6 ± 3.5 | 18.3 ± 6.3 | 123.7 |
| **13** |  | 3.0 ± 1.6 | 21.5 ± 2.7 | 35.1 ± 2.3 | 44.0 ± 8.7 | 25.1 |
| **14** |  | 12.0 ± 3.3 | 24.6 ± 5.9 | 34.0 ± 6.3 | 55.0 ± 3.8 | 17.4 |
| **15** |  | 0.0 | 10.5 ± 4.1 | 14.1 ± 4.6 | 38.7 ± 5.7 | 48.5 |

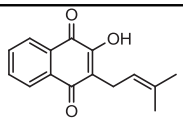
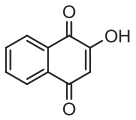
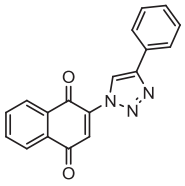
Positive control: crystal violet ($IC_{50}$ = 31 μM); negative control: DMSO.

and carboxylic acid (1.5 mmol) at 70–80 °C. After stirring for more than 20 min, the resulting mixture was cooled, extracted with ethyl acetate, the organic phase was washed with $NaHCO_3$, dried over $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure. The product was purified by flash chromatography.

*4.2.1. (E)-2-(Heptadec-8-enyl)-3-hydroxynaphthalene-1,4-dione (**6**)*

Compound **6** was obtained as yellow oil, 8% yield. IR (KBr, $cm^{-1}$): $\nu$ 3382, 2923, 2854, 1650, 725. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 0.84 (3H, t, $J$ = 6.0 Hz), 1.00–1.40 (22H, m), 1.98 (4H, m), 2.57 (2H, t, $J$ = 9.0 Hz), 5.31 (2H, t, $J$ = 6.0 Hz), 7.68 (2H, td, $J$ = 6.0 Hz e 3.0 Hz),

**Table 3**
Biological activity of lapachol (**1**), lawsone (**5**) and triazole (**16**) against bloodstream trypomastigote form of *T. cruzi*.

| Structures | | $IC_{50}$ (μM) | Ref. |
|---|---|---|---|
| (**1**) |  | 410.8 ± 53.5 | [28] |
| (**5**) |  | >2500 | [29] |
| (**16**) |  | 10.9 ± 1.8 | [26] |

8.06 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR(75 MHz, $CDCl_3$) $\delta$: 14.08 ($CH_3$), 22.65 ($CH_2$), 27.17 (2 $CH_2$), 28.28 ($CH_2$), 29.00–30.00 (9 $CH_2$), 31.88 ($CH_2$), 124.81 (C), 126.01 (CH), 126.74 (CH), 129.44 (C), 129.81 (CH), 129.89 (CH), 132.79 (CH), 132.94 (C), 134.77 (CH), 153.00 (C), 181.45 (C=O), 184.67 (C=O). MS (EI) $m/z$ (rel. int.,%): 188.0 (100) 384 (10); 410 (76) $[M]^{+\bullet}$.

*4.2.2. (E)-2-(Heptadec-8-enyl)-3-methylnaphthalene-1,4-dione (**7**)*

Compound **7** was obtained as yellow oil, 67% yield. IR (KBr, $cm^{-1}$): $\nu$ 2923, 2854, 1658, 717. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.83 (3H, t, $J$ = 6.0 Hz), 1.0–1.5 (22H, m), 1.97 (4H, m), 2.14 (3H, s), 2.58 (2H, t, $J$ = 9.0 Hz), 5.29 (4H, tl, $J$ = 6.0 Hz), 7.62 (2H, td, $J$ = 6.0 Hz e 3.0 Hz), 8.00 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 12.52 ($CH_3$), 14.03 ($CH_3$), 27.02 ($CH_2$), 27.09 ($CH_2$), 27.14 ($CH_2$), 28.68 ($CH_2$), 29.0–30.0 (9 $CH_2$), 31.84 ($CH_2$), 126.06 (CH), 126.16 (CH), 129.65 (CH), 129.89 (CH), 132.07 (C), 132.11 (C), 133.14 (CH), 133.17 (CH), 142.95 (C), 147.43 (C), 185.19 (C=O), 184.53 (C=O). MS (EI) $m/z$ (rel. int.,%): 187 (100), 408 (70) $[M]^{+\bullet}$.

*4.2.3. (E)-2-(Heptadec-8-enyl)naphthalene-1,4-dione (**8**)*

Compound **8** was obtained as yellow oil, 40% yield. IR (KBr, $cm^{-1}$): $\nu$ 2923, 2850, 1666, 721. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.84 (3H, t, $J$ = 6.0 Hz), 1.0–1.5 (24H, m), 1.55 (2H, m), 1.99 (4H, sl), 2.53 (2H, t, $J$ = 9.0 Hz), 5.31 (2H, tl, $J$ = 6.0 Hz), 6.76 (1H, s), 7.69 (2H, td, $J$ = 6.0 Hz e 3.0 Hz), 8.00 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.09 ($CH_3$), 22.65 ($CH_2$), 27.13 ($CH_2$), 27.18 ($CH_2$), 27.97 ($CH_2$), 29.11 ($CH_2$), 29.0–30.0 (8 $CH_2$), 31.87 ($CH_2$), 125.97 (CH), 126.55 (CH), 129.69 (CH), 129.98 (CH), 132.06 (C), 132.28 (C), 133.55 (CH), 133.57 (CH), 134.67 (CH), $\delta$ 151.94 (C), $\delta$ 185.22 (2C=O). MS (EI) $m/z$ (rel. int.,%): 173 (100), 368 (10), 394 (40) $[M]^{+\bullet}$. HRMS (ESI) Calcd for $[C_{27}H_{38}O_2 + Na]^+$: 417.2771, Found: 417.2770.

*4.2.4. 2-((8E,11Z)-Heptadeca-8,11-dienyl)-3-hydroxynaphthalene-1,4-dione (**9**)*

Compound **9** was obtained as yellow oil, 39% yield. IR (KBr, $cm^{-1}$): $\nu$ 3382, 2923, 2854, 1662, 1650, 725. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.85 (3H, t, $J$ = 6.0 Hz), 1.00–1.50 (16H, m), 1.98 (4H, m), 2.57 (2H, t, $J$ = 9.0 Hz), 2.74 (2H, m), 5.31 (4H, m), 7.68 (2H, td, $J$ = 6.0 Hz e 3.0 Hz), 8.10 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.08 ($CH_3$), 22.65 ($CH_2$), 23.33 ($CH_2$), 25.59 ($CH_2$), 27.17 (2$CH_2$), 28.26 ($CH_2$), 29.00–30.00 (5$CH_2$), 31.86 ($CH_2$), 124.81 (C), 126.01 (CH), 126.73 (CH), 127.90 (CH), 127.92 (CH), 129.81 (CH), 129.88 (CH), 130.10 (C), 132.81 (CH), 132.90 (C), 134.78 (CH), 153.01 (C), 181.45 (C=O), 184.70 (C=O). MS (EI) $m/z$ (rel. int.,%): 55 (100), 69 (60), 187 (60), 408 (15) $[M]^{+\bullet}$. Anal. Calcd for $C_{27}H_{36}O_3$: C, 79.37; H, 8.88; O, 11.75. Found: C, 79.56; H, 8.86.

*4.2.5. 2-((8E,11E)-Heptadeca-8,11-dienyl)-3-methylnaphthalene-1,4-dione (**10**)*

Compound **10** was obtained as yellow oil, 13% yield. IR (KBr, $cm^{-1}$): $\nu$ 2923, 2854, 1658, 717. $^1$H NMR(300 MHz, $CDCl_3$) $\delta$: 0.83 (3H, t, $J$ = 6.0 Hz), 1.00–1.50 (16H, m), 1.99 (4H, m), 2.14 (3H, s), 2.58 (2H, t, $J$ = 6.0 Hz), 2.73 (2H, m), 5.32 (4H, m), 7.63 (2H, td, $J$ = 6.0 Hz e 3.0 Hz), 8.00 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 12.50 ($CH_3$), 14.02 ($CH_3$), 22.48 ($CH_2$), 22.59 ($CH_2$), 25.53 ($CH_2$), 26.99 ($CH_2$), 27.10 (2$CH_2$), 28.00–30.00 (5 $CH_2$), 31.82 ($CH_2$), 126.04 (CH), 126.14 (CH), 127.81 (CH), 127.94 (CH), 129.92 (CH), 130.06 (CH), 132.05 (C), 132.09 (C), 133.14 (CH), 133.17 (CH), 142.94 (C), 147.40 (C), 184.51 (C=O), 185.17 (C=O). MS (EI) $m/z$ (rel. int.,%): 55 (35), 81 (19), 187 (100), 406 (15) $[M]^{+\bullet}$. Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; O, 7.87. Found: C, 82.79; H, 9.49.

*4.2.6. 2-((8E,11E)-Heptadeca-8,11-dienyl)naphthalene-1,4-dione (**11**)*

Compound **11** was obtained as yellow oil, 20% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.85 (3H, t, $J$ = 6.0 Hz), 1.00–1.50 (16H, m), 2.00 (2H, m), 2.54 (2H, m), 2.74 (2H, m), 5.33 (4H, m), 6.75 (1H, s), 7.65 (2H, td, $J$ = 6.0 Hz e 3.0 Hz), 8.00 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.03 ($CH_3$), 22.53 ($CH_2$), 25.57 ($CH_2$), 27.00 ($CH_2$), 27.14 (2$CH_2$), 27.95 ($CH_2$), 29.00–30.00 (5 $CH_2$), 31.85 ($CH_2$), 126.09 (CH), 126.51 (CH), 127.84 (CH), 127.97 (CH), 129.81 (C), 129.97 (CH), 130.12 (CH), 132.17 (C), 133.17 (CH), 133.50 (CH), 134.62 (CH), 147.10 (C), 185.06 (C=O), 185.14 (C=O). MS (EI) $m/z$ (rel. int.,%): 55 (84), 81 (49), 95 (35), 160 (26), 173 (100), 197 (30), 392 (10) $[M]^{+\bullet}$. Anal. Calcd for $C_{27}H_{36}O_2$: C, 82.61; H, 9.24; O, 8.15. Found: C, 82.89; H, 9.41.

*4.2.7. 2,3-Diheptylnaphthalene-1,4-dione (**12**)*

Compound **12** was obtained as yellow oil, 40% yield. IR (KBr, $cm^{-1}$): $\nu$ 2954, 2854, 1658, 721. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.84 (6H, t, $J$ = 6.0 Hz), 1.17–1.47 (20H, m), 2.55 (4H, t, $J$ = 6.0 Hz), 7.61 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz), 8.00 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.01 (2$CH_3$), 22.58 (2$CH_2$), 26.98 (2$CH_2$), 28.99 (2$CH_2$), 29.58 (2$CH_2$), 30.03 (2$CH_2$), 31.68 (2$CH_2$), 126.05 (2CH), 132.16 (2C), 133.12 (2CH), 147.11 (2C), 185.02 (2C=O). MS (EI) $m/z$ (rel. int.,%): 186 (39), 187 (78), 199 (33), 213 (13), 255 (47), 270 (16), 354 (100) $[M]^{+\bullet}$. Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67; O, 9.03. Found: C, 81.44; H, 9.71.

*4.2.8. 2,3-Bis(4-phenylbutyl)naphthalene-1,4-dione (**13**)*

Compound **14** was obtained as yellow oil, 41% yield. IR (KBr, $cm^{-1}$): $\nu$ 2931, 1658, 721. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 1.53 (4H, q, $J$ = 9.0 Hz e 6.0 Hz), 1.77 (4H, q, $J$ = 9.0 Hz e 6.0 Hz), 2.61–2.69 (8H, m), 7.17–7.32 (12H, m), 7.67 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz), 8.10 (2H, dd, $J$ = 6.0 Hz e 3.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 26,81 (2$CH_2$), 29,12 (2$CH_2$), 31,70 (2$CH_2$), 35,56 (2$CH_2$), 125,67 (2CH), 126,08 (2CH), 128,22 (4CH), 128,29 (4CH), 132,07 (2C), 133,20 (2CH), 142,09 (2C), 146,89 (2C), 184,93 (2C=O). MS (EI) $m/z$ (rel. int.,%): 91 (100), 131 (48), 173 (10) 187 (50), 289 (30), 422 (50) $[M]^{+\bullet}$. HRMS (ESI) Calcd for $[C_{30}H_{30}O_2 + Na]^+$: 445.2145, Found: 445.2142.

*4.2.9. 3-(4-Methoxyphenyl)-3-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl) propanoic acid (**14**)*

Compound **14** was obtained as yellow oil, 52% yield. IR (KBr, $cm^{-1}$): $\nu$ 2958, 2835, 1658, 1704. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 2.31 (3H, s), 3.24–3.46 (2H, dd, $J$ = 15.0 Hz e 6.0 Hz), 3.73 (3H, s), 4.72 (1H, tl, $J$ = 9.0 Hz), 6.80 (2H, d, $J$ = 9.0 Hz), 7.23 (2H, d, $J$ = 9.0 Hz), 7.61–7.64 (2H, m), 7.93–8.02 (2H, m). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 12.92 ($CH_3$), 37.15 ($CH_2$), 39.93 (CH), 55.16 ($CH_3$), 113.96 (2CH), 126.11 (CH), 126.35 (CH), 128.71 (2CH), 131.70 (C), 132.24 (2C),

133.32 (CH), 133.47 (CH), 144.79 (C), 146.67 (C), 158.30 (C), 177.82 (C=O), 184.69 (C=O), 185.33 (C=O). MS (EI) *m/z* (rel. int.,%): 135 (100), 304 (75), 320 (37), 335 (3), 350 (3) $[M]^{+\bullet}$. Anal. Calcd for $C_{21}H_{18}O_5$: C, 71.99; H, 5.18; O, 22.83. Found: C, 72.08; H, 5.09.

*4.2.10. 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-3-(4-methoxyphenyl) propanoic acid (**15**)*

Compound **15** was obtained as red oil, 30% yield. IR (KBr, $cm^{-1}$): $\nu$ 3421, 2927, 2838, 1662. $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 2.88–3.08 (2H, dd, $J$ = 15.0 Hz e 6.0 Hz), 3.72 (3H, s), 4.76 (1H, tl, $J$ = 6.0 Hz), 6.77 (1H, sl), 6.81 (2H, d, $J$ = 9.0 Hz), 7.20 (2H, d, $J$ = 9.0 Hz), 7.64–7.68 (2H, m), 7.97–8.01 (2H, m). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$: 39.21 (CH), 38.30 ($CH_2$), 55.16 ($CH_3$), 114.27 (2CH), 125.96 (CH), 126.80 (CH), 128.98 (2CH), 130.07 (CH), 131.37 (C), 131.72 (C), 132.12 (C), 133.71 (CH), 133.76 (CH), 152.03 (C), 158.76 (C), 176.45 (C=O), 184.04 (C=O), 185.12 (C=O). MS (EI) *m/z* (rel. int.,%): 247 (26), 261 (18), 277 (100), 290 (43), 318 (30), 336 (**8**) $[M]^{+\bullet}$. Anal. Calcd for $C_{20}H_{16}O_5$: C, 71.42; H, 4.79; O, 23.78. Found: C, 71.66; H, 4.86.

## 5. Trypanocidal assay

The bioassays were made using the blood of infected Swiss albino mice, which was collected by cardiac puncture at the peak of parasitemic infection (7th day of infection for Y strain). The infected blood was diluted with the blood of healthy mice to achieve a concentration of $10^6$ trypomastigote forms $ml^{-1}$. The compounds (**6**–**15**) solutions were prepared in dimethyl sulfoxide (DMSO) and were added into the infected mouse blood to provide concentrations of (0.5), (2.0), (8.0) and (32.0) μM, respectively. The plates were incubated at 4 °C for 24 h. Afterwards, the trypanocidal activity was evaluated by counting the trypomastigote forms of the remaining parasites, following the method described [30–32]. The bioassays were made in triplicate on microtiter plates (96 wells), which contained 200 μl of mixture per well. Negative and positive controls containing either DMSO or crystal violet at $IC_{50}$ 31 μM were run in parallel. The activities of the compounds were expressed as $IC_{50}$ values, corresponding to the concentration that causes lysis on 50% of the parasites.

## Acknowledgments

The authors would like to thank FUNDECT/MS, PROPP-UFMS and CNPq-Brasil, for providing financial support and infrastructure. We also owe a debt of gratitude to Dr. Roberto da Silva Gomes (UFMS) and Professor Paulo Olivato (USP) for the Elemental Analysis and Mass Spectroscopy experiments.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.11.034.

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