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Novel naphthoquinone derivatives and evaluation of their trypanocidal and leishmanicidal activities†

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Herein, we report the synthesis of 12 new naphthoquinone derivatives, 6 substituted 1,4-naphthoquinones and 6 heterocycle-fused naphthoquinones, as well as evaluation of their trypanocidal and leishmanicidal activities. Compounds **11a** and **13a** were active against the amastigote stage of *T. cruzi* and showed low cytotoxic effects. With respect to leishmanicidal assays, all compounds were inactive against the promastigote stages of *L. chagasi* and *L. braziliensis*.

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† Electronic supplementary information (ESI) available: Copies of ^{1}H, ^{13}C NMR spectra and HRMS spectra. See DOI: 10.1039/c4ob01869a

Dênis Pires de Lima obtained B. Sc. in Pharmacy at Federal University of Minas Gerais (UFMG) – Brazil (1986) and Ph.D. in Science, field of Organic Chemistry, at UFMG (1994). During the Ph.D. program, took two years of fellowship at the University of Alberta (Edmonton – Canada) in the Department of Chemistry . Post-Doctoral fellow at the University of Liverpool (1998) in the field of Biotransformation of Organic Compounds under the supervision of Stanley M. Roberts and Andrew Carnell. Presently working as Associate Professor IV and research advisor for master's and doctoral degree programs at the Institute of Chemistry at UFMS. Experienced mainly in the following subjects, related to organic synthesis: (i) synthesis of potential cytotoxic phenolic compounds (ii) synthesis of phenolic lipids and (iii) chemical transformations of bioactive natural products. Associate editor of the new peer-reviewed Electronic Journal of Chemistry (Orbital) of the Federal University of Mato Grosso do Sul and has been a reviewer of Química Nova (ISSN 0100-4042), Molecules (ISSN 1420-3049), European Journal of Medicinal Chemistry (ISSN 0223-5234), Bioorganic & Medicinal Chemistry (ISSN 09680896), Archiv der Pharmazie (ISSN 1521-4184), and Journal of the Brazilian Chemical Society (ISSN 0103-5053). Specialties: Organic Synthesis, Natural Products Transformation, Medicinal Chemistry.

Introduction

Chagas disease is a potentially fatal disease caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*),[1] and one of the most important endemic ones.[2] It is endemic in 21 countries of Latin America, and an estimate of 8 million people are infected all over the world.[1] Migration to developed countries has been a factor of dissemination of such illness and changing its epidemiological profile.[3,4] The therapy for Chagas disease has been considered unsatisfactory.[5] The treatment is applied during the acute phase of the disease for drastically reducing the parasitaemia, which may nearly result in a cure, but is controversial for applying in the chronic phase due to the lack of proof of efficiency in this phase.[6] Currently, benznidazole and nifurtimox – emerged in the late 1970s – are the only drugs used in the treatment of Chagas disease,[1] although they are associated with several side effects.[5,7]

The Leishmaniases are diseases associated with several clinical syndromes caused by protozoan parasites from *Leishmania* species[8,9] that are transmitted to humans and other vertebrates by the bite of the infected female phlebotomine sandflies Diptera (genera *Phlebotomus* and *Lutzomya*).[10,11] They are endemic in more than 98 countries, and close to 20 species of *Leishmania* may infect any species of vertebrate hosts.[9] There are three forms of Leishmaniasis (depending on the species involved in the infection): visceral,[12] cutaneous[13] and mucocutaneous.[10,14] The treatment of the disease varies depending on several factors, including type, species of the parasite and geographic localization.[14] Although there is a certain similarity among the different leishmaniases,[15] it is necessary to find the best, most effective and least toxic treatments for each situation.[16] The pentavalent antimonial salts, meglumine antimoniate and sodium stibogluconate, as well as miltefosine, paromomycin, amphotericin B and pentamidine are the available options.[8,16,17]

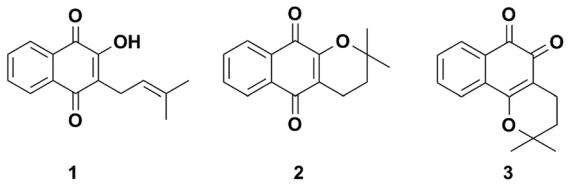


Fig. 1 Chemical structures of lapachol (**1**), α-lapachone (**2**) and β-lapachone (**3**).

The naphthoquinones are natural substances found in different families of plants, including *Bignoniaceae* and *Verbanaceae*.[18,19] These substances are aromatic cyclic α,β-dienones with a basic skeleton of naphthalene.[20] Lapachol (**1**), α-lapachone (**2**) and β-lapachone (**3**) (Fig. 1) are well known naphthoquinones in the medicinal chemistry studies.[18,21] Such compounds, besides being obtained from natural sources, can be easily synthesized,[21–23] and these qualities favor the synthesis of several other substances with potential pharmacological activities, including the development of drug candidates against neglected and other types of diseases.[10]

Taking into account that an important focus of our research group is to seek new drug candidates to treat neglected diseases and considering that naphthoquinones are reported to show antiprotozoal activity,[24] we have been preparing a series of derivatives of naphthoquinones.[20] Here, we report the results of the synthesis of 12 new compounds and their evaluation for trypanocidal and leishmanicidal activities.

Results and discussion

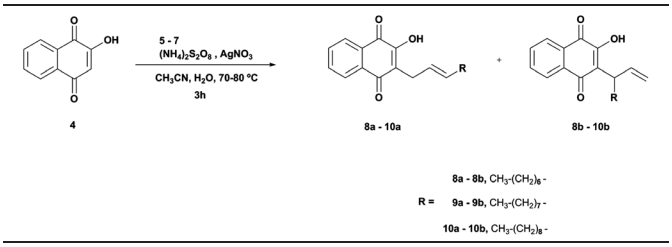
Chemistry

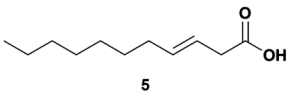
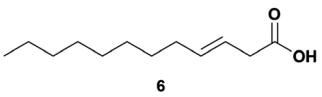
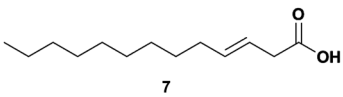
Synthesis of substituted 1,4-naphthoquinones. The alkylation of the quinones is possible due to the formation of radicals through the decarboxylation of carboxylic acids with ions of silver and ammonium persulfate.[25] This procedure enables the synthesis of several types of functionalized quinones.[26–28]

By performing the procedure described by Liu and coworkers,[27] we inserted different carbogenic chains using the β,γ unsaturated carboxylic acids (**5–7**) into the 2-hydroxy-1,4-naphthoquinone (lawsone, **4**), which resulted in the novel substituted 1,4-naphthoquinones (**8a–10a; 8b–10b**) (Table 1).

Synthesis of heterocyclic naphthoquinones. Lewis acids are widely used in organic synthesis due to their efficiency in forming carbon-heteroatom bonds.[30] Cyclization reactions have been developed using alkenyl derivatives of lawsone as the starting material in order to prepare six-member pyrane compounds. In this reaction, both *ortho*- and *para*-quinone are obtained.[31] Fu and coworkers[32] described the ability of indium bromide(III) ($InBr_3$) as a catalyst for the synthesis of α- and β-lapachones by the cyclization of lapachol. According to the authors, $InBr_3$ is a "green Lewis acid" that leads to excellent yields and may be reused without the loss of activity without causing damage to the environment. Thus, we applied this procedure[32] in the preparation of the new heterocyclic naphthoquinones, **11a–13a** and **11b–13b** (Table 2), from the intramolecular cyclization of the substituted 1,4-naphthoquinones **8a–10a**.

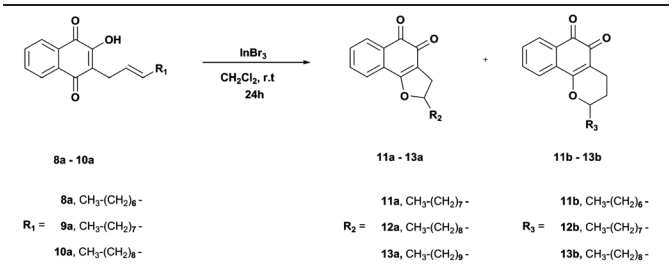
Table 1 Lawsone (**4**) derivatives[a]



Entry	Carboxylic acid	Compound	Yield (%)
1		**8a**	22
		8b	5
2		**9a**	20
		9b	6
3		**10a**	24
		10b	4

[a] The compounds **8a–10a** and **8b–10b** were obtained with low yields (Table 1). However, this was in some way expected, as the literature reports that the insertion of functionalized long-chain groups generally results in a synthetic problem.[20,27,29]

Table 2 Heterocycle-fused naphthoquinones



Entry	Substituted 1,4-naphthoquinones	Compound	Yield (%)
1	**8a**	**11a**	26
		11b	26
2	**9a**	**12a**	25
		12b	20
3	**10a**	**13a**	22
		13b	25

The structures of the synthesized compounds were confirmed *via* spectroscopic techniques such as ^1H, ^{13}C and two-dimensional NMR, infrared spectroscopy and high-resolution mass spectrometry.

Biological activity

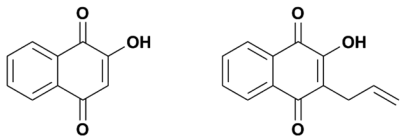
All the synthesized compounds were tested *in vitro* against the amastigote stage of *T. cruzi* and promastigote stages of

L. chagasi and *L. braziliensis*. The cytotoxicity assay was carried out under fibroblast LLCMK2 cells.

Trypanocidal activity

The trypanocidal activity of several lapachol and lapachone derivatives has been reported in literature.[2,18,20] The biological activity of the naphthoquinones is mainly related to the formation of semi-quinone radicals and reactive-oxygen species (ROS) responsible for the oxidative cellular stress.[33] According to Table 3, from the 12 compounds tested against the amastigote stage of *T. cruzi*, 6 resulted in parasite lysis, and the compound **11a** showed the best trypanocidal activity (IC_{50} 20.7 μM).

The increase in the linear unsaturated side chain may affect the biological activity, as can be verified by, although small, the activity of the compound **10a** compared to the inactivity of compounds **8a** and **9a**. Pinto and coworkers[34] found that lawsone (**4**) remained inactive in the trypanocidal activity in the trypomastigote stage when compared to its derivate, C-allyl-lawsone (**14**) (Fig. 2). This suggests that the addition of the aliphatic side chain increases the lipophilicity of the compound resulting in a greater trypanocidal activity, which might be associated with better penetration through the plasmatic membrane of the parasite.[18,34]



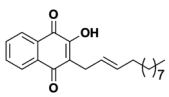
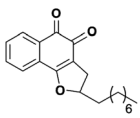
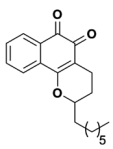
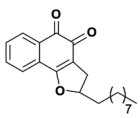
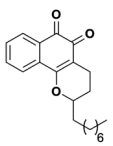
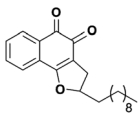
(**4**) (**14**)

ID_{50} > 2500 μM ID_{50} 330.7 ± 62.4 μM

Fig. 2 Comparison of the trypanocidal activity of the compounds tested by Pinto and coworkers.[34]

The trypanocidal activity of compound **10a** against the amastigote stage of *T. cruzi* may be considered unimportant when compared to that of **1**[34] and the substituted 1,4-naphthoquinones **15** and **16** reported in our previous work,[20] which showed excellent trypanocidal activities, reinforcing the

Table 3 Activity of the compounds against the amastigote stage of *T. cruzi*

Compound		Concentration (μM) × % lysis (±SD[b])				
		0.5	2.0	8.0	32.0	IC_{50} (μM)
	**10a**	0.7 ± 0.3	13.2 ± 2.4	16.7 ± 2.1	15.4 ± 5.0	7.7×10^3
	**11a**	10.4 ± 0.0	22.2 ± 2.4	27.4 ± 4.6	61.0 ± 3.8	20.7
	**11b**	10.4 ± 0.0	11.93 ± 0.6	17.6 ± 1.9	20.5 ± 3.6	21.7×10^3
	**12a**	0.3 ± 0.3	1.1 ± 0.1	10.2 ± 0.2	11.7 ± 0.8	1.36×10^3
	**12b**	1.0 ± 0.0	1.1 ± 0.1	1.1 ± 0.1	2.1 ± 1.1	1.06×10^{10}
	**13a**	12.8 ± 2.4	11.4 ± 0.9	26.4 ± 10.4	45.9 ± 3.2	46.8
Benznidazole[a]		1.0 ± 0.9	2.5 ± 3.0	26.4 ± 2.1	63.4 ± 9.0	19.93

[a] Reference drug. [b] Standard deviation.

Table 4 Comparison of the trypanocidal activity among the compounds **1**, **10a**, **15** and **16**

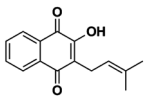
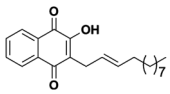
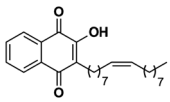
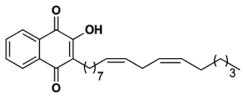
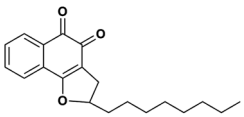
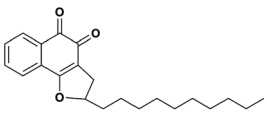
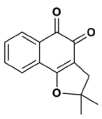
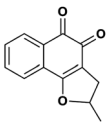
Compound		IC_{50} (μM)	Ref.
	**1**	410.8 ± 53.5	34
	**10a**	7.7×10^3	—
	**15**	10.6	20
	**16**	7.8	20

Table 5 Comparison of the trypanocidal activity among the compounds **11a**, **13a**, **17** and **18**

Compound		IC_{50} μM	Ref.
	**11a**	20.7	—
	**13a**	46.8	—
	**17**	>4800	34
	**18**	641 ± 38	36

structure–activity relationship data described in the literature (Table 4).[18,34]

The position and the number of unsaturation of the side chain may also affect the biological activity of the compounds.[20,35]

ortho-Quinones are known for having high trypanocidal activity when compared with *para*-quinone isomers.[18,36] β-lapachone (**3**) is the most studied and promising molecule of the lapachol group.[10] Nor-β-lapachone (**17**) (IC_{50} > 4800 μM) remained inactive compared to **3** (IC_{50} 391.5 ± 16.5 μM) when tested against the trypomastigote stage of *T. cruzi*.[34] The compounds **11a** and **13a** (Table 3) were the most effective as trypanocidal agents among the examined compounds, when compared to **17** [34] and 2-Methyl-2,3-dihydronaphtho[1,2-*b*]-furan-4,5-dione (**18**),[36] showing that the size of the side chain favored the trypanocidal activity (Table 5).

Compound **11a** showed similar activity to the reference drug (Table 3), suggesting that it is a promising trypanocidal agent. Therefore, studies related to toxicity were also carried out in order to compare with benznidazole (Table 6).

Table 6 Cytotoxic effects of the compounds against LLCMK2 cells[a]

Compound	IC_{50} (μM)	CC_{50} (μM)	SI
10a	7.7×10^3	39.89	5.2×10^{-3}
11a	20.7	2.2×10^3	106.3
11b	21.7×10^3	1.8×10^3	8.3×10^2
12a	1.36×10^3	2.5×10^3	1.83
12b	1.06×10^{10}	443.5	4.2×10^{-8}
13a	46.8	1.7×10^3	36.32
Benznidazole	19.93	323.8	16.8

[a] SI = CC_{50}/IC_{50}.

Cytotoxicity assays

All compounds were tested using fibroblast LLCMK2 cells, and the results of CC_{50} are presented in Table 6, including the SI value.

As observed in Table 6, the most active compounds (**11a** and **13a**) assayed against *T. cruzi* showed low cytotoxic effects against fibroblast LLCMK2 cells with a high value of SI. This fact confirms the trypanocidal potential of the compounds **11a** and **13a** and that both compounds are interesting for new evaluations, mainly for the *in vivo* model of Chagas disease.

Leishmanicidal activity

Parasites of the genus *Leishmania* have their specific metabolic features, which may result in differences in activity among the compounds.[37] All compounds showed to be inactive against the promastigote stages of *L. chagasi* and *L. braziliensis*.

In the evaluation against *L. braziliensis*, compounds **11a**, **11b** and **12b** promoted parasite lysis, but the leishmanicidal activity of such compounds were not significant, considering the high IC_{50} obtained in the assays (Table 7). The remaining compounds were inactive against this parasite.

Lapachol (**1**) was active against the promastigote stage of the wild-type strains of *L. chagasi* (IC_{50} 34.72 μM),[17] *L. major* (IC_{50} 33.0 ± 2.6 μM)[38] and *L. braziliensis* (IC_{50} 11.9 ± 6.9 μg mL^{-1}).[37] However, the long side chain led to inactivity in the studied series (**8a–10a** and **8b–10b**) against the promastigote stages of the two *Leishmania* species.

According to Guimarães and coworkers,[17] the β-lapachone (**3**) (IC_{50} 0.67 μM) and nor-β-lapachone (**17**) (IC_{50} 1.14 μM) showed excellent leishmanicidal activities against the promastigote stage of the wild-type strain of *L. chagasi*. Again, the nature of the substituent affected the bioactivity, as the new compounds **11a–13a** and **11b–13b** were inactive against the promastigote stages *of L. chagasi* and *L. braziliensis*.

Table 7 Leishmanicidal activity against the promastigote stage of *L. braziliensis*

Compound	Concentration (μM) × % lysis (±SD)[a]				IC_{50} (μM)
	0.5	2.0	8.0	32.0	
11a	20.1 ± 6.1	22.2 ± 7.4	23.5 ± 2.3	32.8 ± 3.4	4.7×10^3
11b	14.3 ± 6.7	17.8 ± 4.5	23.0 ± 0.8	29.0 ± 6.6	1.9×10^3
12b	15.1 ± 9.8	23.2 ± 8.6	27.8 ± 9.1	28.7 ± 4.8	3.6×10^3

[a] Standard deviation.

Conclusion

Twelve new naphthoquinone derivatives were synthesized and tested as trypanocidal and leishmanicidal compounds. The most effective derivatives against the amastigote stage of *T. cruzi* were **11a** and **13a**, and importantly, they exhibited low cytotoxic effects to fibroblast LLCMK2. Compound **11a** was found as a promising trypanocidal agent and new studies – especially related to its toxicity – would be worth performing, and it may possibly show less side effects than the current drugs.

In the assays of leishmanicidal activity, all the compounds were inactive against *L. chagasi*, and in general showed an insignificant activity against *L. braziliensis*.

Experimental part

General remarks

The compounds **5–7** were synthesized according to the procedures described in the literature.[39] All the reagents were purchased from Sigma-Aldrich. The purification of the compounds was performed *via* chromatography column (CC) using silica gel Merck 60 (230–400 mesh), preparative silica gel plates GF – 500 microns Uniplat™ and a mixture of hexane and ethyl acetate for the elution. All the reactions were monitored by thin-layer chromatography with silica gel Merk 60. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in a DPX-300 Bruker and calibrated with residual nondeuterated solvent as an internal reference. Chemical shifts are reported in *ppm* using TMS as the internal standard (δ = 0 ppm), and the coupling constant (J) is expressed in Hertz. Mass spectra (EI, 70 eV) were performed on a mass spectrometer coupled to gas chromatography Shimadzu GCMS QP2010 Plus. The main fragments were described as a relation between atomic mass units and the load (m/z) and relative abundance in percentage of the intensity of the base peak. The electron-impact high-resolution mass spectrometry (EI-HRMS) was acquired using Kratos MS 50G. Infrared spectra were recorded on a Perkin-Elmer model 783 in a KBr cell for liquid (film) or KBr pellets for solids and the absorption wave numbers expressed in cm^{-1}. Melting points were determined by MQAPF-301 model equipment.

General preparation of 8a–10a and 8b–10b

The lawsone (**4**) alkenylation that resulted on the substituted 1,4-naphthoquinones (**8a–10a** and **8b–10b**) was performed, as described in the literature.[27] A solution of $(NH_4)_2S_2O_8$ (1 g) in H_2O (10 mL) was added dropwise to a solution of $AgNO_3$ (0.25 g), H_2O (10 mL), CH_3CN (20 mL), **4** (0.2 g) and the respective carboxylic acid **5**, **6** or **7** (1.5 mmol) at 70–80 °C. After 30 minutes, the crude reaction was cooled and extracted with ethyl acetate. The organic layer was washed with H_2O, dried with anhydrous $MgSO_4$, filtered off and the solvent was evaporated under reduced pressure. The obtained residue was purified by flash chromatography. The isomeric mixture of **a** and **b** were purified on preparative TLC using hexane and ethyl acetate 9 : 1 as the eluent.

(*E*)-2-(Dec-2-enyl)-3-hydroxynaphthalene-1,4-dione (8a). Compound **8a** was obtained as orange oil, 22% yield. IR (KBr, cm^{-1}): ν_{max} 3440, 2962–2854, 1670–1647, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.83 (3H, t, J = 6.0 Hz, CH_3, **H-20**), 1.00–1.50 (2H, m, CH_2, **H-19**), 1.00–1.50 (2H, m, CH_2, **H-18**), 1.00–1.50 (6H, m, CH_2, **H15-H17**), 1.95 (2H, q, J = 15.0 Hz and J = 6.0 Hz, CH_2, **H-14**), 3.28 (2H, d, J = 6.3 Hz, CH_2, **H-11**), 5.35–5.65 (1H, m, CH, **H-13**), 5.35–5.65 (1H, m, CH, **H-12**), 7.32 (1H, br s, **OH**), 7.65 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-7**), 7.75 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-6**), 8.06 (1H, dd, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-8**), 8.11 (1H, dd, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-5**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.05 (CH_3, **C-20**), 22.60 (CH_2, **C-19**), 26.41 (CH_2, **C-11**), 29.00–30.00 ($3CH_2$, **C15-C17**), 31.79 (CH_2, **C-14**), 32.46 (CH_2, **C-18**), 122.82 (C, **C-3**), 124.69 (CH, **C-12**), 126.07 (CH, **C-8**), 126.81 (CH, **C-5**), 129.44 (2C, **C9-C10**), 132.86 (CH, **C-13**), 133.05 (CH, **C-7**), 134.87 (CH, **C-6**), 152.84 (C-OH, **C-2**), 181.64 (C=O, **C-1**), 184.34 (C=O, **C-4**). MS (EI) m/z 312 ($M^{+\bullet}$, 20%), 213 (100), 188 (27), 159 (7), 55 (9). HRMS (EI) m/z $[M]^{+\bullet}$ calcd for $C_{20}H_{24}O_3$: 312.1725, found: 312.1725.

2-(Dec-1-en-3-yl)-3-hydroxynaphthalene-1,4-dione (8b). Compound **8b** was obtained as orange oil, 5% yield. IR (KBr, cm^{-1}): ν_{max} 3371, 2954–2854, 1666–1650, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.84 (3H, t, J = 6.0 Hz, CH_3, **H-20**), 1.00–1.50 (2H, m, CH_2, **H-19**), 1.00–1.50 (2H, m, CH_2, **H-18**), 1.00–1.50 (6H, m, CH_2, **H15-H17**), 1.79 (2H, q, J = 16.8 Hz and J = 8.1 Hz, CH_2, **H-14**), 3.80 (1H, q, J = 15.9 Hz and J = 8.7 Hz, CH, **H-11**), 4.99 (1H, dd, J_{cis} = 10.2 Hz, J_{gem} = 1.2 Hz, CH_2, **H-13**), 5.09 (1H, dd, J_{trans} = 17.1 Hz, J_{gem} = 1.2 Hz, CH_2, **H-13**), 6.18 (1H, ddd, J_{trans} = 17.1 Hz, J_{cis} = 9.3 Hz, J_{vic} = 8.7 Hz, CH, **H-12**), 7.44 (1H, br s, **OH**), 7.64 (1H, td, J_o = 7.5 Hz, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-7**), 7.72 (1H, td, J_o = 7.5 Hz, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-6**), 8.04 (1H, dd, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-8**), 8.09 (1H, dd, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-5**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.06 (CH_3, **C-20**), 22.62 (CH_2, **C-19**),

27.00–30.00 (3CH_2, **C15-C17**), 31.82 (CH_2, **C-18**), 32.33 (CH_2, **C-14**), 40.41 (CH, **C-11**), 115.40 (CH_2, **C-13**), 125.70 (C, **C-3**), 126.01 (CH, **C-8**), 126.97 (CH, **C-5**), 129.22 (C, **C-9**), 132.83 (CH, **C-7**), 133.01 (C, **C-10**), 134.98 (CH, **C-6**), 139.07 (CH, **C-12**), 152.68 (C-OH, **C-2**), 181.73 (C═O, **C-1**), 184.18 (C═O, **C-4**). MS (EI) *m/z* 312 ($M^{+\bullet}$, 61%), 241 (61), 213 (100), 188 (34), 159 (66), 55 (87). HRMS (EI) *m/z* $[M]^{+\bullet}$ calcd for $C_{20}H_{24}O_3$: 312.1725, found: 312.1726.

(*E*)-2-(Undec-2-enyl)-3-hydroxynaphthalene-1,4-dione (9a). Compound **9a** was obtained as orange oil, 20% yield. IR (KBr, cm^{-1}): ν_{max} 3317, 2954–2854, 1662–1616, 1600–1550, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, t, J = 6.0 Hz, CH_3, **H-21**), 1.00–1.50 (2H, m, CH_2, **H-20**), 1.00–1.50 (2H, m, CH_2, **H-19**), 1.00–1.50 (8H, m, CH_2, **H15-H18**), 1.94 (2H, q, J = 12.0 Hz and J = 6.0 Hz, CH_2, **H-14**), 3.27 (2H, d, J = 6.6 Hz, CH_2, **H-11**), 5.35–5.65 (1H, m, CH, **H-13**), 5.35–5.65 (1H, m, CH, **H-12**), 7.37 (1H, br s, **OH**), 7.64 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-7**), 7.74 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-6**), 8.05 (1H, dd, J_o = 7.5 Hz, J_m = 0.9 Hz, CH, **H-8**), 8.10 (1H, dd, J_o = 7.5 Hz, J_m = 0.9 Hz, CH, **H-5**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.04 (CH_3, **C-21**), 22.61 (CH_2, **C-20**), 26.37 (CH_2, **C-11**), 29.00–30.00 (4CH_2, **C15-C-18**), 31.85 (CH_2, **C-14**), 32.45 (CH_2, **C-19**), 122.79 (C, **C-3**), 124.67 (CH, **C-12**), 126.03 (CH, **C-8**), 126.74 (CH, **C-5**), 129.41 (2C, **C9-C10**), 132.83 (CH, **C-13**), 133.00 (CH, **C-7**), 134.83 (CH, **C-6**), 152.83 (C-OH, **C-2**), 181.56 (C═O, **C-1**), 184.35 (C═O, **C-4**). MS (EI) *m/z* 326 ($M^{+\bullet}$, 0.8%), 213 (100), 188 (27), 159 (6), 55 (45). HRMS (EI) *m/z* $[M]^{+\bullet}$ calcd for $C_{21}H_{26}O_3$: 326.1881, found: 326.1879.

2-(Undec-1-en-3-yl)-3-hydroxynaphthalene-1,4-dione (9b). Compound **9b** was obtained as orange oil, 6% yield. IR (KBr, cm^{-1}): ν_{max} 3317, 2954–2854, 1666–1650, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.84 (3H, t, J = 6.0 Hz, CH_3, **H-21**), 1.00–1.50 (2H, m, CH_2, **H-20**), 1.00–1.50 (2H, m, CH_2, **H-19**), 1.00–1.50 (8H, m, CH_2, **H15-H18**), 1.79 (2H, q, J = 15.0 Hz and J = 9.0 Hz, CH_2, **H-14**), 3.80 (1H, q, J = 15.9 Hz and J = 8.4 Hz, CH, **H-11**), 5.00 (1H, dd, J_{cis} = 10.2 Hz, J_{gem} = 0.9 Hz, CH_2, **H-13**), 5.11 (1H, dd, J_{trans} = 17.1 Hz, J_{gem} = 0.9 Hz, CH_2, **H-13**), 6.18 (1H, ddd, J_{trans} = 17.1 Hz, J_{cis} = 9.6 Hz, J_{vic} = 8.4 Hz, CH, **H-12**), 7.42 (1H, br s, **OH**), 7.65 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-7**), 7.76 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-6**), 8.04 (1H, dd, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-8**), 8.10 (1H, dd, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-5**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.08 (CH_3, **C-21**), 22.63 (CH_2, **C-20**), 27.00–30.00 (4CH_2, **C15-C18**), 31.83 (CH_2, **C-19**), 32.33 (CH_2, **C-14**), 40.41 (CH, **C-11**), 115.39 (CH_2, **C-13**), 125.69 (C, **C-3**), 126.00 (CH, **C-8**), 126.96 (CH, **C-5**), 129.21 (C, **C-9**), 132.83 (CH, **C-7**), 133.00 (C, **C-10**), 134.97 (CH, **C-6**), 139.07 (CH, **C-12**), 152.68 (C-OH, **C-2**), 181.72 (C═O, **C-1**), 184.17 (C═O, **C-4**). MS (EI) *m/z* 326 ($M^{+\bullet}$, 22%), 241 (2), 213 (100), 188 (31), 159 (7), 55 (11). HRMS (EI) *m/z* $[M]^{+\bullet}$ calcd for $C_{21}H_{26}O_3$: 326.1881, found: 326.1877.

(*E*)-2-(Dodec-2-enyl)-3-hydroxynaphthalene-1,4-dione (10a). Compound **10a** was obtained as orange oil, 24% yield. IR (KBr, cm^{-1}): ν_{max} 3355, 2919–2850, 1654–1643, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.84 (3H, t, J = 6.0 Hz, CH_3, **H-22**), 1.00–1.50 (2H, m, CH_2, **H-21**), 1.00–1.50 (2H, m, CH_2, **H-20**), 1.00–1.50 (10H, m, CH_2, **H15-H19**), 1.94 (2H, q, J = 12.0 Hz and J = 6.0 Hz, CH_2, **H-14**), 3.27 (2H, d, J = 6.3 Hz, CH_2, **H-11**), 5.35–5.65 (1H, m, CH, **H-13**), 5.35–5.65 (1H, m, CH, **H-12**), 7.38 (1H, br s, **OH**), 7.64 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-7**), 7.74 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-6**), 8.04 (1H, d, J_o = 7.5 Hz, CH, **H-8**), 8.10 (1H, d, J_o = 7.5 Hz, CH, **H-5**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.07 (CH_3, **C-22**), 22.63 (CH_2, **C-21**), 26.39 (CH_2, **C-11**), 29.00–30.00 (5CH_2, **C15-C19**), 31.85 (CH_2, **C-14**), 32.46 (CH_2, **C-20**), 122.78 (C, **C-3**), 124.67 (CH, **C-12**), 126.04 (CH, **C-8**), 126.77 (CH, **C-5**), 129.41 (2C, **C9-C10**), 132.83 (CH, **C-13**), 133.02 (CH, **C-7**), 134.83 (CH, **C-6**), 152.84 (C-OH, **C-2**), 181.60 (C═O, **C-1**), 184.30 (C═O, **C-4**). MS (EI) *m/z* 340 ($M^{+\bullet}$, 17%), 213 (100), 188 (34), 159 (11), 55 (24). HRMS (EI) *m/z* $[M]^{+\bullet}$ calcd for $C_{22}H_{28}O_3$: 340.2038, found: 340.2039.

2-(Dodec-1-en-3-yl)-3-hydroxynaphthalene-1,4-dione (10b). Compound **10b** was obtained as orange oil, 4% yield. IR (KBr, cm^{-1}): ν_{max} 3371, 2954–2850, 1666–1650, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, t, J = 6.0 Hz, CH_3, **H-22**), 1.00–1.50 (2H, m, CH_2, **H-21**), 1.00–1.50 (2H, m, CH_2, **H-20**), 1.00–1.50 (10H, m, CH_2, **H15-H19**), 1.79 (2H, q, J = 15.0 Hz and J = 9.0 Hz, CH_2, **H-14**), 3.80 (1H, q, J = 15.9 Hz and J = 8.4 Hz, CH, **H-11**), 5.00 (1H, dd, J_{cis} = 9.3 Hz, J_{gem} = 0.9 Hz, CH_2, **H-13**), 5.10 (1H, dd, 1H, J_{trans} = 17.1 Hz, J_{gem} = 0.9 Hz, CH_2, **H-13**), 6.18 (1H, ddd, J_{trans} = 17.1 Hz, J_{cis} = 9.3 Hz, J_{vic} = 8.4 Hz, CH, **H-12**), 7.43 (1H, br s, **OH**), 7.65 (1H, td, 1H, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-7**), 7.76 (1H, td, J_o = 7.5 Hz, J_m = 1.5 Hz, CH, **H-6**), 8.05 (1H, dd, J_o = 7.5 Hz, J_m = 0.9 Hz, CH, **H-8**), 8.10 (1H, dd, J_o = 7.5 Hz, J_m = 0.9 Hz, CH, **H-5**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.08 (CH_3, **C-22**), 22.65 (CH_2, **C-21**), 27.00–30.00 (5CH_2, **C15-C19**), 31.84 (CH_2, **C-20**), 32.33 (CH_2, **C-14**), 40.41 (CH, **C-11**), 115.38 (CH_2, **C-13**), 125.70 (C, **C-3**), 125.99 (CH, **C-8**), 126.95 (CH, **C-5**), 129.22 (C, **C-9**), 132.82 (CH, **C-7**), 132.99 (C, **C-10**), 134.96 (CH, **C-6**), 139.07 (CH, **C-12**), 152.70 (C-O, **C-2**), 181.72 (C═O, **C-1**), 184.16 (C═O, **C-4**). MS (EI) *m/z* 340 ($M^{+\bullet}$, 64%), 241 (73), 213 (100), 188 (40), 159 (54), 55 (91). HRMS (EI) *m/z* $[M]^{+\bullet}$ calcd for $C_{22}H_{28}O_3$: 340.2038, found: 340.2040.

General preparation of 11a–13a and 11b–13b

The syntheses of the heterocyclic naphthoquinones (**11a–13a** and **11b–13b**) from intramolecular cyclization of the substituted 1,4-naphthoquinones (**8a–10a**) were performed according to the literature.[32] Substituted 1,4-naphthoquinones **8a**, **9a** or **10a** (100 mg) were added to a stirred solution of $InBr_3$ (200 mg) in CH_2Cl_2 (30 mL) and kept under stirring at room temperature for 24 h. The reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous $MgSO_4$, filtered off and the solvent was evaporated under reduced pressure. The obtained residue was purified by preparative silica gel chromatography plate using a mixture of hexane and ethyl acetate 5 : 1 as the eluent.

2-Octyl-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (11a). Compound **11a** was obtained as an orange solid, 26% yield. mp: 95.6–100 °C. IR (KBr, cm^{-1}): ν_{max} 2954–2850, 1697–1608, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.86 (3H, t, J = 6.0 Hz, CH_3, **H-20**), 1.10–1.50 (2H, m, CH_2, **H-19**), 1.10–1.50

(2H, m, CH_2, **H-18**), 1.10–1.50 (2H, m, CH_2, **H-17**), 1.10–1.50 (2H, m, CH_2, **H-16**), 1.10–1.50 (2H, m, CH_2, **H-15**), 1.40–1.60 (2H, m, CH_2, **H-14**), 1.65–1.81 (1H, m, CH_2, **H-13**), 1.82–2.00 (1H, m, CH_2, **H-13**), 2.77 (1H, dd, J = 15.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 3.21 (1H, dd, J = 15.0 Hz and J = 9.0 Hz, CH_2, **H-11**), 5.10 (1H, dddd, J = 9.0 Hz, J = 9.0 Hz, J = 6.0 Hz and J = 6.0 Hz, CH, **H-12**), 7.52–7.60 (1H, m, CH, **H-7**), 7.60–7.67 (2H, m, CH, **H5-H6**), 8.05 (1H, d, J_o = 7.5 Hz, CH, **H-8**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.06 (CH_3, **C-20**), 22.60 (CH_2, **C-19**), 24.92 (CH_2, **C-14**), 29.14 (CH_2, **C-15**), 29.27 (CH_2, **C-17**), 29.38 (CH_2, **C-16**), 31.82 (CH_2, **C-11**), 31.77 (CH_2, **C-18**), 36.02 (CH_2, **C-13**), 88.44 (CH, **C-12**), 115.30 (C, **C-3**), 124.48 (CH, **C-5**), 127.63 (C, **C-10**), 129.33 (CH, **C-8**), 130.71 (C, **C-9**), 131.86 (CH, **C-7**), 134.43 (CH, **C-6**), 169.87 (C, **C-4**), 175.43 (C═O, **C-2**), 181.29 (C═O, **C-1**). MS (EI) m/z 312 ($M^{+\bullet}$, 22%), 213 (10), 183 (18), 159 (100), 55 (19). HRMS (EI) m/z $[M]^{+\bullet}$ calcd for $C_{20}H_{24}O_3$: 312.1725, found: 312.1727.

2-Heptyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (11b). Compound **11b** was obtained as an orange solid, 26% yield. mp: 72.1–74.4 °C. IR (KBr, cm^{-1}): ν_{max} 2943–2854, 1697–1600, 1600–1500, 729. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 6.0 Hz, CH_3, **H-20**), 1.20–1.40 (2H, m, CH_2, **H-19**), 1.20–1.40 (2H, m, CH_2, **H-18**), 1.20–1.40 (2H, m, CH_2, **H-17**), 1.20–1.40 (2H, m, CH_2, **H-16**), 1.40–1.60 (2H, m, CH_2, **H-15**), 1.60–1.80 (1H, m, CH_2, **H-12**), 1.65–1.90 (2H, m, CH_2, **H-14**), 2.00–2.12 (1H, m, CH_2, **H-12**), 2.42 (1H, ddd, J = 18.0 Hz, J = 9.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 2.68 (1H, ddd, J = 18.0 Hz, J = 9.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 4.22 (1H, dddd, J = 12.0 Hz, J = 7.5 Hz, J = 6.0 Hz and J = 3.0 Hz, CH, **H-13**), 7.49 (1H, td, J_o = 7.8 Hz, J_m = 1.5 Hz, CH, **H-7**), 7.63 (1H, td, J_o = 7.8 Hz, J_m = 1.5 Hz, CH, **H-6**), 7.76 (1H, dd, J_o = 7.8 Hz, J_m = 0.9 Hz, CH, **H-5**), 8.04 (1H, dd, J_o = 7.8 Hz, J_m = 0.9 Hz, CH, **H-8**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.05 (CH_3, **C-20**), 17.95 (CH_2, **C-11**), 22.59 (CH_2, **C-19**), 25.27 (CH_2, **C-15**), 25.94 (CH_2, **C-12**), 29.14 (CH_2, **C-17**), 29.39 (CH_2, **C-16**), 31.74 (CH_2, **C-18**), 34.73 (CH_2, **C-14**), 79.03 (CH, **C-13**), 113.92 (C, **C-3**), 123.90 (CH, **C-5**), 128.59 (CH, **C-8**), 130.00 (C, **C-9**), 130.62 (CH, **C-7**), 132.40 (C, **C-10**), 134.78 (CH, **C-6**), 162.87 (C, **C-4**), 178.58 (C═O, **C-2**), 179.72 (C═O, **C-1**). MS (EI) m/z 312 ($M^{+\bullet}$, 4%), 213 (5), 185 (4), 159 (100), 55 (9). HRMS (EI) m/z $[M + 2H]^{+\bullet}$ calcd for $C_{20}H_{24}O_3$: 314.1881, found: 314.1879.

2-Nonyl-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (12a). Compound **12a** was obtained as an orange solid, 25% yield. mp: 90.5–95.8 °C. IR (KBr, cm^{-1}): ν_{max} 2954–2854, 1697–1608, 1600–1500, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, t, J = 9.0 Hz, CH_3, **H-21**), 1.10–1.50 (2H, m, CH_2, **H-20**), 1.10–1.50 (2H, m, CH_2, **H-19**), 1.10–1.50 (2H, m, CH_2, **H-18**), 1.10–1.50 (2H, m, CH_2, **H-17**), 1.10–1.50 (2H, m, CH_2, **H-16**), 1.10–1.50 (2H, m, CH_2, **H-15**), 1.40–1.60 (2H, m, CH_2, **H-14**), 1.62–1.81 (1H, m, CH_2, **H-13**), 1.82–2.00 (1H, m, CH_2, **H-13**), 2.78 (1H, dd, J = 15.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 3.22 (1H, dd, CH_2, J = 15.0 Hz and J = 9.0 Hz, CH_2, **H-11**), 5.11 (1H, dddd, J = 9.0 Hz, J = 9.0 Hz, J = 6.0 Hz and J = 6.0 Hz, CH, **H-12**), 7.50–7.60 (1H, m, CH, **H-7**), 7.60–7.67 (2H, m, CH, **H5-H6**), 8.06 (1H, d, J_o = 7.2 Hz, CH, **H-8**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.07 (CH_3, **C-21**), 22.64 (CH_2, **C-20**), 24.94 (CH_2, **C-14**), 29.24 (CH_2, **C-15**), 29.30 (CH_2, **C-18**), 29.45 ($2CH_2$, **C16-C17**), 31.84 (CH_2, **C-19**), 31.85 (CH_2, **C-11**), 36.04 (CH_2, **C-13**), 88.46 (CH, **C-12**), 115.34 (C, **C-3**), 124.49 (CH, **C-5**), 127.67 (C, **C-10**), 129.38 (CH, **C-8**), 130.77 (C, **C-9**), 131.88 (CH, **C-7**), 134.43 (CH, **C-6**), 169.87 (C, **C-4**), 175.48 (C═O, **C-2**), 181.32 (C═O, **C-1**). MS (EI) m/z 326 ($M^{+\bullet}$, 34%), 213 (9), 185 (13), 159 (100), 55 (14). HRMS (EI) m/z $[M]^{+\bullet}$ calcd for $C_{21}H_{26}O_3$: 326.1881, found: 326.1880.

2-Octyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (12b). Compound **12b** was obtained as an orange solid, 20% yield. mp: 64.5–67.7 °C. IR (KBr, cm^{-1}): ν_{max} 2923–2854, 1693–1600, 1600–1500, 721. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 9.0 Hz, CH_3, **H-21**), 1.20–1.40 (2H, m, CH_2, **H-20**), 1.20–1.40 (2H, m, CH_2, **H-19**), 1.20–1.40 (2H, m, CH_2, **H-18**), 1.20–1.40 (2H, m, CH_2, **H-17**), 1.20–1.40 (2H, m, CH_2, **H-16**), 1.40–1.60 (2H, m, CH_2, **H-15**), 1.60–1.80 (1H, m, CH_2, **H-12**), 1.60–1.95 (2H, m, CH_2, **H-14**), 2.00–2.15 (1H, m, CH_2, **H-12**), 2.42 (1H, ddd, J = 18.0 Hz, J = 9.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 2.68 (1H, ddd, J = 18.0 Hz, J = 9.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 4.22 (1H, dddd, J = 12.0 Hz, J = 6.0 Hz, J = 6.0 Hz and J = 3.0 Hz, CH, **H-13**), 7.47 (1H, td, J_o = 7.5 Hz, J_m = 0.9 Hz, CH, **H-7**), 7.64 (1H, td, J_o = 7.5 Hz, J_m = 0.9 Hz, CH, **H-6**), 7.76 (1H, d, J_o = 7.5 Hz, CH, **H-5**), 8.04 (1H, d, J_o = 7.5 Hz, CH, **H-8**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.09 (CH_3, **C-21**), 17.97 (CH_2, **C-11**), 22.65 (CH_2, **C-20**), 25.30 (CH_2, **C-15**), 25.98 (CH_2, **C-12**), 29.22 (CH_2, **C-18**), 29.46 ($2CH_2$, **C16-C17**), 31.82 (CH_2, **C-19**), 34.76 (CH_2, **C-14**), 79.06 (CH, **C-13**), 113.95 (C, **C-3**), 123.92 (CH, **C-5**), 128.65 (CH, **C-8**), 130.04 (C, **C-9**), 130.67 (CH, **C-7**), 132.43 (C, **C-10**), 134.81 (CH, **C-6**), 162.93 (C, **C-4**), 178.63 (C═O, **C-2**), 179.77 (C═O, **C-1**). MS (EI) m/z 326 ($M^{+\bullet}$, 3%), 213 (5), 185 (4), 159 (100), 55 (11). HRMS (EI) m/z $[M + 2H]^{+\bullet}$ calcd for $C_{21}H_{26}O_3$: 328.2038, found: 328.2037.

2-Decyl-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione (13a). Compound **13a** was obtained as an orange solid, 22% yield. mp: 106.7–109 °C. IR (KBr, cm^{-1}): ν_{max} 2954–2850, 1697–1608, 1600–1500, 721. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.86 (3H, t, J = 6.0 Hz, CH_3, **H-22**), 1.10–1.50 (2H, m, CH_2, **H-21**), 1.10–1.50 (2H, m, CH_2, **H-20**), 1.10–1.50 (2H, m, CH_2, **H-19**), 1.10–1.50 (2H, m, CH_2, **H-18**), 1.10–1.50 (2H, m, CH_2, **H-17**), 1.10–1.50 (2H, m, CH_2, **H-16**), 1.10–1.50 (2H, m, CH_2, **H-15**), 1.40–1.60 (2H, m, CH_2, **H-14**), 1.65–1.82 (1H, m, CH_2, **H-13**), 1.82–2.00 (1H, m, CH_2, **H-13**) 2.77 (1H, dd, J = 15.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 3.21 (1H, dd, J = 15.0 Hz and J = 9.0 Hz, CH_2, **H-11**), 5.11 (1H, dddd, J = 9.0 Hz, J = 9.0 Hz, J = 6.0 Hz and J = 6.0 Hz, CH, **H-12**), 7.52–7.60 (1H, m, CH, **H-7**), 7.60– 7.67 (2H, m, CH, **H5-H7**), 8.05 (1H, d, J_o = 7.5 Hz, CH, **H-8**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.07 (CH_3, **C-22**), 22.64 (CH_2, **C-21**), 24.93 (CH_2, **C-14**), 29.28 ($2CH_2$, **C-15** and **C-19**), 29.43 (CH_2, **C-18**), 29.49 (CH_2, **C-17**), 29.53 (CH_2, **C-16**), 31.83 (CH_2, **C-20**), 31.85 (CH_2, **C-11**), 36.03 (CH_2, **C-13**), 88.46 (CH, **C-12**), 115.32 (C, **C-3**), 124.49 (CH, **C-5**), 127.65 (C, **C-10**), 129.37 (CH, **C-8**), 130.75 (C, **C-9**), 131.87 (CH, **C-7**), 134.43 (CH, **C-6**), 169.87 (C, **C-4**), 175.46 (C═O, **C-2**), 181.30 (C═O, **C-1**). MS (EI) m/z 340 ($M^{+\bullet}$, 45%), 213 (10), 188 (11), 159 (100), 55 (16). HRMS (EI) m/z $[M]^{+\bullet}$ calcd for $C_{22}H_{28}O_3$: 340.2038, found: 340.2042.

2-Nonyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (13b). Compound **13b** was obtained as an orange solid, 25% yield. mp: 64.7–66.7 °C. IR (KBr, cm^{-1}): ν_{max} 2923–2850, 1693–1604, 1600–1500, 721. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 9.0 Hz, CH_3, **H-22**), 1.20–1.40 (2H, m, CH_2, **H-21**), 1.20–1.40 (2H, m, CH_2, **H-20**), 1.20–1.40 (2H, m, CH_2, **H-19**), 1.20–1.40 (2H, m, CH_2, **H-18**), 1.20–1.40 (2H, m, CH_2, **H-17**), 1.20–1.40 (2H, m, CH_2, **H-16**), 1.40–1.60 (2H, m, CH_2, **H-15**), 1.60–1.80 (1H, m, CH_2, **H-12**), 1.60–1.95 (2H, m, CH_2, **H-14**), 2.00– 2.12 (1H, m, CH_2, **H-12**), 2.41 (1H, ddd, J = 18.0 Hz, J = 9.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 2.68 (1H, ddd, J = 18.0 Hz, J = 9.0 Hz and J = 6.0 Hz, CH_2, **H-11**), 4.22 (1H, dddd, J = 12.0 Hz, J = 6.0 Hz, J = 6.0 Hz and J = 3.0 Hz, CH, **H-13**), 7.47 (1H, td, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-7**), 7.64 (1H, td, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-6**), 7.75 (1H, dd, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-5**), 8.02 (1H, dd, J_o = 7.5 Hz, J_m = 1.2 Hz, CH, **H-8**). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.07 (CH_3, **C-22**), 17.96 (CH_2, **C-11**), 22.63 (CH_2, **C-21**), 25.27 (CH_2, **C-15**), 25.95 (CH_2, **C-12**), 29.26 (CH_2, **C-19**), 29.42 (CH_2, **C-18**), 29.49 ($2CH_2$, **C16-C17**), 31.85 (CH_2, **C-20**), 34.73 (CH_2, **C-14**), 79.03 (CH, **C-13**), 113.92 (C, **C-3**), 123.90 (CH, **C-5**), 128.60 (CH, **C-8**), 130.00 (C, **C-9**), 130.63 (CH, **C-7**), 132.39 (C, **C-10**), 134.78 (CH, **C-6**), 162.88 (C, **C-4**), 178.59 (C=O, **C-2**), 179.73 (C=O, **C-1**). MS (EI) m/z 340 ($M^{+\bullet}$, 6%), 213 (4), 185 (5), 159 (100), 55 (13). HRMS (EI) m/z $[M + 2H]^{+\bullet}$ calcd for $C_{22}H_{28}O_3$: 342.2194, found: 342.2190.

Bioassays

Trypanocidal activity. Step 1: The compounds **8a,b–13a,b** underwent a screening evaluation for direct analysis of the compounds on the strains of cells infected with *T. cruzi* in a single concentration, allowing the monitoring of the effects on the trypomastigotes and amastigotes stages in a sole system. Compounds were evaluated at a concentration of 3.8 μM and compared to the reference drug, benznidazole, at the same concentration. Compounds that showed a trypanocidal effect similar or better (**10a**, **11a**, **11b**, **12a**, **12b**, **13a**) than benznidazole at the tested concentration, were submitted to a second evaluation step to determine the IC_{50} (concentration of drug that reaches the inhibition of 50% of the parasites).[40]

Step 2: Previously, LLCMK2 cells were infected with approximately 10^6 trypomastigote bloodstream stage of *T. cruzi* of CL Brener strain (clone B5) obtained from the blood of infected *Swiss* mice at the peak of parasitaemia. After 15 days, large amounts of trypomastigotes were obtained from the supernatant of these cultures as a result of the lysis of cells filled with parasites. The supernatant obtained from these cultures containing the trypomastigote stage together with some cells was centrifuged (115 *g* for 8 min, 10 °C), and the sediment, containing mostly the cells, was discarded, and the supernatant was re-centrifuged (1620 *g* for 30 min 10 °C). Then, the pellet was resuspended in a supplemented RPMI 1640 medium, and the purified trypomastigotes were submitted to counting on a Neubauer hemocytometer to adjust the quantity of parasites in the experimental procedures. Assays were carried out in 96-well plates where LLCMK2 cells were cultivated (2.5×10^3 cells per well). Trypomastigote stages of CL Brener strain (clone B5), obtained from cultivation, were added in a 1 : 10 ratio and incubated for 24 h at 37 with 5% CO_2. The wells were then washed with PBS to remove the extracellular trypomastigote stage, and the tested compounds (**10a**, **11a**, **11b**, **12a**, **12b**, **13a**) were added, resulting in the final concentrations of 0.5; 2.0; 8.0 and 32.0 μM. The plates were incubated for 5 days at 37 °C in a 5% CO_2 atmosphere. After this period, to each well was added 10 μL solution of FluoReporter lacZ/Galactosidase quantitative Kit (Life Technologies), and the plates were re-incubated for 30 min. until the fluorescent labeling of β-galactosidase produced by the parasites occurred. The colorimetric reaction was quantified by fluorescence spectrophotometer (BIOTEK) at 386 nm excitation and 448 nm emission. After the readings, the percentages of parasite lysis, caused by the formulations, were determined from the following formula.

$$\% \text{ Lysis} = 100 - \{[(X - \text{PC})/(\text{NC} - \text{PC})] \times 100\},$$

where X = optical density value of the samples; PC = optical density value of the positive controls; NC = optical density value of the negative controls.

The trials had positive control wells containing only the culture medium and as negative controls, a treatment with 1.6% DMSO. All assays were performed in triplicate. All samples were compared with benznidazole and evaluated at the same concentrations.

Cytotoxicity assays. Previously cultured LLCMK2 cells were trypsinized and centrifuged at 1000 rpm for 5 min. The cell pellet was resuspended in complete RPMI medium (without phenol red), and cell concentration was adjusted to 1×10^6 cells mL^{-1}. Then, 200 μL of the cellular suspension was plated onto a 96-well plate. After 24 hours, the medium was replaced by RPMI with different concentrations of the compounds, which were assayed (0.5, 2.0, 8.0 and 32.0 μM). The plate was incubated for 96 h at 37 °C, with humid atmosphere and 5% CO_2. All the assays were made in triplicate.

CEDEX XS Analyzer (Roche Applied Science) was used to determine the viability and cellular toxic effects of the compounds using the trypan blue exclusion method.

The cytotoxicity to 50% of the cells (CC_{50}) was obtained by a nonlinear regression method, using the software GraphPad Prism, v.6.0 (GraphPad Software, La Jolla California, USA). The Selectivity Index (SI) was calculated by the ratio between the obtained values of CC_{50} and the IC_{50}, respectively (SI = CC_{50}/IC_{50}).

Leishmanicidal activity. An aliquot of 400 μL of compounds **8a,b–13a,b** in concentrations of 0.5; 2.0; 8.0 and 32.0 μM applied in triplicate on a 96-well plate were added to a culture with the promastigote stages *of Leishmania* (*L. chagasi* and *L. braziliensis*), previously adjusted to 1×10^6 cells mL^{-1}. As positive control, only culture medium was used, and parasites with 1.5% DMSO were used as negative control. The plate was incubated in a humidified atmosphere at 22 °C for 72 h. After this period, the viability test of the promastigotes was performed through XTT tetrazol salt, a compound of the yellowish coloring compound easily incorporated by viable cells and reduced by mitochondrial dehydrogenases. When reduced, the XTT

formazan is converted to an orange-colored compound. The test measures the amount of formazan formed by spectrophotometry, in which the absorbance value is directly proportional to the number of viable cells in the medium. 50 mL of a solution of XTT with PMS (Phenazine methosulfate) in the proportion of 1 mg mL^{-1} XTT to 0.001 mg mL^{-1} PMS was added, and the plate was incubated at 37 °C in a humidified 5% CO_2 atmosphere for 4 h, protected from light. At the end, the reading of the plate was performed in a spectrophotometer (Biotek) at 450 nm.[41,42] Data were processed as described in the previous item. The cytotoxicity percentage was calculated by the following formula:

$$\% \text{ Cytotoxicity} = 1 - [(Y - N)/(N - P)] \times 100$$

where Y = optical density reading of cells and wells with different concentrations of the compounds; N = optical density reading of cells in wells with 1.5% DMSO; P = optical density reading of the wells with culture medium.

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