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Short communication

Synthesis and anti-*Trypanosoma cruzi* activity of β-lapachone analogues

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ABSTRACT

The available chemotherapy for Chagas disease, caused by *Trypanosoma cruzi*, is unsatisfactory; therefore, there is an intense effort to find new drugs for the treatment of this disease. In our laboratory, we have analyzed the effect on bloodstream trypomastigotes of 16 new naphthoquinone analogues of β-lapachone modified in the pyran ring, aiming to find a new prototype with high trypanocidal activity. The new compounds presented a broad spectrum of activity, and five of them presented IC_{50}/24 h in the range of 22–63 μM, whereas β-lapachone had a higher value of 391.5 ± 16.5 μM.

1. Introduction

Trypanosoma cruzi is the etiologic agent of Chagas disease, an endemic parasitosis in Latin America with 12–14 million people infected [1]. Acute infections are usually asymptomatic, but the ensuing chronic *T. cruzi* infections have been associated with high rates of morbidity and mortality. Recently, Chagas disease has also been recognized as an opportunistic disease in HIV-infected individuals [2] in addition to being reported throughout the world due to international immigration [3]. At present, the only two nitro derivative drugs, introduced in the 1960s and 1970s, are available for the treatment of chagasic patients: nifurtimox (Lampit®) and benznidazole (Rochagan® or Radanil®). They are effective for acute infections, but their undesirable side effects and controversial use for chronic patients have been forcing the abandonment of the treatment [4,5]. Due to these inadequacies, an intense research program has been directed to find alternative drugs for the treatment of chagasic patients [5]. As also noticed in other neglected sicknesses, drugs for Chagas disease are not of interest for pharmaceutical industries [6].

Naphthoquinones are present in several families of higher plants and are considered privileged structures in medicinal chemistry [7]. In folk medicine, plants containing naphthoquinones are usually employed for the treatment of different diseases [8]. Great interest is present in the study and mechanisms of the action of compounds with quinoidal structures, in addition to their widespread presence in nature, for several different roles in organisms. Naphthoquinones serve as vital links in the electron transport chains in metabolic pathways, participating in multiple biological oxidative processes [9], leading to a depletion of the levels of NADH and/or NAD(P)H and ATP levels [10]. Among the natural naphthoquinones, β-lapachone (Fig. 1) has been used in popular medicine for centuries against several diseases [11]. Its trypanocidal activity [12] was investigated as a possible candidate for the treatment of Chagas disease, but research with this compound has been discarded due to its high human cytoxicity. In this regard, its adverse cytoxicity was explored at micromolar concentrations against a variety of *in vitro* tumour cells revealing potential compounds for cancer therapy. Indeed, β-lapachone is in clinical trials with the name ARQ501 for cancer chemotherapy [13]. New derivatives or analogues of this compound are of great interest. Indeed, it has been demonstrated that trypanocidal activities change by selectively transforming the *ortho*-quinone moieties. Recently, many derivatives and analogues of β-lapachone

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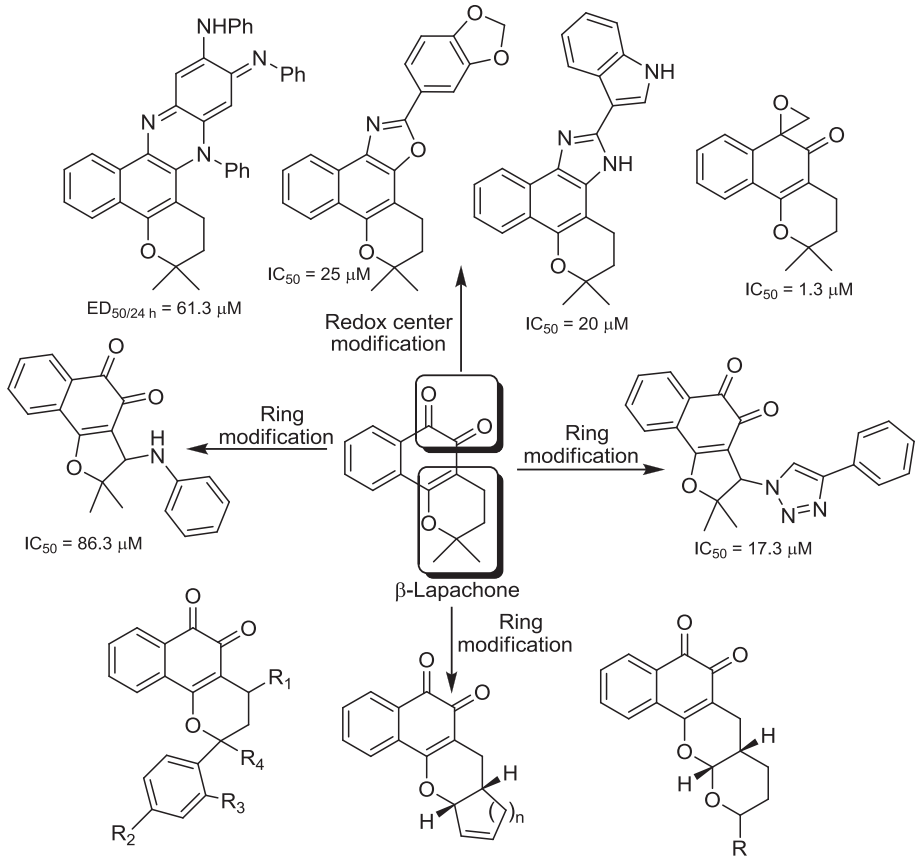


Fig. 1. Chemical structures of potent trypanocidal compounds structurally related to β-lapachone.

with significant trypanocidal activity have been reported by our group. Some of them have been obtained by modification by replacing the *ortho*-quinone moiety with heterocycles (phenazine, oxazoles, imidazoles [14], oxyranes [15]) and others by modification of the size of the ring and introductory substituents such as 1,2,3-triazole (Fig. 1) [16]. The data described in the literature clearly show that the *ortho*-naphthoquinone framework is important, and the pyran ring has important significance for the development of new substances with promising trypanocidal activity [17].

2. Results and discussion

2.1. Chemistry

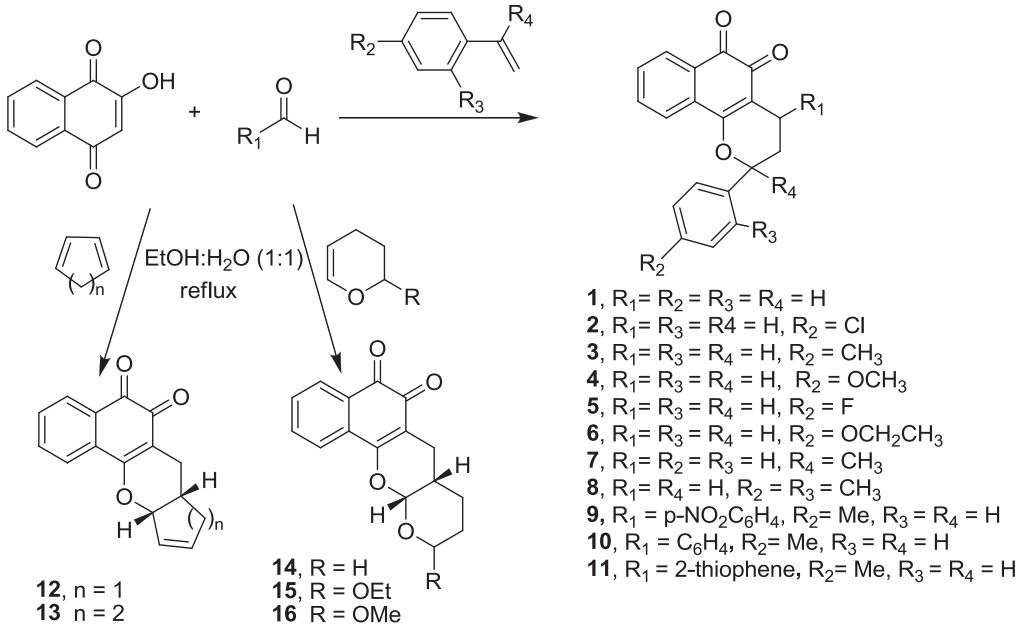
In a previous communication, we reported the synthesis of six β-lapachone analogues by Knoevenagel condensation of 2-hydroxy-1,4-naphthoquinone with paraformaldehyde or arylaldehydes followed by a hetero-Diels–Alder reaction with substituted styrenes in aqueous ethanol media, generating six β-lapachone analogues [18]. In the present work, 16 derivatives were synthesized and assayed against the infective bloodstream form of *T. cruzi*.

Due to the easy access to natural sources of quinones from Brazilian flora and the facile synthetic routes previously developed by our group (AVP and VFF) [19], naphthoquinones were used as the starting points for screening trypanocidal drugs.

The best methodology for the synthesis of pyran-naphthoquinones is sequential reactions of *ortho*-quinone methides (*o*-QMs) followed by an intramolecular hetero-Diels–Alder reaction. *o*-QMs are useful reactive intermediates in organic synthesis and are involved in a large number of chemical reactions and biological processes [20]. They are usually unstable intermediates that must be generated *in situ*; however, some of them may be sufficiently stable to be isolated, depending on their structural arrangement [21]. The first example of the generation and use of an *o*-QM in intramolecular a hetero-Diels–Alder reaction was reported by Brougidou et al. [22]. Following this discovery, many studies demonstrated that these hetero-diene moieties are suitable for [4 + 2] cycloadditions with a wide range of dienophiles. In this context, our group reported the reaction of citronellal with 2-hydroxy-1,4-naphthoquinone (lawsone), in which the *o*-QM intermediate was generated *in situ* by a Knoevenagel reaction that upon the hetero-Diels–Alder cycloaddition formed tetracyclic α- and β-pyranonaphthoquinones [23]. The Nair's group reported in a series of papers the study of the reactivity and use of several *o*-QMs [24], overcoming the limitation regarding the use of aldehydes with dienophiles in the same structure, and it was used for the synthesis of several derivatives of α- and β-lapachone and other heterocyclic compounds. Despite its scope, this three-component reaction is still limited to the use of formaldehyde, whereas aromatic aldehydes do not react under these conditions, and most of them produce xanthenes instead of Diels–Alder adducts.

Following this synthetic protocol, the β-naphthoquinones were prepared in one step by an improved methodology recently reported by our group. Compounds **1**–**16** were obtained from methylene and aryl *o*-quinone methides (*o*-QMs) generated *in situ* by the Knoevenagel condensation of lawsone with paraformaldehyde or arylaldehydes followed by a hetero-Diels–Alder reaction with different dienophiles in ethanol/water (1:1) under reflux (Scheme 1).

The reaction produced a mixture of α and β isomers in good chemical yields (Table 1). The separation of compounds was carried



Scheme 1. Synthetic route for preparing the β-lapachone analogues (**1**–**16**).

out on silica gel column chromatography. For reactions that produced disubstituted naphthoquinones, the crude product was composed of *syn*- and *anti*-diastereoisomers. In most cases, the proportion of the *anti*-isomer was higher than the *syn*-isomer.

The structures of the synthesized compounds were confirmed by spectroscopic techniques, such as ^{1}H and ^{13}C NMR, infrared spectroscopy, and HRMS (ESI).

The ^{1}H NMR spectra mainly showed signals between 5.07 and 5.35 ppm for the β compounds for the hydrogen presented in the *ortho*-carbon of the chromogenic ring, which appeared as a doublet of a doublet. The aromatic region in the ^{1}H NMR spectrum was used for differentiating the α- and β-naphthoquinones. The aromatic hydrogens of the β-naphthoquinones are split into four signals, while those in the α-naphthoquinones are only split into two signals due to the symmetry of the structures. The ^{13}C NMR spectra also showed the formation of the desired products by the signals between 75.0 and 79.9 ppm due to the differences at carbon 1 of the chromogenic ring.

Table 1
Activity of the compounds against bloodstream trypomastigote forms of *T. cruzi*.

Compound	IC_{50}/24 h[a] (μM)
1	56.0 ± 5.3
2	20.2 ± 4.0
3	51.4 ± 14.9
4	121.4 ± 24.7
5	97.6 ± 11.8
6	132.2 ± 19.2
7	122.9 ± 25.3
8	687.5 ± 71.3
9	>4000
10	849.2 ± 79.8
11	1859.4 ± 201.3
12	62.6 ± 5.5
13	161.1 ± 19.6
14	35.4 ± 12.2
15	330.9 ± 45.3
16	880.3 ± 71.9
Benznidazole	103.6 ± 0.6
β-Lapachone	391.5 ± 16.5

[a] Mean ± standard deviation of at least three independent experiments.

For the reaction that used α-methyl styrene as the dienophile (**7**), the signal in the region between 5.00 and 6.00 ppm corresponding to the hydrogen present on the *ortho*-carbon of the chromogenic ring was not observed. However, the quaternary carbon was observed at 100.6 ppm in the NMR of ^{13}C-APT. The *cis*-configuration of compounds **12**–**16** was determined based on the coupling constants in the range of 3.9 Hz between the pyran hydrogens.

2.2. Trypanocidal activity of prepared compounds

The compounds **1**–**16** were assayed for the first time against bloodstream trypomastigotes of *T. cruzi* and were more active than β-lapachone (IC_{50}/24 h = 391.5 ± 16.5 μM), a very well-known trypanocidal natural product (Table 1) [25].

In general, naphthoquinones derived from formaldehyde presented better results than those from arylaldehydes, indicating that the presence of a large group significantly decreases the trypanocidal activity. Analyzing the relative potency of the tested compounds in comparison to the benznidazole, excellent lytic activities were noted for compounds **1**, **2**, **3**, **12** and **14** (Fig. 2), which were 2- to 5-fold more active, while **4**–**7** displayed activity similar to that of the standard drug (IC_{50}/24 h = 103.6 ± 0.6 μM) [26].

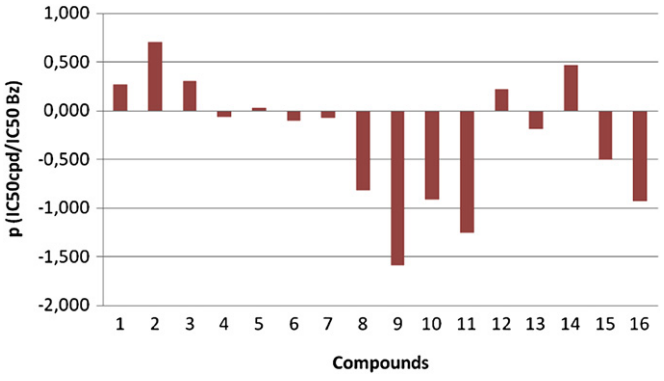


Fig. 2. Graphic representation of the relative potency of the naphthoquinones to benznidazole.

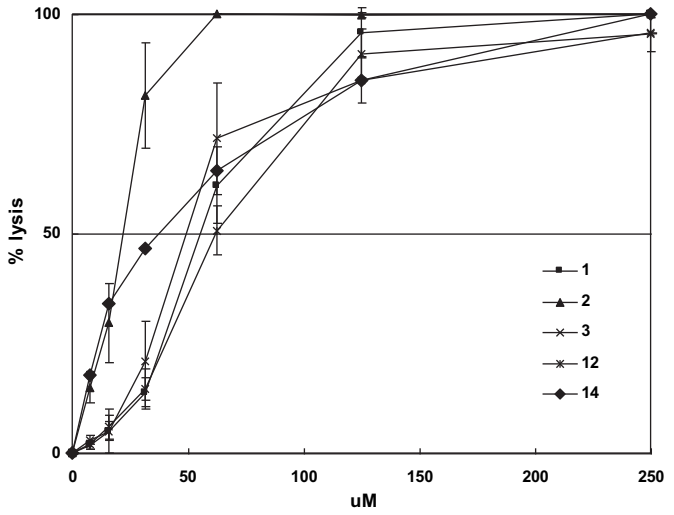


Fig. 3. Graphic representation of the effect of the most active naphthoquinones against trypomastigotes of *T. cruzi*.

Fig. 3 depicts the dose-dependent effect of the most active derivatives against bloodstream trypomastigotes of *T. cruzi*, displaying compound **2**, containing a chlorine atom in position 4 of the aryl group, with the highest activity. The nonsubstituted (**1**) and the methyl (**3**) analogues were both two times less active than **2**, while the substitution with a fluorine (**5**), methoxy (**4**) or ethoxy (**6**) group led to a decrease of 4 fold in the trypanocidal activity. Interestingly, the addition of a methyl group in position 2 of the aryl group led to a dramatic reduction of such activity. The exchange of hydrogen by the methyl group in the R_4 position decreased the activity, as shown by the result of compound **7**. In addition, when R_1 is an aromatic radical as in **9**–**11**, there was a marked decrease in activity. However, the synthesis of naphthoquinone fused with two pyran rings was obtained, and another prototype with excellent activity was found, as is the case for compound **14**. However, the inclusion of the groups at position 2 in the second pyran ring dramatically decreased the activity, as seen in **15** (ethoxy) and **16** (methoxy). Comparing the results of activity of new 1,2-naphthoquinones obtained in this study with the IC_{50} previously described for naphthoquinones of literature, we obtained compounds with activity similar or better than those described in the literature with the advantage of obtaining potentially active compounds by a simple synthetic route in a single step in good yields.

3. Conclusion

Plants containing naphthoquinones are still employed in folk medicine, and their molecular structure endows them with redox properties that could interfere in biological oxidative processes. Due to the previously described lytic effect upon bloodstream trypomastigotes of *T. cruzi*, 16 new derivatives were prepared and assayed against the parasite. The compounds presented a broad spectrum of activity, presenting five derivatives IC_{50}/24 h in the range of 20 to higher than 4000 μM, compound **2** being the most active. These analogues appear as interesting new prototype compounds, and experiments are currently underway in our laboratory to investigate their mode of action, especially for free radical generation, possibly opening new perspectives to the development of more potent and selective trypanocidal drugs. The most active compounds will be further investigated in relation to their mode of action and *in vivo* effect.

4. Experimental section

4.1. General procedures

Melting points were observed on a Fischer Jones and are uncorrected. Analytical grade solvents were used. Reagents were purchased from Aldrich or Acros Chemical Co. Column chromatography was performed on silica gel 60 (Merck 70–230 mesh). Yields refer to purified compounds obtained by chromatography techniques and confirmed by spectroscopic data. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm. E. Merck silica gel plates (60F-254) using UV light as visualizing agent and either an ethanolic solution of sulphate. Infrared spectra were recorded on a Perkin–Elmer FT-IR Spectrum One spectrophotometer, calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene. NMR spectra were recorded on a Varian Unity Plus VXR (300 MHz) equipment in DMSO-d_6 and $CDCl_3$ solutions and tetramethylsilane was used as the internal standard ($\delta = 0$ ppm). High resolution mass spectra (HRMS) were recorded on an MICROMASS Q-TOF MICRO Mass spectrometer using ESI-TOF (electrospray ionization-time of flight).

4.2. General preparation of ***1*–*16***

To a round-bottom flask equipped with a magnetic stirring bar lawsone was dissolved (1 mmol) with water (10 mL) and ethanol (10 mL). Then, the appropriate aldehyde (8 mmol for paraformaldehyde or 3 mmol for arylaldehydes) was added. Styrenes substituted (3 mmol) was added drop-wise and reaction mixture was stirred under reflux until consumption of the starting material. The ethanol was removed under reduced pressure, ethyl acetate was added in the residue and mixture was washed with saturated aqueous solution of sodium bicarbonate. The combined organic extracts washed with water, and dried over anhydrous sodium sulphate, was filtered and concentrated under pressure reduced. The residual crude product was purified via silica-gel chromatography, using gradient mixture of hexane–ethyl acetate.

4.2.1. 2-Phenyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (*1*)

Compound **1** was obtained as an orange solid, 23% yield, m.p. = 161–163 °C. IR (KBr, cm^{-1}): ν 1696, 1647, 1605,1573, 1397, 1300, 1280, 1232, 1158, 1093, 922, 700; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.08 (1H, dddd, $J = 2.7$, 3.2, 5.6 and 13.8 Hz, H-3a), 2.33 (1H, dddd, $J = 3.4$, 6.3, 7.4 and 13.8 Hz, H-3b), 2.60 (1H, ddd, $J = 3.2$, 6.3 and 8.8 Hz, H-4a), 2.76 (1H, ddd, $J = 3.4$, 5.6 and 8.8 Hz, H-4b), 5.27 (1H, dd, $J = 2.7$ and 7.4 Hz, H-2), 7.39–7.46 (5H, m, 2-*phenyl*), 7.53 (1H, ddd, $J = 1.0$, 7.4 and 8.6 Hz, H-8), 7.64 (1H, ddd, $J = 1.4$, 7.6 and 9.1 Hz, H-9), 7.83 (1H, dd, $J = 1.0$ and 7.6 Hz, H-10), 8.01 (1H, dd, $J = 1.4$ and 7.6 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.2 (C-4), 28.2 (C-3), 79.9 (C-2), 113.8 (C-4a), 123.9 (C-10), 125.6 (C-7), 125.7 (C-4′-*phenyl*), 128.4 (C-8), 128.5 (C-2′-*phenyl*), 128.6 (C-3′-*phenyl*), 129.8 (C-6a), 130.6 (C-9), 131.9 (C-1′-*phenyl*), 139.2 (C-10a), 162.7 (C-10b), 178.7 and 179.0 (C-5 and C-6). HRMS (ESI) calcd for $C_{19}H_{14}O_3$: 290.0943, Found: 290.0944.

4.2.2. 2-(4-Chlorophenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (*2*)

Compound **2** was obtained as an orange solid, 25% yield, m.p. = 143–145 °C. IR (KBr, cm^{-1}): ν 1697, 1647, 1574, 1607, 1393, 1291, 1092, 923, 774, 725; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.01 (1H, dddd, $J = 2.4$, 3.5, 5.8 and 12.9 Hz, H-3a), 2.32 (1H, dddd, $J = 3.3$, 6.5, 7.8 and 12.9 Hz, H-3b), 2.63 (1H, ddd, $J = 3.5$, 6.5 and 8.9 Hz, H-4a); 2.74 (1H, ddd, $J = 3.3$, 5.8 and 8.8 Hz, H-4b); 5.24 (1H, dd, $J = 2.4$ and 7.8 Hz, H-2), 7.36–7.45 (4H, m, H-ar), 7.53 (1H, ddd, $J = 1.2$, 7.5 and 8.5 Hz, H-8), 7.64 (1H, ddd, $J = 1.4$, 7.5 and 9.0 Hz, H-9), 7.83 (1H, dd,

J = 1.2 and 7.8 Hz, H-10), 8.01 (1H, dd, J = 1.9 and 8.0 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.2 (C-4), 28.2 (C-3), 79.2 (C-2), 113.8 (C-4a), 123.8 (C-10), 127.1 (C-7), 128.6 (C-8), 128.8 (C-2′), 129.8 (C-6a), 130.7 (C-3′), 131.9 (C-9′), 134.2 (C-4′), 134.8 (C-1′), 137.7 (C-10a), 162.5 (10b), 178.3 and 179.2 (C-5 and C-6). HRMS (ESI) calcd for $C_{21}H_{18}O_3$: 324.0553, Found: 324.8102.

4.2.3. 2-p-tolyl-3,4-Dihydro-2H-benzo[h]chromene-5,6-dione (**3**)

Compound **3** was obtained as an orange solid, 20% yield, m.p. = 165–167 °C. IR (KBr, cm^{-1}): ν 1697, 1647, 1605, 1590, 1572, 1393, 1301, 1280, 1158, 1076, 922, 771; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.06 (1H, dddd, J = 2.4, 3.6, 5.6 and 12.7 Hz, H-3a), 2.31 (1H, dddd, J = 3.1, 6.2, 7.8 and 12.7 Hz, H-3b), 2.40 (3H, s, CH_3), 2.59 (1H, ddd, J = 3.6, 6.2 and 8.7 Hz, H-4a), 2.77 (1H, ddd, J = 3.1, 5.6 and 8.7 Hz, H-4b), 5.24 (1H, dd, J = 2.4 and 7.8 Hz, H-2), 7.25 (2H, d, J = 7.7 Hz, H-*meta tolyl*), 7.32 (2H, d, J = 7.7 Hz, H-*ortho tolyl*), 7.51 (1H, ddd, J = 1.4, 7.5 and 8.7 Hz, H-8), 7.62 (1H, ddd, J = 1.4, 7.5 and 9.0 Hz, H-9), 7.81 (1H, dd, J = 1.2 and 7.8 Hz, H-10), 8.01 (1H, dd, J = 1.4 and 7.5 Hz, H-7); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.7 (C-4), 21.4 (CH_3), 28.6 (C-3), 80.4 (C-2), 114.3 (C-4a), 124.3 (C-10), 126.1 (C-7), 128.9 (C-8), 129.7 (C-2′-*phenyl*), 130.2 (C-6a), 131.0 (C-3′-*phenyl*), 132.4 (C-9′), 135.1 (C-1′-*phenyl*), 136.7 (C-4′-*phenyl*), 138.7 (C-10a), 163.2 (C-10b), 178.8 and 179.8 (C-5 and C-6). HRMS (ESI) calcd for $C_{20}H_{16}O_3$: 304.1099, Found: 304.1012.

4.2.4. 2-(4-Methoxyphenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**4**)

Compound **4** was obtained as an orange solid, 17% yield, m.p. = 155–157 °C. IR (KBr, cm^{-1}): ν 1605, 1572, 1525, 1280, 1249, 1170, 921, 774; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.11 (1H, dddd, J = 2.4, 3.6, 5.7 and 13.0 Hz, H-3a); 2.31 (1H, dddd, J = 3.2, 6.2, 7.8 and 13.0 Hz, H-3b); 2.61 (1H, ddd, J = 3.6, 6.2 and 8.7 Hz, H-4a), 2.76 (1H, ddd, J = 3.2, 5.7 and 8.7 Hz, H-4b), 3.85 (3H, s, OCH_3), 5.20 (1H, dd, J = 2.4 and 7.8 Hz, C-2), 6.96–6.99 (2H, m, H-ar), 7.34–7.37 (2H, m, H-ar), 7.51 (1H, ddd, J = 1.4, 7.5 and 8.7 Hz, H-8), 7.60 (1H, ddd, J = 1.4, 7.5 and 9.0 Hz, H-9), 7.80 (1H, dd, J = 1.2 and 7.8 Hz, H-10), 8.08 (1H, dd, J = 1.4 and 7.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.4 (C-4), 28.0 (C-3), 55.2 (OCH_3), 79.9 (C-2), 113.8 (C-4a), 114.0 (C-10), 123.9 (C-7), 127.2 (C-8), 128.5 (C-2′), 129.8 (C-6a), 130.6 (C-3′), 131.2 (C-9′), 132.0 (C-10a), 134.7 (C-1′), 159.6 (C-4′), 162.9 (C-10b), 178.4 and 179.4 (C-5 and C-6). HRMS (ESI) calcd for $C_{21}H_{18}O_3$: 320.1049, Found: 320.3596.

4.2.5. 2-(4-Fluorophenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**5**)

Compound **5** was obtained as an orange solid, 26% yield, m.p. = 142–147 °C. IR (KBr, cm^{-1}): ν 1573, 1607, 1513, 1394, 1280, 1229, 1156, 922; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.07 (1H, dddd, J = 2.4, 3.2, 5.6 and 12.8 Hz, H-3a), 2.34 (1H, dddd, J = 3.1, 6.5, 7.8 and 12.8 Hz, H-3b), 2.61 (1H, ddd, J = 3.2, 6.5 and 8.7 Hz, H-4a), 2.75 (1H, ddd, J = 3.1, 5.6 and 8.7 Hz, H-4b), 5.24 (1H, dd, J = 2.4 and 7.8 Hz, H-2), 7.15 (2H, t, J = 8.5 Hz, 2CHar-F), 7.39–7.44 (2H, m, H-ar), 7.53 (1H, ddd, J = 1.2, 7.2 and 8.7 Hz, H-8), 7.64 (1H, ddd, J = 1.4, 7.8 and 9.2 Hz, H-9), 7.83 (1H, dd, J = 1.1 and 6.5 Hz, H-10), 8.01 (1H, dd, J = 1.1 and 6.3 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.3 (C-4), 28.3 (C-3), 79.4 (C-2), 113.8 (C-4a), 115.4 and 115.7 (d, J = 21.4 Hz, C-3′), 123.8 (C-10), 127.5 (C-7), 127.6 (C-8), 128.6 (C-1′), 129.8 (C-6a), 130.7 (C-3′), 131.8 (C-2′), 134.7 (C-9′), 135.7 (C-4′), 137.7 (C-10a), 162.6 (C-10b), 178.4 and 179.2 (C-5 and C-6). HRMS (ESI) calcd for $C_{21}H_{18}O_3$: 308.0849, Found: 308.3243.

4.2.6. 2-(4-Ethoxyphenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**6**)

Compound **6** was obtained as an orange solid, 22% yield, m.p. = 145–147 °C. IR (KBr, cm^{-1}): ν 1610, 1642, 1601, 1390, 1300, 1365, 939. ^{1}H NMR ($CDCl_3$, 300 MHz): 1.45 (3H, dd, J = 1.8 and 6.2 Hz, OCH_2CH_3), 2.15 (1H, dddd, J = 2.1, 3.7, 6.1 and 12.5 Hz, H-3a), 2.22 (1H, dddd, J = 3.5, 6.5, 7.3 and 12.5 Hz, H-3b), 2.58 (1H, ddd, J = 3.7, 6.5 and 9.1 Hz, H-4a), 2.69 (1H, ddd, J = 3.5, 6.1 and 9.1 Hz H-4b), 4.09 (2H, dd, J = 1.8 and 6.2 Hz, OCH_2CH_3), 5.11 (1H, dd, J = 2.1 and 7.3 Hz, H-2), 7.05–7.15 (2H, m, H-ar), 7.40–7.47 (2H, m, H-ar), 7.53 (1H, ddd, J = 1.5, 7.7 and 9.2 Hz, H-8), 7.59 (1H, ddd, J = 1.5, 7.7 and 9.2 Hz, H-9), 7.83 (1H, dd, J = 1.2 and 7.7 Hz, H-10), 8.12 (1H, dd, J = 1.5 e 7.7 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.4 (CH_3), 19.4 (C-4), 27.0 (C-3), 64.2 (OCH_2CH_3), 74.5 (C-2), 115.8 (C-4a), 115.0 (C-10), 124.9 (C-7), 125.1 (C-8), 129.2 (C-2′), 129.6 (C-6a), 130.2 (C-3′), 132.4 (C-9′), 131.9 (C-10a), 134.5 (C-1′), 160.6 (C-4′), 163.1 (C-10b), 179.4 and 180.2 (C-5 and C-6). HRMS (ESI) calcd for $C_{21}H_{18}O_4$: 334.1205, Found: 334.1502.

4.2.7. 2-Methyl-2-phenyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**7**)

Compound **7** was obtained as an orange solid, 20% yield, m.p. = 155–157 °C. IR (KBr, cm^{-1}): ν 1606, 1647, 1603, 1387, 1305, 1359, 919. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.78 (3H, s, CH_3), 2.05 (1H, ddd, J = 3.1, 5.3 and 12.9 Hz, H-3a), 2.24 (1H, ddd, J = 3.0, 6.0 and 12.9 Hz, H-3b), 2.42 (1H, ddd, J = 3.1, 6.0 and 7.9 Hz, H-4a), 2.60 (1H, ddd, J = 3.0, 5.3 and 7.9 Hz, H-4b), 7.29–7.38 (5H, m, 2-*phenyl*), 7.55 (1H, ddd, J = 1.2, 7.5 and 9.0 Hz, H-8), 7.71 (1H, ddd, J = 1.2, 7.5 and 9.0 Hz, H-9), 8.00 (1H, dd, J = 1.0 and 7.5 Hz, H-10), 8.10 (1H, dd, J = 1.0 and 7.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.5 (C-4), 29.4 (CH_3), 31.1 (C-3), 82.9 (C-2), 114.2 (C-4a), 124.1 (C-10), 124.2 (C-7), 127.8 (C-4′-*phenyl*), 129.0 (C-8), 129.1 (C-2′-*phenyl*), 131.0 (C-3′-*phenyl*), 135.2 (C-1′-*phenyl*), 130.3 (C-6a), 132.4 (C-10a), 143.2 (C-9), 161.9 (10b), 178.6 and 179.7 (C-5 and C-6). HRMS (ESI) calcd for $C_{20}H_{16}O_3$: 304.1099, Found: 304.3501.

4.2.8. 2-(2,4-Dimethylphenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**8**)

Compound **8** was obtained as an orange solid, 27% yield, m.p. = 164–167 °C. IR (KBr, cm^{-1}): ν 1694, 1646, 1603, 1574, 1394, 1283, 1231, 1160, 914, 728. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.07 (1H, dddd, J = 2.4, 2.8, 5.6 and 11.8 Hz, H-3a), 2.31 (1H, dddd, J = 3.7, 6.5, 7.8 and 11.8 Hz, H-3b), 2.36 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.63 (1H, ddd, J = 2.8, 6.5 and 8.0 Hz, H-4a), 2.85 (1H, ddd, J = 3.7, 5.6 and 8.0 Hz, H-4b), 5.35 (1H, dd, J = 2.4 and 7.8 Hz, C-2), 7.08–7.13 (2H, m, H-ar), 7.33 (1H, d, J = 7.8 Hz, H-ar), 7.51 (1H, ddd, J = 0.9, 7.5 and 8.7 Hz, H-8), 7.61 (1H, ddd, J = 1.4, 7.5 and 9.0 Hz, H-9), 7.71 (1H, dd, J = 0.9 and 7.8 Hz, H-10), 8.06 (1H, dd, J = 1.4 and 7.5 Hz, H-7). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.2 (CH_3), 19.2 (C-4), 21.2 (CH_3), 27.5 (C-3), 77.9 (C-2), 114.2 (C-4a), 124.3 (C-10), 125.8 (C-7), 127.3 (C-4′-*phenyl*), 128.9 (C-8), 130.9 (C-2′-*phenyl*), 131.8 (C-3′-*phenyl*), 134.6 (C-1′-*phenyl*), 130.2 (C-6a), 132.4 (C-10a), 163.5 (10b), 178.7 and 179.8 (C-5 and C-6). HRMS (ESI) calcd for $C_{21}H_{18}O_3$: 318.1256, Found: 318.3792.

4.2.9. 4-(4-Nitrophenyl)-2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**9**)

Compound **9** was obtained as an orange solid, 24% yield, m.p. = 232–235 °C. IR (KBr, cm^{-1}): 1696, 1645, 1600, 1570, 1513, 1344, 1287, 1233, 1168, 1091, 912, 736, 702. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (1H, dt, J = 2.4 and 14.4 Hz, H-3a *syn isomer*) and 2.27 (1H, dt, J = 11.2 and 14.4 Hz, H-3a *anti isomer*), 2.38 and 2.39 (3H, s, CH_3), 2.55 (1H, ddd, J = 5.9, 12.0 and 14.4 Hz, H-3b *syn isomer*) and 2.61 (1H, ddd, J = 2.2, 7.1 and 14.4 Hz, H-3b *anti isomer*), 4.31 (1H, dd, J = 7.1 and 11.0 Hz, H-4 *anti isomer*) and 4.48 (1H, dd, J = 2.4 and 5.9 Hz, H-4 *syn isomer*), 5.07 (1H, dd, J = 2.4 and 12.0 Hz, H-2 *syn isomer*) and 5.31 (1H, dd, J = 2.2 and 11.2 Hz, H-2 *anti isomer*), 7.25 (1H, d, J = 8.1 Hz, H-*meta p-tolyl*), 7.35 (1H, d, J = 8.1 Hz, H-*ortho p-tolyl*), 7.38 and 7.47 (1H, d, J = 8.8 Hz, H-*ortho 4-nitrophenyl*), 7.56–7.94 (1H, m, H-7), 7.56–7.94 (1H, m, H-8), 8.11 (1H, d,

J = 8.8 Hz, H-*meta 4-nitrophenyl*), 8.09–8.23 (1H, m, H-9), 8.09–8.23 (1H, m, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1 (CH_3), 34.9 and 38.4 (C-4), 36.7 and 39.8 (C-3), 80.0 (C-2), 115.9 (C-4a), 123.8 and 123.9 (C-3′ *4-nitrophenyl*), 124.0 (C-2′ *4-nitrophenyl*), 125.9 and 126.0 (C-3′ *p-tolyl*), 127.7 (C-8), 128.6 (C-7), 128.8 and 129.0 (C-2′ *p-tolyl*), 129.5 (C-10), 130.3 (C-6a), 130.4 (C-1′ *p-tolyl*), 131.3 (C-9), 131.7 (C-4′ *p-tolyl*), 135.0 (C-10a), 138.9 (C-4′ *4-nitrophenyl*), 151.1 (C-1′ *4-nitrophenyl*), 164.9 (C-10b), 177.6 (C-5), 178.7 (C-6). HRMS (ESI) calcd for $C_{26}H_{19}NO_5H^+$: 426.1336, Found: 426.4483.

4.2.10. Phenyl-2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**10**)

Compound **10** was obtained as an orange solid, 33% yield, m.p. = 92–95 °C. IR (KBr, cm^{-1}): 1696, 1653, 1600, 1568, 1382, 1284, 1231, 1164, 1086, 909, 767, 699. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13–2.37 (1H, m, H-3a), 2.38 and 2.39 (3H, s, CH_3), 2.59 (1H, ddd, *J* = 2.0, 7.3 and 14.4 Hz, H-3b), 4.20 (1H,dd, *J* = 7.1 and 11.2 Hz, H-4 *anti isomer*) and 4.42 (1H, dd, *J* = 1.7 and 5.4 Hz, H-4 *syn isomer*), 5.15 (1H, dd, *J* = 2.7 and 12.0 Hz, H-2 *syn isomer*) and 5.24 (1H, dd, *J* = 1.7 and 12.0 Hz, H-2 *anti* isomer), 7.13–7.38 (4H, m, *p-tolyl*), 7.13–7.38 (5H, m, Ph), 7.52–7.60 (1H, m, H-7), 7.64–7.70 (1H, m, H-8), 7.88–7.92 (1H, m, H-9), 8.07–8.10 (1H, m, H-6 *anti isomer*) and 8.12–8.15 (1H, m, H-6 *syn isomer*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1 (CH_3), 34.6 and 38.5 (C-4), 40.6 (C-3), 80.1 (C-2), 117.5 (C-4a), 124.5 (C-3′ Ph), 126.0 and 126.1 (C-3′ *p-tolyl*), 126.3 (C-2′ Ph), 126.7 and 126.8 (C-4′ Ph), 128.4 (C-8), 128.5 and 128.6 (C-7), 129.2 and 129.3 (C-2′ *p-tolyl*), 130.2 (C-6a), 130.8 (C-10), 132.1 (C-1′ *p-tolyl*), 134.8 (C-9), 135.6 (C-4′ *p-tolyl*), 138.6 (C-10a), 143.3 (C4′Ph), 164.1 (C-10b), 178.0 (C-5), 179.1 (C-6). HRMS (ESI) calcd for $C_{26}H_{20}O_3H^+$: 381.1485, Found: 381.4503.

4.2.11. 4-(Thiophen-2-yl)-2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione (**11**)

Compound **11** was obtained as an orange solid, 33% yield, m.p. = 83–87 °C. IR (KBr, cm^{-1}): 1696, 1652, 1599, 1568, 1383, 1284, 1231, 1170, 1086, 907, 818, 722, 697. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28–2.43 (1H, m, H-3a), 2.40 (3H, s, CH_3), 2.68 (1H, ddd, 2.1, 7.1 and 14.4 Hz, H-3b), 4.57 (1H, dd, *J* = 7.1 and 11.0 Hz) and 4.65 (1H, dd, *J* = 1.0 and 3.2 Hz) H-4 conformers, 5.23 (1H, dd, *J* = 1.9 and 11.5 Hz) and 5.35 (1H, dd, *J* = 4.4 and 10.0 Hz) H-2 conformers, 6.89 and 6.90 (1H, dd, *J* = 1.0 and 3.4 Hz, H-3′ *thiophen-2-yl* conformers), 6.86 and 6.95 (1H, dd, *J* = 3.7 and 5.1 Hz, H-4′ *thiophen-2-yl* conformers), 7.06 and 7.18 (1H, dd, *J* = 1.2 and 5.1 Hz, H-5′ *thiophen-2-yl* conformers), 7.24 and 7.25 (1H, d, *J* = 8.0 Hz, H-*meta* conformers), 7.30 and 7.36 (1H, d, *J* = 8.0 Hz, H-*ortho* conformers), 7.54 and 7.57 (1H, td, *J* = 1.2 and 7.6 Hz, H-8 conformers), 7.65 and 7.67 (1H, dt, *J* = 1.2 and 7.6 Hz, H-9 conformers), 7.90 (1H, d, *J* = 7.6 Hz, H-10), 8.09 and 8.13 (1H, dd, *J* = 1.5 and 7.6 Hz, H-7 conformers). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (CH_3), 30.1 and 33.4 (C-4), 37.1 and 40.9 (C-3), 76.6 and 80.0 (C-2), 115.2 and 117.2 (C-4a), 122.7 and 123.9 (C-5′ *thiophen-2-yl*), 124.5 and 124.6 (C-3′ *p-tolyl*), 125.0 (C-8), 126.0 and 126.1 (C-2′ *p-tolyl*), 126.4 and 126.9 (C-10), 128.6 and 128.7 (C-7), 129.3 (C-4′ *thiophen-2-yl*), 130.2 and 130.3 (C-6a), 130.9 and 131.0 (C-9), 131.7 and 131.9 (C-1′ *p-tolyl*), 134.7 and 134.8 (C-3′ *thiophen-2-yl*), 135.3 and 135.7 (C-4′ *p-tolyl*), 138.5 and 138.6 (C-10a), 146.2 and 146.7 (C-2′ *thiophen-2-yl*), 163.0 and 163.4 (C-10b), 177.7 and 178.0 (C-5), 179.0 (C-6). HRMS (ESI) calcd for $C_{24}H_{18}O_3SH^+$: 387.1049, Found: 387.4789.

4.2.12. (7aS,10aR)-7a,8-Dihydrobenzo[h]cyclopenta[b]chromene-5,6(7H,10aH)-dione (**12**)

Compound **12** was obtained as an orange solid, 15% yield, m.p. = 146–147 °C. IR (KBr, cm^{-1}): 1738, 1716, 1599, 1284, 1036, 1024, 942, 720. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.23–2.32 (1H, m, H-5), 2.47–2.54 (1H, m, H-5), 2.66–2.95 (3H, m, H-4 and H-4a), 5.48–5.50 (1H, m, H-1a), 6.28–6.31 (1H, m H-3), 6.18–6.21 (1H, m H-2), 7.68–7.73 (1H, m, ArH), 7.85–7.87 (2H, m, ArH), 8.01–8.08 (1H, m, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.7 (C-5), 34.5 (C-4a), 37.9 (C-4), 85.0 (C-1a), 112.5 (C-5a), 124.4 (C-3), 135.7 (C-2), 128.5, 131.2, 131.5 and 135.7 (C-ar), 130.3 (C-7a), 132.6 (C-11a), 163.6 (C-11b), 178.4 and 179.8 (C-5 and C-6). HRMS (ESI) calcd for $C_{16}H_{12}O_3$: 252.0786, Found: 252.0897.

4.2.13. (7aS,11aR)-7,7a,8,9-Tetrahydro-5H-benzo[c]xanthene-5,6(11aH)-dione (**13**)

Compound **13** was obtained as an orange solid, 20% yield, m.p. = 142–143 °C. IR (KBr, cm^{-1}): 1604, 1649, 1500, 1204, 1033, 1012, 930, 715. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.62–1.69 (3H, m, H-4 and H-5), 2.17–2.30 (2H, m, H-5 and H-5a), 2.66 (1H, dd, *J* = 6.5 and 11.0 Hz, H-6), 2.50 (1H, dd, *J* = 6.5 and 11.0 Hz, H-6), 4.77 (1H, t, *J* = 3.9 Hz, H-1a), 5.93–5.99 (1H, m, H-3), 6.04–6.09 (1H, m, H-3), 7.49 (1H, ddd, *J* = 1.2, 7.5 and 9.0 Hz, H-8), 7.63 (1H, ddd, *J* = 1.2, 7.5 and 9.0 Hz, H-9), 7.80 (1H, dd, *J* = 0.9 and 7.5 Hz, H-10), 8.05 (1H, dd, *J* = 0.9 and 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.5 (C-4), 23.6 (C-5), 24.7 (C-6), 29.3 (C-5a), 73.8 (C-1a), 112.2 (C-6a), 124.3 (C-3), 124.7 (C-2), 128.8, 130.8, 134.1 and 135.0 (C-ar), 130.2 (C-8a), 132.4 (C-12a), 161.6 (C-12b), 179.9 (C-7 and C-8). HRMS (ESI) calcd for $C_{17}H_{14}O_3$: 266.0943, Found: 266.3040.

4.2.14. (7aS,11aR)-7a,8,9,10-Tetrahydrobenzo[h]pyrano[2,3-b]chromene-5,6(7H,11aH)-dione (**14**)

Compound **14** was obtained as an orange solid, 12% yield, m.p. = 153–155 °C. IR (KBr, cm^{-1}): 1606, 1646, 1588, 1278, 1040, 1020, 950. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54–1.88 (5H, m, H-6, H-5 and H-4), 2.30 (1H, d, *J* = 2.6 Hz, H-5a), 2.52–2.67 (1H, m, H-6a), 3.82–4.02 (2H, m, H-3), 5.58 (1H, d, *J* = 2.6 Hz, H-1a), 7.53 (1H, ddd, *J* = 1.2, 7.5 and 8.7 Hz, H-8), 7.67 (1H, ddd, *J* = 1.2, 7.5 and 9.0 Hz, H-9), 7.90 (1H, dd, *J* = 0.9 and 7.5 Hz, H-10), 8.08 (1H, dd, *J* = 0.9 and 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.3 (C-4 and C-5), 23.6 (C-6), 30.4 (C-5a), 62.1 (C-3), 98.7 (C-1a), 111.5 (C-6a), 129.9 (C-8a), 131.4 (C-12a), 123.9, 128.6, 130.7 and 134.8 (C-ar), 160.9 (C-12b), 178.6 and 179.1 (C-7 and C-8). HRMS (ESI) calcd for $C_{16}H_{14}O_4$: 270.0892, Found: 270.1897.

4.2.15. (7aS,11aS)-10-Ethoxy-7a,8,9,10-tetrahydrobenzo[h]pyrano[2,3-b]chromene-5,6(7H,11aH)-dione (**15**)

Compound **15** was obtained as an orange solid, 20% yield, m.p. = 150–152 °C. IR (KBr, cm^{-1}): 1604, 1653, 1580, 1269, 1032, 920. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (1H, 3H, dd, *J* = 1.9 and 6.5 Hz, $OCH_2\underline{CH_3}$), 1.55–1.91 (5H, m, H-6, H-5 and H-4), 2.26 (1H, d, *J* = 2.5 Hz, H-5a), 2.50–2.57 (1H, m, H-6a), 3.79–4.00 (2H, m, H-3), 4.05 (2H, dd, *J* = 1.9 and 6.5 Hz, $O\underline{CH_2}CH_3$), 5.65 (1H, d, *J* = 2.7 Hz, H-1a), 7.55 (1H, ddd, *J* = 1.3, 7.5 and 8.9 Hz, H-8), 7.67 (1H, ddd, *J* = 1.3, 7.5 and 8.9 Hz, H-9), 7.92 (1H, dd, *J* = 0.9 and 7.5 Hz, H-10), 8.12 (1H, dd, *J* = 0.9 and 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.1 (C-4 and C-5), 23.5 (C-6), 32.0 (C-5a), 60.0 (C-3), 95.7 (C-1a), 111.7 (C-6a), 130.9 (C-8a), 131.3 (C-12a), 120.9, 126.6, 132.7 and 133.9 (C-ar), 162.0 (C-12b), 178.5 and 179.5 (C-7 and C-8). HRMS (ESI) calcd for $C_{18}H_{18}O_5$: 314.1154, Found: 314.2209.

4.2.16. (7aS,11aS)-10-Methoxy-7a,8,9,10-tetrahydrobenzo[h]pyrano[2,3-b]chromene-5,6(7H,11aH)-dione (**16**)

Compound **16** was obtained as an orange solid, 16% yield, m.p. = 149–152 °C. IR (KBr, cm^{-1}): 1606, 1650, 1563, 1245, 1020, 919. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65–1.98 (5H, m, H-6, H-5 and H-4), 2.23 (1H, d, *J* = 2.1 Hz, H-5a), 2.55–2.61 (1H, m, H-6a), 3.80–4.03 (2H, m, H-3), 3.86 (3H, s, $O\underline{CH_3}$), 5.68 (1H, d, *J* = 2.1 Hz, H-1a), 7.51 (1H, ddd, *J* = 1.1, 7.5 and 9.1 Hz, H-8), 7.70 (1H, ddd, *J* = 1.1, 7.5 and 9.1 Hz, H-9), 7.95 (1H, dd, *J* = 0.8 and 7.5 Hz, H-10), 8.15 (1H, dd, *J* = 0.8 and 7.5 Hz, H-7). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.1 (C-4 and

C-5), 23.5 (C-6), 35.0 (C-5a), 58.3 (C-3), 96.8 (C-1a), 110.7 (C-6a), 131.7 (C-8a), 132.3 (C-12a), 121.6, 125.6, 133.7 and 133.9 (C-ar), 165.0 (C-12b), 178.3 and 178.9 (C-7 and C-8). HRMS (ESI) calcd for $C_{17}H_{16}O_5$: 300.0998, Found: 300.1006.

4.3. Trypanocidal assays

Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO), with the final concentration of the latter in the experiments never exceeding 0.1%. Preliminary experiments showed that at concentrations up to 0.5%, DMSO has no deleterious effect on the parasites. Bloodstream trypomastigotes from the Y strain were obtained at the peak of parasitaemia 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 in the presence of 10% of mouse blood. This suspension (100 μL) was added in the same volume of each compound previously prepared at twice the desired final concentrations. Cell counts were performed in Neubauer chamber and the trypanocidal activity was expressed as IC_{50}, corresponding to the concentration that leads to lysis of 50% of the parasites.

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