Full Paper

Synthesis of α- and β-Pyran Naphthoquinones as a New Class of Antitubercular Agents

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A series of α- and β-pyran naphthoquinones (lapachones) have been synthesized and evaluated for their *in-vitro* antibacterial activity against *Mycobacterium tuberculosis* strain H37Rv (ATCC 27294) using the Alamar-Blue susceptibility test; the activity was expressed as the minimum inhibitory concentration (MIC) in μg/mL. The synthetic methodology consisted of the formation of methylene and aryl *o*-quinone methides (*o*-QMs) generated by Knoevenagel condensation of 2-hydroxy-1,4-naphthoquinone with formaldehyde and arylaldehydes. These *o*-QMs then undergo facile hetero Diels–Alder reactions with dienophiles in aqueous ethanol media. Some naphthoquinones exhibited inhibition with MIC values of 1.25 μg/mL, similar to that of pharmaceutical concentrations currently used in tuberculosis treatment. These results justify further research into the value of these quinones as part of an original treatment for tuberculosis.

Keywords: Lapachones / Naphthoquinones / Synthesis / Tuberculostatics

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Introduction

Naphthoquinones are an important class of quinones found in many synthetic and natural products with a wide range of pharmacological activities, such as trypanocidal [1], anticancer [2], antibacterial [3], antifungal [4], leishmanicidal [5], and antimicrobial [6]. Despite the large number of compounds described in the literature, few studies have been described measuring the efficacy of naphthoquinones against tuberculosis [7].

The α- (**1**) and β-lapachones **2** are described in the literature as important naphthoquinones derived from lapachol **3** (Fig. 1). β-Lapachone, one of the most important derivatives of lapachol [8], presents biological activities against various pathogens and, most significantly, also exhibits anticancer activity [9, 10] and trypanocidal properties [11–13]. In view of the importance and variety of biological activities of β-lapachone **2** against various pathogens, it may be an excellent model compound for the development of more selective and more active agents. Perhaps this is why β-lapachone has been one of the most studied naphthoquinones in recent years [14–20].

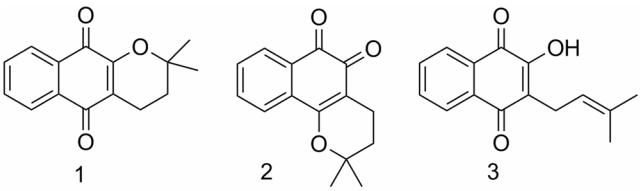

Figure 1. Structures of α- (**1**), β-lapachone **2** and lapachol **3**.

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Abbreviations: multiple drug resistent tuberculosis (MDR-TB); *o*-quinone methides (*o*-QMs); tuberculosis (TB)

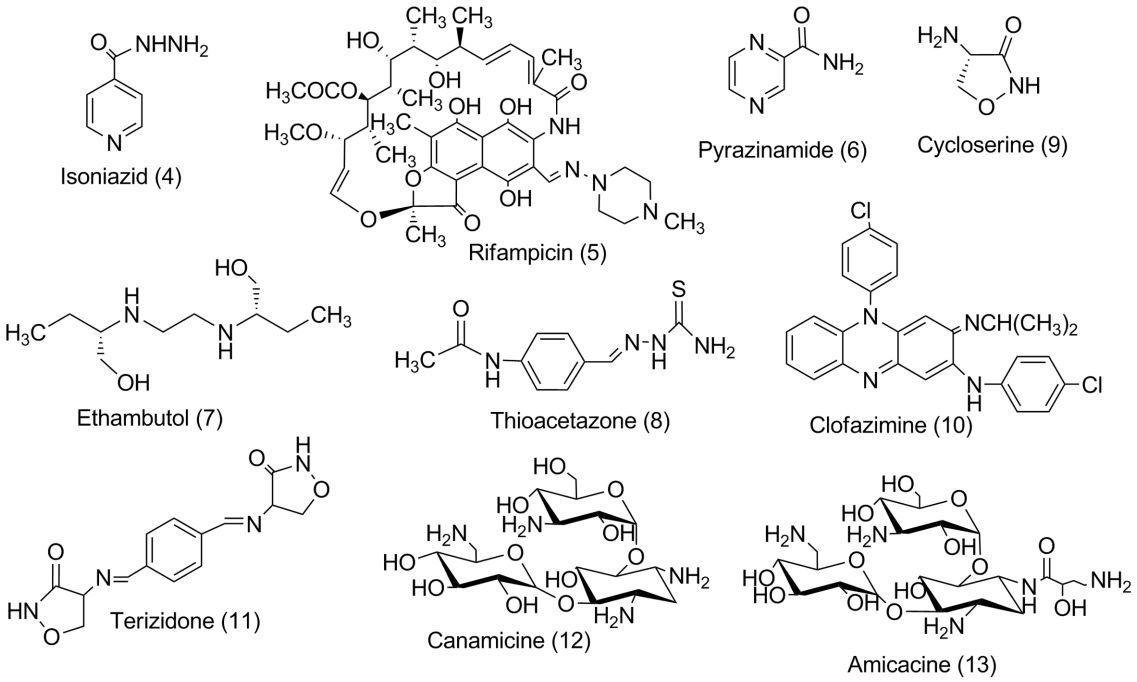
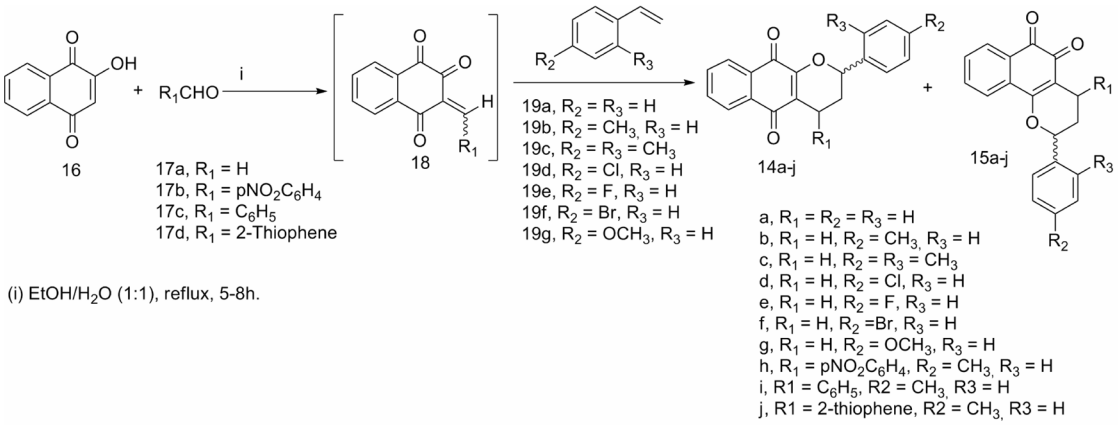


Figure 2. Structures of isoniazid **4**, rifampicin **5**, pyrazinamide **6**, ethambutol **7**, thioacetazone **8**, cycloserine **9**, clofazimine **10**, terizidone **11**, canamicine **12**, and amicacine **13**.

Numerous biological activities of β-lapachone **2** are linked to its ability to generate intracellular reactive oxygen species [21, 22]. For instance, **2** is a potent cytotoxic agent, alone or in combination with other compounds, against several cancer cell lines [23, 24]. The pharmacological mechanism of action remains unclear, despite intense research. Understanding this mechanism requires the recognition that the compound acts on multiple biological targets. In this regard, the trypanocidal, antibacterial, and cytotoxic activities are attributed to the mechanism of formation of reactive oxygen species that cause cellular damage, particularly to DNA [25, 26]. The β-lapachone was semi-synthesized from lapachol **3** by Hooker in 1892. Since then, several other preparations of **2** and analogues have been reported [27 – 30].

Currently, tuberculosis (TB) is a serious worldwide public health problem. In the past, TB was considered a neglected disease because it did not receive significant investment from international pharmaceutical companies for the development of new drugs [31]. While it might be expected that this disease, which was considered a scourge of the 19th and early 20th centuries by developed nations, would have already been eradicated, statistics suggest that one-third of the world's population is currently infected with the TB bacillus, and each year eight million people worldwide develop active TB, of whom about 1.7 million die [32]. Due to the seriousness of the situation, in 1993 the World Health Organization (WHO) declared TB a global emergency in an attempt to increase public awareness of the disease and political will to combat it [33]. Two new factors have contributed to the spread of tuberculosis: the Human Immunodeficiency Virus (HIV) and the emergence of strains of *M. tuberculosis* resistant to one or more drugs (multiple drug resistent – MDR-TB) [34]. It is estimated that one third of the 42 million individuals infected with HIV are co-infected with *M. tuberculosis*, and most people infected with HIV develop TB as the first manifestation of AIDS. As HIV progressively destroys the immune system, there is a greater chance of people infected with the virus developing tuberculosis. The relationship between the two diseases is particularly concentrated in poor countries. In sub-Saharan Africa, for example, about 50% of people with HIV develop TB, and one in three dies as a result of the disease. Furthermore, the increased number of cases of MDR-TB has caused great concern, as it increases the proportion of deaths from TB, and is frequently associated with HIV infection [35]. The presence of multiresistant strains reflects deficiencies in the control of TB, which complicates the treatment and prevention of the disease, contributing to its spread. Although MDR-TB is treatable, it requires a longer course of chemotherapy, with approximately two years of treatment. This entails a high cost, 100 times greater than the treatment of drug-sensitive TB strains (typically conducted in six to nine months) and exposes the patient to increased risk of tox-



Scheme 1. Synthesis of α- and β-pyran naphthoquinones **14a–j** and **15a–j**.

icity. TB-Chemotherapy regimens developed in the 1940s classify available treatments as drugs of first or second choice. The drugs of first choice are isoniazid **4**, rifampicin **5**, pyrazinamide **6**, and ethambutol **7**. The second choice drugs are used when first choices fail, and include thioacetazone **8**, cycloserine **9**, clofazimine **10**, terizidone **11**, canamicine **12**, and amicacine **13** ([36], Fig. 2). However, the emergence of MDR-TB and the indifference on the part of governments, which have invested little in prevention campaigns, in combating the illness, or in the development of new treatments, have precipitated an urgent need for new antitubercular drugs. In light of this goal, our research group has focused on the search for new antitubercular candidates [37].

In this paper, we report our findings on the preparation of α- and β-lapachone derivatives via methylene and aryl *o*-quinone methides (*o*-QMs) generated *in situ* by Knoevenagel condensation of 2-hydroxy-1,4-naphthoquinone with formaldehyde or arylaldehydes, followed by hetero Diels–Alder reaction with substituted styrenes in aqueous ethanol media. We also report results from tests of inhibition of the growth of *M. tuberculosis* strain H37Rv (ATCC 27294).

Table 1. Synthesized α-pyran naphthoquinones.

Entry	R_1	R_2	R_3	Time (h)	Yield (%)
14a	H	H	H	6	75
14b	H	CH_3	H	6	75
14c	H	CH_3	CH_3	6	80
14d	H	Cl	H	4	73
14e	H	F	H	4	74
14f	H	Br	H	4	58
14g	H	OCH_3	H	6	70
14h	4-NO_2Ph	CH_3	H	5	29
14i	Ph	CH_3	H	8	19
14j	2-thiophene	CH_3	H	8	27

Table 2. Synthesized β-pyran naphthoquinones.

Entry	R_1	R_2	R_3	Time (h)	Yield (%)
15a	H	H	H	6	23
15b	H	CH_3	H	6	20
15c	H	CH_3	CH_3	6	17
15d	H	Cl	H	4	25
15e	H	F	H	4	26
15f	H	Br	H	4	19
15g	H	OCH_3	H	6	17
15h	4-NO_2Ph	CH_3	H	5	24
15i	Ph	CH_3	H	8	33
15j	2-thiophene	CH_3	H	8	33

Results and discussion

Chemistry

The preparations of α- and β-pyran naphthoquinones **14a–j** and **15a–j** were carried out in one step by an improved synthetic protocol recently reported by the current authors [38]. Briefly, the Knoevenagel condensation of lawsone **16** with commercially available aldehydes **17a–d**, forming the intermediate **18**, followed by an intermolecular hetero Diels–Alder cycloaddition with styrene derivatives **19a–g** was performed in an ethanol/water mixture (Scheme 1). The naphthoquinones were obtained in a high degree of purity and in good chemical yields with respect to the quantity of aldehydes used. The separations of α- and β-pyran naphthoquinones were carried out via silica gel column chromatography (Tables 1 and 2).

All compounds were identified by IR spectral data, 1-D and 2-D NMR techniques, and by ESI-TOF mass spectrometry. In general, the ^{1}H-NMR spectra mainly showed the signal for the hydrogen presented in the *ortho*-carbon of the chromogenic ring, which appeared as a double of

Table 3. The *in-vitro* activity of compounds **14a–j** in μg/mL (μmol/L) against *M. tuberculosis* H37Rv strain (ATCC 27294) and clogP.

Compound	MIC[c], μg/mL (μmol/L)	cLogP
14a	>100.0 (>344.46)[a]	3.42
14b	>100.0 (>328.58)[a]	3.74
14c	>100.0 (>314.09)[a]	4.05
14d	>100.0 (>307.91)[a]	4.03
14e	>100.0 (>324.36)[a]	3.48
14f	>100.0 (>270.84)[a]	4.12
14g	>100.0 (>312.17)[a]	3.32
14h (*syn*)	>100.0 (>235.06)[a]	5.15
14h (*anti*)	>100.0 (>235.06)[a]	5.15
14i	Nd[b]	5.24
14j (*syn*)	>100.0 (>258.75)[a]	5.08
14j (*anti*)	>100.0 (>258.75)[a]	5.08

[a] MIC > 100.0 μg/mL indicates that the strain is resistant to the tested substance.
[b] Nd = Not detected.
[c] Riphampicin control (MIC = 1.0 μg/mL).

Table 4. The *in-vitro* activity of compounds **15a–j** in μg/mL (μmol/L) against *M. tuberculosis* H37Rv strain (ATCC 27294) and clogP measurements.

Compound	MIC μg/mL (μmol/L)	cLogP
15a	2.5 (8.6)	3.36
15b	1.25 (4.10)	3.68
15c	3.12 (9.80)	4.00
15d	2.5 (7.69)	3.98
15e	2.5 (8.10)	3.42
15f	3.12 (8.45)	4.06
15g	6.25 (19.51)	3.26
15h	2.5 (5.87)	4.36
15i	3.12 (8.20)	5.18
15j	6.25 (16.17)	5.03

doublet signals between 4.99 to 5.35 ppm for the α compounds, and between 5.07 to 5.35 ppm for the β-compounds. The differentiation into derivatives a and β is given mainly by the aromatic region in the ^{1}H-NMR spectrum: in β-pyran naphthoquinones, the aromatic hydrogens are differentiated into four signals, while in the α-pyran naphthoquinones there are only two signals because of the symmetry of this type of compound.

The stereoisomers formation in the Diels–Alder cycloaddition leading to each group of products **14a–j** and **15a–j** are in complete agreement with a [4+2]-cycloaddition of the *o*-QM with the styrene in asynchronous fashion by a zwitterion-like transition state.

For reactions that produced disubstituted naphthoquinones **14h–j** and **15h–j**, a mixture of α- and β-isomers are composed by *syn*- and *anti*-diastereoisomers. In most cases, the amounts of the *anti*-isomers were higher than the *syn*-isomers. Only in the cases of **14h** and **14j** it was possible to separate the *syn*- and *anti*-diastereoisomers by flash column chromatography. The other compounds were used as diastereoisomeric mixtures.

In general, the ^{13}C-NMR spectra of naphthoquinones **14a–j** and **15a–j** were easily distinguished by the signals between 75.0 to 79.9 ppm and 76.6 to 80.4 ppm, respectively, due to differences at carbon-1 of the chromogenic ring.

Pharmacology

The microplate Alamar-Blue Assay was performed as described by Reis *et al.* [39]. The antimycobacterial tests against *M. tuberculosis* ATTC 27294 of the derivatives **14a–j** and **15a–j** were performed using the colorimetric microplate Alamar-Blue[1] assay (MABA) [40], with rifampicin as a positive control (MIC = 1.0 μg/mL). This method consisted of a microdilution in microplates using Alamar Blue as an indicator of cell growth. Alamar Blue is a fluorescent/colorimetric redox indicator that is present in a blue (non-fluorescent) oxidized form in the absence of bacterial growth, while the pink (fluorescent) reduced form indicates the proliferation of bacteria. Compounds showing 90% inhibition in the primary screening were considered active, and were then re-tested at a lower concentration against *M. tuberculosis* (ATCC 27294 H37Rv) in order to determine the actual MIC. The MIC was defined as the lowest drug concentration that prevented a color change from blue (no growth) to pink (growth).

In order to study the hydrophobic properties of these compounds, we calculated values for the log of the octanol/water partition coefficient (cLogP) using the Osiris Property Explorer (www.organic-chemistry.org). These results showed good correlation and proportionality with BACTEC radiometric methods [39]; they are shown in Table 3 and Table 4.

Some of the novel naphthoquinones tested showed high inhibitory activity *in vitro* against *M. tuberculosis* H37Rv (ATCC 27294). While the α-pyran naphthoquinone series **14a–j**, (Table 3) was inactive, the β-pyran naphthoquinone series **15a–j**, (Table 4) were highly active, with MICs in the range of 1.25 to 6.25 μg/mL. The results presented in Table 4 show that compounds **15a**, **15b**, **15d**, **15e**, and **15h**, all of which contain a phenyl group, demonstrated high inhibitory activities, suggesting that the phenyl group is important for *in-vitro* activity.

In order to evaluate this observation, we tested β-lapachone, which has only two methyl groups at the same position of the pyran ring present in the naphthoquinone series. The MIC value obtained for this compound was 12.5 μg/mL, well above the values obtained for compounds in the β-pyran naphthoquinone series. These

results suggest that the presence of a bulky group in position-2 of the pyran ring, such as a phenyl group, is important for antimicrobial activity. Another important observation is that the various substituents of the aromatic rings had similar levels of activity. However, the methyl group in compound **15b** showed the most significant level of inhibition, while an aromatic ring at position-4 of the pyran ring was found to decrease activity.

It is important to remark that lipophilicity was not an important parameter for predicting the activity of α- and β-pyran naphthoquinones. Calculated lipophilicity (cLogP) indicated insignificant differences between the two series, despite marked differences in antimicrobial activity.

Conclusions

Few naphthoquinones are described in the literature as having antitubercular activity. The MICs found for the compounds synthesized in this work are below the 6.25 μg/mL value postulated by the Global Program for the Discovery of New Antituberculosis Drugs as an upper threshold in the evaluation of new candidates for inhibiting *M. tuberculosis*. Therefore, our results indicate that analogues of β-lapachone bearing a bulky moiety in the pyran ring are promising anti-TB agents, and that this activity should be thoroughly investigated.

Experimental

Chemistry

Reagents were purchased from Aldrich (Sigma-Aldrich, Germany) or Acros Chemical Co. (Geel, Belgium), and were used without further purification. Column chromatography was performed with silica gel 60 (Merck 70-230 mesh; Merck, Germany). Analytical thin-layer chromatography was performed with silica gel plates (Merck, TLC silica gel 60 F254), and the plots were visualized using UV light or aqueous solutions of sodium sulfate. Yields refer to chromatographically and spectroscopically homogeneous materials. Melting points were obtained on a Fischer-Johns apparatus (Fischer-Scientific, Pittsburgh, PA, USA), and are uncorrected. Infrared spectra were measured using KBr pellets on a Perkin-Elmer model 1420 FT-IR Spectrophotometer (Perkin-Elmer, Norwalk, CT, USA), calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene. NMR spectra were recorded on a Varian Unity Plus VXR (300 MHz) instrument (Varian Inc., Palo Alto, CA, USA) in DMSO-d_6 and $CDCl_3$ solutions. The chemical shift data were reported in units of δ (ppm) downfield from tetramethylsilane, which was used as an internal standard; coupling constants (*J*) are reported in Hertz, and refer to apparent peak multiplicities. High resolution mass spectra (HRMS) were recorded on MICROMASS Q-TOF Micro Mass spectrometer (Micromass, Manchester, UK) using ESI-TOF (electrospray ionization-time of flight).

*General procedure for the preparation of **14a–j** and **15a–j***

In a round-bottom flask equipped with a magnetic stirring bar, lawsone **16** was dissolved (1 mmol) with water (10 mL) and ethanol (10 mL). Then, the appropriate aldehyde (8 mmol for the formaldehyde and 3 mmol for arylaldehydes) was added. Substituted styrenes (3 mmol) were added dropwise and the reaction mixture was stirred under reflux until total consumption of the starting material. Ethanol was removed under reduced pressure, ethyl acetate was added to the residue and the mixture was washed with a saturated aqueous solution of sodium bicarbonate. The combined organic extracts were washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residual crude product was purified via silica-gel column chromatography, using a gradient mixture of hexane-ethyl acetate.

*2-Phenyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione **14a***

Yellow solid, m.p.: 168 – 170°C; IR (KBr, cm^{-1}) ν: 1679, 1649, 1617, 1260, 1202, 1063, 958, 910, 721; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.15 (1H, dddd, *J* = 2.6, 3.2, 5.7, and 14.0 Hz, H-3a), 2.30 (1H, dddd, *J* = 2.2, 6.2, 6.5, and 14.0 Hz, H-3b), 2.63 (1H, ddd, *J* = 3.2, 6.2, and 13.7 Hz, H-4a), 2.77 (1H, ddd, *J* = 2.2, 5.7, and 13.7 Hz, H-4b), 5.22 (1H, dd, *J* = 2.6 and 6.5 Hz, H-2), 7.32 – 7.40 (5H, m, 2-phenyl), 7.68 (2H, dddd, *J* = 2.0, 7.5, 9.2, and 11.0 Hz, H-8 and H-7), 8.10 (2H, dddd, *J* =2.0, 7.5, 9.2, and 11.0 Hz, H-9 and H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) *d*: 18.3 (C-4), 27.6 (C-3), 78.8 (C-2), 121.4 (C-4a), 125.6 (C-4′-*phenyl*), 125.8 (C-6), 126.1 (C-9), 128.1 (C-2′-*phenyl*), 128.4 (C-3′-*phenyl*), 130.8 (C-9a), 131.7 (C-5a), 132.9 (C-7, C-8), 133.7 (C-H-ar), 139.1 (C-1′), 152.2 (C-10a), 184.0 (C-5 and C-10). HRMS (ESI) calcd. for $C_{19}H_{14}O_3$: 290.0943. Found: 290.0936.

*2-(p-Tolyl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione **14b***

Yellow solid, m.p.: 135 – 137°C; IR (KBr, cm^{-1}) ν: 1677, 1645, 1617, 1595, 1341, 1300, 1258, 1200, 1065, 958, 910, 816, 720; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.06 (1H, dddd, *J* = 2.6, 3.4, 6.0, and 13.0 Hz, H-3a), 2.31 (1H, dddd, *J* = 2.3, 6.4, 6.3, and 13.0 Hz, H-3b), 2.36 (1H, s, CH_3), 2.64 (1H, ddd, *J* = 3.4, 6.4, and 12.7 Hz, H-4a), 2.75 (1H, ddd, *J* = 2.3, 6.0, and 12.7 Hz, H-4b), 5.18 (1H, dd, *J* = 2.6 and 6.3 Hz, H-2), 7.1 (2H, dd, *J* = 7.7 Hz, H-*meta* tolyl), 7.27 (2H, dd, *J* = 7.7 Hz, H-*ortho* tolyl), 7.69 (2H, dddd, *J* = 1.0, 7.3, 9.0, and 10.7 Hz, H-8 and H-7), 8.09 (2H, dddd, *J* = 1.0, 7.3, 9.0, and 10.7 Hz, H-9 and H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) *d*: 18.8 (C-4), 21.4 (CH_3), 28.0 (C-3), 79.2 (C-2), 121.8 (C-4a), 126.1 (C-6), 126.2 (C-9), 126.5 (C-2′), 129.5 (C-3′), 131.3 (C-9a), 133.7 (C-4′), 134.1 (C-1′), 136.3 (C-5a), 138.3 (C-1′), 155.8 (C-10a), 179.6 and 184.0 (C-5 and C-10). HRMS (ESI) calcd. for $C_{20}H_{16}O_3$: 304.1099. Found: 304.1261.

*2-(2,4-Dimethylphenyl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione **14c***

Yellow solid, m.p.: 148 – 152°C; IR (KBr, cm^{-1}) ν: 1673, 1646, 1617, 1590, 1261,1204, 1199, 1063, 961, 825, 718, 676; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.99 (1H, dddd, *J* = 2.4, 3.4, 5.9, and 13.2 Hz, H-3a), 2.25 (1H, dddd, *J* = 2.4, 6.4, 7.8, and 13.2 Hz, H-3b), 2.32 (3H, s, CH_3), 2.34 (3H, s, CH_3), 2.64 (1H, ddd, *J* = 3.4, 6.4, and 13.0 Hz, H-4a), 2.85 (1H, m, ddd, *J* = 2.4, 5.9, and 13.0 Hz, H-4b), 5.25 (1H, dd, *J* = 2.4 and 7.8 Hz, H-2), 7.01 (1H, s, H-*meta* tolyl), 7.06 (1H, d, *J* = 7.8 Hz, H-*ortho* tolyl), 7.30 (1H, d, *J* = 7.8 Hz, H-*meta* tolyl), 7.69

(2H, dddd, *J* = 2.0, 7.8, 9.2 and 10.7 Hz, H-8 and H-7), 8.10 (2H, dddd, *J* = 2.0, 7.6, 9.2, and 10.7 Hz, H-9 and H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 19.2 (CH_3), 19.6 (C-4), 21.3 (CH_3), 27.2 (C-3), 77.0 (C-2), 121.6 (C-4a), 125.9 (C-5′ *ortho* tolyl*)*, 126.2 and 126.5 (C-6 and C-9), 127.3 (C-6′), 131.6 (C-3′ *ortho* tolyl*)*, 131.3 (C-9a), 132.2 (C-5a), 133.3 and 134.1 (C-7 and C-8), 134.7 and 134.9 (C-2′ and C-4′), 138.1 (C-1′), 156.2 (C-10a), 179.6 and 184.0 (C-5 and C-10). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 318.1256. Found: 318.1196.

2-(4-Chlorophenyl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione ***14d***

Yellow solid, m.p.: 130 – 132°C; IR (KBr, cm^{-1}) ν: 1677, 1650, 1620, 1597, 1260, 1202, 1095, 1065, 958, 911, 720; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.03 (1H, dddd, *J* = 2.3, 3.2, 6.2, and 12.9 Hz, H-3a), 2.30 (1H, dddd, *J* = 2.1, 6.3, 6.8, and 12.9 Hz, H-3b), 2.63 (1H, ddd, *J* = 3.2, 6.3, and 12.4 Hz, H-4a), 2.76 (1H, ddd, *J* = 2.1, 6.2, and 12.4 Hz, H-4b), 5.17 (1H, dd, *J* = 2.3 and 6.8 Hz, H-2), 7.29 – 7.39 (4H, m, ArH), 7.70 (2H, dddd, *J* = 2.0, 7.8, 9.2, and 10.9 Hz, H-8 and H-7), 8.10 (2H, dddd, *J* = 2.0, 7.8, 9.2 and 10.9 Hz, H-9 and H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 18.8 (C-4), 28.1 (C-3), 78.5 (C-2), 121.8 (C-4a), 126.3 (C-6), 126.6 (C-9), 127.5 (C-7), 129.1 (C-8), 133.4 (C-2′), 134.2 (C-3′), 131.2 (C-9a), 132.1 (C-4′), 134.4 (C-5a), 138.1 (C-1′); 155.4 (C-10a), 179.5 and 184.4 (C-5 and C-10). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 324.0553. Found: 324.0175.

2-(4-Fluorophenyl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione ***14e***

Yellow solid, m.p.: 171 – 173°C; IR (KBr, cm^{-1}) ν: 1678, 1647, 1618, 1513, 1260, 1201, 1065, 958, 835, 720; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.15 (1H, dddd, *J* = 2.4, 3.3, 6.5, and 13.0 Hz, H-3a), 2.30 (1H, dddd, *J* = 2.1, 6.2, 7.0, and 13.0 Hz, H-3b), 2.75 (1H, ddd, *J* = 3.3, 6.2, and 12.5 Hz, H-4a), 2.80 (1H, ddd, *J* = 2.1, 6.5, and 12.5 Hz, H-4b), 5.16 (1H, dd, *J* = 2.4 and 7.0 Hz, H-2), 7.08 (2H, t, *J* = 8.7, 2C*H*-ar-F), 7.36 – 7.41 (2H, m, 2ArH), 7.70 (2H, dddd, *J* = 1.5, 7.5, 9.2, and 11.0 Hz, H-8 and H-7), 8.10 (2H, dddd, *J* = 1.5, 7.5, 9.2, and 10.9 Hz, H-9 and H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 18.5 (C-4), 27.8 (C-3), 78.3 (C-2), 115.3 and 115.6 (d, *J* = 21.4 Hz, 2C*H*-ar-F), 121.4 (C-4a), 125.9 (C-6), 126.2 (C-9), 127.5 (C-7), 127.6 (C-8), 133.0 (C-2′), 133.8 (C-3′), 130.8 (C-9a), 131.8 (C-5a), 135.0 (C-4′), 155.1 (C-10a), 179.1 and 184.0 (C-5 and C-10). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 308.0849. Found: 308.3238.

2-(4-Bromophenyl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione ***14f***

Yellow solid, m.p.: 167 – 168°C; IR (KBr, cm^{-1}) ν: 1674, 1648, 1619, 1260, 1204, 1067, 909, 720; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.11 (1H, dddd, *J* = 2.4, 3.4, 6.5, and 12.7 Hz, H-3a), 2.27 (1H, dddd, *J* = 2.3, 6.4, 7.0, and 12.7 Hz, H-3b), 2.62 (1H, ddd, *J* = 3.4, 6.4, and 12.6 Hz, H-4a), 2.76 (1H, ddd, *J* = 2.3, 6.5, and 12.6 Hz, H-4b), 5.15 (1H, dd, *J* = 2.4 and 7.0 Hz, H-2), 7.08 (2H, m, 2C*H*-ar-Br), 7.37 – 7.40 (2H, m, 2ArH), 7.71 (2H, dddd, *J* = 1.5, 7.5, 9.2, and 11.0 Hz, H-8 and H-7), 8.09 (2H, dddd, *J* = 1.5, 7.5, 9.2, and 11.0 Hz, H-9 and H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 18.3 (C-4), 27.7 (C-3), 78.1 (C-2), 121.4 (C-4a), 125.9 (C-6), 126.2 (C-9), 127.4 (C-7), 131.3 (C-8), 131.7 (C-2′), 133.0 (C-3′), 133.8 (C-4′), 130.8 (C-9a), 122.1 (C-5a), 138.2 (C-1′), 155.0 (C-10a), 180.0 and 183.9 (C5 and C-10). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 368.0048. Found: 367.9784.

2-(4-Methoxyphenyl)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione ***14g***

Yellow solid, m.p.: 165 – 166°C; IR (KBr, cm^{-1}) ν: 1681, 1645, 1612, 1245, 1194, 1062, 1021, 953, 720; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.10 (1H, dddd, *J* = 2.6, 3.5, 6.4, and 13.0 Hz, H-3a), 2.28 (1H, dddd, *J* = 2.0, 6.1, 6.3, and 13.0 Hz, H-3b), 2.59 (1H, ddd, *J* = 3.5, 6.1, and 12.2 Hz, H-4a), 2.76 (1H, ddd, *J* = 2.0, 6.4, and 12.2 Hz, H-4b), 5.14 (1H, dd, *J* = 2.6 and 6.3 Hz, H-2), 6.89 (2H, d, *J* = 8.3 Hz, ArH), 7.30 (2H, d, *J* = 8.3 Hz, ArH), 7.69 (2H, dddd, *J* = 1.5, 7.5, 9.0, 11.0 Hz, H-8 and H-7), 8.09 (2H, dddd, *J* = 1.5, 7.5, 9.0, 10.9 Hz, H-9 and H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 19.0 (C-4), 28.0 (C-3), 55.5 (O*C*H$_3$), 79.1 (C-2), 114.3 (C-4a), 126.2 (C-6), 126.5 (C-9), 133.5 (C-7), 134.1 (C-8), 131.3 (C-9a), 131.7 (C-5a), 155.8 (C-10a), 159.9 (C-4′), 179.7 and 184.5 (C-5 and C-10). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 320.1049. Found: 320.3606.

4-(4-Nitrophenyl)-2-p-tolyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione, syn-isomer ***14h***

Yellow solid, m.p.: 205 – 207°C; IR (KBr, cm^{-1}) ν: 1677, 1649, 1612, 1516, 1344, 1305, 1266, 1209, 1106, 968, 900, 856, 818, 725; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.25 (1H, dt, *J* = 2.4 and 14.3 Hz, H-3a), 2.35 (3H, s, CH_3), 2.44 (1H, ddd, *J* = 5.7, 11.9, and 14.3 Hz, H-3b), 4.52 (1H, dd, *J* = 1.5 and 5.7 Hz, H-4), 4.99 (1H, dd, *J* = 2.4 and 11.9 Hz, H-2), 7.17 (1H, d, *J* = 8.5 Hz, H-*meta p*-tolyl), 7.22 (1H, d, *J* = 8.5 Hz, H-*ortho p*-tolyl), 7.46 (1H, d, *J* = 8.6 Hz, H-*ortho* 4-nitrophenyl), 7.71 – 7.77 (1H, m, H-7), 7.71 – 7.77 (1H, m, H-8), 8.00 – 8.06 (1H, m, H-9), 8.17 – 8.22 (1H, m, H-6), 8.22 (1H, d, *J* = 8.6 Hz, H-*meta* 4-nitrophenyl); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 21.0 (CH_3), 35.2 (C-4), 36.5 (C-3), 75.0 (C-2), 120.2 (C-4a), 124.0 (C-3′ 4-nitrophenyl), 126.0 (C-9), 126.3 (C-2′ 4-nitrophenyl), 126.5 (C-7), 128.5 (C-3′ *p*-tolyl), 129.3 (C-2′ *p*-tolyl), 131.0 (C-9a), 131.7 (C-5a), 133.3 (C-6), 134.2 (C-8), 135.0 (C-4′ *p*-tolyl), 138.5 (C-1′ *p*-tolyl), 146.8 (C-4′ 4-nitrophenyl), 151.0 (C-1′ 4-nitrophenyl), 156.6 (C-10a), 179.0 (C-10), 183.1 (C-5).

4-(4-nitrophenyl)-2-p-tolyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione, anti-isomer ***14h***

Yellow solid, m.p.: 231 – 234°C; IR (KBr, cm^{-1}) ν: 1677, 1650, 1607, 1511, 1345, 1301, 1266, 1258, 1199, 961, 724; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.25 (1H, dt, *J* = 2.4 and 14.3 Hz, H-3a), 2.35 (3H, s, CH_3), 2.62 (1H, ddd, *J* = 2.2, 7.1, and 14.4 Hz, H-3b), 4.40 (1H, dd, *J* = 7.1 and 10.7 Hz, H-4), 5.20 (1H, dd, *J* = 2.0 and 11.0 Hz, H-2), 7.18 (1H, d, *J* = 8.1 Hz, H-*meta p*-tolyl), 7.30 (1H, d, *J* = 8.1 Hz, H-*ortho p*-tolyl), 7.35 (1H, d, *J* = 8.8 Hz, H-*ortho* 4-nitrophenyl), 7.67 – 7.74 (1H, m, H-7), 7.67 – 7.74 (1H, m, H-8), 7.90 – 7.93 (1H, m, H-9), 8.11 – 8.17 (1H, m, H-6), 8.13 (1H, d, *J* = 8.8 Hz, H-*meta* 4-nitrophenyl); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 21.0 (CH_3), 29.6 (C-3), 38.6 (C-3), 79.2 (C-2), 122.8 (C-4a), 123.9 (C-3′ 4-nitrophenyl), 125.9 (C-9), 126.2 (C-2′ 4-nitrophenyl), 126.4 (C-7), 127.6 (C-3′ *p*-tolyl), 129.3 (C-2′ *p*-tolyl), 130.8 (C-9a), 131.8 (C-5a), 133.3 (C-6), 134.2 (C-8), 134.8 (C-4′ *p*-tolyl), 138.6 (C-1′ *p*-tolyl), 146.4 (C-4′ 4-nitrophenyl), 151.2 (C-1′ 4-nitrophenyl), 157.4 (C-10a), 183.2 (C-10), 186.8 (C-5).

4-Phenyl-2-p-tolyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione ***14i***

Yellow solid, m.p.: 203 – 205°C; IR (KBr, cm^{-1}) ν: 1678, 1647, 1615, 1365, 1336, 1302, 1257, 1194, 1068, 1043, 960, 894, 812, 760, 725, 702; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.13 – 2.32 (1H, m, H-3a), 2.34 and 2.35 (3H, s, CH_3), 2.44 (1H, ddd, *J* = 2.0, 7.3 and 14.4 Hz, H-3b), 4.29 (1H, dd, *J* = 7.3 and 11.0 Hz, H-4 *anti* isomer), 4.46

(1H, dd, *J* = 1.5 and 5.7 Hz, H-4 *syn* isomer), 5.06 (1H, dd, *J* = 3.2 and 11.0 Hz, H-2 *syn* isomer) and 5.12 (1H, dd, *J* = 1.2 and 11.0 Hz, H-2 *anti* isomer), 7.14 – 7.34 (4H, m, *p*-tolyl), 7.14 – 7.34 (5H, m, Ph), 7.63 – 7.72 (1H, m, H-7), 7.63 – 7.72 (1H, m, H-8), 7.91 – 7.94 (1H, m, H-9 *anti* isomer) and 8.02 – 8.05 (1H, m, H-9 *syn* isomer), 8.11 – 8.18 (1H, m, H-6); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 21.4 (CH_3), 35.6 and 39.3 (C-4), 37.3 and 41.2 (C-3), 75.5 and 79.9 (C-2), 125.0 (C-4a), 126.5 and 126.6 (C-9), 126.7 (C-7), 126.8 (C-2′ Ph), 127.1 (C-3′ Ph), 128.0 (C-3′ *p*-tolyl), 128.9 and 129.1 (C-4′ Ph), 129.5 and 129.6 (C-2′ *p*-tolyl), 131.2 (C-9a), 132.5 (C-5a), 133.3 and 133.4 (C-6), 134.3 and 134.4 (C-8), 135.8 (C-4′ *p*-tolyl), 138.6 (C-1′ *p*-tolyl), 143.9 (C-4′ Ph), 157.5 (C-10a), 179.9 (C-10), 183.4 (C-5).

4-(Thiophen-2-yl)-2-p-tolyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione, syn-isomer ***14j***

Yellow solid, m.p.: 123 – 125°C; IR (KBr, cm^{-1}) ν: 1680, 1650, 1614, 1338, 1299, 1261, 1205, 1063, 959, 896, 815, 721; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.22 – 2.39 (2H, m, H-3), 2.36 (3H, s, CH_3), 4.70 (1H, ddd, *J* = 0.7, 1.9 and 4.9 Hz, H-4), 5.23 (1H, dd, *J* = 2.9 and 11.2 Hz, H-2), 6.92 (1H, dt, *J* = 1.0 and 3.4 Hz, H-3′ thiophen-2-yl), 6.96 (1H, dd, *J* = 3.7 and 5.1 Hz, H-4′ thiophen-2-yl), 7.20 (1H, dd, *J* = 1.2 and 5.1 Hz, H-5′ thiophen-2-yl), 7.20 (1H, d, *J* = 8.0 Hz, H-*meta*), 7.28 (1H, d, *J* = 8.0 Hz, H-*ortho*), 7.67 – 7.76 (1H, m, H-7), 7.67 – 7.76 (1H, m, H-8), 8.06 – 8.09 (1H, m, H-9), 8.13 – 8.16 (1H, m, H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 21.5 (CH_3), 30.9 (C-4), 37.4 (C-3), 76.0 (C-2), 122.0 (C-4a), 124.5 (C-5′ thiophen-2-yl), 125.5 (C-9), 126.5 (C-7), 126.7 (C-4′ thiophen-2-yl), 127.3 (C-3′ *p*-tolyl), 129.6 (C-2′ *p*-tolyl), 131.4 (C-4′ *p*-tolyl), 132.3 (C-6), 133.5 (C-8), 134.4 (C-3′ thiophen-2-yl), 136.0 (C-1′ *p*-tolyl), 138.6 (C-2′ thiophen-2-yl), 147.2 (C-11), 176.5 (C-10), 183.6 (C-5).

4-(Thiophen-2-yl)-2-p-tolyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione, anti-isomer ***14j***

Yellow solid, m.p.: 172 – 175°C; IR (KBr, cm^{-1}) ν: 1678, 1649, 1611, 1363, 1333, 1300, 1256, 1192, 1041, 954, 892, 847, 814, 721; ^{1}H-NMR ($CDCl_3$, 300 MH) 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), 7.63 and 7.65 (1H, dt, *J* = 1.2 and 7.6 Hz, H-7 conformers), 7.89 (1H, d, *J* = 7.6 Hz, H-9), 8.09 and 8.13 (1H, dd, *J* = 1.5 and 7.6 Hz, H-6 conformers); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 21.4 (CH_3), 30.9 (C-4), 41.5 (C-3), 79.9 (C-2), 124.4 (C-4a), 124.6 (C-5′ thiophen-2-yl), 126.4 (C-9), 126.5 (C-7), 126.6 (C-4′ thiophen-2-yl), 127.0 (C-3′ *p*-tolyl), 129.6 (C-2′ *p*-tolyl), 131.2 (C-4′ *p*-tolyl), 132.5 (C-6), 133.4 (C-8), 134.4 (C-3′ thiophen-2-yl), 135.5 (C-1′ *p*-tolyl), 138.7 (C-2′ thiophen-2-yl), 146.6 (C-11), 179.8 (C-10), 183.7 (C-5).

2-Phenyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15a***

Orange solid, 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.

2-p-Tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15b***

Orange solid, 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.

2-(2,4-Dimethylphenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15c***

Orange solid, m.p.: 164 – 167°C; IR (KBr, cm^{-1}) ν: 1694, 1646, 1603, 1574, 1394, 1283, 1231, 1034, 998, 775; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.99 (1H, dddd, *J* = 2.4, 3.0, 5.3, and 12.2 Hz, H-3a), 2.26 (1H, dddd, *J* = 2.4, 6.6, 7.8, and 12.2 Hz, H-3b), 2.36 (3H, s, CH_3); 2.38 (3H, s, CH_3), 2.59 (1H, ddd, *J* = 3.0, 6.6, and 8.7 Hz, H-4a), 2.85 (1H, ddd, *J* = 2.4, 5.3, and 8.7 Hz, H-4b), 5.35 (1H, dd, *J* = 2.4 and 7.8 Hz, H-2), 7.10 (2H, d, *J* = 7.8 Hz, H-*ortho* and *meta* tolyl), 7.33 (1H, d, *J* = 7.8 Hz, H-*meta* tolyl), 7.51 (1H, ddd, *J* = 1.2, 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-5′ *ortho* tolyl), 125.8 (C-6 and C-9), 127.3 (C-6′), 128.9 (C-3′ *ortho* tolyl), 130.9 (C-9a), 131.8 (C-5a), 134.6 (C-1′),130.2 (C-6a), 132.4 (C-10a), 135.1 and 138.5 (C-2′ and C-4′), 163.5 (C-10b), 178.7 and 179.8 (C-5 and C-10). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 318.1256. Found: 318.1892.

2-(4-Chlorophenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15d***

Orange solid, 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.

2-(4-Fluorophenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15e***

Orange solid, 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, 2C*H*-ar-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.

2-(4-Bromophenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15f***

Orange solid, m.p.: 169 – 170°C; IR (KBr, cm^{-1}) ν: 1726, 1648, 1604, 1390, 1283, 1073, 922, 775; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.05 (1H, dddd, *J* = 2.4, 3.7, 5.6, and 12.9 Hz, H-3a), 2.32 (1H, dddd, *J* = 3.3, 6.5, 7.8, and 12.9 Hz, H-3b), 2.55 (1H, ddd, *J* = 3.7, 6.5, and 8.8 Hz, H-4a), 2.75 (1H, ddd, *J* = 3.3, 5.6, and 8.8 Hz, H-4b), 5.23 (1H, dd, *J* = 2.4 and 7.8 Hz, H-2), 7.29 – 7.33 (2H, m, H-ar), 7.51 – 7.72 (5H, m, H-8, H-9 and 2H-ar), 7.80 (1H, dd, *J* = 1.1 and 7.8 Hz, H-10), 8.01 (1H, dd, *J* = 1,1 and 7.5 Hz, H-7); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 18.2 (C-4), 28.2 (C-3), 79.3 (C-2), 113.8 (C-4a), 123.8 (C-10), 127.4 (C-7), 127.6 (C-8), 128.7 (C-2′), 129.8 (C-6a), 130.7 (C-3′), 131.8 (C-9′), 134.8 (C-1′), 132.8 (C-4′), 138.2 (C-10a), 162.4 (C-10b), 178.4 and 178.2 (C5 and C-6). HRMS (ESI) calcd. for $C_{21}H_{18}O_3$: 368.0048. Found: 367.9640.

2-(4-Methoxyphenyl)-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15g***

Orange solid, 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-(4-Nitrophenyl)-2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15h***

Orange solid, m.p.: 232 – 235°C; IR (KBr, cm^{-1}) ν: 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-Phenyl-2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15i***

Orange solid, m.p.: 92 – 95°C; IR (KBr, cm^{-1}) ν: 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-(Thiophen-2-yl)-2-p-tolyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione ***15j***

Orange solid, m.p.: 83 – 87°C; IR (KBr, cm^{-1}) ν: 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.

Mycobacterial growth assay

Briefly, 200 μL of sterile de-ionized water was added to all outer-perimeter wells of sterile 96-well plates (Falcon, 3072: Becton Dickinson, Lincoln Park, NJ, USA) to minimize evaporation of the medium in the test wells during incubation. The 96 wells/plate received 100 μL of the Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA) and a serial dilution of the compounds **14a – j** and **15a – j** was made directly on the plate. The final drug concentrations tested were 0.01 to 10.0 μL/mL. Plates were covered, sealed with parafilm, and incubatedat 37°C for five days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar-Blue reagent (Accumed International, Westlake, OH, USA) and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC (minimal inhibition concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

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