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Original article

Synthesis, biological activity, and evaluation of the mode of action of novel antitubercular benzofurobenzopyrans substituted on A ring

Aikaterini Termentzi [a], Inana Khouri [a], Thomas Gaslonde [a], Soizic Prado [b], Brigitte Saint-Joanis [c], Fabienne Bardou [d], Elsa P. Amanatiadou [e], Ioannis S. Vizirianakis [e], Jana Kordulakova [f], Mary Jackson [c,g], Roland Brosch [c], Yves L. Janin [h], Mamadou Daffé [d], François Tillequin [a], Sylvie Michel [a,*]

[a] Laboratoire de Pharmacognosie, Université Paris Descartes, UMR/CNRS 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75006 Paris, France
[b] Molécules de Communication et Adaptation des Micro-organismes, FRE 3206 CNRS/MNHN Muséum National d'Histoire Naturelle, 57 rue Cuvier (CP54), 75005 Paris, France
[c] Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France
[d] CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), Département "Mécanismes Moléculaires des Infections Mycobactériennes", 205 Route de Narbonne, F-31077 Toulouse & Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France
[e] Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, GR-54124 Greece
[f] Department of Biochemistry, Faculty of Natural Sciences, Comenius University, Mlynska dolina, 84215 Bratislava, Slovakia
[g] Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523-1682, USA
[h] Institut Pasteur, Unité de Chimie Organique, URA 2128-CNRS, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

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ABSTRACT

The 8-, 9-, 10-, and 11-halo, hydroxy, and methoxy derivatives of the antimycobacterial 3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran were synthesized by condensation of the diazonium salts of 2-chloroanilines (**13**–**17**) with 1,4-benzoquinone (**18**), reduction of the intermediate phenylbenzoquinones **19**–**22** to dihydroxybiphenyls, cyclisation to halo-2-hydroxydibenzofurans **24**–**27**, and construction of the pyran ring by thermal rearrangement of the corresponding dimethylpropargyl ethers **35**–**38**. Palladium catalyzed nucleophilic aromatic substitution permitted conversion of the halo to the corresponding hydroxy derivatives which were methylated to methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran. All compounds substituted on the A ring were found more potent than the reference compound **1** against *Mycobacterium bovis* BCG and the virulent strain *Mycobacterium tuberculosis* H37Rv. The effect of the most active derivatives on mycolate synthesis was explored in order to confirm the preliminary hypothesis of an effect on mycobacterial cell wall biosynthesis. The linear 9-methoxy-2,2-dimethyl-2*H*-benzofuro[2,3-*g*][1]benzopyran (**46**) exhibiting a good antimycobacterial activity and devoid of cytotoxicity appeared to be the most promising compound.

1. Introduction

Tuberculosis remains a serious and highly contagious infectious disease, which currently represents the second leading cause of death worldwide. Although a renewal of research in this field is currently taking place [1–3], there is still an urgent need for the discovery of novel antitubercular agents. In our continuous search for such compounds, previous work in our group associated two functional "natural like" antimicrobial structures, dibenzofuran and 2,2-dimethylpyran subunits, to successfully create a hybrid compound **1** and its reduced analogue **2**, that showed promising antitubercular activity *in vitro* [4] (Fig. 1). Both compounds appeared interesting as they displayed little cytotoxicity in mammalian VERO cells, stimulating further pharmacomodulation studies in this series. However, as compounds **1** and **2** had no activity *in vivo* [5], further investigation in the benzofurobenzopyran series was made in attempts to improve the activity and/or bioavailability. To this goal, the exploration of the putative mechanism of action of this series at both cellular and molecular levels also appeared as an important issue. In this respect, the specific activity of compounds **1** and **2** against *Mycobacterium* and *Nocardia asteroids* [4], which share in common related cell wall components, suggested that their activity should lie in the perturbation of the biosynthesis of mycolic acids present in the cell walls of both microorganisms.

Previous attempts towards the establishment of structure-activity relationships in the benzofurobenzopyran series led the synthesis of several related compounds (Fig. 2). Replacement of the furan B ring by an ether linkage (**3**), a single carbon–carbon bond, a carbonyl group,

* Corresponding author. Tel.: +33 1 53 73 98 03; fax: +33 1 40 46 96 58.
E-mail address: sylvie.michel@parisdescartes.fr (S. Michel).

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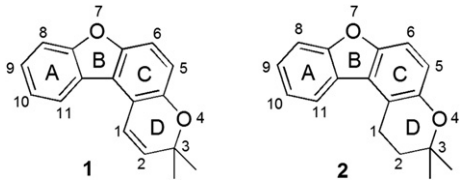


Fig. 1. Chemical structure of 3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**1**) and its reduced analogue (**2**).

a hydroxymethylene group or a methylene gave either inactive or cytotoxic products [5]. Also, several related "seco" compounds including a pyridine ring (**4**), although displaying interesting antimycobacterial activities, were found to be also cytotoxic thus discouraging further research in this direction [6,7]. These data strongly suggested that the tetracyclic benzofurobenzopyran core was an indispensable structural component for the retention of the biological activity.

Modifications, such as bromination (**5**) or acylation on position 5 of C ring of the benzofurobenzopyran skeleton resulted in inactive derivatives [5]. Concerning the substitution on the A ring, acylation at position 10 and nitration at position 8 also gave inactive compounds, against both *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* (**6** & **7**) [5,8]. In contrast, the heterocyclic analogue with a nitrogen atom at position 10 (**8**) was a relatively potent antitubercular agent with MIC_{s95} of 8 μg/ml and 16 μg/ml against *M. smegmatis* and *M. tuberculosis*, respectively [8].

These results suggested to perform a systematic pharmacomodulation on the basic core of **1** and explore various substitutions on each position of the A ring. Consequently, we describe here the synthesis and antimycobacterial activity of a series of 8, 9, 10 and 11-halo, methoxy and hydroxy 3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran. Moreover, their ability to inhibit the mycolate synthesis was investigated, in order to confirm the hypothesis of an effect on mycobacteria cell wall biosynthesis.

2. Results and discussion

2.1. Chemistry

The synthetic methods previously applied for the synthesis of 3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran derivatives do not allow facile modulations of substitutions in the A ring [3–8]. Consequently, a novel access allowing substitutions on all positions of the A ring was envisioned, through the creation of a suitable biphenyl, followed by closure into 2-hydroxydibenzofuran. Construction of the dimethylpyran ring was anticipated at the last step, through thermal rearrangement of a propargylic ether, since this method had previously given satisfactory results in several related series [9–11].

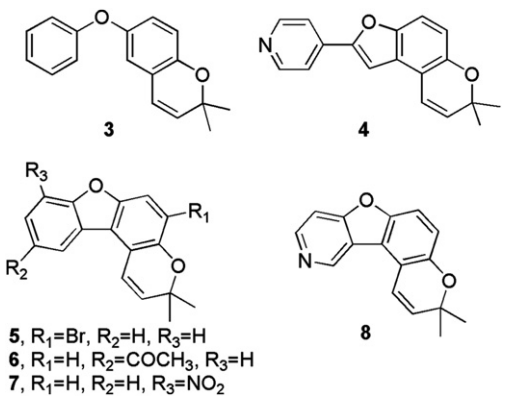


Fig. 2. Previous pharmacomodulations on A, B and C rings.

2.1.1. Synthesis of the biphenyls

2.1.1.1. Synthesis of biphenyls through palladium catalyzed reactions. Several experiments were carried out to get the required biphenyls through Pd catalyzed Suzuki–Miyaura reaction between 2-bromohydroquinone (**9**) and various 2-chlorophenylboronic acid (Scheme 1) [12,13]. The use of Pd/C with different base and solvent systems did not give the expected products. In contrast the use of $Pd(PPh_3)_4$ in toluene:H_2O (5:1), in the presence of Na_2CO_3, with the phenylboronic acid **10** gave the desired 2′-chloro-4′-methoxy[1,1′-biphenyl]-2,5-diol (**11**), (6% yield) and an oxidized product 2-(2-chloro-4-methoxyphenyl)-1,4-benzoquinone (**12**) (19%).

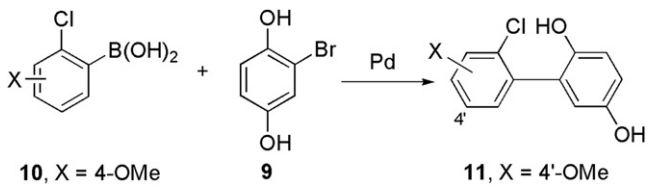
Consequently, the need for large quantities of biphenyls as starting materials led to envisage an alternate synthesis involving a more versatile, efficient and cheaper method (Scheme 2).

*2.1.1.2. Synthesis of biphenyls **24**–**28** through intermediate arylbenzoquinones.* Following a reaction scheme previously developed by Schimmelschmidt [14], the formation of 2-hydroxydibenzofurans conveniently substituted on the C ring was envisioned through a three steps process involving (i) formation of an aryl-1,4-benzoquinone through aromatic nucleophilic substitution of a diazonium salt with *p*-benzoquinone, (ii) reduction to the corresponding *p*-diphenol, and (iii) cyclisation into 2-hydroxybenzofuran in alkaline medium.

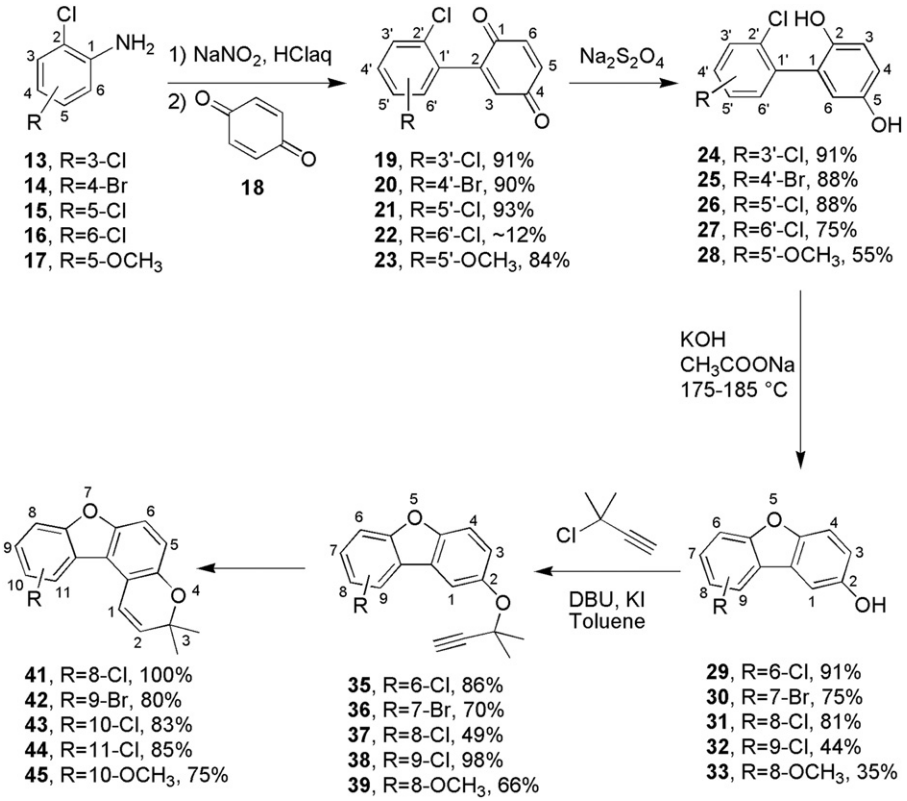
The required diazonium derivatives were conveniently obtained from commercially available 2-chloro-halogeno-anilins (**13**–**17**) and immediately reacted with 1,4-benzoquinone (**18**) in excess (1.2 eq) to give the corresponding 2-phenyl-1,4-benzoquinones **19**–**22** in satisfactory yields (84–98%), with the exception of 2-(2,6-dichlorophenyl)-1,4-benzoquinone (**22**) (12%), due to the known difficulties in the formation of the diazonium salt of 2,6-dichloroanilin [15]. Similarly, the condensation of *p*-benzoquinone with the diazonium salt obtained from 2-chloro-5-methoxyanilin (**17**) successfully afforded 2-(2-chloro-5-methoxyphenyl)-1,4-benzoquinone (**23**) in 84% yield. The arylbenzoquinones **19**–**23** were then reduced to the desired corresponding biphenyls **24**–**28** in 55–91% yield, by treatment with saturated aqueous $Na_2S_2O_4$ (Scheme 2) [14].

2.1.2. Synthesis of dibenzofurans

The use of sodium hydride in different conditions did not permit to observe cyclisation of biphenyls **24**–**28** into the desired dibenzofurans. Thus, the creation of the furan ring was envisioned and successfully achieved through intramolecular aromatic nucleophilic substitution with KOH and NaOAc at high temperature in a sealed tube (Scheme 2) [14]. In some cases significant improvements of the yields were obtained under microwave irradiation. In all cases, the addition of a small amount (~0.2eq) of sodium dithionite permitted to avoid biphenyl oxidation in the course of the reaction. Under optimized conditions, the use of acetonitrile as solvent permitted the formation of 6-chloro-2-hydroxydibenzofuran (**29**), 7-bromo-2-hydroxydibenzofuran (**30**) and 8-chloro-2-hydroxydibenzofuran (**31**) in 92, 76 and 90% yields, respectively. In the case of 9-chloro-2-



Scheme 1. Biphenyl synthesis with palladium catalyst.



Scheme 2. Biphenyl synthesis through the *p*-benzoquinone pathway.

hydroxydibenzofuran (**32**), the best yield (44%) was obtained in water solution. The best results (35% yield) for 2-hydroxy-8-methoxydibenzofuran (**33**) were achieved at solid stage with microwaves. It is interesting to note that, when reaction was carried out on 2′,3′-dichloro[1,1′-biphenyl]-2,5-diol (**24**) at solid stage under microwave irradiation, the formation of the expected compound **29** (12%) was accompanied by that of larger amounts of 2,7-dihydroxydibenzofuran (**34**) (15%) probably resulting from the occurrence of a benzyne intermediate. (Scheme 3).

2.1.3. Formation of the pyran ring

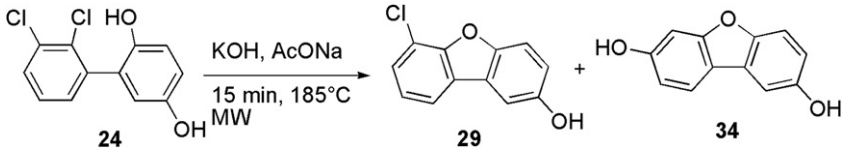
The 3,3-dimethyl-3*H*-benzofuro[3,2-*f*]benzopyrans derivatives **41-45** were prepared by thermal rearrangement of the corresponding propargyl ethers **35**–**39**, obtained by alkylation of the phenolic group of 2-hydroxydibenzofurans **29**–**33** with 3-chloro-3-methylbut-1-yne (Scheme 2) [16]. Purification of the final rearrangement mixtures by column chromatography only permitted to isolate the angular derivatives, except in the case of **39** for which the angular compound **45**, obtained in 75% yield, was accompanied by its linear isomer **46** (16%). (Fig. 3). In the case of compound **34,** which bears two hydroxyl groups, reaction with an excess of 3-chloro-3-methylbut-1-yne led successively to the formation of the dipropargyl ether **40** and the dipyranodibenzofuran **47** (Scheme 4).

2.1.4. Formation of hydroxy- and methoxy-3,3-dimethyl-3H-benzofuro[3,2-f]benzopyrans

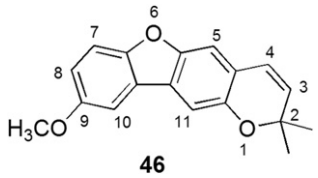
The halides **41**–**44** were converted into the corresponding phenols by nucleophilic aromatic substitution with KOH, catalyzed by a recently described system based on Pd_2dba_3 in the presence of a suitable ligand [17]. (Scheme 5). In the cases of **41** and **43**, the use of 2-di-*tert*-butylphosphino-3,4,5,6-tetramethyl-2′,4′,6′-triisopropyl-1,1′-biphenyl, (ligand **L1**) permitted to obtain phenols **48** and **50** in satisfactory 86–92% yields. The same reaction applied to bromo-compound **42** afforded the corresponding phenol **49** in a moderate 62% yield, together with the dimerization product **52** isolated in 11% yield (Fig. 4). In contrast, the more sterically hindered compound **44** could only be converted into the corresponding phenol when using 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (ligand **L2**). Under optimized conditions, the desired phenol **51** was only obtained in a low 23% yield and was accompanied by 11% of 3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran **1**, resulting from reductive elimination.

Methylation of phenols **48**, **49** and **51** using dimethylsulfate in the presence of sodium hydride at room temperature, gave the corresponding methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*]benzopyrans **53**, **54** and **55** (Scheme 5).

Also, 10-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**58**), the dihydro derivative of compound **50**, was



Scheme 3. Formation of 2,7-dihydroxydibenzofuran (**34**).



46

Fig. 3. Chemical structure of compound **46**.

obtained in three steps from the previously described 10-acetyl-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**6**), (Scheme 6) [5]. Hydrogenation of **6** using Pd/C as catalyst first afforded 10-acetyl-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**56**), which was subsequently converted to ester **57** through Baeyer–Villiger oxidation with *meta*-chloroperbenzoic acid. Final saponification of **57** gave the expected hydroxy-compound **58**.

2.2. Biology

2.2.1. Antimycobacterial activity and cytotoxicity

The antimycobacterial activities of the novel benzofurobenzopyrans were screened on *Mycobacterium bovis* BCG and on the virulent strain *M. tuberculosis* H37Rv using the Microdilution Resazurin Assay (MRA) [18]. Results are expressed as MIC_{95} (Minimal Inhibitory Concentration), which is the amount of compound required for >95% inhibition of bacterial growth. Isoniazid (INH) was used as positive control. The cytotoxicity of all final products was also evaluated on mammalian VERO cells, using doxorubicin (DOX) as control. The results are presented in Table 1.

As a general rule all compounds substituted on the A ring were found more potent than the reference compound **1**, and more active against *M. tuberculosis* than against *M. bovis*, indicating that substitution of benzofurobenzopyran core by hydroxy, methoxy or halogen groups enhanced the antimycobacterial activity.

The good activity (MIC_{95} from 23.5 to 95 μM) of hydroxylated compounds (**48**–**51**), was, unfortunately, accompanied by a strong *in vitro* cytotoxicity against VERO cells (from 3 to 9 μM) suggesting a possible contribution to the antitubercular (anti-TB) activity.

The present study confirmed the results previously observed for compound **1** and **2** indicating that the 1-2-double bond is not indispensable for anti-TB activity [1]. Indeed, dihydro compound **58** (MIC_{95} = 46 μM) was as active as its counterpart **50** (MIC_{95} = 45 μM), with a 1,2-double bond.

Halogenated (**41, 42, 44**) compounds bearing chloro or bromo substituents in positions 8, 9, and 11, respectively, exhibited an increased potency, in comparison with the hit compound **1**. In contrast, the 10-chloroderivative **43** was surprisingly totally devoid of activity against *M. tuberculosis*, but the activity against *M. bovis* was conserved. The most interesting compound in this series was the 9-bromocompound **42**, with a MIC_{95} = 37 μM against *M. tuberculosis*. Furthermore, this compound failed to show any toxic activity on VERO cells, with an IC_{50} = 3500 μM and a selectivity index of 93.

Methoxylated compounds (**53, 54, 45, 55**) were also found more active than the hit compound **1** against *M. tuberculosis* with MIC_{95} from 45 to 89 μM. The 9-methoxylated derivative **54** was the less active (MIC_{95} = 89 μM). The others benzofurobenzopyrans, substituted on the 8, 10, 11 positions (**53, 45, 55**), exhibited similar anti-TB activity, with $MICs_{95}$ values of 45 μM. Compound **55**, with an IC_{50} of 280 μM on VERO cells, was the most interesting compound of this series. The anti-TB activity of the 9-methoxylated linear isomer **46** was similar to that of the angular one **45** (MIC_{95} = 89 μM), with a total lack of toxicity against mammalian cells (IC_{50} = 7400 μM), and a selectivity index of 83.

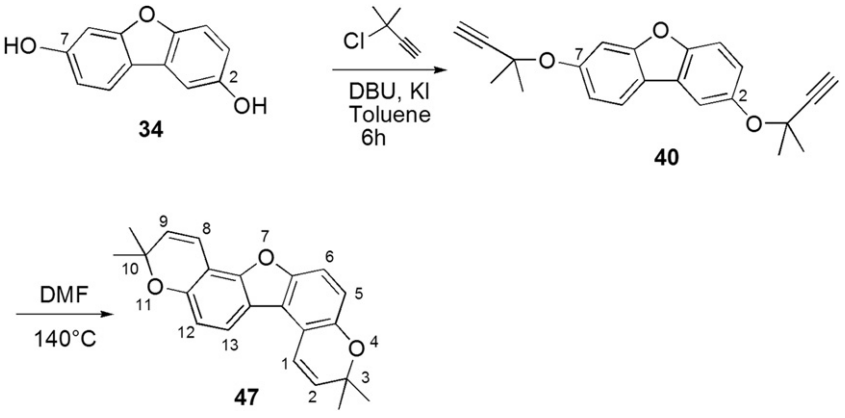
From a structure activity viewpoint, it appeared that the electronic effects of the substituents in the A ring played a dramatic role on the antimycobacterial activity, as exemplified by compounds substituted at C-10: **6**, **56**, **43**, **50**, **58**, and **45**. Indeed, the potency was significantly decreased when the A ring was substituted by an electron-withdrawing group ($R = CH_3CO-$) as illustrated by products **6** (MIC_{95} > 1742 μM) and **56** (MIC_{95} > 340 μM). In contrast, substitution by an electron-donating group, such as a hydroxy (*i.e.*, **50**: MIC_{95} = 47 μM, **58**: MIC_{95} = 46.5 μM) or methoxy group (*i.e.*, **45**: MIC_{95} = 45 μM) resulted in a significantly increased potency.

Finally, the dimer **52** and the complex compound **47**, bearing two pyran rings fused onto the dibenzofuran basic core, displayed no significant activity against both *M. tuberculosis* and *M. bovis*.

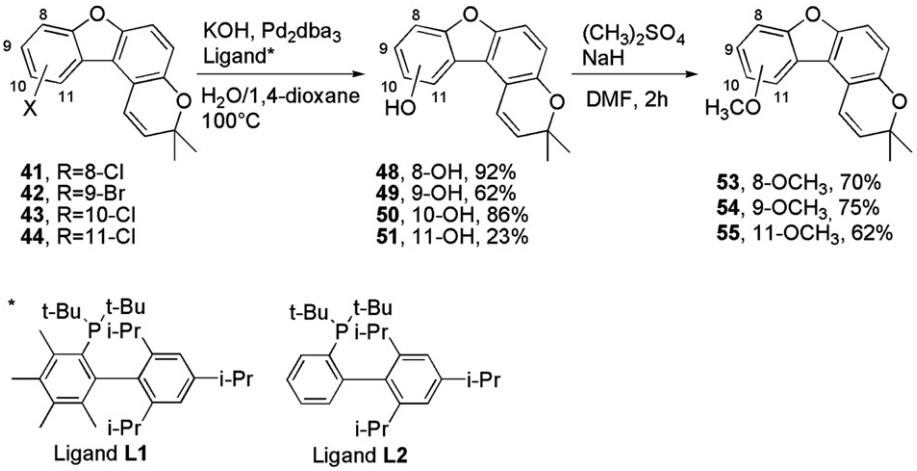
2.2.2. Inhibition of mycolates biosynthesis

Preliminary results permitted to establish that the lead compound **1** did not act on nucleic acids or protein biosynthesis. In addition, the activity on *Nocardia asteroides*, as well as preliminary results (unpublished results), suggested that compound **1** should affect the biosynthesis of mycolates and lipids present in *Mycobacterium* cell wall [19].

Mycolic acids (MAs) are major components of the lipid-rich envelope of the *Mycobacterium* genus, playing a key role in both the architecture and permeability of the mycobacterial envelope [20,21]. Furthermore, the biosynthesis pathway leading to mycolic acids is the target of the most effective antituberculous drug isoniazid (INH) [22–25].



Scheme 4. Synthesis of compound **47**.



Scheme 5. Functionalization of the halogenobenzofurobenzopyrans to hydroxyl- and methoxyl- benzofurobenzopyrans.

Extensive characterization of fatty acid synthesis in *Mycobacteria* has demonstrated the role of two fatty acid synthases (FAS), namely FAS-I and FAS-II systems [26,27] : FAS-I (a multifunctional polypeptide type-I FAS) performs *de novo* synthesis of small fatty acids from acetate producing acyl chains (C_{16}, C_{18}, and C_{22-26}) [26,28,29] and FAS-II (type-II FAS), consisting of discrete monofunctional proteins, unable to perform *de novo* synthesis, preferentially extend the FAS-I products to a mixture of longer homologous acids [26]. FAS-II elongates the FAS-I products to give meromycolate precursors, which are further modified and condensed with FAS-I products to form mycolic acids, very long chain (C_{60}–C_{90}) α-alkylated β-hydroxylated fatty acids [14,27,30–33].

The most widespread class of mycolic acids, found both in *M. tuberculosis* and *M. smegmatis*, is the so-called α-mycolates one. *M. tuberculosis* is further characterized by the presence of keto-mycolates and methoxymycolates, the latest, generally typifying slow-growing pathogenic species. In the case of *M. smegmatis*, a rapidly-grower and non-pathogen of the genus *Mycobacterium*, cell wall contains, in addition to α-mycolate, epoxymycolic acids and the shorter chain α'-mycolates [20,34]. *M. smegmatis* is often used as a model for the study of *M. tuberculosis* mycolic acid biosynthesis pathway due to the similarity of FAS-I and FAS-II systems in the different mycobacterial organisms [35].

The effect of the benzofurobenzopyran derivatives in the mycolate biosynthesis was performed on the *M. smegmatis* mycolic acid synthesis, according to the protocol of Vaubourgeix et al [36]. Compound **1**, halogenated compounds **41**, **42** and **44** and all methoxylated molecules (**45**, **46**, and **53**–**55**) were chosen among different samples for *in vitro* evaluation of the mycolate synthesis. The selection of the molecules was made on the basis of better activity and relatively low cytotoxicity toward mammalian cell lines. The concentrations applied were chosen equal to the MIC_{95} for all compounds. For this purpose, the cell-free system, which contains all the enzyme machinery producing mycolic acid, was treated with the radioactive [1-^{14}C]_acetate and the inhibition of each molecule was evaluated on the basis of reduced incorporation of radioactivity in comparison to the control. Thiolactomycine (TLM), a thiolactone antibiotic inhibiting both the FAS-II system and the elongation steps leading to the synthesis of α-mycolate and epoxymycolate, was used as a comparison standard at two different concentrations, active in cell-free extracts [37]. The percentage of inhibition was determined for the different classes of mycolates: epoxy-, α-prim, α-mycolates and fatty acids, compared with the control.

Results are presented in Fig. 5.

Treatment with TLM inhibited the synthesis of all types of mycolates and accumulated fatty acids. Among the different samples tested a similar inhibition profile was observed with the linear methoxylated molecule **46**.

All other angular molecules tested strongly inhibited the synthesis of epoxy-mycolates (>60%). In the case of methoxylated molecules (**53**, **54**, **45**, and **55**) the inhibition approximately reached 90%.

Leading unsubstituted compound **1** strongly inhibited the synthesis of the epoxy-mycolates and induced an accumulation α′-mycolates and fatty acids at its MIC. Treatment with this molecule did not affect the synthesis of α-mycolates.

Compounds methoxylated at positions 8 and 10 (**53** and **45**) showed the same inhibition profile, strongly accumulating fatty acids and slightly accumulating α′-mycolates. The synthesis of α-mycolates was practically unaffected by these two compounds.

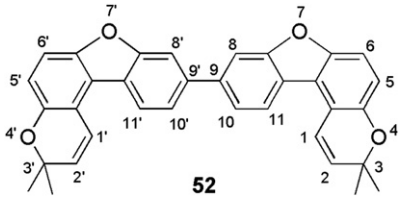
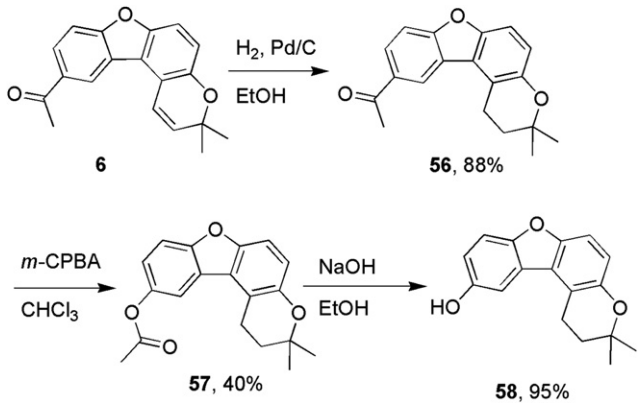


Fig. 4. Chemical structure of compound **52**.



Scheme 6. Synthesis of 10-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**58**).

Table 1
In vitro activity of all final products against *M. tuberculosis* and *M. bovis* and cytotoxicity on VERO cells.

	MIC95[a] *M. bovis*	MIC95[a] *M. tuberculosis*	IC50[a] (VERO cells)	Select Index[b]
INH	4.4	1.8	>100	–
DOX	–	–	3.6	–
1	100	100	320	3.2
6	–	>1712	–	–
56	>340	>340	–	–
Halogenated				
41	>350	87.5	100	1.2
42	150	37.5	3500	93.3
43	100	353	290	0.8
44	>350	43.8	100	2.3
Hydroxylated				
48	94	23.5	3.3	0.1
49	94	23.5	9.2	0.4
50	79	47	5	0.1
51	94	95	9.1	0.1
58	140	46.5	18	0.4
Methoxylated				
53	89	45	100	2.2
54	>360	89	100	1.1
45	89	45	100	2.2
55	89	45	280	6.2
46	89	89	7400	83.2
47	>300	>300	5000	–
52	>200	>200	8600	–

All tests were performed two times in duplicate.
[a] All the values provided are in μM.
[b] The selective index was calculated as the ration of IC50 in VERO cells to MIC95 in *Mycobacterium tuberculosis*.

The 11-methoxylated derivative **55** induced a weaker accumulation of fatty acids. In contrast, 9-methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**54**) had a different inhibition profile: while it practically did not affect the synthesis of fatty acids and α-mycolates, it significantly enhanced the synthesis of α′-mycolates (90%).

8-Chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**41**) and 9-bromo-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**42**) inhibited the synthesis of epoxy-mycolates, approximately by 65% and induced a strong accumulation of fatty acids. The 9-bromo derivative also inhibited the synthesis of α-mycolates by 25%. The 11-chlorinated compound **44** had a different inhibition profile: it strongly inhibited the synthesis of epoxy-mycolates and only induced a moderate inhibition of the syntheses of α′-mycolates, α-mycolates, and fatty acids.

As a general rule, angular benzofurobenzopyrans seem to inhibit the biosynthesis of the epoxy-mycolates of the mycobacteria cell wall and their antimycobacterial effect should be attributed to this property. The mechanism of action of 9-methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**54**) is different, since this compound induces a strong accumulation of α′-mycolates. Interestingly the inhibition profile of the linear methoxy isomer **46** is similar with that of thiolactomycine, causing accumulation of fatty acids, with parallel inhibition of all types of mycolates. These results strongly suggest that the mechanisms of action of linear and angular benzofurobenzopyran are different.

3. Conclusion

Benzofurobenzopyrans, bearing halogen, hydroxy, and methoxy substituents at positions 8, 9, 10, and 11 on the A ring, were prepared in order to determine the structural requirements indispensable to observe antimycobacterial activity in this series. Target compounds were synthesized with satisfactory yields through a novel access allowing substitutions on all positions of the A ring.

The biological results suggest that the substitution on the A ring by an electron-withdrawing group lead to inactive compounds (e.g.: **6**, **7**, **56**). On the contrary, substitution by electron-donating hydroxy or methoxy groups significantly enhances the antimycobacterial activity. It should be noted that in the case of hydroxylation, the enhancement of the antimycobacterial activity is accompanied by a significant increase of the cytotoxicity against mammalian cells. Among halogenated compounds, 9-bromo-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**42**) appears as the most interesting derivative, molecule with increased antitubercular potency and significantly decreased cytotoxicity (selectivity index 93 for VERO cells).

Finally, 9-methoxy-2,2-dimethyl-2*H*-benzofuro[2,3-*g*][1]benzopyran (**46**) might be considered of special interest. Indeed, this linear compound associates a good antitubercular potency with a reduced toxicity towards mammalian cells, suggesting to further explore structure-activity relationships in the benzofuro[2,3-*g*][1]benzopyran in a near future.

All compounds were shown to interact with the mycobacterial cell wall lipid biosynthesis. However, whereas angular 3,3-dimethyl-

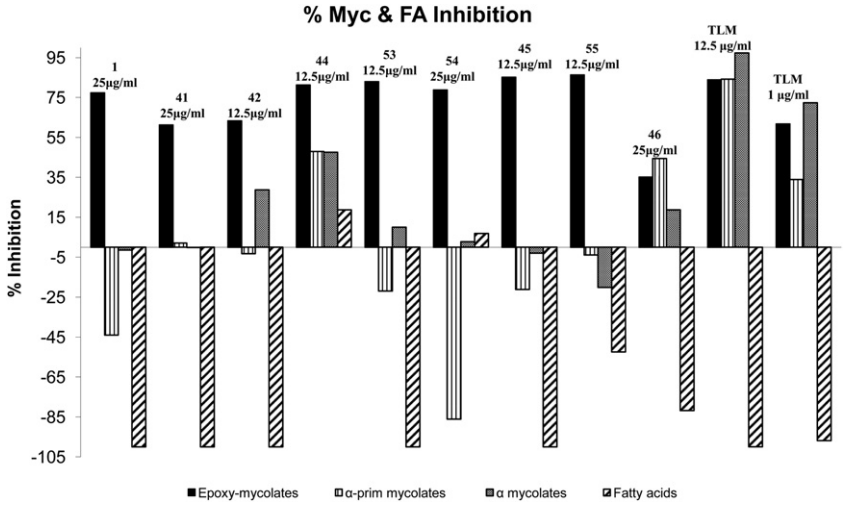


Fig. 5. The mycolic and fatty acid inhibition profile of the synthesized benzofurobenzopyrans. TLM was used as a positive control in two concentrations. Experiment was performed twice in duplicates. The data presented above indicate the mean values obtained by two independent measurements.

3*H*-benzofuro[3,2-*f*][1]benzopyran inhibit the synthesis of the epoxy-mycolates, the linear analogue **46** was shown to act through a different mechanism of action close to that of thiolactomycine, causing accumulation of fatty acids and inhibition of all types of mycolates.

4. Experimental section

4.1. Chemistry

Column chromatographies were performed on Merck silica gel (20–45 μm or 35–70 μm). Thin layer chromatographies were performed using Merck TLC Silica gel 60 F_{254}. IR spectra were obtained from potassium bromide pellets on a Nicolet-510 instrument. UV spectra were recorded in spectroscopic grade MeOH on a Jenway 6705 spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer and ^{13}C NMR on a Bruker Avance 300 (75 MHz) spectrometer. When necessary, the structures of the novel compounds were insured and the signals unambiguously assigned by 2D NMR techniques: ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{13}C–^{1}H HMQC, and ^{13}C–^{1}H HMBC. These experiments were performed using standard Bruker microprograms on a Bruker Avance 400 spectrometer. Mass spectra were recorded with a ZQ 2000 spectrometer using electron spray ionization. Microwave experiments were realized on a Biotage apparatus Initiator™ 2.0. The temperature was set at 185 °C and the level of absorbance at "Very High" in the case of solid phase reactions.

4.1.1. 2′-Chloro-4′-methoxy[1,1′-biphenyl]-2,5-diol (**11**) and 2-(2-chloro-4-methoxyphenyl)-1,4-benzoquinone (**12**)

A biphasic mixture of 2-chloro-4-methoxyphenylboronic acid (**10**) (100 mg, 0.53 mmol), 2-bromohydroquinone (**9**) (91.5 mg, 0.48 mmol), $Pd(PPh_3)_4$ (17 mg, 14.7 nmol), and Na_2CO_3 (308 mg, 2.9 mmol) in toluene:H_2O (5:1) (10 mL) was stirred under argon at 80 °C for 20 h. The reaction mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2) gave successively 2-(2-chloro-4-methoxyphenyl)-1,4-benzoquinone (**12**) (22 mg, 19%) and 2′-chloro-4′-methoxy[1,1′-biphenyl]-2,5-diol (**11**) (7 mg, 6%).

*4.1.1.1. 2-(2-Chloro-4-methoxyphenyl)-1,4-benzoquinone (**12**).* TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.80. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 8.5 Hz, 1H, H-6′), 7.02 (d, J = 2.5 Hz, 1H, H-3′), 6.90 (d, J = 10.0 Hz, 1H, H-6), 6.87 (dd, J = 2.5, 8.4 Hz, 1H, H-5′), 6.84 (dd, J = 2.5, 10.0 Hz, 1H, H-5), 6.79 (d, J = 2.5 Hz, 1H, H-3), 3.85 (s, 1H, $-OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.4 (C-4), 185.5 (C-1), 161.1 (C-4′), 145.7 (C-2), 136.9 (C-6), 136.4 (C-5), 134.9 (C-3), 134.0 (C-2′), 131.6 (C-6′), 124.4 (C-1′), 115.4 (C-3′), 112.9 (C-5′), 55.7 ($-OCH_3$). MS (ESI) m/z: 249 $(M + H)^+$ (^{35}Cl), 251 $(M + H)^+$ (^{37}Cl).

*4.1.1.2. 2′-Chloro-4′-methoxy[1,1′-biphenyl]-2,5-diol (**11**).* TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/EtOAc, 9:1, Rf = 0.68. ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (d, J = 8.5 Hz, 1H, H-6′), 7.03 (d, J = 2.5 Hz, 1H, H-3′), 6.89 (dd, J = 2.5, 8.5 Hz, 1H, H-5′), 6.76 (d, J = 8.5 Hz, 1H, H-3), 6.69 (dd, J = 3.0, 8.5 Hz, 1H, H-4), 6.60 (d, J = 3.0 Hz, 1H, H-6), 3.85 (s, 1H, $-OCH_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 159.5 (C-4′), 149.4 (C-5), 146.8 (C-2), 134.2 (C-2′), 132.3 (C-6′), 129.1 (C-1′), 126.7 (C-1), 117.5 (C-6), 116.2 (C-3), 115.7 (C-4), 114.8 (C-3′), 112.9 (C-5′), 55.5 ($-OCH_3$). MS (ESI) m/z: 251 $(M + H)^+$ (^{35}Cl), 253 $(M + H)^+$ (^{37}Cl).

4.1.2. 2-(2,3-Dichlorophenyl)-1,4-benzoquinone (**19**)

In a typical experiment, a solution of $NaNO_2$ (0.45 g, 6.5 mmol) in water (1.6 mL) was added to a solution of 2,3-dichloroanilin (**13**) (1.00 g, 6.1 mmol) in 9% aqueous hydrochloric acid solution (6.5 mL), and the mixture was stirred at 0–5 °C for 1.5 h to ensure the formation of the diazonium salt. The diazonium salt solution was slowly added to a water solution (54 mL) of 1,4-benzoquinone (**18**) (0.80 g, 7.4 mmol) and sodium acetate (1.2 g, 14.6 mmol). Isolation of the precipitate by filtration, followed by thorough washing of with water (3 × 10 mL) gave 2-(2,3-dichlorophenyl)-1,4-benzoquinone (**19**) (1.40 g, 91%) as a yellow amorphous powder. TLC Silica gel 60 F_{254}, CH_2Cl_2/C_6H_{12} 80:20, Rf = 0.62. IR (KBr) ν_{max} (cm^{-1}): 3070, 3065, 2960, 2918, 1657, 1599, 1450, 1410, 1334, 1291, 1283, 1100, 909, 863, 839, 777, 711. UV λ_{max} (nm) (log ε) (MeOH): 210 (4.45), 246 (4.16), 299 (3.48). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.1 Hz, 1H, H-4′), 7.34 (t, J = 8.1 Hz, 1H, H-5′), 7.17 (d, J = 8.1 Hz, 1H, H-6′), 6.96 (d, J = 10.2 Hz, 1H, H-6), 6.92 (dd, J = 1.6, 10.24 Hz, 1H, H-5), 6.82 (d, J = 1.6 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.1 (C-4), 184.7 (C-1), 146.0 (C-2), 136.8 (C-6), 136.5 (C-5), 134.9 (C-3), 134.6 (C-3′), 133.8 (C-2′), 131.6 (C-1′), 131.5 (C-4′), 128.6 (C-6′), 127.5 (C-5′). MS (ESI) m/z: 251 $(M - H)^-$ (^{35}Cl, ^{35}Cl), 253 $(M - H)^-$ (^{35}Cl, ^{37}Cl), 255 $(M - H)^-$ (^{37}Cl, ^{37}Cl).

4.1.3. 2-(4-Bromo-2-chlorophenyl)-1,4-benzoquinone (**20**)

The procedure described for the preparation of **19**, applied to 4-bromo-2-chloroanilin (**14**) (1.00 g, 4.9 mmol) and 1,4-benzoquinone (**18**) (0.63 g, 5.8 mmol), gave **20** (1.30 g, 90%) as a yellow amorphous solid. TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.89. IR (KBr) ν_{max} (cm^{-1}): 3081, 3065, 2918, 1648, 1590, 1466, 1346, 1287, 1089, 1062, 976, 929, 836, 762. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.65), 217sh (4.53), 298 (3.71). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 1.7 Hz, 1H, H-3′), 7.54 (dd, J = 1.7, 8.2 Hz, 1H, H-5′), 7.14 (d, J = 8.2 Hz, 1H, H-6′), 6.96 (d, J = 10.1 Hz, 1H, H-6), 6.92 (dd, J = 2.3, 10.1 Hz, 1H, H-5), 6.84 (d, J = 2.3 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.0 (C-4), 184.7 (C-1), 145.1 (C-2), 136.8 (C-6), 136.5 (C-5), 135.2 (C-3), 134.1 (C-2′), 132.6 (C-3′), 131.7 (C-6′), 131.3 (C-1′), 130.2 (C-5′), 124.1 (C-4′). MS (ESI^-) m/z: 295 $[M - H]^-$ (^{35}Cl, ^{79}Br), 297 $[M - H]^-$ (^{35}Cl, ^{81}Br or ^{37}Cl, ^{79}Br), 299 $[M - H]^-$ (^{37}Cl, ^{81}Br).

4.1.4. 2-(2,5-Dichlorophenyl)-1,4-benzoquinone (**21**)

The procedure described for the preparation of **19**, applied to 2,5-dichloroanilin (**15**) (2.00 g, 12.3 mmol) and 1,4-benzoquinone (**18**) (1.60 g, 14.8 mmol), gave **21** (2.80 g, 93%) as a yellow amorphous solid. TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.74. IR (KBr) ν_{max} (cm^{-1}): 3085, 3050, 1653, 1466, 1330, 1275, 1096, 993, 925, 839, 816, 426. UV λ_{max} (nm) (log ε) (MeOH): 208 (4.46), 215 (4.39), 229sh (3.96), 253sh (4.03), 292 (3.45). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.6 Hz, 1H, H-3′), 7.41 (dd, J = 2.28, 8.6 Hz, 1H, H-4′), 7.28 (d, J = 2.2 Hz, 1H, H-6′), 6.96 (d, J = 10.1 Hz, 1H, H-6), 6.92 (dd, J = 2.2, 10.1 Hz, 1H, H-5), 6.85 (d, J = 2.2 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.9 (C-4), 184.6 (C-1), 144.9 (C-2), 136.8 (C-6), 136.5 (C-5), 135.3 (C-3), 133.7 (C-1′), 132.8 (C-5′), 131.5 (C-2′), 131.0 (C-3′), 130.7 (C-4′), 130.5 (C-6′). MS (ESI) m/z: 253 $(M + H)^+$ (^{35}Cl, ^{35}Cl), 255 $(M + H)^+$ (^{35}Cl, ^{37}Cl), 257 $(M + H)^+$ (^{37}Cl, ^{37}Cl).

4.1.5. 2-(2-Chloro-5-methoxyphenyl)-1,4-benzoquinone (**23**)

The procedure described for the preparation of **19**, applied to 2-chloro-5-methoxyanilin (**17**) (1.00 g, 6.3 mmol) and 1,4-benzoquinone (**18**) (0.82 g, 7.6 mmol), gave **23** (1.40 g, 84%) as a yellow amorphous solid. TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.51. IR (KBr) ν_{max} (cm^{-1}): 3069, 3059, 2976, 2945, 1653, 1594, 1567, 1474, 1275, 1224, 1170, 1100, 1030, 921, 824, 644. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.51), 288 (3.71), 234sh (4.20). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.9 Hz, 1H, H-3′), 6.94 (dd, J = 3.00, 8.9 Hz, 1H, H-4′), 6.92 (d, J = 10.1 Hz, 1H, H-6), 6.87 (dd, J = 2.1, 10.1 Hz, 1H, H-5), 6.80 (d, J = 2.1 Hz, 1H, H-3), 6.76 (d, J = 3.0 Hz, 1H, H-6′), 3.82 (s, 3H, $-OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.3 (C-4), 185.0 (C-1), 158.2 (C-5′), 146.1 (C-2), 136.8 (C-6), 136.4 (C-5), 135.0 (C-3), 133.1 (C-1′), 130.6 (C-3′), 124.3 (C-2′), 116.3 (C-4′), 116.2 (C-6′), 55.7 (C–OCH_3). MS (ESI) m/z: 247 $(M - H)^-$ (^{35}Cl), 249 $(M - H)^-$ (^{37}Cl).

*4.1.6. 2′,3′-Dichloro[1,1′-biphenyl]-2,5-diol (**24**)*

A saturated aqueous solution of $Na_2S_2O_4$ (200 mL) was added to a solution of 2-(2,3-dichlorophenyl)-1,4-benzoquinone (**19**) (1.00 g, 4.0 mmol) in Et_2O (400 mL). The mixture was vigorously stirred for 1.5 h. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crystallization from toluene gave 2′,3′-dichloro[1,1′-biphenyl]-2,5-diol (**24**) (0.92 g, 91%) as white prisms, m.p. 151–152 °C. TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.17. IR (KBr) ν_{max} (cm^{-1}): 3307, 3054, 3027, 1509, 1457, 1407, 1353, 1310, 1244, 1189, 1034, 814, 777, 757. UV λ_{max} (nm) (log ε) (MeOH): 217 (4.49), 305 (3.67). ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (dd, J = 1.8, 7.7 Hz, 1H, H-4′), 7.30 (t, J = 7.7 Hz, 1H, H-5′), 7.24 (dd, J = 1.8, 7.7 Hz, 1H, H-6′), 6.75 (d, J = 8.7 Hz, 1H, H-3), 6.70 (dd, J = 2.8, 8.7 Hz, 1H, H-4), 6.55 (d, J = 2.8 Hz, 1H, H-6). ^{13}C NMR (75 MHz, CD_3OD) δ 149.6 (C-5), 146.9 (C-2), 140.5 (C-1′), 132.5 (C-3′), 132.1 (C-2′), 129.9 (C-4′), 128.9 (C-6′), 127.1 (C-1), 126.8 (C-5′), 116.5 (C-6), 116.1 (C-4), 115.7 (C-3). MS (ESI) *m/z*: 253 $(M-H)^-$ (^{35}Cl, ^{35}Cl), 255 $(M-H)^-$ (^{35}Cl, ^{37}Cl), 257 $(M-H)^-$ (^{37}Cl, ^{37}Cl), 507 $(2M-H)^-$.

*4.1.7. 4′-Bromo-2′-chloro[1,1′-biphenyl]-2,5-diol (**25**)*

Sodium dithionite reduction of 2-(4-bromo-2-chlorophenyl)-1,4-benzoquinone (**20**) (0.90 g, 3.0 mmol), under the conditions described for the reduction of **19** to **24**, gave 4′-bromo-2′-chloro [1,1′-biphenyl]-2,5-diol (**25**) (0.90 g, 88%), as whitish prisms, m.p. 172–173 °C (toluene). TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.15. IR (KBr) ν_{max} (cm^{-1}): 3303, 3073, 3032, 1583, 1508, 1477, 1462, 1370, 1306, 1186, 1131, 1084, 1069, 808, 788, 768. UV λ_{max} (nm) (log ε) (MeOH): 212 (4.50), 217 (4.55), 236sh (4.14), 306 (3.73). ^{1}H NMR (400 MHz, CD_3OD) δ 7.66 (d, J = 1.9 Hz, 1H, H-3′), 7.49 (dd, J = 1.9, 8.2 Hz, 1H, H-5′), 7.23 (d, J = 8.2 Hz, 1H, H-6'), 6.75 (d, J = 8.7 Hz, 1H, H-3), 6.70 (dd, J = 2.8, 8.7 Hz, 1H, H-4), 6.56 (d, J = 2.8 Hz, 1H, H-6). ^{13}C NMR (75 MHz, CD_3OD) δ 149.6 (C-5), 147.0 (C-2), 137.2 (C-1′), 134.6 (C-2′), 133.0 (C-6′), 131.4 (C-3′), 129.4 (C-5′), 126.1 (C-1), 120.6 (C-4′), 116.7 (C-6), 116.1 (C-3), 115.7 (C-4). MS (ESI) *m/z*: 297 $(M-H)^-$ (^{35}Cl, ^{79}Br), 299 $(M-H)^-$ (^{35}Cl, ^{81}Br or ^{37}Cl, ^{79}Br), 301 $(M-H)^-$ (^{37}Cl, ^{81}Br).

*4.1.8. 2′,5′-Dichloro[1,1′-biphenyl]-2,5-diol (**26**)*

Sodium dithionite reduction of 2-(2,5-dichlorophenyl)-1,4-benzoquinone (**21**) (2.50 g, 9.9 mmol), under the conditions described for the reduction of **19** to **24**, gave 2′,5′-dichloro[1,1′-biphenyl]-2,5-diol (**26**) (2.20 g, 88%), as white prisms, m.p. 163 °C (toluene). TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.14. IR (KBr) ν_{max} (cm^{-1}): 3529, 3389, 3062, 3034, 1516, 1474, 1446, 1353, 1298, 1201, 1092, 1026, 827, 807, 797, 772. UV λ_{max} (nm) (log ε) (MeOH): 210 (4.45), 216 (4.45), 307 (3.63). ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (d, J = 8.9 Hz, 1H, H-3′), 7.32 (dd, J = 2.5, 8.9 Hz, 1H, H-4′), 7.32 (d, J = 2.5 Hz, 1H, H-6′), 6.76 (d, J = 8.7 Hz, 1H, H-3), 6.71 (dd, J = 2.8, 8.7 Hz, 1H, H-4), 6.57 (d, J = 2.8 Hz, 1H, H-6). ^{13}C NMR (75 MHz, CD_3OD) δ 149.6 (C-5), 147.0 (C-2), 139.6 (C-1′), 132.1 (C-5′), 131.7 (C-2′), 131.3 (C-6′), 130.3 (C-3′), 128.1 (C-4′), 126.0 (C-1), 116.6 (C-6), 116.2 (C-3), 115.9 (C-4). MS (ESI) *m/z*: 253 $(M-H)^-$ (^{35}Cl, ^{35}Cl), 255 $(M-H)^-$ (^{35}Cl, ^{37}Cl), 257 $(M-H)^-$ (^{37}Cl, ^{37}Cl).

*4.1.9. 2′,6′-Dichloro[1,1′-biphenyl]-2,5-diol (**27**)*

A solution of $NaNO_2$ (1.90 g, 27 mmol) in water (3.2 mL) was added to a solution of 2,6-dichloroanilin (**16**) (4.00 g, 24.6 mmol) in water (88 mL) and concentrated aqueous solution of hydrochloric acid (12 *N*, 45 mL). The mixture was stirred at 0–5 °C for 3 h to ensure the formation of the diazonium salt. The diazonium salt solution was then slowly added to a solution of 1,4-benzoquinone (**18**) (3.20 g, 29.6 mmol) in water (108 mL) containing sodium acetate (4.8 g, 59 mmol). After 24 h stirring at room temperature, the solid precipitate was isolated by filtration to obtain crude 2-(2,6-dichlorophenyl)-1,4-benzoquinone (**22**) (0.74 g, ~12%). The crude material was dissolved in Et_2O (400 mL) and stirred for 2.5 h with a saturated aqueous solution of $Na_2S_2O_4$ (200 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Crystallization from toluene gave 2′,6′-dichloro[1,1′-biphenyl]-2,5-diol (**27**) (0.56 g, 9% overall yield), as white prisms, m.p. 146–147 °C. TLC Silica gel 60 F_{254}, CH_2Cl_2, Rf = 0.13. IR (KBr) ν_{max} (cm^{-1}): 3490, 3346, 3031, 1559, 1497, 1450, 1427, 1189, 808, 793, 777, 730, 718. UV λ_{max} (nm) (log ε) (MeOH): 209 (4.53), 303 (3.69). ^{1}H NMR (400 MHz, CD_3OD) δ 7.43 (d, J = 8.0 Hz, 2H, H-5′, H-3′), 7.29 (t, J = 8.0 Hz, 1H, H-4′), 6.77 (d, J = 8.7 Hz, 1H, H-3), 6.72 (dd, J = 2.8, 8.7 Hz, 1H, H-4), 6.48 (d, J = 2.8 Hz, 1H, H-6). ^{13}C NMR (75 MHz, CD_3OD) δ 149.7 (C-5), 147.1 (C-2), 136.9 (C-1′), 135.4 (C-2′, C-6′), 128.9 (C-4′), 127.5 (C-3′, C-5′), 124.8 (C-1), 116.5 (C-6), 116.1 (C-4), 115.9 (C-3). MS (ESI) *m/z*: 253 $(M-H)^-$ (^{35}Cl, ^{35}Cl), 255 $(M-H)^-$ (^{35}Cl, ^{37}Cl), 257 $(M-H)^-$ (^{37}Cl, ^{37}Cl).

*4.1.10. 2′-Chloro-5′-methoxy[1,1′-biphenyl]-2,5-diol (**28**)*

Sodium dithionite reduction of 2-(2-chloro-5-methoxyphenyl)-1,4-benzoquinone (**23**) (1.00 g, 4.0 mmol), under the conditions described for the reduction of **19** to **24**, gave 2′-chloro-5′-methoxy [1,1′-biphenyl]-2,5-diol (**28**) (0.55 g, 55%), as white needles, m.p. 129–130 °C (toluene). TLC Silica gel 60 F_{254}, CH_2Cl_2/AcOEt 90:10, Rf = 0.55. IR (KBr) ν_{max} (cm^{-1}): 3408, 3003, 2968, 2944, 2840, 1594, 1575, 1511, 1466, 1450, 1314, 1221, 1170, 1021, 1170, 1022, 802, 783. UV λ_{max} (nm) (log ε) (MeOH): 211 (4.45), 216 (4.46), 294 (3.73). ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (d, J = 8.6 Hz, 1H, H-3′), 6.89 (dd, J = 2.9, 8.6 Hz, 1H, H-4′), 6.86 (d, J = 2.9 Hz, 1H, H-6′), 6.74 (d, J = 8.7 Hz, 1H, H-3), 6.68 (dd, J = 2.8, 8.7 Hz, 1H, H-4), 6.57 (d, J = 2.8 Hz, 1H, H-6), 3.80 (s, 3H, $-OCH_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 158.1 (C-5′), 149.5 (C-5), 146.9 (C-2), 138.6 (C-1′), 129.6 (C-3′), 127.4 (C-1), 124.8 (C-2′), 116.9 (C-6), 116.8 (C-3), 116.1 (C-4), 115.3 (C-6′), 113.9 (C-4′), 54.6 ($C-OCH_3$). MS (ESI) *m/z*: 249 $(M-H)^-$ (^{35}Cl), 251 $(M-H)^-$ (^{37}Cl).

*4.1.11. 6-Chloro-2-hydroxydibenzofuran (**29**)*

Potassium hydroxyde (66 mg, 1.2 mmol), sodium acetate (83 mg, 1 mmol), and sodium dithionite (10 mg, 0.07 mmol) were added to a solution of 2′,3′-dichloro[1,1′-biphenyl]-2,5-diol (**24**) (100 mg, 0.40 mmol) in acetonitrile (4 mL) in a 35 mL tube, which was sealed under vacuum and heated for 30 min at 175–180 °C under stirring. The reaction mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/cyclohexane, 9:1) gave 6-chloro-2-hydroxydibenzofuran (**29**) (79 mg, 91%) as an amorphous white solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.46. IR (KBr) ν_{max} (cm^{-1}): 3276, 3080, 2933, 1477, 1450, 1420, 1224, 1193, 1170, 1142, 848, 812, 792, 743. UV λ_{max} (nm) (log ε) (MeOH): 219sh (4.55), 212 (4.47), 247sh (4.19), 258 (4.12), 282sh (3.99), 291 (4.15), 324 (3.71). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.59 (s, 1H, -O*H*), 7.85 (d, J = 7.9 Hz, 1H, H-9), 7.46 (d, J = 8.9 Hz, 1H, H-4), 7.45 (d, J = 7.9 Hz, 1H, H-7), 7.36 (d, J = 2.6 Hz, 1H, H-1), 7.28 (t, J = 7.8 Hz, 1H, H-8), 7.00 (dd, J = 2.6, 8.9 Hz, 1H, H-3). ^{13}C NMR (75 MHz, DMSO-*d6*) δ 153.7 (C-2), 152.3 (C-5a), 150.2 (C-4a), 126.6 (C-7), 126.2 (C-6), 124.5 (C-9b), 123.1 (C-8), 118.9 (C-9), 116.5 (C-9a), 116.0 (C-3), 111.7 (C-4), 105.6 (C-1). MS (ESI) *m/z*: 217 $(M-H)^-$ (^{35}Cl), 219 $(M-H)^-$ (^{37}Cl).

*4.1.12. 2,7-Dihydroxydibenzofuran (**34**)*

Potassium hydroxide (200 mg, 3.6 mmol), sodium acetate (83 mg, 1 mmol), sodium dithionite (10 mg, 0.07 mmol), and 2′,3′-dichloro[1,1′-biphenyl]-2,5-diol (**24**) (100 mg, 0.4 mmol) were thoroughly mixed in a 10 mL microwave vial. The vial was sealed and left for 15 min at 185 °C in the microwave apparatus, with the absorption level set at "Very High". The reaction mixture was diluted with water (20 mL) and extracted with dichloromethane

(3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2) successively afforded 6-chloro-2-hydroxydibenzofuran (**29**) (10 mg, 12%) and 2,7-dihydroxydi benzofuran (**34**) (11.5 mg, 15%), as an amorphous whitish solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.04. IR (KBr) ν_{max} (cm^{-1}): 3202, 3105, 3031, 2930, 1645, 1595, 1470, 1443, 1326, 1210, 1186, 1143, 1104, 812. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.21), 307 (3.54). ^1H NMR (400 MHz, CD_3OD) δ 7.70 (d, J = 8.4 Hz, 1H, H-9), 7.29 (d, J = 8.8 Hz, 1H, H-4), 7.24 (d, J = 2.6 Hz, 1H, H-1), 6.91 (d, J = 2.1 Hz, 1H, H-6), 6.81 (dd, J = 2.6, 8.8 Hz, 1H, H-3), 6.80 (dd, J = 2.1, 8.4 Hz, 1H, H-8). ^{13}C NMR (75 MHz, CD_3OD) δ 158.3 (C-7), 157.6 (C-5a), 153.0 (C-4a), 150.3 (C-2), 125.1 (C-9b), 120.5 (C-9), 116.4 (C-9a), 112.9 (C-8), 110.8 (C-4), 110.8 (C-3), 104.6 (C-1), 97.6 (C-6). MS (ESI) *m/z*: 199 $(M - H)^-$.

4.1.13. 7-Bromo-2-hydroxydibenzofuran (**30**)

The procedure described for the preparation of **29** from **24** in a sealed tube, applied to 4′-bromo-2′-chloro[1,1′-biphenyl]-2,5-diol (**25**) (100 mg, 0.34 mmol) gave 7-bromo-2-hydroxydibenzofuran (**30**) (60 mg, 75%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.24. IR (KBr) ν_{max} (cm^{-1}): 3256, 3100, 2956, 2925, 1477, 1438, 1415, 1219, 1184, 1170, 905, 804. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.56), 220sh (4.45), 248 (4.25), 257 (4.26), 295 (4.19), 320 (3.74). ^1H NMR (400 MHz, CD_3OD) δ 7.85 (d, J = 8.3 Hz, 1H, H-9), 7.74 (d, J = 1.7 Hz, 1H, H-6), 7.46 (dd, J = 1.7, 8.3 Hz, 1H, H-8) 7.40 (d, J = 8.9 Hz, 1H, H-4), 7.36 (d, J = 2.6 Hz, 1H, H-1), 6.98 (dd, J = 2.6, 8.9 Hz, 1H, H-3). ^{13}C NMR (75 MHz, CD_3OD) δ 157.1 (C-5a), 153.6 (C-2), 150.4 (C-4a), 125.4 (C-8), 123.9 (C-9a), 123.6 (C-9b), 121.4 (C-9), 119.6 (C-7), 115.7 (C-3), 114.5 (C-6), 111.5 (C-4), 105.3 (C-1). MS (ESI) *m/z*: 261 $(M - H)^-$ (^{79}Br), 263 $(M - H)^-$ (^{81}Br), 523 $(2M\text{-}H)^-$ (^{79}Br), 525 $(2M\text{-}H)^-$ (^{81}Br).

4.1.14. 8-Chloro-2-hydroxydibenzofuran (**31**)

The procedure described for the preparation of **29** from **24** in a sealed tube, applied to **26** (100 mg, 0.40 mmol) gave 8-Chloro-2-hydroxydibenzofuran (**31**) (70 mg, 81%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.37. IR (KBr) ν_{max} (cm^{-1}): 3233, 2925, 2855, 1598, 1470, 1442, 1419, 1166, 1065, 866, 804, 750, 695. UV λ_{max} (nm) (log ε) (MeOH): 209 (4.49), 244 (4.15), 254 (4.10), 298 (4.12), 329sh (3.57). ^1H NMR (400 MHz, CD_3OD) δ 7.94 (d, J = 2.1 Hz, 1H, H-9), 7.50 (d, J = 8.8 Hz, 1H, H-6), 7.42 (dd, J = 2.1, 8.8 Hz, 1H, H-7), 7.41 (d, J = 8.8 Hz, 1H, H-4), 7.36 (d, J = 2.6 Hz, 1H, H-1), 6.99 (dd, J = 2.5, 8.8 Hz, 1H, H-3). ^{13}C NMR (75 MHz, CD_3OD) δ 155.2 (C-5a), 153.5 (C-2), 150.9 (C-4a), 127.5 (C-8), 126.6 (C-7), 125.9 (C-9a), 123.7 (C-9b), 120.0 (C-9), 116.1 (C-3), 112.3 (C-6), 111.6 (C-4), 105.4 (C-1). MS (ESI) *m/z*: 217 $(M - H)^-$ (^{35}Cl), 219 $(M - H)^-$ (^{37}Cl).

4.1.15. 9-Chloro-2-hydroxydibenzofuran (**32**)

Potassium hydroxyde (300 mg, 5.4 mmol), sodium acetate (83 mg, 1 mmol), sodium dithionite (28 mg, 0.19 mmol), and water (150 μL) were added to a solution of 2′,6′-dichlorobiphenyl-2,5-diol (**27**) (100 mg, 0.40 mmol) in acetonitrile (4 mL) in a 35 mL tube, which was sealed under vacuum and heated for 2.5 h at 175–180 °C under stirring. The reaction mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to give, by crystallization from cyclohexane, 9-chloro-2-hydroxydibenzofuran (**32**) (37 mg, 44%), as white needles, m.p. 113–115 °C. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.47. IR (KBr) ν_{max} (cm^{-1}): 3292, 2957, 2925, 2851, 1477, 1420, 1227, 1193, 1162, 933, 808, 777, 744. UV λ_{max} (nm) (log ε) (MeOH): 209 (4.50), 217 (4.45), 240 (4.24), 257 (4.08), 282sh (3.95), 290 (4.09), 325 (3.72). ^1H NMR (400 MHz, CD_3OD) δ 7.71 (d, J = 2.3 Hz, 1H, H-1), 7.45 (d, J = 8.2 Hz, 1H, H-6), 7.41 (d, J = 9.0 Hz, 1H, H-4), 7.38 (t, J = 8.2 Hz, 1H, H-7), 7.28 (d, J = 7.8 Hz, 1H, H-8), 7.01 (dd, J = 2.5, 8.8 Hz, 1H, H-3). ^{13}C NMR (75 MHz, CD_3OD) δ 157.3 (C-5a), 153.2 (C-2), 150.1 (C-4a), 127.6 (C-9), 127.2 (C-7), 123.2 (C-9a), 122.6 (C-8), 122.1 (C-9b), 115.8 (C-3), 111.3 (C-4), 109.7 (C-6), 107.4 (C-1). MS (ESI) *m/z*: 217 $(M - H)^-$ (^{35}Cl), 219 $(M - H)^-$ (^{37}Cl).

4.1.16. 8-Methoxy-2-hydroxydibenzofuran (**33**)

The procedure described for the preparation of **29** and **34** from **24** by microwave irradiation, applied to 2′-chloro-5′methoxybiphenyl-2,5-diol (**28**) (100 mg, 0.40 mmol) gave, after chromatography on silica gel (CH_2Cl_2), 8-methoxy-2-hydroxybenzofuran (**33**) (30 mg, 35%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2, Rf = 0.15. IR (KBr) ν_{max} (cm^{-1}): 3381, 3097, 3062, 3000, 2930, 1599, 1485, 1459, 1435, 1186, 1155, 1034, 839, 816, 793. UV λ_{max} (nm) (log ε) (MeOH): 213 (4.41), 217 (4.49), 256 (3.89), 304 (4.13), 328 (3.62). ^1H NMR (400 MHz, CD_3OD) δ 7.41 (d, J = 2.6 Hz, 1H, H-9), 7.38 (d, J = 8.9 Hz, 1H, H-6), 7.34 (d, J = 8.9 Hz, 1H, H-4), 7.34 (d, J = 2.8 Hz, 1H, H-1), 7.0 (dd, J = 2.6, 8.9 Hz, 1H, H-7), 6.93 (dd, J = 2.5, 8.9 Hz, 1H, H-3), 3.86 (s, 3H, $-OCH_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 155.8 (C-8), 152.8 (C-4a), 151.5 (C-5a), 151.0 (C-2), 124.9 (C-9b), 124.7 (C-9a), 115.1 (C-3), 114.8 (C-7), 111.5 (C-6), 111.3 (C-4), 105.3 (C-1), 103.2 (C-9), 55.0 ($C-OCH_3$). MS (ESI) *m/z*: 213 $(M - H)^-$.

4.1.17. 6-Chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**35**)

Potassium iodide (119 mg, 0.72 mmol) and DBU (164 μL, 1.1 mmol) were added to a solution of 6-chloro-2-hydroxydibenzofuran (**29**) (120 mg, 0.55 mmol) in anhydrous toluene (4 mL) under argon. 3-Chloro-3-methylbut-1-yne (166 μL, 1.1 mmol) was added and the reaction mixture was stirred for 2 h at 65 °C under argon. The reaction was quenched with water (10 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography over silica gel (cyclohexane/CH_2Cl_2, 9:1) gave 6-chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**35**) (134 mg, 86%) as an amorphous white solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.52. IR (KBr) ν_{max} (cm^{-1}): 3300, 2984, 2926, 2852, 1474, 1443, 1190, 1171, 1140, 960, 890, 867, 781, 735. UV λ_{max} (nm) (log ε) (MeOH): 210 (4.65), 218 (4.78), 255 (4.27), 285 (4.34), 310 (3.80). ^1H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 7.7 Hz, 1H, H-9), 7.82 (d, J = 2.4 Hz, 1H, H-1), 7.60 (d, J = 8.9 Hz, 1H, H-4), 7.49 (d, J = 7.7 Hz, 1H, H-7), 7.41 (dd, J = 2.4, 8.9 Hz, 1H, H-3), 7.31 (t, J = 7.8 Hz, 1H, H-8), 2.66 (s, 1H, H-3′), 1.74 (s, 6H, H-1′a, 1′b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6 (C-5a), 152.0 (C-4a), 151.5 (C-2), 127.2 (C-7), 126.5 (C-6), 124.3 (C-9b), 123.4 (C-8), 123.2 (C-3), 119.1 (C-9), 117.1 (C-9a), 114.3 (C-1), 111.9 (C-4), 86.1 (C-2′), 74.2 (C-3′), 73.2 (C-1′), 29.6 (C-1′a, 1′b). MS (ESI) *m/z*: 285 $(M + H)^+$ (^{35}Cl), 287 $(M + H)^+$ (^{37}Cl), 591 $(2M + Na)^+$ (^{35}Cl), 593 $(2M + Na)^+$ (^{37}Cl).

4.1.18. 7-Bromo-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**36**)

The procedure described for the preparation of **35** from **29**, in a procedure involving 7-bromo-2-hydroxydibenzofuran (**30**) (236 mg, 0.89 mmol), DBU (269 μL, 1.77 mmol), potassium iodide (192 mg, 1.16 mmol), and 3-chloro-3-methylbut-1-yne (273 μL, 1.78 mmol) in anhydrous toluene (6 mL) gave 7-bromo-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**36**) (205 mg, 70%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.56. IR (KBr) ν_{max} (cm^{-1}): 3296, 3089, 3062, 2984, 2930, 2850, 1632, 1595, 1474, 1464, 1418, 1186, 1169, 1139, 1124, 1049, 949, 918, 883, 858, 820. UV λ_{max} (nm) (log ε) (MeOH): 218 (4.43), 246 (4.10), 256 (4.16), 294 (4.18), 303sh (4.08). ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.1 Hz, 1H, H-9), 7.80 (d, J = 2.3 Hz, 1H, H-1), 7.76 (d, J = 1.1 Hz, 1H, H-6), 7.51 (d, J = 8.8 Hz, 1H, H-4), 7.49 (dd, J = 1.4, 8.2 Hz, 1H, H-8), 7.39 (dd, J = 2.3, 8.8 Hz, 1H, H-3), 2.64 (s, 1H, H-3′), 1.77 (s, 1H, H-1′a, 1′b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.1 (C-5a), 152.9 (C-4a),

151.5 (C-2), 125.9 (C-8), 123.8 (C-9a), 122.9 (C-3), 121.6 (C-9), 120.3 (C-7), 115.3 (C-6), 115.1 (C-9b), 114.0 (C-1), 111.6 (C-4), 86.3 (C-2′), 74.1 (C-3′), 73.4 (C-1′), 29.6 (C-1′a, 1′b). MS (ESI) *m/z*: 329 $(M + H)^+$ (^{79}Br), 331 $(M + H)^+$ (^{81}Br).

*4.1.19. 8-Chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**37**)*

The procedure described for the preparation of **35** from **29**, in a procedure involving 8-chloro-2-hydroxydibenzofuran (**31**) (250 mg, 1.15 mmol), DBU (342 μL, 2.30 mmol), potassium iodide (248 mg, 1.50 mmol), and 3-chloro-3-methylbut-1-yne (346 μL, 2.30 mmol) in anhydrous toluene (4 mL) gave 8-chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**37**) (160 mg, 49%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.69. IR (KBr) ν_{max} (cm^{-1}): 3270, 3241, 2984, 2925, 2852, 1477, 1435, 1166, 1135, 1108, 886, 820, 808, 691. UV λ_{max} (nm) (log ε) (MeOH): 209sh (4.63), 218 (4.80), 253 (4.22), 292 (4.28). ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 1.7 Hz, 1H, H-9), 7.81 (d, J = 2.1 Hz, 1H, H-1), 7.51 (d, J = 8.9 Hz, 1H, H-4), 7.50 (d, J = 8.7 Hz, 1H, H-6), 7.43 (dd, J = 1.9, 8.7 Hz, 1H, H-7), 7.39 (dd, J = 2.3, 8.9 Hz, 1H, H-3), 2.67 (s, 1H, H-3′), 1.74 (s, 6H, H-1′a, 1′b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.2 (C-5a), 153.2 (C-4a), 151.2 (C-2), 128.0 (C-8), 127.2 (C-7), 125.9 (C-9a), 123.5 (C-9b), 123.3 (C-3), 120.5 (C-9), 114.1 (C-1), 112.7 (C-4), 111.7 (C-6), 86.1 (C-2′), 74.3 (C-3′), 73.5 (C-1′), 29.6 (C-1′a, 1.b). MS (ESI) *m/z*: 285 $(M + H)^+$ (^{35}Cl), 287 $(M + H)^+$ (^{37}Cl).

*4.1.20. 9-Chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**38**)*

The procedure described for the preparation of **35** from **29**, in a procedure involving 9-chloro-2-hydroxydibenzofuran (**32**) (180 mg, 0.82 mmol), DBU (246 μL, 1.50 mmol), potassium iodide (178 mg, 1.03 mmol), and 3-chloro-3-methylbut-1-yne (250 μL, 1.50 mmol) in anhydrous toluene (8 mL) gave 9-chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran were obtained (**38**) (226 mg, 98%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.72. IR (KBr) ν_{max} (cm^{-1}): 3303, 3081, 2992, 2929, 2855, 1645, 1583, 1469, 1442, 1182, 1162, 1135, 929, 782, 753. UV λ_{max} (nm) (log ε) (MeOH): 210sh (4.60), 222 (4.72), 256 (4.22), 286 (4.28), 312 (3.78). ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 2.4 Hz, 1H, H-1), 7.53 (d, J = 8.9 Hz, 1H, H-4), 7.51 (d, J = 7.8 Hz, 1H, H-6), 7.42 (t, J = 7.8 Hz, 1H, H-7), 7.41 (dd, J = 2.4, 8.9 Hz, 1H, H-3), 7.36 (d, J = 7.5 Hz, 1H, H-8), 2.66 (s, 1H, H-3′), 1.74 (s, 6H, H-1′a, 1′b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3 (C-5a), 152.5 (C-4a), 151.1 (C-2), 128.3 (C-9), 127.4 (C-7), 123.3 (C-8), 123.0 (C-3), 122.5 (C-9a), 116.4 (C-9b), 116.4 (C-1), 111.2 (C-4), 110.1 (C-6), 86.1 (C-2′), 74.2 (C-3′), 73.5 (C-1′), 29.6 (C-1′a, 1′b). MS (ESI) *m/z*: 285 $(M + H)^+$ (^{35}Cl), 287 $(M + H)^+$ (^{37}Cl), 307 $(M + Na)^+$ (^{35}Cl), 309 $(M + Na)^+$ (^{37}Cl), 591 $(2M + Na)^+$ (^{35}Cl), 593 $(2M + Na)^+$ (^{37}Cl).

*4.1.21. 8-Methoxy-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**39**)*

The procedure described for the preparation of **35** from **29**, in a procedure involving 8-methoxy-2-hydroxydibenzofuran (**33**) (70 mg, 0.33 mmol), DBU (100 μL, 0.66 mmol), potassium iodide (71 mg, 0.43 mmol), and 3-chloro-3-methylbut-1-yne (100 μL, 0.65 mmol) in anhydrous toluene (3 mL), with stirring for 16 h at 65 °C under argon gave 8-methoxy-2-(1,1-dimethylpropyn-1-oxy) dibenzofuran (**39**) (60 mg, 66%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 80:20, Rf = 0.72. IR (KBr) ν_{max} (cm^{-1}): 3097, 2918, 2848, 1645, 1594, 1181, 1460, 1176, 1131, 1030, 808. UV λ_{max} (nm) (log ε) (MeOH): 212 (4.60), 217 (4.40), 254 (3.85), 297 (3.89), 326sh (3.43). ^1H NMR (400 MHz, CD_3OD) δ 7.75 (d, J = 2.4 Hz, 1H, H-1), 7.45 (d, J = 8.9 Hz, 1H, H-6), 7.44 (d, J = 8.8 Hz, 1H, H-4), 7.38 (d, J = 2.6 Hz, 1H, H-9), 7.31 (dd, J = 2.4, 8.8 Hz, 1H, H-3), 7.05 (dd, J = 2.7, 8.9 Hz, 1H, H-7), 3.92 (s, 3H, $-OCH_3$), 2.61 (s, 1H, H-3′), 1.69 (s, 6H, H-1′a, 1′b). ^{13}C NMR (75 MHz, CD_3OD) δ 155.7 (C-8), 153.4 (C-4a), 151.6 (C-5a), 150.7 (C-2), 124.9 (C-9b), 124.6 (C-9a), 122.6 (C-3), 115.2 (C-7), 114.1 (C-1), 112.2 (C-6), 111.5 (C-4), 103.7 (C-9), 86.3 (C-2′), 74.0 (C-3′), 73.4 (C-1′), 29.6 (C-1′a, 1′b). MS (ESI) *m/z*: 281 $(M + H)^+$.

*4.1.22. 2,7-Di(1,1-dimethylpropyn-1-oxy)dibenzofuran (**40**)*

The procedure described for the preparation of **35** from **29**, in a procedure applied to 2,7-dihydroxydibenzofurane (**34**) (44 mg, 0.22 mmol), DBU (100 μL, 0.66 mmol), potassium iodide (95 mg, 0.57 mmol), and 3-chloro-3-methylbut-1-yne (100 μL, 0.65 mmol) in anhydrous toluene (2.5 mL), with stirring for 6 h at 65 °C under argon gave 2,7-di(1,1-dimethylpropyn-1-oxy)dibenzofuran (**40**) (50 mg, 69%) as a pale yellow oil. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.50. IR (KBr) ν_{max} (cm^{-1}): UV λ_{max} (nm) (log ε) (MeOH): 217 (4.45), 255 (4.04), 295 (4.05). ^1H NMR (400 MHz, CD_3OD) δ 7.83 (d, J = 8.5 Hz, 1H, H-9), 7.76 (d, J = 2.2 Hz, 1H, H-1), 7.55 (d, J = 1.9 Hz, 1H, H-6), 7.47 (d, J = 8.8 Hz, 1H, H-4), 7.30 (dd, J = 2.2, 8.8 Hz, 1H, H-3), 7.21 (dd, J = 1.9, 8.5 Hz, 1H, H-8). 2.67 (s, 1H, H-3′ or H-3″), 2.63 (s, 1H, H-3″ or H-3″), 1.75 (s, 6H, H-1′a, 1′b or H-1″a, 1″b). ^{13}C NMR (75 MHz, CD_3OD) δ 157.2 (C-5a), 155.7 (C-7), 152.9 (C-4a), 151.1 (C-2), 124.5 (C-9b), 121.5 (C-3), 120.3 (C-9), 119.7 (C-9a), 117.3 (C-8), 113.8 (C-1), 111.2 (C-4), 105.0 (C-6), 86.4 (C-2′ or C-2″), 85.8 (C-2′ or C-2″), 74.3 (C-3′ or C-3″), 73.9 (C-3′ or C-3″), 73.4 (C-1′ or C-1″), 73.1 (C-1′ or C-1″). 29.6 (C-1′a, 1′b or C-1″a, 1″b). MS (ESI) *m/z*: 333 $(M + H)^+$, 355 $(M + Na)^+$.

*4.1.23. 8-Chloro-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**41**)*

A solution of 6-chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**35**) (130 mg, 0.46 mmol) in DMF (5 mL) was heated at 150 °C for 20 h. After removal of the solvent under reduced pressure, purification by column chromatography over silica gel (solvent cyclohexane) gave 8-chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1] benzopyran (**41**) (130 mg, 100%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.66. IR (KBr) ν_{max} (cm^{-1}): 3093, 3038, 2968, 2922, 2855, 1621, 1574, 1474, 1431, 1415, 1361, 1259, 1236, 1217, 1154, 1143, 1119, 1094, 975, 894, 816, 788, 729. UV λ_{max} (nm) (log ε) (MeOH): 210 (4.44), 233 (4.36), 283 (4.06), 295 (4.24), 306 (4.32), 349 (3.68). ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 7.9 Hz, 1H, H-11), 7.48 (d, J = 7.9 Hz, 1H, H-9), 7.43 (d, J = 8.8 Hz, 1H, H-6), 7.30 (t, J = 7.8 Hz, 1H, H-10), 7.08 (d, J = 9.8 Hz, 1H, H-1), 7.01 (d, J = 8.8 Hz, 1H, H-5), 5.90 (d, J = 9.8 Hz, 1H, H-2), 1.55 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6 (C-7a), 151.0 (C-6a), 148.9 (C-4a), 132.5 (C-2), 126.8 (C-9), 126.1 (C-8), 123.3 (C-10), 120.5 (C-11), 119.5 (C-11b), 118.9 (C-1), 117.1 (C-11a), 116.8 (C-5), 115.9 (C-11c), 111.5 (C-9), 75.8 (C-3), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 285 $(M + H)^+$ (^{35}Cl), 287 $(M + H)^+$ (^{37}Cl). HRESIMS calcd. for $C_{17}H_{13}ClO_2$ (^{35}Cl), 285.0679 $(M + H)^+$, found 285.0685; calcd. for $C_{17}H_{13}ClO_2$ (^{37}Cl), 287.0835 $(M + H)^+$, found 287.0664.

*4.1.24. 9-Bromo-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**42**)*

A solution of 7-bromo-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**36**) (200 mg, 0.61 mmol) in DMF (6 mL) was heated at 150 °C for 9 h. The solvent was removed under reduced pressure. Purification by column chromatography over silica gel (solvent cyclohexane) gave 9-bromo-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzo pyran (**42**) (160 mg, 80%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.48. IR (KBr) ν_{max} (cm^{-1}): 3078, 3043, 2979, 2926, 2852, 1478, 1412, 1365, 1274, 1217, 1194, 1155, 963, 887, 805, 741, 716. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.47), 234 (4.33), 279 (4.04), 303 (4.33), 313 (4.41), 349 (3.65). ^1H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 8.4 Hz, 1H, H-11), 7.71 (d, J = 1.7 Hz, 1H, H-8), 7.45 (dd, J = 1.7, 8.3 Hz, 1H, H-10), 7.30 (d, J = 8.8 Hz, 1H, H-6), 7.01 (d, J = 9.8 Hz, 1H, H-1), 6.95 (d, J = 8.7 Hz, 1H, H-5), 5.86 (d, J = 9.8 Hz, 1H, H-2), 1.51 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3 (C-7a), 151.2 (C-6a), 148.8 (C-4a), 132.5 (C-2), 125.8 (C-10), 123.6 (C-11a), 122.9 (C-11), 119.9 (C-9), 119.0 (C-11b), 119.0 (C-1), 116.5 (C-5), 115.7 (C-11c), 115.2 (C-8), 111.2 (C-6), 75.8 (C-3),

27.4 (C-3a, 3b). MS (ESI) *m/z*: 327 $(M - H)^-$ (^{79}Br), 329 $(M - H)^-$ (^{81}Br).

4.1.25. 10-Chloro-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**43**)

A solution of 8-chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**37**) (150 mg, 0.53 mmol) in DMF (5 mL) was heated at 130 °C for 20 h. The solvent was removed under reduced pressure. Purification by column chromatography over silica gel (solvent cyclohexane) gave 10-chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**43**) (125 mg, 83%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.73. IR (KBr) ν_{max} (cm^{-1}): 2980, 2921, 2848, 1482, 1459, 1443, 1416, 1260, 1159, 1089, 964, 801, 781, 708. UV λ_{max} (nm) (log ε) (MeOH): 211 (4.50), 215 (4.50), 281 (3.99), 303 (4.20), 313 (4.36), 352 (3.66). ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2.1 Hz, 1H, H-11), 7.47 (d, J = 8.7 Hz, 1H, H-8), 7.40 (dd, J = 2.1, 8.7 Hz, 1H, H-9), 7.31 (d, J = 8.8 Hz, 1H, H-6), 7.01 (d, J = 9.8 Hz, 1H, H-1), 6.96 (d, J = 8.8 Hz, 1H, H-5), 5.87 (d, J = 9.8 Hz, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3 (C-7a), 151.7 (C-6a), 148.7 (C-4a), 132.6 (C-2), 127.9 (C-10), 126.7 (C-9), 125.8 (C-11a), 121.8 (C-11), 118.9 (C-1), 118.8 (C-11b), 116.9 (C-5), 115.8 (C-11c), 112.7 (C-8), 111.2 (C-6), 75.8 (C-3), 27.3 (C-3a, 3b). MS (ESI) *m/z*: 285 $(M + H)^+$ (^{35}Cl), 287 $(M + H)^+$ (^{37}Cl), 307 $(M + Na)^+$. HRESIMS calcd. for $C_{17}H_{13}ClO_2$ (^{35}Cl), 285.0679 $(M + H)^+$, found 285.0646; calcd. for $C_{17}H_{13}ClO_2$ (^{37}Cl), 287.0835 $(M + H)^+$, found 287.0547.

4.1.26. 11-Chloro-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**44**)

A solution of 9-chloro-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**38**) (200 mg, 0.7 mmol) in DMF (7 mL) was heated at 150 °C for 4 h. The solvent was removed under reduced pressure. Purification by column chromatography over silica gel (solvent cyclohexane) gave 11-chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**44**) (176 mg, 85%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.75. IR (KBr) ν_{max} (cm^{-1}): 3059, 3027, 2969, 2922, 2868, 1460, 1421, 1408, 1368, 1360, 1252, 1213, 1139, 1115, 958, 931, 809, 765, 708. UV λ_{max} (nm) (log ε) (MeOH): 212 (4.42), 237 (4.28), 285 (4.07), 295 (4.20), 306 (4.30), 352 (3.65). ^1H NMR (400 MHz, CD_3OD) δ 7.90 (d, J = 10.0 Hz, 1H, H-1), 7.53 (d, J = 8.0 Hz, 1H, H-8), 7.44 (t, J = 7.9 Hz, 1H, H-9), 7.38 (d, J = 7.8 Hz, 1H, H-10), 7.38 (d, J = 8.6 Hz, 1H, H-6), 7.03 (d, J = 8.8 Hz, 1H, H-5), 5.85 (d, J = 10.0 Hz, 1H, H-2), 1.48 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, CD_3OD) δ 157.9 (C-7a), 151.3 (C-6a), 149.5 (C-4a), 129.8 (C-2), 127.3 (C-9), 126.8 (C-11), 124.5 (C-10), 123.3 (C-11a), 123.1 (C-1), 119.0 (C-11b), 117.7 (C-5), 116.2 (C-11c), 111.3 (C-6), 110.4 (C-8), 74.9 (C-3), 27.0 (C-3a, 3b). MS (ESI) *m/z*: 285 $(M + H)^+$ (^{35}Cl), 287 $(M + H)^+$ (^{37}Cl). HRESIMS calcd. for $C_{17}H_{13}ClO_2$ (^{35}Cl), 285.0679 $(M + H)^+$; found 285.0670 & calcd. for $C_{17}H_{13}ClO_2$ (^{37}Cl), 287.0835 $(M + H)^+$; found 287.0646.

4.1.27. 10-Methoxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**45**) and 9-methoxy-2,2-dimethyl-2H-benzofuro[2,3-g][1]benzopyran (**46**)

A solution of 8-methoxy-2-(1,1-dimethylpropyn-1-oxy)dibenzofuran (**39**) (50 mg, 0.18 mmol) in DMF (2 mL) was heated at 140 °C for 20 h. The solvent was removed under reduced pressure. Purification by column chromatography over silica gel (solvent cyclohexane) successively afforded 10-methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**45**) (37.5 mg, 75%) and 9-methoxy-2,2-dimethyl-2*H*-benzofuro[2,3-*g*][1]benzopyran (**46**) (8 mg, 16%) as white amorphous solids.

4.1.27.1. 10-Methoxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**45**).

TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 70:30, Rf = 0.68. IR (KBr) ν_{max} (cm^{-1}): 3051, 3004, 2956, 2923, 1481, 1446, 1259, 1209, 1193, 1143, 968, 824, 796, 737. UV λ_{max} (nm) (log ε) (MeOH): 217 (4.57), 259 (4.12), 268 (4.11), 287 (3.99), 312sh (4.19), 321 (4.24), 352sh (3.77), 369sh (3.61). ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 2.5 Hz, 1H, H-11), 7.48 (d, J = 9.0 Hz, 1H, H-8), 7.33 (d, J = 8.6 Hz, 1H, H-6), 7.10 (d, J = 9.9 Hz, 1H, H-1), 7.08 (dd, J = 2.5, 9.0 Hz, 1H, H-9), 6.96 (d, J = 8.6 Hz, 1H, H-5), 5.89 (d, J = 9.9 Hz, 1H, H-2), 3.97 (s, 3H, $-OCH_3$), 1.55 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.6 (C-10), 151.9 (C-7a), 151.8 (C-6a), 148.3 (C-4a), 132.1 (C-2), 124.8 (C-11a), 119.8 (C-11b), 119.2 (C-1), 116.1 (C-5), 115.7 (C-11c), 114.4 (C-9), 112.0 (C-8), 111.2 (C-6), 105.8 (C-11), 75.6 (C-3), 56.2 (C—OCH_3), 27.3 (C-3a, 3b). MS (ESI) *m/z*: 279 $(M - H)^-$. HRESIMS calcd. for $C_{18}H_{16}O_3$, 281.1173 $(M + H)^+$, found 281.1170.

4.1.27.2. 9-Methoxy-2,2-dimethyl-2H-benzofuro[2,3-g][1]benzopyran (**46**).

TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 70:30, Rf = 0.63. UV λ_{max} (nm) (log ε) (MeOH): 209sh (4.29), 216 (4.37), 230sh (4.18), 310 (3.92), 331sh (3.78). ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.9 Hz, 1H, H-7), 7.35 (d, J = 2.6 Hz, 1H, H-10), 7.34 (s, 1H, H-11), 7.19 (s, 1H, H-5), 7.05 (dd, J = 2.6, 8.9 Hz, 1H, H-8), 6.50 (d, J = 9.8 Hz, 1H, H-4), 5.80 (d, J = 9.8 Hz, 1H, H-3), 3.95 (s, 3H, $-OCH_3$), 1.52 (s, 6H, H-2a, 2b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5 (C-9), 152.0 (C-5a), 151.8 (C-6a), 148.8 (C-11a), 132.8 (C-3), 124.9 (C-10a), 124.3 (C-10b), 122.9 (C-4), 121.5 (C-4a), 115.0 (C-8), 112.0 (C-7), 108.6 (C-5), 107.3 (C-11), 103.6 (C-10), 76.0 (C-2), 56.1 (C—OCH_3), 27.6 (C-2a, 2b). MS (ESI) *m/z*: 279 $(M - H)^-$. HRESIMS calcd. for $C_{18}H_{16}O_3$, 281.1173 $(M + H)^+$, found 281.1171.

4.1.28. 3,3,10,10-Tetramethyl-3H,10H-furo[3,2-f:5,4-f′]dichromene (**47**)

A solution of 2,7-di(1,1-dimethylpropyn-1-oxy)dibenzofuran (**40**) (50 mg, 0.15 mmol) in DMF (2.5 mL) was heated at 150 °C for 32 h. The solvent was removed under reduced pressure. Purification by column chromatography over silica gel (solvent cyclohexane) gave 3,3,10,10-tetramethyl-3*H*,10*H*-furo[3,2-*f*:5,4-*f*′]dichromene (**47**) (13 mg, 26%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.53. IR (KBr) ν_{max} (cm^{-1}): 3054, 2970, 2924, 2850, 1642, 1431, 1280, 1213, 1202, 1159, 1108, 1050, 805, 727. UV λ_{max} (nm) (log ε) (MeOH): 205 (4.39), 232 (4.44), 246 (4.42), 284 (4.41), 292 (4.43), 333 (4.04). ^1H NMR (400 MHz, CD_3OD) δ 7.72 (d, J = 8.4 Hz, 1H, H-13) 7.30 (d, J = 8.7 Hz, 1H, H-6), 7.05 (d, J = 9.8 Hz, 1H, H-1), 6.91 (d, J = 9.8 Hz, 1H, H-8), 6.87 (d, J = 8.7 Hz, 1H, H-5), 6.84 (d, J = 8.4 Hz, 1H, H-12), 5.85 (d, J = 9.8 Hz, 1H, H-2), 5.79 (d, J = 9.8 Hz, 1H, H-9), 1.53 (s, 12H, H-3a, 3b, 10a, 10b). ^{13}C NMR (75 MHz, CD_3OD) δ 153.2 (C-7a), 152.3 (C-11a), 151.3 (C-6a), 148.5 (C-4a), 131.8 (C-2), 130.5 (C-9), 121.5 (C-13), 120.2 (C-13b), 119.4 (C-1), 117.7 (C-13a), 116.0 (C-8), 115.1 (C-13c), 114.4 (C-5), 112.0 (C-12), 110.7 (C-6), 106.7 (C-7b), 76.7 (C-10), 75.6 (C-3), 27.8 (C-10a, 10b), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 333 $(M + H)^+$. HRESIMS calcd. for $C_{22}H_{20}O_3$, 333.1485 $(M + H)^+$, found 333.1499.

4.1.29. 8-Hydroxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**48**)

A solution of 8-chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**41**) (50 mg, 0.18 mmol), Pd_2dba_3 (3.3 mg, 3.6×10^{-3} mmol), 2-di-*tert*-butylphosphino-3,4,5,6-tetramethyl-2′,4′,6′-triisopropyl-1,1′-biphenyl (6.9 mg, 14.4×10^{-3} mmol), and KOH (29.5 mg, 0.53 mmol) in 1,4-dioxane/water 1:1 (1.5 mL) was heated at 100 °C under stirring for 8 h in a Schlenk tube previously evacuated and back-filled with argon. The reaction mixture was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography over silica gel (solvent: cyclohexane/dichloromethane 1:0 to 1:1) gave 8-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**48**) (42 mg, 92%) as

a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.1. IR (KBr) ν_{max} (cm^{-1}): 3373, 3066, 3034, 2978, 2971, 2921, 1594, 1485, 1431, 1252, 1197, 1158, 1115, 987, 909, 808, 793, 734. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.29), 216 (4.32), 241 (4.38), 269 (3.85), 293 (4.06), 305 (4.07), 346 (3.60), 360sh (3.53). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.5 Hz, 1H, H-11), 7.35 (d, J = 8.4 Hz, 1H, H-6), 7.25 (t, J = 8.5 Hz, 1H, H-10), 7.11 (d, J = 10.0 Hz, 1H, H-1), 7.05 (d, J = 8.5 Hz, 1H, H-9), 6.98 (d, J = 8.4 Hz, 1H, H-5), 5.89 (d, J = 10.0 Hz, 1H, H-2), 5.50 (s, 1H, -O*H*), 1.55 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.0 (C-6a), 148.6 (C-4a), 144.9 (C-7a), 141.2 (C-8), 132.2 (C-2), 125.8 (C-11a), 123.5 (C-10), 120.2 (C-11b), 119.2 (C-1), 116.1 (C-5), 115.9 (C-11c), 114.2 (C-11), 113.2 (C-9), 111.2 (C-6), 75.8 (C-3), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 265 $(M - H)^-$. HRESIMS calcd. for $C_{17}H_{14}O_3$, 267.1017 $(M + H)^+$, found 267.1017.

4.1.30. 9-Hydroxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**49**) and 3,3,3′,3′-tetramethyl-9,9′-bi(3H-benzofuro[3,2-f]chromene) (**52**)

The procedure described for the preparation of **48** from **41**, applied to 9-bromo-3,3-dimethyl-3*H*-benzofuro[3,2-*f*]benzopyran (**42**) (120 mg, 0.36 mmol), Pd_2dba_3 (6.6 mg, 7.2×10^{-3} mmol), 2-di-*tert*-butylphosphino-3,4,5,6-tetramethyl-2′,4′,6′-triisopropyl-1,1′-biphenyl (13.8 mg, 29×10^{-3} mmol), and KOH (59 mg, 1.06 mmol) in 1,4-dioxane/water 1:1 (3.4 mL), with heating for 3 h at 100 °C, successively afforded after extraction and column chromatography over silica gel (solvent: cyclohexane/dichloromethane 1:0 to 1:1) 9-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**49**) (60 mg, 62%) and 3,3,3′,3′-tetramethyl-9,9′-bi(3*H*-benzofuro[3,2-*f*]chromene) (**52**) (20 mg, 11%) as white amorphous solids.

4.1.30.1. 9-Hydroxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**49**).

TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.11. IR (KBr) ν_{max} (cm^{-1}): 3373, 3076, 2964, 2921, 1637, 1630, 1442, 1422, 1255, 1142, 1113, 1080, 961, 817, 807, 718. UV λ_{max} (nm) (log ε) (MeOH): 208 (4.38), 215sh (4.25), 233 (4.27), 253 (4.20), 271 (3.94), 282 (3.99), 313sh (4.22), 321 (4.25). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.5 Hz, 1H, H-11), 7.26 (d, J = 8.6 Hz, 1H, H-6), 7.03 (d, J = 9.8 Hz, 1H, H-1), 7.02 (d, J = 2.3 Hz, 1H, H-8), 6.85 (d, J = 8.6 Hz, 1H, H-5), 6.85 (dd, J = 2.3, 8.5 Hz, 1H, H-10), 5.84 (d, J = 9.8 Hz, 1H, H-2), 5.11 (s, 1H, -O*H*), 1.45 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9 (C-7a), 155.3 (C-9), 151.9 (C-4a), 148.9 (C-6a), 132.0 (C-2), 122.6 (C-11), 120.0 (C-11b), 119.3 (C-1), 119.2 (C-11a), 115.3 (C-11c), 114.6 (C-5), 111.2 (C-10), 110.8 (C-6), 98.8 (C-8), 75.9 (C-3), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 265 $(M - H)^-$. HRESIMS calcd. for $C_{17}H_{14}O_3$, 265.0861 $(M - H)^-$, found 265.0864.

4.1.30.2. 3,3,3′,3′-Tetramethyl-9,9′-bi(3H-benzofuro[3,2-f]chromene) (**52**).

TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 70:30, Rf = 0.40. IR (KBr) ν_{max} (cm^{-1}): 3042, 2976, 2925, 2855, 1485, 1435, 1414, 1271, 1254, 1188, 1158, 1112, 964, 807. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.72), 237sh (4.49), 334 (4.52). ^{1}H NMR (400 MHz, CD_3OD) δ 8.08 (d, J = 8.2 Hz, 2H, H-11, H-11′), 7.86 (d, J = 1.6 Hz, 2H, H-8, H-8′), 7.68 (dd, J = 1.6, 8.2 Hz, 2H, H-10, H-10′), 7.35 (d, J = 8.7 Hz, 2H, H-6, H-6′), 7.14 (d, J = 9.8 Hz, 2H, H-1, H-1′), 6.96 (d, J = 8.7 Hz, 2H, H-5, H-5′), 5.89 (d, J = 9.8 Hz, 2H, H-2, H-2′), 1.54 (s, 12H, H-3a, 3b, H-3′a, 3′b). ^{13}C NMR (75 MHz, CD_3OD) δ 157.7 (C-7a, C-7'a), 151.6 (C-6a, C-6′a), 148.6 (C-4a, C-4′a), 139.9 (C-9, C-9′), 132.3 (C-2, C-2′), 123.8 (C-11a, C-11′a), 122.3 (C-11, C-11′), 122.2 (C-10, C-10′), 119.5 (C-11b, C-11′b), 119.3 (C-1, C-1′), 116.1 (C-5, C-5′), 115.7 (C-11c, C-11′c), 111.1 (C-6, C-6′), 110.4 (C-8, C-8′), 75.8 (C-3, C-3′), 27.4 (C-3a, 3b, C-3′a, 3′b). MS (ESI) *m/z*: 497 $(M - H)^-$. HRESIMS calcd. for $C_{34}H_{26}O_4$, 499.1902 $(M + H)^+$, found 499.1881.

4.1.31. 10-Hydroxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**50**)

The procedure described for the preparation of **48** from **41**, applied to 10-chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**43**) (100 mg, 0.36 mmol), Pd_2dba_3 (6.6 mg, 7.2×10^{-3} mmol), 2-di-*tert*-butylphosphino-3,4,5,6-tetramethyl-2′,4′,6′-triisopropyl-1,1′-biphenyl (13.8 mg, 29×10^{-3} mmol), and KOH (59 mg, 1.06 mmol) in 1,4-dioxane/water 1:1 (3.0 mL), with heating for 24 h at 100 °C, gave 10-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**50**) (80 mg, 86%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.26. IR (KBr) ν_{max} (cm^{-1}): 3401, 3097, 3058, 2976, 2926, 2871, 1474, 1424, 1194, 1140, 1112, 967, 852, 809, 743. UV λ_{max} (nm) (log ε) (MeOH): 209 (4.34), 259 (4.29), 304 (3.87), 325sh (3.63), 335sh (3.52). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 2.6 Hz, 1H, H-11), 7.41 (d, J = 8.8 Hz, 1H, H-8), 7.28 (d, J = 8.8 Hz, 1H, H-6), 7.01 (d, J = 9.8 Hz, 1H, H-1), 6.95 (dd, J = 2.6, 8.8 Hz, 1H, H-9), 6.93 (d, J = 8.8 Hz, 1H, H-5), 5.84 (d, J = 9.8 Hz, 1H, H-2), 1.51 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.9 (C-10), 151.9 (C-6a), 151.1 (C-7a), 148.2 (C-4a), 132.2 (C-2), 125.0 (C-11a), 120.9 (C-11b), 119.1 (C-1), 116.3 (C-5), 115.8 (C-11c), 114.9 (C-9), 112.1 (C-8), 111.1 (C-6), 107.8 (C-11), 75.7 (C-3), 27.3 (C-3a, 3b). MS (ESI) *m/z*: 265 $(M - H)^-$, 531 $(2M-H)^-$. HRESIMS calcd. for $C_{17}H_{14}O_3$, 265.0861 $(M - H)^-$, found 265.0875.

4.1.32. 11-Hydroxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**51**)

A solution of 11-chloro-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**44**) (160 mg, 0.56 mmol), Pd_2dba_3 (10.6 mg, 11.5×10^{-3} mmol), 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (*t*-BuXPhos) (19.2 mg, 45×10^{-3} mmol), and KOH (95 mg, 1.7 mmol) in 1,4-dioxane/water 1:1 (5 mL) was heated at 100 °C under stirring for 22 h in a Schlenk tube previously evacuated and back-filled with argon. The reaction mixture was extracted with dichloromethane (3×5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography over silica gel (solvent: cyclohexane/dichloromethane 1:0 to 1:1) gave 11-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**51**) (36 mg, 23%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 50:50, Rf = 0.29. IR (KBr) ν_{max} (cm^{-1}): 3318, 2968, 2917, 2851, 1598, 1472, 1423, 1290, 1259, 1143, 1115, 1033, 960, 936, 773, 718. UV λ_{max} (nm) (log ε) (MeOH): 217 (4.36), 242 (4.39), 292 (4.26), 305 (4.20), 346 (3.69), 361 (3.63). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 10.0 Hz, 1H, H-1), 7.33 (d, J = 8.6 Hz, 1H, H-6), 7.31 (t, J = 8.0 Hz, 1H, H-9), 7.18 (d, J = 8.2 Hz, 1H, H-10), 6.98 (d, J = 8.6 Hz, 1H, H-5), 6.70 (d, J = 7.8 Hz, 1H, H-8), 5.81 (d, J = 10.0 Hz, 1H, H-2), 5.64 (s, 1H, -O*H*), 1.53 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9 (C-11), 150.7 (C-6a), 150.0 (C-7a), 148.9 (C-4a), 130.7 (C-2), 127.7 (C-9), 122.5 (C-1), 119.9 (C-11b), 116.1 (C-5), 115.9 (C-11c), 112.5 (C-11a), 110.6 (C-6), 108.6 (C-8), 104.6 (C-10), 75.2 (C-3), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 265 $(M - H)^-$. HRESIMS calcd. for $C_{17}H_{14}O_3$, 265.0861 $(M - H)^-$, found 265.0861.

4.1.33. 10-Acetyl-3,3-dimethyl-1,2-dihydro-3H-benzofuro[3,2-f][1]benzopyran (**56**)

Palladium on charcoal (5% Pd) (4 mg) was added to a solution of 10-acetyl-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**6**) (45 mg, 0.15 mmol) in EtOH (10 ml). The reaction mixture was stirred under hydrogen pressure (4 bar) for 24 h and filtered. After evaporation of the solvent, column chromatography over silica gel (solvent: dichloromethane/cyclohexane 95:5) gave 10-acetyl-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**56**) (40 mg, 88%) as a brown solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/MeOH (9.5/0.5), Rf = 0.55, IR (KBr) ν_{max} (cm^{-1}): 2969, 2922, 1673, 1488, 1447, 1416, 1354, 1248, 1162, 1120, 953, 824, 799. UV λ_{max} (nm) (log ε) (MeOH): 220 (4.26), 244 (4.36), 267 (4.34). ^{1}H NMR (300 MHz,

$CDCl_3$) δ 8.62 (d, J = 2.0 Hz, 1H, H-11), 8.09 (dd, J = 2.0, 9.0 Hz, 1H, H-9), 7.55 (d, J = 9.0 Hz, 1H, H-8), 7.37 (d, J = 9.0 Hz, 1H, H-6), 6.9 (d, J = 9.0 Hz, 1H, H-5), 3.33 (t, J = 7.0 Hz, 2H, H-1), 2.71 (s, 3H, $C\underline{H}_3CO$), 2.12 (t, J = 7.0 Hz, 2H, H-2), 1.41 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.5 ($CH_3\underline{C}O$), 159.6 (C-7a), 150.9 (C-6a), 150.0 (C-4a), 132.1 (C-10), 127.5 (C-9), 125.9 (C-11a), 122.9 (C-11), 121.9 (C-11b), 117.9 (C-5), 115.8 (C-11c), 111.4 (C-8), 110.3 (C-6), 74.0 (C-3), 32.2 (C-2), 26.9 ($\underline{C}H_3CO$), 26.6 (C-3a,3b), 21.1 (C-1). MS (ESI): *m/z* 317 $(M + Na)^+$.

4.1.34. 10-Acetoxy-3,3-dimethyl-1,2-dihydro-3H-benzofuro[3,2-f][1]benzopyran (**57**)

A solution of 10-acetyl-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**56**) (50 mg, 0.17 mmol) and 3-chloroperoxybenzoic acid (625 mg, 3.6 mmol) in $CHCl_3$ (50 mL) was stirred at room temperature for 21 h. The reaction mixture was washed with aqueous sodium hydroxide solution (2 × 25 mL) and water (2 × 25 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure. Column chromatography over silica gel (solvent: dichloromethane/cyclohexane 9:1) gave 10-Acetoxy-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**57**) as a yellow amorphous solid (22 mg, 40%). TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} (9/1), Rf = 0.25. UV λ_{max} (nm) (log ε) (MeOH): 245 (4.22), 256 (4.20), 293(4.23). ^{1}H NMR (300 MHz, $CDCl_3$): 7.69 (dd, J = 0.5, 2.5 Hz, 1H, H-11), 7.55 (dd, J = 0.5, 8.5 Hz, 1H, H-8), 7.34 (d, J = 9.0 Hz, 1H, H-6), 7.15 (dd, J = 2.5, 8.5 Hz, 1H, H-9), 6.95 (d, J = 9.0 Hz, 1H, H-5), 3.21 (t, J = 7.0 Hz, 2H, H-1), 2,37 (s, 3H, $C\underline{H}_3CO$), 1.97 (t, J = 7.0 Hz, 2H, H-2), 1.38 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) 170.1 ($CH_3\underline{C}O$), 154.2 (C-7a), 151.0 (C-6a), 149.5 (C-4a), 145.7 (C-10), 125.7 (C-11a), 122.2 (C-11b), 119.8 (C-9), 117.7 (C-5), 115.3 (C-11c), 115.0 (C-11), 111.9 (C-8), 110.2 (C-6), 73.8 (C-3), 32.2 (C-2), 26.5 (C-3a, 3b), 21.2 ($\underline{C}H_3CO$), 20.8 (C-1). MS (ESI): *m/z* 333 $(M + Na)^+$.

4.1.35. 10-Hydroxy-3,3-dimethyl-1,2-dihydro-3H-benzofuro[3,2-f][1]benzopyran (**58**)

Sodium hydroxide pellets (55 mg, 1.37 mmol) were added to a solution of 10-acetoxy-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**57**) (22 mg, 0.07 mmol) in ethanol (20 mL). The reaction mixture was heated at 50 °C for 2.5 h. After evaporation of the solvent under reduced pressure, the solid residue was partitioned between dichloromethane (20 mL) and water (20 mL). The aqueous layer was acidified by addition of concentrated aqueous HCl solution (12 *N*, 10 mL) and extracted with dichloromethane (20 mL). The organic layer dried over magnesium sulfate, filtered, and evaporated under reduced pressure gave 10-hydroxy-3,3-dimethyl-1,2-dihydro-3*H*-benzofuro[3,2-*f*][1]benzopyran (**58**) as a yellow amorphous solid (17 mg, 95%). TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} (9/1), Rf = 0.25. IR (KBr) ν_{max} (cm^{-1}): 3362; 3229; 2973; 2922; 1595; 1474; 1451; 1424; 1381; 1369; 1193; 1159; 1120; 801. UV λ_{max} (nm) (log ε) (MeOH): 259 (4.05), 303 (4.23), 321 (3.73), 335 (3.64). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (d, J = 2.3 Hz, 1H, H-11), 7.41 (d, J = 8.7 Hz, 1H, H-8), 7.29 (d, J = 8.9 Hz, 1H, H-6), 6.95 (dd, J = 2.3, 8.7 Hz, 1H, H-9), 6.92 (d, J = 8.9 Hz, 1H, H-5), 3.39 (t, J = 6.8 Hz, 2H, H-1), 1.9 (t, J = 6.8 Hz, 2H, H-2), 1.3 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.5, 151.1, 151 (C-6a, C-7a, C-10), 125.8 (C-11a), 122 (C-11b), 117 (C-5), 115.2 (C-11c), 114.4 (C-9), 111.8 (C-8), 110.1 (C-6), 108.0 (C-11), 73.8 (C-3), 32.3 (C-2), 26.5 (C-3a, 3b), 20.8 (C-1). MS (ESI): *m/z* 267 $(M + H)^+$. HRESIMS *m/z* calcd for $C_{17}H_{16}O_3$, 267.1095 $(M + H)^+$, found 267.1020.

4.1.36. 8-Methoxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**53**)

A solution of 8-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**48**) (30 mg, 0.11 mmol) in anhydrous DMF (1.5 mL) was poured into a flask containing NaH (13.5 mg, 0.56 mmol). Dimethylsulfate (21 μL, 0.22 mmol) was slowly added and the reaction was stirred at room temperature for 1 h. The reaction mixture was quenched with water (10 mL) and extracted with dichloromethane (3 × 10 mL). The organic layers were concentrated under reduced pressure. Chromatography over silica gel (solvent: cyclohexane/dichloromethane 1:0 to 1:1) gave 8-methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**53**) (22 mg, 70%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 80:20, Rf = 0.57. IR (KBr) ν_{max} (cm^{-1}): 3058, 2976, 2933, 2836, 1626, 1582, 1497, 1483, 1463, 1423, 1271, 1208, 1185, 1158, 1115, 1065, 980, 901, 851, 808, 793, 734. UV λ_{max} (nm) (log ε) (MeOH): 219 (4.47), 240 (4.49), 267 (3.98), 293 (4.16), 305 (4.15), 331 (3.63), 345 (3.65), 361sh (3.54). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 7.9 Hz, 1H, H-11), 7.40 (d, J = 8.7 Hz, 1H, H-6), 7.30 (t, J = 7.9 Hz, 1H, H-10), 7.12 (d, J = 9.8 Hz, 1H, H-1), 7.03 (d, J = 8.0 Hz, 1H, H-9), 6.97 (d, J = 8.7 Hz, 1H, H-5), 5.88 (d, J = 9.8 Hz, 1H, H-2), 4.11 (s, 3H, $-OCH_3$), 1.54 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.9 (C-7a), 148.6 (C-4a), 146.0 (C-6a), 145.7 (C-8), 132.1 (C-2), 125.8 (C-11a), 123.2 (C-10), 119.7 (C-11b), 119.2 (C-1), 116.1 (C-5), 115.7 (C-11c), 114.3 (C-11), 111.4 (C-6), 108.9 (C-9), 75.7 (C-3), 56.2 ($C-OCH_3$), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 303 $(M + Na)^+$. HRESIMS calcd. for $C_{18}H_{16}O_3$, 281.1173 $(M + H)^+$, found 281.1174.

4.1.37. 9-Methoxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**54**)

The procedure described for the preparation of **53** from **48**, applied to 9-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**49**) (40 mg, 0.15 mmol), NaH (18 mg, 0.75 mmol), and dimethylsulfate (28 μL, 0.29 mmol), gave 9-methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**54**) (31.5 mg, 75%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 80:20, Rf = 0.69. IR (KBr) ν_{max} (cm^{-1}): 3053, 2966, 2922, 2838, 1637, 1501, 1419, 1275, 1150, 1112, 1079, 964, 827, 807, 715. UV λ_{max} (nm) (log ε) (MeOH): 207 (4.46), 235 (4.30), 253 (4.25), 271 (4.00), 281 (4.04), 321 (4.33), 350sh (4.02). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 8.7 Hz, 1H, H-11), 7.27 (d, J = 8.7 Hz, 1H, H-6), 7.07 (d, J = 2.3, 1H, H-8), 7.04 (d, J = 9.8 Hz, 1H, H-1), 6.93 (dd, J = 2.3, 8.8 Hz, 1H, H-10), 6.85 (d, J = 8.7 Hz, 1H, H-5), 5.83 (d, J = 9.8 Hz, 1H, H-2), 3.92 (s, 3H, $-OCH_3$), 1.51 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.6 (C-9), 158.4 (C-7a), 151.2 (C-6a), 148.5 (C-4a), 131.9 (C-2), 122.4 (C-11), 120.0 (C-11b), 119.4 (C-1), 117.5 (C-11a), 115.03 (C-11c), 114.4 (C-11), 110.8 (C-6), 96.4 (C-8), 75.6 (C-3), 55.7 ($-OCH_3$), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 281 $(M + H)^+$, 303 $(M + Na)^+$, 583 $(2M + Na)^+$. HRESIMS calcd. for $C_{18}H_{16}O_3$, 281.1173 $(M + H)^+$, found 281.1174.

4.1.38. 11-Methoxy-3,3-dimethyl-3H-benzofuro[3,2-f][1]benzopyran (**55**)

The procedure described for the preparation of **53** from **48**, applied to 11-hydroxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**51**) (20 mg, 0.08 mmol), NaH (9.5 mg, 0.38 mmol), and dimethylsulfate (15.5 μL, 0.16 mmol), gave 11-methoxy-3,3-dimethyl-3*H*-benzofuro[3,2-*f*][1]benzopyran (**55**) (13 mg, 62%) as a white amorphous solid. TLC Silica gel 60 F_{254}, Solv. CH_2Cl_2/C_6H_{12} 70:30, Rf = 0.45. IR (KBr) ν_{max} (cm^{-1}): 3104, 2976, 2925, 2836, 1594, 1496, 1470, 1463, 1423, 1375, 1279, 1256, 1212, 1154, 1119, 1088, 967, 804, 773, 718. UV λ_{max} (nm) (log ε) (MeOH): 214 (4.42), 238 (4.40), 282sh (4.13), 293 (4.31), 305 (4.28), 345 (3.73), 360 (3.64). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 10.0 Hz, 1H, H-1), 7.43 (t, J = 8.2 Hz, 1H, H-9), 7.33 (d, J = 8.7 Hz, 1H, H-6), 7.22 (d, J = 8.2 Hz, 1H, H-8), 6.97 (d, J = 8.7 Hz, 1H, H-5), 6.83 (d, J = 8.2 Hz, 1H, H-10), 5.80 (d, J = 10.0 Hz, 1H, H-2), 4.09 (s, 3H, $-OCH_3$), 1.53 (s, 6H, H-3a, 3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3 (C-7a), 154.4 (C-11), 150.7 (C-6a), 148.9 (C-4a), 130.4 (C-2), 127.9 (C-9), 122.8 (C-1), 121.5 (C-11b), 115.9 (C-11c), 115.9 (C-5), 113.5 (C-11a), 110.6 (C-6), 104.8 (C-8),

103.7 (C-10), 75.0 (C-3), 55.5 (C–OCH_3), 27.4 (C-3a, 3b). MS (ESI) *m/z*: 281 $(M + H)^+$, 303 $(M + Na)^+$. HRESIMS calcd. for $C_{18}H_{16}O_3$, 281.1173 $(M + H)^+$, found for 281.1170.

4.2. Biology

4.2.1. MIC determinations

MICs were determined using the Microdilution Resazurin Assay (MRA) [18]. Resazurin salt powder (Sigma) was prepared at 0.01% (wt/vol) in distilled water, sterilized by filtration through a 0.22 μm membrane and stored at 4 °C until used. Drug stock solutions were prepared in dimethylsulfoxide (DMSO) at concentration of 10 mg/mL and frozen until used. The inocula were prepared from *M. bovis* BCG or *M. tuberculosis* H37Rv strains grown in 7H9, supplemented with 10% ADC enrichment (Difco). Two microliters (one in case of *M. tuberculosis*) of two-fold serial dilutions of each drug were prepared in 200 μL of 7H9 medium (100 μL for *M. tuberculosis*) directly in 96-well plates at concentrations from 100 μg/mL to 0.1 μg/mL (500 μg/mL to 0.9 μg/mL for *M. tuberculosis*). Growth controls containing DMSO and isoniazid (from 1 μg/mL to 1 ng/mL were also included. The plates were covered, sealed and incubated at 37 °C. After 8 days for *M. bovis* or 6 days for *M. tuberculosis*, 30 μL of resazurin solution was added to each well and plates were allowed to incubate at 37 °C for an additional 24 h. A change from blue to pink indicates the reduction of resazurin and therefore bacterial growth. The MIC was defined as the lowest drug concentration that prevented this color change.

4.2.2. Cytotoxicity evaluation

A cell line, which grows attached to tissue culture plates, was used for the determination of the antiproliferative activity of the synthesized benzofuranobenzopyrans. Monkey kidney epitheliar VERO cell cultures were maintained in Dulbecco's Modified Engle's medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin 100 μg/ml). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and maintained at densities that permitted logarithmic growth. For the evaluation of cytotoxicity of the synthesized compounds, cells at their logarithmic phase of growth were removed from culture and seeded in 24-well plate cultures at cell density 2×10^{-4} cells/ml. Cells then were allowed to attach in plates for 3 h before the addition of any compound. All compounds were diluted in dimethylsulfoxide (DMSO), whereas the final concentration of the solvent in cell culture did not exceed the level of 0.4% v/v. That solvent concentration caused no measurable growth inhibition for both cell lines. Chemical molecules were added in cultures at different concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and/or 10^{-4} M. Also, the known antituberculosis drug isoniazid and the cytotoxic doxorubicin were tested in VERO cell cultures and served as controls. Cell growth was then assessed after 48 h by counting cell number through the use of a hemocytometer [38]. All experiments were carried out for each molecule two times duplicate and the mean values obtained were used to calculate the 50% inhibition concentration (IC_{50}) i.e. the concentration of the compound capable to inhibit 50% of the cell growth. The selective index was calculated as the ration of IC_{50} in VERO cells to MIC_{95} in *M. tuberculosis*.

4.2.3. Evaluation of the inhibition of mycolic acid biosynthesis

The ability of all final products to inhibit the mycolic acid (MA) biosynthesis was evaluated *in vitro*, on a cell-free extract of *M. smegmatis* mc^2155 according to the protocol of Vaubourgeix et al., 2009 [36].

4.2.3.1. Preparation of cell wall extracts. Freshly grown bacteria were washed with 10 mM potassium phosphate buffer, pH 7.0, and resuspended in 50 mM β-mercaptoethanol (buffer A). Bacteria were lysed by cell distruption, by two passages through a One-shot Cell Disrupter (Constant Systems Ltd.) at 0.8 kbar. The crude extract was centrifuged for 15 min at 3000 × *g* at 4 °C. The very dense layer at the surface of the supernatant, consisting of cell wall and membrane fragments [39], was removed, resuspended in buffer A and homogenized with a syringe (needle size 0.7 × 30 mm). The protein concentration of the extracts was determined with the DC method (Bio-Rad) for insoluble proteins by boiling in 0.5 N NaOH at 100 °C for 10 min.

4.2.3.2. Evaluation of the activity of all final products to inhibit MA biosynthesis in vitro. MA biosynthesis was assayed *in vitro* in a reaction medium containing 100 mM potassium phosphate buffer, pH 7.0, 3 mM $MgCl_2$, 7 mM $KHCO_3$, 7 mM ATP, 0.7 mM CoASH and 500 μg of cell wall extract proteins in a total volume of 500 μL. Mixtures were first incubated in the presence or absence of the compounds (10 mM solutions in DMSO, such as the final solvent concentration in the reaction medium is 5% v/v). Reaction mixtures were incubated at 37 °C for 15 min and then 50 μM of [1-^{14}C]_acetate (PerkinElmer Life Sciences) were added for the MA and fatty acid biosynthesis. Reaction mixtures were incubated at 37 °C for 60 min. The reactions were stopped by saponification and the MA profiles were analyzed by TLC.

Results were expressed as % inhibition of mycolate and fatty acid synthesis in comparison to the control. The concentrations of the compounds were equal to their antituberculosis MICs determined for *M. tuberculosis*.

[1-^{14}C]_acetate (specific activity, 56.60 mCi/mmol) was purchased from PerkinElmer Life Sciences.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmech.2010.09.048.

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