Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 2177–2186
Bioorganic & Medicinal Chemistry

Synthesis and antimycobacterial evaluation of benzofurobenzopyran analogues

Soizic Prado,[a,b] Yves L. Janin,[a,*] Brigitte Saint-Joanis,[b] Priscille Brodin,[b] Sylvie Michel,[c] Michel Koch,[c] Stewart T. Cole,[b] François Tillequin[c] and Pierre-Etienne Bost[a]

[a]*Laboratoire de Chimie Organique, URA 2128 CNRS-Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France*
[b]*Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France*
[c]*Laboratoire de Pharmacognosie de l'Université René Descartes, UMR/CNRS 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75006 Paris, France*

Received 25 October 2006; revised 25 November 2006; accepted 8 December 2006
Available online 12 December 2006

Abstract—We recently reported that 3,3-dimethyl-3*H*-benzofuro[3,2,*f*][1]-benzopyran and its hydrogenated analogue are selective in vitro inhibitors of mycobacterial growth. However, their lack of in vivo activity on a murine model of *Mycobacterium tuberculosis* infection due to their poor bioavailability led to a structure–activity relationship investigation. We wish to report here the preparation of some structural analogues along with their biological effect on the growth of *Mycobacterium smegmatis*, *M. tuberculosis*, as well as on VERO cells for the most active compound.

1. Introduction

The antimycobacterial activity of the 3,3-dimethyl-3*H*-benzofuro[3,2,*f*][1]-benzopyran (**1**), or its dihydro analogue **2**, was measured[1] in view of the antibacterial activity reported for the chromene-containing structures of various compounds[2–5] and that of the less structurally related antimycobacterial agent;[6–8] the cytotoxic[9] and hepatotoxic[10] usnic acid (**3**) (Fig. 1). From our results, a survey of other antimycobacterials related to compound **1** pointed out the series of dibenzofurans or dibenzothiophenes reported in the 50's for their in vitro antimycobacterial activity.[11–14] Among them, the sulfate **4** was reported to be the most active in vitro but, similarly to **1** or **2**, these compounds were found inactive in vivo in the mouse model. On the other hand, compounds **1** and **2** display a most remarkable selectivity of action on the *Mycobacterium* genus.[1] For this reason, biological studies have been undertaken in order to elucidate their mechanism of action and identify a potential biological target specific for *Mycobacteria*. We also started a structure–activity relationship study. This synthetic work not only attempts to obtain analogues with an in vivo antimycobacterial effect but also to generate active compounds suitable for a biochemical target identification using affinity chromatography. This paper describes the synthesis of various compounds structurally related to **1** and the first elements of the structure–activity relationship obtained.

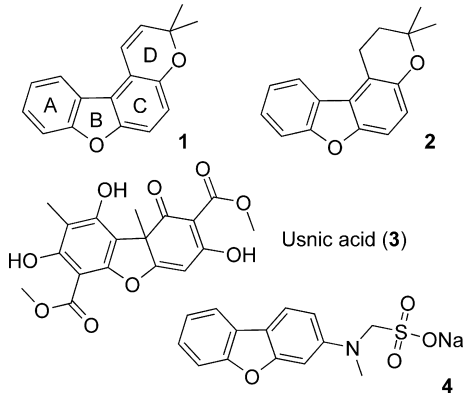


Figure 1.

Keywords: Antimycobacterials; Benzofuro[3,2,*f*][1]-benzopyran; Structure–activity relationship study; *Mycobacterium tuberculosis*.
* Corresponding author. Tel.: +33 6 85 42 49 47; fax: +33 1 45 68 84 04; e-mail: yljanin@pasteur.fr

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.12.009

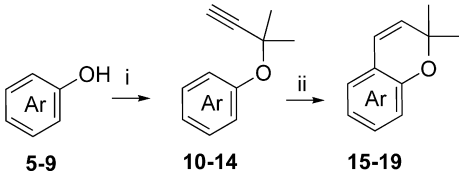
2. Chemistry

In many instances the synthesis of the analogues described here started from commercially available phenols. As depicted on Scheme 1, from the hydroxy-bearing compounds **5**–**9**, the corresponding propargylic ethers **10**–**14** were made using the remarkable copper chloride-catalysed method.[15,16] *Note*: for the structures of some of the numbered synthetic intermediates not depicted on the schemes, see the Experimental part. This was followed by a thermal treatment[17–19] in boiling dichlorobenzene which provided, via a Claisen cyclization, the chromene-containing compounds **15**–**19** as depicted in Figure 2.

From the unsymmetrical 3-phenylphenol (**9**), the Claisen cyclization of the corresponding propargylic ether **14** led to a mixture of the corresponding mixture of chromenes **19a** and **19b** which could not be separated in our hands (data not shown). As described below, this two-component mixture was evaluated anyway.

Since analogue **16** turned out to be biologically interesting, we prepared from it alcohol **20** or the further reduced compound **21** by treatment with either sodium borohydride or ammonium formate in the presence of palladium over charcoal. The use of a montmorillonite K10-catalysed reaction[20,21] between allylbromide (**22**) and, in our case, 4-hydroxybenzophenone (**6**) provided the chromane analogue **23**. Following a preparation of the previously described 7-hydroxychromane **24**,[22] we synthesized analogue **25**, bearing an internal hydrogen bond between the carbonyl function and the chromene C–D ring system via a Friedel-Craft reaction with benzoyl chloride. This internal hydrogen bond was introduced in an attempt to reduce the single bond rotation existing in structures **16**, **17**, and **23**. A possible occurrence of an equilibrium between a putative, biologically active, flat conformation (the one depicted) and an inactive one (the opposite) was the reason behind this part of our work. Moreover, we further demonstrated the potential for chemical diversity in this series of analogues and prepared the nitrogen-bearing derivative **27**. This was achieved via a condensation reaction between the aldehyde-bearing chromene **26** (made from 4-hydroxybenzaldehyde using the two-step preparation depicted in Scheme 1) and 3-pyridylmagnesiumchloride.[23] (Scheme 2).

Further hydroxy-bearing compounds were prepared prior to the construction of the chromene ring according to the synthetic path depicted in Scheme 1. From benzoquinone (**28**), using modified procedures,[24–27] involving the Michael addition of enamines **29** followed by a cyclization under acidic conditions, we prepared the phenol **30**. Attempts to use more functionalized enamines were not very successful and further work is still ongoing. It is noteworthy that such reactions are related to the Nenitzescu hydroxyindole preparation from benzoqui-



Scheme 1. Reagents and condition: (i) $ClC(Me)_2CCH$, DBU, MeCN, $CuCl_2$; (ii) 1,3-dichlorobenzene, reflux.

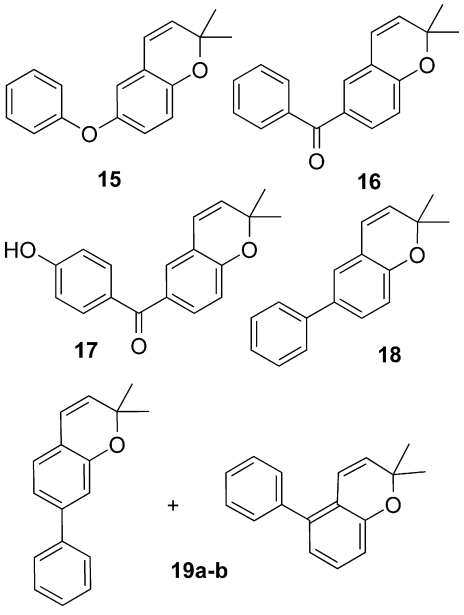
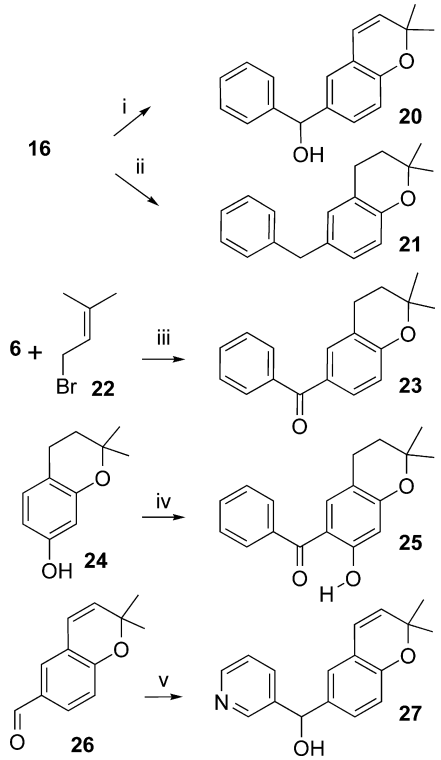


Figure 2.

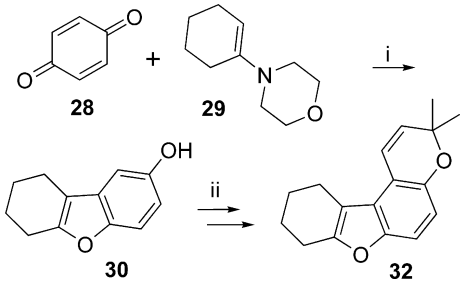


Scheme 2. Reagents: (i) $NaBH_4$, EtOH; (ii) NH_3, HCO_2H/Pd/C, EtOH; (iii) K10, CH_2Cl_2; (iv) C_6H_5COCl, $AlCl_3$, CH_2Cl_2; (v) 3-pyridylMgCl, THF.

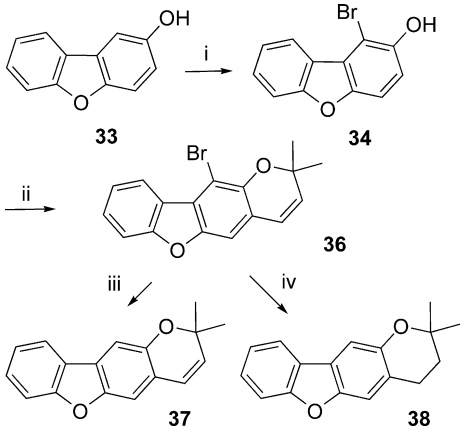
none and enamines[28] which sometimes proceeds in modest yield and often requires extensive optimisation of the reaction conditions.[29] From this phenol, analogue **32** was obtained using the usual route (see Scheme 1). Traces of the isomeric chromene were observed in the course of its preparations and could be removed during the purification steps (Scheme 3).

The preparation of compound **1**, via the cyclization of the corresponding propargylic ether, always takes place with the occurrence of traces of the linear analogues **37** and we could remove these traces to below detectable levels by recrystallization.[1] However, in order to remove any ambiguity concerning its improbably powerful antimycobacterial property, we devised an unequivocal synthetic access to compound **37**. The bromination of 2-hydroxydibenzofuran (**33**) enabled the preparation of pure 5-bromo derivative **34** from which, through the usual route depicted in Scheme 1, the brominated linear analogue **36** was obtained. Hydrogenation of **36** using palladium over charcoal and one or two equivalents of ammonium formate allowed the preparation of the linear analogue **37** or the further reduced chromane-bearing compound **38** (Scheme 4).

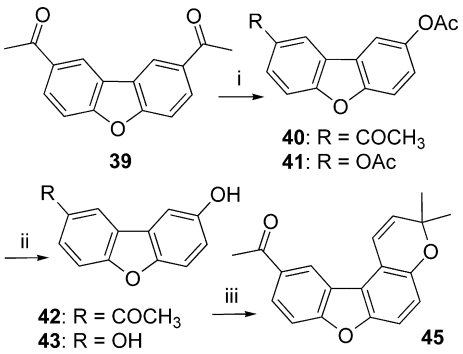
From the previously described[30] 2,8-diacyldibenzofuran (**39**), a limited Baeyer–Villinger reaction led to the 2-acetyl-8-acyl derivative (**40**) along with a proportion of the corresponding 2,8-diacetyl derivative (**41**). The following hydrolysis of the mixture of these esters provided a mixture of 2-hydroxydibenzofurans **42** and **43** which



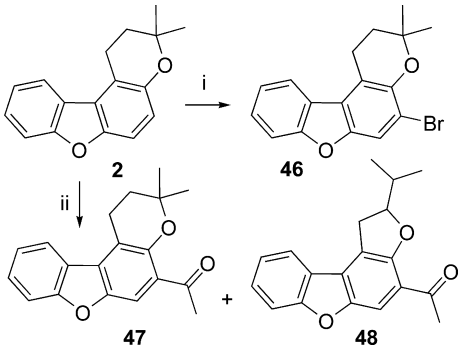
Scheme 3. Reagents: (i) a—toluene, b—H_2SO_4; (ii) see Scheme 1.



Scheme 4. Reagents and condition: (i) NBS, EtOH; (ii) see Scheme 1; (iii) or (iv) NH_3, HCO_2H (1 or 2 equiv), Pd/C, EtOH, reflux.



Scheme 5. Reagents: (i) 3-$ClPhCO_3H$, CF_3CO_2H, CH_2Cl_2; (ii) MeONa, MeOH; (iii) see Scheme 1.



Scheme 6. Reagents: (i) NBS, EtOH; (ii) CH_3COCl, $AlCl_3$, CH_2Cl_2.

could be separated. However, if the propargylic ether **44** could be obtained from **42** (and transformed into analogues **45**) alkylation trials with 2,8-dihydroxydibenzofuran (**43**) only led to extensive decomposition (Scheme 5).

As the chromane analogue **2** is as effective on *Mycobacterium tuberculosis* growth in vitro as compound **1**, we undertook some chemical transformation of **2** aiming at introducing substituents on its ring C. Such chemistry is not possible on compound **1** because of the reactive chromene double bond.[1] Bromination of **2** gave the 5-bromo derivative **46** in good yield. On the other hand, the aluminium chloride-catalysed acylation of **2** gave the 11-acyl analogue **47** along with the bisfuran derivative **48** resulting from a Lewis acid-catalysed pyran rearrangement into a furan ring.[31,32] The separation of compounds **47** and **48** turned out to be difficult and could only be achieved in low yield (Scheme 6).

3. Biological results and discussion

The antimycobacterial activity was screened on the fast growing saprophyte *Mycobacterium smegmatis* mc^2155 and on the virulent strain *M. tuberculosis* H37Rv for all synthesized compounds, using the new Microdilution Resazurin Assay (MRA).[33] The minimal inhibitory concentration (MIC_{95}) is defined as the amount of compound required for >95% inhibition of bacterial growth. The compounds found to be the most active

Table 1. In vitro biological assays

Compound	MIC_{95} (μg/mL) *M. smegmatis*	MIC_{95} (μg/mL) *M. tuberculosis*	IC_{50} (VERO Cells)
Isoniazid	12	0.1	>500
1	10	10	80
2	10	10	100
7	62	125	ND
15	70	>500	ND
16	20	40	32
17	32	62	ND
18	>500	>500	ND
19a,b	>500	>500	ND
20	20	20	16
21	>500	30	16
23	50	30	75
25	4	62	ND
27	62	62	ND
30	31	500	ND
32	>500	120	ND
33	ND	25	ND
36	120	30	10
37	20	35	ND
38	40	>500	ND
39	5	10	16
42	>500	8	16
43	15	15	16
45	>500	>500	ND
46	>500	>500	ND
47	>500	>500	ND
48	>500	>500	ND

ND, not determined.

against *M. tuberculosis* were further evaluated for their cytotoxicity on mammalian VERO cell line growth using the dimethylthiazolyldiphenyl tetrazolium (MTT)-based assay.

Many propargylic ether intermediates not shown in Table 1 (compounds **10**, **13**, **14**, **31**, and **44**) were tested on *M. smegmatis* and *M. tuberculosis* but their antimycobacterial activity turned out to be above 100 μg/mL. Other tricyclic intermediates were found to be almost as active as the pyranodibenzofuran **1**. For instance, 2-hydroxydibenzofuran (**33**) and the dibenzofurans **39**, **42**, and **43** displayed antimycobacterial activities. This is in accordance with the results previously reported for the mono-substituted series of dibenzofurans such as the sulfate derivative **4**[11–14] or more recently reported carbazoles.[34] However and also in accordance with these previously reported results, compounds **39**, **42**, and **43** turned out to be also cytotoxic for VERO cells. Of note is the drastic difference of effect on *M. tuberculosis* or *M. smegmatis* observed for some analogues (see compounds **21**, **30**, **38**, and **42**). These contrasting results, which were confirmed at least by two experiments, are of interest for the design of biological assays on *Mycobacterium* in general and deserve further investigations. Aside from the fact that the two bacteria have different biochemistry and physiology, it is probably important to mention that an assay on *M. smegmatis* takes 2 days, whereas an assay on *M. tuberculosis* requires at least 6 days. We suggest that this time factor explains some of the differences observed. In the case of the most lipophilic compounds, a slow crystallization out of the growth medium over 6 days would result in loss of biological effect. The replacement of the furan B ring was attempted by an ether linkage (compound **15**), or a single carbon–carbon bond (compound **18** and the mixture **19a** and **19b**). This was not successful from a biological point of view. In a different approach, the furan ring was replaced by a carbonyl group (compounds **16**, **17**, and **23**), a hydroxymethylene (compounds **20** and **27**) or a methylene (compound **21**). This met with a certain degree of success. However, as shown in Table 1, the most active compounds were evaluated for their cytotoxicity on VERO cells and turned out to be cytotoxic at the same concentration range, contrary to the pyranodibenzofurans **1** and **2**. For this reason, further work on this type of tricyclic analogues was stopped. The recent report on the activity of various acetophenones[35,36] was of interest in the light of the antimycobacterial activity we measured for 4,4'-dihydroxybenzophenone (**7**) or compounds **16**, **23**, and **25**. The linear tetracyclic analogues **36**–**38**, which had been prepared in order to lift any doubt concerning trace amounts of the linear isomer **37** at the origin of the measured antimycobacterial activities for compound **1**, turned out to be somewhat less active. An important difference of activity on *M. tuberculosis* was observed between compounds **37** and **38** but this may just reflect a slow precipitation of compound **38** out of the culture media of *M. tuberculosis* as mentioned above. The biological results for the few angular tetracyclic analogues **45**–**47** made so far are disappointing as they illustrate a very narrow structure–activity relationship for this series. However, the recurrent problem of water solubility is acute for all these compounds; the estimated CLogP of compound **1** is actually 5.5. The substituents so far introduced (bromine or acetyl) on the benzofuro[3,2,*f*][1]-benzopyran structure do not improve this physical characteristic. These results lead to the conclusion that there is a need to devise original synthetic access to analogues of **1** and **2** bearing more diverse substituents thereby providing improved water solubility. From the chemistry point of view, two other syntheses of the core structure of **1** or **2** were reported.[37,38] On the other hand, the design of preparations of polyfunctionalized hydroxydibenzofurans along with methods to circumvent the recurrent problem of the unequivocal cyclization of asymmetric propargylic ethers into chromenes (as depicted in Fig. 1) would still be quite useful. A recent report of dibenzofuran synthesis[39] should be mentioned here as well as our recent work on the synthesis of 2,2-dimethylchromenes from various salicylaldehydes.[40]

4. Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400 and 100 MHz, respectively. Most often, two-dimensional experiments were performed in order to further demonstrate structural assignments. Shifts (δ) are given in ppm with respect to the TMS signal and coupling constants (J) are given in Hertz. Column chromatography was performed over Merck silica gel 60 (0.035–0.070 mm), using a solvent pump operating at pressure between 2 and 7 bar

(25–50 mL/mn) and an automated collecting system driven by a UV detector set to 254 nm unless stated otherwise. Mass spectra were obtained on an Agilent 1100 series LC/MSD system using an atmospheric electrospray ionization system, a C18 column, a gradient of water and either acetonitrile containing 0.07% of either formic acid or water and methanol containing 0.07% ammonium formate.

General procedure for synthesis of propargylic ethers

To a solution of the phenol (1 equiv) in anhydrous acetonitrile (50 mL for 5.5 mmol; dried over 4A molecular sieve) under argon and cooled using an ice bath were added DBU (1.3 equiv) and copper chloride dihydrate (0.01 equiv). The solution was maintained at 0 °C and chlorobutyne (1.3 equiv) was added. After stirring for 5 h, the mixture was concentrated at reduced pressure and the residue was partitioned between water and toluene. The organic fraction was washed with hydrochloric acid 1 N, sodium hydroxide 1 N, sodium hydrogenocarbonate 1 N and brine and dried over anhydrous sodium sulfate. After concentration to dryness, the residue was purified as described below.

General procedure for the cyclization of the propargylic ethers

Chromene was obtained from propargylic ethers by heating them in 1,2-dichlorobenzene (100 mL or 7 mmol) for 12 h. Purification of the residue, obtained after concentration to dryness, was made by a chromatography over silica gel and led to the chromene-containing compounds described below.

1-(2-Methylbut-3-yn-2-yloxy)-4-phenoxybenzene (10). Using the general procedure described above and after a chromatography over silica gel (cyclohexane/dichloromethane, 98:2), compound **10** was obtained as an oil (0.4 g, 60%) from 4-phenoxyphenol (**5**). LC/MS (ES) *m/z* 253 $[M+H]^+$. ^1H ($CDCl_3$): 7.35 (m, 2H); 7.21 (m, 2H); 7.09 (dd, 1H, $J = 7$ and 1); 7.02 (d, 2H, $J = 8$); 6.96 (d, 2H, $J = 9$); 2.57 (s, 1H); 1.66 (s, 6H). ^{13}C ($CDCl_3$): 158.3; 152.9; 151.6; 130.0; 123.8; 123.2; 119.9; 118.5; 86.6; 74.1; 73.1; 29.9.

(4-(2-Methylbut-3-yn-2-yloxy)phenyl)(phenyl)methanone (11). Using the procedure described above, propargylic ether **11** was obtained from 4-hydroxybenzophenone (**6**) after a chromatography over silica gel (cyclohexane/dichloromethane, 6:4) as an oil (75%). LC/MS (ES) *m/z* 279 $[M-H]^-$. ^1H ($CDCl_3$): 7.78 (m, 4H); 7.58 (m, 1H); 7.49 (m, 2H); 7.31 (m, 2H); 2.66 (s, 1H); 1.74 (s, 6H). ^{13}C ($CDCl_3$): 196.1; 160.1; 138.5; 132.3; 132.2; 131.6; 130.2; 128.6; 119.5; 85.6; 75.2; 72.2; 30.1; 30.0.

(4-Hydroxyphenyl)(4-(2-methylbut-3-yn-2-yloxy)phenyl)-methanone (12). Using the procedure described above, propargylic ether **12** was obtained from 4,4′-dihydroxybenzophenone (**7**) after a chromatography over silica gel (cyclohexane/dichloromethane, 6:4) as an oil (75%). LC/MS (ES) *m/z* 263 $[M-H]^-$ weak. ^1H ($CDCl_3$): 7.78 (m, 4H); 7.29 (m, 2H); 6.91 (m, 2H); 2.69 (s, 1H, s); 1.74 (s, 6H). ^{13}C ($CDCl_3$): 195.2; 160.2; 159.7; 133.0; 132.2; 131.9; 130.8; 119.8; 115.5; 85.7; 75.0; 72.7; 29.9.

3-(2-Methylbutyn-2-yloxy)biphenyl (13). Using the general procedure described above and after a chromatography over silica gel (cyclohexane/dichloromethane, 95:5), compound **13** was obtained as a pale oil (1.1 g, 68%) from 4-phenylphenol (**8**). LC/MS (ES) *m/z* 237 $[M+H]^+$. ^1H ($CDCl_3$): 7.80 (m, 2H); 7.66 (m, 2H); 7.54 (m, 3H); 7.43 (m, 2H); 2.70 (s, 1H); 1.85 (s, 6H). ^{13}C ($CDCl_3$): 155.5; 141.4; 141.3; 136.0; 127.8; 127.3; 122.1; 86.7; 75.4; 73.7; 30.1.

3-(2-Methylbut-3-yn-2-yloxy)biphenyl (14). Using the general procedure described above and after a chromatography over silica gel (cyclohexane/dichloromethane, 99.5:0.5), compound **14** was obtained from 3-phenylphenol (**9**) as a pale oil (1.1 g, 69%). LC/MS (ES) *m/z* 237 $[M+H]^+$. ^1H ($CDCl_3$): 7.62 (m, 2H); 7.46 (m, 3H); 7.54 (m, 3H); 7.35 (m, 2H); 7.23 (m,1H); 2.62 (s, 1H); 1.71 (s, 6H). ^{13}C ($CDCl_3$): 156.3; 142.5; 141.3; 129.5; 127.9; 127.5; 127.2 (three signals); 122.1; 121.9; 91.0; 74.4; 75.8; 30.1.

2,2-Dimethyl-6-phenoxy-2*H*-chromene (15). From compound **10**, using the general procedure described above, compound **15** was obtained after chromatography over silica gel (cyclohexane/dichloromethane, 95:5) as an oil 0.19 g, (75%). LC/MS (ES) *m/z* 253 $[M+H]^+$. ^1H ($CDCl_3$): 7.33 (m, 2H); 7.07 (dd, 1H, $J = 7$ and 1); 6.98 (m, 2H); 6.81 (d, 2H, $J = 3$); 6.78 (s, 1H); 6.71 (d, 2H, $J = 3$); 6.30 (d, 1H, $J = 9$); 5.67 (d, 1H, $J = 9$); 1.47 (s, 6H). ^{13}C ($CDCl_3$): 158.8; 150.4; 149.4; 132.0; 129.9; 122.8; 122.6; 122.4; 120.6; 118.0; 117.8; 117.5; 75.5; 30.0.

(2,2-Dimethyl-2*H*-chromen-6-yl)(phenyl)methanone (16). From compound **11**, using the procedure described above, chromene **16** was obtained after chromatography over silica gel (cyclohexane/dichloromethane, 50:50) as an oil (88%). LC/MS (ES) *m/z* 265 $[M+H]^+$. ^1H ($CDCl_3$): 7.75 (m, 1H); 7.49–7.60 (m, 6H); 6.83 (dd, 1H, $J = 1.2$ and 9.8); 6.37 (d, 1H, $J = 9.8$); 5.69 (dd, 1H, $J = 1.4$ and 9.8); 1.5 (s, 6H). ^{13}C ($CDCl_3$): 195.9; 157.6; 138.7; 132.7; 132.2; 131.6; 130.6; 130.1; 129.2; 128.6; 122.1; 121.0; 116.3; 77.9; 28.8.

(2,2-Dimethyl-2*H*-chromen-6-yl)(phenyl)methanone (17). From compound **12**, using the procedure described above, chromene **17** was obtained after chromatography over silica gel (dichloromethane/ethanol, 98:02) as a yellow amorphous solid (58%). LC/MS (ES) *m/z* 279 $[M-H]^-$. ^1H ($CDCl_3$): 7.75 (m, 2H); 7.62 (dd, 1H, $J = 9$ and 2); 7.50 (d,1H, $J = 2$); 7.18 (s, 1H, OH); 6.94 (m, 2H); 6.84 (d, 1H, $J = 9$); 6.35 (d, 1H, $J = 9$); 5.70 (d, 1H, $J = 9$); 1.5 (s, 6H). ^{13}C ($CDCl_3$): 195.8; 160.6; 157.4; 133.0; 132.6; 131.6; 130.9; 130.6; 129.2; 122.0; 121.0; 116.4; 115.6; 77.9; 28.7.

2,2-Dimethyl-6-phenyl-2*H*-chromene (18). Cyclization of compound **13** under conditions described above gave compound **18** as an oil (0.35 g, 37%) after chromatography over silica gel (cyclohexane/dichloromethane, 98:2).

LC/MS (ES) *m/z* 235 $[M+H]^+$. ^1H ($CDCl_3$): 7.45 (m, 2H); 7.41 (m, 2H); 7.37 (dd, 1H, $J = 8$ and 2); 7.32 (dd, 1H, $J = 7$ and 1); 7.23 (d, 1H, $J = 2$); 6.87 (d, 1H, $J = 8$); 6.41 (d, 1H, $J = 9$); 5.68 (d, 1H, $J = 9$); 1.48 (s, 6H). ^{13}C ($CDCl_3$): 152.9; 141.3; 134.3; 131.5; 129.0; 128.1; 127.0 (three signals); 125.4; 121.8; 122.7; 121.8; 116.9; 75.5; 28.4.

(2,2-Dimethyl-2*H*-chromen-6-yl)(phenyl)methanol (20). Compound **16** (1.07 g, 4.05 mmol) was dissolved in ethanol (80 mL) and sodium borohydride (0.23 g, 6.07 mmol) was added. This was stirred at room temperature overnight and concentrated to dryness. The residue was dissolved in dichloromethane, the organic phase was washed with water, dried over sodium sulfate and concentrated to dryness to give a quantitative yield of **20**. Solid, LC/MS (ES) *m/z* 249 $[M-OH]^-$, 266 $[M-OH + NH_3]^+$. ^1H ($CDCl_3$): 7.49–7.60 (m, 4H); 7.10 (dd, 1H, $J = 2.2$ and 8.2); 7.00 (d, 1H, $J = 2.2$); 6.75 (d, 1H, $J = 8.2$); 6.30 (d, 1H, $J = 9.8$); 5.77 (s, 1H); 5.61 (d, 1H, $J = 9.8$); 2.18 (s br, 1H); 1.43 (s, 6H). ^{13}C ($CDCl_3$): 152.8; 144.3; 136.7; 131.4; 128.8; 127.9; 127.8; 126.9; 125.1; 122.7; 121.6; 116.6; 76.7; 76.2; 28.5; 28.4.

6-Benzyl-2,2-dimethylchroman (21). Compound **16** (0.15 g, 0.56 mmol), ammonium formate (0.5 g, 8.5 mmol) and 10% palladium over charcoal (0.03 g, 0.03 mmol) were refluxed in ethanol (40 mL) for 1 h. The suspension was filtered and concentrated to dryness. The residue was purified by chromatography over silica gel (cyclohexane/dichloromethane, 7:3) to give compound **21** (0.13 g, 85%) as a solid. LC/MS (ES) *m/z* 267 $[M+H]^+$. ^1H ($CDCl_3$): 7.60 (m, 2H); 7.23 (m, 3H); 6.96 (m, 2H); 6.75 (m, 1H); 3.93 (s, 2H); 2.71 (t, 2H, $J = 6.7$); 1.82 (t, 2H, $J = 6.7$); 1.30 (s, 6H). 13C ($CDCl_3$): 152.7; 142.2; 132.5; 130.1; 129.3; 128.8; 128.2; 126.3; 121.1; 117.5; 74.4; 41.5; 33.3; 27.3; 22.9.

(2,2-Dimethyl-2*H*-chroman-6-yl)(phenyl)methanone (23). 4-Hydroxybenzophenone (**6**) (0.5 g, 2.52 mmol), 1-bromo-3-methylbut-2-ene (0.64 mL, 5.04 mol) and montmorillonite K10 (10 g) were stirred in dichloromethane (50 mL) overnight. The suspension was filtered and concentrated to dryness. The residue was purified by chromatography over silica gel (cyclohexane/dichloromethane, 4:6 to 1:1) to give a small fraction of pure compound **23** which had to be further sublimated (0.04 g, 7%). LC/MS (ES) *m/z* 267 $[M+H]^+$. ^1H ($CDCl_3$): 7.78 (m, 1H); 7.60 (m, 2H); 7.50 (m, 2H); 6.84 (d, 1H, $J = 8.5$); 2.84 (t, 2H, $J = 6.7$); 1.87 (t, 2H, $J = 6.7$); 1.39 (s, 6H). ^{13}C ($CDCl_3$): 196.2; 158.8; 138.9; 132.9; 132.0; 131.8; 130.1; 129.5; 128.5; 121.2; 117.4; 75.9; 32.9; 27.3; 22.7.

2,2-Dimethylchroman-7-ol (24). Resorcinol (10.08 g, 91 mmol) and 2-methylbut-3-en-2-ol (4.76 mL, 91.0 mmol) were refluxed in 80% formic acid (50 mL) for 4 h as previously described.[22] The solution was concentrated, diluted in water, made basic with solid sodium hydrogen carbonate and extracted with dichloromethane. The organic layer were washed with water, dried over sodium sulfate, and concentrated to dryness. The residue was purified by chromatography over silica gel (cyclohexane/dichloromethane, 3:7 to pure dichloromethane) to give, in order of elution, the following not quite expected[22] four compounds. Compound **2,2,8,8-tetramethyl-3,4,9,10-tetrahydro-*2H,8H*-pyrano[*2,3-f*]chromene**: 3% yield, oil. ^1H ($CDCl_3$): 6.81 (d, 1H, $J = 8.3$); 6.35 (d, 1H, $J = 8.3$); 2.71 (t, 2H, $J = 6.7$); 2.62 (t, 2H, $J = 6.8$); 1.77 (m, 4H); 1.33 (m, 12H). 13C (CDCl3): 153.2; 152.2; 111.7; 109.9; 108.9; 74.3; 73.9; 33.3; 32.8; 27.5; 27.1; 22.4; 17.5. Compound **2,2,8,8-tetramethyl-2,3,4,6,7,8-hexahydropyrano[3,2-g]chromene**: 3.2% yield, solid. ^1H ($CDCl_3$): 1.32 (s, 12H); 6.74 (s, 1H); 6.24 (d, 1H, $J = 8.3$); 2.69 (t, 4H, $J = 6.7$); 1.77 (t, 4H, $J = 6.7$). ^{13}C ($CDCl_3$): 153.3; 129.7; 113.0; 105.1; 74.2; 33.5; 27.2; 22.2. Compound **2,2-dimethylchroman-5-ol**: 4.6% yield, solid. ^1H ($CDCl_3$): 6.97 (m, 1H); 6.43 (dd, 1H, $J = 1.0$ and 8.2); 6.34 (dd, 1H, $J = 1.0$ and 7.9); 4.70 (s, 1H); 2.68 (t, 2H, $J = 6.8$); 1.83 (t, 2H, $J = 6.8$); 1.35 (s, 6H). ^{13}C ($CDCl_3$): 155.5; 154.2; 127.5; 110.3; 108.8; 106.3; 74.3; 32.5; 27.0; 17.2. Compound **24**: LC/MS (ES) *m/z* 179 $[M+H]^+$ 23% yield, wax. ^1H ($CDCl_3$): 6.92 (d, 1H, $J = 8.1$); 6.36 (dd, 1H, $J = 2.5$ and 8.1); 6.29 (d, 1H, $J = 2.5$); 4.59 (m, 1H); 2.71 (t, 2H, $J = 6.7$); 1.79 (t, 2H, $J = 6.7$); 1.34 (s, 6H). ^{13}C ($CDCl_3$): 155.2; 130.5; 113.7; 107.7; 104.2; 74.8; 33.3; 27.3; 22.1.

7-Hydroxy-2,2-dimethylchroman-6-yl)(phenyl)methanone (25). Compound **24** (0.26 g, 1.4 mmol) and benzoyl chloride (0.2 mL, 0.0017 mol) were dissolved in dichloromethane (50 mL, distilled over P_2O_5). Dry aluminium chloride (0.23 g, 1.7 mmol) was added and the reaction mixture was protected from humidity by a calcium guard and stirred for an hour. Water was added and this was extracted with dichloromethane. The organic layer were dried over sodium sulfate and concentrated to dryness. A first chromatography over silica gel eluting with a mixture of dichloromethane and cyclohexane, 7:3, provided a fraction (0.21 g) containing a mixture of isomeric compounds. This was recrystallized in methanol (20 mL) to yield pure compound **25** (0.088 g, 24%). LC/MS (ES) *m/z* 283 $[M+H]^+$. ^1H ($CDCl_3$): 12.27 (s, 1H); 7.58 (m, 5H); 7.30 (s, 2H); 6.43 (s, 1H); 2.67 (t, 2H, $J = 6.8$); 1.82 (t, 2H, $J = 6.8$); 1.38 (s, 6H). ^{13}C ($CDCl_3$): 200.3; 164.2; 161.9; 139.1; 135.5; 131.6; 129.2; 128.7; 113.6; 113.0; 105.2; 76.4; 33.1; 27.4; 22.1.

2,2-Dimethyl-6-formyl-2*H*-chromene (26). Using the above-mentioned protocol 4-(2-methylbut-3-yn-2-yloxy)benzaldehyde, precursor to compound **26**, was obtained from 4-formylphenol (5.0 g, 41 mmol) after purification by chromatography over silica gel (cyclohexane/dichloromethane, 50:50, v/v) as a pale oil (3.70 g, 44.79%). LC/MS (ES) *m/z* 189 $[M+H]^+$. ^1H ($CDCl_3$): 9.92 (s, 1H); 7.84 (m, 2H); 7.34 (m, 2H); 2.67 (s, 1H); 1.73 (s, 6H). ^{13}C ($CDCl_3$): 191.3; 161.6; 131.2 (two signals); 131.0; 119.7 (two signals); 85.3; 75.3; 72.8; 29.8. This propargylic ether was cyclized using the above-mentioned protocol to give, after purification by chromatography over silica gel (cyclohexane/dichloromethane, 70:30 v/v), compound **26** as a pale oil (1.59 g, 51.4%). LC/MS (ES) *m/z* 268 $[M+H]^+$. ^1H ($CDCl_3$): 8.65 (d, 1H, $J = 1$); 8.53 (dd, 1H, $J = 4$ and 1); 7.73

(m, 1H); 7.28 (m, 1H); 7.10(dd, 1H, $J = 8$ and 2); 6.98 (d, 1H, $J = 2$); 6.77 (d, 1H, $J = 8$); 6.30 (d, 1H, $J = 9$); 5.81 (s; 1H); 5.65 (d, 1H, $J = 9$); 1.49 (s, 6H). ^{13}C ($CDCl_3$): 153.2; 149.1; 148.6; 139.6; 135.7; 134.4; 131.6; 127.8; 125.0; 123.7; 122.4; 121.8; 116.8; 74.2; 76.8; 28.4.

(2,2-Dimethyl-2*H*-chromen-6-yl)-pyridin-3-yl-methanol (27). A 2 M isopropyl magnesium chloride solution in THF (1.58 mL, 3.16 mmol) was added under argon to a solution of 3-bromopyridine (0.5 g, 3.16 mmol) in anhydrous THF (16 mL, distilled over sodium). The reaction mixture was stirred at room temperature for 1 h. The chromene **26** was then added and the reaction mixture was stirred for 2 h. The solution was evaporated to dryness and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. Purification by chromatography of the residue over silica gel (cyclohexane/dichloromethane, 5:5) gave compound **27** as an amorphous solid (30%). LC/MS (ES) *m*/*z* 268 $[M+H]^+$. ^1H ($CDCl_3$): 8.65 (d, 1H, $J = 1$); 8.53 (dd, 1H, $J = 5$ and 1); 7.73 (m, 1H); 7.28 (m, 1H); 7.10 (dd, 1H, $J = 8$ and 2); 6.98 (d, 1H, $J = 2$); 6.77 (d, 1H, $J = 8$); 6.30 (d, 1H, $J = 9$); 5.81 (s, 1H); 5.65 (d, 1H, $J = 9$); 1.45 (s, 6H). ^{13}C ($CDCl_3$): 153.2; 149.07; 148.6; 139.6; 135.7; 134.4; 131.6; 127.8; 125.0; 123.7; 122.4; 121.8; 116.8; 76.8; 74.2; 28.4.

6,7,8,9-Tetrahydrodibenzo[*b*,*d*]furan-2-ol (30). To a solution of benzoquinone (**28**) (1 g, 9.25 mmol) in toluene (50 mL) at 0 °C was added drop-wise the commercially available enamine **29** (2 g, 12 mmol). The reaction mixture was stirred at 0 °C for 4 h. The solid precipitate was filtered off and washed with toluene and this was dried in a desiccator (3.1 g). It was then dispersed in H_2SO_4 70% (16 mL) and the suspension stirred at room temperature for 3 h. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. A purification of the residue by chromatography over silica gel (cyclohexane/dichloromethane, 5:5) gave compound **30** as an amorphous solid (0.73 g, 42%). LC/MS (ES) *m*/*z* 189 $[M+H]^+$. ^1H ($CDCl_3$): 7.25 (d, 1H, $J = 8$); 6.85 (d, 1H, $J = 3$); 6.73 (dd, 1H, $J = 8$ and 3); 4.73 (s, 1H); 2.75 (m, 1H); 2.58 (m, 1H); 1.95 (m, 1H); 1.93 (m, 1H). ^{13}C ($CDCl_3$):155.7; 151.5; 149.7; 130.1; 113.2; 111.4; 104.3; 27.3; 23.9; 23.3; 20.8.

8-(2-Methylbut-3-yn-2-yloxy)-1,2,3,4-tetrahydrodibenzo[*b*,*d*]furan (31). Using the general procedure for synthesis of propargylic ethers described above compound **31** was obtained from **30** (1 g, 5.32 mmol) as an oil in 37% yield. LC/MS (ES) *m*/*z* 255 $[M+H]^+$. ^1H ($CDCl_3$): 7.31 (d, 1H, $J = 8$); 7.26 (d, 1H, $J = 2$); 7.10 (dd, 1H, $J = 8$ and 2); 2.75 (m, 1H); 1.97 (m, 1H); 1.87 (m, 1H); 1.97 (m, 1H); 1.77 (s, 1H); 1.69 (s, 3H). ^{13}C ($CDCl_3$):155.2; 151.4; 151.3; 129.4; 119.1; 113.5; 112.6; 110.7; 91.7; 87.0; 74.4; 73.3; 30.0; 23.9; 23.3; 23.0; 20.3.

3,3-Dimethyl-8,9,10,11-tetrahydro-benzofuro[3,2-*f*][*1*]benzopyran (32). Compound **32** was obtained as a solid (0.2 g, 40%) from ether propargylic ether **31** (0.59 g, 1.97 mmol) using the general procedure for the cyclization of the propargylic ethers described above and was purified by chromatography over silica gel (cyclohexane/dichloromethane, 98:2). LC/MS (ES) *m*/*z* 255 $[M+H]^+$. ^1H ($CDCl_3$): 7.12 (d, 1H, $J = 8$); 6.77 (d, 1H, $J = 9$); 6.68 (d, 1H, $J = 9$); 5.65 (d, 1H, $J = 8$); 2.81 (m, 1H); 2.73 (m, 1H); 1.92 (m, 2H,); 1.49 (s, 6H). ^{13}C ($CDCl_3$):155.5; 149.8; 148.5; 131.1; 130.7; 119.9; 114.6; 114.1; 113.8; 113.0; 111.4; 75.8; 27.5; 24.4; 23.9.

1-Bromo-dibenzofuran-2-ol (34). To a solution of 2-hydroxydibenzofuran (**33**) (5 g, 27.00 mmol) in ethanol (50 mL), *N*-bromosuccinimide (4.8 g, 40.7 mmol) was added and this was stirred overnight. The solvent was then evaporated to dryness and the residue purified by chromatography over silica gel (cyclohexane/dichloromethane, 5:5). A recrystallization of the chromatographic fraction containing **34** and a dibrominated side-product in methanol gave compound **34** as white crystals (4.5 g, 63%). LC/MS (ES) *m*/*z* 261, 263 $[M-H]^-$. ^1H ($CDCl_3$): 8.45 (d, 1H, $J = 8$); 7.61 (d, 1H, $J = 8$); 7.53 (dd, 1H, $J = 8$ and 2); 7.51 (d, 1H, $J = 9$); 7.43 (dd, 1H, $J = 8$ and 2); 7.19 (d, 1H, $J = 9$); 5.5 (s, 1H, OH). ^{13}C ($CDCl_3$):157.2; 151.0; 148.8; 128.1; 124.4; 124.1; 122.8; 122.4; 115.1; 112.0; 111.9; 102.7; 75.8.

1-Bromo-2-(2-methylbut-3-yn-2-yloxy)dibenzo[*b*,*d*]furan (35). Following the general procedure described above a quantitative yield of compound **35** was obtained from compound **34** as an oil. LC/MS (ES) *m*/*z* 329,331 $[M+H]^+$. ^1H ($CDCl_3$): 8.60 (dd, 1H, $J = 8$ and 2); 7.75 (d, 1H, $J = 8$); 7.57 (d, 1H, $J = 8$); 7.53 (dd, 1H, $J = 8$ and 2); 7.49 (d, 1H, $J = 8$); 7.42 (dd, 1H, $J = 8$ and 2); 2.61 (s, 1H); 1.71 (s, 6H). ^{13}C ($CDCl_3$): 157.2; 152.8; 151.3; 125.1; 124.8; 123.1; 122.2; 121.5; 111.9; 111.8; 110.5; 86.7; 73.3; 30.0.

1-Bromo-2,2-dimethyl-2*H*-benzofuro[2,3-*g*][*1*]benzopyran (36). Cyclization according to the general procedure, after chromatography over silica gel (cyclohexane), gave a quantitative yield of compound **36** as an oil. LC/MS (ES) *m*/*z* 327; 330 $[M-H]^-$. ^1H ($CDCl_3$): 8.50 (d, 1H, $J = 8$); 7.52 (d, 1H, $J = 8$); 7.48 (dd, 1H, $J = 8$ and 2); 7.37 (dd, 1H, $J = 8$ and 2); 6.45 (d, 1H, $J = 9$); 5.80 (d, 1H, $J = 9$); 1.40 (s, 6H). ^{13}C ($CDCl_3$): 157.2; 151.3; 146.3; 133.1; 127.8; 125.0; 124.1; 122.9; 122.1; 122.8; 111.7; 107.9; 104.0; 75.8; 27.7.

2,2-Dimethyl-2*H*- benzofuro[2,3-*g*][*1*]benzopyran (37). To a solution of **36** (0.15 g, 0.46 mmol) in ethanol (50 mL) were added 10% Pd/C (0.02 g, 0.02 mmol) and ammonium formate (0.03 g, 0.46 mmol). The reaction mixture was heated at reflux during 5 h. After chromatography over silica gel (cyclohexane/dichloromethane, 98:2), compound **37** (0.045 g, 40%) was obtained as a white amorphous solid. LC/MS (ES) *m*/*z* 251 $[M+H]^+$. ^1H ($CDCl_3$): 7.88 (d, 1H, $J = 8$); 7.55 (d, 1H, $J = 8$); 7.45 (dd, 1H, $J = 8$ and 2); 7.36 (s, 1H); 7.33 (dd, 1H, $J = 8$ and 2); 7.19 (s, 1H); 6.49 (d, 1H, $J = 8$); 5.78 (d, 1H, $J = 8$); 1.51 (s, 6H). ^{13}C ($CDCl_3$): 157.3; 151.5; 149.3; 132.5; 217.2; 127.1; 127.7; 122.8; 122.5; 212.8; 120.9; 112.1; 111.5; 108.2; 74.5; 28.0.

2,2-Dimethyl-3,4-dihydro-2*H*-benzofuro[2,3-*g*][*1*]benzopyran (38). To a solution of **36** (0.40 g, 1.22 mmol) in ethanol (50 mL) were added 10% Pd/C (0.06 g, 0.06 mmol) and ammonium formate (0.15 g, 2.44 mmol). The reaction mixture was heated at reflux during 5 h. After filtration, the filtrate was concentrated to dryness and purified by chromatography over silica gel (cyclohexane/dichloromethane, 98:2) yielding compound **38** (0.11 g, 35%) as a white amorphous solid. LC/MS (ES) *m/z* 253 $[M+H]^+$. ^1H ($CDCl_3$): 7.90 (d, 1H, $J = 8$); 7.52 (d, 1H, $J = 8$); 7.42 (dd, 1H, $J = 8$ and 2); 7.35 (s, 1H); 7.29 (dd, 1H, $J = 8$ and 2); 7.29 (s, 1H); 3.00 (t, 2H, $J = 6$); 1.91 (t, 2H, $J = 6$); 1.45 (s, 3H); 1.40 (s, 3H). ^{13}C ($CDCl_3$): 157.0 (C6a); 150.9; 150.4; 127.1; 124.9; 123.7; 122.6; 121.6; 120.8; 111.8; 111.4; 108.2; 74.5; 33.2; 27.3; 23.8.

1-(8-Hydroxydibenzo[*b*,*d*]furan-2-yl)ethanone (42). The 2,8-diacyldibenzofuran (**39**)[30] (1 g, 3.96 mmol) and trifluoroacetic acid (0.89 mL, 11.9 mmol) were dissolved in dichloromethane (100 ml). Compound 3-chloroperbenzoperoxoic acid (0.89 g, 3.96 mmol) was added and the solution was stirred for 3 days. The resulting solution was diluted in dichloromethane, washed with 1 N sodium hydroxide, brine and dried over sodium sulfate. The residue, containing a mixture of unreacted **39**, monoacetate **40**, and bisacetate **41**, was heated to reflux for 15 min in a 1 N methanolate solution (50 mL). This was concentrated to dryness and dispersed in water. The aqueous phase was washed with dichloromethane (to remove compound **39**) and made acidic using 2 N hydrochloric acid. This was then extracted with ethyl acetate. The organic layer was washed with brine to neutrality, dried over sodium sulfate and concentrated to dryness. The residue was purified by chromatography over silica gel (dichloromethane/ethanol, 99.5:0.5) yielding compounds **42** (0.22 g, 24%) and 2,8-dihydroxydibenzofuran (**43**) (0.14 g, 19%; previously described[30]). Compound **42**: LC/MS (ES) *m/z* 225 $[M-H]^-$. ^1H (DMSO-d_6): 9.57 (s, 1H); 8.74 (d, 1H, $J = 1.6$); 8.09 (dd, 1H, $J = 1.1$ and 8.6); 7.72 (d, 1H, $J = 8.6$); 7.55 (m, 2H); 7.00 (dd, 1H, $J = 2.6$ and 8.8); 2.7 (s, 3H). ^{13}C (DMSO-d_6): 198.0; 159.6; 154.8; 150.8; 133.0; 128.5; 125.0; 124.8; 123.2; 117.3; 115.0; 112.5; 107.3; 27.7.

1-(8-(2-methylbut-3-yn-2-yloxy)dibenzo[*b*,*d*]furan-2-yl)-ethanone (44). Using the above-mentioned protocol, the propargylic ether**44** was obtained from compound **42** after a purification by chromatography over silica gel (cyclohexane/dichloromethane, 95:5) as a pale oil (0.04 g, 14.3%). (LC/MS): not ionized by electrospray. ^1H ($CDCl_3$): 8.57 (1H, $J = 2$); 8.11 (dd, 1H, $J = 2$ and 8); 7.86 (d, 1H, $J = 2$); 7.60 (d, 1H, $J = 8$); 7.50 (1H, $J = 8$); 7.49 (1H, $J = 2$ and 8); 2.73 (s, 1H); 2.64 (s, 1H); 1.70 (s, 6H). ^{13}C (400 MHz, $CDCl_3$): 196.3; 158.6; 152.2; 150.5; 131.3; 127.0; 123.8; 122.9; 122.2; 120.6; 113.1; 110.7; 110.5; 85.0; 73.3; 72.4; 28.6; 25.7.

1-(3,3-Dimethylbut-3*H*-benzofuro[3,2-*f*]chromen-10-yl)-ethanone (45). Compound **45** was obtained from ether **44** using the general procedure described above after purification by chromatography over silica gel (cyclohexane/dichloromethane, 50:50) as white crystals (0.006 g, 38%). LC/MS (ES) *m/z* 293 $[M+H]^+$. ^1H ($CDCl_3$): 8.64 (1H, $J = 2$); 8.11 (dd, 1H, $J = 2$ and 8); 7.60 (d, 1H, $J = 8$); 7.34 (d, 1H, $J = 8$); 7.15 (1H, $J = 9$); 6.98 (1H, $J = 8$); 5.91 (d, 1H, $J = 9$); 2.73 (s, 1H); 1.70 (s, 6H). ^{13}C ($CDCl_3$): 197.6; 160.1; 152.1; 149.3; 133.1; 132.7; 128.1; 125.3; 123.3; 119.5; 117.3; 116.5; 111.9; 111.7; 76.3; 27.7; 27.2.

5-Bromo-3,3-dimethyl-2,3-dihydro-1*H*-benzofuro[3,2-*f*]chromene (46). Chromane **2** (0.32 g, 1.3 mmol) was dissolved in ethanol (40 mL). *N*-bromosuccinimide (0.27 g, 1.57 mmol) was added and the solution was stirred for an hour. This was concentrated to dryness and the residue was purified by chromatography over silica gel (cyclohexane/dichloromethane, 97:3) to yield compound **46** (0.38 g, 88%). LC/MS (ES) *m/z* 331, 333 $[M+H]^+$. ^1H ($CDCl_3$): 7.96 (d, 1H, $J = 7.7$); 7.66 (s, 1H); 7.56 (d, 1H, $J = 8.1$); 7.47 (m, 1H); 7.35 (m, 1H); 3.28 (t, 2H, $J = 6.8$); 2.01 (t, 2H, $J = 6.8$); 1.45 (s, 6H). ^{13}C ($CDCl_3$): 157.1; 150.0; 146.6; 127.2; 125.1; 123.0; 122.5; 122.3; 114.3; 112.1; 111.3; 75.5; 32.6; 26.9; 21.8.

Acylation of compound, preparation of 1-(3,3-dimethyl-2,3-dihydro-*1H*-benzofuro[*3,2-f*]chromen-5-yl)ethanone (47) and **1-(2-isopropyl-1,2-dihydro-benzo[*d*]benzo[*1,2-b; 4,3-b′*]difuran-4-yl)-ethanone (48)**. In a flask protected from moisture by a calcium chloride guard, acetic anhydride (0.11 mL, 1.2 mmol) was dissolved in dichloromethane (30 mL) and dry aluminium chloride (0.16 g, 1.2 mmol) was added. This was stirred for 10 min and compound **2** (0.2 g, 0.79 mmol) was added. The resulting red solution was stirred for 24 h and concentrated to dryness. The residue was purified by chromatography over silica gel eluting with dichloromethane-cyclohexane, 3:7, to yield a fraction containing a mixture of **47** and **48** (0.12 g). This was recrystallized in methanol twice to provide pure compound **48** (0.01 g, 4.3% yield). LC/MS (ES) *m/z* 295 $[M+H]^+$. ^1H ($CDCl_3$): 7.92 (s, 1 H); 7.88 (d, 1H, $J = 7.5$); 7.53 (m, 2H); 7.38 (m, 1H); 3.64 (dd, 1H, $J = 9.2$ and 16.0); 3.33 (dd, 1H, $J = 8.9$ and 16.0); 2.74 (s, 3H); 2.12 (sept, 1H, $J = 6.7$); 1.16 (d, 3H, $J = 6.7$); 1.11 (d, 3H, $J = 6.7$). ^{13}C ($CDCl_3$): 197.1; 158.7; 157.2; 151.3; 129.1; 126.2; 123.5; 123.1; 122.6; 121.1; 119.4; 112.4; 109.6; 90.5; 33.9; 32.1; 31.6; 18.6; 18.4.

The mother liquors were concentrated to dryness and the residue subjected to a second chromatography over silica gel using a finer type (20–45 μm). This allowed the isolation of a small portion of quasi-pure compound **47** (0.01 g; 4.3% yield; 95% pure). LC/MS (ES) *m/z* 295 $[M+H]^+$. ^1H ($CDCl_3$): 7.99 (d, 1H, $J = 7.6$); 7.83 (s, 1H); 7.53 (m, 1H); 7.51 (m, 1H); 7.36 (m, 1H); 3.29 (t, 2H, $J = 6.9$); 2.72 (s, 3H); 2.04 (t, 2H, $J = 6.9$); 1.48 (s, 6H). ^{13}C ($CDCl_3$): 200.3; 158.2; 149.9; 149.8; 128.2; 128.0; 126.8; 124.7; 122.3; 123.0; 116.5; 112.3; 110.9; 75.2; 32.7; 32.2; 27.1; 21.5.

4.1. MIC determinations

MICs were determined using the new Microdilution Resazurin Assay (MRA).[33] 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 for a week. Drug stock solutions were prepared in dimethylsulfoxide (DMSO) at concentration of 50 mg/mL and frozen until used. The inocula were prepared from *M. tuberculosis* H37Rv and *M. Smegmatis* mc^2155 strains grown in Dubos medium supplemented with 10% ADC enrichment (Difco). One microlitre of twofold serial dilutions of each drug were prepared in 100 μL of Dubos medium directly in 96-well plates at concentrations from 500 to 0.9 μg/mL. 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 48 h for *M. smegmatis* 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 reduction of resazurin and therefore bacterial growth. The MIC was defined as the lowest drug concentration that prevented this colour change. The optical density of each well was measured at 530–630 nm using a multi-well plate reader. The 95% inhibition concentrations for the most active compounds (MIC_{95} <15 μg/mL) were determined by curve fitting.

4.2. Cytotoxicity evaluation

VERO cell lines were maintained in DMEM 4.5 g/L Glutamax I with PYR.NA (Invitrogen) supplemented with 10% foetal calf serum (FCS), penicillin–streptomycin solution 1% at 37 °C in air with 5% CO_2. Proliferating cells were seeded in 96-well microtitration plates at a density of 6×10^3 cells/ml, which were further incubated for 24 h at 37 °C under 5% CO_2 in air before each assay. Various concentrations of solutions of compounds in 1% DMSO were added and then incubated for 48 h under the above conditions. At the end of incubation, 20 μL of dimethylthiazolyldiphenyl tetrazolium bromide solution (MTT, Sigma) (5 mg/mL) was then added to each well and further incubated for 4 h at 37 °C to allow the formation of formazan. The crystals of formazan were then dissolved with 100 μL of a freshly prepared solution of sodium dodecyl sulfate (SDS) 10% (15 mL) and HCl 1 N (150 μL). The optical density of each well was measured at 595 nm using a multi-well plate reader. The 50% inhibition concentration was then determined by curve fitting.

Acknowledgments

We thank Sanofi-Aventis as well as Pfizer for very generous donations of scientific equipment. This work received the financial support of the Institut Pasteur (GPH-5, DVPI) and the European Community (LHSP-CT-2005-018923).

References and notes

1. Prado, S.; Ledeit, H.; Michel, S.; Koch, M.; Darbord, J. C.; Cole, S. T.; Tillequin, F.; Brodin, P. *Bioorg. Med. Chem.* **2006**, *14*, 5423.
2. Fukushima, T.; Tanaka, M.; Gohara, M. Patent JP 2000 63,376 *see Chem. Abstr.* **2000**, 132, 179673r.
3. Nicolaou, K. C.; Roecker, A. J.; Barluenga, S.; Pfefferkorn, J. A.; Cao, G. Q. *ChemBioChem* **2001**, *2*, 460.
4. Babu, K. S.; Raju, B. C.; Praveen, B.; Kishore, K. H.; Murty, U. S.; Rao, J. M. *Heterocyclic Commun.* **2003**, *9*, 519.
5. Brown, C. W.; Shengquan, L.; Klucik, J.; Berlin, K. D.; Brennan, P. J.; Kaur, D.; Benbrook, D. M. *J. Med. Chem.* **2004**, *47*, 1008.
6. Sawada, T.; Aono, M.; Asakawa, S.; Ito, A.; Awano, K. *J. Antibiot.* **2000**, *53*, 959.
7. Krishna, D.; Venkataramana, D. *Drug Metabol. Dispos.* **1992**, *20*, 909.
8. Ingolfsdottir, K.; Chung, G. A. C.; Skulason, V. G.; Gissurarson, S. R.; Vilhelmsdottir, M. *Eur. J. Pharm. Sci.* **1998**, *6*, 141.
9. Mayer, M.; O'Neill, M. A.; Murray, K. E.; Santos-Magalhaes, N. S.; Carneiro-Leao, A. M.; Thompson, A. M.; Appleyard, V. C. *Anticancer Drugs* **2005**, *16*, 805.
10. Sanchez, W.; Maple, J. T.; Burgart, L. J.; Kamath, P. S. *Mayo Clin. Proc.* **2006**, *81*, 541.
11. Shibata, S.; Natori, S.; Kawakami, T.; Okanon, M.; Tsuchimoto, Y. *Chem. Pharm. Bull.* **1954**, *2*, 45.
12. Natori, S.; Ito, M. *Chem. Pharm. Bull.* **1957**, *5*, 553.
13. Natori, S. *Chem. Pharm. Bull.* **1957**, *5*, 539.
14. Natori, S. *Chem. Pharm. Bull.* **1957**, *5*, 548.
15. Bell, D.; Davies, M. R.; Green, G.; Mann, I. S. *Synthesis* **1995**, 707.
16. Godfrey, J. D.; Mueller, R. H.; Sedergran, T. C.; Soundararajan, N.; Colandrea, V. J. *Tetrahedron Lett.* **1994**, *35*, 6405.
17. Iwai, I.; Ide, J. *Chem. Pharm. Bull.* **1963**, *10*, 926.
18. Iwai, I.; Ide, J. *Chem. Pharm. Bull.* **1963**, *11*, 1042.
19. Hlubucek, J.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1971**, *24*, 2347.
20. Dintzner, M. R.; Lyons, T. W.; Akroush, M. H.; Wucka, P.; Rzepka, A. T. *Synlett* **2005**, 785.
21. Dintzner, M. R.; McClelland, K. M.; Morse, K. M.; Akroush, M. H. *Synlett* **2004**, 2028.
22. Wang, Q.; She, X.; Ren, X.; Ma, J.; Pann, X. *Tetrahedron Assym.* **2004**, *15*, 29.
23. Trécourt, F.; Breton, G.; Bonnet, V.; Mongin, F.; Marsais, F.; Quéguiner, G. *Tetrahedron* **2000**, *56*, 1349.
24. Zav'yalov, S. I.; Kondrat'eva, G. V.; Gunar, G. I. *Izv. Akad. Nauk. SSSR, Ser. Khim* **1964**, 2086, See *Chem. Abstr.* 62, 7713g.
25. Domschke, G. *Z. Chem.* **1964**, *4*, 29, See *Chem. Abstr.* 60, 7973f.
26. Shvedov, V. I.; Grinev, A. N. *Zh. Organ. Khim.* **1965**, *1*, 1125, See *Chem. Abstr.* 63, 13245.
27. Domschke, G. *J. Prakt. Chem.* **1966**, *32*, 144.
28. Allen, G. R., Jr. *Org. React.* **1973**, *20*, 337.
29. Li, J.; Cook, J. M. Nenitzescu indole synthesis. In *Name Reactions in Heterocyclic Chemistry (Comprehensive Name Reactions);* Li, J. J. Ed., 2004; pp 145–153.
30. Albrecht, W. L.; Fleming, R. W.; Horgan, S. W.; Mayer, G. D. *J. Med. Chem.* **1977**, *20*, 365.
31. Srebnik, M.; Mechoulam, R. *Tetrahedron* **1983**, *40*, 3839.
32. Youn, S. W.; Eom, J. I. *J. Org. Chem.* **2006**, *71*, 6705.
33. Palomino, J.-C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. *Antimicrob. Agents Chemother.* **2002**, *46*, 2720.
34. Choi, T. A.; Czerwonka, R.; Frohner, W.; Krahl, M. P.; Reddy, K. R.; Franzblau, S. G.; Knolker, H. J. *ChemMedChem* **2006**, *1*, 812.
35. Rajabi, L.; Courreges, C.; Montoya, J.; Aguilera, R. J.; Primm, T. P. *Lett. Appl. Microbiol.* **2005**, *40*, 212.

36. Dwivedi, N.; Tewari, N.; Tiwari, V. K.; Chaturvedi, V.; Manju, Y. K.; Srivastava, A.; Giakwad, A.; Sinha, S.; Tripathi, R. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4526.
37. Grigg, R.; Kongkathip, N.; Kongkathip, B.; Luangkamin, S.; Dondas, H. A. *Tetrahedron* **2001**, *57*, 7965.
38. Huang, Q.; Fazio, A.; Dai, G.; Campo, M. A.; Larock, R. C. *J. Am. Chem. Soc.* **2004**, *126*, 7460.
39. Campeau, L. C.; Parisien, M.; Jean, A.; Fagnou, K. *J. Am. Chem. Soc.* **2006**, *128*, 581.
40. Prado, S.; Janin, Y. L.; Bost, P. E. *J. Heterocyclic Chem.* **2006**, *43*, 1605.