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Benzofuro[3,2-*f*][1]benzopyrans: A new class of antitubercular agents

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Abstract—Alkylation of 2-hydroxydibenzofuran with 3-chloro-3-methyl-1-butyne, followed by Claisen rearrangement, gave access to 3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]-benzopyran. Several derivatives modified at the pyran 1,2-double bond were prepared, including the corresponding dihydro compound and (±)-*cis*-diol, which was converted into diacetate and cyclic carbonate upon acylation. Both 3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran and 1,2-dihydro-3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran displayed significant activities when tested against *Mycobacterium tuberculosis* H37Rv and *Beijing* strains, with MIC_{99} in the range of 1–10 μg/ml. Further biological studies demonstrated good activities against drug-resistant mycobacterial strains. These compounds appear as promising specific antitubercular agents, since they exhibited neither significant cytotoxicity against mammal cells, nor effect on the growth of various bacteria and fungi.

1. Introduction

Tuberculosis (TB) remains a leading infectious disease accounting for more than 2 million deaths and 8 million new cases each year in the world.[1] Though widely prescribed, the live vaccine BCG fails to prevent pulmonary tuberculosis in adults and to confer any protection in African countries and India partly due to environmental mycobacterial interference.[2] The human immunodeficiency virus pandemic and the decline of the health-care systems also contribute to this increase in tuberculosis incidence in less-developed countries and in the former Soviet Union. The current chemotherapy was established more than 30 years ago and its outcome strongly depends on following the drug regimen for 6 months or more.[3] Thus, the resurgence of the disease, inappropriate regimens or inadequate compliance to the directly observed therapy short course (DOTS) further entail the rise of multiple drug-resistant strains of tuberculosis (MDR-TB). When not restrained, MDR-TB spreads in the population with the same trend as the drug-sensitive disease.[4,5] Very few new treatments have been developed since the introduction of rifampicin (RIF) in 1971, even though there has been significant advances in drug development technologies.[6,7] For instance, the nitroimidazopyran derivative (PA-824) and a diarylquinoline derivative (R207910) are currently undergoing preclinical and clinical trials, respectively.[8,9] Thus, there is a pressing need for the development of a new class of antimycobacterial agents in order to fight resistance and shorten the duration of therapy.

Natural products play a major role in drug discovery, as a unique source of original structures, which can provide models for future drug design. In the field of antitubercular agents, the lichen dibenzofuran derived secondary metabolite usnic acid (**1**) has been shown to display an interesting activity,[10–12] but its weak potency did not permit its further development as an antimycobacterial drug.[13,14] Simple dibenzofurans also occur in higher plants, where they often act as antifungal phytoalexins.[15–17] In this context, it appeared interesting to synthesize potential antitubercular natural product-like hybrids,[18,19] including a dibenzofuran basic core and

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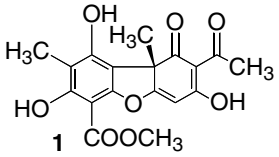
an additional fused dimethylpyran ring. Indeed, the fused dimethylpyran system, which arises in nature from the condensation of a phenol with an active isoprene unit, appears as a 'privileged substructure'[20] present in numerous bioactive compounds exemplified by the antifungal benzopyran methylripariochromene A (**2**),[21] the antibacterial pyranoflavanone 5-methyllupinifoliol (**3**),[22] and the antitumor acridone alkaloid acronycine (**4**).[23] Additionally, the double bond of the fused dimethylpyran ring constitutes a latent site of chemical diversity, which can be easily modified by oxidation or reduction, permitting facile variation of molecular lipophilicity in the course of structure–activity relationship studies. Angular fusion of the pyran ring onto the dibenzofuran system, encountered in most bioactive polycyclic structures,[20,23] appeared particularly desirable, leading to consider 3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (**5**) as a key synthetic target.



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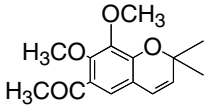

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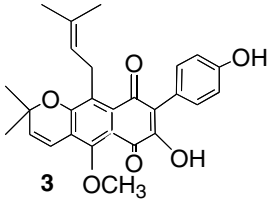

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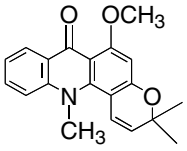

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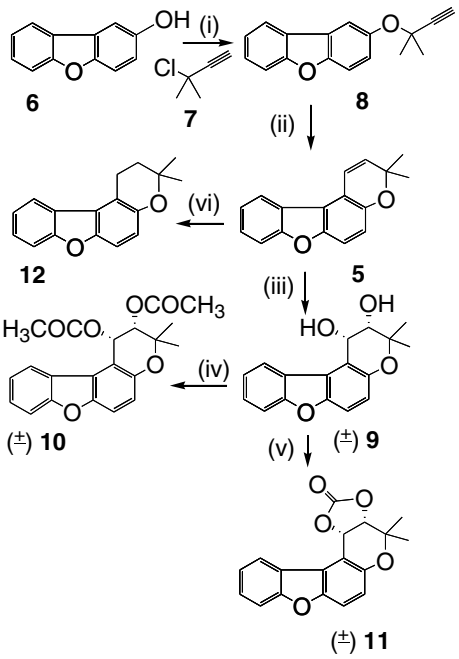
2. Chemistry

Based on previous synthetic experiments in the acronycine series, the synthesis of **5** was envisioned through Claisen rearrangement of an intermediate dimethylpropargyl ether.[24] Thus, treatment of commercially available 2-hydroxydibenzofuran (**6**) with 3-chloro-3-methylbut-1-yne (**7**)[25] in alkaline medium gave the required 2-(1,1-dimethyl-propargyloxy)-dibenzofuran (**8**). Thermal cyclization, performed by heating **8** under reflux for 12 h in 1,3-dichlorobenzene, afforded the desired 3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (**5**) in 46% yield. The (±)-*cis*-diol **9** was conveniently obtained in 89% yield by catalytic osmium tetroxide oxidation of **5**, using *N*-methylmorpholine *N*-oxide to regenerate the oxidative agent.[26] Treatment of **9** with excess acetic anhydride gave the corresponding diacetate **10**. In a similar way, acylation of the diol **9** with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux afforded almost quantitatively the cyclic carbonate **11**.[27] Finally, hydrogenation of **5** in ethanol, using Pd–C (10%) as catalyst, led to 1,2-dihydro-3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (**12**) in almost quantitative yield (Scheme 1).



Scheme 1. Syntheses of 3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran derivatives **5** and **9**–**12**. Reagents and conditions: (i) DBU/$CuCl_2$, $2H_2O/CH_3CN$/5 h/rt; (ii) 1,3-dichlorobenzene/Rx/12 h; (iii) OsO_4/NMMO/*t*-BuOH–THF–H_2O_2/48 h/rt; (iv) Ac_2O/C_5H_5N/48 h/rt; (v) *N*,*N*′-carbonyldiimidazole/2-butanone/Rx/2 h; (vi) H_2/Pd–C (10%)/18 h/rt.

Table 1. In vitro activity against *Mycobacterium smegmatis* mc^2155 and *Mycobacterium tuberculosis* H37Rv of pyranodibenzofuran derivatives

Compound	Clog *P*	MIC_{99} (μg/ml) on *M. smegmatis*	MIC_{99} (μg/ml) on *M. tuberculosis*[a]
8	5.3	12	NI
5	5.5	5	5
9	3.4	30	60
12	5.6	1	5
10	4.8	NI	NI
11	3.3	NI	NI
INH	−0.7	13	0.1

MIC is the minimal concentration (μg/ml) of a compound resulting in a 99% decrease in the number of colony-forming units of mycobacterial cultures compared to control (DMSO only). NI, no inhibition observed at the highest concentration tested (1 mg/ml). Data are representative of three independent experiments and standard deviation is within 10% of the MIC_{99}. Log *P* (Clog *P*) represents hydrophobicity and was determined using the ChemDrawPro software.

[a] MIC of compounds **9** and **15** were also determined by Bactec 460 (Becton Dickinson) method as previously described[31] and are 10 and 5 μg, respectively.

3. Biology

The biological activities of novel pyranodibenzofuran derivatives **5**–**12** were investigated on a wide range of bacteria. The antimycobacterial activity was screened on the fast growing saprophyte *Mycobacterium smegmatis* mc^2155 and on the virulent strain *Mycobacterium tuberculosis* H37Rv for all synthesized compounds. This was achieved using a liquid culture, which was then plated on solid media to determine the exact number of colony-forming units (CFU). The minimal inhibitory concentration (MIC_{99}) is defined as the amount of compound required for >99% inhibition of bacterial growth

Table 2. In vitro Activity against a whole range of *Mycobacteria* of compounds **8**, **5**, and **12**

Mycobacterial strains of *M. tuberculosis* complex	MIC_{99} (μg/ml)			
	8	**5**	**12**	INH
M. tuberculosis				
H37Rv	>500	5	5	0.1
Beijing	>500	2	10	0.1
H37Ra	>500	10	10	1
INH resistant	ND	5	1	5
RIF resistant	ND	5	1	0.1
M. bovis BCG	>500	10	10	1
M. microti	>500	1	ND	0.1

ND, not determined.

Table 3. In vitro activity of compounds **5** and **12** against Gram-positive and Gram-negative bacteria and fungi

Strains		MIC_{99} (μg/ml)	
		5	**12**
Gram-negative	*E. coli*	>600	>500
	K. oxytoca	>600	ND
	P. aeruginosa	>500	>500
Gram-positive	*S. aureus*	>100	ND
	E. faecalis	>500	>500
	P. pneumoniae	>500	ND
Actinomycetes	*Strp. subspec.*	> 500	ND
	C. jeikeium	>150	> 150
	N. asteroides	15	15
Fungus	*C. albicans*	>600	ND

Ampicillin (10 μg/ml) was used as positive control.

Table 4. Cytotoxicity of compounds **5** and **12**

Cell type	**5**		**12**	
	IC_{50} (μg/ml)	SI	IC_{50} (μg/ml)	SI
VERO cells	80	15	100	20
Macrophages	80	15	>100	>20

SI = IC_{50}/MIC_{99}.

(Table 1). Results of the most promising compounds on various strains of the *M. tuberculosis* complex, including the more virulent *Beijing* strain and other drug-resistant strains, are shown in Table 2. To further assess the specificity of this new series, compounds were also screened for activity against a large range of bacteria and fungi as well as for cytotoxicity (Tables 3 and 4).

4. Results and discussion

From the results shown in Table 1, an inhibitory activity on *M. smegmatis* mc^2155 of the 2,2-dimethyl-propargylic ether **8** (MIC_{99} of 12 μg/ml), equivalent in this test to the control antibiotic isoniazid (INH), was found. Of even more interest, the 3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]-benzopyran **5** and its reduced analogue **12** with an MIC_{99} of 5 μg/ml are actually more potent than INH. The diol **9** exhibited a lower level of activity ranking between 30 and 60 μg/ml. Diesters **10** and **11**, bearing bulky substituents on the pyran ring, were devoid of activity at concentrations up to 1 mg/ml.

The promising results obtained in the course of this screen led us to investigate the effect of this series of compounds on different *M. tuberculosis* strains such as H37Rv. The results shown in Table 2 demonstrate that compound **5** as well as the dihydro derivative **12** have a good inhibition activity on the growth of *M. tuberculosis* with an MIC_{99} (less than 5 μg/ml) that is comparable to that of the first-line anti-TB drugs.[3] In contrast, no effect on *M. tuberculosis* was observed for compound **8**, even at doses as high as 500 μg/ml. Functionalization aiming to improve the solubility in biocompatible solvents, such as a dihydroxylation of the pyranodibenzofuran ring and subsequent conversion into esters (compounds **9**–**11**), resulted in a complete loss of inhibition of *M. tuberculosis* growth. Therefore, inhibitory activity of this series of compounds correlates with an increased value of the lipophilicity index (C log *P*), in good agreement with previous observations for major antitubercular drugs.[28] Altogether these results show a good correlation between activity on *M. smegmatis* and on *M. tuberculosis*. In addition, similar MIC_{99} values were obtained for compounds **5** and **12** on other mycobacterial strains such as the attenuated strains *M. tuberculosis* H37Ra, *Mycobacterium microti* as well as BCG (Table 2). The effect of compounds **5** and **12** on drug-resistant strains was measured, to further investigate their potential value when combined with existing regimens. Similar MIC_{99} values were found for both compounds on rifampicin- and isoniazid-resistant strains and on sensitive H37Rv strain (Table 2).

The general antibacterial screening shown in Table 3 demonstrates that compounds **5** and **12** have no effect on Gram-positive and Gram-negative bacteria at the maximum concentration tested. However, a significant inhibitory effect was observed on the *Actinomycete Nocardia asteroides*, whose cell wall structure is closely related to that of *Mycobacterium*.[29] Conversely, no inhibition was observed on *Streptomyces* and *Corynebacterium* species. Among the plausible explanations for these results, a specific biological target within *Mycobacteria* for these novel derivatives appears as an attractive hypothesis. Preliminary results (data not shown) suggest that compounds **5** and **12** inhibit the biosynthesis of mycolic acids present in *Mycobacteria* and *Nocardia* cell walls.

The results displayed in Table 4 show that a low cytotoxicity (IC_{50} >50 μg/ml) was observed in mammalian VERO cells for compounds **5** and **12**. In addition, toxicity was assessed on macrophages and was found to be higher than 80 μg/ml.

5. Conclusion

Benzofuro[3,2-*f*][1]benzopyrans, readily synthesized from commercially available 2-hydroxydibenzofuran, appear as a new class of inhibitors active against resistant strains of *M. tuberculosis*, which might be able to shorten the current tuberculosis treatment. Both compounds **5** and **12** meet the criteria required for further tuberculosis drug development, with MICs measured on *M. tuberculosis* below 6.25 μg/ml and Selectivity

Indexes above 10.[30] Their potent and remarkable specificity strongly suggests a novel biological target restricted to *Mycobacteria*. Current studies are focused toward its identification.

6. Experimental

6.1. Chemistry

Melting points were determined on a hot stage Reichert microscope and are uncorrected. Mass spectra (MS) were recorded with a Nermag R-10-10C spectrophotometer using desorption-chemical ionization (90 eV) (DCI-MS; reagent gas: NH_3). UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR Spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets on a Perkin-Elmer 257 instrument. ^1H NMR spectra were run at 400 MHz and ^{13}C NMR spectra at 75 MHz, using Bruker Avance-400 and AC-300 spectrometers, respectively. The experiments were conducted at 20 °C. The chemical shifts are reported in parts per million (δ), relative to solvent peaks as internal standards (δ: $CDCl_3$: 7.27 (^1H), 77.0 (^{13}C); DMSO-d_6: 2.49 (^1H), 40.6 (^{13}C)). The coupling constant (J) values are given in hertz. When necessary, the signals were unambiguously assigned by 2D NMR techniques: ^1H–^1H COSY, ^1H–^1H NOESY, ^{13}C–^1H HMQC, and ^{13}C–^1H HMBC. These experiments were performed using standard Bruker microprograms. Elemental analyses were determined by the Microanalyse Service of the Pierre and Marie Curie University, Paris. Results were in agreement with calculated values ±0.4%. Column chromatography was performed on Merck silica gel 35–70 μM or 20–45 μM, with an overpressure of 300 mbars. 2-Hydroxydibenzofuran (**6**) was purchased from Aldrich Chemical Company (Steinheim, Germany). C log *P* values were determined by the predictive software ChemDrawPro 7.0.

6.2. 2-(1,1-Dimethyl-propargyloxy)-dibenzofuran (8)

DBU (1.68 ml, 16.2 mmol) and copper chloride dihydrate (3.0 mg, 0.20 mmol) were added to a solution of 2-hydroxydibenzofuran (**6**) (3.00 g, 16.2 mmol) in anhydrous acetonitrile (6 ml) at 0 °C under argon. 3-Chloro-3-methylbut-1-yne (**7**) (2.50 ml, 16.2 mmol) was added at 0 °C. After stirring for 5 h at room temperature, the mixture was evaporated under reduced pressure and the residue was partitioned between water (20 ml) and toluene (20 ml). The organic layer was washed with 1 M aqueous HCl (20 ml), 1 M aqueous NaOH (20 ml), 1 M aqueous $NaHCO_3$ (20 ml), and brine (20 ml), dried over $MgSO_4$, and evaporated under reduced pressure to give **8** as a pale yellow oil (3.40 g, 85%). IR (KBr) ν_{max} (cm^{-1}): 2992, 2918, 2358, 2334, 1593, 1481, 1444, 1175, 1137, 747. UV λ (nm) ($\log\varepsilon$) (MeOH): 211 (4.06), 251 (3.59), 287 (3.49).^1H NMR (400 MHz, $CDCl_3$): δ 7.98 (dd, 1H, J = 8, 2 Hz, H-9), 7.81 (d, 1H, J = 2 Hz, H-1), 7,60 (d, 1H, J = 9 Hz, H-4), 7.50 (m, 2H, H-6, H-7), 7.35 (m, 2H, H-3, H-8), 2.60 (s, *CH*≡C), 1.53 (s, 6H, 2× CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.2 (C-2), 153.1 (C-5a), 151.4 (C-4a), 127.5 (C-7), 124.8 (C-9a), 124.7 (2C, C-9, C-8), 121.1 (C-6), 114.6 (C-4), 112.1 (C-1), 111.9 (C-9b), 111.7 (C-3), 86.6 (HC≡*C*), 74.3 (*HC*≡C), 73.82 (*C*(CH_3)$_3$), 30.0 (2× CH_3). MS (DCI/NH_3) *m*/*z*: 251 $[MH]^+$. Anal. ($C_{17}H_{14}O_2$): C, H.

6.3. 3,3-Dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (5)

A solution of **8** (3.65 g 14.6 mmol) in 1,3-dichlorobenzene (100 ml) was heated under reflux for 12 h. After removal of the solvent under reduced pressure, purification by chromatography over silica gel (solvent: 98:2 (cyclohexane/dichloromethane, followed by crystallization in methanol gave **5** as white needles (1.67 g, 46%), mp: 101 °C. IR (KBr) ν_{max} (cm^{-1}): 2975, 1424, 1146, 810. UV λ (nm) ($\log\varepsilon$) (MeOH): 276 (4.37), 295 (4.67), 305 (4.73).^1H NMR (400 MHz, $CDCl_3$): δ 8.01 (dd, 1H, J = 8, 2 Hz, H-11), 7.57 (dd, 1H, J = 8, 1 Hz, H-8), 7,45 (td, 1H, J = 8, 2 Hz, H-9), 7.35 (m, 2H, H-6, H-10), 7.11 (d, 1H, J = 9 Hz, H-1), 6.95 (d, 1H, J = 9 Hz, H-5), 5.85 (d, 1H, J = 9 Hz, H-2), 1.53 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.1 (C-7a), 151.2 (C-6a), 148.6 (C-4a), 132.2 (C-2), 126.9 (C-9), 124.5 (C-11a), 123.0 (C-11), 122.6 (C-10), 120.65 (C-11b), 119.4 (C-1), 116.1 (C-5), 115.8 (C-11c), 111.9 (C-8), 111.2 (C-6), 75.8 (C-3), 27.7 (CH_3), 27.5 (CH_3). MS (DCI/NH_3) *m*/*z*: 251 $[MH]^+$. Anal. ($C_{17}H_{14}O_2$): C, H.

6.4. (±)-*cis*-1,2-Dihydro-1,2-dihydroxy-3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (9)

Pyranodibenzofuran **5** (0.05 g, 1.98 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol, 1.25 ml) and 4-methylmorpholine *N*-oxide monohydrate (0.23 g, 1.98 mmol) in 10:3:1 *t*-BuOH/THF/H_2O (8.9 ml). The reaction mixture was stirred at room temperature for 48 h. After addition of saturated aqueous solution of sodium bisulfite (20 ml), the mixture was stirred for 1 h and then extracted with dichloromethane (5× 20 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography (solvent: 99:1–95:5 cyclohexane/ethyl acetate) to give **9** (0.50 g, 89%) as a white amorphous solid. IR (KBr) ν_{max} (cm^{-1}): 3331, 2929, 1429, 1038, 930. UV λ (nm) ($\log\varepsilon$) (MeOH): 254 (3.84), 290 (3.90), 314 (3.43), 325 (3.45). ^1H NMR (400 MHz, $CDCl_3$): δ 8.40 (dd, 1H, J = 8, 1 Hz, H-11), 7.56 (dd, 1H, J = 8, 1 Hz, H-8), 7.46 (td, 1H, J = 8, 1 Hz, H-9), 7.44 (d, 1H, J = 9 Hz, H-6), 7.36 (td, 1H, J = 8, 1 Hz, H-10), 6.97 (d, 1H, J = 9 Hz, H-5), 5.28 (dd, 1H, J = 9, 5 Hz, H-1), 3.92 (dd, 1H, J = 9, 5 Hz, H-2), 2.96 (d, 1H, J = 9 Hz, D_2O exch., OH-1), 2.44 (d, 1H, J = 9 Hz, D_2O exch., OH-2), 1.53 (s, 3H, CH_3), 1.39 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.9 (C-7a), 151.1 (C-6a), 147.9 (C-4a), 127.0 (C-9), 124,5 (C-11a), 122.4 (C-11), 122.2 (C-10), 119.8 (C-11b), 117.1 (C-5), 116.1 (C-11c), 112.7 (C-8), 111.4 (C-6), 77.6 (C-3), 71.8 (C-2), 64.4 (C-1), 23.4 (CH_3), 23.1 (CH_3). MS (DCI/NH_3) *m*/*z*: 285 $[MH]^+$. Anal. ($C_{17}H_{16}O_4$): C, H.

6.5. (±)-*cis*-1,2-Diacetoxy-1,2-dihydro-3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (10)

Acetic anhydride (4 ml) was added to a solution of **9** (0.05 g, 0.17 mmol) in dry pyridine (4 ml). After stirring at room temperature for two days, the mixture was poured onto cold water (10 ml). The precipitate was filtered, washed with water (2× 5 ml), and dried in vacuo over P_2O_5 to give **10** (0.045 g, 70%) as white crystals, mp: 189 °C (from CH_2Cl_2/MeOH). IR (KBr) ν_{max} (cm^{-1}): 2986, 1734, 1374, 1240, 1188, 1093, 808. UV λ (nm) ($\log\varepsilon$) (MeOH): 253 (3.38), 291 (3.35), 326 (3.03). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (dd, 1H, J = 8, 1 Hz, H-11), 7.53 (dd, 1H, J = 8, 1 Hz, H-8), 7.50 (d, 1H, J = 9 Hz, H-6), 7.44 (dd, 1H, J = 8, 1 Hz, H-9), 7.28 (td, 1H, J = 8, 1 Hz, H-10), 7.01 (d, 1H, J = 9 Hz, H-5), 6.71 (d, 1H, J = 5 Hz, H-1), 5.45 (d, 1H, J = 5 Hz, H-2), 2.10 (s, 3H, $OCOCH_3$), 2.08 (s, 3H, $OCOCH_3$), 1.49 (s, 3H, CH_3), 1.45 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9 (O–*CO*–CH_3), 156.8 (O–*CO*–CH_3), 157.0 (C-7a), 151.0 (C-6a), 149.5 (C-4a), 127.2 (C-9), 123.5 (C-11a), 123.0 (C-11), 121.9 (C-10), 123.0 (C-11b), 117.5 (C-5), 114.0 (C-8), 112.0 (C-6), 110.4 (C-11c), 75.6 (C-3), 71.7 (C-1), 64.5 (C-2), 21.5 (O–CO–*CH*$_3$), 20.6 (O–CO–*CH*$_3$), 25.9 (2C, 2× CH_3). MS (DCI/NH_3) *m*/*z* = 369 $[MH]^+$. Anal. ($C_{21}H_{20}O_6$): C, H.

6.6. (±)-*cis*-1,2-Dihydro-3,3-dimethyl-1,3-dioxolo[4,5-*h*]benzofuro[3,2-*f*][1]benzopyran (11)

To a solution of **9** (0.03 g, 0.1 mmol) in 2-butanone (2 ml), *N*,*N*′-carbonyldiimidazole (0.08 g, 0.50 mmol) was added. The reaction mixture was refluxed for 2 h under argon. After cooling, 5% aqueous $NaHCO_3$ (20 ml) was added. The mixture was extracted with EtOAc (3× 20 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (solvent: dichloromethane, then 99:1–96:4 dichloromethane/acetone) to give **11** (0.01 g, 33%) as white needles, mp: 215 °C (from MeOH). IR (KBr) ν_{max} (cm^{-1}): 2990, 2933, 1802, 1626, 1175, 1029, 745. UV λ (nm) ($\log\varepsilon$) (MeOH): 219 (3.95), 253 (3.23), 262 (2.65), 291 (3.32), 312 (2.99). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (dd, 1H, J = 8, 1 Hz, H-11), 7.59 (dd, 1H, J = 8, 1 Hz, H-8), 7.55 (d, 1H, J = 9 Hz, H-6), 7.51 (td, 1H, J = 8, 1 Hz, H-9), 7.39 (td, 1H, J = 8, 1 Hz, H-10), 7.02 (d, 1H, J = 9 Hz, H-5), 6.23 (d, 1H, J = 8 Hz, H-1), 4.88 (d, 1H, J = 8 Hz, H-2), 1.60 (s, 3H, CH_3), 1.40 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.1 (O–CO–O), 153.8 (C-7a), 151.3 (C-6a), 148.5 (C-4a), 127.8 (C-9), 123.5 (C-11a), 123.0 (C-11), 122.9 (C-11b), 122.7 (C-10), 117.6 (C-5), 114.7 (C-8), 111.9 (C-6), 109.7 (C-11c), 78.8 (C-3), 74.9 (C-2), 70.4 (C-1), 22.8 (CH_3), 22.5 (CH_3). MS (DCI/NH_3) *m*/*z* = 311 $[MH]^+$. Anal. ($C_{18}H_{14}O_5$): C, H.

6.7. 1,2-Dihydro-3,3-dimethyl-3*H*benzofuro[3,2-*f*][1]benzopyran (12)

To a solution of **5** (0.060 g 0.25 mmol) in ethanol (15 ml) was added Pd–C (10%, 7 mg). The resulting mixture was flushed under H_2 (1 atm) at room temperature for 18 h. After filtration, the solution was concentrated to dryness to give compound **12** (0.059 g, 94%) as white needles. mp: 124 °C (from MeOH). IR (KBr) ν_{max} (cm^{-1}): 2971, 2920, 1638, 1420, 1266, 1168. UV λ (nm) ($\log\varepsilon$) (MeOH): 217 (3.68), 223 (3.77), 290 (3.87), 328 (3.85). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, 1H, J = 8 Hz, H11), 7.58 (d, 1H, J = 8 Hz, H8), 7,48 (t, 1H, J = 8 Hz, H9), 7.37 (m, 2H, H6, H10), 6.98 (d, 1H, J = 9 Hz, H5), 3.28 (t, 2H, J = 4 Hz, H1), 2.02 (t, 2H, J = 4 Hz, H2), 1.42 (s, 6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.1 (C7a), 150.6 (C6a), 149.62 (C4a), 126.7 (C9), 125.59 (C11a), 122.7 (2C, C11, C10), 122.5 (C11b), 123.0 (C11), 117.5 (C5), 115.7 (C11c), 111.9 (C8), 111.2 (C6), 74.1 (C3), 32.7 (C2), 26.9 (2C, 2CH_3), 21.4 (C1). MS (DCI/NH_3) *m*/*z*: = 253 $[MH]^+$. Anal. ($C_{17}H_{16}O_2$): C, H.

6.8. Methods for cytotoxicity evaluation

Raw cell line was maintained in RPMI 1640 medium without L-GLU supplemented with 10% Fetal calf serum (FCS), penicillin–streptomycin solution 1% at 37 °C in air with 5% CO_2. VERO cell lines were maintained in D-MEM without L-GLU supplemented with 10% Fetal 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 5× 10^5 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 **9** and **15** (10 μl/well) in 1.25% dimethyldisulfoxide (DMSO) were added and then incubated for 24 h under the above conditions. At the end of incubation, 20 μl of dimethylthiazolyl diphenyl tetrazolium bromide solution (MTT) (7.5 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 an acidic solution (sodium dodecyl sulfate (SDS) 10% and HCl 10 mM). The optical density of each well was measured at 595 nm using a multi-well plate reader. The values given are the average means of six replicates. The 50% inhibition concentration was determined by curve fitting.

6.9. Bacterial strains

Escherichia coli (ATCC11775), *Corynebacterium jeikeium* (ATCC43734), *Klebsiella oxytoca* (29C1104), *Nocardia asteroides* (ATCC19247), *Staphylococcus aureus* (18C2204), and *Pneumococcus pneumoniae* (15C5004) strains are from the Collection de l'Institut Pasteur, Paris, France. *Streptomyces coelicolor* (M15), *albus*, *avidinii*, and *avermitilis* (ATCC31267), *Pseudomonas aeruginosa* (13C3104), and *Enterococcus faecalis* (14C1104) strains were kindly given by Philippe Mazodier and Laurent Marsollier. *M. smegmatis* mc^2155, *M. tuberculosis* H37Rv (Institut Pasteur, Paris, France), GC1237 Beijing strains (Carlos Martin), *Mycobacterium bovis* BCG (Pasteur), and *M. microti* (MYC-942272). MDR-TB strains (2003/1762 and 2004/0499) were kindly given by Dr. Véronique Vincent (Laboratoire de Référence des Mycobactéries, Institut Pasteur, Paris, France).

6.10. Methods for evaluation of inhibitory activities of compounds against bacteria

Effect of the compounds was assessed on *C. jeikeium*, *E. coli*, *E. faecalis*, *K. oxytoca*, *N. asteroides*, *S. aureus*, *P. aeruginosa*, and *Streptomyces* in vitro growth in liquid or solid medium. Compounds were solubilized in dimethylsulfoxide (DMSO) and 100 μl of the appropriate dilution was added to a 5 ml appropriate culture with predetermined bacterium inoculums. Quantification of the bacteria was carried out by plating on appropriate agar serial 5-fold dilutions in medium, and colony-forming units (CFUs) were ascertained after 24–48 h according to the strains. Effects on *Candida albicans* were kindly measured by Pr. Darbord (Faculté des Sciences Pharmaceutiques et Biologiques, Paris).

6.11. Methods for evaluation of inhibitory activities of compounds against *Mycobacteria*

Effect of the compounds was assessed on mycobacterial strain's in vitro growth in liquid medium. Compounds were solubilized in DMSO and 100 μl of the appropriate dilution was added to a 5 ml culture with predetermined mycobacterium inocula. For the assays on the rapid growing mycobacterium *M. smegmatis*, optical density of the culture in Luria–Bertani medium at 595 nm was measured after two-day growth at 37 °C. The effect on *M. tuberculosis* strains and MDR-TB was monitored after three-week growth in Middlebrook 7H9 broth at 37 °C. Quantification of the *Mycobacteria* was carried out by plating on 7H11 agar serial 5-fold dilutions in medium, and colony-forming units (CFUs) ascertained after two-week growth. The minimal inhibitory concentration (MIC) given is the concentration of compound for which 99% of the bacterial growth is inhibited.

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References and notes

1. Frieden, T. R.; Sterling, T. R.; Munsiff, S. S.; Watt, C. J.; Dye, C. *Lancet* **2003**, *362*, 887.
2. Fine, P. E. *Lancet* **1995**, *346*, 1339.
3. Blumberg, H. M.; Burman, W. J.; Chaisson, R. E.; Daley, C. L.; Etkind, S. C.; Friedman, L. N.; Fujiwara, P.; Grzemska, M.; Hopewell, P. C.; Iseman, M. D.; Jasmer, R. M.; Koppaka, V.; Menzies, R. I.; O'Brien, R. J.; Reves, R. R.; Reichman, L. B.; Simone, P. M.; Starke, J. R.; Vernon, A. A. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 603.
4. Di Perri, G.; Bonora, S. J. *Antimicrob. Chemother.* **2004**, *54*, 593.
5. Cohen, T.; Murray, M. *Nat. Med.* **2004**, *10*, 1117.
6. Ballell, L.; Field, R. A.; Duncan, K.; Young, R. J. *Antimicrob. Agents Chemother.* **2005**, *49*, 2153.
7. Tripathi, R. P.; Tewari, N.; Dwivedi, N.; Tiwari, V. K. *Med. Res. Rev.* **2005**, *25*, 93.
8. Andries, K.; Verhasselt, P.; Guillemont, J.; Gohlmann, H. W.; Neefs, J. M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; de Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. *Science* **2005**, *307*, 223.
9. Stover, C. K.; Warrener, P.; VanDevanter, D. R.; Sherman, D. R.; Arain, T. M.; Langhorne, M. H.; Anderson, S. W.; Towell, J. A.; Yuan, Y.; McMurray, D. N.; Kreiswirth, B. N.; Barry, C. E.; Baker, W. R. *Nature* **2000**, *405*, 962.
10. Stoll, A. *Experientia* **1947**, *3*, 111.
11. Klosa, J. *Pharmazie* **1953**, *8*, 435.
12. Savisz, V. P.; Litvinov, M. A.; Moissejeva, E. N. *Planta Med.* **1960**, *8*, 191.
13. Müller, K. *Appl. Microbiol. Biotechnol.* **2001**, *56*, 9.
14. Ingólfsdóttir, K.; Chung, G. A. C.; Skúlason, V. G.; Gissurarson, S. R.; Vihelmdóttir, M. *Eur. J. Pharm. Sci.* **1998**, *6*, 141.
15. Kokubun, T.; Harborne, J. B.; Eagles, J.; Waterman, P. G. *Phytochemistry* **1995**, *39*, 1033.
16. Kokubun, T.; Harborne, J. B.; Eagles, J.; Waterman, P. G. *Phytochemistry* **1995**, *39*, 1039.
17. Carotenuto, A.; Fattorusso, E.; Lanzotti, V.; Magno, S. *Eur. J. Org. Chem.* **1998**, *4*, 661.
18. Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996.
19. Mehta, G.; Singh, V. *Chem. Soc. Rev.* **2002**, *31*, 324.
20. Nicolaou, K. C.; Pfefferkorn, J. A.; Roecker, A. J.; Cao, G.-Q.; Barluenga, S.; Mitchell, H. J. *J. Am. Chem. Soc.* **2000**, *122*, 9939.
21. Bandara, B. M. R.; Hewage, C. H.; Karunaratne, V.; Wannigama, G. P.; Adikaram, N. K. B. *Phytochemistry* **1992**, *31*, 1983.
22. Rao, E. V.; Sridhar, P.; Rao, B. V. L. N.; Ellaiah, P. *Phytochemistry* **1999**, *50*, 1417.
23. Tillequin, F.; Michel, S.; Skaltsounis, A.-L.. In Pelletier, S. W., Ed.; Alkaloids: Chemical and Biological Perspectives; Elsevier: New York, 1998; Vol. 12, pp 1–102.
24. Costes, N.; Le Deit, H.; Michel, S.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Renard, P.; Léonce, S.; Guilbaud, N.; Kraus-Berthier, L.; Pierré, A.; Atassi, Gh. *J. Med. Chem.* **2000**, *43*, 2395.
25. Hennion, G. F.; Boiselle, A. P. *J. Org. Chem.* **1961**, *26*, 725.
26. Rheenen, V. V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *23*, 1973.
27. Elomri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pierré, A.; Guilbaud, N.; Léonce, S.; Kraus-Berthier, L.; Rolland, Y.; Atassi, Gh. *J. Med. Chem.* **1996**, *39*, 4762.
28. Barry, C. E., 3rd; Slayden, R. A.; Sampson, A. E.; Lee, R. E. *Biochem. Pharmacol.* **2000**, *59*, 221.
29. Barry, C. E., 3rd; Lee, R. E.; Mdluli, K.; Sampson, A. E.; Schroeder, B. G.; Slayden, R. A.; Yuan, Y. *Prog. Lipid Res.* **1998**, *37*, 143.
30. http://www.taacf.org. Tuberculosis Antimycobacterial Acquisition and Coordinating Facility (TAACF).
31. Rastogi, N.; Bauriaud, R. M.; Bourgoin, A.; Carbonnelle, B.; Chippaux, C.; Gevaudan, M. J.; Goh, K. S.; Moinard, D.; Roos, P. *Antimicrob. Agents Chemother.* **1995**, *39*, 638.