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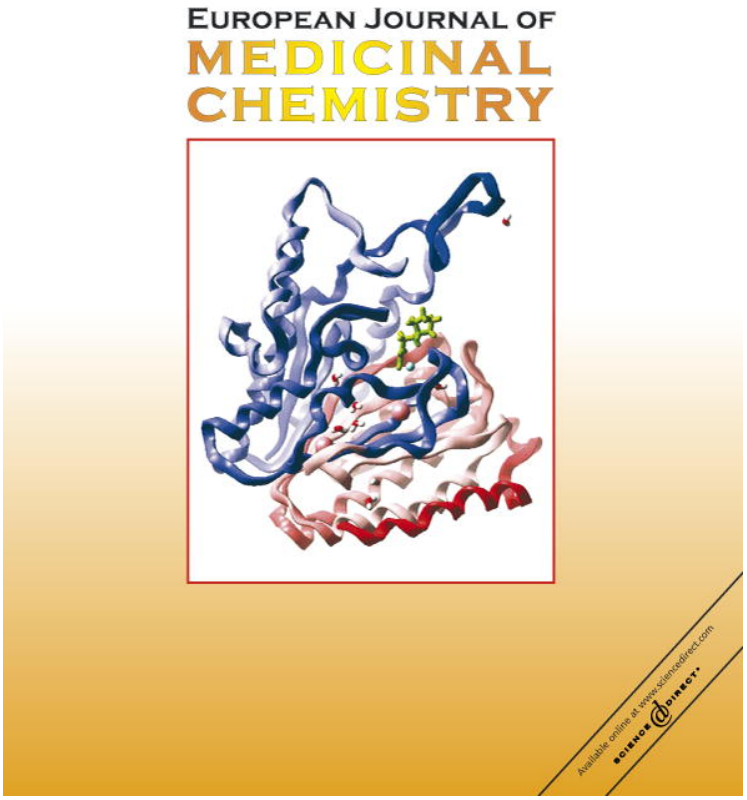

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Short communication

Synthesis and antimicrobial activity of pyranobenzoquinones related to the pyranonaphthoquinone antibiotics

S.H. Lagorio[a], D.A. Bianchi[a], E.G. Sutich[b], T.S. Kaufman[a,*]

[a] *Instituto de Química Orgánica de Síntesis (CONICET-UNR) and Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina*
[b] *Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina*

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Abstract

The synthesis and antimicrobial activity of isochromane-type analogs of the pyranonaphthoquinone antibiotics are reported. Isochromane derivatives with (**17a**, **b**) and without (**22a**, **b**) a C-4 hydroxyl moiety and their corresponding quinones (**19a** and **23**), were prepared. Both quinones exhibited antimicrobial activity against *Staphylococcus aureus*, *Bacillus atrophaeus* and *Streptococcus agalactiae*, while the related isochromanes were inactive. The results suggest that the quinone moiety is important for biological activity while the C-4 hydroxyl may not be essential.
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Keywords: Pyranobenzoquinones; Pyranonaphthoquinone analogs; Antimicrobial activity

1. Introduction

The pyranonaphthoquinones are a complex family of polysubstituted natural products which have been isolated from bacteria, fungi, aphids and higher plants [1]. They carry a characteristic 1*H*-naphtho[2,3-*c*]pyran-5,10-dione framework (**1a**).

Many members of this group display the basic skeleton (psychorubin, **1b**), while compounds like eleutherin (**2**) exhibit little functionalization (Fig. 1). Key structural features of others include the presence of additional heterocyclic rings such as in mederrhodin B (**3**), marticin (**4**) and the spiroketalic griseusin B (**5**). However, compounds like frenolicin B (**6a**) and kalafungin (**6b**, the enantiomer of nanaomycin D) contain an additional γ-lactone ring fused to the dihydropyran moiety, while others, exemplified by deoxyfrenolycin (**7a**) and nanaomycin A (**7b**), exhibit only an acetic acid side chain bound to C-3. In addition, the arizonins A2 (**8a**) and B2 (**8b**) and granaticinic acid (**9**) are examples of γ-hydroxyacids, the open forms of γ-lactones; aphid pigments such as protoaphin *fb* (**10**) are functionalized dimers of the common structural unit, which display hydroxyl groups at C-4 and C-4′.

The pyranonaphthoquinones have been found to possess an ample array of interesting properties including antibiotic, antiparasitic, antiviral, antitumor and anti-platelet aggregatory [2–4]. Due to their wide therapeutic potential and structural diversity [5–11], synthetic organic chemists have been continuously interested in these compounds during the last quarter of century.

Surprisingly, however, little is known about the structure of their pharmacophore. Nanaomycin D and related compounds have been proposed to act as bioreductive alkylating agents [12–15], and a series of experiments with several polycyclic pyranonaphthoquinones of the naphthocyclinone family, suggested that the minimal structure **11** (Fig. 2) is required for activity [16,17]. On the other hand, Omura et al. [18] have shown that deoxyfrenolicin (**7a**) is less active than frenolicin B (**6a**) against molds and yeasts, this being suggestive that their oxygen functionality attached to C-4 may also be linked to bioactivity [18,19].

Interestingly, benzoisochromane-5,8-diones of general structure **12** (Fig. 2) have been prepared by the oxa-

* Corresponding author.
E-mail address: kaufman@iquios.gov.ar (T.S. Kaufman).

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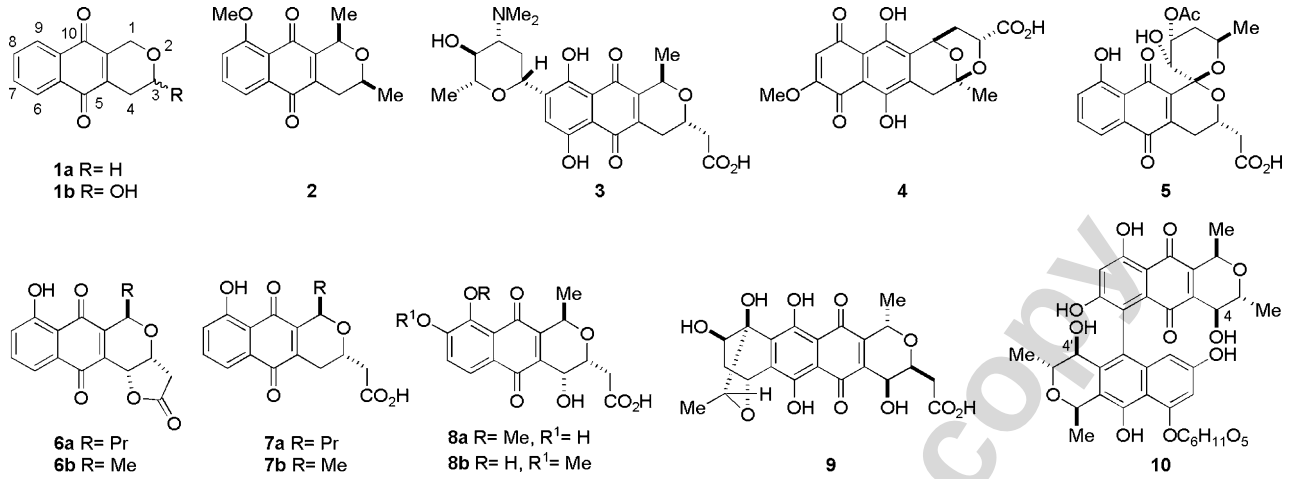


Fig. 1. Examples of structural diversity among the pyranonaphthoquinones.

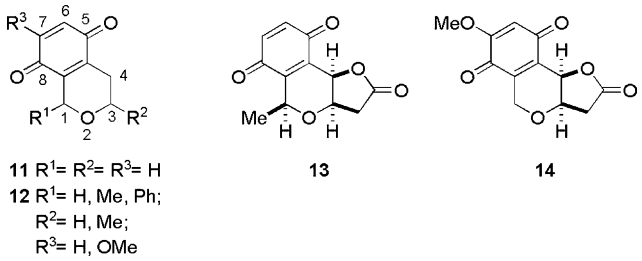


Fig. 2. Synthetic pyranobenzoquinones and the proposed pharmacophore of the pyranonaphthoquinone antibiotics.

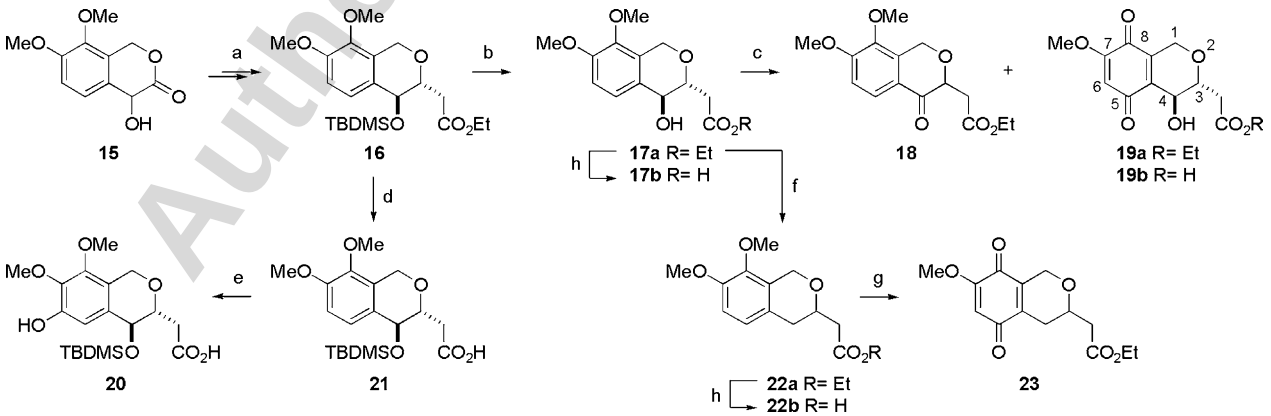
Pictet-Spengler condensation of 1,4-dimethoxy-β-phenethyl alcohols with aldehydes followed by oxidative demethylation of the resultant benzopyrans [20], a Michael addition/cyclization sequence between 2-(1-hydroxyalkyl)-1,4-benzoquinones and imines or enamines [21], and by other means [22], and tricyclic lactones such as **13** (Fig. 2) have also been synthesized [23], but surprisingly their activity has not been tested.

Recently, we reported the elaboration of 3,3a-dihydro-5*H*-furo[3,2-*c*]isochromene-2,6,9(9bH)-trione **14** from commercially available 2,3-dimethoxytoluene through the intermediacy of lactone **15** (Scheme 1) [24–26], employing an acid-catalyzed lactonization and a Wittig-oxa-Michael sequence for isochromane ring formation and functionalization. We also demonstrated that trione **14**, which is the key structural element of a number of biologically important pyranonaphthoquinones, exhibited antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* ATCC 29213.

In continuation of our interest in the elaboration of simplified partial analogs of the pyranonaphthoquinone antibiotics and in the recognition of the minimum structural features required for their biological activity, herein we focus on the role of the alcoholic functionality associated to the C-4 methinic carbon attached to the quinone moiety and wish to report the syntheses of isochromane ester and acid derivatives **17a**, **b** and **22a**, **b** (Scheme 1) and their corresponding quinone esters **19a** and **23** (Scheme 1) from the known α-hydroxy-lactone **15**, as well as results of their antimicrobial activities against *S. aureus*, *Bacillus atrophaeus* and *Streptococcus agalactiae*.

2. Synthesis

According to Scheme 1, synthesis of γ-hydroxyester **17a** from lactone **15** was carried out in approximately 60% overall yield, through the intermediacy of silyl ether **16**. The structure of **16** was assigned after a careful NMR spectral study of the



Scheme 1. *Reagents and conditions*: a: See Ref. [24]; b: TBAF, THF, RT, 1 h (90%); c: AgO, 6 N HNO_3, 7 min. (**18**, 34%; **19a**, 32%); d: LiOH, THF/MeOH, 0 °C, 1 h (70%); e: AgO, 6 N HNO_3, 7 min (16%); f: ZnI_2, $NaCNBH_3$, $ClCH_2CH_2Cl$,))), RT, 4 h (69%); g: AgO, 6 N HNO_3, 7 min (32%); h: LiOH, THF/MeOH, 0 °C, 1 h (94%).

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latter, NMR analyses (including selective decoupling and NOE experiments) of a series of related compounds including **14** and **17a** [24], and literature precedents [27]. Next, silver(II) oxide mediated oxidation [28–31] of **17a** provided quinone **19a** in 32% yield, as a pale yellowish oil, together with 34% of the related ketone **18**, the structure of which was corroborated by comparison with the product resulting from the PCC/Al_2O_3 oxidation of **17a**. Yields of **19a** did not improve despite changing reaction parameters such as time, temperature and work-up conditions. Submission of **17a** to reaction with zinc iodide and sodium cyanoborohydride under ultrasound promotion, provided 69% of the required product **22a** [32], demonstrating the excellent selectivity of this reagent combination for the deoxygenation of benzylic alcohols [33,34]. Finally, the sequence was completed with the oxidation of **22a** with silver (II) oxide, which furnished quinone **23** in 32% yield.

Acids **17b** and **22b** were conveniently obtained by LiOH-mediated hydrolysis; surprisingly, however, they were unable to withstand the strong acidic conditions of the silver(II) oxidation step and completely decomposed, perhaps by pyranic oxygen protonation, followed by loss of CO_2, concomitant heterocyclic ring opening and further oxidation. On the other hand, mild hydrolysis of **16** furnished 70% of acid **21**, which upon oxidation with the silver(II) oxide-nitric acid reagent gave 16% of phenol **20**, together with recovered starting material. This outcome was interpreted as being a result of steric hindrance by the bulky silyl ether moiety and precluded further exploration of this strategy as a means of accessing quinone-acid **19b** through quinone formation and subsequent desilylation. Therefore, synthesis of **19b** by this or an alternative route was not pursued.

3. Results and discussion

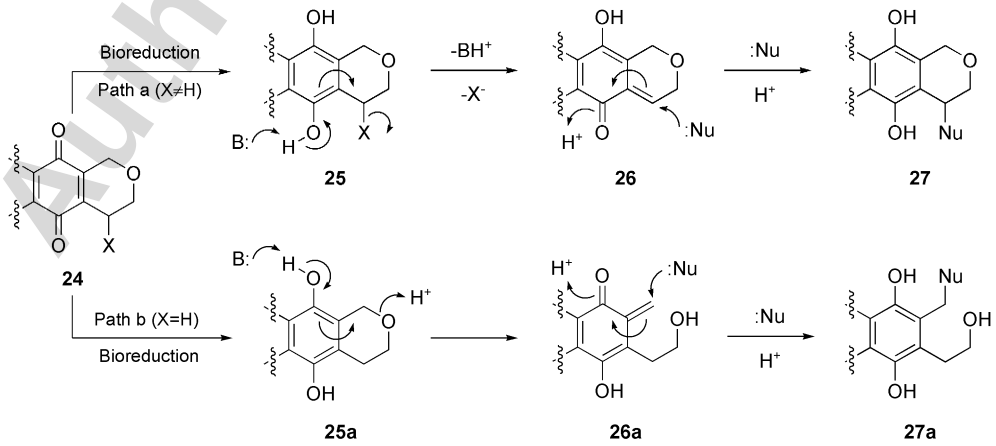
Isochromanes **17a,b** and **22a,b** and quinones **19a** and **23** were submitted to the antimicrobial disk assay on agar plates against *S. aureus* ATCC 29213, *B. atrophaeus* (*B. subtilis* spp. *Niger* ATCC 9372) and *S. agalactiae*. In this test, the isochromanes were inactive, while the quinones displayed moderate inhibition (Table 1). For the sake of comparison, a commercial disk containing 10 μg of ampicillin was included, giving inhibition zones of 26, 44 and 40 mm against *S. aureus*, *B. atrophaeus* and *S. agalactiae*, respectively. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of the quinones were 256 and 12.8 μg ml^{-1}, respectively.

Simple 1,4-benzoquinone derivatives are widespread in nature and many have shown to possess antibacterial, antifungal and related activities [35–41]. Interestingly, in many cases small structural changes have demonstrated to profoundly affect their potency. Moore et al. [12–15] have proposed (Scheme 2) that quinones having a leaving group (X) attached to a methylene or methine carbon directly associated to the quinone moiety, such as in **24** can undergo reduction, with further elimination (**25→26**) under the action of a base (Path a). This transformation furnishes the reactive Michael acceptor **26**, which might ultimately be attacked by suitable nucleophiles, undergoing alkylation (**26→27**). Thus, the pyranonaphthoquinones can act as alkylating agents upon bioreduction, in a mode of action greatly resembling that of the antitumor drug mitomycin C [12].

Table 1
Antimicrobial activity of quinones **19a** and **23**

	S. aureus			*B. atrophaeus*			*S. agalactiae*		
Compounds[a]	10 μg	30 μg	70 μg	10 μg	30 μg	70 μg	10 μg	30 μg	70 μg
19a[b]	7	12	15	–	–	18	–	–	13
23[b]	11	12	13	12	15	17	9	15	17
Ampicillin[b]	26	ND	ND	44	ND	ND	40	ND	ND

[a] Disks (diameter = 6 mm) were charged with 10, 30 and 70 μg of the quinones.
[b] Diameters of the inhibition zones are in mm. ND = not determined.



Scheme 2. Speculative proposed mechanism for the bioreductive alkylation [12].

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The observed results suggest that the quinone moiety seems to be required for antimicrobial activity; however, a C-4 hydroxyl group might not be essential for the antimicrobial activity. Therefore, it is likely that alternative bioreductive alkylation mechanisms may be operative among pyranonaphthoquinones devoid of leaving groups, like a free alcohol or a γ-butyrolactone associated to their respective quinone moieties, such as natural compounds **1**–**3** and **7a**, **b**. Among these compounds, a mechanism which involves the opening of the heterocyclic ring of **24**, might be proposed. Path b of Scheme 2 illustrates the base-promoted formation of the alternate Michael acceptor **26a** upon bioreductive alkylation of **24** (X = H) to intermediate **25a**. As in Path a, attack to **26a** by suitable external nucleophiles could provide the alkylated product **27a**.

In conclusion, we have synthesized two isochromane derivatives with and without a C-4 hydroxyl group and their corresponding pyranobenzoquinones, as simplified analogs of the pyranonaphthoquinone antibiotics, and have tested their antimicrobial activity against *S. aureus*, *B. atrophaeus* and *S. agalactiae*. The aromatic analogs were inactive, while both quinones displayed growth inhibition haloes and similar MIC and MBC values. These results suggest that this hydroxyl group may not be essential for the antibiotic activity.

4. Experimental

4.1. Chemistry

The melting point (uncorrected) was taken on an Ernst Leitz Wetzlar model 350 hot-stage microscope. Fourier transform infrared (FT-IR) spectra were determined with a Shimadzu Prestige 21 infrared spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were acquired with a Bruker AC200-E spectrometer (200.13 MHz for ^{1}H), employing $CDCl_3$ as solvent; chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard (* = assignments may be exchanged) and coupling constants (*J*) are expressed in Hertz. High-resolution mass spectral data were obtained from the Kent Mass Spectrometry Unit 1 (Kent, UK). The homogeneity of the compounds was monitored by ascending thin layer chromatography, on silicagel-coated aluminum plates (Merck, art. 5554). All new compounds gave single spots, when run in different hexanes/EtOAc solvent systems. Detection of the spots was done by exposure of the plates to UV light (254 nm), followed by spraying with ethanolic *p*-anisaldehyde/sulfuric acid reagent and careful heating for better selectivity. Flash column chromatographies were carried out with silica gel 60 H and eluted with hexanes/EtOAc employing gradient techniques.

4.1.1. (±)-trans-2-(4-Hydroxy-7,8-dimethoxy-3,4-dihydro-1H-isochromen-3-yl)acetic acid ethyl ester (**17a**)

A 1 M solution of Bu_4NF (0.25 ml) in THF was added to a solution of silyl ether **16** (232 mg, 0.57 mmol) in THF (2.2 ml). After stirring 30 min at room temperature, the solvent was evaporated under reduced pressure and the residue was purified by chromatography furnishing **17a** (153 mg, 91%) as a pale yellow oil. IR (neat, ν): 3446, 2941, 2816, 1732, 1611, 1498, 1323, 1283, 1230, 1123, 1074, 971, 804 and 719 cm^{-1}; ^{1}H NMR (*δ*): 1.34 (t, 3 H, *J* = 7.0, CH_2CH_3), 2.60 (dd, 1 H, *J* = 8.6 and 15.5, CH_2CO_2Et), 2.95 (dd, 1 H, *J* = 3.7 and 15.5, CH_2CO_2Et), 3.40 (bs, 1 H, $w_{1/2}$ = 20, OH), 3.84 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.93 (ddd, 1 H, *J* = 3.7, 4.6 and 8.6, H-3), 4.20 (q, 2 H, *J* = 7.0, OCH_2CH_3), 4.41 (bd, 1 H, *J* = 4.6, H-4), 4.74 (d, 1 H, *J* = 15.9, $ArCH_2O$), 4.94 (d, 1 H, *J* = 15.9, $ArCH_2O$), 6.89 (d, 1 H, *J* = 8.6, H-6) and 7.27 (d, 1 H, *J* = 8.6, H-5); ^{13}C NMR (δ): 14.01 (OCH_2CH_3), 37.95 (CH_2CO_2Et), 55.66 (OCH_3-7), 59.95 (OCH_3-8), 60.61 (OCH_2CH_3), 64.25 (C-1), 68.68 (C-3), 76.20 (C-4), 111.44 (C-6), 122.04 (C-5), 128.63* (C-8a), 129.54* (C-4a), 143.57 (C-7), 151.19 (C-8) and 171.46 (CH_2CO_2Et). HRMS- Observed *m/z* = 296.12628; $C_{15}H_{20}O_6$ requires *m/z* = 296.12599.

4.1.2. (±)-trans-2-(4-Hydroxy-7-methoxy-5,8-dioxo-3,4,5,8-tetrahydro-1H-isochromen-3-yl)-acetic acid ethyl ester (**19a**)

AgO (160 mg, 1.28 mmol) was added to a stirred solution of **17a** (95 mg, 0.32 mmol) in dioxane (6 ml). After 2 min at room temperature, 6 M HNO_3 (0.8 ml) was added dropwise. The reaction was quenched with $CHCl_3$–H_2O (3:1, 10 ml) after stirring 7 min. The reaction products were extracted with $CHCl_3$ and the combined organic extracts were washed with H_2O (5 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography, yielding **18** (32 mg, 34%) as an oil. IR (neat, ν): 3500, 2926, 2852, 1733, 1684, 1593, 1531, 1495, 1361, 1284, 1177 and 1042 cm^{-1}; ^{1}H NMR (*δ*): 1.27 (t, 3 H, *J* = 7.1, CH_2CH_3), 2.56 (dd, 1 H, *J* = 7.8 and 16.4, CH_2CO_2Et), 3.12 (dd, 1 H, *J* = 7.8 and 16.4, CH_2CO_2Et), 3.84 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 4.19 (q, 2 H, *J* = 7.1, OCH_2CH_3), 4.54 (dd, 1 H, *J* = 3.2 and 7.8 H-3), 4.82 (d, 1 H, *J* = 15.8, $ArCH_2O$), 5.16 (d, 1 H, *J* = 15.8, $ArCH_2O$), 6.95 (d, 1H, *J* = 8.6, H-6) and 7.83 (d, 1H, *J* = 8.6, H-5); ^{13}C NMR (*δ*): 14.02 (OCH_2CH_3), 35.76 (CH_2CO_2Et), 55.79 (OCH_3-7), 60.43 (OCH_3-8), 60.70 (OCH_2CH_3), 63.36 ($ArCH_2O$), 75.25 (C-3), 111.19 (C-6), 122.91* (C-8a), 124.01* (C-4a), 143.20 (C-7), 156.98 (C-8), 170.77 (CH_2CO_2Et) and 192.86 (C-4); HRMS- Observed *m/z* = 294.11019; $C_{15}H_{18}O_6$ requires *m/z* = 294.11034. Increasing solvent polarity furnished **19a** as a yellowish oil. IR (neat, ν): 3509, 2978, 2935, 1733, 1659, 1630, 1608, 1495, 1370, 1286, 1233, 1177 and 1037 cm^{-1}; ^{1}H NMR (*δ*): 1.28 (t, 3 H, *J* = 7.0, CH_2CH_3), 2.56 (dd, 1 H, *J* = 8.8 and 15.8, CH_2CO_2Et), 2.93 (dd, 1 H, *J* = 3.3 and 15.8, CH_2CO_2Et), 3.82 (bs, 1 H, OH), 3.84 (s, 3 H, OCH_3), 3.91 (ddd, 1 H, *J* = 3.3, 4.2 and 8.8, H-3), 4.19 (q, 2 H, *J* = 7.0, OCH_2CH_3), 4.49 (d, 1 H, *J* = 19.3, $ArCH_2O$), 4.55 (bd, 1 H, *J* = 4.2, H-4), 4.64 (d, 1 H, *J* = 19.3, $ArCH_2O$) and 5.90 (s, 1 H, H-6); ^{13}C NMR (*δ*): 14.06 (OCH_2CH_3), 37.46 (CH_2CO_2Et), 56.35 (OCH_3), 60.63 (OCH_2CH_3), 63.07 (C-1), 65.00 (C-4), 74.54 (C-3), 107.36 (C-6), 139.06* (C-8a), 139.14* (C-4a), 158.73 (C-7), 170.61 (CH_2CO_2Et), 180.32 (C-8) and 187.89 (C-5). HRMS- Observed *m/z* = 296.08986; $C_{14}H_{16}O_7$ requires *m/z* = 296.08961.

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*4.1.3. (±)-(7,8-Dimethoxy-isochroman-3-yl)-acetic acid ethyl ester (**22a**)*

ZnI_2 (351 mg, 1.10 mmol) and $NaCNBH_3$ (70 mg, 1.10 mmol) were successively added to a solution of alcohol **17a** (153 mg, 0.52 mmol) in 1,2-dichloroethane (5 ml). The resulting mixture was submitted to ultrasound irradiation at room temperature during 6 h. Then, the reaction was diluted with brine (10 ml) and the products were extracted with EtOAc (4 × 25 ml). The combined organic extracts were washed with brine (10 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by chromatography, furnishing **22a** (100 mg, 69%) as an oil; IR (neat, ν): 2940, 2835, 1739, 1495, 1368, 1276, 1159, 1046, 967 and 801 cm^{-1}; ^1H NMR (δ): 1.28 (t, 3 H, $J = 7.1$, CH_2CH_3), 2.55 (dd, 1 H, $J = 5.4$ and 15.3, CH_2CO_2Et) 2.71 (dd, 1 H, $J = 10.9$ and 15.3, CH_2CO_2Et), 2.65-2.75 (m, 2 H, H-4), 3.80 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 4.02-4.12 (m, 1 H, H-3), 4.19 (q, 2 H, $J = 7.1$, OCH_2CH_3), 4.71 (d, 1 H, $J = 15.8$, $ArCH_2O$), 5.00 (d, 1 H, $J = 15.8$, $ArCH_2O$) and 6.78 (s, 2 H, H-5 and H-6); ^{13}C NMR (δ): 13.96 (OCH_2CH_3), 32.58 (C-4), 40.76 (CH_2CO_2Et), 55.62 (OCH_3-7), 59.82 (OCH_3-8), 60.29 (OCH_2CH_3), 64.56 (C-1), 70.98 (C-3), 111.00 (C-6), 123.60 (C-5), 125.72* (C-8a), 128.01* (C-4a), 144.35 (C-7), 150.10 (C-8) and 170.75 (CH_2CO_2Et). HRMS- Observed $m/z = 280.13095$; $C_{15}H_{20}O_5$ requires $m/z = 280.13108$.

*4.1.4. (±)-(7-Methoxy-5,8-dioxo-3,4,5,8-tetrahydro-1H-isochromen-3-yl)-acetic acid ethyl ester (**23**)*

AgO (160 mg, 1.28 mmol) was added to a stirred solution of **22a** (88 mg, 0.31 mmol) in dioxane (6 ml). After 2 min at room temperature, 6 M HNO_3 (0.8 ml) was added dropwise. The reaction was quenched with $CHCl_3/H_2O$ (3:1, 10 ml) after stirring 7 min. The reaction products were extracted with $CHCl_3$ and the combined organic extracts were washed with H_2O (5 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography, yielding **23** (28 mg, 32%) as a white solid, m.p.: 129–131 °C (hexane/EtOAc). IR (neat, ν): 2984, 2887, 1732, 1670, 1655, 1639, 1491, 1400, 1343, 1240, 1156, 1095, 1008 and 859 cm^{-1}; ^1H NMR (δ): 1.28 (t, 3 H, $J = 7.1$, CH_2CH_3), 2.20–2.60 (m, 2 H, H-4), 2.57 (dd, 1 H, $J = 5.7$ and 15.8, CH_2CO_2Et), 2.70 (dd, 1 H, $J = 2.9$ and 15.8, CH_2CO_2Et), 3.82 (s, 3 H, OCH_3), 3.88–4.03 (m, 1 H, H-3), 4.19 (q, 2 H, $J = 7.1$, OCH_2CH_3), 4.40 (d, 1 H, $J = 15.8$, $ArCH_2O$), 4.69 (d, 1 H, $J = 15.8$, $ArCH_2O$) and 5.90 (s, 1 H, H-6); ^{13}C NMR (δ): 14.01 (OCH_2CH_3), 26.99 (C-4), 40.32 (CH_2CO_2Et), 56.09 (OCH_3), 60.64 (OCH_2CH_3), 62.70 (C-1), 69.94 (C-3), 106.94 (C-6), 138.10* (C-8a), 139.66* (C-4a), 158.30 (C-7), 170.27 (CH_2CO_2Et), 180.08 (C-8) and 185.62 (C-5). HRMS-Observed $m/z = 280.09432$; $C_{14}H_{16}O_6$ requires $m/z = 280.09469$.

4.2. Antimicrobial activity on agar plates

Bacterial suspensions (10^8 CFU ml^{-1}) were prepared and spread over Mueller–Hinton agar plates. After 3 min at room temperature, 6 mm disks containing ampicillin (10 μg, DIFCO) or the test compounds (prepared by adding 10, 30 and 70 μg of the test compounds as a solution in MeOH and allowing to dry at room temperature) were placed at distances of 24 mm to each other and incubated for 2 h at room temperature and 24 h at 37 °C, when inhibition haloes were determined in triplicate.

4.3. MIC and MBC

Working solutions (512 μg ml^{-1}) were prepared by dilution of stock solutions (1000 μg ml^{-1} in MeOH) with Mueller–Hinton broth. The bacterial innoculum was a 1/10 dilution of a bacterial suspension (grown overnight in brain hearth broth), adjusted to a value of 0.5 in the Mc Farland turbidity scale. Serial dilutions of the working solutions were added to different tubes containing the bacterial suspension and the tubes were incubated during 24 h at 37 °C. MIC is the minimum concentration of the substance which avoids production of turbidity, while MBC is the minimum concentration of the tested substance which kills at least 99% of the bacterial population.

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