Short communication

Synthesis and antimicrobial activity of pyranobenzoquinones related to the pyranonaphthoquinone antibiotics

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Abstract

The synthesis and antimicrobial activity of isochromane-type analogs of the pyranonaphthoquinone antibiotics are reported. Isochromane derivatives with (17\textsubscript{a}, b) and without (22\textsubscript{a}, b) a C-4 hydroxyl moiety and their corresponding quinones (19\textsubscript{a} 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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1. Introduction

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

Many members of this group display the basic skeleton (psychorubin, 1\textsubscript{b}), 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 (6\textsubscript{a}) and kalafungin (6\textsubscript{b}, the enantiomer of nanaomycin D) contain an additional γ-lactone ring fused to the dihydropyran moiety, while others, exemplified by deoxyfrenolicin (7\textsubscript{a}) and nanaomycin A (7\textsubscript{b}), exhibit only an acetic acid side chain bound to C-3. In addition, the arizonins A2 (8\textsubscript{a}) and B2 (8\textsubscript{b}) and granaticinic acid (9) are examples of γ-hydroxyacids, the open forms of γ-lactones; aphid pigments such as protoaphin \textsubscript{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 (7\textsubscript{a}) is less active than frenolicin B (6\textsubscript{a}) 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-
Pictet-Spengler condensation of 1,4-dimethoxy-β-phenethyl alcohols with aldehydes followed by oxidative demethylation of the resultant benzopyrans, a Michael addition/cyclization sequence between 2-(1-hydroxyalkyl)-1,4-benzoquinones and imines or enamines, and tricycle lactones such as 13 (Fig. 2) have also been synthesized, but surprisingly their activity has not been tested. Recently, we reported the elaboration of 3,3a-dihydro-5H-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), employing an acid-catalyzed lactonization and a Wittig-oxa-Michael sequence for isochromane ring formation and functionalization. We also demonstrated that triene 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](image-url)

**Scheme 1. Reagents and conditions:** a: See Ref. [24]; b: TBAF, THF, RT, 1 h (90%); c: AgO, 6 N HNO₃, 7 min. (18, 34%; 19a, 32%); d: LiOH, THF/MeOH, 0 °C, 1 h (70%); e: AgO, 6 N HNO₃, 7 min (16%); f: ZnI₂, NaCNBH₃, ClCH₂CH₂Cl, RT, 4 h (69%); g: AgO, 6 N HNO₃, 7 min (32%); h: LiOH, THF/MeOH, 0 °C, 1 h (94%).
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/Al2O3 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 CO2, 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 alkylation 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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S. aureus</th>
<th>B. atrophaeus</th>
<th>S. agalactiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>19a</td>
<td>10 μg</td>
<td>30 μg</td>
<td>70 μg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>23b</td>
<td>12</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>26</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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.
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 antibiotics with and without a C-4 hydroxyl group and their corresponding pyranobenzoquinones, 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 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 1H NMR and 13C NMR spectra were acquired with a Bruker AC200-E spectrometer (200.13 MHz for 1H), employing CDCl3 as solvent; chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard (*δ* 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 ethanol 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 Bu3NF (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, v): 3446, 2941, 2816, 1732, 1611, 1498, 1323, 1283, 1230, 1123, 1074, 971 and 719 cm−1; 1H NMR (δ): 1.34 (t, 3 H, J = 7.0, CH3CH2), 2.60 (dd, 1 H, J = 8.6 and 15.5, CH2CO2Et), 2.95 (dd, 1 H, J = 3.7 and 15.5, CH2CO2Et), 3.40 (bs, 1 H, w1/2 = 20, OCH3), 3.84 (s, 3 H, OCH3), 3.89 (s, 3 H, OCH3), 3.93 (ddd, 1 H, J = 3.7, 4.6 and 8.6–3.3), 4.20 (q, 2 H, J = 7.0, OCH2CH3), 4.41 (bd, 1 H, J = 4.6, H-4), 4.74 (d, 1 H, J = 15.9, ArCH2O), 4.94 (d, 1 H, J = 15.9, ArCH2O), 6.89 (d, 1 H, J = 8.6, H-6) and 7.27 (d, 1 H, J = 8.6, H-5); 13C NMR (δ): 14.01 (OCH2CH3), 37.95 (CH2CO2Et), 55.66 (OCH3), 59.95 (OCH2-8), 60.61 (OCH2CH3), 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 (CH2CO2Et). HRMS- Observed *m/z* = 296.12628; C14H16O6 requires *m/z* = 296.12599.

4.1.2. (±)-trans-2-(4-Hydroxy-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 HNO3 (0.8 ml) was added dropwise. The reaction was quenched with CHCl3–H2O (3:1, 10 ml) after stirring 7 min. The reaction products were extracted with CHCl3, and the combined organic extracts were washed with H2O (5 ml), dried over Na2SO4 and concentrated under reduced pressure. The residue was purified by chromatography, yielding 18 (32 mg, 34%) as an oily IR. IR (neat, v): 3500, 2926, 2852, 1733, 1684, 1593, 1531, 1495, 1361, 1284, 1177 and 1042 cm−1; 1H NMR (δ): 1.27 (t, 3 H, J = 7.1, CH3CH2), 2.56 (dd, 1 H, J = 7.8 and 16.4, CH2CO2Et), 3.12 (dd, 1 H, J = 7.8 and 16.4, CH2CO2Et), 3.84 (s, 3 H, OCH3), 3.94 (s, 3 H, OCH3), 4.19 (q, 2 H, J = 7.1, OCH2CH3), 4.54 (dd, 1 H, J = 3.2 and 7.8 H-3), 4.82 (d, 1 H, J = 15.8, ArCH2O), 5.16 (d, 1 H, J = 15.8, ArCH2O), 6.95 (d, 1 H, J = 8.6, H-6) and 7.83 (d, 1 H, J = 8.6, H-5); 13C NMR (δ): 14.02 (OCH2CH3), 35.76 (CH2CO2Et), 55.79 (OCH3), 60.43 (OCH3), 68.07 (OCH3CH3), 63.36 (ArCH2O), 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 (CH2CO2Et) and 192.86 (C-4); HRMS- Observed *m/z* = 294.11019; C14H16O6 requires *m/z* = 294.11034. Increasing solvent polarity furnished 19a as a yellowish oil. IR (neat, v): 3509, 2978, 2935, 1733, 1659, 1630, 1459, 1370, 1286, 1233, 1177 and 1037 cm−1; 1H NMR (δ): 1.28 (t, 3 H, J = 7.0, CH3CH2), 2.56 (dd, 1 H, J = 8.8 and 15.8, CH2CO2Et), 2.93 (dd, 1 H, J = 3.3 and 15.8, CH2CO2Et), 3.82 (bs, 1 H, OH), 3.84 (s, 3 H, OCH3), 3.91 (ddd, 1 H, J = 3.3, 4.2 and 8.8, H-3), 4.19 (q, 2 H, J = 7.0, OCH2CH3), 4.49 (d, 1 H, J = 19.3, ArCH2O), 4.55 (bd, 1 H, J = 4.2, H-4), 4.64 (d, 1 H, J = 19.3, ArCH2O) and 5.90 (s, 1 H, H-6); 13C NMR (δ): 14.06 (OCH2CH3), 37.46 (CH2CO2Et), 56.35 (OCH3), 60.63 (OCH2CH3), 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 (CH2CO2Et), 180.32 (C-8) and 187.89 (C-5). HRMS- Observed *m/z* = 296.08986; C14H16O6 requires *m/z* = 296.08961.
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$_2$SO$_4$ 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, 1732, 1670, 1655, 1641, 1584, 1462, 1384, 1354, 1276, 1159, 1046, and 967 cm$^{-1}$; $^1$H NMR (δ): 1.28 (t, 3 H, $J$ = 7.1, CH$_2$CH$_3$), 2.55 (dd, 1 H, $J$ = 5.4 and 15.3, CH$_2$CO$_2$Et) 2.71 (dd, 1 H, $J$ = 10.9 and 15.3, CH$_2$CO$_2$Et), 2.62–2.75 (m, 2 H, H-4), 3.10 (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$_2$CH$_3$), 4.71 (d, 1 H, $J$ = 15.8, ArCH$_2$O) 5.00 (d, 1 H, $J$ = 15.8, ArCH$_2$O) and 6.78 (s, 2 H, H-5 and H-6); $^{13}$C NMR (δ): 13.96 (OCH$_2$CH$_3$), 32.58 (C-4), 40.32 (CH$_2$CO$_2$Et), 62.70 (C-1), 69.94 (C-3), 106.94 (C-2O) and 180.08 (C-2O). 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$_2$O (3:1, 10 ml) after stirring 7 min. The reaction products were extracted with CHCl$_3$ and the combined organic extracts were washed with brine (5 ml), dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Brine (10 ml), dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was purifed by chromatography, furnishing 23 (28 mg, 32%) as a white solid, m.p.: 131 °C.

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 inoculum 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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