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Synthesis of steroidal quinones and hydroquinones from bile acids by Barton radical decarboxylation and benzoquinone addition. Studies on their cytotoxic and antifungal activities

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ABSTRACT

Twelve new hydroquinones and quinones (**4a–c** to **7a–c**) derived from free or peracetylated bile acids were prepared by a Barton decarboxylation reaction, with subsequent trapping of the resulting free radical by benzoquinone. All new compounds were completely characterized by 2D NMR techniques and screened for antifungal and cytotoxic activity. One of the new hydroquinones (**7b**) showed promising results against the human pancreatic ductal carcinoma cell line PANC1, with similar cytotoxic activity as the commercial chemotherapy drug doxorubicin.

1. Introduction

Marine natural products are well known for their novel structures and biological activities [1]. However, many of these fascinating compounds suffer a severe drawback in terms of availability. In many cases, these are minor metabolites produced by slow-growing and delicate marine invertebrates such as sponges or soft corals. Large scale collections of these invertebrates are generally not possible for practical and ecological reasons. Aquaculture of these *phyla* of marine invertebrates, although much progress has been made, in most cases is still not a reliable alternative for the production of bioactive chemicals. All these factors contribute to a lack of available substances for *in vivo* bioassays, and generally stop the development of new drugs derived from marine organisms at the discovery stage. As a result, only a few marine-derived drugs have reached the market.

We have recently started a project on synthetic modifications of abundant and easily accessible natural starting materials such as bile acids and plant terpenoids. One of the aims of this project is to incorporate to these starting natural substances, structural fragments which are frequently found in bioactive marine natural products, and to evaluate their biological activities. A careful revision of the literature on marine natural products revealed several privileged structures, which are generally present in bioactive compounds. One of such structures is a quinone or hydroquinone moiety attached to a terpenoid skeleton [2]. There are several examples of this kind of compounds in the literature, but the best known are avarol and avarone, a sesquiterpenoid-derived quinone–hydroquinone pair originally isolated from the sponge *Dysidea avara*, in which the privileged structure is attached to a drimane skeleton [3]. Avarol and avarone display powerful antitumor activity [4–6], a fact that has triggered the development of several synthetic strategies and SAR studies for these compounds [7–9]. On the other hand, there are many examples of natural quinones and hydroquinones that display antifungal activity, making the incorporation of these structural fragments a good choice in the preparation of new antifungals [10,11].

In one of the most successful avarol syntheses, the quinone fragment was incorporated by means of a Barton decarboxylation reaction on an adequate carboxylic acid, with subsequent trapping of the resulting free radical by benzoquinone [12]. The Barton decarboxylation reaction of *O*-acyl esters formed from carboxylic acids and *N*-hydroxy-2-thiopyridone is a versatile tool that can be used for functional group conversions and the formation of carbon-carbon bonds [13]. The N–O bond of Barton esters can be cleaved either in thermal or photolytic conditions to generate a

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carboxyl radical and a pyridine-2-thyil radical. The carboxyl radical then undergoes CO_2 loss to generate an alkyl radical which can be conveniently used to produce a variety of products. If the generated radical reacts with a conveniently substituted alkene, quinone or aromatic group, a new carbon-carbon bond will be formed. In the steroid field, the Barton reaction has been previously used for the preparation of a brominated derivative of a cholanic acid which was finally used for the synthesis of a one-carbon homolog of the starting compound [14].

In the present work, the same strategy of reference [12] was applied to a series of readily available bile acids. In this way, twelve new quinone or hydroquinone derivatives of free or peracetylated cholic, lithocholic and chenodeoxycholic acids were synthesized. The peracetylated bile acid derivatives were of particular interest since peracetylated bile acids, probably of microbiological origin, have been isolated from several marine invertebrates, where they are believed to be involved in the chemical defense of these organisms [15,16]. As such, the peracetylated quinones can be considered as hybrids of avarone and acetylated bile acids. All new compounds were completely characterized by 2D NMR techniques and screened for antifungal and cytotoxic activity. One of the new hydroquinones showed promising results against human pancreatic carcinoma cell line PANC1 (see Fig. 1).

2. Experimental

2.1. General

Bile acids were obtained from commercial sources and used as such or recrystallized prior to use when necessary. Peracetylated compounds were obtained by standard procedures, using Ac_2O/DMAP/Py. Solvents were distilled for chromatography; CH_2Cl_2 was distilled from phosphorous pentoxide. NMR spectra were recorded on Bruker AC-200 (200.13 MHz) and Bruker Avance II (500.13 MHz) spectrometers, using the signals of residual non-deuterated solvents as internal reference. All 2D NMR experiments (COSY, DEPT-HSQC, HMBC, and NOESY) were performed using standard pulse sequences. HRMS were acquired on a Bruker micrOTOF-Q II spectrometer. UV spectra were obtained on a Hewlett Packard 8453 spectrophotometer and IR spectra were obtained on an FT-IR Nicolet Magna 550 instrument. TLC was carried out on Merck Sílicagel 60 F_{254} plates. TLC plates were sprayed with 2% vainillin in concentrated H_2SO_4. Merck Silicagel (230–400 mesh) was used for column chromatography. Solid phase extraction was performed with reversed-phase silica gel SPE cartridges (Thermo Scientific).

2.2. Chemistry

2.2.1. Preparation of thiohydroxamic esters (**2a–c**)

In a flask protected from light with aluminum foil at 0 °C, peracetylated acid **1a** (26 mg, 0.062 mmol) and 2-mercaptopyridine N-oxide (39.6 mg, 0.311 mmol) in 1 mL of dry dichloromethane were added. A solution of DCC (17 mg, 0.082 mmol) in 1 mL of CH_2Cl_2 was then added and the mixture was stirred for 2 h at room temperature. The solvent was removed by evaporation and the obtained residue was purified by solid phase extraction on reversed-phase silicagel, eluting with methanol: water (7:3) to remove excess 2-mercaptopyridine N-oxide and then acetone to recover the Barton ester. The acetone fraction was concentrated carefully, with protection from light and water bath temperature below 30 °C, to afford 28 mg of thiohydroxamate ester **2a** (yield 87%). ^{1}H NMR (200 MHz, $CDCl_3$): 7.70(1H, dd, J = 8.4, 1.5 Hz, H-6″), 7.56(1H, dd, J = 7.0, 0.9 Hz, H-3″), 7.21(1H, br t, J = 8.4 Hz, H-5″), 6.64(1H, td, J = 7.0, 1.5 Hz, H-4″), 4.88(1H, br s, H-7), 4.59(1H, m, H-3), 2.71(2H, m,H-23), 2.06(3H, s, Ac), 2.03 (3H, s, Ac), 0.99(3H, d, J = 5.5 Hz, H-21), 0.93(3H, s, H-19), 0.67(3H, s, H-18).

Using the same procedure described above for compound **2a**, reaction of 163 mg of peracetylated acid **1b** in 4 mL of dichloromethane afforded 197.5 mg of thiohydroxamic ester **2b** (yield 96%). ^{1}H NMR (200 MHz, $CDCl_3$): 7.69(1H, d, J = 8.4 Hz, H-6″), 7.57(1H, d, J = 7.1 Hz, H-3″), 7.21(1H, t, J = 8,4 Hz, H-5″), 6.63(1H, t, J = 7.1 Hz, H-4″), 4.72(1H, m, H-3), 2.70(2H, m, H-23), 2.03 (3H, s, H-1′), 0.98(3H, d, J = 6,0 Hz, H-21), 0.93(3H, s, H-19), 0.66(3H, s, H-18).

Using the same procedure described above for compound **2a**, reaction of 114 mg of peracetylated acid **1c** in 2 mL of dichloromethane afforded 115.6 mg of thiohydroxamic ester **2c** (yield 84%). ^{1}H NMR (200 MHz, $CDCl_3$): 7.69(1H, dd, J = 8.4, 1.8 Hz, H-6″), 7.56(1H, dd, J = 6.8, 1.5 Hz, H-3″), 7.21(1H, ddd, J = 8,4 Hz, 6.8, 1.5 Hz, H-5″), 6.64(1H, ddd, J = 6.8, 6.8, 1.6 Hz, H-4″),

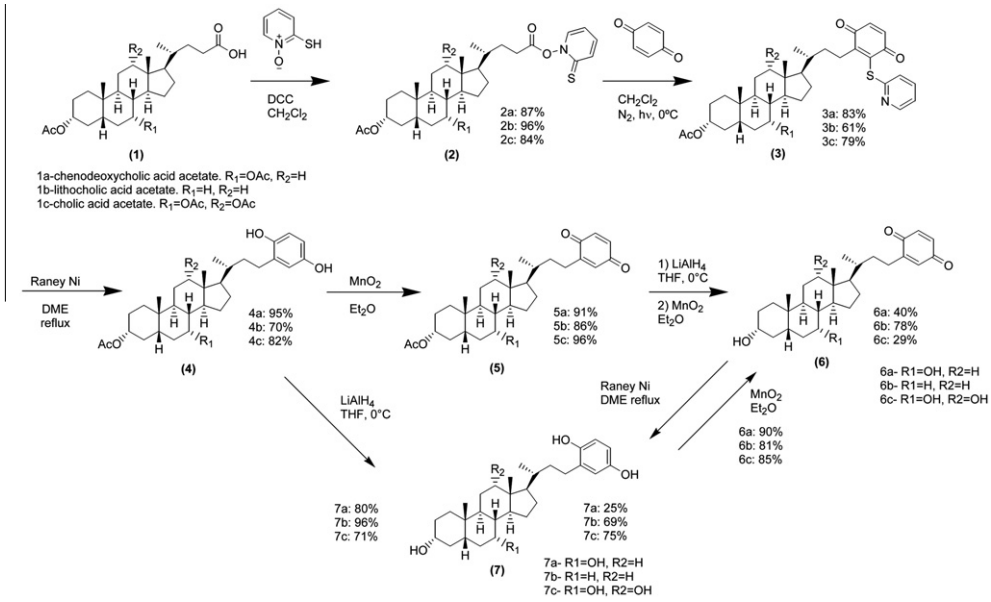

Fig. 1. Synthesis of quinones and hydroquinones derived from bile acids.

5.11(1H, br s, H-11), 4.91(1H, br s, H-7), 4.58(1H, m, H-3), 2.70(2H, m, H-23), 2.15(3H, s, Ac-11), 2.09(3H, s, Ac-3), 2.04(3H, s, Ac-7), 0.92(6H, br s, H21, H-19), 0.75(3H, s, H-18).

2.2.2. Decarboxylation and quinone addition – preparation of quinones (**3a–c**)

Thiohydroxamate ester **2a** (27.8 mg, 0.047 mmol) and p-benzoquinone (76.3 mg, 0.706 mmol) were dissolved in 4 mL of dry dichloromethane under nitrogen atmosphere at 0 °C. The reaction mixture was subsequently irradiated using a single 300 W tungsten lamp, while maintaining the temperature at 0 °C in an ice bath. Solvent evaporation followed by solid-phase extraction of the residue on reversed-phase using methanol: water (1:1) and then acetone as eluants, afforded a crude product which could be used as such or further purified by column chromatography on silica using ciclohexane: ethyl acetate (8:2), yielding 25.5 mg (83%) of pure quinone **3a**. ^{1}H NMR (200 MHz, $CDCl_3$): 8.31(1H, br d, J = 4.9 Hz, H-6″), 7.57(1H, td, J = 7.9, 1.8 Hz, H-4″), 7.34(1H, br d, J = 7.9 Hz, H-3″), 7.05(1H, dd, J = 7.9, 5.1 Hz, H-5″), 6.82(2H, br s, H-5′, H-6′), 4.87(1H, br s, H-7), 4.59(1H, m, H-3), 2.84(1H, td, J = 11.9, 5.3 Hz, H-23a), 2.67(1H, td, J = 11.3, 5.1 Hz, H-23b), 2.05(3H, s, Ac), 2.03(3H, s, Ac), 1.04(3H, d, J = 6.6 Hz, H-21), 0.93(3H, s, H-19), 0.63(3H, s, H-18).

Using the same procedure described above for compound **3a**, reaction of 83.1 mg of thiohydroxamic ester **2b** resulted in 53.8 mg of quinone **3b** (yield 61%). ^{1}H NMR (200 MHz, $CDCl_3$): 8.32(1H, br d, J = 4.4 Hz, H-6″), 7.57(1H, td, J = 7.7, 2.2 Hz, H-4″), 7.34(1H, br d, J = 8.0 Hz, H-3″), 7.04(1H, ddd, J = 7.3, 5.1, 1.1 Hz, H-5″), 6.81(2H, br s, H5′, H-6′), 4.72(1H, br s, H-3), 2.85H, td, J = 11.7, 4.8 Hz, H-23a), 2.66(1H, td, J = 11.7, 4.4 Hz, H-23b), 2.02(3H, s, Ac), 1.03 (3H, d, J = 6.2 Hz, H-21), 0.93(3H, s, H-19), 0.63(3H, s, H-18).

Using the same procedure described above, reaction of 19.3 mg of thiohydroxamic ester **2c** resulted in 16.7 mg of quinone **3c** (yield 79%). ^{1}H NMR ($CDCl_3$, 200 MHz): 8.31(1H, d, J = 4.7 Hz, H-6″), 7.58(1H, dd, J = 7.8, 7.7 Hz, H-4″), 7.35(1H, d, J = 7.8 Hz, H-3″), 7.05(1H, dd, J = 7.7, 4.7 Hz, H-5″), 6.81(2H, br s, H-5′, H-6′), 5.08(1H, br s, H-11), 4.91(1H, br s, H-7), 4.57(1H, m, H-3), 2.12(3H, s, Ac-11), 2.09(3H, s, Ac-3), 2.05(3H, s, Ac-7), 0.93(3H, d, J = 5.5 Hz, H-21), 0.91(3H, s, H-19), 0.71(3H, s, H-18).

2.2.3. Reduction to steroidal peracetylated hydroquinones (**4a–c**)

*2.2.3.1. 23-Hydroquinoyl-24-nor-5β-cholane-3α,7α-diyl diacetate (**4a**).* A solution of quinone **3a** (42.8 mg, 0.066 mmol) in 2 mL of DME was added to a suspension of an excess of activated Raney Nickel in 2 mL of DME and refluxed for 30 min while stirring. The reaction mixture was then filtered over a small pad of celite and the solvent was evaporated to obtain a crude residue which could be used as such or further purified by column chromatography on silica using ciclohexane: ethyl acetate (7:3), yielding 44.8 mg (95%) of pure hydroquinone **4a**. ^{1}H NMR (500 MHz, $CDCl_3$): 6.63(1H, d, J = 8.3 Hz, H-6′), 6.61(1H, d, J = 3.0 Hz, H-3′), 6.54(1H, dd, J = 8.3, 3.0 Hz, H-5′), 4.88(1H, br q, J = 3 Hz, H-7), 4.59(1H, m, H-3), 4.48(1H, s, OH), 4.4(1H, s, OH), 2.62(1H, ddd, J = 13.9, 11.6, 5.2 Hz, H-23a), 2.41(1H, ddd, J = 13.9, 11.3, 5.7 Hz, H-23b), 2.06(3H, s, Ac), 2.04(3H, s, Ac), 1.04(3H, d, J = 6.6 Hz, H-23), 0.93(3H, s, H-19), 0.66(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 563.3355 (calc. for $C_{33}H_{48}NaO_6^+$,

Table 1
^{13}C NMR (125 MHz) data for compounds **4a–c**, **5a–c**, **6a–c**, **7a–b**.

C	4a[a]	4b[a]	4c[a]	5a[a]	5b[a]	5c[a]	6a[a]	6b[a]	6c[b]	7a[b]	7b[b]
1	34.8	34.8	34.9	35.0	35.3	34.9	35.2	35.5	35.4	35.9	35.1
2	26.7	32.4	27.1	26.9	32.5	27.1	30.8	30.8	30.7	36.6	30.4
3	74.0	74.6	74.3	74.3	74.6	74.3	72.2	72.1	72.2	72.9	69.9
4	34.5	27.1	34.8	34.9	26.8	34.8	40.1	36.7	40.0	40.5	36.3
5	40.8	42.1	41.1	41.1	42.1	41.1	41.6	42.3	41.6	43.2	40.1
6	31.3	28.5	31.4	31.5	28.5	31.5	34.8	28.4	34.9	31.4	26.9
7	71.2	26.5	71.0	71.4	26.5	70.9	68.7	26.6	68.5	69.1	26.1
8	37.8	35.2	37.9	38.0	36	38.0	39.6	36.0	39.8	40.6	35.4
9	33.9	40.6	29.1	34.2	40.6	29.1	33.0	40.6	26.9	34.1	41.5
10	34.0	32.1	34.5	34.8	34.8	34.5	35.5	34.8	34.7	36.2	34.2
11	20.6	21.0	25.8	20.8	21.1	25.8	20.7	21.0	27.7	21.8	20.4
12	39.4	40.4	75.3	39.6	40.3	75.6	39.8	40.4	73.1	41.1	39.7
13	42.7	42.9	45.3	42.9	43	45.3	42.9	43.0	46.7	43.7	42.3
14	50.3	56.7	43.5	50.4	56.7	43.6	50.6	56.7	42.2	51.5	56.1
15	23.5	24.4	23.0	23.7	24.4	23.0	23.9	24.4	23.3	24.6	23.8
16	28.0	27.2	27.3	28.2	27.2	27.4	28.4	27.4	25.7	29.3	27.8
17	55.8	56.2	47.6	55.7	56.1	47.4	55.7	56.0	47.2	57.5	55.6
18	11.5	12.3	12.4	11.9	12.2	22.8	11.9	12.2	12.8	12.2	11.9
19	22.6	23.5	22.7	22.8	23.5	12.4	22.9	23.6	22.7	23.4	23.3
20	35.6	36.2	35.2	35.7	35.9	35.2	35.9	35.9	35.7	37.1	35.2
21	18.6	18.9	18.2	18.7	18.7	18.0	18.7	18.7	17.8	19.3	18.6
22	35.9	36.1	36.1	34.2	34.4	34.2	34.4	34.4	34.4	37.5	35.9
23	26.5	26.8	26.8	25.7	25.9	25.7	25.9	25.9	26.0	27.9	26.3
1′	147.1	147.5	147.5	187.7	187.7	187.7	187.7	187.7	182.2	149.1	147.4
2′	130.3	130.7	130.6	150.4	150.7	138.2	150.6	132.5	150.5	131.9	129.7
3′	116.6	116.9	116.9	132.4	132.5	132.7	132.5	188.1	132.5	117.5	116.2
4′	149.1	149.5	149.8	187.9	188.1	188.0	188.1	150.7	182.4	151.0	149.6
5′	113.1	113.3	113.3	136.4	136.5	136.5	136.4	136.4	136.5	113.7	112.6
6′	115.9	116.1	116.1	136.9	137	137.0	137.0	137.1	137.0	116.6	115.3
3-Ac	170.6	170.9	170.8	170.8	170.9	170.7					
	21.4	21.7	21.8	21.6	21.7	21.8					
7-Ac	170.4		170.7	170.6		170.6					
	21.5		21.7	21.8		21.6					
12-Ac			170.9			170.8					
			21.6			21.7					

[a] In $CDCl_3$.
[b] In CD_3OD.

563.3349). IR (film, cm^{-1}): 3420.8, 2940.4, 2881.2, 1742.6, 1722.9, 1518.8, 1459.6, 1393.8, 1249.0, 1202.9, 1038.4, 755.4. α_D ($CHCl_3$, c = 0.29) = +4.83°. UV ($CHCl_3$, 1/M cm): ε_{241} = 1209, ε_{293} = 2260.

*2.2.3.2. 23-Hydroquinoyl-24-nor-5β-cholane-3α-yl acetate (**4b**).* Using the same procedure described above for compound **3a**, reaction of 8.7 mg of quinone **3b** resulted in 5.0 mg of hydroquinone **4b** (yield 70%). ^{1}H NMR (500 MHz, $CDCl_3$): 6.63(1H, d, J = 8.3 Hz, H-6′), 6.61(1H, d, J = 3.0 Hz, H-3′), 6.54(1H, dd, J = 8.3, 3.0 Hz, H-5′), 4.72(1H, m, H-3), 4.48(1H, s, OH), 4.4(1H, s, OH), 2.62(1H, ddd, J = 13.9, 11.6, 5.2 Hz, H-23a), 2.41(1H, ddd, J = 13.9, 11.3, 5.7 Hz, H-23b), 2.06(3H, s, Ac), 2.04(3H, s, Ac), 1.04(3H, d, J = 6.6 Hz, H-23), 0.93(3H, s, H-19), 0.66(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 505.3285 (calc. for $C_{31}H_{46}NaO_4$, 505.3294). IR (film, cm^{-1}): 3394.5, 2940.4, 2868.0, 1709.7, 1510.0, 1466.2, 1393.0, 1281.9, 1189.0, 1031.8, 775.1. α_D ($CHCl_3$, c = 0.45) = 14.66°. UV ($CHCl_3$, cm^{-1}): ε_{250} = 276.3, ε_{288} = 330.

*2.2.3.3. 23-Hydroquinoyl-24-nor-5β-cholane-3α,7α,12α-triyl triacetate (**4c**).* Using the abovementioned procedure, reaction of 37.3 mg of quinone **3c** afforded 25.9 mg of hydroquinone **4c** (Yield 82%). ^{1}H NMR ($CDCl_3$, 500 MHz): 6.61(1H, d, J = 2.95 Hz, H-3′), 6.63(1H, d, J = 8,5 Hz, H-6′), 6.54(1H, dd, J = 8.5, 2.95 Hz, H-5′), 5.11(1H, br dd, J = 2.6, 2.8 Hz, H-12), 4.91(1H, br dd, J = 2.9, 5.7 Hz, H-7), 4.58(1H, m, H-3), 2.62(1H, ddd, J = 13.5, 11.9, 4.8 Hz, H-23a), 2.39(1H, ddd, J = 13.5, 11.2, 5.3 Hz, H-23b), 2.15(3H, s, Ac-12), 2.08(3H, s, Ac-3), 2.05(3H, s, Ac-7), 0.93(3H, d, J = 4.6 Hz, H-21), 0.92(3H, s, H-19), 0.72(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 621.3419 (calc. for $C_{35}H_{50}NaO_8$, 621.3398), $[M+NH_4]^+$ 616.3860 (calc. for $C_{35}H_{54}NO_8$, 616.3844). IR (film, cm^{-1}): 3401.1, 2947.0, 2861.4, 1742.6 br, 1643.9d, 1466.2d, 1387.2d, 1255.6 br, 1038.4, 768.6. α_D (MeOH, c = 0.28) = +45.4°. UV (MeOH, 1/M cm): ε_{229} = 961, ε_{296} = 888.

2.2.4. Oxidation to peracetilated steroidal quinones (5a-c)

*2.2.4.1. 23-(1,4-benzoquinoyl)-24-nor-5β-cholane-3α7α-diyl diacetate (**5a**).* A solution of hydroquinone **4a** (44.3 mg, 0.082 mmol) in 5 mL of ethyl ether was treated with an excess of MnO_2 and strirred at 25 °C for 30 min. The reaction mixture was then filtered over a small pad of celite yielding a crude product after solvent removal. Purification by column chromatography on silica using ciclohexane:ethyl acetate (9:1) afforded 40 mg (91%) of pure quinone **5a**. ^{1}H NMR (500 MHz, $CDCl_3$): 6.76 (1H, d, J = 10.1 Hz, H-6′), 6.71(1H, dd, J = 10.1, 2.4 Hz, H-5′), 6.55(1H, br d, J = 2.4 Hz, H3′), 4.88(1H, br s, H-7), 4.59(1H, m, H-3), 2.49(1H, ddd, J = 15.0, 12.0, 3.8 Hz, H-23a), 2.29(1H, ddd, J = 15.0, 11.8, 4.9, H-23b), 2.06(3H, s, Ac), 2.04(3H, s, Ac), 1.00(3H, d, J = 6.6 Hz, H-21), 0.94(3H, s, H-19), 0.64(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 561.3206 (calc. for $C_{33}H_{46}NaO_6^+$, 561.3187), $[M+NH_4]^+$ 556.3656 (calc. for $C_{33}H_{50}NO_6^+$, 556.3633). IR (film, cm^{-1}): 2947.0, 2881.2, 1736.0, 1663.6, 1380.6, 1262.2, 1025.2, 906.8, 768.6. α_D ($CHCl_3$, c = 1.24) = +7.58°. UV ($CHCl_3$, 1/M cm): ε_{254} = 3096.8, ε_{288} = 535.2.

*2.2.4.2. 23-(1,4-Benzoquinoyl)-24-nor-5β-cholane-3α-yl acetate (**5b**).* Using the same procedure described above, reaction of 34.6 mg of hydroquinone **4b** resulted in 31.4 mg of quinone **5b** (Yield 86%).^{1}H NMR (500 MHz, $CDCl_3$): 6.75(1H, d, J = 10.1 Hz, H-6′), 6.71(1H, dd, J = 10.1, 2.6 Hz, H-5′), 6.55(1H, m, H-3′), 4.72(1H, m, H-3), 2.49(1H, dddd, J = 15.1, 11.3, 4.7, 1.4 Hz, H-23a), 2.29(1H, dddd, J = 15.41, 11.1, 5.2, 1.2 Hz, H-23b), 2.03 (3H, s, Ac). 0.93(3H, s, H-19), 0.99(3H, d, J = 6.6 Hz, H-21), 0.65(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): See Table 1. ESI-MS m/z $[M+Na]^+$ 503.3127 (calc. for $C_{31}H_{44}NaO_4$, 503.3137), $[M+H]^+$ 481.3324 (calc. for $C_{31}H_{45}O_4$, 481.3318). α_D ($CHCl_3$, c = 1.28) = +28.63°. IR (film, cm^{-1}): 2933.8, 2868.0, 1749.2, 1670.2, 1459.6, 1367.5, 1249.0, 1038.4, 906.8, 748.8. UV ($CHCl_3$, cm^{-1}): ε_{254} = 2071.4, ε_{324} = 658.6.

*2.2.4.3. 23-(1,4-Benzoquinoyl)-24-nor-5β-cholane-3α,7α,12α-triyl triacetate (**5c**).* Using the same procedure described above, reaction of 25.9 mg of hydroquinone **4c** resulted in 24.8 mg of quinone **5c** (Yield 96%). ^{1}H NMR ($CDCl_3$, 500 MHz): 6.75(1H, d, J = 10.1 Hz, H-6′), 6.72(1H, dd, J = 10.1, 2.4 Hz, H-5′), 6.54(1H, d, J = 2.4 Hz, H-3′), 5.10(1H, dd, J = 2.85, 2.8 Hz, H-12), 4.91(1H, dd, J = 2.74, 5.4 Hz, H-7), 4.57(1H, s, H-3), 2.48(1H, dddd, J = 15.1, 11.5, 5.9, 1.4 Hz, H-23a), 2.26(1H, dddd, J = 15.1, 11.2, 5.1, 1.3 Hz, H23-b), 2.15(3H, s, Ac-12), 2.05(3H, s, Ac-7), 2.09(3H, s, Ac-3), 0.92(3H, s, H-18), 0.89(3H, d, J = 6.6 Hz, H-21), 0.74(3H, s, H-19). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 619.3264 (calc. for $C_{35}H_{48}NaO_8$, 619.3241), $[M+NH_4]^+$ 614.3708 (calc. for $C_{35}H_{52}NO_8$, 614.3687). IR (film, cm^{-1}): 2953.6, 2874.6, 1729, 1663.6, 1472.8d, 1380.6, 1262.2, 1031.8, 755.4. α_D ($CHCl_3$, c = 0.62) = +51.1°.UV ($CHCl_3$, 1/M cm): ε_{301} = 504, ε_{320} = 498, ε_{260} = 2648.

*2.2.5. Cleavage of acetates – preparation of quinones (**6a–c**)*

*2.2.5.1. 23-(1,4-Benzoquinoyl)-3α,7α-dihydroxy-24-nor-5β-cholane (**6a**).* To a solution of quinone **5a** (16.3 mg, 0.0303 mmol) in 1 mL of dry THF at 0 °C, $LiAlH_4$ (200 mg, excess) was added, and the reaction mixture was strirred for 30 min in an ice/brine bath. Methanol, water and 1 M aqueous hydrochloric acid were sequentially added in order to destroy the excess of $LiAlH_4$, and the mixture was then extracted twice with EtOAc. The combined organic phases were washed with $NaHCO_3$ (ss) and water, and finally evaporated at reduced pressure. The residue was redissolved in 4 mL of ethyl ether and treated with an excess of MnO_2. The reaction mixture was stirred at 25 °C for 30 min, filtered through a small pad of celite and the solvent was removed by evaporation at reduced pressure. The crude product was purified by column chromatography on silica using a ciclohexane:ethyl acetate gradient to give 4.5 mg of quinone **6a** (Yield 40%). ^{1}H NMR (500 MHz, $CDCl_3$): 6.76(1H, d, J = 10.0 Hz, H-6′), 6.71(1H, dd, J = 10.0, 2.5 Hz, H-3′), 6.56(1H, dt, J = 2.4, 1.3 Hz, H-3′), 3.86(1H, br s, H-7), 3.47(1H, m, H-3), 2.49(1H, dddd, J = 15.1, 11.3, 4.5, 1.4 Hz, H-23a), 2.30(1H, dddd, J = 15.1, 11.1, 5.1, 1.3 Hz, H-23b), 0.91(3H, s, H-19), 1.00(3H, d, J = 6.9 Hz, H-19), 0.67(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 477.2960 (calc. for $C_{29}H_{42}NaO4$, 477.2981). IR (film, cm^{-1}): 3394.5, 2940.4, 2868.0, 1663.6, 1492.5, 1084.5, 906.8, 768.6. α_D ($CHCl_3$, c = 0.84) = +5.59° UV ($CHCl_3$, 1/M cm): ε_{248} = 2890, ε_{256} = 2960, ε_{294} = 916.3.

*2.2.5.2. 23-(1,4-Benzoquinoyl)-3α-hydroxy-24-nor-5β-cholane (**6b**).* Using the same procedure described above for compound **6a**, reaction of.11.8 mg of quinone **5b** resulted in 8.4 mg of quinone **6b** (yield 78%). ^{1}H NMR (500 MHz, $CDCl_3$): 6.75(1H, d, J = 10.2 Hz, H-6′), 6.71(1H, dd, J = 10.1, 2.5, H-5′), 6.56(1H, br s, J = 2.5 Hz, H-3′), 3.63(1H, m, H-3), 2.49(1H, dddd, J = 15.1, 11.2, 4.8, 1.4 Hz, H-23a), 2.30(1H, dddd, J = 15.1, 11.2, 5.2. 1.1 Hz, H-23b), 0.92(3H, s, H-19), 0.99(3H, d, J = 6.6 Hz, H-21), 0.65(3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 461.3035 (calc. for $C_{29}H_{42}NaO_3$, 461.3026) $[M+NH_4]^+$ 456.3475 (calc. for $C_{29}H_{46}NO_3$, 456.3472). α_D ($CHCl_3$, c = 0.43) = +12.1°. IR (film, cm^{-1}): 3355.0, 2947.0, 2874.6, 1663.6, 1466.2w, 1301.6d, 1038.4, 762.0 m. UV ($CHCl_3$, 1/M cm): ε_{258} = 2982, ε_{289} = 1185, ε_{324} = 548.

*2.2.5.3. 23-(1,4-Benzoquinoyl)-3α,7α,12α-trihydroxy-24-nor-5β-cholane (**6c**).* Using the same procedure described above for compound **6a**, reaction of 25.0 mg of quinone **5c** resulted in 5.7 mg of quinone **6c** (yield 29%) after purification by preparative TLC. ^{1}H NMR (500 MHz, CD_3OD): 6.56(1H, dt, J = 2.4, 1.4 Hz, H-3′), 6.75(1H, d, J = 10.1 Hz, H-6′), 6.71(1H, dd, J = 10.1, 2.5 Hz, H-5′),

4.00(1H, br t, J = 2.6 Hz, H-12), 3.86(1H, br dd, J = 2.7, 5.7 Hz, H-7), 3.46(1H, m, H-3), 2.50(1H, dddd, J = 15.2, 11.7, 4.8, 1.5 Hz, H-23a), 2.32(1H, dddd, J = 15.2, 10.9, 5.4, 1.0 Hz, H-23b), 1.06(3H, d, J = 6.5 Hz, H-21), 0.90(3H, s, H-19), 0.70(3H, s, H-18). ^{13}C NMR (125 MHz, CD_3OD): see Table 1. ESI-MS m/z $[M+Na]^+$ 493.2925 (calc. for $C_{29}H_{42}NaO_5$, 493.2930). IR (film, cm^{-1}): 3369.5, 2944.2, 2863.5, 1727.1, 1661.1, 1631.8, 1463.1, 1389.8, 1272.5, 1206.5, 1081.9. UV (MeOH, 1/M cm): ε_{209} = 5500, ε_{253} = 1190, ε_{294} = 857, ε_{332} = 395.

*2.2.6. Reduction to steroidal hydroquinones (**7a–c**)*

*2.2.6.1. 23-Hydroquinoyl-3α,7α-dihydroxy-24-nor-5β-cholane (**7a**).* Using the same procedure described previously for the preparation of compound **4a**, reaction of quinone **6a** (8.6 mg, 0.019 mmol) in 1 mL of CH_2Cl_2 resulted, after preparative TLC chromatography, in 2.2 mg of hydroquinone **7a** (yield 25%). ^{1}H NMR (500 MHz, CD_3OD): 6.56(1H, d, J = 8.5 Hz, H-6′), 6.51 (1H, d, J = 3.2 Hz, H-3′), 6.42(1H, dd, J = 8.5, 3.1 Hz, H-5′), 3.80(1H, br s, H-7), 3.37(1H, m, H-3), 2.60(1H, d, J = 13.4 Hz, H-23), 2.40(1H, d, J = 13.4 Hz, H-5″), 2.27(1H, ddd, J = 13, 12, 11 Hz, H-4a), 0.93(3H, s, H-19), 1.04(3H, d, J = 6.6 Hz, H-21), 0.68(3H, s,H-18). ^{13}C NMR (125 MHz, CD_3OD): see Table 1. ESI-MS m/z $[M+Na]^+$ 479.3135 (calc. for $C_{29}H_{44}NaO_4^+$, 479.3132). IR (film, cm^{-1}): 3374.8, 2933.8, 2868.0, 1729(d), 1459.6, 1216.1, 1077.9, 755.4. α_D (MeOH, c = 0.2) = +1.5°. UV (MeOH, 1/M cm): ε_{226} = 1003, ε_{252} = 746, ε_{295} = 920.

*2.2.6.2. 23-Hydroquinoyl-3α-hydroxy-24-nor-5β-cholane (**7b**).* Using the same procedure, reaction of 8.4 mg of quinone **6b** resulted in 5.8 mg of hydroquinone **7b** (yield 69%). ^{1}H NMR (500 MHz, $CDCl_3$): 6.43(1H, br d, J = 2.95 Hz,H-3′), 6.53(1H, d, J = 8.5 Hz, H-6′), 6.35(1H, dd, J = 8.5, 2.95 Hz, H-5′), 3.36(1H, m, H-3), 2.48(1H, m, H-23a), 2.29(1H, ddd, J = 13.4, 10.9, 6.2 Hz, H-23b), 0.87(3H, s, H-19), 0.96(3H, d, J = 6.5 Hz, H-21), 0.60(3H, s, H-18), 8.48(1H, s, OH-4′), 8.41(1H, s, OH-1′), 4.45(1H, OH-3). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. ESI-MS m/z $[M+Na]^+$ 463.3189 (calc. for $C_{29}H_{44}NaO_3$, 463.3183). IR (film, cm^{-1}): 3374.8, 2933.8, 2861.4, 1466.2, 1038.4, 769. α_D (MeOH, c = 0.22) = +19.1°. UV (MeOH, 1/M cm): ε_{223} = 1006, ε_{295} = 934.

*2.2.6.3. 23-Hydroquinoyl-3α,7α,12α-trihydroxy-24-nor-5β-cholane (**7c**).* Using the same procedure, reaction of 5.7 mg of quinone **6c** resulted in 4.3 mg of hydroquinone **7c** (yield 75%). ^{1}H NMR (200 MHz, CD_3OD): 6.50(1H, d, J = 2.6 Hz, H-3′), 6.53(1H, d, J = 9.0 Hz, H-6′), 6.40(1H, dd, J = 8.7, 3.0 Hz, H-5′), 3.89(1H, br s, H-12), 3.28(1H, m, H-3), 2.51(1H, m, H-23a), 2.33(1H, m, H-23b), 0.98(3H, d, J = 6.0 Hz, H-21), 0.79(3H, s, 1H-19), 0.59(3H, s, H-18).ESI-Q-TOF: 490.35412). ESI-MS m/z $[M+Na]^+$ 495.3090 (calc. for $C_{29}H_{44}NaO_5$, 495.3086), $[M+NH_4]^+$ 490.3541 (calc. for $C_{29}H_{48}NO_5$, 490.3533). IR (film, cm^{-1}): 3406.1, 2914.9, 2856.2, 1463.1, 1382.5, 1206.5, 1089.2. UV (MeOH, 1/M cm): ε_{211} = 2392, ε_{227} = 1080, ε_{293} = 770.

*2.2.7. Reduction of **4a–c** to **7a–c***

Using the same procedure described in Section 2.2.5 for the reduction of quinones **5a–c**, treatment of hydroquinones **4a–c** with $LiAlH_4$ in THF produced the corresponding deacetylated hydroquinones **7a–c** (yields: **7a**: 80%, **7b**: 96%, **7c**: 71%).

*2.2.8. Oxidation of **7a–c** to **6a–c***

Using the same procedure described in Section 2.2.4 for the oxidation of hydroquinones **4a–c**, oxidation of hydroquinones **7a–c** with MnO_2 in Et_2O gave the corresponding deacetylated quinones **6a–c** (yields: **7a**: 90%, **7b**: 81%, **7c**: 85%).

2.3. Cytotoxicity evaluation

The effect of the different compounds on cell growth was assayed on log phase unsynchronized monolayers of two different cell lines: LM3 (murine mammary adenocarcinoma) and PANC1 (human ductal pancreatic carcinoma) [17]. LM3 cells were cultured at 37 °C in plastic flasks in MEM medium (Gibco; InvitrogenCorp, Carlsbad, Calif) supplemented with 10% FCS, 2 mM L-glutamine and 80 μg/ml Gentamicin. PANC1 cells were cultured at 37 °C in RPMI 1640 (Gibco; InvitrogenCorp, Carlsbad, Calif) supplemented with 10% FCS, 2 mM L-glutamine and 80 μg/ml Gentamicin. Both cells lines were cultured in a humidified air atmosphere with 5% CO_2. Serial passages were made by treatment of confluent monolayers with 0.25% trypsin and 0.02% EDTA in Ca^{+2} and Mg^{+2}-free PBS. Cells were periodically determined to be mycoplasma free by the Hoechst's method.

The bioassays were carried on as follows: 3×10^3 LM3 cells/well or 4×10^3 PANC1 cells/well in complete medium were seeded in 96 multiwell plates. After 24 h, cells received serial doses of the compounds (0.01–100 μM) or vehicle (DMSO) in medium plus 2% SFB for 3 days. As control, the cytotoxic activity of doxorubicin was also tested. Medium with freshly added compounds was changed every two days. Viability was assessed by reduction of the tetrazolium salt (MTS) to the formazan product in viable cells (Cell Titer 96 TM, Promega Corp) as calculated by the 492/620 nm absorbance ratio. IC (Inhibitory Concentration)$_{50}$ was defined as the concentration of the compound required for 50% cell growth inhibition.

2.4. Antifungal activity evaluation

2.4.1. Microorganisms and media

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC), Rockville, MD, USA *Candida albicans* ATCC 10231 and *Cryptococcus neoformans* ATCC 32264 were used.

Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C and maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and sub-cultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures and adjusted to $1–5 \times 10^3$ cells with colony forming units (CFU)/mL [18].

2.4.2. Antifungal susceptibility tests

Minimum Inhibitory Concentration (MIC) of each compound was determined by using broth microdilution techniques according to the guidelines of the National Committee for Clinical Laboratory Standards (CLSI) for yeasts (M27-A3) [18]. MIC values were determined in RPMI-1640 (Sigma, St Louis, Mo, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 35 °C in a moist, dark chamber, and MICs were visually recorded at 48 h.

For the assay, stock solutions of pure compounds were two-fold diluted with RPMI from 256–0.98 μg/mL (final volume = 100 μl) and a final DMSO concentration ⩽1%. A volume of 100 μl of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. Amphotericin B, fluconazole, and itraconazole (Sigma, St. Louis, MO, USA), were used as positive controls.

3. Results and discussion

In the present work, Barton esters **2a–c** were prepared from peracetylated chenodeoxicholic, lithocholic and cholic acids respectively, by reaction with 2-mercaptopyridine N-oxide and DCC in dry CH_2Cl_2 at room temperature. The yields obtained with

the free bile acids as starting materials were considerably lower. The choice of acetate as protective group was decided on the basis of its presence in many marine natural products and the abovementioned interest in the peracetylated bile acid derivatives as "natural product like" compounds. The general synthetic route would provide sequential access to both free and peracetylated quinones and hydroquinones. In order to obtain reasonable yields of Barton esters, an excess of 2-mercaptopyridine N-oxide had to be employed. A quick purification of the labile thiohydroxamate esters, with minimal handling and efficient removal of the excess of 2-mercaptopyridine N-oxide, was then mandatory. This could be achieved by SPE on reversed phase cartridges. An initial elution with MeOH: H2O (7:3) completely removed the excess of 2-mercaptopyridine N-oxide, allowing the recovery of the thiohydroxamate esters by a subsequent elution of the cartridge with acetone. These esters were immediately used without further purification.

Barton esters **2a**–**c** were then subjected to photolytic decarboxylation in the presence of benzoquinone as radical trap, by irradiation with a 300 W tungsten lamp at 0° in CH_2Cl_2 under N_2 atmosphere to yield the corresponding 2-thiopyridyl 1,2 quinone adducts **3a**–**c** [19]. The excess of benzoquinone was removed quickly and efficiently by SPE on reversed-phase by elution with MeOH:H_2O (1:1), and then adducts **3a**–**c** were recovered by subsequent elution of the SPE cartridge with acetone. As expected, 2-thioprydil sulfides of the decarboxylated bile acids were the main byproducts of this reaction, but in most cases the crude adducts could be used as such without further purification by column chromatography. Reductive desulfurization of adducts **3a**–**c** with Raney Ni in CH_2Cl_2 yielded crude hydroquinones **4a**–**c** in fairly good yields. These products were oxidized to the corresponding quinones **5a**–**c** by MnO_2 in ether.

Cleavage of the acetate groups was the most problematic step of the syntheses. Initial attempts under basic conditions (4% KOH in dioxane:methanol or K_2CO_3 in methanol:water) produced decomposition of the compounds and led to complex mixtures. The enhanced acidity of H-23s, vicinal to the quinone group, may be one of the causes of this unstability under basic conditions [20]. For this reason, acidic and neutral conditions were also tested. Transesterification in the presence of KCN in methanol under reflux also produced complex mixtures [21], as well as the use of $(Bu_3Sn)_2O$ in a variety of solvents (MeOH, EtOH, and toluene) under reflux [22,23].

Lewis acids were also tested: $ZnCl_2$ in MeOH under reflux failed, while the use of $Sc(OTf)_3$ under the same conditions only produced the deprotection of the C-3 hydroxyl in compound **5a**, leaving the C-7 acetate intact. The use of microwave heating with $Sc(OTf)_3$ on compound **5b** produced 50% of **6b**, while under the same conditions, compound **5c** only produced 20% of the C-3 deacetyl derivative, leaving C-7 and C-12 acetates intact.

All these unproductive attempts, led to the use of a strong reducing agent such as $LiAlH_4$ in THF for the deprotection of quinones **5a**–**c**. This reaction gave mixtures of the deacylated quinones and hydroquinones which, for ease of purification were oxidized with MnO_2 to obtain the fully deacetylated quinones **6a-c**, with a good yield for compound **6b** (78% for both steps) and relatively lower yields for compounds **6a** and **6c**. Finally, compounds **6a**–**c** were reduced to the corresponding fully deacylated hydroquinones **7a**–**c** with Raney Ni in DME, using basically the same technique as for the preparation of compounds **3a**–**c**.

In order to circumvent the low-yielding deprotection step, compounds **7a**–**c** could be prepared directly from **4a**–**c** by reduction with $LiAlH_4$ in THF with good yields. Compounds **6a-c** could then be prepared from **7a**–**c** by MnO_2 oxidation.

The effect on cell growth of compounds **4a**–**c**, **5a**–**c**, **6a**–**c** and **7a**–**c**, was tested against two different cell lines: LM3 (murine mammary adenocarcinoma) and PANC1 (human ductal pancreatic carcinoma). LM3 is an aggressive murine tumor cell line with high metastatic capacity to lung, originally developed at the Institute of Oncology A.H. Roffo [17]. The well known *in vivo* behavior of this cell line makes it a good model for further studies. On the other hand, PANC1 is a pancreatic carcinoma cell line that is quite resistant to chemotherapeutic agents. For comparison, doxorubicin was used as positive standard. Of the twelve tested compounds, only the free hydroquinones **7a**–**c** displayed some cytotoxic activity. The results are shown in Table 2, and indicate that only compound **7b** (the free hydroquinone derived from lithocholic acid) showed remarkable cytotoxic activity against both cell lines. PANC1 is a very resistant tumor cell line as shown by the high IC50 value for doxorubicin. Compound **7b** was as active as doxorubicin against this cell line. Interestingly the corresponding hydroquinones derived from the other two bile acids (**7a** and **7c**) also displayed cell cytotoxic activity but with IC50 values larger than 50 μM, while lithocholic acid was also tested for comparison, and displayed IC50 values larger than 100 μM.

Table 2
IC50 values (μM) for the *in vitro* screening against tumor cell lines.

Compound	**PANC1** (pancreas)	**LM3** (murine)
7a	>50	>50
7b	12.1 ± 7.5	5.6 ± 1.0
7c	>50	>50
Doxorubicin	20.2 ± 3.3	0.48 ± 0.1
Lithocholic acid	>100	>100

All the abovementioned compounds were also tested for antifungal activity against *C. albicans* and *C. neoformans*. Unfortunately, in all cases the MIC50 values were higher than 400 μM showing that these compounds are not good promissory antifungal compounds for further research. Nevertheless, this lack of activity against fungal cells indicates a selective toxicity of these steroidal quinones and hydroquinones against tumor cell lines enhancing their importance as cytotoxic entities.

4. Conclusion

In the present work, 12 new quinones and hydroquinones derived from free or peracetylated bile acids were synthesized. All the compounds were fully characterized by spectroscopical techniques and studies of their cytotoxic and antifungal activities were performed. One of the new compounds, **7b**, showed good IC50 values against both tested tumor cell lines, especially against the quimioresistant PANC1 cell line, where **7b** was twice as active as doxorubicin. In general, the yields were very good except for the deacetylation step. However, the reduction of **5b**, precursor of the most bioactive compound **7b**, with $LiAlH_4$ proceeded with a fairly good yield. On the other hand, direct reduction of acetylated hydroquinones **4a**–**c** with $LiAlH_4$ gave better yields of compounds **7a**–**c**, which could then be oxidized to **6a**–**c** with MnO_2. These exciting preliminary results will lead to the preparation of a library of derivatives of compound **7b**, and to a more extensive and thorough exploration of the scope and mechanism of action of this novel substance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2011.09.012.

References

[1] Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2009;26:170–244 [and references therein].
[2] Gordaliza M. Cytotoxic terpene quinones from marine sponges. Mar Drugs 2010;8:2849–70.
[3] Minale L, Riccio R, Sodano G. Avarol a novel sesquiterpenoid hydroquinone with a rearranged drimane skeleton from the sponge *Dysidea avara*. Tetrahedron Lett 1974;15:3401–4.
[4] Keyzers RA, Davies-Coleman MT. Anti-inflammatory metabolites from marine sponges. Chem Soc Rev 2005;34:355–65.
[5] Yasuhara-Bell J, Lu Y. Marine compounds and their antiviral activities. Antiviral Res 2010;86:231–40.
[6] Sladić D, Gašić MJ. Reactivity and biological activity of the marine sesquiterpene hydroquinone avarol and related compounds from sponges of the order Dictyoceratida. Molecules 2006;11:1–33.
[7] Sarma AS, Chattopadhyay P. Synthetic studies of trans-clerodane diterpenoids and congeners: stereocontrolled total synthesis of (+/−)-avarol. J Org Chem 1982;47:1727–31 [corrected 1982;47:5427].
[8] An J, Wiemer DF. Stereoselective synthesis of (+)-avarol, (+)-avarone, and some nonracemic analogues. J Org Chem 1996;61:8775–9.
[9] Ling T, Xiang AX, Theodorakis EA. Enantioselective total synthesis of avarol and avarone. Angew Chem Int Ed Engl 1999;38:3089–91.
[10] Nakamura M, Kakuda T, Qi J, Hirata M, Shintani T, Yoshioka Y, et al. Novel relationship between the antifungal activity and cytotoxicity of marine-derived metabolite xestoquinone and its family. Biosci Biotechnol Biochem 2005;69:1749–52.
[11] Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, et al. Natural products – antifungal agents derived from plants. J Asian Nat Prod Res 2009;11:621–38.
[12] Ling T, Poupon E, Rueden E, Kim SH, Theodorakis EA. Unified synthesis of quinone sesquiterpenes based on a radical decarboxylation and quinone addition reaction. J Am Chem Soc 2002;124:12261–7.
[13] Saraiva MF, Couri MRC, Le Hyaric M, De Almeida MV. The Barton ester free-radical reaction: a brief review of applications. Tetrahedron 2009;65:3563–72.
[14] Barton DH, Crich D, Motherwell WB. New and improved methods for the radical decarboxylation of acids. Chem Commun 1983:939–41.
[15] Chen, W. C., Sheu, J. H., Fang, L. S., Hu, W. P., Sung, P. J. 3α,7α,12α-Triacetoxy-5β-cholanic acid, a steroid from the Formosan soft coral Alcyonium sp. (alcyoniidae) Nat. Prod. Res. 2006; 20: 748-753.
[16] Sung PJ, Fang LS, Chen YP, Chen WC, Hu WP, Ho CL, et al. 5β-Steroids from the gorgonian coral *Junceella fragilis* (Ellisellidae). Biochem Syst Ecol 2006;34:64–70.
[17] Bal De Kier Joffe E, Puricelli LI, Vidal Del MCC, de Lustig ES. Characterization of two murine mammary adenocarcinoma tumors with different metastatic ability. J Exp Clin Cancer Res 1983;2:151–60.
[18] CLSI, Clinical and laboratory standards institute document M27-A3, vol. 28. 3rd ed. Pennsylvania: Wayne Ed., NCCLS; 2008; p. 1–25.
[19] Barton DH, Bridon D, Zard S. The invention of radical chain reactions. Part XIV: A decarboxylative radical addition to quinones. Tetrahedron 1987;43: 5307–14.
[20] Jurd L, Roitman J, Wong R. Quinones and quinone methides—IV: dimerization reactions of 2-phenylmethyl-5-methoxy-1,4-benzoquinones. Tetrahedron 1979;35:1041–54.
[21] Mori K, Tominaga M, Takigawa T, Matsui M. A mild transesterification method. Synth Commun 1973:790–1.
[22] Salomon C, Mata E, Mascaretti O. Scope and mechanism of deprotection of carboxylic esters by bis(tributyltin) oxide. J Org Chem 1994;59:7259–66.
[23] Furlan R, Mata E, Mascaretti O. Cleavage of carboxylic esters effected by organotin oxides and hydroxides under classical heating and microwave irradiation. A comparative study. Tetrahedron Lett 1996;37:5229–32.