Hydroquinone derivatives from the marine-derived fungus Gliomastix sp.†

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Eight new hydroquinone derivatives, gliomastins A–D (1–4), 9-β-O-methylgliomastin C (5), acremonin A 1-β-d-glucopyranoside (6), gliomastin E 1-β-d-glucopyranoside (7), and 6'-O-acetyl-isohomoarbutin (8), together with seven known analogues were isolated from the marine-derived fungus Gliomastix sp. Their structures were elucidated by extensive spectroscopic analysis including 1D and 2D NMR measurements aided by DFT NMR calculations as well as MS data. TDDFT-ECD and OR calculations were performed to determine the absolute configurations of 1 and the aglycones of 6 and 7. Compound 1 features a novel skeleton, biogenetically derived from a Diels–Alder reaction between derivatives of 11 and 13. Compound 2 represents a rare sulfur-containing alkaloid from the known hydroquinone 13. Compounds 1, 10 and 12 showed strong cytotoxicity against the LS178Y mouse lymphoma cell line with IC_{50} values of 1.8, 1.0 and 1.1 μM, respectively. Compound 3 exhibited moderate antitubercular activity against Mycobacterium tuberculosis with a MIC value of 12.5 μM.

Introduction

Marine-derived microorganisms are attracting considerable attention owing to their high potential for producing new bioactive secondary metabolites. Unique and stressful marine habitats, such as those characterized by extreme pressure, changing levels of salinity or temperature, may have a great impact on fungal biological activity. One of the most diverse marine ecosystems are coral reefs. In recent years, numerous bioactive secondary metabolites derived from coral-associated fungi have been reported. Examples include the prenylated polyketides teritrems A–C, which were isolated from the fungus Echinogorgia aurantiaca, which exhibit pronounced acetylcholinesterase inhibitory activity. Hydroanthraquinones and anthraquinone dimers, which were isolated from the soft coral-derived fungus Alternaria sp., showed cytotoxicity against PC-3 and HCT-116 tumor cell lines. In addition, the structurally unique 14-membered resorcylic acid lactones cochliomycins A–C, which exhibit antifouling activity, were isolated from the gorgonian-derived fungus Cochlidiopus lunatus.

As a part of our ongoing exploration of bioactive metabolites from fungi,6,11 examination of the Red Sea derived hard coral Stylophora sp. collected in Egypt, afforded different fungal strains from the coral tissues, one of which was identified as Gliomastix sp. So far only few secondary metabolites have been reported from the genus Gliomastix. The crude extract of the solid rice culture of Gliomastix sp. in this study showed cytotoxic activity against the LS178Y mouse lymphoma cell line with inhibition rate of 69.1% at a dose of 10 μg mL⁻¹. Chromatographic workup of the fungal extract afforded fifteen hydroquinone derivatives including eight new natural products (1–8). Herein, we report the isolation, structure elucidation as well as cytotoxic and antimicrobial activity of the isolated compounds.

Results and discussion

Compound 1 was isolated as a yellow amorphous powder. Its molecular formula was determined to be C_{32}H_{32}O_{8} by HRESIMS, indicating twelve degrees of unsaturation. The ^{13}C NMR...
data of 1 (Table 1) together with HSQC and HMBC spectra revealed the presence of 22 carbons, including two carbonyl groups ($\delta_C$ 202.3 and 200.5), twelve olefinic or aromatic carbons ($\delta_C$ 110–151) and eight aliphatic carbons (two quaternary carbons at $\delta_C$ 59.8 and 47.6, two methines at $\delta_C$ 48.0 and 46.8, two methylenes at $\delta_C$ 36.9 and 36.5, and two methyls at $\delta_C$ 24.5 and 23.5). The $^1$H NMR spectrum of 1 (Table 1) displayed five olefinic protons at 6.73 (d, H-2), 6.50 (d, H-3), 5.68 (br s, H-8), 6.73, 6.50, and 5.68.

Table 1  NMR data for compounds 1 and 2

| Position | $\delta_C$, type | $\delta_H$ (J in Hz) | $\delta_C$, type$^b$ | $\delta_H$ (J in Hz) |
|----------|-----------------|--------------------|-----------------|--------------------|
| 1        | 202.3, C        | 6.73, d (10.4)     | 129.8, C        |
| 2        | 140.5, CH       | 6.50, d (10.4)     | 124.4, C        |
| 3        | 142.2, CH$^b$   | 6.73, d (10.4)     | 124.4, C        |
| 4        | 200.5, C        | 112.8, CH          | 155.0, C        |
| 5        | 59.8, C         | 6.57, d (2.6)      | 117.0, CH       |
| 6        | 47.6, C         | 6.57, d (2.6)      | 132.6, C        |
| 7        | 36.5, CH$_2$    | 2.66, dd (11.9, 10.6) | 35.1, CH$_2$    |
|          |                 | 1.97, dd (11.9, 8.8) | 2.80, dd (14.1, 1.6) |
| 8        | 48.0, CH        | 3.31$^c$           | 81.6, CH        |
| 9        | 145.8, C        | 4.66, s            | 73.4, C         |
| 10       | 111.7, CH$_2$   | 1.77, s            | 25.9, CH$_3$    |
| 11       | 23.5, CH$_3$    | 6.40, d (8.5)      | 24.3, CH$_3$    |
| 1'       | 148.3, C        | 1.77, s            | 167.6, C        |
| 2'       | 117.1, CH       | 6.40, d (8.5)      | 30.8, CH$_3$    |
| 3'       | 114.5, CH       | 6.34, dd (8.5, 2.9)| 3.45, d (14.5)  |
| 4'       | 150.9, C        | 4.68, s            | 3.22, d (14.5)  |
| 5'       | 117.3, CH$^b$   | 6.21, d (2.9)      | 3.45, d (14.5)  |
| 6'       | 130.8, C$^c$    | 3.97, br s         | 3.22, d (14.5)  |
| 7'       | 46.8, CH$^b$    | 5.68, br s         | 3.22, d (14.5)  |
| 8'       | 122.7, CH$^b$   | 5.68, br s         | 3.22, d (14.5)  |
| 9'       | 133.8, C        | 5.68, br s         | 3.22, d (14.5)  |
| 10'      | 36.9, CH$_2$    | 2.97, d (17.7)     | 2.97, d (17.7)  |
| 11'      | 24.5, CH$_3$    | 2.28, d (17.7)     | 2.28, d (17.7)  |

$^a$ Recorded at 600 MHz for $^1$H and 150 MHz for $^{13}$C in CD$_3$OD. $^b$ Data extracted from HSQC and HMBC spectra. $^c$ Overlapped with water peak.
possess a tricyclic skeleton in addition to an aromatic ring. The NMR calculations were performed on the arbitrarily chosen energy conformers in a 21 kJ mol⁻¹ energy window, respectively. These conformers were reoptimized at the B3LYP/6-31+G(d,p) level and NMR shift values were computed for conformers over 1% Boltzmann population at the mPW1PW91/6-311+G(d,p) level.¹⁷ In accordance with the experimental NOE data, computed ¹³C-NMR chemical shifts of most carbons suggest 8⁰ relative configuration, while good agreement between experimental and computed ¹H-NMR chemical shifts of the 8⁰ epimer compared to that of the 8R epimer allowed unambiguous assignment of the relative configuration of 1 as (5S*,6R*,8S*,7'R*).

To determine the absolute configuration of 1, the solution TDDFT-ECD method was applied on the arbitrarily chosen (5S,6R,8S,7'R)-1 enantiomer.¹⁸,¹⁹ The 14 MMFF conformers obtained in the conformational search of the NMR calculations were reoptimized at the B3LYP/6-31G(d), B97D/TZVP,¹⁸,¹⁹ PCM/MeCN and CAM-B3LYP/TZVP²³,²⁴ PCM/MeCN levels and ECD computations were performed for the low-energy conformers with various functionals (B3LYP, BH&HLYP, CAM-B3LYP, PBEO) combined with the TZVP basis set. Computed ECD spectra obtained at all of the applied combination of levels gave moderate to good mirror-image agreement with the experimental spectrum, allowing determination of the absolute configuration of 1 as (5R,6S,8R,7'S) (Fig. 2).

Gliomastin A (1) features a new carbon skeleton, which could be derived from two co-isolated known hydroquinone derivatives aceromin A (11)²⁴ and F-11334A₁ (13).²⁵,²⁶ A biosynthetic pathway from the latter two compounds to 1 via Diels–Alder reaction is proposed (Fig. 3).

The molecular formula of compound 2 was established as C₁₄H₁₇NO₅S by HRESIMS, requiring six degrees of unsaturation. In the ¹H NMR spectrum of 2 (Table 1), two meta-coupled aromatic protons at δH 6.65 (d, J = 2.6 Hz, H-3) and 6.57 (d, J = 2.6 Hz, H-5), one oxygenated methine at δH 3.46 (dd, J = 10.2, 1.6 Hz, H-8), one isolated methylene at δH 3.45 (dd, J = 14.5 Hz,
H-2’a) and 3.22 (dd, J = 14.5 Hz, H-2’b), one methylene at δH 2.80 (dd, J = 14.1, 1.6 Hz, H-7a) and 2.66 (dd, J = 14.1, 10.2 Hz, H-7b), and two methyl groups at δH 1.25 (s, Me-10) and 1.24 (s, Me-11) were observed. The COSY correlations between H-7a/H-8 and H-7b/H-8 as well as the HMBC correlations from Me-10 and Me-11 to C-8 (δC 81.6) and C-9 (δC 71.6) indicated the presence of a 2,3-dihydroxy-3-methyl-butenyl moiety in compound 2 (Fig. 4). The attachment of this moiety to a meta-coupled benzene ring at the C-6 position was confirmed by the HMBC correlations from H-7a and H-7b to C-1 (δC 129.8), C-5 (δC 117.0) and C-6 (δC 132.6), from H-3 to C-1, C-4 (δC 155.0) and C-5, and from H-5 to C-1, C-3 (δC 112.8), C-4 and C-7 (δC 35.1). C-4 was suggested to be substituted with a hydroxy group due to its high chemical shift. The HMBC correlations from H-2’a and H-2’b to C-2 (δC 124.4) and C-1’ (δC 167.6), combined with its molecular formula, indicated that the isolated methylene CH2-2’ was linked to the benzene ring via an amide bond and a sulfur atom at the C-1 and C-2 positions to form an additional ring. Thus, the structure of 2 was determined, representing a rare sulfur-containing alkaloid, of which the co-isolated known hydroquinone derivative F-11334A1 (13) could be the precursor.

Gliomastin C (3) was recovered as a white amorphous powder, possessing the molecular formula C11H12O3 as determined by HRESIMS. Its 1H NMR data (Table 2) exhibited three aromatic protons at δH 7.25 (dd, J = 8.8 Hz, H-2), 6.95 (dd, J = 2.5 Hz, H-5) and 6.76 (dd, J = 8.8, 2.5 Hz, H-3), which are characteristic signals of an ABX ring system. Together with the HMBC correlations from H-2 to C-4 (δC 151.4) and C-6 (δC 130.3), from H-3 to C-1 (δC 149.9) and C-5 (δC 106.4), and from H-5 to C-1 and C-3 (δC 113.0), a benzene ring in 3 was established (Fig. 4). Furthermore, the HMBC correlations from Me-10/11 to C-8 (δC 166.3) and C-9 (δC 69.1), from OH-9 (δH 4.32) to C-8, C-9 and C-10/11 (δC 29.4), and from H-7 (δH 6.52, s) to C-1, C-6 and C-8 indicated the nature of a 3-hydroxy-3-methyl-buteryl moiety and its attachment to C-6. A hydroxy group at C-4 and an oxygen bridge between C-1 and C-8 were assigned in consideration of the molecular formula and the chemical shifts of the corresponding carbons. Thus, the structure of gliomastin C (3) was elucidated as shown.

Gliomastin D (4) shared the same molecular formula as gliomastin C (3). The UV spectra and NMR data (Table 2) of 4 were also comparable to those of 3. Three aromatic protons belonging to an ABX coupling system at δH 7.29 (d, H-2), 6.98 (d, H-5) and 6.80 (dd, H-3) were found in addition to a singlet olefinic methine at δH 6.63 (H-7) and two singlet methyls at δH 1.55 (Me-10 and 11). Analysis of the 1D and 2D NMR spectra of 4 revealed that the two compounds were structurally similar except for the position of the hydroxy group and the oxygen bridge. The upfield shifted C-8 (~4.4 ppm) and downfield shifted C-9 (+4.7 ppm) in 4 compared to those of 3 suggested the

Table 2 NMR data for compounds 3–5

| Position | δC, type | δH (J in Hz) | δC, type | δH (J in Hz) | δC, type | δH (J in Hz) |
|----------|----------|--------------|----------|--------------|----------|--------------|
| 1        | 149.9, C  |              | 150.1, C |              | 150.8, C |              |
| 2        | 111.7, CH | 7.25, d (8.8)| 112.0, CH | 7.29, d (8.8)| 112.1, CH | 7.25, d (8.8)|
| 3        | 113.0, CH | 6.76, dd (8.8, 2.5)| 113.6, CH | 6.80, dd (8.8, 2.5)| 114.0, CH | 6.74, dd (8.8, 2.5)|
| 4        | 154.1, C |              | 154.2, C |              | 154.3, C |              |
| 5        | 106.4, CH | 6.95, d (2.5)| 106.4, CH | 6.98, d (2.5)| 106.7, CH | 6.91, d (2.5)|
| 6        | 130.3, C |              | 129.9, C |              | 130.2, C |              |
| 7        | 100.7, CH | 6.52, s      | 104.4, CH | 6.63, s      | 105.0, CH | 6.61, s      |
| 8        | 166.3, C |              | 161.9, C |              | 161.5, C |              |
| 9        | 69.1, C  |              | 73.8, C  |              | 74.9, C  |              |
| 10       | 29.4, CH3 | 1.57, s     | 25.6, CH3 | 1.55, s     | 25.6, CH3 | 1.59, s     |
| 11       | 29.4, CH3 | 1.57, s     | 25.6, CH3 | 1.55, s     | 25.6, CH3 | 1.59, s     |
| OH-4     |          | 8.03, s     |          | 8.10, s     |          |              |
| OH-9     |          | 4.32, s     |          |              |          |              |
| OMe-9    |          |              |          | 51.3, CH3   | 3.10, s   |              |

*Recorded at 600 MHz for 1H and 150 MHz for 13C in CD3COCD3. bRecorded at 600 MHz for 1H and 150 MHz for 13C in CD2OD.*
presence of a pyran ring and a hydroxy group at C-8 in 4 instead of a furan ring.

Compound 5 was isolated as a white amorphous powder with the molecular formula C_{12}H_{14}O_{3} as established by HRESIMS, containing an additional CH unit compared to glio- mastin C (3). The UV absorption spectrum and NMR data (Table 2) of 5 resembled those of 3 except for the appearance of an additional methoxy group at δ_{C} 51.3 and δ_{H} 3.10, which was further confirmed to be located at C-9 by the HMBC correlation from the protons of the methoxy group to C-8 (δ_{C} 74.9). Thus, compound 5 was elucidated as 9-O-methylgliomastin C.

The HRESIMS of compound 6 displayed a pseudomolecular ion peak at m/z 361.1255 [M + Na]^{+}, corresponding to the molecular formula C_{17}H_{22}O_{7}. Positive and negative ESI mass spectra showed fragment peaks at m/z 177 [M + H − 162]^{+} and 175 [M − H − 162]^{−}, respectively, which suggested the presence of a hexose residue in the molecule. The UV and NMR data (Table 3) of 6 were similar to those of acremonin A 4-O-β-D-glucopyranoside. Acid hydrolysis of 6 yielded two products, one of which was confirmed to be the co-isolated hydroquinone acremonin A (11) by comparison of their NMR data and optical rotation values. The other product was determined as β-D-glucopyranosyl by TLC analysis and optical rotation measurement compared to a known standard as well as by the glucopyranoside.

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Since the absolute configuration of acremonin A (11) is unknown in literature, ECD calculations were carried out for the arbitrarily chosen (S) enantiomer of the aglycone 11. DFT reoptimization of the initial 15 MMFF conformers yielded 8 low-energy conformers at both the B3LYP/6-31G(d) in vacuo and the B97D/TZVP PCM/MeCN levels of theory. ECD spectra computed at various levels for these conformers gave nice mirror-image agreement with the experimental spectrum (Fig. 6). In some of the higher-energy conformers (e.g. conformers C and D, Fig. 7), the isopropenyl adopted a different orientation from that of the lowest-energy conformer, which is reflected in a near mirror image ECD of the conformers. Considering the relatively small computed energy differences of the conformers, this might be a possible source of error (energy difference between conformers A and C is less than 1 kJ mol^{-1}). Therefore OR

### Table 3 NMR data for compounds 6–8

| Position | δ_{C} (δ_{H} (J in Hz)) | δ_{C} (δ_{H} (J in Hz)) | δ_{C} (δ_{H} (J in Hz)) |
|----------|-------------------------|-------------------------|-------------------------|
| 1        | 146.6, C                | 148.6, C                | 150.4, C                |
| 2        | 118.2, CH 6.69, d (8.8) | 116.8, CH 6.80, d (8.7) | 119.2, CH 6.92, d (8.7) |
| 3        | 116.2, CH 6.54, d (8.8) | 115.4, CH 6.50, d (8.7) | 113.7, CH 6.52, dd (8.7, 3.0) |
| 4        | 147.2, C                | 151.0, C                | 153.8, C                |
| 5        | 132.8, C                | 136.9, C                | 118.1, CH 6.58, d (3.0) |
| 6        | 129.8, C                | 131.4, C                | 131.0, C                |
| 7        | 36.8, CH₂ 3.55, dd (13.3, 5.7) | 36.3, CH₂ 2.28, dd (16.1, 7.2) | 16.7, CH₃ 2.21, s |
| 8        | 48.9, CH 3.99, dd (5.7, 2.5) | 82.2, CH 3.99, t (7.2) | 64.7, CH₂ 4.37, dd (11.8, 2.3) |
| 9        | 146.8, C                | 48.4, C                 | 75.2 CH 3.51, m         |
| 10       | 110.6, CH₂ 4.84, s      | 26.0, CH₂ 1.36, s       | 172.7, C 2.03, s        |
| 11       | 20.4, CH₁ 1.78, s       | 20.0, CH₁ 1.20, s       | 20.7, CH₃ 1.18, s       |
| 1'       | 102.1, CH 4.88, d (7.7) | 104.1, CH 4.69, d (7.6) | 104.3, CH 4.66, d (7.6) |
| 2'       | 75.0, CH 3.36, m        | 75.0, CH 3.41, m        | 75.1, CH 3.43, m        |
| 3'       | 77.9, CH 3.42, m        | 78.2, CH 3.42, m        | 78.0, CH 3.44, m        |
| 4'       | 71.4, CH 3.36, m        | 71.5, CH 3.35, m        | 71.7, CH 3.35, m        |
| 5'       | 78.2 CH 3.37, m         | 78.0 CH 3.32, m         | 75.2 CH 3.51, m         |
| 6'       | 62.6, CH₂ 3.89, dd (12.2, 1.8) | 62.7, CH₂ 3.87, dd (12.0, 2.1) | 64.7, CH₂ 4.37, dd (11.8, 2.3) |
| OAc-6'   | 3.69, dd (12.2, 5.2)    | 3.68, dd (12.0, 5.5)    | 4.25, dd (11.8, 6.6)    |

* Recorded at 600 MHz for 1H and 150 MHz for 13C in CD_{3}OD. Data extracted from HSQC and HMBC spectra.
calculations were also performed on the low-energy gas-phase conformers and on those reoptimized at the B97D/TZVP PCM/MeCN level (8 conformers over 2% also at this level).\textsuperscript{25,28} The OR calculations performed at various levels for both sets of conformers were in line with the ECD results confirming the previous assignment. However, the sign of the OR values of the individual conformers were influenced by the orientation of the isopropenyl group such as in the case of ECD. The (R) absolute configuration of the calculations was also confirmed by the biosynthetic relationship of 1 and 11.

Compound 7 was obtained as a white solid. It possesses the molecular formula C\textsubscript{17}H\textsubscript{24}O\textsubscript{8} as established by analysis of the HRESIMS data. The UV spectrum and in MeCN (black line) compared with the PBE0/TZVP PCM/MeCN ECD spectra computed for the B97D/TZVP PCM/MeCN optimized low-energy conformers of (S)-11 (average of 8 conformers/2, red line) and (R)-11 (average of 8 conformers/2, blue line). Bars represent computed rotational strength values of (S)-11 (conformer A/2).

Fig. 6

Compound 7 was obtained as a white solid. It possesses the molecular formula C\textsubscript{17}H\textsubscript{24}O\textsubscript{8} as established by analysis of the HRESIMS data. The UV spectrum and in MeCN (black line) compared with the PBE0/TZVP PCM/MeCN ECD spectra computed for the B97D/TZVP PCM/MeCN optimized low-energy conformers of (S)-11 (average of 8 conformers/2, red line) and (R)-11 (average of 8 conformers/2, blue line). Bars represent computed rotational strength values of (S)-11 (conformer A/2).

Fig. 6

Fig. 7 Structures and populations of the low-energy (\(\geq 2\%\)) B97D/TZVP PCM/MeCN conformers of (S)-11.

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The nature of the benzene ring was established by the HMBC correlations from H-2 to C-4 (\(\delta_C\) 151.0) and C-6 (\(\delta_C\) 131.4) and from H-3 to C-1 (\(\delta_C\) 148.6) and C-5 (\(\delta_C\) 136.9) (Fig. 5). Analysis of the hydrolysis products of 7 confirmed the presence of a glucopyranose residue, which was determined to be attached to C-1 by the HMBC correlation from H-1\textsuperscript{+} to C-1. However, compared to 6, the signals of the isopropenyl group were replaced by two singlet methyl groups at \(\delta_H\) 1.35 (s, Me-10) and 1.20 (s, Me-11) as well as by an oxygenated methine at \(\delta_H\) 82.2 and \(\delta_H\) 3.99 (CH-8). The COSY correlations between H-7a/H-8 and H-7b/H-8 together with the HMBC correlations from Me-10 and Me-11 to C-5, C-8 and C-9 (\(\delta_C\) 48.4), and from H-7a and H-7b to C-1, C-5 and C-6 indicated the presence of a further cyclopentene ring with two methyls at C-9 and a hydroxy group at C-8, which was fused to the benzene ring. Thus, the structure of 7 was elucidated as shown. Its aglycone was obtained following hydrolysis of 7, representing a new hydroquinone derivative, for which the name gliomastin E is proposed.

For the configurational assignment of the aglycone gliomastin E, the solution TDDFT-ECD calculation protocol was carried out on the arbitrarily chosen (S) enantiomer. Reoptimization of the initial 23 MMFF conformers resulted in 13 and 15 low-energy (\(\geq 2\%\)) conformers at the B3LYP/6-31G(d) \textit{in vacuo} and the B97D/TZVP PCM/MeCN levels. ECD spectra computed at various levels for both sets of conformers resembled the 273 and the 198 nm transitions of gliomastin E suggesting (S) absolute configuration (Fig. 8). Since the 273 nm positive Cotton effect (CE) could not be reproduced by any of the applied combination, OR calculations performed \textit{in vacuo} and in MeOH (similarly to 11) were applied to prove the (S) absolute configuration.\textsuperscript{28} OR values computed at all of the applied combinations of levels resulted in small positive overall optical rotations in the range from +10.2 to +18.4, while the experimental value was +15.2, which verified the ECD results.

The molecular formula C\textsubscript{17}H\textsubscript{24}O\textsubscript{8} was deduced for compound 8 from the HRESIMS data. The UV spectrum and
All isolated compounds (1–15) were evaluated for their cytotoxicity against the L5178Y murine lymphoma cell line (Table 4). Compounds 1, 10, 12 and 13 showed significant cytotoxic activity with IC50 values of 1.8, 1.0, 1.1 and 3.0 μM, respectively, which are below that of the positive control kahalalide F. Compared to the two precursors 11 and 13, compound 1 demonstrated stronger cytotoxicity while the sulfur-containing alkaloid 2 was inactive compared to its precursor 13. Compounds 10 and 11 showed strong cytotoxicity while their glycosides 9, 8 and 6 were inactive. The ether bridge in 14 and 15 led to total loss of cytotoxicity compared to 13.

In addition, all compounds were tested for their antitubercular activity against Mycobacterium tuberculosis and for their antibacterial activities against different strains of pathogenic bacteria (Table 4). Only compounds 10 and 12 exhibited antibacterial activity but they were also cytotoxic. Compound 3, which lacked cytotoxicity, showed antitubercular activity with a MIC value of 12.5 μM. Comparison of the antitubercular activity of 3 with those of 4, 5, 14 and 15 indicated the importance of the furan ring and of the free hydroxy group at C-9.

In conclusion, our investigation of secondary metabolites from the marine-derived fungus Gliomastix sp. led to the isolation of fifteen hydroquinone derivatives including eight new natural products (1–8). Among the latter, the dimer possesses a novel carbon skeleton, which could be derived from derivatives of 11 and 13 through a Diels–Alder reaction. Compound 1 also exhibited significant cytotoxicity against the L5178Y mouse lymphoma cell line with an IC50 value of 1.8 μM. Compound 2 is a rare sulfur-containing alkaloid. TDDFT-ECD and OR calculations were performed to determine the absolute configurations of 1 and the aglycones of 6 and 7. All isolated compounds were tested for their cytotoxic and antimicrobial activity and the structure–activity relationships were discussed.

Experimental section

General procedures

Optical rotations were recorded on a PerkinElmer-241 MC polarimeter. 1H, 13C and 2D NMR spectra were measured on a Bruker Avance DMX 600 NMR spectrometer. A Finnigan LCQ NMR data (Table 3) of 8 were almost identical to those of isohomoarbutin (9), except for the appearance of an additional acetox group (δH 2.03, δC 20.7 and 172.7) in 8. This acetox group was determined to be located at C-6 on the basis of the HMBC correlations from H-6′a and H-6′b (δH 4.37 and 4.25) to the carbonyl carbon of the acetox group. The remaining structure of 8 was confirmed to be the same as 9 by detailed analysis of the 2D NMR spectra of 8 (Fig. 5). The hexose residue was determined to be β-D-glucopyranose by the coupling constant (7.6 Hz) of the anomeric proton (H-1′) and analysis of the acid hydrolysis products of 8 in comparison with a known standard. Owing to the data mentioned above, compound 8 was elucidated as 6′-O-acetyl-isorhamnatin.

By comparison with the literature data, the remaining known compounds were identified as isohomoarbutin (9), 2-methyl-1,4-benzenedi (10), acremomin A (11), prenylhydroquinone (12), E-1134A2 (13), (R)-2-(2-hydroxyprop-2-yl)-2,3-dihydro-5-hydroxybenzofuran (14), and 2,2-dimethylchroman-3,6-diol (15).

Table 4 Cytotoxicity, antitubercular and antibacterial activities of 1, 3, 10–13

| Compd | L5178Y (μM) | S. aureus ATCC 25923 | S. aureus ATCC 700699 | E. faecalis ATCC 29212 | E. faecalis ATCC 51299 | E. faecium ATCC 35667 | E. faecium ATCC 700221 |
|-------|-------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 1     | 1.8         | —                   | —                   | —                   | —                   | —                   | —                   |
| 3     | —           | 12.5                | —                   | —                   | —                   | —                   | —                   |
| 10    | 1.0         | 12.5                | 25                  | 6.25                | 12.5                | 12.5                | 12.5                |
| 11    | 9.6         | 25                  | —                   | 12.5                | —                   | —                   | —                   |
| 12    | 1.1         | 12.5                | 25                  | 12.5                | 6.25                | 6.25                | 12.5                |
| 13    | 3.0         | 25                  | —                   | 12.5                | —                   | —                   | —                   |
| Positive control | 4.3b | <0.64c | 1.56d | 1.56d | 0.008e | 0.008e | 0.008e |

IC50 or MIC > 50 μM. KAkalalide F as positive control. Rifampicin as positive control. Mosifloxacin as positive control. Ciprofloxacin as positive control.
Deca XP Thermoquest spectrometer was used to record mass spectra while a FTHRMS-Orbitrap (Thermo Finnigan) mass spectrometer was utilized to obtain HRESIMS data. The Dionex P580 HPLC system was coupled to a photodiode array detector (UVD340S) and the analytical column (125 × 4 mm, L x i.d.) was prefilled with Eurospher-10 C18 (Knauer, Germany). Semi-preparative HPLC separation was performed using a photodiode array detector and Eurosphere 100 C18 column using MeOH as eluent to obtain six subfractions (F4-1–F4-6), of which F4-5 (80 mg) was purified using semi-preparative RP-HPLC with 60% MeOH–H2O as mobile phase to yield compounds 10 (42.3 mg), 11 (3.6 mg) and 12 (13.0 mg).

F5 (120 mg) was further fractionated using a Sephadex LH-20 column (80 × 4 cm) with MeOH as eluent to give five subfractions (F5-1–F5-5). Subfraction F5-1 (34 mg) was subjected to semi-preparative RP-HPLC with 38% MeOH–H2O to yield 15 (14.0 mg) and 14 (3.2 mg). Subfraction F5-2 (14 mg) was purified by semi-preparative RP-HPLC using 50% MeOH–H2O to give 5 (1.5 mg). Subfraction F5-3 (10 mg) was separated by semi-preparative RP-HPLC using 57% MeOH–H2O to afford 3 (0.9 mg) and 4 (2.4 mg). Subfraction F5-4 (15 mg) was purified by semi-preparative RP-HPLC with 64% MeOH–H2O as mobile phase to yield 1 (3.5 mg).

Following similar procedures, compound 13 (7.4 mg) was obtained from fraction F8, by column chromatography over a Sephadex LH-20 column (100 × 5 cm) with MeOH as eluent, followed by purification using semipreparative RP-HPLC with 34% MeOH–H2O as eluent.

F10 (235 mg) was separated into five subfractions (F10-1–F10-5) by a Sephadex LH-20 column (80 × 4 cm) using 100% MeOH as eluent. Subfraction F10-3 (35 mg) was then subjected to semi-preparative RP-HPLC eluted with 40% MeOH–H2O to yield 9 (1.8 mg) and 8 (3.0 mg) while subfraction F10-4 (13 mg) was purified by semi-preparative RP-HPLC with 50% MeOH–H2O to afford 2 (0.8 mg).

Compounds 7 (2.2 mg) and 6 (3.0 mg) were obtained from fraction F12 (150 mg) using a Sephadex LH-20 column (80 × 4 cm) with MeOH for elution followed by semi-preparative HPLC separation with 35% MeOH–H2O as eluent.

Gliomastin A (1). Yellow amorphous powder; [α]D20 20–6 (c 0.37, MeOH); UV (MeOH) λmax: 212 and 296 nm; ECD (MeCN, λ0 (Δε) c 0.35 mM) 392 (+0.46), 372sh (+0.34), 331 (−0.18), 272sh (+0.27), 255 (−0.60), 228sh (−2.88), 215sh (−3.75), 197 (−7.91) nm; 1H NMR and 13C NMR data, see Table 1; HRESIMS [M + Na] + 373.1412 (calcd for C17H22O7Na, 373.1410).

Gliomastin B (2). White solid; [α]D20 24 (c 0.08, MeOH); UV (MeOH) λmax: 211, 243 and 292 nm; 1H NMR and 13C NMR data, see Table 1; HRESIMS [M + H] + m/z 284.0950 (calcd for C13H14NO4S, 284.0951).

Gliomastin C (3). White amorphous powder; UV (MeOH) λmax: 203, 249 and 294 nm; 1H NMR and 13C NMR data, see Table 2; HRESIMS [M + Na] + m/z 215.0677 (calcd for C11H12O2Na, 215.0679).

Gliomastin D (4). White amorphous powder; UV (MeOH) λmax: 206, 249 and 295 nm; 1H NMR and 13C NMR data, see Table 2; HRESIMS [M − H2O + H] + m/z 175.0751 (calcd for C11H12O2Z, 175.0754).

9-O-Methylgliomastin C (5). White amorphous powder; UV (MeOH) λmax: 206, 249 and 295 nm; 1H NMR and 13C NMR data, see Table 2; HRESIMS [M + Na] + m/z 268.0870 (calcd for C17H22O2Na, 268.0870).

Acremonin A 1-O-β-D-glucopyranoside (6). White amorphous powder; [α]D20 30 (c 0.31, MeOH); UV (MeOH) λmax: 206 and 298 nm; 1H NMR and 13C NMR data, see Table 3; HRESIMS [M + Na] + m/z 361.1255 (calcd for C17H22O2Na, 361.1258).

Gliomastin E 1-O-β-D-glucopyranoside (7). White solid; [α]D20 22 (c 0.33, MeOH); UV (MeOH) λmax: 201 and 281 nm; 1H NMR and 13C NMR data, see Table 3; HRESIMS [M + Na] + m/z 379.1361 (calcd for C17H22O2Na, 379.1363).
6'-O-Acetyl-isohomoarbutin (8). White amorphous powder; [α]D20 +10 [c 0.31, MeOH]; UV (MeOH) λmax 201, 214 and 283 nm; 1H NMR and 13C NMR data, see Table 3; HRESIMS [M + Na]+ m/z 351.1053 (calcd for C15H20O4Na, 351.1050).

Acromonin A (11). [α]D20 +138 [c 0.37, MeOH]; ECD {MeCN, λmax (Δε) c 0.71 mM} 286sh (+0.30), 227sh (+1.90), 200 (+13.49) nm.

Gliomastin E (aglycone of 7). [α]D20 +15 [c 0.05, MeOH]; ECD {MeCN, λmax (Δε) c 0.70 mM} 273 (+0.24), 247 (−0.26), 198 (±3.01) nm.

Acid hydrolysis

Compounds 6–8 (1 mg each) were separately hydrolysed with 2 N HCl (2.0 mL) at 90 °C for 4 h. After cooling, the solution was partitioned with EtOAc (3 mL × 3) and H2O. The EtOAc phase containing the aglycone was dried under reduced pressure. The water phase containing the sugar moiety, was then examined using TLC analysis with D-glucose as an authentic standard (Sigma-Aldrich, Germany). Two different eluting systems were used including DCM–MeOH–H2O (6 : 4 : 1) and EtOAc–MeOH–H2O (10 : 4 : 1), along with methanol–sulphuric acid as spraying reagent.

Cytotoxicity assay

The MTT method was performed to test the cytotoxicity of the compounds against the L5178Y mouse lymphoma cell line (European Collection of Authenticated Cell Cultures, Catalogue No. 8711908) as described previously.26 Kahalalide F was used as positive control and media with 0.1% DMSO was used as negative control.

Antibacterial assay

The antibacterial assay was performed using the broth microdilution method. Following the recommendations of the Clinical and Laboratory Standards Institute (CLSI), the MIC values against S. aureus ATCC 25923, S. aureus ATCC 700699, E. faecalis ATCC 29212, E. faecalis ATCC 51299, E. faecium ATCC 35667, E. faecium ATCC 700221 and Mycobacterium tuberculosis strain H37Rv were determined.27 Mexilofloxin (for S. aureus strains), ciprofloxacin (for E. faecalis and E. faecium strains) and rifampicin (for Mycobacterium tuberculosis strains) were used as positive controls.

Computational section

Mixed torsional/low-frequency mode conformational searches were carried out by means of the Macromodel 9.9.223 software using the Merck Molecular Force Field (MMFF) with an implicit solvent model for CHCl3.28 Geometry reoptimizations were carried out at the B3LYP/6-31G(d) level in vacuo, the B3LYP/6-31+G(d,p) level in vacuo, the B97D/TZVP level and the CAMB3LYP/TZVP levels with the PCM solvent model for MeCN or MeOH. TDDFT ECD calculations and OR calculations were run with various functionals (B3LYP, BH&HLYP, CAM-B3LYP, PBE0) and the TZVP basis set as implemented in the Gaussian 09 package with the same or no solvent model as in the preceding DFT optimization step.29 NMR calculations were performed at the mPW1PW91/6-311+G(2d,p) level.30 ECD spectra were generated as sums of Gaussians with 3000 and 3300 cm⁻¹ widths at half-height (corresponding to ca. 12 and 13 nm at 200 nm), using dipole-velocity-computed rotational strength values.31,32 Computed ECD spectra were shifted by –48 (B3LYP/TZVP for the B3LYP/6-31G(d) in vacuo conformers) for 1, +6 (PBE0/TZVP PCM/McCN for the B97D/TZVP PCM/McCN conformers) for 11 and –3 (B3LYP/TZVP for the B3LYP/6-31G(d) in vacuo conformers) for gliomastin E. Computed C-NMR data were corrected with I = 185.4855 and S = −1.0306 and H-NMR data with I = 31.8996 and S = −1.0734.33 Boltzmann distributions were estimated from the ZPVE-corrected B3LYP/6-31G(d) energies in the B3LYP/6-31G(d) gas-phase calculations, and from the uncorrected B3LYP/6-31+G(d,p), B97D/TZVP and CAM-B3LYP/TZVP energies in the other cases. The MOLEKEL software package was used for visualization of the results.34

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