Tetrahedron 65 (2009) 2898–2904

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Oxygenated cembranoids of the decaryiol type from the Indonesian soft coral *Lobophytum* sp.

Ernesto Fattorusso [a], Adriana Romano [a], Orazio Taglialatela-Scafati [a,*], Carlo Irace [b], Carmen Maffettone [b], Giorgio Bavestrello [c], Carlo Cerrano [d]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli 'Federico II', Via D. Montesano 49, 80131 Napoli, Italy
[b] Dipartimento di Farmacologia Sperimentale, Università di Napoli 'Federico II', Via D. Montesano 49, 80131 Napoli, Italy
[c] Dipartimento di Scienze del Mare, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
[d] Dipartimento per lo Studio del Territorio e delle sue Risorse, Università di Genova, Corso Europa 26, 16132 Genova, Italy

ARTICLE INFO

Article history:
Received 27 November 2008
Received in revised form 13 January 2009
Accepted 5 February 2009
Available online 11 February 2009

Keywords:
Soft corals
Cembranoids
Decaryiol
Growth inhibition

ABSTRACT

Three novel cembrane diterpenoids, decaryiols B–D (**5**–**7**), characterized by a bicyclic skeleton of the decaryiol-type, have been isolated from the Indonesian soft coral *Lobophytum* sp., along with three known cembranoids. The stereostructures of these metabolites have been established through extensive NMR spectroscopic analysis, application of the modified Mosher method, and chemical conversion. Cembranoids obtained from *Lobophytum* sp. (**2**–**7**) and six semisynthetic derivatives (**9**–**14**) prepared from decaryiol were tested for cell growth inhibitory activity against three different cell lines. *O*-Methyl decaryiol (**10**) exhibited a significant and selective activity against glioma cell lines.

1. Introduction

In the course of our ongoing screening for bioactive metabolites from marine organisms,[1–3] we have been recently studying the chemical composition of marine invertebrates from the Indonesian coasts,[4,5] held as one of the richest biodiversity hot spots of the oceans. In this context, we had the opportunity to analyze a specimen of the soft coral *Lobophytum* sp. (Alcyonacea, Alcyoniidae), which belongs to one of the dominant families of reef dwelling octocorals in the Indo-Pacific reefs.[6] From this organism, collected along the island of Siladen, in the Bunaken Marine Park of Manado (North Sulawesi, Indonesia), we have very recently reported the isolation of a complex heptacyclic alkaloid belonging to the zoanthamine class, named lobozoanthamine (**1**),[7] which is the first alkaloid from soft corals belonging to the genus *Lobophytum*. We have now investigated the terpene fraction of the organic extract obtained from this organism and a series of known (**2**–**4**) and new (**5**–**7**) cembranoids have been isolated. Herein we report the stereostructural characterization of the three new cembranoids decaryiols B–D (**5**–**7**); in addition, the cell growth inhibitory activity of compounds **2**–**7** and of six semisynthetic derivatives (**9**–**14**) has been evaluated against three different cell lines, including C6 and HeLa, two tumor cell lines.

2. Stereostructure elucidation of decaryiols B–D (5–7)

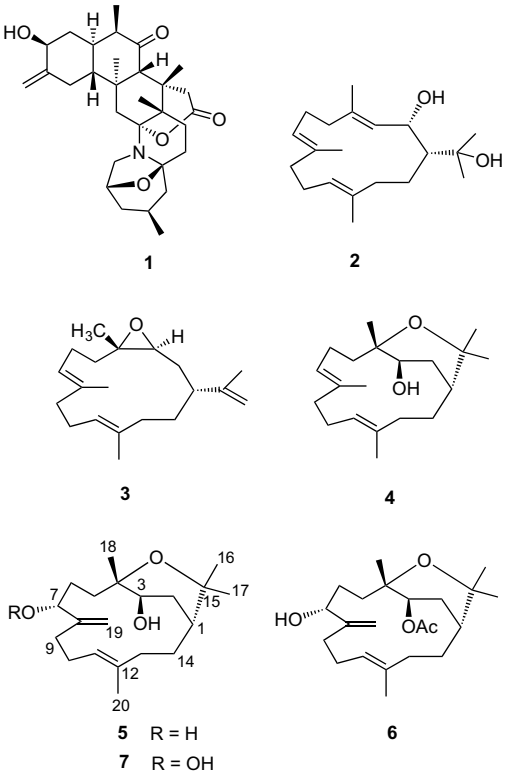
Colonies of *Lobophytum* sp. (750 g wet weight) have been repeatedly extracted with MeOH and $CHCl_3$ at room temperature and the obtained material has been partitioned between water and EtOAc. The organic extract (2.0 g) has been chromatographed by MPLC over silica gel eluting with a gradient system of increasing polarity from *n*-hexane to EtOAc, and the obtained fraction have been further purified by analytical HPLC (*n*-hexane/EtOAc mixtures). Fractions eluted with *n*-hexane/EtOAc 9:1 afforded epoxycembrene (**3**); fractions eluted with *n*-hexane/EtOAc 8:2 afforded 2-hydroxynephthenol (**2**) and decaryiol C (**6**); fractions eluted with *n*-hexane/EtOAc 7:3 contained pure decaryiol (**4**); fractions eluted with *n*-hexane/EtOAc 6:4 afforded decaryiol D (**7**), while fractions eluted with *n*-hexane/EtOAc 4:6 afforded decaryiol B (**5**).

The structures of the known metabolites 2-hydroxynephthenol (**2**),[8] epoxycembrene (**3**),[9] and decaryiol (**4**)[10] were assigned through the comparison of obtained spectral data with those reported in the literature.

Decaryiol B (**5**) was isolated as an optically active colorless amorphous solid, with the molecular formula $C_{20}H_{34}O_3$ deduced from HR-EIMS. ^1H ($CDCl_3$, Table 1) and ^{13}C ($CDCl_3$, Table 2) NMR

* Corresponding author. Tel.: +39 081 678509; fax: +39 081 678552.
E-mail address: scatagli@unina.it (O. Taglialatela-Scafati).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.02.008



spectra of **5** were analyzed with the help of a 2D HSQC NMR experiment, allowing the association of all the proton signals with those of the directly attached carbon atoms. Thus, decaryiol B (**5**) must include one geminally diprotonated double bond (δ_H 5.04, br s and 5.01, br s; δ_C 110.7), one monoprotonated double bond (δ_H 5.29, dd, J=10.2 and 4.1 Hz; δ_C 126.7), two oxygenated methines (δ_H 3.66, δ_C 71.5; δ_H 4.14, δ_C 78.8), and two unprotonated oxygenated carbon atoms (δ_C 75.6 and 75.9). These data, combined with the four degrees of unsaturation implied by the molecular formula, suggested a bicyclic structure for **5**. The 2D ^{1}H–^{1}H COSY NMR spectrum of **5** provided information to group the proton multiplets into the three spin systems *A*–*C* (highlighted in bold in Fig. 1). These subunits were connected with the help of some key 2D g-HMBC NMR cross-peaks (see Fig. 1). In particular, H-19a showed cross-peaks with C-9 and the sp^2 C-8, while H-19b showed cross-peaks with C-7 and C-8, thus joining the subunits *A* and *B*. Analogously, the ^2J and ^3J cross-peaks from H_3-20 (with C-12, C-11, and C-13) were useful to join subunits *B* and *C*, while the ^2J and ^3J cross-peaks from H_3-18 (with C-4, C-3, and C-5) indicated the oxygenated C-4 as a connection point between subunits *A* and *C*. A 14-membered ring skeleton, typical of cembrane diterpenoids, was thus defined. Finally, the series of cross-peaks exhibited by H_3-16 and H_3-17 indicated that both C-16 and C-17 must be linked at the unprotonated and oxygenated C-15, which is in turn attached at C-1.

At this stage, in order to define the planar structure of decaryiol B (**5**) two data needed to be taken into account: (i) the presence of four oxygenated carbons (^{13}C NMR data) with only three oxygen atoms in the molecular formula, (ii) the presence of a fourth unsaturation degree. Both these evidences could be explained with the presence in **5** of an ether bridge connecting two of the oxygenated carbons and two hydroxyl groups. In order to define the carbon atoms linked to these functionalities, an aliquot of decaryiol B (**5**) was subjected to acetylation reaction in standard conditions, obtaining the diacetylated compound **8** (Scheme 1). Analysis of 1D and 2D NMR spectra for compound **8** revealed that acetylation had

Table 1
^{1}H (500 MHz) NMR data of decaryiol B (**5**), C (**6**), and D (**7**) in $CDCl_3$

Pos.	**5**	**6**	**7**
	δ_H, mult., J in Hz	δ_H, mult., J in Hz	δ_H, mult., J in Hz
1	1.29, m	1.34, m	1.29, m
2a	2.38[a]	2.46[a]	2.39[a]
2b	2.35[a]	2.41[a]	2.33[a]
3	3.66, dd, 8.7, 2.1	4.81, dd, 9.1, 2.3	3.65, dd, 8.3, 2.5
5a	1.33, m	1.33, m	1.36, m
5b	1.08, m	1.16, m	1.07, br dd, 13.3, 7.5
6a	1.71, m	1.76, m	1.69[a]
6b	1.67, m	1.69, m	1.66[a]
7	4.14, dd, 9.9, 4.1	4.13, dd, 9.9, 4.1	4.34, dd, 10.7, 3.8
9a	2.33[a]	2.30[a]	2.24[a]
9b	2.11, m	2.19, m	2.10[a]
10a	2.50, m	2.50, m	2.50, m
10b	2.22, m	2.23, m	2.25[a]
11	5.29, dd, 10.2, 4.1	5.30, d, 10.2, 4.1	5.26, dd, 10.2, 4.1
13a	2.08[a]	2.06[a]	2.06[a]
13b	2.05[a]	2.00[a]	1.90, br dd, 13.3, 10.5
14a	1.79, m	1.80, m	1.61, m
14b	1.64, m	1.74, m	1.49, m
16	1.22, s	1.25, s	1.20, s
17	1.19, s	1.15, s	1.15, s
18	1.18, s	1.18, s	1.14, s
19a	5.04, br s	5.04, br s	5.19, br s
19b	5.01, br s	5.00, br s	5.12, br s
20	1.61, s	1.60, s	1.61, s
–OAc		2.08, s	
–OOH			7.78, s

[a] Overlapped with other signals.

Table 2
^{13}C (125 MHz) NMR data of decaryiol B (**5**), C (**6**), and D (**7**)[a]

Pos.	**5**	**6**	**7**
	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	35.0, CH	34.9, CH	34.9, CH
2	32.3, CH_2	33.0, CH_2	31.4, CH_2
3	71.5, CH	75.9, CH	71.3, CH
4	75.6, C	76.1, C	75.6, C
5	36.6, CH_2	34.7, CH_2	36.4, CH_2
6	28.9, CH_2	37.0, CH_2	31.2, CH_2
7	78.8, CH	78.8, CH	91.2, CH
8	154.0, C	154.1, C	150.0, C
9	29.7, CH_2	30.1, CH_2	31.0, CH_2
10	31.5, CH_2	32.2, CH_2	34.2, CH_2
11	126.7, CH	126.8, CH	126.6, CH
12	134.7, C	134.0, C	135.3, C
13	36.9, CH_2	36.8, CH_2	36.9, CH_2
14	28.6, CH_2	32.2, CH_2	29.3, CH_2
15	75.9, C	70.0, C	75.9, C
16	24.0, CH_3	23.1, CH_3	25.7, CH_3
17	31.1, CH_3	28.8, CH_3	30.9, CH_3
18	25.9, CH_3	24.5, CH_3	23.8, CH_3
19	110.7, CH_2	110.0, CH_2	110.3, CH_2
20	15.6, CH_3	16.2, CH_3	16.8, CH_3
–OAc		170.9, C	
		21.0, CH_3	

[a] Data taken in $CDCl_3$.

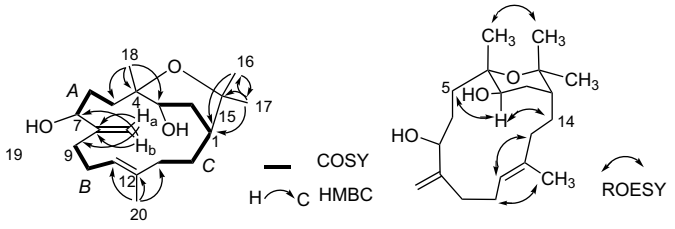
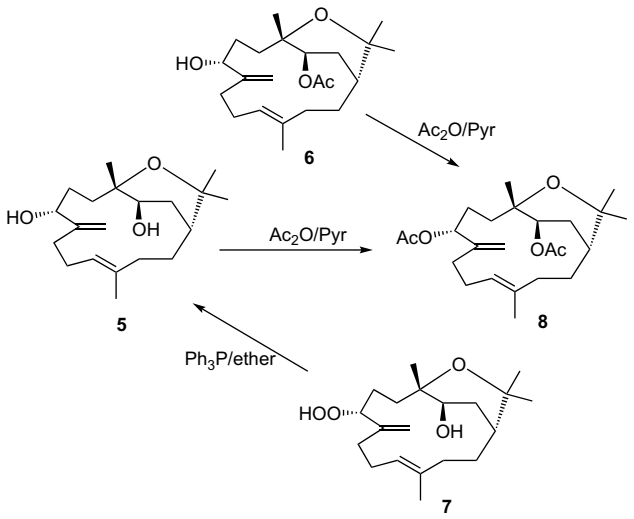


Figure 1. COSY, $^{2,3}J_{H\rightarrow C}$ HMBC, and ROESY correlations for decaryiol B (**5**).



Scheme 1. Chemical transformations used to deduce the absolute configuration of decaryiols C (**6**) and D (**7**).

occurred at both C-3 and C-7 hydroxyls, indicating, consequently, that C-4 and C-15 must be connected by the ether linkage.

The *E* geometry of the Δ^{11} double bond and the relative configuration around the oxygen-containing six-membered ring were deduced based on the ROESY cross-peaks indicated in Figure 1. In particular, the axial H-3 (dd, J=8.7, 2.1 Hz) correlated with both H_2-14 and H_2-5.

The presence of two secondary alcohol groups within the structure of decaryiol B (**5**) offered the possibility to determine its absolute configuration through application of the Mosher's method.[11] Thus, treatment of **5** with (−)-(*R*)- and (+)-(*S*)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) chloride in dry pyridine and DMAP afforded the (*S*)-MTPA diester **5a** and the (*R*)-MTPA diester **5b**, respectively (Scheme 2). Analysis of the $\Delta\delta$ ($\delta_S-\delta_R$) values according to the Mosher's method in the light of the Riguera analysis for 1,*n*-diols[12] assigned the *R* configuration at both C-7 and C-3. Absolute configuration at C-1 and C-4 was consequently deduced on the basis of the assigned relative geometry around the six-membered ring and, therefore, the complete stereostructure of decaryiol B (**5**) was established.

Decaryiol C (**6**), $C_{22}H_{36}O_4$ from HR-EIMS, was isolated as a colorless amorphous solid. ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectroscopic data of **6** were quite similar to those of decaryiol B (**5**) and, in agreement with molecular formula, the single difference appeared to be the presence of an acetyl unit (δ_H 2.08, s; δ_C 21.0 and 170.9) in the structure **6**. Interpretation of 2D NMR spectra (COSY, HSQC, and g-HMBC) allowed the complete assignment of the NMR data and identified C-3 as the site of attachment for the acetyl unit (HMBC cross-peak from H-3 to the acetyl carbonyl). Accordingly, a downfield shift for H-3 (δ_H 4.81 instead of 3.66) and surrounding protons (for H_2-2, δ_H 2.46 and 2.41 instead of 2.38 and 2.35) was observed.

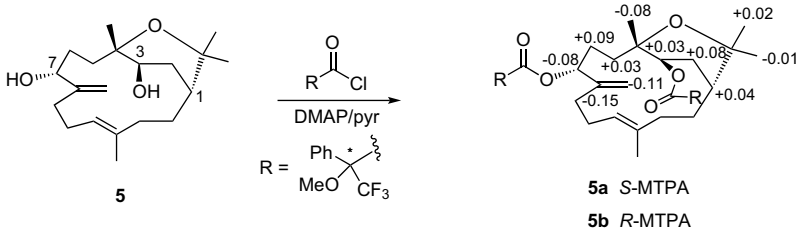
Decaryiol D (**7**) was isolated as a colorless solid with the molecular formula $C_{20}H_{34}O_4$, containing an additional oxygen atom compared to that of decaryiol B (**5**). This difference was explained by the presence of a hydroperoxyl group in place of the hydroxyl group at C-7 on the basis of the following spectral evidence: (i) decaryiol B (**5**) and D (**7**) featured parallel spin systems with very similar proton resonances (Table 1) except for H-7 (δ_H 4.34 in **7** instead of 4.14 in **5**); (ii) ^{13}C NMR spectra of the two molecules (Table 2) were practically superimposable apart from a marked downfield shift at C-7 (δ_C 91.2 in **7** instead of 78.8 in **5**); (iii) finally, g-HMBC spectrum of **7**, allowing the attachment of all the partial moieties, confirmed the assignment of decaryiol D planar structure as the 7-hydroperoxyl analogue of decaryiol B.

Absolute configurations at the stereogenic carbons of decaryiol C (**6**) and D (**7**) were assigned as those of the corresponding carbons of decaryiol B (**5**) on the basis of the transformations depicted in Scheme 1. Briefly, upon treatment with Ac_2O/pyridine, decaryiol C (**6**) gave the same diacetyl derivative **8** previously obtained from **5**, while treatment of decaryiol D (**7**) with triphenylphosphine in ether afforded decaryiol B (**5**).

3. Semisynthetic derivatives of decaryiol (4) and pharmacological activity

Diterpenoids of the cembrane family constitute a large group of natural products, isolated from both marine and terrestrial sources. In particular, the occurrence of cembranoids in gorgonians and alcyonarians (soft corals) of the genera *Lobophytum*, *Sinularia*, *Sarcophyton*, and *Clavularia* has been recently reported in an increasing number of examples.[13–15] A chemical defense role against other reef organisms[16] has been attributed to these molecules and significant anti-inflammatory,[17] cytotoxic,[18] and antimicrobial[19] activities have been discovered for members of the cembrane class. In this context, a few years ago, Koenig and coworkers reported that decaryiol (**4**) exhibited a concentration-dependent inhibition of cell growth[20] through a specific arrest of the cell cycle in the G2/M phase. Therefore, the isolation of three new decaryiol analogues during this work appeared to be particularly interesting, since it allowed exploration of the effect on the growth inhibition activity of the structural changes shown by **5**–**7**.

Thus, the cembranoids obtained during the present study (**2**–**7**) have been evaluated for cell growth inhibitory activity against three different cell lines, H9c2 (cardiac myoblasts), C6 (glioma), and HeLa (epithelial carcinoma), the last two being tumor cell lines. Results obtained (expressed as IC_{50} in μM) are reported in Table 3. Although decaryiol (**4**) had been reported to be strongly active against the HM02 (gastric adenocarcinoma) cell line,[20] it proved to be practically inactive against the two tumor cell lines chosen for our investigation and only moderately active against the H9c2 cell line. On the other hand, one of the new molecules isolated during the present study, namely decaryiol D (**7**), was significantly active against the C6 glioma cell line. The inactivity of the closely related decaryiol B (**5**) is noteworthy and seems to indicate that the growth inhibitory activity of decaryiol D (**7**) should be attributed



Scheme 2. Application of the modified Mosher's method for secondary alcohols on the MTPA esters of decaryiol B (**5a** and **5b**). $\Delta\delta$ ($\delta_S-\delta_R$) are given in parts per million.

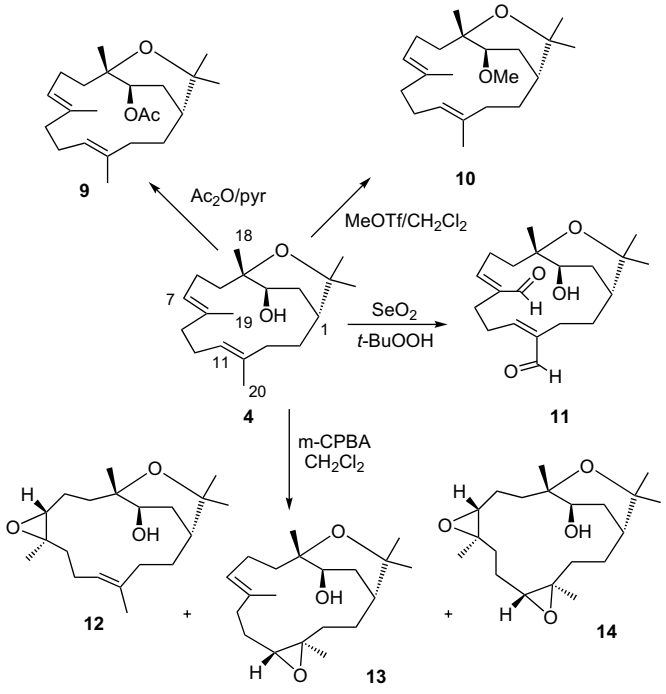
Table 3
Cell growth inhibition[a]

Compounds	IC_{50}, μM (C6 cell line)	IC_{50}, μM (H9c2 cell line)	IC_{50}, μM (HeLa cell line)
2-Hydroxynephthenol (**2**)	n.a.[b]	170±20	n.a.
Epoxycembrene (**3**)	44±5	125±12	n.a.
Decaryiol (**4**)	n.a.	85±15	n.a.
Decaryiol B (**5**)	n.a.	n.a.	n.a.
Decaryiol C (**6**)	n.a.	n.a.	n.a.
Decaryiol D (**7**)	40±3	150±15	n.a.
Decaryiol acetate (**9**)	56±5	165±15	190±14
Methyl decaryiol (**10**)	8±2	120±20	n.a.
Decaryiol dialdehyde (**11**)	n.a.	n.a.	n.a.
Decaryiol Epoxides (**12**–**14**)	n.a.	n.a.	n.a.

[a] IC_{50} values are expressed as mean±SEM, (n=24).
[b] n.a. indicates not active (IC_{50}≥200 μM).

exclusively to the presence of the hydroperoxy functionality in this molecule.

The availability of relatively high amounts of decaryiol (about 100 mg) constituted an attractive chance to further investigate this point and to extend the structure–activity relationships through the preparation of semisynthetic analogues. To this aim, decaryiol (**4**) was subjected to the reactions summarized in Scheme 3 to obtain derivatives **9**–**14**. Thus, treatment with Ac_2O/pyr afforded decaryiol acetate **9**, while treatment with methyl triflate provided *O*-methyl decaryiol **10**. Selenium dioxide/*tert*-butylhydroperoxide[21] achieved chemoselective oxidation of the allylic methyls affording the dialdehyde derivative **11** as the major product, while the monoepoxide derivatives **12** and **13** and the diepoxide **14** were obtained as major compounds upon treatment with *meta*-chloroperbenzoic acid (minor diastereomers of **12**–**14** were not characterized). The structures of these semisynthetic derivatives were secured through MS and NMR (1D and 2D) spectroscopic analysis; assignment of their NMR resonances is reported in the Experimental section. In particular, the relative orientation of the newly created stereogenic centers of the epoxidic derivatives **12**–**14** was assigned on the basis of some diagnostic ROESY cross-peaks (H_2-6/H_3-19 for **12** and **14**; H_2-10/H_3-20 for **13** and **14**; H-7/H-11 for **14**; H-7/H_3-18 for **12** and **14**).



Scheme 3. Preparation of semisynthetic derivatives of decaryiol.

The semi-synthetic cembranoids **9**–**14** have been then evaluated for cell growth inhibitory activity against the same cell lines (H9c2, C6, and HeLa, Table 3). Surprisingly, one of these derivatives, namely *O*-methyl decaryiol (**10**), proved to be significantly active against C6, glioma cell line, with an IC_{50} of 8 μM and an interesting selectivity, since it was practically inactive against HeLa and more than ten times less active against the non-tumor H9c2 cell line.

These results indicate that also subtle structural changes on the macrocyclic cembranoid scaffold of decaryiol can dramatically affect the activity and the selectivity as cell growth inhibitors. In view of its selective action and of the reported resistance of glioma cell lines to chemotherapy,[22] the activity of *O*-methyl decaryiol (**10**) would deserve further investigation.

4. Experimental section

4.1. General experimental procedures

Optical rotations ($CHCl_3$) were measured at 589 nm on a Perkin–Elmer 192 polarimeter. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^1H connectivities were determined by the COSY experiment; one-bond heteronuclear ^1H–^{13}C connectivities by the HSQC experiment; two- and three-bond ^1H–^{13}C connectivities by gradient-HMBC experiments optimized for a $^{2,3}J$ of 9 Hz. Through-space ^1H connectivities were evidenced by using a ROESY experiment with a mixing time of 500 ms. ESI-MS spectra were performed on a LCQ Finnigan MAT mass spectrometer. Medium pressure liquid chromatography was performed on a Büchi apparatus using a silica gel (230–400 mesh) column; HPLC were achieved on a Knauer apparatus equipped with a refractive index detector and analytical LUNA (Phenomenex) SI60 (250×4 mm) columns.

4.2. Animal material, extraction and isolation

Colonies of *Lobophytum* sp. (750 g wet weight) were collected in March 2006 in the Bunaken Marine Park of Manado along the coasts of the small island of Siladen (North Sulawesi, Indonesia) at a depth of 2–5 m. A small voucher sample is deposited at the Dipartimento per lo Studio del Territorio e delle sue Risorse, Università di Genova. The colonies were repeatedly extracted with MeOH and $CHCl_3$ at room temperature. The obtained material was partitioned between water and EtOAc and the organic extract (2.0 g) chromatographed by MPLC over silica gel using an eluent gradient system of increasing polarity from *n*-hexane to EtOAc. Fractions eluted with *n*-hexane/EtOAc 9:1 were further purified by HPLC (*n*-hexane/EtOAc 9: 1) to afford epoxycembrene (**3**, 6.2 mg). Fractions eluted with *n*-hexane/EtOAc 8:2 were further purified by HPLC (*n*-hexane/EtOAc 75: 25) to afford 2-hydroxynephthenol (**2**, 4.5 mg) and decaryiol C (**6**, 1.8 mg). Fractions eluted with *n*-hexane/EtOAc 7:3 contained pure decaryiol (**4**, 105.0 mg); fractions eluted with *n*-hexane/EtOAc 6:4, further purified by HPLC (*n*-hexane/EtOAc 7:3), afforded decaryiol D (**7**, 2.8 mg), while fractions eluted with *n*-hexane/EtOAc 4:6, further purified by HPLC (*n*-hexane/EtOAc 1:1), afforded decaryiol B (**5**, 3.2 mg).

4.3. Decaryiol B (5)

Colorless amorphous solid, mp 75–76 °C; $[\alpha]_D$ −13.4 (c 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 2; (+) ESI-MS m/z 345 $[M+Na]^+$; HREI-MS found 322.2518, $C_{20}H_{34}O_3$ requires 322.2508.

4.4. Acetylation of decaryiols B (5) and C (6) to the diacetate 8

Compound **5** (1.0 mg, 0.0031 mmol) was dissolved in dry pyridine (0.2 mL) and treated with Ac_2O (0.3 mL). After standing overnight, the reaction was worked up by addition of methanol, water (ca. 1 mL), and EtOAc (ca. 2 mL). The organic phase was washed sequentially with 2 M H_2SO_4, satd $NaHCO_3$, and brine. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by HPLC (*n*-hexane/EtOAc 85:15) to afford the diacetate **8** (1.1 mg, 0.0027 mmol, 87%). When the same procedure was applied to decaryiol C (**6**, 0.9 mg), we obtained 0.9 mg of the same diacetate **8**. Colorless amorphous solid, mp 80–81 °C; $[\alpha]_D$ −10.5 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.30 (1H, dd *J* 10.2, 4.1, H-11), 5.19 (1H, br s, H-19a), 5.15 (1H, br s, H-19b), 4.92 (1H, dd, *J* 9.5, 3.8, H-7), 4.85 (1H, dd, *J* 9.1, 2.3, H-3), 2.50 (1H, ov, H-10a), 2.45 (1H, ov, H-2a), 2.43 (1H, ov, H-2b), 2.41 (1H, ov, H-9a), 2.37 (1H, ov, H-9b), 2.33 (1H, ov, H-10b), 2.10 (3H, s, OAc), 2.08 (3H, s, OAc), 2.06 (1H, ov, H-13a), 2.02 (1H, ov, H-13b), 1.92 (1H, m, H-6a), 1.80 (1H, m, H-14a), 1.75 (1H, ov, H-6b), 1.74 (1H, ov, H-14b), 1.60 (3H, s, H-20), 1.42 (1H, m, H-5a), 1.35 (1H, m, H-1), 1.25 (1H, m, H-5b), 1.25 (3H, s, H-16), 1.19 (3H, s, H-18), 1.15 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 170.9 (OAc), 170.7 (OAc), 155.2 (C-8), 134.0 (C-12), 126.8 (C-11), 111.0 (C-19), 79.5 (C-7), 76.3 (C-4), 76.0 (C-3), 71.0 (C-15), 37.4 (C-6), 34.8 (C-1), 34.7 (C-5), 33.0 (C-2), 32.2 (C-10), 32.2 (C-14), 28.8 (C-17), 24.6 (C-18), 23.1 (C-16), 21.0 (OAc×2), 16.3 (C-20); ESI-MS *m*/*z* 429 $[M+Na]^+$. HREI-MS found 406.2730; $C_{24}H_{38}O_5$ requires 406.2719.

4.5. Formation of MTPA diesters 5a and 5b

A sample of **5** (0.8 mg, 0.0025 mmol) was dissolved in dry pyridine (1 mL) and then (−)-(*R*)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) chloride (5 μL) and a spatula tip of DMAP were added to the solution, which was then stirred overnight at room temperature. After dilution with EtOAc, the solution was evaporated in vacuo and the residue purified by HPLC (SI60, EtOAc/*n*-hexane 1:9) to obtain the *S*-MTPA diester **5a** (1.0 mg, 0.0013, 53%). Colorless amorphous solid; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 7.53 (4H, m, MTPA phenyl), 7.41 (6H, m, MTPA phenyl), 5.30 (1H, dd, *J* 10.2, 4.1, H-11), 5.22 (1H, br s, H-19a), 5.15 (1H, br s, H-19b), 5.01 (1H, dd, *J* 9.5, 3.8, H-7), 4.92 (1H, dd, *J* 9.1, 2.3, H-3), 3.55 (6H, s, 2×OMe), 2.55 (1H, ov, H-2a), 2.50 (1H, ov, H-10a), 2.49 (1H, ov, H-9a), 2.48 (1H, ov, H-2b), 2.35 (1H, ov, H-9b), 2.30 (1H, ov, H-10b), 2.05 (2H, m, H-13), 1.98 (1H, m, H-6a), 1.80 (1H, ov, H-14a), 1.80 (1H, ov, H-6b), 1.75 (1H, ov, H-14b), 1.60 (3H, s, H-20), 1.42 (1H, m, H-5a), 1.40 (1H, m, H-1), 1.30 (1H, m, H-5b), 1.25 (3H, s, H-16), 1.20 (3H, s, H-18), 1.15 (3H, s, H-17); ESI-MS *m*/*z* 777 $[M+Na]^+$. When a second sample was treated with (+)-(*S*)-MTPA chloride and subjected to the same procedure, the *R*-MTPA diester **5b** (0.9 mg, 0.012 mmol, 48%) was obtained. Colorless amorphous solid; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 7.53 (4H, m, MTPA phenyl), 7.38 (6H, m, MTPA phenyl), 5.33 (1H, ov, H-19a), 5.30 (1H, ov, H-11), 5.25 (1H, br s, H-19b), 5.09 (1H, dd, *J* 9.5, 3.8, H-7), 4.89 (1H, dd, *J* 9.1, 2.3, H-3), 3.58 (6H, s, 2×OMe), 2.64 (1H, m, H-9a), 2.50 (1H, ov, H-10a), 2.47 (1H, ov, H-2a), 2.40 (1H, ov, H-2b), 2.40 (1H, ov, H-9b), 2.30 (1H, ov, H-10b), 2.05 (2H, m, H-13), 1.89 (1H, m, H-6a), 1.80 (1H, ov, H-14a), 1.75 (1H, ov, H-14b), 1.72 (1H, ov, H-6b), 1.60 (3H, s, H-20), 1.39 (1H, m, H-5a), 1.36 (1H, m, H-1), 1.27 (1H, m, H-5b), 1.28 (3H, s, H-18), 1.23 (3H, s, H-16), 1.16 (3H, s, H-17); ESI-MS *m*/*z* 777 $[M+Na]^+$.

4.6. Decaryiol C (6)

Colorless amorphous solid, mp 86–87 °C; $[\alpha]_D$ +2.2 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 2; (+) ESI-MS *m*/*z* 387 $[M+Na]^+$; HREI-MS found 364.2627, $C_{22}H_{36}O_4$ requires 364.2614.

4.7. Decaryiol D (7)

Colorless amorphous solid; $[\alpha]_D$ −7.0 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 2; (+) ESI-MS *m*/*z* 361 $[M+Na]^+$; HREI-MS found 338.2466, $C_{20}H_{34}O_4$ requires 338.2457.

4.8. Reduction of decaryiol D (7) to decaryiol B (5)

Compound **7** (1.0 mg, 0.0030 mmol) and triphenylphosphine (2.0 mg) were dissolved in ether (1 mL) and stirred at room temperature for 6 h. Then, after removal of the solvent, the residue was purified by silica gel HPLC (SI60, *n*-hexane/EtOAc 55:45) to give pure decaryiol B (**5**, 0.7 mg, 0.0022 mmol, 72%).

4.9. Acetylation of decaryiol (4)

Compound **4** (6.0 mg, 0.019 mmol) was dissolved in dry pyridine (0.25 mL) and treated with Ac_2O (0.25 mL). After standing overnight, the reaction was worked up as above described for decaryiol B to obtain decaryiol acetate (**9**, 5.8 mg, 0.017 mmol, 90%). Colorless amorphous solid, mp 83–84 °C; $[\alpha]_D$ +31.0 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.49 (1H, dd, *J* 11.5, 5.8, H-3), 5.34 (1H, d, *J* 9.5, H-7), 5.04 (1H, d, *J* 11.1, H-11), 2.60 (1H, m, H-6a), 2.50 (1H, m, H-10a), 2.33 (2H, m, H-9), 2.05 (1H, overlapped, H-10b), 2.03 (2H, m, H-13), 2.00 (3H, s, OAc), 1.86 (1H, overlapped, H-6b), 1.84 (1H, overlapped, H-5a), 1.82 (1H, overlapped, H-2a), 1.57 (3H, s, H-19), 1.56 (3H, s, H-20), 1.56 (1H, overlapped, H-5b), 1.55 (1H, overlapped, H-1), 1.30 (1H, overlapped, H-14a), 1.28 (1H, overlapped, H-2b), 1.16 (3H, s, H-16), 1.12 (3H, s, H-18), 1.10 (3H, s, H-17), 0.89 (1H, m, H-14b); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 170.8 (OAc), 134.0 (C-12), 133.1 (C-8), 127.7 (C-7), 126.9 (C-11), 76.3 (C-4), 76.1 (C-3), 71.1 (C-15), 38.7 (C-5), 36.6 (C-9), 34.8 (C-1), 33.0 (C-2), 32.1 (C-14), 28.8 (C-17), 25.2 (C-10), 24.6 (C-18), 23.8 (C-6), 23.1 (C-16), 21.0 (OAc), 16.3 (C-20), 15.2 (C-19); ESI-MS *m*/*z* 371 $[M+Na]^+$. HREI-MS found 348.2666; $C_{22}H_{36}O_3$ requires 348.2664.

4.10. Methylation of decaryiol (4)

Decaryiol (**4**, 34.0 mg, 0.11 mmol) was dissolved in dry CH_2Cl_2 and an excess of 2,6-di-*tert*-butylpyridine (150 μL) and methyl triflate (60 μL) were added. The reaction mixture was left under stirring at 0 °C overnight and then partitioned against satd $NaHCO_3$ solution. The organic phase, dried over Na_2SO_4, was purified by HPLC (SI60 *n*-hexane/EtOAc 87:13) affording *O*-methyl decaryiol (**10**, 28.0 mg, 0.087 mmol, 79%) in the pure state. Colorless amorphous solid, mp 77–78 °C; $[\alpha]_D$ +22.0 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.25 (1H, d, *J* 9.2, H-7), 4.87 (1H, d, *J* 10.4, H-11), 3.77 (1H, dd, *J* 11.8, 5.4, H-3), 3.40 (3H, s, OMe), 2.61 (1H, m, H-6a), 2.51 (1H, m, H-10a), 2.33 (2H, m, H-9), 2.04 (1H, overlapped, H-10b), 2.03 (2H, m, H-13), 1.89 (1H, overlapped, H-5a), 1.89 (1H, overlapped, H-6b), 1.87 (1H, overlapped, H-2a), 1.58 (3H, s, H-19), 1.57 (3H, s, H-20), 1.53 (1H, overlapped, H-5b), 1.51 (1H, overlapped, H-1), 1.28 (1H, overlapped, H-2b), 1.16 (3H, s, H-16), 1.14 (1H, overlapped, H-14a), 1.12 (3H, s, H-18), 1.10 (3H, s, H-17), 0.93 (1H, m, H-14b); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 134.0 (C-12), 133.3 (C-8), 127.6 (C-7), 126.9 (C-11), 76.3 (C-4), 75.1 (C-3), 71.1 (C-15), 52.1 (OMe), 38.2 (C-5), 36.6 (C-9), 34.4 (C-1), 32.1 (C-2), 32.0 (C-14), 28.8 (C-17), 25.2 (C-10), 24.6 (C-18), 23.3 (C-6), 23.1 (C-16), 16.3 (C-20), 15.2 (C-19); ESI-MS *m*/*z* 343 $[M+Na]^+$. HREI-MS found 320.2725; $C_{21}H_{36}O_2$ requires 320.2715.

4.11. Allylic oxidation of decaryiol (4)

SeO_2 (24.4 mg) was added to dry CH_2Cl_2 (300 μL) and *tert*-butylhydroperoxide (100 μL) and left under stirring for 30 min at

room temperature. Then, decaryiol (**4**, 25 mg, 0.082 mmol) was added to the solution and the reaction mixture left under stirring at room temperature for 24 h. Then, the mixture was partitioned between water and ethylacetate and the organic phase has been purified by HPLC (*n*-hexane/EtOac 1:1) to obtain as major product decaryiol dialdehyde **11** (14 mg, 0.042 mmol, 51%). Colorless amorphous solid; $[\alpha]_D$ +15.0 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 10.21 (1H, s, H-19), 10.02 (1H, s, H-20), 6.73 (1H, br d, *J* 9.2, H-7), 6.17 (1H, br d, *J* 10.4, H-11), 3.85 (1H, overlapped, H-10a), 3.82 (1H, overlapped, H-3), 3.45 (1H, m, H-6a), 2.84 (1H, m, H-9a), 2.77 (1H, m, H-13a), 2.41 (1H, br d, *J* 8.5, H-10b), 2.30 (2H, overlapped, H-9b), 2.30 (1H, overlapped, H-6b), 2.00 (1H, m, H-5a), 1.77 (1H, overlapped, H-2a), 1.73 (1H, overlapped, H-13), 1.71 (1H, overlapped, H-5b), 1.44 (1H, overlapped, H-1), 1.42 (1H, overlapped, H-2b), 1.26 (1H, m, H-14a), 1.22 (3H, s, H-16), 1.20 (3H, s, H-17), 1.10 (3H, s, H-18), 1.03 (1H, m, H-14b); ESI-MS *m*/*z* 357 $[M+Na]^+$. HREI-MS found 334.2139; $C_{20}H_{30}O_4$ requires 334.2144.

4.12. Epoxidation of decaryiol (4)

Decaryiol (**4**, 29 mg, 0.1 mmol) was dissolved in dry CH_2Cl_2 (400 μL) and an excess of *m*-chloroperbenzoic acid (1.63 mmol) was added to the solution, which was left under stirring overnight at room temperature. The reaction mixture was then partitioned between $CHCl_3$ and satd $NaHCO_3$ aqueous solution. The organic phase was then purified by HPLC (SI60 *n*-hexane/EtOAc 6:4) to obtain decaryiol-7,8-epoxide (**12**, 5.2 mg, 0.016 mmol, 16%), decaryiol-11,12-epoxide (**13**, 3.4 mg, 0.011 mmol, 11%), and decaryiol diepoxide (**14**, 3.8 mg, 0.011 mmol, 11%). *Decaryiol-7,8-epoxide (**12**)*. Colorless amorphous solid; $[\alpha]_D$ +29.0 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.02 (1H, d, *J* 10.4, H-11), 3.67 (1H, dd, *J* 11.8, 7.7, H-3), 2.82 (1H, d, *J* 6.7, H-7), 2.45 (1H, m, H-10a), 2.12 (1H, overlapped, H-10b), 2.11 (2H, overlapped, H-13), 1.98 (1H, m, H-2a), 1.89 (1H, m, H-6a), 1.86 (1H, overlapped, H-5a), 1.66 (1H, overlapped, H-6b), 1.64 (1H, overlapped, H-5b), 1.61 (3H, s, H-20), 1.60 (1H, overlapped, H-1), 1.45 (2H, m, H-9), 1.45 (1H, overlapped, H-14a), 1.34 (1H, m, H-2b), 1.25 (3H, s, H-19), 1.19 (3H, s, H-16), 1.17 (3H, s, H-18), 1.14 (3H, s, H-17), 1.02 (1H, m, H-14b); ESI-MS *m*/*z* 345 $[M+Na]^+$. *Decaryiol-11,12-epoxide (**13**)*. Colorless amorphous solid; $[\alpha]_D$ +17.0 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.54 (1H, br d, *J* 7.0, H-7), 4.10 (1H, dd, *J* 10.8, 6.0, H-3), 2.56 (1H, d, *J* 8.9, H-11), 2.44 (1H, m, H-6a), 2.32 (2H, m, H-9), 2.15 (1H, overlapped, H-6b), 2.02 (2H, overlapped, H-13), 1.79 (1H, m, H-5a), 1.70 (1H, overlapped, H-2a), 1.65 (3H, s, H-19), 1.64 (1H, overlapped, H-5b), 1.62 (1H, overlapped, H-10a), 1.55 (1H, overlapped, H-10b), 1.53 (1H, overlapped, H-1), 1.45 (1H, overlapped, H-14a), 1.34 (1H, m, H-2b), 1.28 (3H, s, H-20), 1.24 (3H, s, H-18), 1.17 (3H, s, H-16), 1.11 (3H, s, H-17), 1.02 (1H, m, H-14b); ESI-MS *m*/*z* 345 $[M+Na]^+$. *Decaryiol diepoxide (**14**)*. Colorless amorphous solid; $[\alpha]_D$ +31.0 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.73 (1H, dd, *J* 10.3, 5.2, H-3), 2.97 (1H, d, *J* 6.8, H-7), 2.65 (1H, d, *J* 7.2, H-11), 2.23 (1H, m, H-9a), 2.08 (2H, m, H-13), 1.98 (1H, overlapped, H-10a), 1.97 (1H, overlapped, H-6a), 1.86 (1H, overlapped, H-5a), 1.84 (1H, m, H-2a), 1.79 (1H, overlapped, H-10b), 1.66 (1H, overlapped, H-6b), 1.62 (1H, overlapped, H-9b), 1.61 (1H, overlapped, H-5b), 1.48 (1H, overlapped, H-14a), 1.43 (1H, overlapped, H-2b), 1.41 (1H, overlapped, H-1), 1.32 (3H, s, H-20), 1.30 (3H, s, H-19), 1.23 (3H, s, H-16), 1.20 (3H, s, H-18), 1.15 (3H, s, H-17), 1.08 (1H, m, H-14b); ESI-MS *m*/*z* 361 $[M+Na]^+$.

4.13. Cell culture and evaluation of cell growth inhibitory activity

Inhibitory activity of compounds **2**–**7** and **9**–**14** was investigated on rat glioma cells (C6), HeLa (epithelial carcinoma), and H9c2 (cardiac myoblasts) by evaluation of cell growth and viability. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing high glucose (4.5 g/L) and supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere. Cells were washed, collected by trypsine and then inoculated in a 96-microwell culture plates at density of 10^4 cells/well. Cells were allowed to grow for 24 h, then the medium was replaced with fresh medium and cells were treated for further 48 h with test compounds. In detail, 1 or 2 μL of DMSO solutions containing the test compounds at different concentrations were added to cell culture medium; 1 or 2 μL of DMSO alone (vehicle) were added into control cells. After incubations, the medium was removed and the cells were washed twice with PBS buffer solution and then incubated with a trypsin-EDTA solution at 37 °C for 5 min. Trypsin was inactivated by re-suspending the cells in medium containing 10% FBS. The cells were pelleted and re-suspended in PBS. Viable cells, cells that excluded 0.4% trypan blue, were then counted with a hemocytometer. Concurrently, cell viability was evaluated with an MTT assay procedure, which measures the level of mitochondrial dehydrogenase activity using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) as substrate.[23] After treatments with the test compounds, the medium was removed and the cells were incubated with 20 μL/well of an MTT solution (5 mg/mL) for 1 h in a humidified 5% CO_2 incubator at 37 °C. The incubation was stopped by removing the MTT solution and adding 100 μL/well of DMSO to solubilize the formazan.[24] The absorbance was monitored at 550 nm by using a Perkin–Elmer LS 55 Luminescence Spectrometer. Statistical significance among the means was determined by the ANOVA followed by the Newman–Keuls test. The calculation of the concentration of the test compounds required to inhibit the net increase in the 48 h cell count and viability by 50% (IC_{50}) is based on plots of data carried out in triplicate and repeated three times. IC_{50} values are expressed as mean±SEM.

Acknowledgements

This research was partially conduced during the Master Course 'Tropical Marine Biodiversity and Natural Products' of Università Politecnica delle Marche. Mass and NMR spectra were recorded at 'Centro di Servizio Interdipartimentale di Analisi Strumentale', Università di Napoli 'Federico II'.

References and notes

1. Fattorusso, E.; Parapini, S.; Campagnuolo, C.; Basilico, N.; Taglialatela-Scafati, O.; Taramelli, D. *J. Antimicrob. Chemother.* **2002**, *50*, 883–888.
2. Fattorusso, E.; Taglialatela-Scafati, O.; Ianaro, A.; Di Rosa, M. *Tetrahedron* **2000**, *56*, 7959–7967.
3. Fattorusso, C.; Campiani, G.; Catalanotti, B.; Persico, M.; Basilico, N.; Parapini, S.; Taramelli, D.; Campagnuolo, C.; Fattorusso, E.; Romano, A.; Taglialatela-Scafati, O. *J. Med. Chem.* **2006**, *49*, 7088–7094.
4. Fattorusso, E.; Romano, A.; Taglialatela-Scafati, O.; Bavestrello, G.; Bonelli, P.; Calcinai, B. *Tetrahedron Lett.* **2006**, *47*, 2197–2200.
5. Fattorusso, E.; Romano, A.; Taglialatela-Scafati, O.; Achmad, M. J.; Bavestrello, G.; Cerrano, C. *Tetrahedron* **2008**, *64*, 3141–3146.
6. Benayahu, Y.; Jeng, M.-S.; Perkol-Finkel, S.; Dai, C.-F. *Zool. Stud.* **2004**, *43*, 548–560.
7. Fattorusso, E.; Romano, A.; Taglialatela-Scafati, O.; Achmad, M. J.; Bavestrello, G.; Cerrano, C. *Tetrahedron Lett.* **2008**, *49*, 2189–2192.
8. Tursch, B.; Braekman, J. C.; Daloze, D. *Bull. Soc. Chim. Belg.* **1975**, *84*, 767–774.
9. Liu, Z.; Li, W. Z.; Li, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 95–100.
10. Carmely, S.; Groweiss, A.; Kashman, Y. *J. Org. Chem.* **1981**, *46*, 4279–4284.
11. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
12. Freire, F.; Seco, J. M.; Quinoa, E.; Riguera, R. *J. Org. Chem.* **2005**, *70*, 3778–3790.
13. Zhang, W.; Krohn, K.; Ding, J.; Miao, Z.-H.; Zhou, X.-H.; Chen, S.-H.; Pescitelli, G.; Salvadori, P.; Kurtan, T.; Guo, Y.-W. *J. Nat. Prod.* **2008**, *71*, 961–966.
14. Cheng, S.-Y.; Wen, Z.-H.; Chiou, S.-F.; Hsu, C.-H.; Wang, S.-K.; Dai, C.-F.; Chiang, M. Y.; Duh, C.-Y. *Tetrahedron* **2008**, *64*, 9698–9704.
15. Chen, S.-H.; Guo, Y.-W.; Huang, H.; Cimino, G. *Helv. Chim. Acta* **2008**, *91*, 873–880.

16. Coll, J. C.; Bowden, B. F.; Tapiolas, D. M.; Willis, R. H.; Djura, P.; Streamer, M.; Trott, L. *Tetrahedron* **1985**, *41*, 1085–1092.
17. Radhika, P.; Rao, P. R.; Archana, J.; Rao, N. K. *Biol. Pharm. Bull.* **2005**, *28*, 1311–1313.
18. Coval, S. J.; Patton, R. W.; Petrin, J. M.; James, L.; Rothofsky, M. L.; Lin, S. L.; Patel, M.; Reed, J. K.; McPhil, A. T.; Bishop, W. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 909–912.
19. Badria, F. A.; Guirguis, A. N.; Perovic, S.; Steffen, R.; Muller, W. E. G.; Schroder, H. C. *Toxicology* **1997**, *131*, 133–143.
20. Gross, H.; Kehraus, S.; Nett, M.; Konig, G. M.; Beil, W.; Wright, A. D. *Org. Biol. Chem.* **2003**, *1*, 944–949.
21. Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526–5528.
22. Denecke, J.; Fiedler, K.; Hacker-Klom, U.; Molenkamp, G.; Jurgens, H.; Wolff, J. E. A. *Anticancer Res.* **1997**, *17*, 4531–4534.
23. Hansen, M. B.; Nielsen, S. E.; Berg, K. *J. Immunol. Methods* **1989**, *119*, 203–210.
24. Irace, C.; Scorziello, A.; Maffettone, C.; Pignataro, G.; Matrone, C.; Adornetto, A.; Santamaria, R.; Annunziato, L.; Colonna, A. *J. Neurochem.* **2005**, *95*, 1321–1331.