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Tetrahedron
Tetrahedron 60 (2004) 2517–2524

Structure and cytotoxicity of new metabolites from the sponge *Mycale cecilia*

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Received 21 October 2003; revised 14 January 2004; accepted 16 January 2004

Abstract—The chemical study of the sponge *Mycale cecilia* has led to the isolation of 14 new pyrrole-containing metabolites. Mycalazals 3–13 are pyrrole-2-carbaldehydes possessing at C-5 hydrocarbon side chains of different length and/or number of unsaturations. Mycalenitriles 1–3 are 5-cyanoalkylpyrrole-2-carbaldehydes. The structures of the new compounds were established mainly by NMR and MS spectroscopic analysis. The location of the double bond in mycalazal-4, -8, and -11 was determined by MS analysis of the corresponding bis(methylthio) derivatives. Mycalazals have shown activity as growth inhibitors of several tumor cell lines, in particular the LNcaP cell line, being mycalazal-8 the most active metabolite.
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1. Introduction

Although heterocyclic compounds are widely distributed in nature, those containing a simple pyrrole nucleus are among the less frequently found, despite that pyrrole is a structurally simple five-membered ring. Other than tetrapyrrole pigments, pyrrole-containing natural products have scarcely been encountered in the marine environment, with distribution limited principally to specific species of algae, sponges, bryozoans, tunicates and mollusca.[1] Furthermore, the enormous reactivity of pyrroles in electrophilic substitution reactions, has been claimed to explain the high incidence of halogenated derivatives among marine pyrroles.[2]

Sponges of the *Mycale* genus have been source of novel nitrogenous metabolites belonging to very diverse structural types. Thus, while one of the first accounts on this genus describes the isolation of the nucleosides mycalisines,[3] likely of symbiotic origin, other species are characterized by containing highly cytotoxic compounds of the mycalamides family.[4–6] Another relevant group of *Mycale* metabolites is formed by macrocyclic compounds as the mycalolides,[7–11] thiomycalolides,[12] pateamine[13] and peluroside,[14] all of them also displaying a potent cytotoxic activity. On the other hand, several species of this genus have shown to contain complex mixtures of pyrrole-containing metabolites.[15–17]

In our continuing search for cytotoxic metabolites from marine invertebrates, we have studied specimens of the sponge *Mycale cecilia*, collected along the coasts of Sinaloa in the Gulf of California (Mexico). Bioassay guided isolation yielded fourteen new metabolites, the mycalazals 3–13 (**1**–**11**) and mycalenitriles 1–3 (**12**–**14**), together with the known compounds **15**–**17**,[16] **18**,[18] **19**,[16] **20** and **21**,[19] and **22**.[16]

2. Results and discussion

Specimens of *M. cecilia* were collected by hand using SCUBA, lyophilized and exhaustively extracted with acetone. After evaporation of the solvent under reduced pressure the residue was partitioned between H_2O and Et_2O and the organic extract subjected to column chromatography. Fractions eluted with hexane/Et_2O (8:2) and hexane/Et_2O (7:3) showed cytotoxicity against P-388, A-549 and HT-29 tumor cell lines, and were subjected to repeated separations on reversed phase HPLC to yield, in order of elution, mycalazals 3–13 (**1**–**11**) and mycalenitriles 1–3 (**12**–**14**).

Keywords: Natural products; Sponges; Pyrroles; Structure determination; Cytotoxicity.
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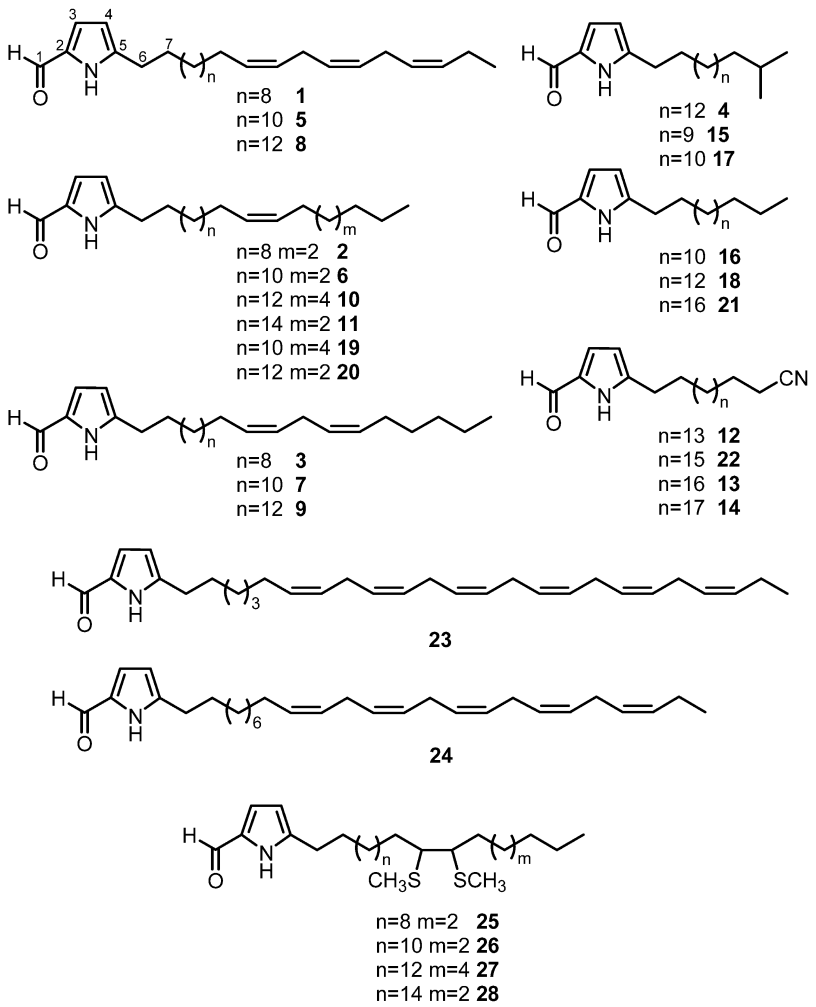
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.01.056



The metabolites **1**–**14** displayed NMR spectra closely similar to those of mycalazals 1 and 2 (**23** and **24**),[15] indicating that all the new compounds possessed a pyrrole-2-carbaldehyde nucleus, but substituted at C-5 with different side chains. For the sake of clarity, in the following discussion compounds have been grouped by the number of unsaturations in the side chain.

2.1. Compounds 1, 5, and 8

Mycalazal-3 (**1**) was isolated as a colorless oil whose molecular formula $C_{26}H_{41}NO$ was established by HRMS. The ^1H and ^{13}C NMR spectra showed, in addition to the signals of the 2,5-disubstituted pyrrole and the formyl group (Table 1), signals attributable to an unbranched polyolefinic chain. In particular, the ^1H NMR multiplet centered at 5.36 as well as six doublets in the ^{13}C NMR spectrum at δ 132.2, 130.6, 128.5, 128.4, 127.9, and 127.3, were assigned to three disubstituted double bonds. These data, together with two ^1H NMR multiplets at δ 2.06 (4H) and 2.81 (4H) attributable to two allylic and two bis-allylic methylenes, respectively, were in agreement with a sequence of three methylene interrupted double bonds. An all-*cis* geometry was proposed for the olefinic system based on the chemical shift of the bis-allylic carbon signals in the ^{13}C NMR spectrum at δ 25.6 and 25.5. Furthermore, the chemical shift of one of the allylic methylenes at δ 20.5 indicated that the series of three methylene interrupted double bonds started at ω-3.[20] Finally, taking into account the molecular formula of **1**, it was deduced that the olefinic system had to be connected to C-5 of the pyrrole nucleus through a sequence of ten methylenes. These gave rise to the ^1H NMR signals at δ 2.64 (t, 2H, J=7.7 Hz, H-6), 1.65 (q, 2H, J=7.4 Hz, H-7), and 1.25 (bs, 16H, H-8 to H-15). It was therefore proposed structure **1** for mycalazal-3.

Mycalazal-7 (**5**) and mycalazal-10 (**8**) displayed NMR spectra almost identical to those previously described for mycalazal-3 (**1**), which indicated that compounds **5** and **8** also were pyrrole-2-carbaldehydes possessing a side chain with a sequence of three methylene interrupted double bonds that started at ω-3. This structural assignment was further confirmed by the cross peak observed in the HMBC spectrum of **5** between the olefinic carbon signal at δ 131.9 and the signal at δ 0.97 (t, 3H, J=7.5 Hz), due to the methyl at the end of the chain. These data, together with the molecular formulae $C_{28}H_{45}NO$ and $C_{30}H_{49}NO$ obtained from HRMS of compounds **5** and **8**, respectively, indicated that the side chain of each compound was elongated in two and four methylenes, respectively, with respect to that of **1**.

2.2. Compounds 3, 7, and 9

Mycalazal-5 (**3**) was isolated as a colorless oil of molecular

Table 1. NMR data for compounds **1**–**3** and **5**–**11**[a–c]

	1		**2**		**3**[d]		**5**[d]		**6**		**7**[d]		**8**		**9**[d]		**10/11**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	178.1	9.37	178.1	9.37	178.1	9.37	178.1	9.35	178.1	9.37	178.1	9.36	178.1	9.35	178.1	9.36	177.9	9.37
2	131.8	—	131.8	—	131.8	—	131.8	—	131.8	—	131.8	—	131.8	—	131.8	—	131.8	—
3	122.3	6.89	122.4	6.89	122.3	6.89	122.6	6.89	122.7	6.89	122.7	6.89	122.8	6.89	122.6	6.89	122.5	6.89
4	109.4	6.07	109.4	6.07	109.4	6.07	109.4	6.07	109.4	6.07	109.5	6.07	109.6	6.07	109.5	6.07	109.5	6.07
5	142.8	—	142.9	—	142.9	—	143.1	—	143.3	—	143.2	—	143.3	—	143.2	—	143.0	—
6	27.9	2.64	27.9	2.64	28.2	2.64	27.9	2.65	27.9	2.65	27.9	2.65	27.9	2.65	27.8	2.65	27.9	2.64
7	28.9	1.65	28.9	1.65	29.1	1.64	28.9	1.65	28.9	1.65	28.9	1.64	28.9	1.64	28.9	1.64	29.0/28.9	1.65
8–15	29.6–28.9	1.25	29.8–29.0	1.25	29.9–29.5	1.25	29.7–29.2	1.25	29.8–29.2	1.25	29.8–29.2	1.25	29.7–29.2	1.25	29.2–29.7	1.25	29.8–29.2	1.25
16	27.2	2.06	27.2	2.02	27.6[e]	2.03	29.7–29.2	1.25	29.8–29.2	1.25	29.8–29.2	1.25	29.7–29.2	1.25	29.2–29.7	1.25	29.8–29.2	1.25
17	127.9	5.36	129.9	5.35	128.3	5.35	29.7–29.2	1.25	29.8–29.2	1.25	29.8–29.2	1.25	29.7–29.2	1.25	29.2–29.7	1.25	29.8–29.2	1.25
18	130.6	5.36	129.9	5.35	130.5	5.35	27.2	2.06	27.2	2.02	27.2	2.03	29.7–29.2	1.25	29.2–29.7	1.25	29.8–29.2	1.25
19	25.6	2.81	27.2	2.02	25.9	2.77	127.6	5.35	129.9	5.35	127.9	5.36	29.7–29.2	1.25	29.2–29.7	1.25	29.8–29.2	1.25
20	128.5[e]	5.36	29.8–29.0	1.25	128.3	5.35	130.4	5.35	129.9	5.35	130.2	5.36	27.2	2.07	27.2	2.03	27.2/29.8–29.2	2.01/1.25
21	128.4[e]	5.36	29.8–29.0	1.25	130.5	5.35	25.6[e]	2.80	27.2	2.02	25.6	2.77	127.6	5.34	127.9	5.35	129.9/29.8–29.2	5.35/1.25
22	25.6	2.81	31.8	1.25	27.5[e]	2.03	128.2[f]	5.35	29.8–29.2	1.25	127.9	5.36	130.4	5.34	130.2	5.35	129.9/27.2	5.35/2.01
23	127.3	5.36	22.6	1.25	29.5–29.9	1.25	128.3[f]	5.35	29.8–29.2	1.25	130.2	5.36	25.6[e]	2.80	25.6	2.77	27.2/129.9	2.01/5.35
24	132.2	5.36	14.1	0.88	31.8	1.25	25.5[e]	2.80	31.8	1.25	27.2	2.03	128.2[f]	5.34	127.9	5.35	29.8–29.2/129.9	1.25/5.35
25	20.5	2.06			22.9	1.25	127.1	5.35	22.6	1.25	29.8–29.2	1.25	128.3[f]	5.34	130.2	5.35	29.8–29.2/27.2	1.25/2.01
26	14.3	0.98			14.4	0.88	131.9	5.35	14.1	0.88	31.5	1.25	25.5[e]	2.80	27.2	2.03	29.8–29.2	1.25
27							20.5	2.06			22.6	1.25	127.1	5.34	29.2–29.7	1.25	29.8–29.2	1.25
28							14.3	0.97			14.1	0.89	131.9	5.34	31.5	1.25	31.9/31.8	1.25
29													20.5	2.07	22.6	1.25	22.7	1.25
30													14.3	0.97	14.1	0.89	14.1	0.88
NH		9.10		9.30		9.08		9.48		9.47		9.57		9.40		9.50		9.19

[a] ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400 MHz and 100 MHz, respectively.
[b] Assignments were aided by gHSQC experiments.
[c] Multiplicity and coupling constant: H-3 (dd, J=3.8, 2.5 Hz), H-4 (dd, J=3.8, 2.5 Hz), H-6 (t, J=7.7 Hz), H-7 (q, J=7.4 Hz), CH_3 at the end of the chain (t, J=7.5 Hz for **1**, **5**, **8**; J=6.8 Hz for **2**, **3**, **6**, **7**, **9**, **10/11**).
[d] Assignments were aided by gHMBC experiments.
[e,f]Values with the same superscripts in the same column may be interchanged.

formula $C_{26}H_{43}NO$, as determined by HRMS. Since the pyrrole-2-carbaldehyde moiety accounted for five carbons, four unsaturations, and all the heteroatoms of the molecular formula, it was concluded that compound **3** contained a C_{21} diunsaturated hydrocarbon side chain. The double bonds gave rise to a ^1H NMR multiplet centered at δ 5.35 that was correlated in the HSQC spectrum with the olefinic carbon signals at δ_C 130.5 (2C, d) and 128.3 (2C, d). Furthermore, it was readily inferred that the two double bonds were separated by a methylene upon observation of the NMR signals at δ_H 2.77 and δ_C 25.9, this latter chemical shift being typical of a *cis* configuration of the double bonds. Finally, the analysis of the HMBC spectrum allowed to establish the location of the olefinic system in the chain. In particular, the proton signal at δ 0.88 (t, 3H, J=6.8 Hz) showed two and three bonds correlations with the methylene carbon signals at δ 22.9 and 31.8, respectively, while this latter signal, in turn, was correlated with the allylic protons signal at δ 2.03 (Fig. 1). It was therefore concluded that the olefinic system started at ω-6 and structure **3** was proposed for mycalazal-5.

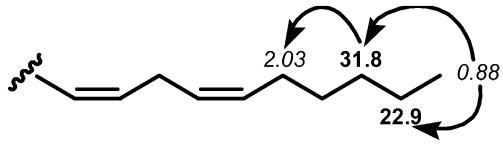


Figure 1. Selected HMBC correlations observed for compound **3**.

The molecular formulae $C_{28}H_{47}NO$ and $C_{30}H_{51}NO$ established for mycalazal-9 (**7**) and mycalazal-11 (**9**), respectively, together with the close similarity of their NMR data with those of the previously described mycalazal-5 (**3**), clearly indicated that the three compounds were members of the same homologous series and that differed in the number of methylenes linking the pyrrole nucleus and the olefinic system. Therefore, structures **7** and **9** were proposed for mycalazal-9 and mycalazal-11, respectively.

2.3. Compounds 2, 6, and 10/11

The NMR spectra of mycalazal-4 (**2**) indicated that it was another pyrrole-2-carbaldehyde metabolite containing a linear unsaturated side chain. In particular, from the molecular formula $C_{24}H_{41}NO$, established by HRMS, it was inferred the presence of nineteen carbon atoms and one double bond in the side chain. However, none of the NMR data allowed to assign the position of this unsaturation along the chain. Furthermore, the fragments observed in the low-resolution mass spectra did not provide unambiguous information, due to double bond migration in the ionization process. It was therefore decided to perform an oxidative cleavage of the double bond with $HIO_4/RuCl_3$ as described by Sharpless[21] with the aim to characterize the two resulting carboxylic acids. Although this procedure has been previously employed by us with good results,[22] in this occasion all attempts to perform the reaction were unfruitful. At room temperature an untreatable complex mixture of compounds were obtained, while lowering temperature led to the recovery of the starting material together with minor amounts of the corresponding diol and α-hydroxyketo derivatives. Finally, the location of the double bond in compound **2** could be deduced from the bis(methylthio) derivative **25** obtained by treatment of **2** with Me_2S_2.[23] The ^1H NMR spectrum of **25** was closely similar to that of compound **2**, except by the absence of the signals due to olefinic and allylic protons, appearing in turn the signals attributable to the methylthio groups (δ 2.10, s, 6H) and their geminal protons (δ 2.69, m, 2H). The low resolution mass spectrum of **25** showed the molecular ion peak at m/z 453 $(C_{26}H_{47}NOS_2)^+$ and two intense peaks at m/z 308 and 145 due to fragments resulting from the easy cleavage of the bond between carbons bearing the methylthio groups. The peak at m/z 308 was due to the fragment containing the pyrrole nucleus $(C_{18}H_{30}NOS)^+$ while the peak at m/z 145 arose from the fragment containing the methyl group at the end of the chain $(C_8H_{17}S)^+$ (Fig. 2). These data unambiguously established that the methylthio groups in **25** were attached to C-17 and C-18 and therefore it was concluded that the double bond in **2** was located at Δ^{17}.

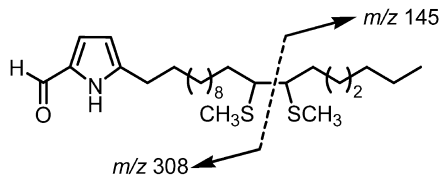


Figure 2. MS fragmentation of compound **25**.

An inspection of the NMR data of mycalazal-8 (**6**) together with its molecular formula $C_{26}H_{45}NO$ were consistent with the presence of a monounsaturated side chain elongated in two methylenes with respect to that of mycalazal-4 (**2**). Derivatization of **6** with Me_2S_2 and subsequent analysis of the corresponding bis(methylthio) derivative **26** by mass spectrometry, as described above, allowed to locate the double bond in **6** at Δ^{19}.

Mycalazal-12 (**10**) and mycalazal-13 (**11**) were obtained as an inseparable mixture. In fact, this mixture showed a single peak in GC–MS analysis and all the spectroscopic data accounted for a single compound possessing a C_{25} monounsaturated hydrocarbon side chain. However, when the procedure to locate the double bond was applied, the presence of two isomeric bis(methylthio) derivatives, **27** and **28**, became apparent during the GC–MS analysis (t_R 57.3 and 57.9 min, respectively). It was deduced that the methylthio groups in **27** were linked to C-21 and C-22 upon observation in its mass spectrum of two peaks at m/z 364 and 173 attributed to the fragments $(C_{22}H_{38}NOS)^+$ and $(C_{10}H_{21}S)^+$, respectively. On the other hand, the mass spectrum of **28** showed peaks at m/z 392 $(C_{24}H_{42}NOS)^+$ and 145 $(C_8H_{17}S)^+$. Following a similar rationale to that employed for compound **2**, it was determined that mycalazal-12 (**10**) and mycalazal-13 (**11**) were constitutional isomers which differed in the position of the double bond, Δ^{21} for **10** and Δ^{23} for **11**.

2.4. Compound 4

Two distinctive features of the ^1H NMR spectrum of mycalazal-6 (**4**) were the absence of signals due to olefinic protons and the presence of a doublet at δ 0.86 which integrated for 6H. These data indicated that in compound **4**

the pyrrole-2-carbaldehyde nucleus was substituted at C-5 with an aliphatic chain possessing a terminal isopropyl group. These data together with the molecular formula $C_{23}H_{41}NO$, established by HRMS, allowed to characterize mycalazal-6 (**4**) as the 5-(16-methylheptadecyl)pyrrole-2-carbaldehyde.

2.5. Compounds 12–14

Mycalenitrile-1 (**12**) was isolated as a colorless oil whose molecular formula, $C_{23}H_{38}N_2O$, indicated the presence of an additional nitrogen atom in the structure. The presence of the pyrrole-2-carbaldehyde nucleus was confirmed upon observation of the NMR signals at δ_H 9.33 (CHO), 9.20 (NH), 6.90 (dd, J=3.9, 2.3 Hz, H-3), 6.08 (dd, J=3.9, 2.3 Hz, H-4), and δ_C 177.5 (CHO), 142.9 (C-2), 131.8 (C-5), 123.1(C-3), and 109.8 (C-4). The remaining signals of the ^{1}H NMR spectrum were attributable to a series of methylenes within an aliphatic chain, although no signal could be found due to a methyl or an isopropyl group at the end of the chain. In fact, the ^{13}C NMR spectrum showed a downfield signal at δ 119.9 (s) that together with the IR absorption 2246 cm^{-1} were assigned to a nitrile group located at the end of the side chain. All these data led us to propose structure **12** for mycalenitrile-1.

Mycalenitrile-2 (**13**) and mycalenitrile-3 (**14**) possessed IR and NMR spectra almost superimposable with those of mycalenitrile-1 (**12**) above described, indicating that the three compounds had to differ only in the length of the chain. Thus, the molecular formulae obtained from HRMS allowed to establish that compound **13** ($C_{26}H_{44}N_2O$) possessed a side chain elongated in three methylenes with respect to that of **12** while in compound **14** ($C_{27}H_{46}N_2O$) the side chain contained four additional methylenes with respect to that of **12**.

From a biosynthetic point of view, different precursors and mechanisms have been proposed to explain the formation of a pyrrole nucleus in marine metabolites. Thus, while the central pyrrole ring present in a series of marine alkaloids could arise from the condensation of DOPA or tyrosine units,[24] the origin of the pyrrole system in stevensine has been traced to the amino acids ornithine and proline.[25] The structures of mycalazols,[15] mycalazals and mycalenitriles, combine a pyrrole moiety with long hydrocarbon chains reminiscent of those of fatty acids. These features suggest a mixed biogenesis pathway in which pyrrole-2-carboxylic acid (or equivalent), derived from ornithine and/or proline,[25] could be acylated at C-5 with different long chain acyl units.

2.6. Cytotoxic activity

All the metabolites isolated from *M. cecilia* were tested against a panel of tumor cell lines in bioassays directed to detect in vitro cytotoxicity (see Section 3). In general, all the metabolites showed activity as growth inhibitors of various cell lines. The more significant GI_{50} values (<5 μg/mL) are presented in Table 2. The cell lines LNcaP, IGROV, and SK-MEL28 were affected by most of the compounds of the class of mycalazals. These results could suggest a general selectivity of mycalazals as growth inhibitors of the cell lines above mentioned. Furthermore, the higher levels of activity for all the tested compounds were observed against the LNcaP cell line, being compound **6** the most active (GI_{50}=0.2 μg/mL). In addition, compounds **1**, **2**, **5**, **15**, **16**, and **17** showed a significant cytostatic effect on this cell line with TGI (total growth inhibition) values of 3.3, 2.6, 2.8, 3.1, 2.9, and 3.0 μg/mL, respectively. On the other hand, compounds of the mycalenitrile class showed a mild but highly selective activity against the cell lines PANC1, LOVO and HELA cell lines (Table 2).

The high number of metabolites isolated prompted us to analyze possible relationships between the structural features of the side chain of mycalazals (length, number of double bonds, and/or position of the unsaturations) and the observed activity, in particular concerning to the growth inhibition effect of mycalazals on the LNcaP line. A comparison of the activity of compounds **1**, **3**, **6**, and **21**, all of them possessing a C_{21} side chain, showed that the higher

Table 2. Cytotoxicity assay results for the compounds isolated from *Mycale cecilia* (GI_{50} values in μg/mL)

GI_{50}	A	B	C	D	E	F	G	H	I
1	1.8	4.8		3.8					
2	1.3	3.0		2.7		5.0			
3		3.9	4.1	3.9			3.1	2.0	3.6
4			4.8	3.5			3.6	3.2	4.1
5	1.5	3.0		2.9	3.6			4.2	
6	0.2	2.7		3.2			4.5		
7	3.0	4.5		4.2					
8	1.5	3.2		3.8					
9	3.8	4.8							
10/11	4.2								
12							4.8	2.4	
13								3.9	4.7
14									4.3
15	1.6			3.9		3.2		4.2	
16	1.6			2.3				3.6	
17	1.6			4.1		3.3		4.4	
18			4.7	4.1		4.3	3.9	2.1	3.9
19/20	2.8	4.8							
21									4.4
22								4.5	4.4

A: LN-caP, B: IGROV, C: SK-BR3; D: SK-MEL-28, E: A-549, F: K-562, G: PANC1, H: LOVO, I: HELA.

activity is associated to the presence of one double bond at ω-7 (compound **6**). When three double bonds are present (**1**) the activity decreases and it is lost for compounds possessing two (**3**) or no (**21**) double bond. The higher level of activity showed by compounds **5** and **8** with respect to **7** and **9** again points out that compounds with triunsaturated side chains are more active than the corresponding diunsaturated ones. The GI_{50} values observed for compounds **1**, **5**, **8**, displaying C_{21}, C_{23}, and C_{25} triolefinic side chains, respectively, were closely similar without regard of the length of the chain. However, this trend was not observed for the remaining mycalazals.

3. Experimental

3.1. General experimental procedures

IR spectra were recorded with a Genesis Series FT IR Mattson spectrophotometer, and UV spectra were registered on a Philips PU 8710 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Varian INOVA 400 spectrometer using $CDCl_3$ as solvent. Proton chemical shifts were referenced to the residual $CHCl_3$ signal at δ 7.26. ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ 77.0. $^1H-^1H$-COSY, HMQC and HMBC were performed using standard VARIAN pulse sequences. Low resolution mass spectra were recorded on an Finningan Voyager GC8000^{top} spectrometer. High resolution electronic impact mass spectra were recorded on a VG Autospec spectrometer. Column chromatography was carried out using Merck Silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrosorb RP-18 (Merck) column using a differential refractometer RI-71. All solvents were spectral grade or distilled prior to use.

3.2. Collection, extraction and isolation procedure

The sponge *Mycale cecilia* was collected by hand using SCUBA in the Gulf of California and liophylized (298.84 g). The material was extracted with acetone (9 L) and the solvent concentrated under reduced pressure to give a residue that was partitioned between H_2O and Et_2O. The Et_2O extract (7 g) was chromatographed on a silica gel column using solvents of increasing polarities from hexane to Et_2O and, subsequently $CHCl_3$/MeOH (8:2) and MeOH. The fraction eluted with hexane/Et_2O (8:2) was subjected to repeated reversed HPLC separations using MeOH as eluent to afford 5-(13′-methyltetradecyl)pyrrole-2-carbaldehyde (**15**, 13.1 mg, 0.0044%), 5-pentadecylpyrrole-2-carbaldehyde (**16**, 57.9 mg, 0.019%), 5-(14′-methylpentadecyl)-pyrrole-2-carbaldehyde (**17**, 5.8, 0.002%), 5-heptadecylpyrrole-2-carbaldehyde (**18**, 1.5 mg, 4.92×10^{-4}%), mycalazal-3 (**1**, 5.1 mg, 0.0017%), mycalazal-4 (**2**, 7.4 mg, 0.0025%), mycalazal-5 (**3**, 1.9 mg, 6.29×10^{-4}%), mycalazal-6 (**4**, 1.5 mg, 5.02×10^{-4}%), mycalazal-7 (**5**, 10.1 mg, 0.0034%), mycalazal-8 (**6**, 16.0 mg, 0.0054%), mycalazal-9 (**7**, 8.5 mg, 0.0028%), mycalazal-10 (**8**, 9.2 mg, 0.0032%), a mixture of (14′*Z*)-5-tricos-14′-enylpyrrole-2-carbaldehyde and (16′*Z*)-5-tricos-16′-enylpyrrole-2-carbaldehyde (**19/20**, 46.5 mg, 0.016%), 5-heneicosylpyrrole-2-carbaldehyde (**21**, 1.0 mg, 0.0003%), mycalazal-11 (**9**, 8.3 mg, 0.0029%),and a mixture of mycalazal-12 and mycalazal-13 (**10/11**, 15.7 mg, 0.0053%). Fractions from the general chromatography eluted with hexane/Et_2O (7:3) yielded, after purification on HPLC eluting with CH_3CN/MeOH (7:3), mycalenitrile-1 (**12**, 6.5 mg; 0.0022%), 5-(19′-cyanononadecyl)pyrrole-2-carbaldehyde (**22**, 26.8 mg, 0.009%), mycalenitrile-2 (**13**, 4.8 mg; 0.0016%), and mycalenitrile-3 (**14**, 8.2 mg, 0.0028%). Final purification of all these compounds was accomplished by HPLC on reversed phase mode using solvents of various proportions of either H_2O in MeOH or H_2O in CH_3CN.

3.2.1. Mycalazal-3 (1). Colorless oil, IR (film) 3257, 2924, 2850, 1666, 1496, 770 cm^{-1}; UV (MeOH) 204 (ε 14,688), 248 (ε 3437), 300 (ε 15,397) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m*/*z* (rel. int.) 383 (9.5), 354 (8.3), 150 (19.1), 136 (11.5), 122 (77.4), 108 (100), 94 (54.4); HREIMS Obsd. *m*/*z*=383.3188 $(M)^+$, $C_{26}H_{41}NO$ requires *m*/*z*=383.3187.

3.2.2. Mycalazal-4 (2). Amorphous powder, IR (film) 3255, 2920, 2850, 1664, 1496, 770 cm^{-1}; UV (MeOH) 202 (ε 7585), 249 (ε 2344), 300 (ε 11,764) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m*/*z* (rel. int.) 359 (38.0), 330 (24.1), 316 (9.9), 288 (10.0), 274 (11.7), 260 (13.8), 150 (42.5), 136 (29.6), 122 (45.2), 108 (100), 94 (43.5); HREIMS Obsd. *m*/*z*=359.3183 $(M)^+$, $C_{24}H_{41}NO$ requires *m*/*z*=359.3188

3.2.3. Mycalazal-5 (3). Colorless oil, IR (film) 3262, 2924, 2852, 1644, 1496, 770 cm^{-1}; UV (MeOH) 204 (ε 9254), 248 (ε 2392), 300 (ε 11,150) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m*/*z* (rel. int.) 385 (31.5), 356 (20.5), 342 (8.2), 328 (11.0), 314 (11.0), 150 (39.7), 122 (97.3), 108 (100), 94 (54.8); HREIMS Obsd. *m*/*z*=385.3342 $(M)^+$, $C_{26}H_{43}NO$ requires *m*/*z*=385.3345

3.2.4. Mycalazal-6 (4). Amorphous powder, IR (film) 3248, 2920, 2850, 1633, 774 cm^{-1}; UV (MeOH) 203 (ε 6260), 249 (ε 2078), 300 (ε 9786) nm; ^1H NMR δ 9.36 (s, 1H, H-1), 9.20 (s, 1H, NH), 6.89 (dd, *J*=3.8, 2.5 Hz, 1H, H-3), 6.07 (dd, *J*=3.8, 2.5 Hz, 1H, H-4), 2.65 (t, *J*=7.7 Hz, 2H, H-6), 1.65 (q, *J*=7.4 Hz, 2H, H-7), 1.51 (sept, *J*=6.6 Hz, 1H, H-21), 1.25 (broad signal, 24H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18 and H-19), 1.13 (m, 2H, H-20), 0.86 (d, *J*=6.5 Hz, 6H, H-22 and H-23); EIMS (70 eV) *m*/*z* (rel. int.) 347 (45.5), 304 (34.0), 150 (43.5), 136 (25.5), 122 (74.9), 108 (100), 94 (43.4); HREIMS Obsd. *m*/*z*=347.3182 $(M)^+$, $C_{23}H_{41}NO$ requires *m*/*z*=347.3188.

3.2.5. Mycalazal-7 (5). Colorless oil, IR (film) 3263, 2924, 2853, 1645, 1496, 770 cm^{-1}; UV (MeOH) 204 (ε 9254), 248 (ε 2392), 300 (ε 11,150) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m*/*z* (rel. int.) 411 (16.6), 382 (13.7), 150 (11.3), 136 (10.7), 122 (64.9), 108 (83.4), 94 (100); HREIMS Obsd. *m*/*z*=411.3501 $(M)^+$, $C_{28}H_{45}NO$ requires *m*/*z*=411.3499.

3.2.6. Mycalazal-8 (6). Amorphous powder, IR (film) 3248, 2920, 2850, 1644, 1496, 773 cm^{-1}; UV (MeOH) 202 (ε 7803), 248 (ε 2317), 300 (ε 11,290) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m*/*z* (rel. int.) 387 (38.3), 358

(18.9), 344 (22.2), 330 (26.0), 150 (53.0), 136 (21.9), 122 (59.5), 108 (100), 94 (52.0); HREIMS Obsd. *m/z*=387.3501 $(M)^+$, $C_{26}H_{45}NO$ requires *m/z*=387.3500.

3.2.7. Mycalazal-9 (7). Colorless oil, IR (film) 3259, 2924, 2853, 1644, 1496, 717 cm^{-1}; UV (MeOH) 201 (ε 14,167), 248 (ε 4056), 300 (ε 21,159) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m/z* (rel. int.) 413 (62.7), 384 (37.3), 150 (44.0), 136 (34.7), 122 (100), 108 (97.3), 94 (96.0); HREIMS Obsd. *m/z*=413.3658 $(M)^+$, $C_{28}H_{47}NO$ requires *m/z*=413.3653.

3.2.8. Mycalazal-10 (8). Colorless oil, IR (film) 3252, 2922, 2850, 1661, 1496, 772 cm^{-1}; UV (MeOH) 204 (ε 11,921), 248 (ε 3692), 300 (ε 16,903) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m/z* (rel. int.) 439 (20.8), 410 (15.5), 150 (43.0), 136 (29.6), 122 (75.1), 108 (99.8), 94 (100); HREIMS Obsd. *m/z*=439.3814 $(M)^+$, $C_{30}H_{49}NO$ requires *m/z*=439.3803.

3.2.9. Mycalazal-11 (9). Colorless oil, IR (film) 3263, 2923, 2852, 1644, 1496, 772 cm^{-1}; UV (MeOH) 202 (ε 6044), 248 (ε 1796), 301 (ε 7633) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m/z* (rel. int.) 441 (39.5), 412 (23.3), 398 (9.3), 384 (11.6), 150 (35.0), 136 (14.0), 122 (90.7), 108 (100), 94 (51.2); HREIMS Obsd. *m/z*=441.3971 $(M)^+$, $C_{30}H_{51}NO$ requires *m/z*=441.3943.

3.2.10. Mycalazal-12/13 (10/11). Amorphous powder, IR (film) 3251, 2953, 2850, 1639, 1457, 770 cm^{-1}; UV (MeOH) 202 (ε 7458), 248 (ε 2750), 301 (ε 12,728) nm; ^1H and ^{13}C NMR (see Table 1); EIMS (70 eV) *m/z* (rel. int.) 443 (40.5), 400 (9.0), 386 (9.2), 150 (50.9), 136 (22.1), 122 (61.0), 108 (100); HREIMS Obsd. *m/z*=443.4127 $(M)^+$, $C_{30}H_{53}NO$ requires *m/z*=443.4124.

3.2.11. Mycalenitrile-1 (12). Colorless oil, IR (film) 3250, 2246, 1644, 1490, 780 cm^{-1}; UV (MeOH) 204 (ε 6025), 248 (ε 2544), 300 (ε 10,125) nm; ^1H NMR δ 9.33 (s, 1H, H-1), 9.20 (s, 1H, NH), 6.90 (dd, *J*=3.9, 2.3 Hz, 1H, H-3), 6.08 (dd, *J*=3.9 and 2.3 Hz, 1H, H-4), 2.64 (t, *J*=7.7 Hz, 2H, H-6), 2.33 (t, *J*=7.1 Hz, 2H, H-22), 1.65 (m, 4H, H-7 and H-21), 1.43 (m, 2H, H-20), 1.25 (broad signal, 24H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18 and H-19); ^{13}C NMR δ 177.5 (s, C-1), 142.9 (s, C-2), 131.8 (s, C-5), 123.1 (d, C-3), 119.9 (s, C-23), 109.8 (d, C-4), 28.7 and 29.1-29.7 (t, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19), 28.8 (t, C-7), 28.6 (t, C-20), 27.9 (t, C-6); 25.3 (t, C-21), 17.1 (t, C-22); EIMS (70 eV) *m/z* (rel. int.) 358 (35.6), 329 (15.5), 150 (41.5), 136 (20.0), 122 (56.1), 108 (100), 94 (43.5); HREIMS Obsd. *m/z*=358.2978 $(M)^+$, $C_{23}H_{38}N_2O$ requires *m/z*=358.2984.

3.2.12. Mycalenitrile-2 (13). Colorless oil, IR (film) 3255, 2246, 1660, 1490, 780 cm^{-1}; UV (MeOH) 204 (ε 6028), 248 (ε 2714), 300 (ε 11,788) nm; ^1H NMR δ 9.38 (s, 1H, H-1), 6.88 (dd, *J*=3.8, 2.5 Hz, 1H, H-3), 6.07 (dd, *J*=3.8, 2.5 Hz, 1H, H-4), 2.63 (t, *J*=7.8 Hz, 2H, H-6), 2.33 (t, *J*=7.2 Hz, 2H, H-25), 1.65 (m, 4H, H-7 and H-24), 1.43 (m, 2H, H-23), 1.25 (broad signal, 30H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21 and H-22); ^{13}C NMR δ 178.1 (s, C-1), 142.9 (s, C-2), 131.8 (s, C-5), 122.9 (d, C-3), 119.9 (s, C-26), 109.4 (d, C-4), 28.7 and 29.1-29.7 (t, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-22), 28.9 (t, C-7), 28.6 (t, C-23), 27.9 (t, C-6); 25.4 (t, C-24), 17.1 (t, C-25); EIMS (70 eV) *m/z* (rel. int.) 400 (16.9), 371 (15.5), 150 (43.0), 136 (29.6), 122 (75.1), 108 (99.8), 94 (100); HREIMS Obsd. *m/z*=400.3460 $(M)^+$, $C_{26}H_{44}N_2O$ requires *m/z*=400.3453.

3.2.13. Mycalenitrile-3 (14). Colorless oil, IR (film) 3250, 2920, 2848, 2242, 1646, 1490, 780 cm^{-1}; UV (MeOH) 203 (ε 5950), 248 (ε 2512), 300 (ε 10,000) nm; ^1H NMR δ 9.36 (s, 1H, H-1), 9.20 (s, 1H, NH), 6.89 (dd, *J*=3.8, 2.5 Hz, 1H, H-3), 6.07 (dd, *J*=3.8, 2.5 Hz, 1H, H-4), 2.64 (t, *J*=7.7 Hz, 2H, H-6), 2.33 (t, *J*=7.1 Hz, 2H, H-26), 1.65 (m, 4H, H-7 and H-25), 1.43 (m, 2H, H-24), 1.25 (broad signal, 32H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-22 and H-23); ^{13}C NMR δ 178.1 (s, C-1), 142.9 (s, C-2), 131.8 (s, C-5), 122.9 (d, C-3), 119.9 (s, C-27), 109.4 (d, C-4), 28.7 and 29.1-29.7 (t, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-22, C-23), 28.9 (t, C-7), 28.6 (t, C-24), 27.9 (t, C-6); 25.4 (t, C-25), 17.1 (t, C-26); EIMS (70 eV) *m/z* (rel. int.) 439 (20.8), 410 (15.5), 150 (43.0), 136 (29.6), 122 (75.1), 108 (99.8), 94 (100); HREIMS Obsd. *m/z*=414.3610 $(M)^+$, $C_{27}H_{46}N_2O$ requires *m/z*=414.3591.

3.3. Location of the double bond in compounds 2, 6 and 10/11

To a solution of mycalazal-5 (**2**, 1.1 mg, 3.06×10^{-3} mmol) in dimethyl disulfide (0.5 mL) was added iodine (4.7 mg, 0.019 mmol) at room temperature. After 3.5 h of stirring the reaction was quenched with 5% $Na_2S_2O_3$ (1 mL) and extracted with n-hexane (3×1 mL). The combined extracts were concentrated under reduced pressure yielding 0.8 mg (1.77×10^{-3} mmol) of the bis(methylthio) derivative **25**.

3.3.1. Compound 25. Amorphous powder, ^1H NMR δ 9.36 (s, 1H, H-1), 9.21 (s, 1H, NH), 6.88 (dd, *J*=3.5, 2.3 Hz, 1H, H-3), 6.07 (dd, *J*=3.5, 2.3 Hz, 1H, H-4), 2.69 (m, 2H, H-17, H-18), 2.64 (t, *J*=7.6 Hz, 2H, H-6), 2.10 (s, 6H, 2×SCH_3), 1.63 (m, 6H, H-7, H-16 and H-19), 1.25 (broad signal, 24H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-20, H-21, H-22 and H-23), 0.86 (d, *J*=6.4 Hz, 3H, H-24); EIMS (70 eV) *m/z* (rel. int.) 453 (8.5), 308 (81.4), 232 (84.3), 145 (85.7), 108 (88.5), 94 (35.5), 80 (100).

Application of this procedure to 4.6 mg (0.012 mmol) of mycalazal-8 (**6**) yielded 5.19 mg (0.011 mmol) of the bis(methylthio) derivative **26**.

3.3.2. Compound 26. Amorphous powder, ^1H NMR δ 9.36 (s, 1H, H-1), 9.21 (s, 1H, NH), 6.88 (dd, *J*=3.5, 2.3 Hz, 1H, H-3), 6.07 (dd, *J*=3.5, 2.3 Hz, 1H, H-4), 2.69 (m, 2H, H-19, H-20), 2.64 (t, *J*=7.6 Hz, 2H, H-6), 2.10 (s, 6H, 2×SCH_3), 1.63 (m, 6H, H-7, H-18 and H-21), 1.25 (broad signal, 28H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-22, H-23, H-24 and H-25), 0.86 (d, *J*=6.4 Hz, 3H, H-26); EIMS (70 eV) *m/z* (rel. int.) 481 (0.2), 336 (79.7), 260 (92.0), 145 (47.7), 108 (91.3), 94 (42.2), 80 (100).

Application of this same experimental procedure to the

mixture of mycalazal-12 and mycalazal-13 (**10/11**) (3.8 mg, 8.58×10^{-3} mmol) yielded the corresponding mixture of the bismethylthio derivatives **27** and **28**.

3.3.3. Compounds 27 and 28. Amorphous powder, ^{1}H NMR δ 9.37 (s, 2H, H-1), 9.14 (s, 2H, NH), 6.88 (dd, J=3.5, 2.3 Hz, 2H, H-3), 6.07 (dd, J=3.5, 2.3 Hz, 2H, H-4), 2.69 (m, 4H, H-21 and H-22/H-23 and H-24), 2.64 (t, J=7.6 Hz, 4H, H-6), 2.10 (s, 12H, 4×SCH_3), 1.63 (m, 12H, H-7, H-20 and H-23/H-7, H-22 and H-25), 1.25 (broad signal, 56H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-22, H-23, H-24 and H-25/H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-26, H-27, H-28 and H-29), 0.86 (d, J=6.4 Hz, 6H, H-26).

The reaction mixture was analyzed by CG–MS and the results were as follows:

Compound **27**: t_R [min]=57.3, EIMS (70 eV) *m/z* (rel. int.) 364 (40.0, $(C_{22}H_{38}NOS)^+$), 288 (45.7), 173 (57.1, $(C_{10}H_{21}S)^+$), 108 (67.1), 94 (41.4), 80 (100).

Compound **28**: t_R [min]=57.9, EIMS (70 eV) *m/z* (rel. int.) 392 (40.0, $(C_{24}H_{42}NOS)^+$), 316 (51.0), 145 (64.3, $(C_8H_{17}S)^+$), 108 (70.1), 94 (37.1), 80 (100).

3.4. Cytotoxicity assays

All the compounds isolated from *M. cecilia* were tested against the following human tumor cell lines: DU-145 (prostate carcinoma), LN-caP (prostate carcinoma), IGROV (ovarian adenocarcinoma), SK-BR3 (breast adenocarcinoma), SK-MEL-28 (melanoma), A-549 (lung adenocarcinoma), K-562 (chronic myelogenous leukemia), PANC-1 (pancreas carcinoma), HT-29 (colon adenocarcinoma), LOVO (colon adenocarcinoma), LOVO-DOX (colon adenocarcinoma resistant to doxorubicin), and HELA (cervix epithelial adenocarcinoma).

Acknowledgements

This research was supported by grants from MCYT (research project PPQ2001-0020) and from Junta de Andalucía (FQM-285). M. C. S. acknowledges a fellowship from MCYT. Cytotoxicity assays were performed through a cooperation agreement with PharmaMar S. A.

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