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New Cytotoxic Metabolites from the Sponge *Mycale micracanthoxea*

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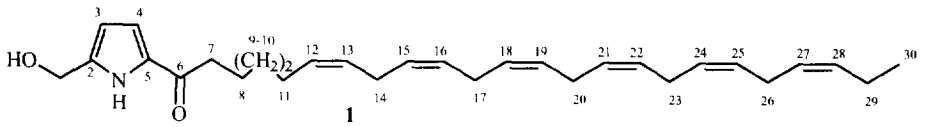
Abstract: The sponge *Mycale micracanthoxea* contains fourteen new compounds: a series of twelve 5-acyl-2-hydroxymethylpyrroles, named mycalazols 1-12 (**1-12**), together with two 5-alkylpyrrole-2-carbaldehydes, mycalazal 1 (**13**) and mycalazal 2 (**14**). The structures were elucidated by interpretation of spectral data. In general, the new compounds showed significant *in vitro* cytotoxicity.

Sponges of *Mycale* genus have been a source of natural compounds of varied structures. Mycalamides, mycalolides, rotalins, mycalisines, polybrominated C-15 acetogenins, brominated isocoumarins and norsesterterpene cyclic peroxides are examples of marine natural products isolated from this genus some of which have shown interesting activities.[2]

As a part of our project directed towards the search of antitumoral compounds from marine origin we have undertaken the study of the sponge *Mycale micracanthoxea*, an incrusting orange-ochre sponge typical of the north-eastern Atlantic and also found in the sourthern coast of Spain representing the southernmost limit of the geographical range of this species.[3] The chemical study of *M. micracanthoxea* has led to the isolation and characterization of fourteen new pyrroles, twelve of them (**1-12**) are 5-acyl-2-hydroxymethylpyrroles, and the remaining two (**13-14**) are 5-alkylpyrrole-2-carbaldehydes.

Specimens of *Mycale micracanthoxea* were collected by hand using SCUBA and immediately frozen. The less polar material of an acetone extract was chromatographed on Si gel. Futher purification using reversed phase HPLC of selected fractions led to the isolation of a series of 5-acyl-2-hydroxymethylpyrroles named, in order of elution, mycalazol 1 (**1**, 0.016 % dry wt), mycalazol 2 (**2**, 0.016 % dry wt), mycalazol 3 (**3**, 0.024 % dry wt), mycalazol 4 (**4**, 0.045 % dry wt), mycalazol 5 (**5**, 0.016 % dry wt), mycalazol 6 (**6**, 0.008 % dry wt), mycalazol 7 (**7**, 0.008 % dry wt), mycalazol 8 (**8**, 0.006 % dry wt), mycalazol 9 (**9**, 0.020 % dry wt), mycalazol 10 (**10**, 0.045 % dry wt), mycalazol 11 (**11**, 0.008 % dry wt) and mycalazol 12 (**12**, 0.008 % dry wt).

Mycalazol 1 (**1**) was isolated as a colorless oil. The molecular formula, $C_{30}H_{43}NO_2$, was obtained from the high resolution mass measurement. The presence of a disubstituted pyrrole nucleus was deduced from the ^{1}H NMR signals at δ 10.44 (brs, N-H), 6.89 (dd, J=3.8 and 2.7, 1H) and 6.14 (dd, J=3.8 and 2.7, 1H) and the ^{13}C NMR signals at δ 139.7 (s), 131.6 (s), 117.6 (d) and 108.6 (d). The value of the coupling constant of 3.8 Hz between the pyrrolic protons indicated a 2,5-disubstitution pattern.[4] A singlet on the ^{1}H NMR spectrum at δ 4.72 (s, 2H) together with a triplet on the ^{13}C NMR spectrum at δ 57.9 (t) were assigned to a primary



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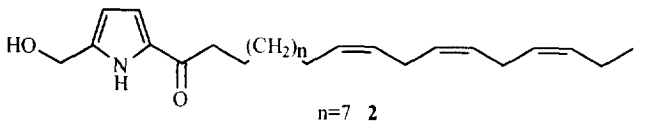

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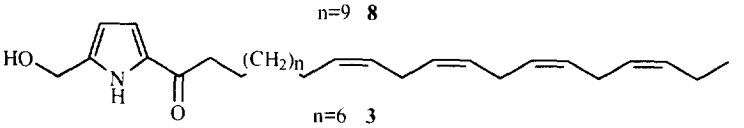
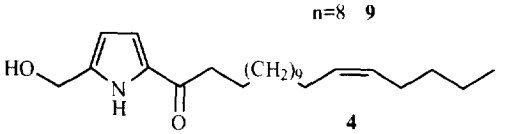
n=9 **8**



n=6 **3**
n=8 **9**



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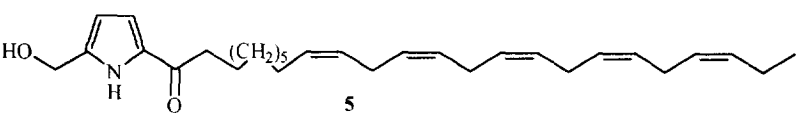

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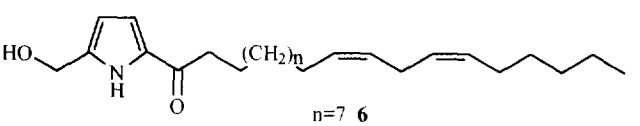
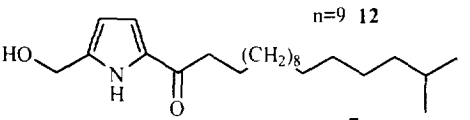


n=7 **6**
n=9 **12**



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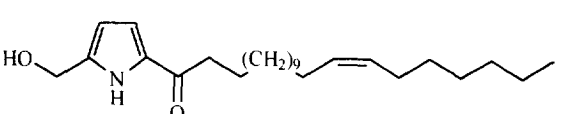

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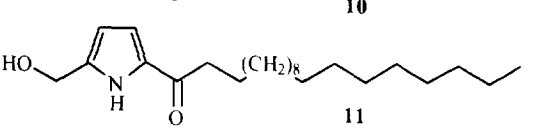

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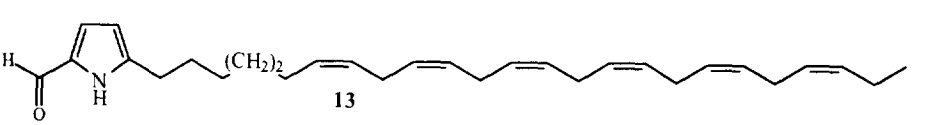

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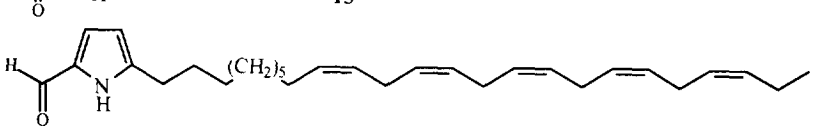

14

alcohol. The IR absorption at 1683 cm^{-1} and the ^{13}C NMR signal at δ 191.6 (s) were due to a carbonyl group conjugated with the pyrrole nucleus. An intense signal on the ^{1}H NMR at δ 5.73 (m, 12H) assigned to twelve olefinic protons was coupled, as indicated by the COSY spectrum, to the signals at δ 2.80 (m, 10H) and 2.07 (m, 4H) indicating a sequence of six methylene interrupted double bonds. These data together with the triplet at δ 0.97 (t, J=9.6, 3H) that was coupled with the signal at δ 2.07 indicated that the six double bonds system started at ω-3, as ascertained by the ^{13}C NMR triplet at 20.5 (t) that was correlated in the HETCOR with the 2.07 proton signal. The *Z* geometry of the double bonds, which is a characteristic of all the unsaturated side chain mycalazols, was deduced by the strong IR absorption at 723 cm^{-1} and the chemical shift of the bisallylic methylene carbons between 25.6 and 27.5 ppm.[5] The presence of a sequence of five methylenes linking the carbonyl group and the double bonds system was established by the signals at δ 2.77 (t, J=7.6, 2H), 1.73 (tt, J=7.6 and 7.6, 2H), assigned to the methylenes α and β to the carbonyl group, and the broad signal at δ 1.25-1.39 (m, 4H) coupled in the COSY spectrum with the allylic methylene protons signal at δ 2.07. It was therefore proposed structure **1** for mycalazol 1.

Mycalazol 5 (**5**) was isolated as a colorless oil. The molecular formula $C_{30}H_{45}NO_2$, established by the study of the molecular ion peak at *m/z* 451 and the ^{13}C NMR and DEPT spectra, and the similarities on the rest of the spectroscopic data indicated that **5** was a dihydroderivative of mycalazol 1 (**1**). The sequence of the double bonds was similarly deduced upon observation of the ^{1}H NMR signal at δ 2.81 (m, 8H) due to four bisallylic methylenes. The location of the double bonds of the acylic chain was established as ω-3 based on the coupling observed in the COSY spectrum between the methyl signal at δ 0.97 (t, J=7.6, 3H) and the methylene protons signal at δ 2.06 (m, 4H) and of this latter with the olefinic protons signal at δ 5.37 (m, 10H), and the presence of a triplet at δ 20.6 in the ^{13}C MNR spectrum. All these data indicated that mycalazol 5 (**5**) was the 12,13-dihydroderivative of mycalazol 1 (**1**).

Mycalazol 9 (**9**) was isolated as a colorless oil. The analysis of the spectroscopic data and the molecular formula $C_{30}H_{47}NO_2$, deduced from the HRMS, clearly indicated based on a similar rationale as followed above that **9** was the 12,13-15,16-tetrahydroderivative of mycalazol 1 (**1**).

Mycalazol 3 (**3**), isolated as a colorless oil, possessed the molecular formula $C_{28}H_{43}NO_2$ as ascertained by the HRMS. The spectroscopic data of **3** were very similar to those of mycalazol 9 (**9**). The only difference in the ^{13}C NMR spectra were the signals between δ 29.7 and 29.5 that in mycalazol 3 (**3**) were assigned to six methylene groups. It was therefore proposed structure **3** for mycalazol 3.

Mycalazol 2 (**2**) and mycalazol 8 (**8**) were isolated as colorless oils. The HRMS indicated the molecular formulae $C_{26}H_{41}NO_2$ and $C_{28}H_{45}NO_2$ for **2** and **8**, respectively. The fragmentation patterns observed in the mass spectra of both compounds were quite similar the only difference being the molecular ion peak at *m/z* 399 and 427 for **2** and **8** respectively and, therefore, indicating that **8** presented an acyl chain elongated in two methylene groups with respect to that of **2**. A comparison of the spectral data of both compounds with those of mycalazol 1 (**1**) suggested that the unsaturations of the molecular formula in each compound accounted, four

to the 5-acyl substituted pyrrole nucleus and the three remaining to three double bonds. The sequence of three methylene interrupted double bonds starting at position ω-3 in **2** was deduced by the ^{1}H NMR signals at δ 2.80 (m, 4H), assigned to two bisallylic methylene groups, at δ 2.06 (m, 4H) due to the allylic methylene groups and at δ 0.97 (t, J=7.6, 3H) of the methyl group. A similar rationale led to the establishment of the structure of the acyl chain in **8**. All these data are in agreement with the proposed structures for mycalazol 2 (**2**) and mycalazol 8 (**8**).

Mycalazol 6 (**6**) and mycalazol 12 (**12**) were isolated as colorless oils and had the molecular formulae $C_{26}H_{43}NO_2$ and $C_{28}H_{47}NO_2$, respectively, as indicated by the high resolution mass measurements. The ^{1}H NMR spectra of both compounds presented the typical signals of the 2,5-disubstituted pyrrole nuclei and the singlets due to the methylene groups of the primary alcohol. The molecular formulae and the examination of the ^{1}H and ^{13}C NMR indicated that both compounds posessed an acyl chain with two double bonds. The presence of a bisallylic methylene in **6** was deduced upon observation of the ^{13}C NMR signal at δ 25.6 (t) and the ^{1}H NMR signal at δ 2.80 (m, 2H). A comparison of the ^{13}C NMR data of **6** with those of arachidonic acid methyl ester[6] supported that the unsaturation started at ω-6 position and therefore the double bonds must be located in ω-6 and ω-9 positions, as ascertained by the fragmentation observed in the mass spectrum. The two carbon-carbon double bonds of the acyl chain in **12** were located following a similar rationale. These data defined structures **6** and **12** for mycalazol 6 and mycalazol 12, respectively.

Mycalazol 4 (**4**) and mycalazol 10 (**10**) were isolated as white crystals of molecular formulae $C_{24}H_{41}NO_2$ and $C_{26}H_{45}NO_2$, respectively, suggesting that the difference in the structure between both compounds consisted in two methylene groups as in the three pairs of mycalazols above described. The analysis of the ^{1}H and ^{13}C NMR data of **4** and **10** indicated that the acylic chain in both compounds contained a single carbon-carbon double bond. The location of the double bond was established by a careful study of the MS spectra. In particular, the loss of twelve units of mass observed between the fragments at *m/z* 318 and 306 in both compounds indicated that the double bond was located at C-19,C-20.[7] Therefore structures **4** and **10** were proposed for mycalazol 4 and mycalazol 10, respectively.

Mycalazol 7 (**7**) was obtained as an amorphous powder of molecular formula $C_{23}H_{41}NO_2$ as deduced from the high resolution mass measurement. The ^{1}H NMR spectrum showed the H-3 and H-4 signals of a pyrrole nucleus at δ 6.86 (dd, J=3.5 and 2.8, 1H) and 6.12 (dd, J=3.5 and 2.8, 1H). Furthermore, the ^{13}C NMR signals at 191.7 (s) and 57.9 (t) indicated that **7** possessed the 5-acyl-2-hydroxymethylpyrrole structure which accounts for the four degrees of unsaturation of the molecule. All the ^{13}C NMR signals attributable to the side chain were triplets excepting the doublet at δ 27.9 and the quartet at δ 22.6 indicating the presence of a 16-methylheptadecanoyl side chain. These data were in agreement with structure **7** for mycalazol 7.

Mycalazol 11 (**11**) was isolated as an amorphous powder. The molecular formula $C_{24}H_{43}NO_2$, deduced by HRMS, together with the ^{1}H and ^{13}C NMR data indicated that the structure of **11** was very similar to that of mycalazol 7 (**7**). The main difference on the ^{13}C NMR spectrum of **11** was the absence of the doublet due

to the CH of an isopropyl group indicating that the acyl chain in **11** was unbranched. These data could be acommodated by structure **11**.

A less polar fraction of the general chromatography contained a mixture of 5-alkylpyrrole-2-carbaldehydes. After HPLC separation on reversed phase, mycalazal 1 (**13**, 0.024 % dry wt) and mycalazal 2 (**14**, 0.008 % dry wt) were isolated. The high unstableness of both compounds prevented better spectroscopic data from being obtained.

Mycalazal 1 (**13**) was obtained as a colorless oil. The mass spectrum showed the molecular ion peak at *m/z* 433. Both ^{1}H NMR and ^{13}C NMR spectra contained the signals of a 2,5-disubstituted pyrrole nucleus. The presence of an aldehyde conjugated with the pyrrole nucleus was deduced from the ^{13}C NMR signal at δ 178.1 (s), the ^{1}H NMR signal at 9.37 (s, 1H) as well as from the IR absorption at 1650 cm^{-1}. The absence of other carbonyl signals in the ^{13}C NMR spectrum together with the presence of twelve doublets between δ 132.0 and δ 127.0 and the ^{1}H NMR signal at δ 5.37 (m, 12H) indicated the presence of an alkenyl chain with six double bonds. The ^{1}H NMR signal at δ 2.83 (m, 10H) was assigned to five bisallylic methylene groups. A methyl proton signal at δ 0.97 (t, J=7.4, 3H) which showed a cross peak in the COSY spectrum with the allylic methylene signals at δ 2.06 indicated that the unsaturations started at ω-3 position. These spectroscopic data together with the ^{13}C NMR triplets at δ 29.4, 29.1, 28.9, 27.8, 27.2 and 25.5 indicated the presence of a pentacosa-7,10,13,16,19,22-hexaenyl side chain and therefore structure **13** was proposed for mycalazal 1.

Mycalazal 2 (**14**) was isolated as a colorless oil. The similarities observed in the spectroscopic data of **14** with those of mycalazal 1 (**13**) and the molecular ion peak at *m/z* 435 in the mass spectrum of **14** indicated that this compound was a dihydroderivative of mycalazal 1 (**13**). In the ^{1}H NMR spectrum the signal at δ 2.83 (m, 8H) accounted for only four bisallylic methylenes and the signal at δ 0.97 (t, J=7.4, 3H) showed coupling in the COSY spectrum with the allylic methylenes signal at δ 2.06 (m, 4H) indicating that the unsaturations started at ω-3 position and that **14** must be the 12,13-dihydroderivative of mycalazal 1 (**13**).

Table 1. Cytotoxicity Data (ED_{50}, μg/mL) of the Mycalazols 1-12 (1-12) and Mycalazal 2 (14).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**14**
P 388	2	2	2	2	2	1	1	2	2	2	2.5	1	2
SCHABEL	2	2	2	2	2	1	1	2	2	2	2.5	2	2
A 549	2	2	2	2	2	1	1	2	2	2	1	1	5
HT 29	2	5	2.5	2.5	2.5	1	2	5	2	2.5	10	10	5
MEL 28	2.5	5	2.5	2.5	2.5	2	2	2.5	2	2.5	10	5	5

The new compounds isolated from *M. micracanthoxea* were tested against P388 and SCHABEL mice lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma and MEL28 human melanoma cell

lines to detect *in vitro* cytotoxicity. The results of ED_{50} are summarized in Table 1. In general the new compounds showed cytotoxicity against the five cell lines above mentioned, being the mycalazols 6 (**6**), 7 (**7**) and 12 (**12**) the more active compounds. Mycalazal 1 (**13**) was inactive in these tests probably due to the high unstableness of this aldehyde.

EXPERIMENTAL SECTION

General: IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer and UV spectra were recorded on a Philips PU 8710 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR were made on a Varian Unity 400 at 400 MHz and 100 MHz respectively, using $CDCl_3$ as solvent. The resonance of residual chloroform at δ_H 7.26 and δ_C 77.0 were used as internal reference for ^{1}H and ^{13}C spectra, respectively. Mass spectra were recorded on a VG 12250 or on Kratos MS 80RFA spectrometer. In High Performance Liquid Chromatography separations LiChrosorb RP-18 was used in reversed phase mode using a differential refractometer. Assignments with identical superscripts may be interchanged. All solvents were spectral grade or were distilled from glass prior to use.

Collection, Extraction and Purification: The sponge *Mycale microchantoxea* (24.5 g, dry weight) was collected by hand using SCUBA in Algeciras Bay in September 1994, and was kept frozen until its extraction. The frozen tissue was extracted exhaustively with acetone at room temperature. The filtered Me_2CO solution was evaporated under reduced pressure and the aqueous residue was extracted with Et_2O (3 x 250 mL). The solvent was evaporated to give an oil residue (0.85 g) which was chromatographed on a SiO_2 column using solvents of increasing polarity from hexane to diethyl-ether and, subsequently, chlorofom-methanol (8:2). Fractions eluted with 40% ether in hexane were subjected to reversed phase HPLC separation on a preparative LiChrosorb RP-18 column eluting with MeOH/H_2O (93:7) to afford mycalazol 1 (**1**, 4 mg, 0.0016% dry weight), mycalazol 2 (**2**, 4 mg, 0.0016% dry weight), mycalazol 3 (**3**, 6 mg, 0.0024% dry weight), mycalazol 4 (**4**, 11 mg, 0.0045% dry weight), mycalazol 5 (**5**, 4 mg, 0.016% dry weight), mycalazol 6 (**6**, 2 mg, 0.008% dry weight), mycalazol 7 (**7**, 2 mg, 0.008% dry weight), mycalazol 8 (**8**, 1.5 mg, 0.006% dry weight), mycalazol 9 (**9**, 5 mg, 0.020% dry weight), mycalazol 10 (**10**, 11 mg, 0.044% dry weight), mycalazol 11 (**11**, 1.9 mg, 0.008% dry weight), mycalazol 12 (**12**, 2 mg, 0.008% dry weight). Final purification of the mycalazols 1-12 was accomplished by HPLC on reversed phase mode using solvents of various proportions of H_2O in CH_3CN. Fractions of the general chromatography eluted with 20% ether in hexane yielded, after purification by HPLC on a preparative LiChrosorb RP-18 column eluting with acetonitrile, mycalazal 1 (**13**, 6 mg, 0.024% dry weight) and mycalazal 2 (**14**, 2 mg, 0.008% dry weight).

Mycalazol 1 (1): Colorless oil; IR (film) 3200-3500, 2924, 2853, 1688, 1635, 1047, 723 cm^{-1}; UV (Methanol) 201 (ε 4170), 247 (ε 1581), 294 (ε 7656) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.44 (br s, -NH), 6.89 (dd, 1H, J = 3.8, 2.7 Hz, H-4), 6.14 (dd, 1H, J = 3.8, 2.7 Hz, H-3), 5.37 (m, 12H, H-12, H-13, H-15, H-16, H-18, H-19, H-21, H-22, H-24, H-25, H-27 and H-28), 4.72 (s, 2H, -CH_2OH), 3.23 (br s, -OH), 2.80 (m, 10H, H-14, H-17, H-20, H-23 and H-26), 2.77 (t, 2H, J = 7.6 Hz, H-7), 2.07 (m, 4H, H-11 and H-29), 1.73 (tt, 2H, J = 7.6, 7.6 Hz, H-8), 1.25-1.39 (broad signal, 4H, H-9 and H-10), 0.97 (t, 3H, J = 7.6 Hz, H-30); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.6 (s, C-6), 139.7 (s, C-5), 132.0 (d, C-28), 131.6 (s, C-2), 130.1 (d, C-13), 128.6 (d, C-25)[a], 128.5 (d, C-24)[a], 128.3 (d, C-22)[a], 128.2 (d, C-21)[a], 128.1 (2×d, C-18 and C-19)[a], 127.9 (d, C-16)[a], 127.8 (2×d, C-12 and C-15)[a], 127.0 (d, C-27), 117.6 (d, C-4), 108.6 (d, C-3), 57.9 (t, -CH_2OH), 37.8 (t, C-7), 29.4

(t, C-10)[b], 29.1 (t, C-9)[b], 27.1 (t, C-11), 25.6 (4×t, C-17, C-20, C-23 y C-26)[c], 25.5 (t, C-14)[c], 25.4 (t, C-8)[c], 20.5 (t, C-29), 14.2 (q, C-30); EIMS (70 eV) *m/z* (rel. int.) 449 (0.26), 361 (6.7), 330 (4.7), 152 (20.4), 139 (100), 105 (40); HREIMS Obsd. *m/z*= 449.3320 $(M)^+$, $C_{30}H_{43}NO_2$ requires *m/z*= 449.3294.

Mycalazol 2 (2): Colorless oil; IR (film) 3300-3500, 2925, 2850, 1650, 1035, 723 cm^{-1}; UV (Methanol) 202 (ε 8490), 238 (ε 6912), 296 (ε 16584) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.44 (br s, -NH), 6.89 (dd, 1H, *J* = 3.8, 2.6 Hz, H-4), 6.14 (dd, 1H, *J* = 3.8, 2.6 Hz, H-3), 5.37 (m, 6H, H-17, H-18, H-20, H-21, H-23 and H-24), 4.72 (s, 2H, -CH_2OH), 3.23 (br s, -OH), 2.80 (m, 4H, H-19 and H-22), 2.77 (t, 2H, *J* = 7.6 Hz, H-7), 2.06 (m, 4H, H-25 and H-16), 1.71 (tt, 2H, *J* = 7.0, 7.0 Hz, H-8), 1.25-1.33 (broad signal, 14H, H-9, H-10, H-11, H-12, H-13, H-14 and H-15), 0.97 (t, 3H, *J* = 7.6 Hz, H-26); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.8 (s, C-6), 139.6 (s, C-5), 132.0 (d, C-24), 131.7 (s, C-2), 130.4 (d, C-18), 128.3 (d, C-20)[a], 128.2 (d, C-21)[a], 127.7 (d, C-17), 127.2 (d, C-23), 117.6 (d, C-4), 108.6 (d, C-3), 58.0 (t, -CH_2OH), 37.9 (t, C-7), 29.7 (t, C-15)[b], 29.6 (t, C-14)[b], 29.5 (3×t, C-13, C-12 and C-11)[b], 29.4 (t, C-10)[b], 29.3 (t, C-9)[b], 27.3 (t, C-16), 25.7 (t, C-22)[c], 25.6 (t, C-19)[c], 25.5 (t, C-8)[c], 20.6 (t, C-25), 14.3 (q, C-26); EIMS (70 eV) *m/z* (rel. int.) 399 (2.10), 384 (0.9), 370 (3.9), 152 (12.6), 139 (100); HREIMS Obsd. *m/z*= 399.3123 $(M)^+$, $C_{26}H_{41}NO_2$ requires *m/z*= 399.3137.

Mycalazol 3 (3): Colorless oil; IR (film) 3300-3500, 2924, 2850, 1635, 1044, 721 cm^{-1}; UV (Methanol) 206 (ε 11045), 246 (ε 2332), 295 (ε 10106) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.57 (br s, -NH), 6.90 (dd, 1H, *J* = 3.8, 2.5 Hz, H-4), 6.15 (dd, 1H, *J* = 3.8, 2.5 Hz, H-3), 5.37 (m, 8H, H-16, H-17, H-19, H-20, H-22, H-23, H-25 and H-26), 4.73 (s, 2H, -CH_2OH), 3.40 (br s, -OH), 2.81 (m, 6H, H-18, H-21 and H-24), 2.76 (t, 2H, *J* = 7.6 Hz, H-7), 2.06 (m, 4H, H-27 and H-15), 1.71 (tt, 2H, *J* = 7.6, 7.6 Hz, H-8), 1.25-1.36 (broad signal, 12H, H-9, H-10, H-11, H-12, H-13 and H-14), 0.97 (t, 3H, *J* = 7.4 Hz, H-28); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.9 (s, C-6), 139.9 (s, C-5), 132.0 (d, C-26), 131.6 (s, C-2), 130.4 (d, C-17), 128.5 (d, C-23)[a], 128.4 (d, C-22)[a], 128.0 (d, C-20)[a], 127.9 (d, C-19)[a], 127.6 (d, C-16), 127.0 (d, C-25), 117.8 (d, C-4), 108.6 (d, C-3), 57.9 (t, -CH_2OH), 37.8 (t, C-7), 29.6 (t, C-14)[b], 29.5 (2×t, C-13 and C-12)[b], 29.4 (2×t, C-11 and C-10)[b], 29.3 (t, C-9)[b], 27.2 (t, C-15), 25.6 (2×t, C-21 and C-18)[c], 25.5 (2×t, C-24 and C-8)[c], 20.5 (t, C-27), 14.3 (q, C-28); EIMS (70 eV) *m/z* (rel. int.) 425 (4.2), 408 (1.3), 394 (1.4), 356 (1.3), 276 (0.4), 152 (9.9), 139 (100); HREIMS Obsd. *m/z*= 425.3281 $(M)^+$, $C_{28}H_{43}NO_2$, requires *m/z*= 425.3294.

Mycalazol 4 (4): White crystals; mp (MeOH)= 68-70°C; IR (film) 3300-3500, 2930, 2850, 1640, 1044, 723 cm^{-1};UV (Methanol) 206 (ε 10492), 252 (ε 1106), 292 (ε 13105) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.80 (br s, -NH), 6.91 (dd, 1H, *J* = 3.8, 2.6 Hz, H-4), 6.15 (dd, 1H, *J* = 3.8, 2.6 Hz, H-3), 5.35 (m, 2H, H-19 and H-20), 4.73 (s, 2H, -CH_2OH), 3.74 (br s, -OH), 2.77 (t, 2H, *J* = 7.6 Hz, H-7), 2.01 (m, 4H, H-18 and H-21), 1.72 (tt, 2H, *J* = 7.2, 7.2 Hz, H-8), 1.25-1.32 (broad signal, 22H, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-22 and H-23), 0.88 (br t, 3H, *J* = 6.8 Hz, H-24); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 192.1 (s, C-6), 140.2 (s, C-5), 131.6 (s, C-2), 129.9 (d, C-20)[a], 129.8 (d, C-19)[a], 118.0 (d, C-4), 108.6 (d, C-3), 57.9 (t, -CH_2OH), 37.5 (t, C-7), 31.9 (t, C-22), 29.7-29.3 (9×t, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16 and C-17), 27.2 (t, C-18)[b], 25.6 (2×t, C-21 and C-8)[b], 22.7 (t, C-23), 14.1 (q, C-24); EIMS (70 eV) *m/z* (rel. int.) 375 (1.4), 332 (0.3), 318 (2.5), 306 (1.0), 152 (16.9), 139 (100); FABMS *m/z* (int. rel.) 398 ($(M+23)^+$, 100); HREIMS Obsd. *m/z*= 375.3142 $(M)^+$, $C_{24}H_{41}NO_2$ requires *m/z*= 375.3137.

Mycalazol 5 (5): Colorless oil; IR (film) 3300-3500, 2924, 2850, 1640, 1044, 723 cm^{-1};UV (Methanol) 204 (ε 11230), 250 (ε 2122), 296 (ε 10003) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.00 (br s, -NH), 6.88 (dd, 1H, *J* = 3.8, 2.8 Hz, H-4), 6.15 (dd, 1H, *J* = 3.8, 2.8 Hz, H-3), 5.37 (m, 10H, H-15, H-16, H-18, H-19, H-21, H-22,

H-24, H-25, H-27 and H-28), 4.73 (d, 2H, J = 6.0 Hz, -CH_2OH), 2.81 (m, 8H, H-17, H-20 H-23 and H-26), 2.75 (t, 2H, J = 7.6 Hz, H-7), 2.60 (br t, J = 6.0 Hz, -OH), 2.06 (m, 4H, H-29 and H-14), 1.71 (tt, 2H, J = 7.6, 7.6 Hz, H-8), 1.25-1.33 (broad signal, 10H, H-9, H-10, H-11, H-12 and H-13), 0.97 (t, 3H, J = 7.6 Hz, H-30); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.8 (s, C-6), 139.7 (s, C-5), 132.0 (d, C-28), 131.7 (s, C-2), 130.4 (d, C-16), 128.6 (2×d, C-21 and C-18)[a], 128.2 (2×d, C-24 and C-22)[a], 127.9 (2×d, C-25 and C-19)[a], 127.6 (d, C-15), 127.0 (d, C-27), 117.6 (d, C-4), 108.6 (d, C-3), 57.9 (t, -CH_2OH), 37.8 (t, C-7), 29.6 (t, C-13)[b], 29.4 (3×t, C-12, C-11 and C-10)[b], 29.3 (t, C-9)[b], 27.3 (t, C-14), 25.7 (2×t, C-20 and C-17)[c], 25.6 (3×t, C-26, C-23 and C-8)[c], 20.6 (t, C-29), 14.3 (q, C-30); EIMS (70 eV) *m/z* (rel. int.) 451 (0.2), 433 (0.3), 420 (1.4), 404 (2.8), 152 (8.7), 139 (100).

Mycalazol 6 (6): Colorless oil; IR (film) 3300-3600, 2943, 2850, 1645, 1040, 723 cm^{-1}; UV (Methanol) 205 (ε 9128), 296 (ε 8968) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.20 (br s, -NH), 6.88 (dd, 1H, J = 3.7, 2.5 Hz, H-4), 6.15 (dd, 1H, J = 3.7, 2.5 Hz, H-3), 5.37 (m, 4H, H-17, H-18, H-20 and H-21), 4.72 (s, 2H, -CH_2OH), 3.23 (br s, -OH), 2.80 (m, 2H, H-19), 2.75 (t, 2H, J = 7.6 Hz, H-7), 2.04 (q, 4H, J = 7.6 Hz, H-22 and H-16), 1.71 (tt, 2H, J = 7.0 Hz, H-8), 1.25-1.34 (broad signal, 20H, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-23, H-24 and H-25), 0.88 (br t, 3H, J = 7.2 Hz, H-26); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.6 (s, C-6), 139.2 (s, C-5), 131.8 (s, C-2), 130.4 (2×d, C-18 and C-21), 127.9 (2×d, C-17 and C-20), 117.3 (d, C-4), 108.5 (d, C-3), 58.0 (t, -CH_2OH), 37.9 (t, C-7), 31.5 (t, C-24), 29.7 (t, C-9)[a], 29.6 (t, C-10)[a], 29.5 (2×t, C-12 and C-11)[a], 29.4 (3×t, C-13, C-14 and C-15)[a], 29.3 (t, C-23)[a], 27.3 (t, C-16)[b], 27.2 (t, C-22)[b], 25.6 (t, C-19)[c], 25.5 (t, C-8)[c], 22.6 (t, C-25), 14.1 (q, C-26); EIMS (70 eV) *m/z* (rel. int.) 401 (6.6), 384 (0.6), 370 (1.8), 358 (0.3), 344 (0.6), 318 (1.7), 304 (1.5), 264 (0.3), 152 (21.6), 139 (100); HREIMS Obsd. *m/z*= 401.3289 $(M)^+$, $C_{26}H_{43}NO_2$ requires *m/z*= 401.3294.

Mycalazol 7 (7): Colorless oil; IR (film) 3300-3500, 2930, 2850, 1640, 1044 cm^{-1}; UV (Methanol) 204 (ε 7452), 294 (ε 9470) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.35 (br s, -NH), 6.86 (dd, 1H, J = 3.5, 2.8 Hz, H-4), 6.12 (dd, 1H, J = 3.5, 2.8 Hz, H-3), 4.70 (s, 2H, -CH_2OH), 3.00 (br s, -OH), 2.73 (t, 2H, J = 7.6 Hz, H-7), 1.69 (tt, 2H, J = 7.4, 7.4 Hz, H-8), 1.69 (qq, 1H, J = 6.6, 6.6 Hz, H-21), 1.23-1.30 (broad signal, 24H, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19 and H-20), 0.83 (d, 6H, J = 6.6 Hz, H-22 and H-23); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.7 (s, C-6), 139.3 (s, C-5), 131.7 (s, C-2), 117.4 (d, C-4), 108.5 (d, C-3), 57.9 (t, -CH_2OH), 39.0 (t, C-20), 37.8 (t, C-7), 29.9 (2×t, C-9 and C-10)[a], 29.7 (2×t, C-11 and C-12)[a], 29.6 (3×t, C-13, C-14 and C-15)[a], 29.5 (t, C-16)[a], 29.4 (2×t, C-17 and C-18)[a], 27.9 (d, C-21), 27.4 (t, C-19), 25.5 (t, C-8), 22.6 (2×q, C-22 and C-23); EIMS (70 eV) *m/z* (rel. int.) 363 (3.6), 332 (3.0), 152 (16.9), 139 (100); HREIMS Obsd. *m/z*= 363.3142 $(M)^+$, $C_{23}H_{41}NO_2$ requires *m/z*= 363.3137.

Mycalazol 8 (8): Colorless oil; IR (film) 3300-3500, 2925, 2850, 1650, 1035, 723 cm^{-1}; UV (Methanol) 201 (ε 9128), 240 (ε 4312), 295 (ε 16348) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.59 (br s, -NH), 6.87 (dd, 1H, J = 3.8, 2.5 Hz, H-4), 6.13 (dd, 1H, J = 3.8, 2.5 Hz, H-3), 5.35 (m, 6H, H-19, H-20, H-22, H-23, H-25 and H-26), 4.71 (s, 2H, -CH_2OH), 3.40 (br s, -OH), 2.80 (m, 4H, H-21 and H-24), 2.74 (t, 2H, J = 7.6 Hz, H-7), 2.05 (m, 4H, H-18 and H-27), 1.70 (tt, 2H, J = 7.5, 7.5 Hz, H-8), 1.25-1.31 (broad signal, 18H, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16 and H-17), 0.96 (t, 3H, J = 7.6 Hz, H-28); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.7 (s, C-6), 139.3 (s, C-5), 132.0 (d, C-26), 131.8 (s, C-2), 130.4 (d, C-20), 128.3 (d, C-23)[a], 128.2 (d, C-22)[a], 127.6 (d, C-19), 127.2 (d, C-25)[a], 117.3 (d, C-4), 108.5 (d, C-3), 58.0 (t, -CH_2OH), 37.9 (t, C-7), 29.7 (4×t, C-17, C-16, C-15 and C-14)[b], 29.6 (t, C-13)[b], 29.5 (2×t, C-12 and C-11)[b], 29.4 (t, C-10)[b], 29.3 (t, C-9)[b], 27.3 (t, C-18), 25.6 (2×t, C-8 and C-24)[c], 25.5 (t, C-21)[c], 20.6 (t, C-27), 14.3 (q, C-28); EIMS (70 eV) *m/z*

(rel int.) 427 (4.4), 410 (2.1), 398 (1.2), 278 (0.3), 152 (21.9), 139 (100); HREIMS Obsd. *m/z*= 427.3455 $(M)^+$, $C_{28}H_{45}NO_2$ requires *m/z*= 427.3450.

Mycalazol 9 (9): Colorless oil; IR (film) 3300-3500, 2924, 2850, 1635, 1044, 724 cm^{-1}; UV (Methanol) 203 (ε 10145), 251 (ε 1580), 293 (ε 13110) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.49 (br s, -NH), 6.90 (dd, 1H, *J* = 3.8, 2.5 Hz, H-4), 6.15 (dd, 1H, *J* = 3.8, 2.5 Hz, H-3), 5.37 (m, 8H, H-18, H-19, H-21, H-22, H-24, H-25, H-27 and H-28), 4.73 (s, 2H, $-CH_2OH$), 3.40 (br s, -OH), 2.81 (m, 6H, H-20, H-23 and H-26), 2.76 (t, 2H, *J* = 7.6 Hz, H-7), 2.06 (m, 4H, H-29 and H-17), 1.71 (tt, 2H, *J* = 7.6, 7.6 Hz, H-8), 1.25-1.36 (broad signal, 16H, H-9, H-10, H-11, H-12, H-13, H-14, H-15 and H-16), 0.97 (t, 3H, *J* = 7.4 Hz, H-30); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.9 (s, C-6), 139.7 (s, C-5), 132.0 (d, C-28), 131.7 (s, C-2), 130.5 (d, C-19), 128.5 (2×d, C-21 and C-22)[a], 128.0 (d, C-24)[a], 127.9 (d, C-25)[a], 127.6 (d, C-18), 127.1 (d, C-27), 117.7 (d, C-4), 108.6 (d, C-3), 57.9 (t, $-CH_2OH$), 37.9 (t, C-7), 29.7 (t, C-9)[b], 29.6 (3×t, C-10, C-11 and C-12)[b], 29.5 (t, C-13)[b], 29.4 (2×t, C-14 and C-15)[b], 29.3 (t, C-16)[b], 27.3 (t, C-17), 25.7 (2×t, C-20 and C-23)[c], 25.6 (2×t, C-26 and C-8)[c], 20.6 (t, C-29), 14.2 (q, C-30); EIMS (70 eV) *m/z* (rel. int.) 453 (30.5), 436 (14.2), 422 (5.9), 384 (4.1), 344 (2.7), 152 (27.0), 139 (100); FABMS *m/z* (rel. int.) 476 $((M+23)^+$, 100); HREIMS Obsd. *m/z*= 453.3618 $(M)^+$, $C_{30}H_{47}NO_2$ requires *m/z*= 453.3642.

Mycalazol 10 (10): White crystals; mp (MeOH)= 74-76°C; IR (film) 3300-3500, 2930, 2850, 1640, 1044, 723 cm^{-1}; UV (Methanol) 204 (ε 10978), 252 (ε 925), 296 (ε 12940) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.90 (br s, -NH), 6.92 (dd, 1H, *J* = 3.8, 2.6 Hz, H-4), 6.14 (dd, 1H, *J* = 3.8, 2.6 Hz, H-3), 5.34 (m, 2H, H-19 and H-20), 4.73 (s, 2H, $-CH_2OH$), 3.70 (br s, -OH), 2.77 (t, 2H, *J* = 7.6 Hz, H-7), 2.01 (m, 4H, H-18 and H-21), 1.71 (tt, 2H, *J* = 7.2, 7.2 Hz, H-8), 1.25-1.32 (broad signal, 26H, 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), 0.88 (br t, 3H, *J* = 6.8 Hz, H-26); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 192.1 (s, C-6), 140.3 (s, C-5), 131.6 (s, C-2), 129.9 (2×d, C-19 and C-20), 118.1 (d, C-4), 108.7 (d, C-3), 57.8 (t, $-CH_2OH$), 37.8 (t, C-7), 31.8 (t, C-24), 29.8-28.9 (11×t, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-22 and C-23), 27.2 (2×t, C-18 and C-21), 25.6 (t, C-8), 22.6 (t, C-25), 14.1 (q, C-26); EIMS (70 eV) *m/z* (rel. int.) 403 (19.3), 372 (6.3), 388 (0.6), 374 (1.27), 332 (0.3), 318 (2.5), 306 (2.0), 152 (11.5), 139 (100); FABMS *m/z* (rel. int.) 426 $((M+23)^+$, 100); HREIMS Obsd. *m/z* = 403.3454 $(M)^+$, $C_{26}H_{45}NO_2$ requires *m/z* = 403.3450.

Mycalazol 11 (11): Amorphous powder; IR (film) 3300-3500, 2930, 2850, 1640, 1044 cm^{-1}; UV (Methanol) 204 (ε 7452), 294 (ε 9470) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.23 (br s, -NH), 6.88 (dd, 1H, *J* = 3.8, 2.4 Hz, H-4), 6.15 (dd, 1H, *J* = 3.8, 2.4 Hz, H-3), 4.72 (s, 2H, $-CH_2OH$), 2.75 (t, 2H, *J* = 7.6 Hz, H-7), 1.71 (tt, 2H, *J* = 7.6, 7.6 Hz, H-8), 1.25-1.32 (broad signal, 30H, 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), 0.88 (t, 3H, *J* = 6.8 Hz, H-24); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.7 (s, C-6), 139.3 (s, C-5), 131.7 (s, C-2), 117.4 (d, C-4), 108.5 (d, C-3), 57.9 (t, $-CH_2OH$), 37.8 (t, C-7), 31.9 (t, C-22), 29.7-29.4 (13×t, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20 and C-21), 25.5 (t, C-8), 22.7 (t, C-23), 14.1 (q, C-24); EIMS (70 eV) *m/z* (rel. int.) 377 (20.33), 360 (2.9), 346 (6.0), 152 (16.9), 139 (100); HREIMS Obsd. *m/z* = 377.3295 $(M)^+$, $C_{24}H_{43}NO_2$ requires *m/z* = 377.3294.

Mycalazol 12 (12): Colorless oil; IR (film) 3300-3600, 2943, 2850, 1645, 1040, 723 cm^{-1}; UV (Methanol) 203 (ε 7852), 295 (ε 8864) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.18 (br s, -NH), 6.86 (dd, 1H, *J* = 3.7, 2.5 Hz, H-4), 6.12 (dd, 1H, *J* = 3.7, 2.5 Hz, H-3), 5.37 (m, 4H, H-19, H-20, H-22 and H-23), 4.70 (s, 2H, $-CH_2OH$), 3.00 (br s, -OH), 2.77 (m, 2H, H-21), 2.75 (t, 2H, *J* = 7.5 Hz, H-7), 2.02 (q, 4H, *J* = 6.8 Hz, H-24 and H-18),

1.69 (tt, 2H, J = 6.8, 6.8 Hz, H-8), 1.25-1.35 (broad signal, 24H, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-25, H-26 and H-27), 0.87 (br t, 3H, J = 6.8 Hz, H-26); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.6 (s, C-6), 139.1 (s, C-5), 131.8 (s, C-2), 130.2 (2×d, C-23 and C-20), 127.9 (2×d, C-19 and C-22), 117.3 (d, C-4), 108.5 (d, C-3), 58.0 (t, -CH_2OH), 37.9 (t, C-7), 31.6 (t, C-26), 29.7 (4×t, C-9, C-10, C-11 and C-12)[a], 29.6 (t, C-13)[a], 29.5 (3×t, C-14, C-15 and C-16)[a], 29.4 (2×t, C-17 and C-25)[a], 27.3 (t, C-18)[b], 27.2 (t, C-24)[b], 25.7 (t, C-21)[c], 25.6 (t, C-8)[c], 22.6 (t, C-27), 14.1 (q, C-28); EIMS (70 eV) m/z (rel. int.) 429 (10.5), 398 (2.3), 400 (2.1), 386 (0.2), 372 (0.5), 358 (0.24), 346 (0.9), 332 (0.51), 152 (22.5), 139 (100); FABMS m/z (rel int.) 452 ($(M+23)^+$, 100); HREIMS Obsd. m/z= 429.3599 $(M)^+$, $C_{28}H_{47}NO_2$ requires m/z= 429.3607.

Mycalazal 1 (13): Colorless oil; IR (film) 3260, 2923, 2690, 1650, 1044, 723 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.37 (s, -CHO), 9.25 (br s, -NH), 6.88 (dd, 1H, J = 3.5, 2.4 Hz, H-3), 6.07 (dd, 1H, J = 3.5, 2.4 Hz, H-4), 5.37 (m, 12H, H-12, H-13, H-15, H-16, H-18, H-19, H-21, H-22, H-24, H-25, H-27 and H-28), 2.83 (m, 10H, H-14, H-17, H-20, H-23 and H-26), 2.64 (t, 2H, J = 7.6 Hz, H-6), 2.04 (m, 4H, H-11 and H-29), 1.64 (tt, 2H, J = 7.6, 7.6 Hz, H-7), 1.34 (broad signal, 6H, H-8, H-9 and H-10), 0.97 (t, 3H, J = 7.4 Hz, H-30); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 178.1 (d, -CHO), 142.6 (s, C-2), 132.0 (d, C-28), 131.9 (s, C-5), 130.2 (d, C-13), 128.6 (d, C-25)[a], 128.5 (d, C-24)[a], 128.3 (d, C-22)[a], 128.2 (2×d, C-21 and C-19)[a], 128.1 (d, C-18)[a], 128.0 (d, C-16)[a], 127.9 (d, C-15)[a], 127.8 (d, C-12)[a], 127.0 (d, C-27), 122.3 (d, C-3), 109.4 (d, C-4), 29.4 (t, C-10)[b], 29.1 (t, C-9)[b], 28.9 (t, C-8)[b], 27.8 (t, C-11), 27.2 (t, C-7), 25.6 (4×t, C-14, C-17, C-20, C-23 and C-26)[c], 25.5 (t, C-6)[c], 20.6 (t, C-29), 14.3 (q, C-30); EIMS (70 eV) m/z (rel. int.) 433 (32.1), 364 (6.0), 324 (5.9), 108 (100).

Mycalazal 2 (14): Colorless oil; IR (film) 3340, 2935, 2850, 1650, 1055, 721 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.37 (s, CHO), 9.22 (bs, -NH), 6.88 (dd, 1H, J = 3.6, 2.4 Hz, H-3), 6.07 (dd, 1H, J = 3.6, 2.4 Hz, H-4), 5.37 (m, 10H, H-15, H-16, H-18, H-19, H-21, H-22, H-24, H-25, H-27 and H-28), 2.83 (m, 8H, H-17, H-20, H-23 and H-26), 2.64 (t, 2H, J = 7.6 Hz, H-6), 2.06 (m, 4H, H-14 and H-29), 1.65 (tt, 2H, J = 7.6, 7.6 Hz, H-7), 1.27 (broad signal, 12H, H-8, H-9, H-10, H-11, H-12 and H-13), 0.97 (t, 3H, J = 7.4 Hz, H-30); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 178.1 (d, CHO), 142.6 (s, C-2), 132.0 (d, C-28), 131.9 (s, C-5), 130.4 (d, C-16), 128.5 (d, C-18)[a], 128.4 (d, C-19)[a], 128.2 (d, C-21)[a], 128.0 (d, C-22)[a], 127.9 (d, C-24)[a], 127.8 (d, C-25)[a], 127.6 (d, C-15)[a], 127.0 (d, C-27), 122.3 (d, C-3), 109.4 (d, C-4), 29.4-28.9 (6×t, C-8, C-9, C-10, C-11, C-12 and C-13)[b], 27.9 (t, C-14), 27.3 (t, C-7), 25.6 (4×t, C-17, C-20, C-23 and C-26)[c], 25.5 (t, C-6)[c], 20.6 (t, C-29), 14.3 (q, C-30); EIMS (70 eV) m/z (rel. int.) 435 (0.3), 366 (1.0), 286 (2.5), 122 (42.1), 108 (100).

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