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Daminin, a bioactive pyrrole alkaloid from the Mediterranean sponge *Axinella damicornis*

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Abstract—The isolation and characterization of the known pyrrole alkaloid agelongine (**6**) and of the new natural product daminin (**7**), the bromine-free analogue of **6**, from a specimen of the marine sponge *Axinella damicornis* is described. Compound **7** showed significant neuroprotective properties. Moreover, for the supply of sufficient material for future medicinal investigations, a short total synthesis of **7** was developed.

1. Introduction

Marine sponges are rich sources of pyrrole alkaloids.[1] These secondary metabolites are of interest because of their structural variety and pharmacological activities. Most of them are characterized by the presence of a short linear aliphatic segment connecting a pyrrole-2-carboxylic acid moiety equipped with different bromine substitution patterns, with a heterocyclic ring, frequently an amino-imidazole unit (Fig. 1). The first member of this group to be discovered, initially in *Agelas oroides* in 1971 and then in various other sponges, was the dibrominated compound oroidin (**1**).[2] This alkaloid **1** shows antimicrobial activity and interacts with muscarinic acetylcholine receptors (mAChR) in rat brain membranes.[3] In the meantime, numerous similar alkaloids have been isolated from Agelasidae, Hymeniacidonidae, and Axinellidae species,[4–9] with diverse structures and interesting biological properties ranging from antiserotonergic and antihistaminic activities to inhibitory effects against EGF receptor kinase.[3,10–14]

More recently, manzacidins A–D (**2**–**5**), from a *Hymeniacidon* sp.[15] and agelongine (**6**), from the sponge *Agelas longissima*,[16] have extended the structural variety of the so far known pyrrole alkaloids in having a 3,4,5,6-tetrahydropyrimidine and a pyridinium ring, respectively, instead of the commonly found imidazole nucleus, and an ester linkage that replaces the usual amidic bond in the central segment. Agelongine (**6**) exhibits antiserotonergic activity on rat stomach fundus strip.

During our search for bioactive substances from Mediterranean sponges in the frame of the NOMATEC (BIOTECmarin) project, which aims at the sustainable development of the Mediterranean resources, we have now chemically investigated the organic extract of a specimen of *Axinella damicornis*. This study led to the renewed isolation of agelongine (**6**), together with the identification of a new alkaloid, daminin (**7**), the first naturally occurring agelongine analogue. In vitro tests on rat cortical cell cultures showed that daminin (**7**) might represent a new therapeutic tool for the treatment of CNS diseases such as Parkinson's and Alzheimer's diseases.[17]

2. Results and discussions

2.1. Isolation and structural elucidation of daminin (7)

Specimens of the sponge *Axinella damicornis* collected in the Bay of Calvi (Corsica) were extracted with methanol

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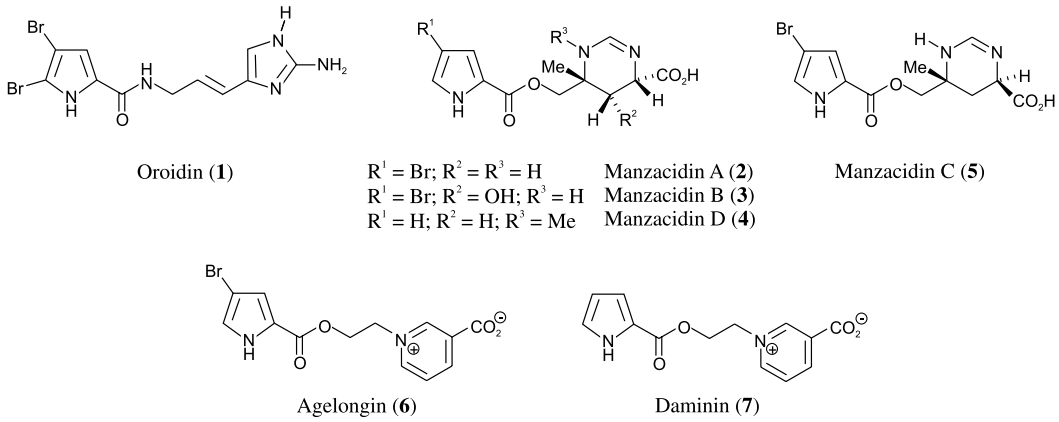

Figure 1. Structures of known pyrrole alkaloids (**1**–**6**) isolated from marine sponges and of the new natural product daminin (**7**).

and, subsequently, with chloroform. The combined extracts were concentrated in vacuo and partitioned between butanol and water. The butanol soluble material was initially subjected to a medium pressure liquid chromatography (MPLC) using a reversed-phase C-18 column. Further separation of the alkaloid-containing fractions was achieved by repeated preparative high performance liquid chromatography (HPLC) on an RP-18 column, thus giving the known agelongine (**6**, 3 mg) and the new compound daminin (**7**, 4 mg), both in a pure form. Compound **6** was identified by comparison of its spectral properties with those reported for agelongine (**6**) isolated from the sponge *Agelas longissima*.[16]

The ESI (positive ions) mass spectrum of daminin (**7**, Fig. 2) exhibited pseudomolecular ion peaks at *m/z* 261 $[M+H]^+$ and 283 $[M+Na]^+$. The molecular formula $C_{13}H_{12}N_2O_4$ was deduced from the HRFABMS (positive ions) of this compound, measured on the peak at *m/z* 261.0890 $[M+H]^+$ (calculated value: *m/z* 261.0875).

The presence of an aromatic ester function and a carboxylate group was suggested by IR absorptions at ν_{max} 1710 and 1646 cm^{-1}, respectively, which was confirmed by the carbonyl resonances in the ^{13}C NMR spectrum of **7** (DMSO-d_6) at δ 161.6 and 159.0 (Table 1).

The ^{13}C NMR spectrum also contained seven sp^2 methines and two sp^2 unprotonated carbons, which indicated the presence of aromatic rings, and furthermore two sp^3 methylene signals. The ^1H NMR spectrum (DMSO-d_6) exhibited ten well separated signals. The lowfield region of the spectrum contained seven signals of aromatic protons and one signal exchangeable with D_2O, while two mutually coupled methylene signals were visible in the central region of the spectrum (see Table 1). Analysis of the NMR spectra, including 2D COSY, ROESY, HSQC, and HMBC, clearly showed **7** to be a close structural analogue of agelongine (**6**). In fact, the spectral data obtained for **7** revealed it to contain the same pyridinium-β-carboxylate moiety as agelongine (**6**) linked through an ethylenoxy bridge to a pyrrole-2-carboxylic acid connected via an ester bond. The carboxylate portion of the ester corresponds to the chemical structure of the known betaine alkaloid pyridinebetaine A.[18] The latter heterocyclic moiety, however, was found to lack a bromine substituent, as clearly indicated by NMR signals of a complete set of three neighboring aromatic protons [δ_H 6.78 (t, J=0.9; 1.9 Hz), H-3; 6.16 (d, J=1.9 Hz), H-4; 7.05 (d, J=0.9 Hz), H-5; δ_C 120.7, C-2; 115.7, C-3; 109.5, C-4; 124.6, C-5], as well as by mass data (see Section 4). Thus, daminin (**7**) was concluded to be the non-brominated parent compound of agelongine (**6**). The NMR analysis also allowed us to assign all of the resonances present in ^{13}C and

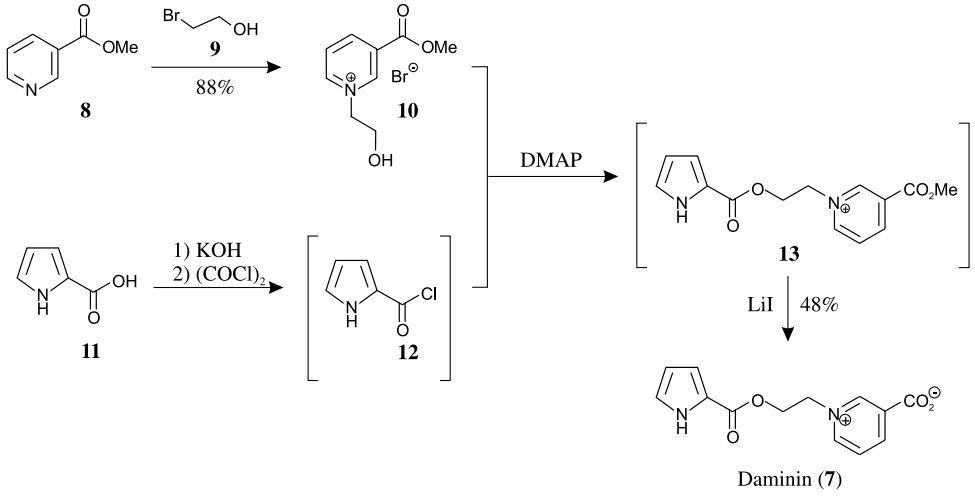

Figure 2. Synthesis of daminin (**7**) from the building blocks **8** and **1**.

Table 1. ^{1}H and ^{13}C NMR data (DMSO-d_6) of compound **7**

No.	δ_H (mult., J Hz)	δ_C	HMBC	COSY
1	12.3 (s)	—		
2	—	120.7		
3	6.78 (t, 0.9, 1.9)	115.7	2	4, 5
4	6.16 (d, 1.9)	109.5	2, 5	3, 5
5	7.05 (d, 0.9)	124.6	2, 3, 4	3, 4
6	—	159.0		
7	4.65 (t, 3.7)	61.8	8	8
8	5.01 (t, 3.7)	58.9	7	7
9	9.4 (s)	145.7	8, 10, 11, 14	11, 13
10	—	140.9		
11	8.78 (d, 7.4)	144.7	13, 14	9, 12
12	8.06 (t, 6.5, 7.4)	126.6	10, 11, 13	11, 13
13	9.04 (d, 6.5)	144.0	11, 12	9, 12
14	—	161.6		

^{1}H NMR spectra, which are reported in Table 1; particularly, the assignments of the pyrrole moiety were in good agreement with those reported for 2-substituted pyrroles.[8,19]

2.2. Synthesis of daminin (7)

A synthetic access (Fig. 2) to the new compound daminin (**7**) was realized by esterification of 1-(2-hydroxyethyl)-3-methoxycarbonyl-pyridinium bromide (**10**) with pyrrole-2-carboxylic acid chloride (**12**). *N*-alkylation of nicotinic acid methyl ester (**8**) using 2-bromoethanol (**9**) delivered the pyridinium salt **10** in 88% yield. The acid chloride **12** was prepared from pyrrole-2-carboxylic acid (**11**) and oxalyl chloride under basic conditions. In situ reaction of **12** with **10** and selective cleavage of the more reactive methyl ester on the electron-poor pyridinium portion of the crude intermediate **13** using lithium iodide gave daminin (**7**) in 48% yield, identical in all chromatographic and spectroscopic respects with the natural product from *A. damicornis*.

2.3. Bioactivities of daminin (7)

2.3.1. Cytostatic/cytotoxic activity of daminin (7). Daminin (**7**) was tested for its cytotostatic/cytotoxic activity against several tumor cell lines, namely murine leukemic lymphoblasts L5178y, rat adrenal pheochromocytoma PC12 cells, and human cervix carcinoma HeLa S3 cells. It displayed no cytotoxic activity against all tested cell lines. In all cases, the IC_{50} was >40 μg/mL.

2.3.2. Influence of L-Glu and $CaCl_2$ on the $[Ca^{2+}]_i$ level in neuronal cells after pre-incubation with daminin (7). Incubation of neurons with 200 μM of L-Glu (glutamic acid) and 2.4 mM $CaCl_2$ resulted in a strong rise in $[Ca^{2+}]_i$. If these components were added 10 min after starting the experiment, the 340/380 nm ratio value increased by 1.537 (305%, Fig. 3). However, if the neurons were pre-incubated for 5 min with 0.5, 1.0 or 3.0 μg/mL of daminin (**7**) a significant decrease of the $[Ca^{2+}]_i$ levels was recorded; after addition of L-Glu and $CaCl_2$ at time 10 min, the $[Ca^{2+}]_i$ level dropped to 58.1% (0.5 μg/mL of **7**), to 65.4% (1 μg/mL of 7) or to 25.1% (3 μg/mL of **7**, Fig. 3). Daminin (**7**) was present from time 5 min up to the end of the experiments (30 min). In the pre-incubation set of experiments neurons were incubated 5–10 min only in the presence of **7** and in the absence of L-Glu/$CaCl_2$. No effect on the $[Ca^{2+}]_i$ was measured (Fig. 3). In a parallel series of experiments it could be shown that daminin (**7**) caused no effect on the $[Ca^{2+}]_i$ level (not presented).

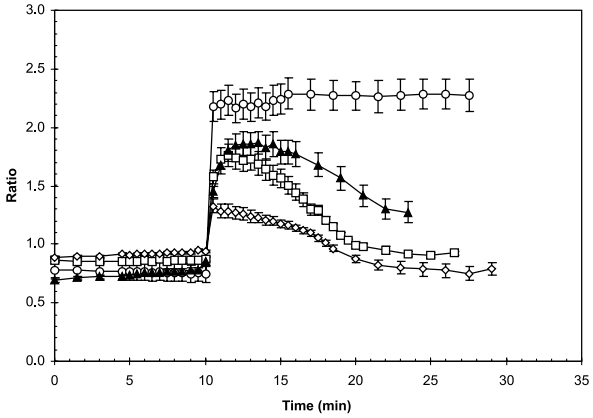

Figure 3. Incubation of the neurons with L-Glu and Ca^{2+} in the absence (○) or in the presence of 0.5 (▲), 1 (□), or 3 (◇) μg/mL of daminin (**7**). Daminin (**7**) was added at time 5 min and remained in the cultures during the entire incubation period. At time 10 min, neurons were stimulated with L-Glu and $CaCl_2$ as described in Experimental. The results are expressed as mean value ($n=35$) plus standard deviation (±SE).

2.3.3. Modulating effect of daminin (7) on the NMDA-caused $[Ca^{2+}]_i$ level. Incubation of neurons with 200 μM of NMDA (*n*-methyl-D-aspartate) and 2.4 mM $CaCl_2$ (here added at time 10 min) resulted in a strong increase in $[Ca^{2+}]_i$; the 340/380 nm ratio value increased by 1.117 (235%). This strong rise was statistically reduced to 63.5% (controls were set to 100) if the cells were pre-incubated with 1 μg/mL of daminin (**7**); Figure 4.

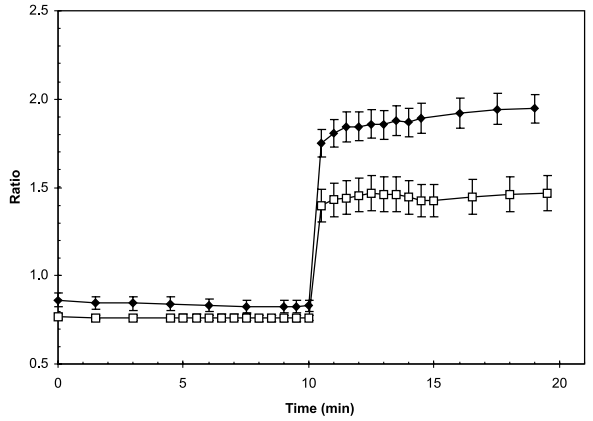

Figure 4. Incubation of the neurons with 200 μM of NMDA and 2.4 mM Ca^{2+} in the absence (◆) or in the presence of 1 (□) μg/mL of **7**. Daminin (**7**) was added at time 5 min and remained in the cultures, while NMDA/Ca^{2+} was added at time 10 min.

3. Conclusion

Daminin (**7**) is one of the relatively few non-brominated pyrrole alkaloids of marine origin. The promising neuroprotective activities of this natural product combined with its very low cytotoxicity and its easy synthetic access presented in this paper make further investigations concerning possible medicinal uses potentially promising.

4. Experimental

4.1. General aspects

ESI mass spectra (positive ions) were performed on an API 2000 mass spectrometer. High resolution FAB mass spectra (glycerol matrix) were taken on a VG Prospec (FISONS) mass spectrometer. NMR experiments were done on a Bruker AMX-500 and AV-400 spectrometers; chemical shifts are referred to the residual solvent signal (DMSO: $\delta_H = 2.49$ ppm, $\delta_C = 39.5$ ppm). Homonuclear (^1H–^1H) and heteronuclear (^1H–^{13}C) connectivities were determined by COSY and HSQC experiments, respectively. Two- and three-bond ^1H–^{13}C connectivities were investigated by HMBC experiments optimized for a $^{2,3}J$ of 10 Hz. Separations by medium-pressure liquid chromatography (MPLC) were performed on a Büchi 861 apparatus with SiO_2 (230–400 mesh) packed columns. High performance liquid chromatography (HPLC) separations were achieved on a Waters 501 apparatus equipped with an RI detector. IR (KBr) spectra were recorded on a Bruker model IFS-48 spectrophotometer.

4.2. Collection, extraction, and isolation

Specimens of the sponge *Axinella damicornis* were collected in November 2001 in the Bay of Calvi (Corsica) and kept frozen until use. For the extraction, the fresh thawed sponge (75.2 g dry weight after extraction) was homogenized and treated at room temperature with methanol (3×600 mL) and, subsequently, with chloroform (3×600 mL). The extracts were concentrated in vacuo and the water-insoluble lipid fraction obtained was treated with BuOH. The BuOH soluble portion was subjected to MPLC on a reversed-phase column eluting with $H_2O \rightarrow MeOH \rightarrow CHCl_3$. Two main alkaloid-containing fractions, eluted with MeOH/H_2O 3:7 (fraction A) and MeOH/H_2O 2:8 (fraction B), were obtained. The more polar fraction A was separated by HPLC on an RP-18 column (Luna, 5 μm, 250×4.6 mm) using MeOH/H_2O 3:7 as the eluent and further purified by HPLC on an C18 column (AQUA, 5 μm, 250×4.6 mm) thus providing **7** (4 mg) in a pure form. Fraction B was separated and purified by consecutive HPLC runs on RP-18 columns (Luna, 5 μm, 250×4.6 mm; Luna, 3 μm, 250×4.6 mm) with MeOH/H_2O 1:1 as the eluent, giving rise to pure **6** (3 mg).

4.2.1. Daminin (7). Amorphous white solid. IR (KBr): ν_{max} 1710, 1646 cm^{-1}. HRFABMS *m/z* $[M+H]^+$ 261.0890 (Calcd for $C_{13}H_{12}N_2O_4$, 261.0875). ^1H (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data: Table 1.

4.2.2. Agelongine (6). The data fully matched those reported in the literature.[16]

4.3. Total synthesis of daminin (7)

4.3.1. Synthesis of 1-(2-hydroxyethyl)-3-methoxycarbonylpyridinium bromide (10). A solution of 1.00 g (7.29 mmol) nicotinic acid methyl ester (**8**) and 5 mL (70.82 mmol) 2-bromoethanol (**9**) in 7 mL of toluene was stirred at 120 °C for 2.5 h. The solvent was removed under reduced pressure and the residue was washed 3 times with 15 mL diethyl ether. The solid was recrystallized from acetonitrile–diethyl ether (1:2) to give 1.17 g (6.41 mmol, 88%) pure **10** as colorless crystals.

Mp 108–109 °C (acetonitrile–diethyl ether); ^1H NMR (400 MHz, CD_3OD) δ 4.07 (t, $J=5.0$ Hz, 2H), 4.09 (s, 3H), 4.89 (t, $J=5.0$ Hz, 2H), 8.31 (dd, $J=8.2$, 6.1 Hz, 1H), 9.12 (ddd, $J=8.2$, 1.4, 1.4 Hz, 1H), 9.25 (ddd, $J=6.1$, 1.4, 1.4 Hz, 1H), 9.57 (bs, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 57.0, 64.3, 68.2, 132.1, 134.7, 149.6, 150.4, 152.2, 166.1. FABMS *m/z* 182.1 $[M+H\text{-}Br]^+$, 445.1 $[2M+H\text{-}Br]^+$.

4.3.2. Synthesis of daminin (7). A suspension of 230 mg (2.07 mmol) pyrrole-2-carboxylic acid potassium salt (**11**) (Aldrich, Germany) and 0.25 mL (2.98 mmol) oxalyl chloride in 5 mL CH_2Cl_2 was stirred at room temperature under N_2. After 4 h the solvent was removed under reduced pressure and the residue was re-dissolved in 5 mL CH_2Cl_2. 262 mg (1.43 mmol) of **10** were added together with a catalytic amount of 4-*N*,*N*-dimethylaminopyridine and the mixture was stirred over night at room temperature. The solvent was removed under reduced pressure and the remaining solid washed with diethyl ether and then dissolved in 4 mL of pyridine. After addition of 900 mg (6.72 mmol) of lithium iodide, the mixture was stirred at 100 °C for 3 h. The solvent was evaporated and the remaining solid was purified by chromatography on a silica gel column with ethyl acetate–MeOH (2:8) as the eluent. After recrystallization of the crude product from EtOH–diethylether (2:1), 177 mg (0.68 mmol, 48%) of pure daminin (**7**) were obtained.

Mp 193 °C (decomposition); all spectral data identical with those of the natural product **7** (see above).

4.4. Cell lines

L5178y cells (ATTCC CRL-1722) were grown in RPMI1640 supplemented with 10 mM Hepes, 10% (v/v) fetal calf serum (FCS) (PAA, Cölbe, Germany) and 0.1% (w/v) Gentamycin. PC12 cells (ATCC CRL-1573) were kept in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) horse serum, 5% (v/v) FCS and 0.1% (w/v) of gentamycin. HeLa S3 cells (ATCC CCL-2.2) were cultured in DMEM supplemented with 10% (v/v) FCS. All cells were routinely passaged once or twice weekly.

4.5. MTT assay

The cell viability was determined using MTT assay.[20] The evaluation was performed in 96-well plates at 595 nm using an ELISA plate reader.

4.6. Calcium measurement on primary neurons

The primary cortical cell culture was prepared from 17 to 18 days old rat embryos following the modified procedure.[21–23] Daminin (**7**) was dissolved in sterile water (stock concentration of 10 mg/mL) and stored at 4 °C. Neurons were exposed to 200 μM of L-glutamic acid (L-Glu) or *N*-methyl-D-aspartic acid (NMDA), both compounds together with

2.4 mM $CaCl_2$, 10 min after the beginning of the recording. The $[Ca^{2+}]_i$ level was measured for 20 (experiment with NMDA) or 30 min (L-Glu). Compound **7** was added to the cultures 5 min prior to the stimulation with L-Glu/$CaCl_2$ or NMDA/$CaCl_2$.

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References and notes

1. Mourabit, A. A.; Potier, P. *Eur. J. Org. Chem.* **2001**, 237–243.
2. Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129–1130.
3. Morales, J. J.; Rodiguez, A. D. *J. Nat. Prod.* **1991**, *54*, 629–631.
4. Kobayaschi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. *Experientia* **1986**, *42*, 1176–1177.
5. Rosa, R.; Silva, W.; Escalona de Motta, G.; Rodriguez, A. D.; Morales, J. J., '; Ortiz, M. *Experientia* **1992**, *48*, 885–887.
6. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron* **1996**, *52*, 13713–13720.
7. Kobayashi, J.; Inaba, K.; Tsuda, M. *Tetrahedron* **1997**, *53*, 16679–16682.
8. Tsuda, M.; Uemoto, H.; Kobayashi, J. *Tetrahedron Lett.* **1999**, *40*, 5709–5712.
9. Uemoto, H.; Tsuda, M.; Kobayashi, J. *J. Nat. Prod.* **1999**, *62*, 1581–1583.
10. Nakamura, H.; Ohizumi, Y.; Kobayashi, J.; Kasei, M. *Tetrahedron Lett.* **1984**, *25*, 2475–2478.
11. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1996**, *37*, 3587–3590.
12. Jahn, T.; König, G. M.; Wright, A. D. *Tetrahedron Lett.* **1997**, *38*, 3883–3884.
13. Cafieri, F.; Carnuccio, R.; Fattorusso, E.; Taglialatela-Scafati, O.; Vallefuoco, T. *Bio. Med. Chem. Lett.* **1997**, *7*, 2283–2288.
14. Fattorusso, E.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **2000**, *41*, 9917–9922.
15. Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574–4576.
16. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O.; Carnuccio, R. *Biol. Med. Chem. Lett.* **1995**, *5*, 799–804.
17. Perović, S.; Böhm, M.; Meesters, E.; Schröder, H. C.; Pergande, G.; Müller, W. E. G. *Mech. Ageing Develop.* **1998**, *101*, 1–19.
18. Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O. *J. Nat. Prod.* **1998**, *61*, 1171–1173.
19. Cheng, Y.; Zhou, J.; Tan, N.; Teng, R.; Lu, Y.; Wang, C.; Zheng, Q. *J. Nat. Prod.* **2002**, *65*, 750–752.
20. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827–4833.
21. Freshney, R. I. Culture of specific cell types. *In Culture of Animal Cells. A Manual of Basic Technique*; A. R. Liss: New York, 1987; pp 257–288.
22. Perović, S.; Schleger, C.; Pergande, G.; Iskric, S.; Ushijima, H.; Rytik, P.; Müller, W. E. G. *Eur. J. Pharmacol. (Mol. Pharmacol. Sec.)* **1994**, *288*, 27–33.
23. Perović, S.; Böhm, M.; Meesters, E.; Meinhardt, A.; Pergande, G.; Müller, W. E. G. *Mech. Ageing Dev.* **1998**, *101*, 1–19.