Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 2283–2285
Tetrahedron Letters

Diazonamides C–E, new cytotoxic metabolites from the ascidian *Diazona* sp.

Rogelio Fernández, María Jesús Martín, Raquel Rodríguez-Acebes, Fernando Reyes *, Andrés Francesch, Carmen Cuevas

Medicinal Chemistry Department, PharmaMar S.A.U., Pol. Ind. La Mina Norte, Avda. de los Reyes, 1, 28770-Colmenar Viejo, Madrid, Spain

Received 14 January 2008; accepted 4 February 2008
Available online 8 February 2008

Abstract

Three new macrocyclic peptides, diazonamides C–E (**1**–**3**), were isolated together with the previously reported diazonamides A (**4**) and B (**5**) from samples of the marine ascidian *Diazona* sp. collected in Indonesia. Their structures were assigned on the basis of detailed analysis of the 1D and 2D NMR and mass spectral data as well as Marfey's analysis of the aminoacid residues. All the new compounds isolated displayed moderate cytotoxicity against a panel of three human tumor cell lines.

Keywords: Marine tunicate; Diazonamide; NMR; Cytotoxicity

The diazonamides are a family of novel macrocyclic peptides with potent cytotoxic activity isolated from the ascidian *Diazona angulata* (originally misidentified as *Diazona chinensis*) by Fenical and co-workers.[1] The first structural proposal by the Fenical's group was later revised and the final structures of the diazonamides A and B were established after reinterpretation of the NMR and X-ray data and total synthesis of diazonamide A (**4**).[2–4] Strong cytotoxic activity has been reported for **4** with IC_{50} values in the nanomolar range.[1,5] The unique structural features and potent biological activity against cancer cells of diazonamide A rendered this compound the ideal target for synthetic studies.

As part of our continuing research into the discovery of new antitumor drugs from marine organisms, diazonamides A and B together with three structurally related compounds, designated as diazonamides C–E (**1**–**3**), were isolated by bioassay-guided fractionation of extracts of a tunicate of the genus *Diazona* collected in Indonesia.[6] Herein, we describe the isolation, structural characterization, and cytotoxic properties of these three new members of the diazonamide structural class.

Two different samples of *Diazona* sp. were extracted with a 1:1 mixture of CH_2Cl_2–MeOH. After reversed-phase VLC on RP-18 silica gel and semipreparative reversed-phase HPLC (RP18, CH_3CN–H_2O + 0.1% TFA gradient, UV detection) of the first of these samples (38 g, wet weight), the new diazonamides C (**1**, 1.0 mg)[7] and D (**2**, 1.1 mg)[8] together with the known compound diazonamide B (**5**) (1.0 mg) were obtained. A similar treatment of a second sample of the tunicate (137 g, wet weight) yielded diazonamides D (**2**, 1.9 mg), E (**3**, 2.6 mg),[9] and A (**4**) (1.3 mg).

The ^1H and ^{13}C NMR spectra of diazonamide C (**1**) (Table 1) were very similar to those of diazonamide A, indicating an identical polycyclic nucleus for both compounds. The major differences were found in the chemical shifts for the methine group C-37 (δ_H/δ_C 3.66/59.6 ppm in **1** versus 3.88/76.9 ppm in **4**) suggesting the replacement of the hydroxyvaline unit of diazonamide A for a valine residue in **1**. A difference of one mass unit in the MS of both compounds corroborated this proposal. Acid hydrolysis of **1**, derivatization with Marfey's reagent,[10] HPLC–MS analysis of the FDAA derivatives, and comparison with authentic

* Corresponding author. Tel.: +34 91 8234527; fax: +34 91 8466001.
E-mail address: jfreyes@pharmamar.com (F. Reyes).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.02.012

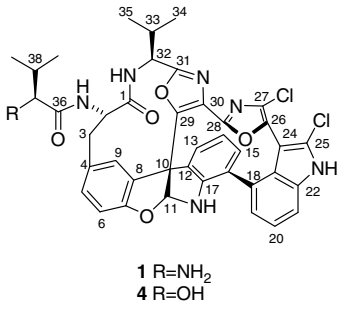
Table 1
NMR data in CD_3OD of diazonamides C–E (**1**–**3**)[a]

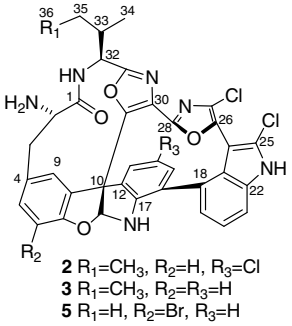
Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	174.4 s		171.0 s		171.2 s	
2	57.7 d	4.59 dd (11.9, 3.5)	56.9 d	3.94 dd (12.0, 3.5)	57.0 d	3.93 dd (11.6, 3.6)
3	38.2 t	3.43 dd (12.7, 11.9)	37.4 t	3.45 dd (12.0, 12.0)	37.6 t	3.45 dd (12.8, 11.6)
		2.78 dd (12.7, 3.5)		2.99 dd (12.0, 3.5)		2.99 dd (12.8, 3.6)
4	129.8 s		127.7 s		127.7 s	
5	131.1 d	7.19 dd (8.0, 1.0)	131.2 d	7.21 dd (8.0, 2.0)	130.9 d	7.20 dd (8.1, 2.0)
6	111.3 d	6.79 d (8.0)	111.6 d	6.83 d (8.0)	110.6 d	6.82 d (8.1)
7	159.8 s		160.2 s		160.2 s	
8	129.7 s		129.5 s		130.2 s	
9	131.2 d	7.35 d (1.0)	131.4 d	7.30 d (2.0)	131.5 d	7.30 d (2.0)
10	62.3 s		62.0 s		62.2 s	
11	106.1 d	6.35 s	106.3 d	6.37 s	106.2 d	6.36 s
12	127.8 s		129.2 s		127.4 s	
13	123.8 d	7.01 dd (7.5, 1.0)	123.9 d	7.03 d (2.0)	123.8 d	6.99 dd (7.5, 1.0)
14	120.9 d	6.69 dd (7.5, 7.5)	125.1 s		120.9 d	6.67 dd (7.5, 7.5)
15	131.2 d	6.87 dd (7.5, 1.0)	130.9 d	6.85 d (2.0)	131.3 d	6.87 dd (7.5, 1.0)
16	123.6 s		124.2 s		123.2 s	
17	151.0 s		150.2 s		151.0 s	
18	131.6 s		130.1 s		131.3 s	
19	122.7 d	7.17 dd (7.2, 1.0)	122.6 d	7.19 dd (7.2, 2.0)	122.7 d	7.20 dd (7.2, 1.8)
20	124.2 d	7.36 dd (8.2, 7.2)	124.3 d	7.38 dd (8.0, 7.2)	124.2 d	7.37 dd (8.2, 7.2)
21	112.2 d	7.47 dd (8.2, 1.0)	112.7 d	7.48 dd (8.0, 2.0)	112.2 d	7.46 dd (8.2, 1.8)
22	136.9 s		136.9 s		136.9 s	
23	127.5 s		127.2 s		127.4 s	
24	97.9 s		99.1 s		98.1 s	
25	141.7 s		141.8 s		141.9 s	
26	130.9 s		130.5 s		130.0 s	
27	129.2 s		129.5 s		129.3 s	
28	155.1 s		155.0 s		155.0 s	
29	128.4 s		128.7 s		128.4 s	
30	155.3 s		154.6 s		155.3 s	
31	163.1 s		162.7 s		162.6 s	
32	56.6 d	4.83 Under solvent	55.6 d	5.10 d (5.5)	55.5 d	5.09 d (5.4)
33	31.5 d	2.19 m	38.6 d	1.98 m	38.6 d	1.98 m
34	18.9 q	1.08 d (6.8)	15.9 q	1.01 d (6.8)	15.9 q	1.01 d (6.8)
35	18.7 q	0.95 d (6.8)	26.5 t	1.51 m, 1.36 m	26.5 t	1.51 m, 1.36 m
36	168.0 s		11.9 q	0.91 t (7.4)	11.9 q	0.91 t (7.4)
37	59.6 d	3.66 d (6.0)				
38	31.9 d	2.16 m				
39	18.1 q	1.03 d (6.8)				
40	18.3 q	1.03 d (6.8)				

[a] Data recorded on a Varian Unity 500 spectrometer.

samples revealed both valine residues to have an *S* configuration. The configuration for the rest of the chiral centers of the molecule was assumed to be the same as in Diazonamide A due to the close similarity in the chemical shifts of the NMR spectra of both compounds.

Diazonamide D (**2**) showed a pseudomolecular ion at *m*/*z* 713.1229 in the mass spectrum with an isotopic cluster for three chlorine atoms, accounting for a molecular formula of $C_{36}H_{27}Cl_3N_6O_4$ for the compound. Its ^1H NMR spectrum (Table 1) confirmed the absence of one of the aminoacid residues present in **1**. The methyl signals at δ 0.91 (t) and 1.01 (d) ppm observed in the ^1H NMR and the analysis of correlations observed in the COSY and HMBC spectra suggested the presence of an isoleucine unit in the molecule rather than the valine found in the rest of the compounds of the series. Marfey's analysis of the





hydrolized compound revealed an *S* configuration for this aminoacid unit. The rest of the chiral centers of the molecule was assumed to have the same relative configuration as in **1** due to the similarity in the ^1H and ^{13}C chemical shifts of both compounds. The upfield shift of proton H-2 in

diazonamide D with respect to **1** was explained by the absence of the valine residue at C-2 in **2**. Two of the chlorine atoms were located at C-25 and C-27 by analogy of the chemical shifts of these carbons with the other compounds of the family. The location of the third chlorine atom at the C-14 position in **2** was confirmed by the analysis of the ^{1}H, ^{13}C, COSY, and HMBC spectra. Indeed, signals for three hydrogen-bearing aromatic systems were observed in the ^{1}H and COSY spectra. The location and substitution pattern of two of these systems at the C-4 to C-9 and C-18 to C-23 positions was evident from the similarity of the ^{1}H and ^{13}C NMR data for these parts of the molecule in **1** and **2** (see Table 1). The third aromatic ring contained two protons with a *m*-coupling as deduced from a *J* value of 2.0 Hz. HMBC correlations from H-13 to C14, C-15, and C-17 and from H-15 to C-13, C-14, and C-17, and a downfield shift of C-14 by 4.2 ppm with respect to **1** corroborated the placement of a chlorine atom at C-14.

The high field region of the ^{1}H NMR spectrum of diazonamide E (**3**) was very similar to that of **2** (Table 1), indicating the presence of an isoleucine residue in the molecule. The major changes in the ^{1}H NMR spectrum of both compounds were found in the aromatic region. Signals for three proton-bearing aromatic rings, two of them 1,2,3-trisubstituted, were observed in the spectrum of **3**, confirming the absence in this molecule of the chlorine atom at C-14 present in **2**. MS measurements, with a pseudomolecular ion at *m*/*z* 679.1615 accounting for a molecular formula of $C_{36}H_{28}Cl_2N_6O_4$ and the presence of two chlorine atoms in the molecule, corroborated this proposal. Marfey's analysis of the acid hydrolysate of the molecule confirmed an *S* configuration for the isoleucine residue.

The cytotoxic activity of the new compounds and diazonamide A was evaluated against a panel of three human tumor cell lines, including lung (A549), colon (HT29), and breast (MDA-MB-231), following a described procedure[11] (Table 2). Moderate cytotoxicity with GI_{50} values in the micromolar range was found for the new compounds, whereas the potent activity previously reported for diazonamide A[1,5] was confirmed with values in the low nanomolar range. The differences found in the activity of **1** compared to **4** clearly suggested that the hydroxyl group at C-37 must play a key role in the cytotoxicity of the latter compound.

In conclusion, three new members of the diazonamide family of alkaloids, all with cytotoxic properties, have been isolated from the tunicate *Diazona* sp. The great difference observed between the cytotoxic activity of these new compounds and that of the previously reported diazonamide A allows to establish some conclusions about structure–activity relationship (SAR) in this structural class, with the hydroxyl group present at C-37 in **4** being essential for its nanomolar cytotoxicity. In addition, these results are further evidence that many marine organisms produce structurally novel chemical entities that may be lethal to cancer cells and are therefore potential new drugs for the treatment of this disease.

Table 2
GI_{50} values (μM) for compounds **1**–**4**

Compound	Cell lines		
	A549	HT29	MDA-MB-231
1	2.2	1.8	2.2
2	2.9	2.9	3.1
3	8.0	5.2	9.0
4	0.029	0.006	0.007

Acknowledgments

The authors thank the assistance of S. García in recording the NMR experiments, S. Bueno and C. de Eguilior for collection of the samples, X. Turón for their taxonomic identification, and L. F. García-Fernandez for performing the cytotoxicity assays. This work was partially supported by the MEC and ESF (Torres Quevedo contract to R. Rodríguez-Acebes).

References and notes

1. Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303–2304.
2. Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4765–4770.
3. Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4770–4773.
4. Nicolaou, K. C.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Bella, M.; Snyder, S. A. *J. Am. Chem. Soc.* **2004**, *126*, 12888–12896.
5. Nicolaou, K. C.; Hao, J.; Reddy, M. V.; Rao, P. B.; Rassias, G.; Snyder, S. A.; Huang, X.; Chen, D. Y.-K.; Brenzovich, W. E.; Giuseppone, N.; Giannakakou, P.; O'Brate, A. *J. Am. Chem. Soc.* **2004**, *126*, 12897–12906.
6. Two samples of *Diazona* sp. were collected at different locations in Indonesia. The first sample (ORMA034977, 38 g) was collected in May 2005 by SCUBA near Manado (0° 32′ 39″ S, 130° 41′ 53″ E). The second of the samples (ORMA043097, 137 g) was collected at Raja Ampat (1° 37′ 44″ N, 124° 45′ 56″ E) in June 2006. The material was identified by Dr. Xavier Turón from the University of Barcelona (Spain). Voucher specimens of both samples are deposited at PharmaMar.
7. Diazonamide C (**1**): amorphous pale yellow solid, $[\alpha]_D^{25}$ −24.1 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 764 $[M+H]^+$, (+)-HRMALDITOFMS *m*/*z* 764.2142 (calcd for $C_{40}H_{36}{}^{35}Cl_2N_7O_5$, 764.2149).
8. Diazonamide D (**2**): amorphous pale yellow solid, $[\alpha]_D^{25}$ −33.5 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 713 $[M+H]^+$, (+)-HRMALDITOFMS *m*/*z* 713.1229 (calcd for $C_{36}H_{28}{}^{35}Cl_3N_6O_4$, 713.1232).
9. Diazonamide E (**3**): amorphous pale yellow solid, $[\alpha]_D^{25}$ −56.8 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 679 $[M+H]^+$, (+)-HRMALDITOFMS *m*/*z* 679.1615 (calcd for $C_{36}H_{29}{}^{35}Cl_2N_6O_4$, 679.1622).
10. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
11. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.