

## ORIGINAL ARTICLE

# Atacamycins A–C, 22-membered antitumor macrolactones produced by *Streptomyces* sp. C38\*

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Three new 22-membered macrolactone antibiotics, atacamycins A–C, were produced by *Streptomyces* sp. C38, a strain isolated from a hyper-arid soil collected from the Atacama Desert in the north of Chile. The metabolites were discovered in our HPLC-diode array screening and isolated from the mycelium by extraction and chromatographic purification steps. The structures were determined by mass spectrometry and NMR experiments. Atacamycins A, B and C exhibited moderate inhibitory activities against the enzyme phosphodiesterase (PDE-4B2), whereas atacamycin A showed a moderate antiproliferative activity against adeno carcinoma and breast carcinoma cells.

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**Keywords:** antitumor agents; atacamycins; hyper-arid soils; 22-membered macrolactone antibiotics; phosphodiesterase 4 inhibitors; *Streptomyces*

## INTRODUCTION

The phylum *Actinobacteria* and particularly the genus *Streptomyces* is an excellent and in-exhausted source for the discovery of novel secondary metabolites with diverse biological activities based on unique pharmacophores.<sup>1,2</sup> High-quality isolates are a pre-requisite to prevent the rediscovery of known compounds as well as new sources for strain isolation.<sup>3</sup> Our focus for strain isolation is based on poorly studied habitats within the extremobiosphere.<sup>4</sup> The Atacama Desert in northern Chile is such an extreme habitat and is acknowledged to be the driest place on earth due to the rainshadow in front of the Andes mountains.<sup>5</sup> It is the oldest continuously arid desert, which has experienced extreme hyper-aridity for at least 150 million years of climatic stability.<sup>6,7</sup> Drees and coworkers<sup>8</sup> has shown that the hyper-arid soils of the Atacama Desert are not sterile but harbor a rich source of culturable bacteria, the majority of which were members of the phylum *Actinobacteria*.

In a recently published study, the isolation of novel members of the order *Actinomycetales* from Atacama Desert soils was reported.<sup>9</sup> In this study, samples were collected at El Tatio (4300 m, geyser field), the Salar de Atacama (2300 m, salt flat hyper-arid) and the Valle de la Luna (2450 m, extreme hyper-arid). In all, 16 selected actinomycete isolates were added to our HPLC-diode array screening program to detect the production of novel secondary metabolites. In all, 3 of them

were members of the genus *Lechevalieria*, 3 were members of the genus *Amycolatopsis* and 10 were members of the genus *Streptomyces*. The strains were cultivated in shake flasks in three different complex media with and without supplementation of sodium chloride, and the extracts were analyzed and evaluated by means of an in-house HPLC-UV-Vis database that contained approximately 950 natural products, mainly antibiotics.<sup>10</sup>

The *Lechevalieria* strains were characterized as novel species (*L. atacamensis*<sup>T</sup>, *L. deserti*<sup>T</sup> and *L. roselyniae*<sup>T</sup>),<sup>11</sup> but in our hands have not produced any secondary metabolites. In contrast, the *Amycolatopsis* isolates produced various secondary metabolites including the known antibiotic 1-hydroxy-4-methoxy-2-naphthoic acid.<sup>12</sup> Nearly all of the *Streptomyces* isolates were potent producers of diverse antibiotics including the anthelmintic LL-F28249 $\alpha$  (nemadectin  $\alpha$ ),<sup>13</sup> a member of the oligomycin group of macrocyclic lactones related to milbemycins and avermectins, and LL-F28249 $\omega$  (nemadectin  $\omega$ , 21-hydroxy-oligomycin A).<sup>14</sup> Other *Streptomyces* isolates produced the anthraquinone antibiotic  $\beta$ -rubromycin,<sup>15</sup> and an uncharacterized tetraene-polyene antibiotic.

The *Streptomyces* strain C38 was of special interest because it synthesized three closely related metabolites that were recovered from the mycelium extract. These compounds were named atacamycins A (1), B (2) and C (3), and their structures are shown in Figure 1.

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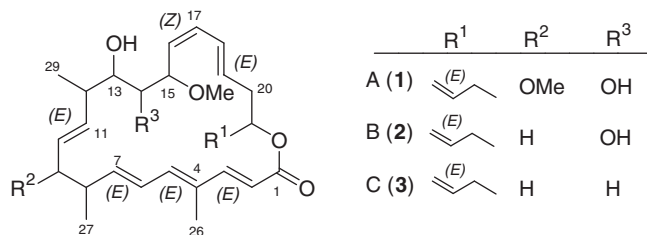
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**Figure 1** Structures of atacamycins A (1), B (2) and C (3).

Their nearly congruent UV spectra were not identical with those of the reference compounds stored in the HPLC-UV-Vis database, and their molecular masses of 500, 470 and 454 Da, respectively, gave no positive identification when referred to the DNP database.<sup>16</sup> This report describes the taxonomy of the producing strain, its fermentation, and the isolation, structure determination and biological activities of the novel polyketide-type compounds.

## MATERIALS AND METHODS

### Producing strain

Hyper-arid soil samples from the Laguna de Chaxa (Salar de Atacama, 23°17'S, 68°10'W, altitude 2300 m) were collected aseptically by one of us (ATB) in November 2004. Strain C38 was recovered from this salt flat and designated as a member of the genus *Streptomyces* based on a nearly complete 16S rRNA gene sequence and associated chemotaxonomic and morphological properties.<sup>9</sup>

### HPLC-diode array screening

Strain C38 was grown in various complex media<sup>3</sup> at the 100-ml shake flask scale with and without the supplementation of 1% NaCl. Samples (10 ml) of the culture broths were taken at 96 and 144 h. After centrifugation, the supernatant was adjusted to pH 5.0 and extracted with an equivalent volume of EtOAc; the mycelial pellet was extracted with a 10-ml MeOH-Me<sub>2</sub>CO (1:1). The organic layers were concentrated, dried *in vacuo* and resuspended in 1 ml MeOH. Aliquots (5 µl) were injected onto an HPLC column (125×3 mm i.d., pre-column 20×3 mm i.d.) packed with 5-µm Nucleosil-100 C-18 (Maisch, Ammerbuch, Germany). The samples were analyzed by linear gradient elution using 0.1% *ortho*-phosphoric acid as solvent A and CH<sub>3</sub>CN as solvent B at a flow rate of 0.85 ml min<sup>-1</sup>. The gradient applied was from 4.5 to 100% solvent B in 15 min with a 3-min hold at 100% solvent B. The evaluation of the chromatograms was done by means of our HPLC-UV-Vis database.<sup>10</sup>

### Fermentation and isolation

Batch fermentations of *Streptomyces* sp. C38 were performed in a 10-l stirred tank fermentor (Biostat S, B. Braun, Melsungen, Germany) in a complex medium that consisted of (per litre tap water) soluble starch 10 g, glucose 10 g, glycerol 10 g, cornsteep powder 2.5 g, bacto peptone 5 g, yeast extract 2 g, NaCl 1 g and CaCO<sub>3</sub> 3 g; the pH was adjusted to 7.3 (5M HCl) before sterilization. The fermentor was inoculated with 5% by volume of a shake culture grown in the same medium at 27 °C in 500 ml Erlenmeyer flasks with a single baffle for 72 h on a rotary shaker at 120 r.p.m. The fermentation was carried out for 7 days with an aeration rate of 0.5 volume air per volume per minute and agitation at 250 r.p.m.

Hyphlo Super-cel (2%) was added to the fermentation broth, which was separated by multiple sheet filtration into culture filtrate and mycelium. The culture filtrate was discarded. The mycelium, which contained compounds 1–3 was extracted three times each with 1 litre MeOH-Me<sub>2</sub>CO (1:1). The extracts were combined, concentrated *in vacuo* to an aqueous residue and re-extracted twice each with 250 ml cyclohexane. The extracts were combined and concentrated *in vacuo* to an oily raw product (3.1 g). Aliquots were dissolved in MeOH and separated on a Sephadex LH-20 column (90×2.5 cm i.d., Amersham, Freiburg, Germany) with MeOH as an eluent at a flow rate of 30 ml h<sup>-1</sup>. Fractions containing 1, 2 and 3, respectively, were separated to pure compounds by preparative reverse-phase HPLC (Reprosil-Pur Basic-C18,

10 µm, 250×20 mm i.d., Maisch) and elution with 0.1% HCOOH-MeOH (linear gradient 75–100% MeOH) at a flow rate of 24 ml min<sup>-1</sup>. The isolation yields of 1, 2 and 3 were 16, 18 and 13 mg, respectively.

### Structure determination

ESI-MS spectra were obtained on a QTRAP 2000 LC-MS/MS spectrometer (Applied Biosystems, Darmstadt, Germany). High-resolution ESI-FT-ICR mass spectra were recorded on an APEX II FTICR mass spectrometer (4.7 T, Bruker-Daltonics, Bremen, Germany) and NMR spectra were recorded on a DRX 500 spectrometer (Bruker, Karlsruhe, Germany) at 500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively. The chemical shifts are given in p.p.m. referred to DMSO-*d*<sub>6</sub> as 2.50 p.p.m. (<sup>1</sup>H) and 39.51 p.p.m. (<sup>13</sup>C). Optical rotation was recorded on a 341 polarimeter (Perkin-Elmer, Überlingen, Germany). Infrared data measurement was carried out on an 881 IR-spectrometer (Perkin-Elmer, Überlingen, Germany).

### Antiproliferative assays

A modified propidium iodide assay was used to determine the cytotoxic activity of compounds 1 and 2 against 42 cell lines derived from solid human tumors. The test procedure has been described elsewhere.<sup>17</sup> Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from the American Type Culture Collection (Rockville, MD, USA), National Cancer Institute (Bethesda, MD, USA) or Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). IC<sub>50</sub> values were determined as the concentration that gave a response half way between the maximum signal (top plateau) and the maximally inhibited signal (bottom plateau); that is the inflection point of the sigmoidal concentration-effect curve as determined by non-linear regression.

### Antibacterial assay

Antimicrobial assays were performed as described earlier by Schneemann *et al.*<sup>18</sup>

### Enzyme inhibition assay

Analysis of the effect of compounds 1–3 on human recombinant cAMP-specific phosphodiesterase (PDE-4B2) was carried out as described earlier.<sup>19</sup>

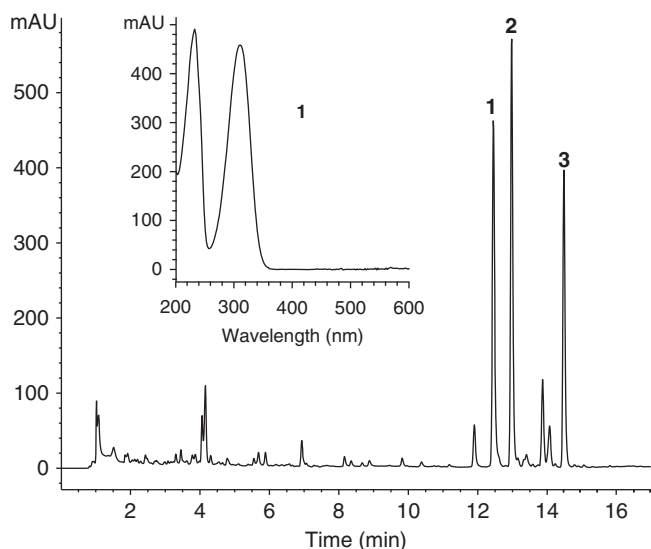
## RESULTS

### Taxonomy of the producing strain

Strain C38 and 21 other strains isolated from the Salar de Atacama sample formed a well-delineated subclade in the *Streptomyces* 16S rRNA gene tree that was supported by a 100% bootstrap value.<sup>9</sup> In addition, isolate C38 produced an extensively branched substrate mycelium, abundant aerial hyphae and whole-organism hydrolysates rich in LL-diaminopimelic acid,<sup>9</sup> properties consistent with its classification in the genus *Streptomyces*.<sup>20</sup>

### Screening, fermentation and isolation

Strain C38 was included in our HPLC-DAD screening together with nine further *Streptomyces* strains isolated from the same hyper-arid soil from the Salar de Atacama. The strains were grown in various complex media as shake flask cultures and extracts from the culture filtrates and mycelia were analyzed by gradient-mode reverse-phase HPLC and diode array monitoring. Three dominant peaks were observed exclusively in the mycelium extract of strain C38 as shown in Figure 2, and found to have nearly congruent UV-vis spectra that indicated a strong structural similarity; the three compounds were named atacamycins A (1), B (2) and C (3). HPLC-ESI-MS analysis revealed molecular masses of 500, 470 and 454 Da, respectively. The unique UV-vis spectra that differed from all the 950 reference compounds stored in our HPLC-UV-Vis database, together with molecular masses that gave no positive identification by DNP database interrogation, indicated the novelty of all the three compounds.



**Figure 2** HPLC analysis of a mycelium extract from *Streptomyces* sp. C38 at a fermentation time of 148 h, monitored at 230 nm. Insert: UV-vis spectrum of **1** (spectra of **2** and **3** are nearly identical).

The biomass production by the strain C38 was increased 1.28-fold when the complex medium was supplemented with 1% NaCl, whereas the production of **1–3** was not influenced.

The productivity obtained in the shake flask cultures was transferred reproducibly to the 10-l fermentor scale. Strain C38 reached at 72 h a maximal biomass of 14 vol% that correlated to a DNA amount of 230  $\mu\text{g ml}^{-1}$ , at which time glucose, starch, glycerol and phosphate were depleted from the medium. The maximal production of compounds **1**, **2** and **3** was observed at a fermentation time of 7 days reaching amounts of 17  $\text{mg l}^{-1}$  atacamycin A (**1**), 20  $\text{mg l}^{-1}$  atacamycin B (**2**) and 14  $\text{mg l}^{-1}$  atacamycin C (**3**) in the mycelium (Figure 1).

Compounds **1–3** were isolated from the mycelium by solvent extraction and purified by chromatography on Sephadex LH-20. Pure compounds were obtained by preparative reverse-phase HPLC as white powders in amounts of 16 mg atacamycin A (**1**) 18 mg atacamycin B (**2**) and 13 mg for atacamycin C (**3**).

### Structure determination

Physico-chemical properties of **1–3** including IR spectroscopic data and optical rotation data are summarized in Table 1. The exact molecular masses were determined measuring molecular ions by high-resolution ESI-FT-ICR-MS, revealing the molecular formulae  $\text{C}_{30}\text{H}_{44}\text{O}_6$  (**1**),  $\text{C}_{29}\text{H}_{42}\text{O}_5$  (**2**) and  $\text{C}_{29}\text{H}_{42}\text{O}_4$  (**3**). The structure elucidation of atacamycins **1–3** was done on the basis of 1D and 2D NMR experiments exemplarily described for derivative **3** as follows. The  $^1\text{H}$ -NMR spectrum of **3** showed signals for 4 methyl, 4 methylene, 5 aliphatic methine, 1 methoxy and 13 olefinic protons (Table 2). One signal was characteristic for a hydroxy group. The  $^{13}\text{C}$ -NMR spectra as well as DEPT spectra revealed the presence of 4 methyl, 1 hydroxymethyl, 4 methylene, 18 methine and 2 quaternary carbon atoms, showing a total of 29 carbon atoms. The correlation of  $^1\text{H}$ -NMR signals to the corresponding  $^{13}\text{C}$ -carbon atoms was carried out in a HSQC NMR experiment. Hence, the structure of **3** was fully elucidated using COSY and HMBC spectra. The  $^1\text{H}$ - $^1\text{H}$  COSY experiment revealed correlations from H-2 to H-3, from H-5 throughout H-12 and from H-13 throughout H-25 of the macrolactone backbone (Figure 3c). No  $^1\text{H}$ - $^1\text{H}$  COSY correlation could be seen from H-12 to H-13, instead the connection was established by HMBC correla-

tions from H-29 to C-13 and from 30-OH to C-12. The COSY fragments containing H-3 and H-5 were connected by the correlation of H-3 to C-5 and C-26, H-5 to C-3 and C-26 and H-26 to C-3, C-4 and C-5 in the HMBC spectra. The connection across the ester moiety was established by HMBC correlations from H-2, H-3 and H-21 to C-1. The protons at H-27, H-29 and 30-OH showed correlation to the macrolactone backbone at H-8, H-12 and H-13, respectively. The connection of the methoxy group to C-15 was established by HMBC correlation from H-32 to C-15. The stereochemistry of the double bonds of **3** was elucidated by means of the selTOCSY and NOESY experiments. The coupling constants of  $J_{2,3}=15.5$  Hz,  $J_{6,7}=15.2$  Hz,  $J_{10,11}=15.8$  Hz,  $J_{16,17}=10.7$  Hz,  $J_{18,19}=14.7$  Hz and  $J_{22,23}=15.7$  Hz revealed 2*E*, 6*E*, 10*E*, 16*Z*, 18*E* and 22*E*, respectively. NOE correlation observed between H-3 and H-5 revealed a 4*E*-configuration.

Compound **2** differs from **3** in the molecular formula by one oxygen atom ( $\Delta m=16$  Da). All recorded NMR spectra are similar to the spectra of **3**, but the proton spectra of **2** instead of a methylene group at C-14, showed a methine at  $\delta_{\text{H}}$  3.04 as well as an additional exchangeable proton at  $\delta_{\text{H}}$  4.30 (Figure 3b). Therefore, it was concluded that **2** is the 14-hydroxy derivative of **3**. Compound **1** has an additional methoxy group compared with **2** ( $\Delta m=30$  Da), which was established by the replacement of the methylene group by a methoxymethine moiety at C-9 (Figure 3a). The coupling from H-28 to C-9 in the HMBC spectrum and the NMR signals for H-28 ( $\delta_{\text{H}}$  3.16) and C-28 ( $\delta_{\text{C}}$  56.0) finally confirmed that **1** is the 9-methoxy derivative of **2**.

### Antibacterial assay

The antibacterial activities of **1–3** were determined against a set of Gram-positive and Gram-negative bacteria. Compounds **1**, **2** and **3** (100  $\mu\text{M}$ ) slightly inhibited the growth of the phytopathogenic strain *Ralstonia solanacearum* DSM 9544 with an inhibition of 41, 58 and 47%, respectively.

### Enzyme inhibition assay

Compounds **1–3** were tested regarding their inhibitory activity against the enzyme phosphodiesterase PDE-4B2. Compound **2** exhibited the strongest activity with an  $\text{IC}_{50}$  value of 1.38  $\mu\text{M}$ . The  $\text{IC}_{50}$  values of **1** and **3** were 2.28 and 4.07  $\mu\text{M}$ , respectively.

### Antiproliferative activity

**1** and **2** were subjected to cytotoxic testing in a panel consisting of 42 different human tumor cell lines, reflecting 15 histotypes. Overall, **1** and **2** showed clear cytotoxic activities with mean  $\text{IC}_{50}$  values of 13.4 and 20.4  $\mu\text{M}$ , respectively (Table 3). Compound **1** was most active in cell lines of colon cancer (CXF DiFi), breast cancer (MAXF 401NL) and uterus cancer (UXF 1138L) with  $\text{IC}_{50}$  values ranging from 2.66 to 5.93  $\mu\text{M}$ , whereas **2** was most active against colon RKO cells ( $\text{IC}_{50}=8.51$   $\mu\text{M}$ ; Table 3).

### DISCUSSION

In all, 21 *Streptomyces* strains isolated from the Salar de Atacama soil formed a well-delineated subclade in the 16S rRNA *Streptomyces* tree.<sup>9</sup> Five of these strains, C01, C19, C38, C40 and C79, were included in our HPLC-DAD screening program for metabolic profiling. Strains C01, C19 and C40 produced LL-F28249 $\alpha$  (nemadectin  $\alpha$ ) and LL-F28249 $\omega$  (nemadectin  $\omega$ ), which are 26-membered macrolactone antibiotics. Only strains C38 and C79 differed in their metabolic profiles; the compounds from strain C38 were characterized in this report as the new 22-membered macrolactone antibiotics atacamycins A–C (**1–3**). In parallel and independently from this report, the

**Table 1** Physico-chemical properties of atacamycins A–C (1–3)

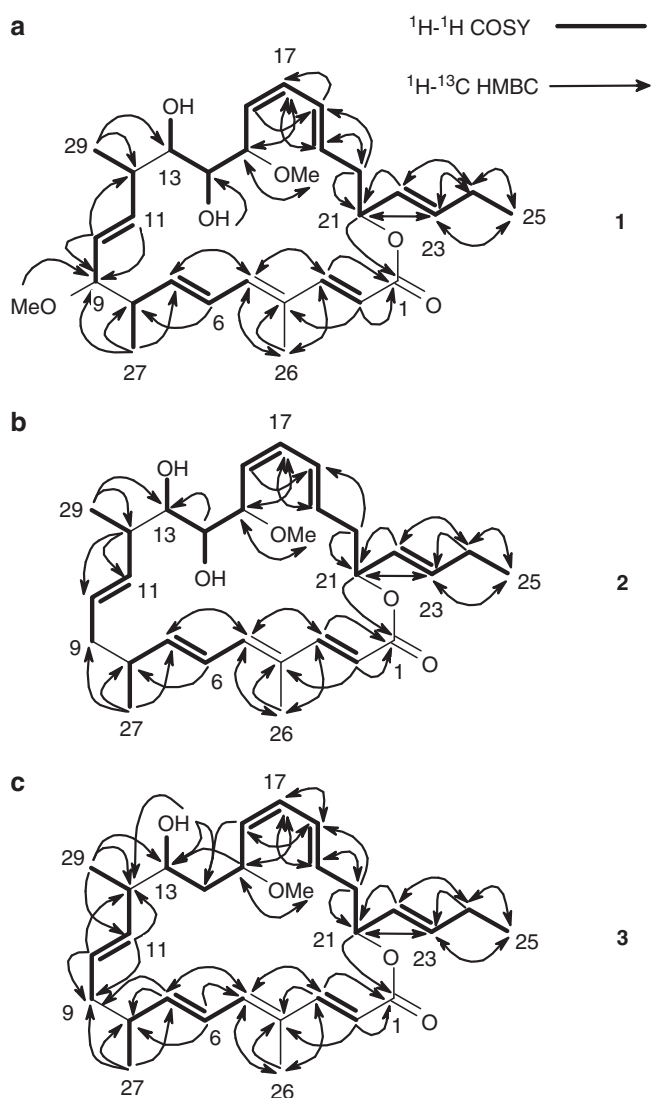
	<b>1</b>	<b>2</b>	<b>3</b>
Appearance	White powder	White powder	White powder
Molecular mass	500.3	470.3	454.3
Molecular formula	C <sub>30</sub> H <sub>44</sub> O <sub>6</sub>	C <sub>29</sub> H <sub>42</sub> O <sub>5</sub>	C <sub>29</sub> H <sub>42</sub> O <sub>4</sub>
ESI-FT-ICR-MS			
Measured	523.30272 [M+Na] <sup>+</sup>	493.29240 [M+Na] <sup>+</sup>	477.29608 [M+Na] <sup>+</sup>
Calculated	523.30301	493.29245	477.29753
UV λ <sub>max</sub> <sup>MeOH</sup> nm (log ε)	221 (4.74), 310 (4.63)	221 (4.69), 308 (4.60)	222 (4.67), 307 (4.55)
[α] <sub>D</sub> <sup>22</sup> (MeOH)	−46.2 (c 0.6)	+3.1 (c 0.1)	−8.0 (c 0.7)
IR ν <sub>max</sub> [cm <sup>−1</sup> ]	3460, 2964, 2930, 2875, 1700, 1606, 1452, 1375, 1263, 1094, 1068, 981	3406, 2963, 2927, 2876, 1710, 1635, 1457, 1378, 1259, 1171, 1050, 977	3443, 2960, 2935, 2873, 1713, 1626, 1457, 1376, 1270, 1171, 1103, 971

**Table 2** <sup>1</sup>H and <sup>13</sup>C-NMR spectroscopic data of atacamycins A–C (1–3) in DMSO-*d*<sub>6</sub>

No.	<b>1</b>		<b>2</b>		<b>3</b>	
	δ <sub>H</sub> , (multiplicity, J in Hz)	δ <sub>C</sub> , (multiplicity, J in Hz)	δ <sub>H</sub> , (multiplicity, J in Hz)	δ <sub>C</sub> , (multiplicity, J in Hz)	δ <sub>H</sub> , (multiplicity, J in Hz)	δ <sub>C</sub> , (multiplicity, J in Hz)
1	—	165.8	—	165.7	—	165.7
2	5.68 (m)	117.0	5.71 (m)	116.5	5.70 (d, 15.5)	133.5
3	7.05 (d, 15.6)	149.1	7.02 (d, 15.5)	148.7	7.02 (d, 15.5)	148.7
4	—	131.9	—	130.7	—	130.9
5	6.07 (d, 11.0)	139.8	6.02 (d, 11.3)	139.3	6.06 (d, 11.2)	139.3
6	6.42 (dd, 11.3, 15.1)	126.1	6.33 (dd, 11.7, 15.6)	122.7	6.33 (dd, 11.2, 15.2)	122.9
7	5.87 (dd, 9.0, 14.8)	145.2	6.13 (dd, 5.7, 15.5)	146.3	6.13 (dd, 5.3, 15.2)	145.8
8	2.61 (m)	41.3	2.32 (m)	33.9	2.32 (m)	34.3
9	3.49 (dd, 3.9, 8.0)	86.0	2.20, 1.70 (m)	41.2	2.23, 1.68 (dt, 13.0, 3.1)	41.2
10	5.23 (8.0, 15.9)	126.2	5.38 (m)	126.9	5.33 (m)	127.7
11	5.66 (dd, 15.7, 8.3)	135.5	5.34 (dd, 15.5, 8.1)	133.7	5.33 (m)	134.2
12	2.72 (m)	35.7	2.62 (m)	37.1	1.97 (m)	43.4
13	3.69 (9.4, 5.2)	72.7	3.56 (m)	72.8	3.72 (s, br)	68.9
14	2.94 (t, 9.0)	74.6	3.04 (t, 9.1)	74.1	1.40 (dd, 4.6, 6.8)	43.3
15	4.40 (d, 10.4)	73.6	4.44 (d, 10.5)	72.8	4.36 (m)	71.2
16	5.51 (t, 10.9)	126.9	5.55 (t, 10.7)	126.3	5.07 (t, 10.7)	129.3
17	6.24 (t, 11.3)	132.8	6.28 (t, 11.1)	131.7	6.18 (t, 10.7)	131.1
18	6.62 (dd, 11.7, 14.7)	128.7	6.64 (dd, 11.7, 14.7)	127.7	6.61 (dd, 10.7, 14.7)	127.6
19	5.68 (m)	117.1	5.71 (m)	134.5	5.68 (m)	132.8
20	2.48 (m)	37.9	2.50 (m)	37.9	2.48 (m)	37.9
21	5.19 (m)	72.8	5.11 (m)	72.3	5.12 (m)	72.6
22	5.48 (dd, 15.6, 6.0)	128.4	5.49 (dd, 15.6, 6.0)	127.4	5.49 (dd, 15.7, 6.0)	127.5
23	5.73 (6.6, 15.7)	134.8	5.74 (m)	134.0	5.74 (m)	134.1
24	2.00 (q, 6.9)	25.2	2.01 (q, 6.8)	24.4	2.01 (q, 7.0)	24.4
25	0.93 (t, 7.3)	13.8	0.94 (t, 7.4)	12.9	0.93 (t, 7.4)	12.8
26	1.79 (s)	12.4	1.80 (s)	11.7	1.80 (s)	11.6
27	0.96 (d, 6.8)	14.2	1.07 (d, 6.6)	18.6	1.07 (d, 6.6)	18.5
28	3.16 (s)	56.0	—	—	—	—
29	1.07 (d, 6.9)	16.6	1.01 (d, 7.2)	18.2	1.00 (d, 6.8)	19.0
30-OH	4.58 (d, 5.2)	—	4.58 (d, 5.8)	—	4.49 (d, 4.6)	—
31-OH	4.56 (d, 8.9)	—	4.30 (d, 9.0)	—	—	—
32	3.13 (s)	55.7	3.13 (s)	55.1	3.11 (s)	54.7

metabolic profile of *Streptomyces* strain C34 was investigated by Marcel Jaspars and coworkers at the University of Aberdeen (personal communication). Strain C34 was isolated from the same soil as strain C38 and they belong to the same 16S rRNA subclade, but differed

slightly but significantly in their 16S rRNA gene sequences (C34: EU551711, C38: EU551719).<sup>9</sup> Interestingly, strain C34 also produced similar 22-membered macrolactone antibiotics, and their structures and biological activities are reported elsewhere.<sup>21</sup>



**Figure 3**  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations of (a) atacamycin A (1), (b) atacamycin B (2) and (c) atacamycin C (3).

The small- and medium-membered macrolides (12–16 ring atoms) are potent inhibitors of bacterial protein biosynthesis and are in use as anti-infective drugs in medicine; for example, erythromycin and its derivatives. Larger than 16-membered macrolides, alternatively named macrolactones are distinguished by a huge variety of biological activities, that include antifungal, insecticide, anthelmintic, antitumor, immunosuppressive or anti-inflammatory action.<sup>22</sup> The 22-membered macrolactones show mainly a cytotoxic activity, as in the case of dictyostatin<sup>23</sup> produced by a marine sponge, respectively, by its microbial symbiont, dolabelides<sup>24</sup> produced by the sea hare *Dolabella auricularia*, and wortmannilactones<sup>25</sup> produced by the fungus *Talaromyces wortmannii*. From ushikulides which were isolated from a *Streptomyces* strain, an immunosuppressant activity was reported.<sup>26</sup>

To the best of our knowledge, this is the first study describing an inhibitory effect of 22-membered macrolactones on the activity of the enzyme phosphodiesterase 4. In this context, the atacamycins and other compounds belonging to the 22-membered macrolactones could be considered as the drug candidates for the treatment of inflammatory diseases, such as chronic obstructive pulmonary diseases.<sup>27</sup> Besides

**Table 3** Antiproliferative activities of atacamycins A (1) and B (2) determined in a panel of 42 human tumor cell lines

Tumor type	Cell line	$IC_{50}$ [ $\mu\text{M}$ ]	
		1	2
Bladder	BXF 1218L	8.37	13.7
	BXF 1352L	17.1	17.3
	T-24	12.0	17.4
Colon	CXF 269L	22.5	25.0
	DIFI	5.93	15.0
	HCT-116	16.8	18.6
	HT-29	27.5	30.0
	RKO	12.4	8.5
Stomach	GXF 251L	13.9	25.6
	MKN45	24.2	27.5
Head and neck	CAL-27	10.0	11.1
Liver	LIXF 575L	17.7	30.0
Lung	NCI-H460	27.5	30.0
	LXFA 289L	16.6	27.5
	LXFA 526L	10.4	20.2
	LXFA 629L	10.1	22.1
	LXFL 1121L	9.04	22.7
	LXFL 529L	7.59	14.3
	LXFL 529L	7.59	14.3
Breast	MAXF 401NL	2.66	11.5
	MCF-7	12.3	22.9
	MDA-MB-231	13.4	15.1
Melanoma	MEXF 1341L	12.5	17.7
	MEXF 276L	14.8	17.1
	MEXF 462NL	9.20	21.4
Ovary	OVXF 899L	30.0	30.0
	NIH:OVCAR-3	13.6	28.0
Pancreas	PAXF 1657L	21.2	20.3
	PAXF 546L	15.1	25.0
	PANC-1	15.3	24.7
Prostate	22Rv1	7.96	19.2
	DU-145	11.1	18.2
	LNCaP	8.46	13.7
	PC3-M	16.6	23.7
	PXF 1118L	14.1	30.0
Mesothelioma	PXF 1752L	27.5	23.2
	PXF 698L	10.5	17.6
	PXF 698L	10.5	17.6
Kidney	RXF 1781L	21.7	30.0
	RXF 393NL	29.1	27.5
	RXF 486L	23.8	26.6
Sarcoma	Saos-2	15.3	17.9
	TE671	10.7	18.1
Uterus	UXF 1138L	5.32	17.0
Mean $IC_{50}$		13.4	20.4

their enzyme inhibitory activity, atacamycins A (1) and B (2) showed a moderate antitumor activity against tumor cell lines, with potency and differential activity of 1 being more pronounced compared with 2.

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