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Structural revision of halipeptins: synthesis of the thiazoline unit and isolation of halipeptin C

Carmela Della Monica,[c] Antonio Randazzo,[b] Giuseppe Bifulco,[a] Paola Cimino,[a] Maurizio Aquino,[c] Irene Izzo,[c] Francesco De Riccardis[c,*] and Luigi Gomez-Paloma[a,*]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte Don Melillo, 84084 Fisciano (SA), Italy*
[b]*Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy*
[c]*Dipartimento di Chimica, Università di Salerno, Via S. Allende, 84081 Baronissi (SA), Italy*

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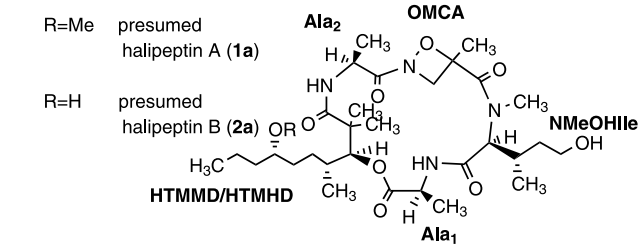
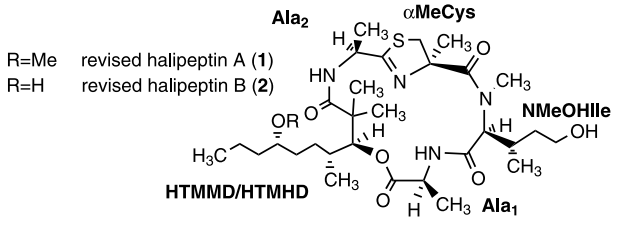
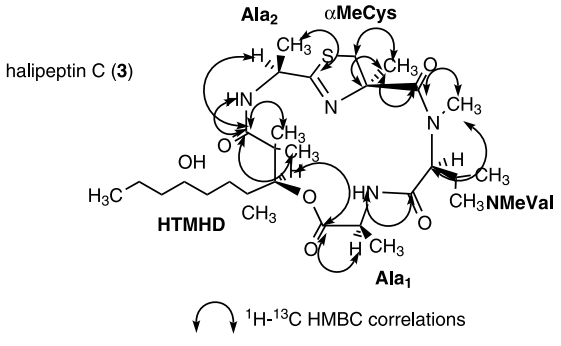
Abstract—The structural revision of the anti-inflammatory marine metabolites halipeptin A (**1**) and B (**2**) along with the isolation of the new related product halipeptin C (**3**) are reported. In particular, the heterocyclic portion of the molecule, incorrectly assigned as an oxazetidine ring, has now been characterised as a thiazoline unit by comparison of the spectral data of the natural products (**1**–**3**) with an appropriate synthetic model (**10**). GIAO calculated ^{13}C NMR chemical shifts for oxazetidine and thiazoline model compounds provide additional support to the revised structure.

Halipeptins A (**1**) and B (**2**) are cyclic depsipeptides displaying a potent in vivo anti-inflammatory activity (60% of carrageenan induced edema reduction at a intraperitoneal dose of 0.3 mg/Kg in mice).[1] Their structures feature the presence of common coded amino acid residues (2×L-Ala) along with unusual units, such as the polysubstituted decanoic acid HTMMD, *N*-methyl-δ-hydroxyisoleucine (NMe-δOH-Ile) and the heterocyclic version of an α,α-disubstituted amino acid which was incorrectly identified as a methyloxazetidine–carboxylic acid residue (OMCA) mainly on the basis of NMR and HRFABMS data.

The opportunity of re-examine the spectral data of halipeptins came with the isolation, from the same Vanuatu species of *Haliclona*, of a new minor related compound, named halipeptin C (**3**). Although all the NMR data (see Table 1 and HMBC correlations of **3**) could be readily interpreted assuming that halipeptin C was a derivative of **2** bearing a L-NMeVal in place of the NMe-δOH-Ile residue, HRESIMS data suggested that its molecular formula contained an unexpected sulphur atom. Indeed, the pseudomolecular ion peak of **3** at m/z 605.3360 ($M+Na^+$, 605.33487 calculated for $C_{29}H_{50}N_4NaO_6S$ versus 605.35263 calculated for $C_{29}H_{50}N_4NaO_8$) clearly favoured the molecular formula containing a sulphur in place of two oxygen atoms.







* Corresponding authors. Tel.: +39-089-962811; fax: +39-089-962828 (L.G.P.); Tel.: +39-089-965230; fax: +39-089-965296; e-mail: dericca@unisa.it; gomez@unisa.it

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Table 1. ^{1}H, ^{13}C and ^{15}N NMR data of of halipeptin C (**3**) ($CDCl_3$, 500 MHz)

Residue	δ_H[a], mult., J in Hz	δ_C[a]	^{1}H–^{13}C HMBC
Ala1			
CO		169.6	
α	4.71, quintet, 7.6	49.9	CO
β	1.41, d, 7.6	18.2	Cα, CO
N	7.01, d, 7.6		CO-NMeVal
NMeVal			
CO		169.9	
α	4.98	64.8	
β	2.56, m	26.4	
Me-1	0.93, d, 6.3	17.7	Cα, Cβ, Me-2
Me-2	0.98, d, 6.3	21.0	Cα, Cβ, Me-1
NMe	2.81, s	30.5	Cα, CO-α-MeCys
α-MeCys			
CO		171.9	
α		83.6	
Me-α	1.49, s	23.0	Cα, Cβ, CO
β1	3.31, d, 12.0	43.6	
β2	4.15, d, 12.0		
N			
Ala2			
CN(S)		177.3	
α	4.82, quintet, 7.4	48.8	CO-HTMHD
β	1.50, d, 7.4	22.1	Cα, CO
N	7.23, d, 7.4		CO-HTMHD
HTMHD			
1		173.6	
2		45.6	
Me′-2	1.12, s	25.9	Me″-2, C-1, C-2, C-3
Me″-2	1.20, s	21.9	Me′-2, C-1, C-2, C-3
3	4.71, d, 2.3	82.3	CO-Ala1
4	1.93, m	34.0	
Me-4	0.80, d, 6.5	14.1	C-3
5	1.30, m	32.0	
	1.34, m		
6	1.45, m	34.1	
	1.50, m		
7	3.55, quintet, 5.3	72.0	
8	1.30, m	39.8	
	1.43, m		
9	1.33	17.5	
10	0.91, t, 6.0	14.9	C-8, C-9

[a] Chemical shift values are referred to $CHCl_3$ ($\delta_H = 7.26$) and $^{13}CHCl_3$ ($\delta_C = 77.0$) as internal standards.

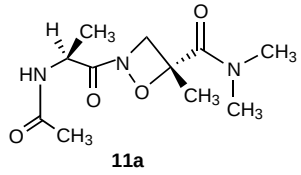
Careful HRESIMS measurements revealed that this was also the case for the parent compound halipeptin A, for which a pseudomolecular ion peak could be found at m/z 649.3628 ($M+Na^+$, 649.3611 calculated for $C_{31}H_{54}N_4NaO_6S$ versus 649.3788 calculated for $C_{31}H_{54}N_4NaO_8$).[†] The new molecular formulas of the halipeptin family indicated that the original assignment of the heterocycle portion as OMCA was incorrect.

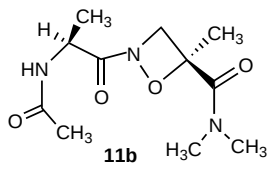
A literature search led to the conclusion that ^{1}H and ^{13}C NMR data of **1**, **2** and **3** were consistent with the presence of a methylthiazoline unit.[2]

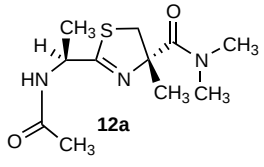
In order to gather conclusive evidences for the structural revision of these molecules (see Table 2 for spectral data) we synthesised a model thiazoline unit (Schemes 1 and 2). The synthesis of Δ^2-thiazoline fragment was based on a four steps preparation of (*R*)-2-methylcysteine hydrochloride (**4**), reported by Pattenden and co-workers in 1993.[3] Esterification of **4** with HCl/MeOH[4] furnished a mixture the desired methyl ester **6** and the corresponding dimeric amino acid **5** in variable ratio (Scheme 1). Reduction[5] of the disulphide bond with PPh_3 gave the expected (*R*)-2-methylcysteine hydrochloride methyl ester (**6**) in 80% overall yield from **4**.

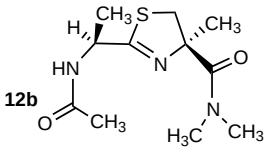
Coupling[3] of **6** with the 2-(*S*)-*t*-butoxycarbonylaminoproprionitrile (**9**), easily prepared in 74% yield from commercially available (L)-alaninamide hydrochloride (**7**) through *N*-Boc protection[6] and efficient dihydration of the amide moiety[7] (Scheme 2), yielded the desired 2-[1-(*S*)-*tert*-butoxycarbonylaminoethyl)]-4-(*R*)-methyl-4,5-dihydrothiazole-4-carboxylic acid methyl ester **10**.[‡]

Final support to the revised structure came from the comparison of GIAO (gauge including atomic orbitals) calculated[8] ^{13}C and ^{15}N NMR chemical shift of four model compounds, representing the two diastereomeric couples of oxazetidine (**11a** and **11b**) and thiazoline (**12a** and **12b**) units, respectively, with those of the natural product **1** (see Table 2), following an approach recently reported by our group.[9]







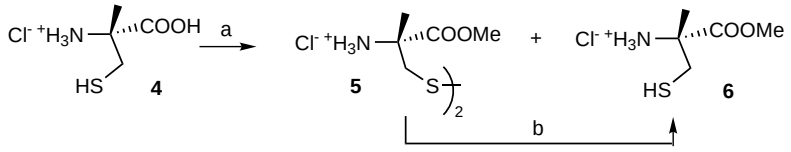


[†] Actually, our first HRMS measurements on **1** gave pseudomolecular ion peaks that were more in agreement with the molecular formula $C_{31}H_{54}N_4O_9$ than with $C_{31}H_{54}N_4O_7S$, leading to a misinterpretation of its NMR data. However, new HRMS data on **1** and **3** were obtained on a superior instrumentation (API QSTAR Pulsar) capable of reaching a resolution of about 20000 in that particular mass range.

[‡] Physical data for compound **10**: $[\alpha]_D = -23$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (1 H, d, $J = 7.3$ Hz, $CHCH_3$), 1.42 (9 H, s, $(CH_3)_3$), 1.50 (3 H, s, CH_3), 3.14 (1 H, d, $J = 11.3$ Hz, CH*H*), 3.75 (1 H, d, $J = 11.3$ Hz, C*H*H), 3.76 (3 H, s, OCH_3), 4.50 (1 H, m, $CHCH_3$), 5.23 (1 H, m, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.43 ($CHCH_3$), 23.80 (CH_3), 28.30 (×3, $(CH_3)_3$), 41.63 (CH_2S), 49.18 (*C*H), 52.81 (OCH_3), 79.76 ($OC(CH_3)_3$), 84.20 ($C(CH_3)CO_2Me$), 154.86 (NCO_2), 173.53 and 174.55 (S*C*=N and *C*O_2Me); HRESIMS: m/z 303.1372 (303.1379 calculated for $C_{13}H_{23}N_2O_4S$).

Table 2. Calculated and experimental ^{13}C chemical shifts values (ppm) of natural and synthetic compounds

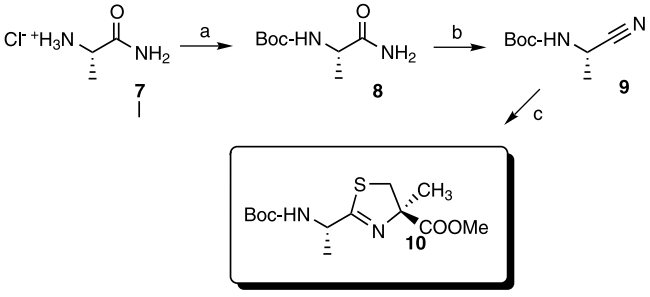
Residue	Calculated ^{13}C chemical shifts values	Residue	Calculated ^{13}C chemical shifts values	Experimental ^{13}C chemical shifts values	
	Oxazetidine unit		Thiazoline unit	Natural compound	Synthetic compound
	11a/**11b**		**12a**/**12b**	**1**	**10**
Ala2		**Ala2**			
CO	182.2/180.4	SC=N	178.2/178.1	177.3	173.5
α	44.2/47.8	α	48.0/47.8	48.5	49.2
β	18.1/18.8	β	22.1/21.5	22.0	20.4
N	−247.1/−247.4	N	−109.4/−107.2	−89.3	–
OMCA		**α-MeCys**			
CO	168.4/170.6	CO	174.2/173.6	172.4	174.6
α	77.8/76.1	α	78.0/79.1	83.3	84.2
β	57.0/55.0	β	40.9/43.1	44.2	41.6
Me-α	23.0/22.0	Me-α	29.5/27.7	23.1	23.8



Scheme 1. *Reagents and conditions*: (a) 5.0 equiv. of $SOCl_2$, MeOH, reflux, 12 h, 80%; (b) 4.5 equiv. of PPh_3, DME/MeOH/H_2O, 7:2:1, 90°C, 12 h, 100%.

Notably, this set of data would *slightly* favour the *R* absolute configuration at C-4 of the thiazoline unit, a finding which is not surprising, considering that this configuration is expected from the cyclisation of a *L*-α-methylcystein amino acid residue and taking into account that all the amino acid residues of halipeptins appear to belong to the *L* series.

Extraction and isolation of **3** followed the same protocol used for **1** and **2**.[1,§] Structure elucidation of **3** was straightforward due to the very limited differences existing between **2** and **3** (their ^1H NMR spectra were virtually superimposable, except for few signals). However, besides the opportunity to correct the assignment of the OMCA unit, the structural study of **3** was useful also for stereochemical reasons. In fact, HPLC Marfey analysis[10] of the acidic hydrolysate of **3** allowed us to assign the *N*-MeVal residue to the *L* series, with the important implication that the same absolute configuration is very likely present in the NMe-δOH-Ile of **1** and **2**.



Scheme 2. *Reagents and conditions*: (a) 1.5 equiv. of Boc_2O, 1.5 equiv. of Et_3N, MeOH, 12 h, 90%; (b) 2.2 equiv. of $(CF_3CO)_2O$, 4.4 equiv. of pyridine, THF, 0°C→rt, 3 h, 82%; (c) 1.0 equiv. of **6**, 1.0 equiv. of **9**, 1.0 equiv. of Et_3N, MeOH, 65°C, 12 h, 30%.

§ Halipeptin C (**3**), white amorphous solid, $[\alpha]_D = -30$ (*c* 0.3, $CHCl_3$), was purified on a μ-Bondapack C-18 column (7.8×300 mm) with linear gradient elution, H_2O/CH_3OH, 75:25–0:100 in 30 min (t_R = 20.5 min). HRESIMS: *m*/*z* 605.3360 (M+Na^+, 605.33487 calculated for $C_{29}H_{50}N_4NaO_6S$). For NMR data, see Table 1.

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