Supporting Information

Piperazimycins: Cytotoxic Hexadepsipeptides from a Marine-Derived Bacterium of the Genus *Streptomyces*.

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Table S1 NMR Spectral Data for the Methyl Ester of 1 (4) at 500MHz in CDCl₃

Unit	Position	δ_{H} mult (<i>J</i> ,Hz)	δ_{C} , MeHSQC	HMBC	COSY
α MeSer C=O	1		174.3, C		
OMe	1-OMe	3.75 s	53.6, CH ₃	C1	
α	2		63.1, C		
β	3a	3.81 m	64.1, CH ₂		H3b
	3b	4.14 m			H3a
α Me	4	1.52 s	19.3, CH ₃	C1	
NH-1		7.44 s		C1	
OH-1		3.59 bs			
γ OHPIP1 C=O	5		172.5, C		
α	6	5.18 d(6.5)	50.9, CH	C5,C7,C8,C10	
β	7a	2.03 m	28.5, CH ₂	C5,C6,C8,C9	H6,H7b
	7b	2.36 bd(14.5)		C8,C9	H7a
γ	8	3.77 bs	59.2, CH	C6,C7,C9	OH-2
δ	9a	2.83 bd(13.0)	53.4, CH ₂	C7,C8	H9b,NH-2
	9b	3.03 bs(11.5)		C7,C8	H9a,NH-2
NH-2		4.62 d(12.5)		C8,C10	H9ab
OH-2		5.73		C7,C8	H8
γ ClPIP C=O	10		173.8, C		
α	11	5.72 d(6.0)	51.3, CH	C10,C12,C13,C15	H12a
β	12a	2.08 m	35.2, CH ₂	C10,C11,C13,C14	H11,H12b,H13
	12b	2.48 bd(9.5)		C11,C13,C14	H12a
γ	13	4.08 m	51.5, CH	C11,C12,C14	H12a,H14ab,
δ	14a	2.80 m	54.0, CH ₂	C12,C13	H13,H14b
	14b	3.31 bs(11.5)		C12,C13	H13,H14a
		5.08 d(10.5)		C11,C13,C14,C15	
NH-3					
AMNA C=O	15		172.4, C		
α	16	5.36 m	49.5, CH	C15,C17,C18,C25	H17ab,NH-4
β	17a	2.31 m	34.6, CH ₂	C15,C16,C18,C20	H16,H18
	17b	2.42 m		C15,C16,C20	H16,H18
γ	18	5.39 m	124.9, CH	C16,C17,C21	H17ab,H19
δ	19	6.03 dd(15.0,10.0)	134.5, CH	C17,C20,C21,C22	H18,H20
ϵ	20	5.92 dd(15.5,11.5)	126.7, CH	C17,C18,C19,C21,C22	H19,H21
ζ	21	5.60 dd(15.0,7.0)	141.6, CH	C20,C22,C23,C24	H20
η	22	2.29 m	31.0, CH	C19,C21,C23,C24	H23,H24
θ	23	0.97 d(7.0)	22.2, CH ₃	C19,C22,C24	H22
η -Me	24	0.97 d(7.0)	22.2, CH ₃	C19,C22,C23	H22
NH-4		7.18 d(9.0)		C15,C16,C25,C26	H16
γ OHPIP2 C=O	25		170.8, C		
α	26	5.02 d(6.0)	49.9, CH	C25,C27,C28	
β	27a	1.93 ddd(14.5,7.0,3.0)	30.2, CH ₂	C25,C26,C28,C29	H26,H27b
	27b	2.63 bd(15.0)		C25,C26,C28,C29	H26,H27a,H28
γ	28	3.84 bs	60.9, CH	C26,C27,C29	H27ab,H29a
δ	29a	2.79 m	52.2, CH ₂	C28	H28b,NH-5
	29b	2.93 bd(12.5)		C27,C28	H28
NH-5		5.03 d(11.5)		C26,C28,C29,C30	H29a
OH-3		5.75m		NA	H28
HAAC C=O	30		175.5, C		
	31a	4.21 dd(17.0,4.0)	61.1, CH ₂	C1,C30	H31b, OH-4
	31b	4.35 dd(16.5,5.0)		C1,C30	H31a, OH-4
OH-4		3.01 m	NA	NA	H31ab

All ¹H multiplicities and ³*J*_{HCCH} values determined from 1H NMR in CDCl₃ (500MHz) 25

Table S2 NMR Spectral Data for Piperazimycin B (2) at 500MHz in CDCl₃

Unit	Position	δ_{H} mult (J, Hz)	δ_{C} , DEPT	HMBC	COSY
$\alpha\text{MeSer C=O}$	1		173.6, C		
α	2		62.6, C		
β	3a	3.99 m	64.6, CH ₂	C1,C2	H3a
	3b	4.10 m		C1,C2,C4	H3b
αMe	4	1.60 s	18.7, CH ₃	C1,C2,C3	
NH-1		7.16 s		C1,C2,C4,C5	
OH-1		3.22 bd(9.0)			
Pip C=O	5		170.0, C		
α	6	4.98 bd(5.5)	50.9, CH	C5,C7,C8,C10	H7ab
β	7a	1.68 m	22.8, CH ₂	C5,C6,C8,C9	H6,H7b,H8
	7b	2.18 m		C6,C9	H6,H7a,H8
γ	8a	1.50 bd(14.0)	20.6, CH ₂	C7,C9	H7ab,H9ab
	8b	1.50 bd(14.0)		C7,C9	H7ab,H9ab
δ	9a	2.76 m	47.3, CH ₂	C8	H8,H9b,NH-2
	9b	3.12 bd(13.0)		C7,C8	H8,H9a,NH-2
NH-2		4.11 m		C8	H9ab
$\gamma\text{ClPip C=O}$	10		174.2, C		
α	11	5.89 m	50.3, CH	C10,C12,C13,C15	H12ab
β	12a	2.00 m	36.0, CH ₂	C10,C11,C13,C14	H11,H12b,H13
	12b	2.39 bd(13.5)		C11,C13,C14	H11,H12a,H13
γ	13	4.02 m	51.4, CH	C14	H12ab,H14ab
δ	14a	2.78 td(13.5,10.5)	54.1, CH ₂	C12,C13	H13,H14b,NH-3
	14b	3.32 bd(14.0)		C12,C13	H13,H14a,NH-3
NH-3		5.29 bd(12.5)		C13,C14,C15	H14ab
AMNA C=O	15		171.8, C		
α	16	5.25 m	50.6, CH	C15,C17,C18,C25	H17ab,NH-3
β	17a	2.48 m	36.1, CH ₂	C15,C16,C18,C20	H16,H17b,H18
	17b	2.53 m		C15,C16,C18,C20	H16,H17a,H18
γ	18	5.37 dt(15.0,7.5)	124.7, CH	C16,C17,C19	H17ab,H19
δ	19	5.92 m	134.1, CH	C17,C18,C20,C21,C22	H18,H20
ϵ	20	5.90 m	126.7, CH	C18,C19,C22	H19,H21
ζ	21	5.56 dd(14.5,6.5)	141.3, CH	C20,C22,C23,C24	H20,H22
η	22	2.29 dq(8.0,7.0,7.0)	31.0, CH	C19,C21,C23,C24	H21,H23,H24
θ	23	0.97 d(7.0)	22.3, CH ₃	C21,C22,C24	H22
$\eta\text{-Me}$	24	0.97 d(7.0)	22.3, CH ₃	C21,C22,C23	H22
NH-4		7.60 d(6.5)		C15,C25	H16
$\gamma\text{OHPip C=O}$	25		169.4, C		
α	26	4.95 bd(7.5)	51.0, CH	C25,C27,C28,C30	H27ab
β	27a	2.01 m	28.5, CH ₂	C26	H26,H27b,H28
	27b	2.14 m		C28,C29	H26,H27a,H28
γ	28	3.75 bs	58.6, CH	C26,C29	H27ab,H29ab,OH
δ	29a	2.80 m	53.6, CH ₂	C28	H28,H29b,NH-5
	29b	2.98 d(13.0)		C27,C28	H28,H29a,NH-5
NH-5		4.35 d(13.0)		C28,C29	H29ab
OH-2		6.49 d(7.5)		C27,C28	H28
HAAC C=O	30		169.1, C		
	31a	4.40 d(16.0)	63.0, CH ₂	C1,C30	H31b
	31b	5.44 d(16.0)		C1,C30	H31a

All ¹H multiplicities and ³J_{HCHH} values determined from 1H NMR in CDCl₃ (500MHz) 25°C

Table S3 NMR spectral data for piperazimycin C (3) (500MHz) recorded in CDCl₃ at 25°C

Unit	Position	δ_{H} mult (J,Hz)	δ_{C} , DEPT	HMBC	COSY
α MeSer C=O	1		173.6, C		
α	2		63.0, C		
β	3a	4.04 d(11.5)	64.2, CH ₂	C1	H3b
	3b	4.15 d(11.5)		C1,C2	H3a
α Me	4	1.58 m	18.3, CH ₃	C1,C2,C3	
NH-1		7.57		C1,C5	
OH-1		3.24			
γ OHPIP1 C=O	5		172.3, C		
α	6	5.15 bd(6.5)	51.2, CH	C5,C7,C8	H7ab
β	7a	2.03 m	28.5, CH ₂	C6	H6,H7b
	7b	2.30 bd(8.5)			H7a,H9b
γ	8	3.75 bs	58.8, CH		H7a,H9b,OH
δ	9a	2.85 m	53.8, CH ₂	C8	H9b,NH-2
	9b	3.05 bd(13.5)			H7b,H9a,NH-2
NH-2		4.58 bd(12.5)			H9ab
OH-2		6.27			H8
γ ClPIP C=O	10		174.3, C		
α	11	5.81 bd(6.5)	51.0, CH	C10,C12,C13	H12a
β	12a	2.05 m	35.7, CH ₂	C10,C13	H11, H12b,H13
	12b	2.42 bd(10.5)		C15	H12a,H13
γ	13	3.91 m	51.3, CH		H12ab,H14ab
δ	14a	2.78 m	54.0, CH ₂		H13,NH-3
	14b	3.31 bd(13.5)			H13
NH-3		5.19 bd(13.5)		C5	H14a
AMDA C=O	15		172.2, C		
α	16	5.28 m	50.4, CH	C15,C17,C18,C26	H17ab,NH-4
β	17a	2.48 m	36.2, CH ₂	C15,C16,C18,C20	H16,H18
	17b	2.53 m		C15,C16,C18,C20	H16,H18
γ	18	5.38 m	124.6, CH	C21	H17ab,H19,H21
δ	19	5.94 m	140.3, CH		H18,H20
ϵ	20	5.90 m	134.3, CH		H19,H21
ζ	21	5.50 m	127.9, CH	C20,C22,C24	H18,H19,H20,H22
η	22	2.02 m	38.2, CH	C19,C21,C23,C24,C32	H21,H24
θ	23a	1.30 m	29.6, CH ₂	C19,C22,C24,C32	H22,H24,H32
	23b	1.30 m		C19,C22,C24,C32	H22,H24,H32
θ -Me	32	0.83 t(7.5)	11.8, CH ₃	C22,C23	H23ab,H24
η -Me	24	0.96 d(7.0)	19.8, CH ₃	C19,C22,C23	H22,H23ab,H32
NH-4		7.60		C15,C25	H16
γ OHPIP2 C=O	25		169.5, C		
α	26	4.93 bd(7.5)	50.9, CH	C25,C27,C28	H27a
β	27a	2.03 m	28.5, CH ₂	C26	H26,H27b
	27b	2.17 bd(15.0)			H27a,H29b
γ	28	3.75 bs	58.6, CH		H27ab,OH-3
δ	29a	2.81 m	53.5, CH ₂	C28	NH-5
	29b	2.99 bd(13.5)			NH-5
NH-5		4.36 bd(12.5)		C30	H29ab
OH-3		6.47			
HAAC C=O	30		169.1, C		
	31a	4.40 d(15.5)	63.0, CH ₂	C1,C30	H31b
	31b	5.48 d(15.5)		C1,C30	H31a

All ¹H multiplicities and ³J_{HCH} values determined from 1H NMR in CDCl₃ (500MHz) 25°C

Table S4 $\Delta\delta_{S-R}$ Chemical Shift Values for *tris*-MTPA derivatives of **1 at 500MHz in CDCl₃**

Unit	Position	Tris-(<i>S</i>)-MTPA (δ_H ppm)	Tris-(<i>R</i>)-MTPA (δ_H ppm)	$\Delta\delta_{S-R}$ (ppm)
α MeSer C=O	1	NA	NA	NA
α	2	NA	NA	NA
β	3a	3.99	4.10	-0.11
	3b	5.32	5.19	+0.13
α Me	4	1.30	1.38	-0.08
NH-1		6.82	7.00	-0.18
OMTPA		NA	NA	NA
γ OMTPAPip1	5	NA	NA	NA
α	6	4.58	4.54	+0.04
β	7a	2.00	2.19	-0.19
	7b	2.30	2.30	0.00
γ	8	5.09	5.12	-0.03
δ	9a	3.08	3.06	+0.02
	9b	3.22	3.19	+0.03
NH-2		4.82	4.79	+0.03
OMTPA		NA	NA	NA
γ ClPip C=O	10	NA	NA	NA
α	11	5.73	5.70	+0.03
β	12a	2.01	1.97	+0.04
	12b	2.30	2.33	-0.03
γ	13	4.07	4.08	-0.01
δ	14a	2.78	2.75	+0.03
	14b	3.32	3.29	+0.03
NH-3		5.25	5.	-0.05
AMNA C=O	15	NA	NA	NA
α	16	5.17	5.12	+0.05
β	17a	2.17	2.19	-0.02
	17b	2.23	2.29	-0.06
γ	18	5.47	5.42	+0.05
δ	19	5.95	5.94	+0.01
ϵ	20	6.04	6.00	+0.04
ζ	21	5.63	5.61	+0.02
η	22	2.29	2.28	+0.01
θ	23	0.98	0.96	+0.02
η -Me	24	0.98	0.96	+0.02
NH-4		6.59	6.60	-0.01
γ OMTPAPip2	25	NA	NA	NA
α	26	4.68	4.69	-0.01
β	27a	2.00	2.28	-0.28
	27b	2.30	2.38	-0.08
γ	28	5.04	5.16	-0.12
δ	29a	2.92	2.99	-0.07
	29b	3.12	3.20	-0.08
NH-5		4.62	4.64	-0.02
OMTPA		NA	NA	NA
HAAC C=O	30	NA	NA	NA
	31a	4.44	4.68	-0.24
	31b	5.06	4.97	+0.09

All chemical shift assignments made from interpretation of ¹H and COSY NMR spectral data

Figure S1 CD Spectra for **1-3**

Figure S2 NCI 60 Cell Line Screen Dose Response Curves for **1**

Figure S3 NCI 60 Cell Line Screen Mean Graphs for 1

Figure S4 ^1H NMR Spectrum of Piperazimycin A (**1**) at 500MHz in CDCl_3

Figure S5 ^{13}C NMR Spectrum of Piperazimycin A (**1**) at 500MHz in CDCl_3

Figure S6 DEPT NMR Spectrum of Piperazimycin A (**1**) at 500MHz in CDCl₃

Figure S7 DEPT NMR Spectrum of Piperazimycin A (**1**) at 500MHz in CDCl₃

Figure S8 gCOSY NMR Spectrum of Piperazimycin A (**1**) at 500MHz in CDCl₃

Figure S9 gHMBC NMR Spectrum of Piperazimycin A (**1**) at 500MHz in CDCl₃

Figure S10 ^1H NMR Spectrum of Piperazimycin B (**2**) at 500MHz in CDCl_3

Figure S11 ^{13}C NMR Spectrum of Piperazimycin B (**2**) at 500MHz in CDCl_3

Figure S12 DEPT NMR Spectrum of Piperazimycin B (**2**) at 500MHz in CDCl₃

Figure S13 gHSQC NMR Spectrum of Piperazimycin B (**2**) at 500MHz in CDCl₃

Figure S14 gCOSY NMR Spectrum of Piperazimycin B (**2**) at 500MHz in CDCl₃

Figure S15 gHMBC NMR Spectrum of Piperazimycin B (**2**) at 500MHz in CDCl₃

Figure S16 ^1H NMR Spectrum of Piperazimycin C (**3**) at 500MHz in CDCl_3

Figure S17 ^{13}C NMR Spectrum of Piperazimycin C (**3**) at 500MHz in CDCl_3

Figure S18 ^1H NMR Spectrum of the Methyl Ester of **1** (**4**) at 500MHz in CDCl_3

Figure S19 ^{13}C NMR Spectrum of the Methyl Ester of **1** (**4**) at 500MHz in CDCl_3

Figure S20 MeHSQC NMR Spectrum of the Methyl Ester of **1** (**4**) at 500MHz in CDCl₃

Figure S21 gCOSY NMR Spectrum of the Methyl Ester of **1** (**4**) at 500MHz in CDCl₃

Figure S22 gHMBC NMR Spectrum of the Methyl Ester of **1** (**4**) at 500MHz in CDCl₃

S23 General Experimental Procedures

NMR spectral data were recorded on a 500 MHz NMR spectrometer with the solvent serving as an internal standard [CDCl_3 ; δ_{H} 7.24; δ_{C} 77.0]. High resolution mass spectra were recorded with a FAB ion source and MS/MS fragmentation data were obtained using with electrospray ionization (ESI). IR spectra were recorded as thin films using NaCl plates. Low resolution MS data were acquired using a LCMS system with a reversed phase C_{18} column [5μ ; 4.6 x 100 mm].