Identification of Synthetic Host Defense Peptide Mimics that Exert Dual

Antimicrobial and Anti-Inflammatory Activities

Abhigyan Som [a,b], Nicolás Navasa [b], Avital Percher [a,b], Richard W. Scott[c], Gregory N. Tew [a,b,#], Juan Anguita [b,d,e#]

Departments of [a] Polymer Science & Engineering and [b] Veterinary & Animal Sciences, University of Massachusetts, Amherst, MA 01003

[c] Polymedix Inc., 170 N. Radnor Chester Road, Suite 300, Radnor, PA 19087

[d] CIC bioGUNE, Technological Park of Bizkaia, 48160 Derio, Bizkaia, Spain and [e] Ikerbasque Foundation, 48011 Bilbao, Spain.

Running Title: Antiinflammatory Antimicrobial Oligomers

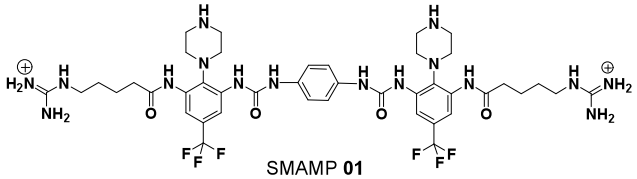
Correspondence:

Juan Anguita, PhD. CIC bioGUNE. Proteomics Unit. Technological Park of Bizkaia. 48160 Derio, Bizkaia, Spain. +34 944 061 311; janguita@cicbiogune.es.

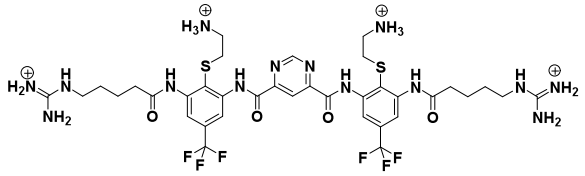
Gregory Tew, PhD. UMass Amherst. Dept of Polymer Sciences and Engineering. 120 Governors Dr. Amherst, MA 01003. (413) 577-1612; tew@mail.pse.umass.edu.

Supporting Information

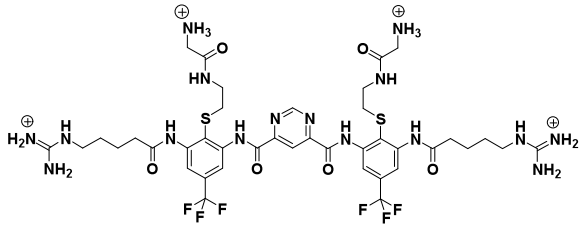
Scheme S1. Chemical structures of SMAMPs 01-03.



SMAMP **01**



SMAMP **02**



SMAMP **03**

Table S1. Antimicrobial activity, cytotoxicity and maximum tolerated dose (MTD) of SMAMPs 01-03.

	MIC_{90}[a] (µg/ml)		Cytotoxicity[b] (EC_{50} in µM)			
	E. coli (ATCC 25922)	*S. aureus* (ATCC 27660)	HC_{50}	3T3	HG2	MTD[c] (mg/kg)
SMAMP 01	1.56	0.195	174	237	206	n.d.
SMAMP 02	0.78	0.195	271	61	178	20
SMAMP 03	0.78	0.195	241	103	160	30

[a]Antimicrobial activity was quantified in terms of minimum inhibitory concentration (MIC); the lowest concentration of SMAMP that inhibits bacterial growth by more than 90%. These values were determined according to the Hancock method for cationic antimicrobial peptides, which is a modification of the classical microbroth dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) (3, 4). [b]HC_{50} are reported as EC_{50} against isolated human erythrocytes; cytotoxicity is reported as EC_{50} against 3T3 cells (embryonic mouse fibroblast line) and HG2 cells (human transformed liver cell line) (1, 2). [c]Maximum tolerated dose in mice; mice were administered a single dose of SMAMP and clinical signs were recorded over a four-day to seven-day period following SMAMP administration. Gross necropsy was performed at the conclusion of the study and MTD values represent the highest dose where no adverse signs were recorded (1, 2).

Table S2. Broad spectrum antimicrobial activity and the cytotoxicity of SMAMPs 01-25.

	MIC_{90} (µg/ml)					Cytotoxicity (EC_{50} in µM)		
SMAMP	**EC**	**SA**	**EF**	**PA**	**KP**	**HC_{50}**	**3T3**	**HG2**
SMAMP 01	1.56	0.195	1.56	6.25	1.56	174	237	206
SMAMP 02	0.78	0.195	0.39	3.13	0.39	271	61	178
SMAMP 03	0.78	0.195	1.56	6.25	1.56	241	103	160
SMAMP 04	12.5	0.39	6.25	6.25	12.5	64.53	-	-
SMAMP 05	0.39	0.195	1.56	25	1.56	526.9	594	843
SMAMP 06	0.78	0.098	0.39	1.56	0.78	114.405	128	145
SMAMP 07	1.56	0.195	-	12.5	0.78	651.4	727	684
SMAMP 08	0.78	0.39	0.78	3.13	1.56	>1000	93	201
SMAMP 09	3.13	0.39	1.56	50	6.25	>1000	221.7	139.6
SMAMP 10	25	-	-	>50	-	>1000	-	-
SMAMP 11	0.78	0.195	0.195	6.25	0.78	167	179	480
SMAMP 12	1.56	0.78	3.13	100	3.13	775.9	822.8	132.1
SMAMP 13	0.78	0.098	0.39	3.13	1.56	94.05	235.3	206
SMAMP 14	0.39	0.39	0.39	25	3.13	<0.4	75	12
SMAMP 15	1.56	0.39	0.78	25	12.5	4	<16	24
SMAMP 16	1.56	1.56	-	25	6.25	9	158	249
SMAMP 17	0.195	0.049	3.13	>50	3.13	-	>1371	>1371
SMAMP 18	0.195	0.098	3.13	12.5	0.195	-	44	81
SMAMP 19	0.78	0.195	0.78	1.56	1.56	13.4	159	98.99
SMAMP 20	12.5	50	12.5	50	25	>1000	-	-
SMAMP 21	6.25	3.13	3.13	50	12.5	-	41	54
SMAMP 22	0.78	0.195	0.39	1.56	1.56	7.8	429.7	>1000
SMAMP 23	1.56	0.39	0.78	12.5	0.78	2.23	44	28
SMAMP 24	1.56	0.195	1.56	50	3.13	>1000	502	531
SMAMP 25	3.13	0.195	0.78	12.5	6.25	26	>2000	>2000

MIC_{90}, minimum inhibitory concentration; EC, *Escherichia coli* ATCC 25922; SA, *Staphylococcus aureus* ATCC 27660; EF, *Enterococcus faecalis* ATCC 29212; PA, *Pseudomonas aeruginosa* ATCC 10145; KP, *Klebsiella pneumoniae* ATCC 13883. HC_{50} are reported as EC_{50} against isolated human erythrocytes; cytotoxicity is reported as EC_{50} against 3T3 cells (embryonic mouse fibroblast line) and HG2 cells (human transformed liver cell line) (1, 2).

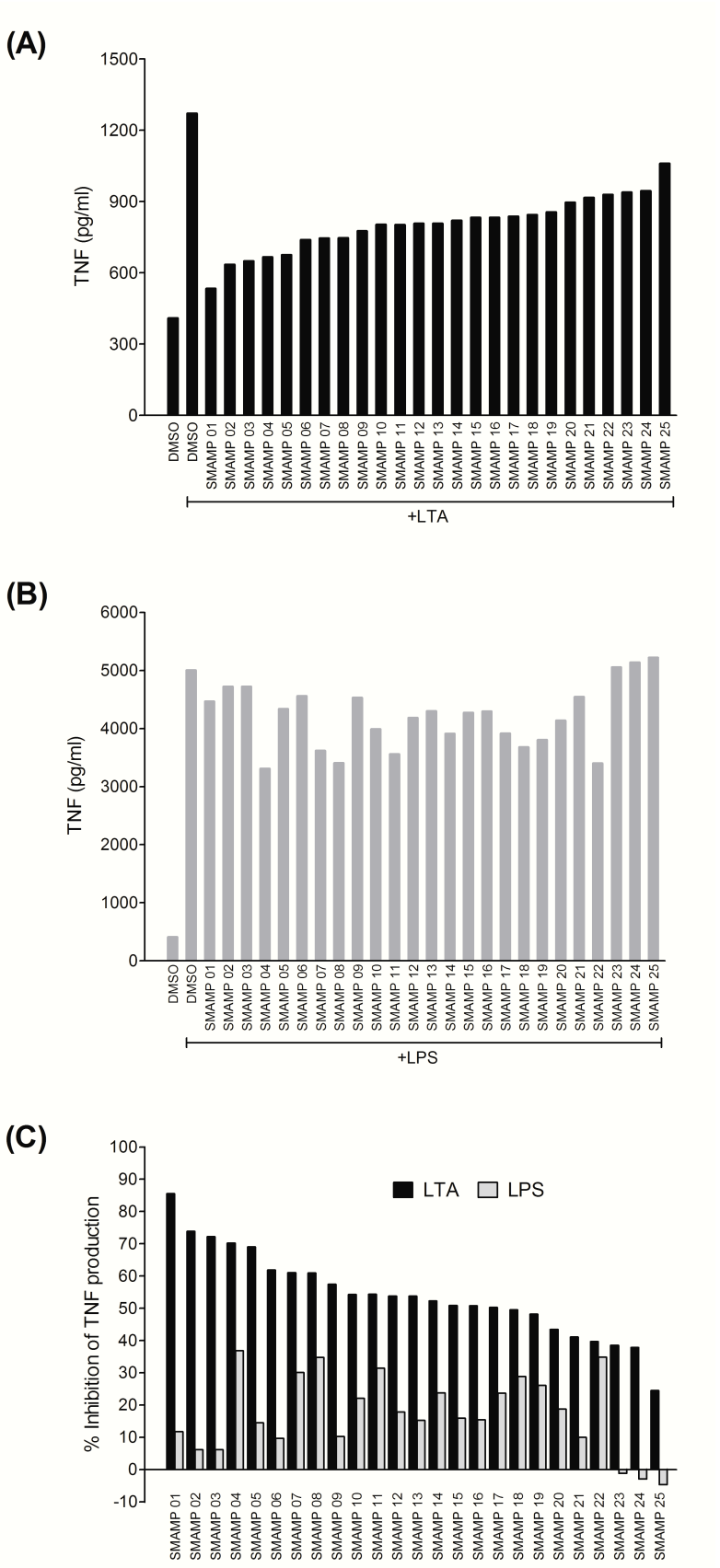

(A)
TNF (pg/ml)
1500
1200
900
600
300
0
DMSO
DMSO
SMAMP 01
SMAMP 02
SMAMP 03
SMAMP 04
SMAMP 05
SMAMP 06
SMAMP 07
SMAMP 08
SMAMP 09
SMAMP 10
SMAMP 11
SMAMP 12
SMAMP 13
SMAMP 14
SMAMP 15
SMAMP 16
SMAMP 17
SMAMP 18
SMAMP 19
SMAMP 20
SMAMP 21
SMAMP 22
SMAMP 23
SMAMP 24
SMAMP 25
+LTA
(B)
TNF (pg/ml)
6000
5000
4000
3000
2000
1000
0
DMSO
DMSO
SMAMP 01
SMAMP 02
SMAMP 03
SMAMP 04
SMAMP 05
SMAMP 06
SMAMP 07
SMAMP 08
SMAMP 09
SMAMP 10
SMAMP 11
SMAMP 12
SMAMP 13
SMAMP 14
SMAMP 15
SMAMP 16
SMAMP 17
SMAMP 18
SMAMP 19
SMAMP 20
SMAMP 21
SMAMP 22
SMAMP 23
SMAMP 24
SMAMP 25
+LPS
(C)
% Inhibition of TNF production
100
90
80
70
60
50
40
30
20
10
0
-10
LTA
LPS
SMAMP 01
SMAMP 02
SMAMP 03
SMAMP 04
SMAMP 05
SMAMP 06
SMAMP 07
SMAMP 08
SMAMP 09
SMAMP 10
SMAMP 11
SMAMP 12
SMAMP 13
SMAMP 14
SMAMP 15
SMAMP 16
SMAMP 17
SMAMP 18
SMAMP 19
SMAMP 20
SMAMP 21
SMAMP 22
SMAMP 23
SMAMP 24
SMAMP 25

Figure S1. *Identification of SMAMPs that thwart the stimulatory capacity of LTA or LPS in RAW264.7 cells.* RAW 264.7 cells (1×10^6 cells/ml) cells were stimulated with (a) LTA (10 µg/ml) (solid bars) or (b) LPS (100 ng/ml) (grey bars) in the presence of 25 different SMAMPs (1.0 µg/ml). TNF production in the supernatants was assessed by ELISA after 12 h of stimulation. 0.01% DMSO was used as control as each SMAMP solution has 0.01% DMSO content. (c) The percentage of TNF production inhibition mediated by SMAMPs was then calculated. Stimulations with 0.01% DMSO (control) and LTA/LPS in the absence of SMAMPs were taken as 100% and 0% inhibition, respectively. RAW 264.7 cells (1×10^6 cells/ml) were pretreated with SMAMPs for 1h before LTA/LPS stimulation.

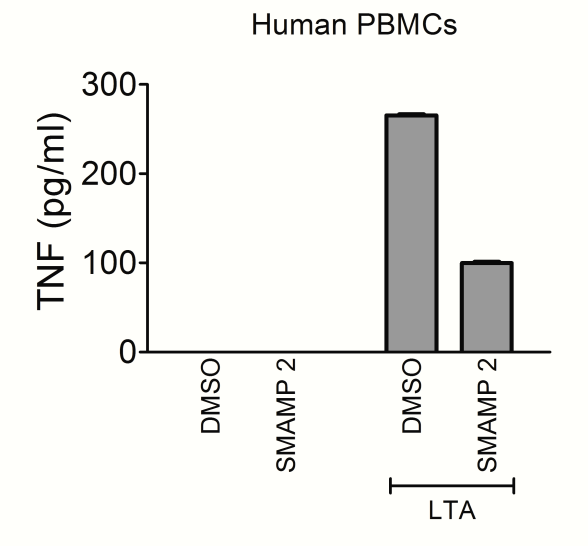


Figure S2. PBMC cells (0.8×10^6 cells/ml) were pre-incubated with SMAMP 2 (5.0 µg/ml) or 0.05% DMSO (control) for 1 h and stimulated with LTA (10 µg/ml) for 20 h. The stimulation supernatants were analyzed for TNF production by ELISA. The data are presented as the average ± s.e.m of triplicate samples.

Supplemental Methods:

Mouse bone marrow derived macrophages (BMDM) cells. Mouse bone marrow cells were collected from the femoral shafts by flushing three times with 1 ml of cold complete RPMI 1640 supplemented with 20% L929-conditioned RPMI 1640. The cell suspensions were cultured in 100 × 15-mm petri dishes (Fisher Scientific, Pittsburgh, PA) in 20% L929-conditioned RPMI 1640 for 8 days at 37°C with 5% CO_2. Following incubation, non-adherent cells were eliminated and the adherent macrophages were scraped, counted, and resuspended in RPMI 1640 medium.

Human peripheral blood mononuclear cells (PBMCs) stimulation with LTA. PBMCs (**(**0.8×10^6 cells) were seeded in 24 well plates in 1 ml of complete RPMI media. After 2 h, cells were incubated with 5 μg/ml SMAMP for 1h, and then stimulated with LTA (10 μg/ml). The cells were incubated for 20 h, and the supernatants were assessed for TNF by ELISA (BD Biosciences) per the manufacturer's instructions (see Figure S2).

Supplementary References:

1. **Choi S, Isaacs A, Clements D, Liu DH, Kim H, Scott RW, Winkler JD, DeGrado WF.** 2009. De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers. Proc. Natl. Acad. Sci. USA. **106:**6968-6973.
2. **Scott R, Degrado W, Tew G.** 2008. De novo designed synthetic mimics of antimicrobial peptides. Curr. Opin. Biotechnol. **19:**620-627.
3. **Steinberg DA, Hurst MA, Fujii CA, Kung AHC, Ho JF, Cheng FC, Loury DJ, Fiddes JC.** 1997. Protegrin-1: A broad-spectrum, rapidly microbicidal peptide with in vivo activity. Antimicrob. Agents Chemother. **41:**1738-1742.
4. **Yan H, Hancock REW.** 2001. Synergistic interactions between mammalian antimicrobial defense peptides. Antimicrob. Agents Chemother. **45:**1558-1560.