Bioorganic & Medicinal Chemistry 20 (2012) 6059–6062

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

'Inverted' analogs of the antibiotic gramicidin S with an improved biological profile

Varsha V. Kapoerchan [a], Annemiek D. Knijnenburg [a], Peter Keizer [a], Emile Spalburg [b], Albert J. de Neeling [b], Roos H. Mars-Groenendijk [c], Daan Noort [c], José M. Otero [a], Antonio L. Llamas-Saiz [d], Mark J. van Raaij [e], Gijs A. van der Marel [a], Herman S. Overkleeft [a], Mark Overhand [a,*]

[a] Bioorganic Synthesis, Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands
[b] Laboratory for Infectious Diseases, National Institute of Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands
[c] Defense, Security and Safety TNO, PO Box 45, 2280 AA Rijswijk, The Netherlands
[d] Unidad de Difracción de Rayos X (RIAIDT), Edificio CACTUS, Campus Vida, Universidad de Santiago de Compostela, E-15782 Santiago de Compostela, Spain
[e] Centro Nacional de Biotecnología (CNB-CSIC), c/Darwin 3, Campus Cantoblanco, E-28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 3 May 2012
Revised 21 August 2012
Accepted 21 August 2012
Available online 31 August 2012

Keywords:
Cyclic peptide
Antibiotics
Hemolysis
Improved properties
MRSA

ABSTRACT

A series of gramicidin S derivatives **4**–**15** are presented that have four ornithine residues as polar protonated side chains and two central hydrophobic amino acids with unaltered turn regions. These peptides were screened against human erthrocytes and our standard panel of Gram negative- and Gram positive bacteria, including four MRSA strains. Based on the antibacterial- and hemolytic data, peptides **13** and **14** have an improved biological profile compared to the clinically applied topical antibiotic gramicidin S.

1. Introduction

Gramicidin S (*cyclo*-(Pro-Val-Orn-Leu-^{D}Phe)$_2$ **1**, Fig. 1) is an amphiphilic cationic antimicrobial peptide natural product with a cyclic beta-hairpin structure. In this cyclic decapeptide the protonated ornithine side chains are positioned at one face of the molecule and the hydrophobic side chains of the valine and leucine residues at the opposing face. The amphiphilic and cationic characteristics of the molecule are essential for its mode of action which involves[1] the disruption of the bacterial membranes. Gramicidin S also disrupts mammalian cell membranes and due to these toxic properties its clinical application is limited to topical infections. Less toxic derivatives are of interest as cationic antimicrobial peptides generally do not lead to highly effective resistance mechanisms.[2]

Numerous synthetic derivatives of gramicidin S have been described in the literature with the aim to obtain compounds with reduced toxicity while retaining antimicrobial activity.[3] A successful strategy in this respect is to extend the cyclic beta-hairpin ring to allow the strategic introduction of additional positive charged residues combined with hydrophobic ones in such a way that an optimal amphiphilicity is obtained.[4] The thus obtained highly charged gramicidin S derivatives with an optimized amphiphilic character were reported to have an increased biological profile.[4b] Another strategy to modulate the amphiphilic characteritics entails the introduction of additional positively charged side chains in the original decapeptide sequence via the replacement of the ornithine residues with two hydrophobic amino acids and the valine and leucine residues with four ornithine residues.[5] An example of such an 'inverted' gramicidin S derivative is compound **2** (Fig. 1).[5c] Using this design strategy we recently reported analog **3** (Fig. 1) that contains two adamantyl glycines as the central bulky hydrophobic amino acid residues.[6] It was shown that compound **3** has noticeably decreased hemolytic properties, as well as a slightly increased antibacterial activity as compared with gramicidin S. In contrast, derivative **2** proved completely inactive. Because of these intriguing findings we decided to make a series of derivatives with varying central hydrophobic amino acid residues (**4**–**15**, Fig. 1) and to explore their antibacterial- and hemolytic properties.

2. Results and discussion

As hydrophobic residues we selected the aliphatic amino acids (*S*)-valine, (*S*)-leucine, (*S*)-*tert*-butylglycine, (*S*)-*tert*-butylalanine,

* Corresponding author. Fax: +31 527 4307.
E-mail address: overhand@chem.leidenuniv.nl (M. Overhand).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.08.038

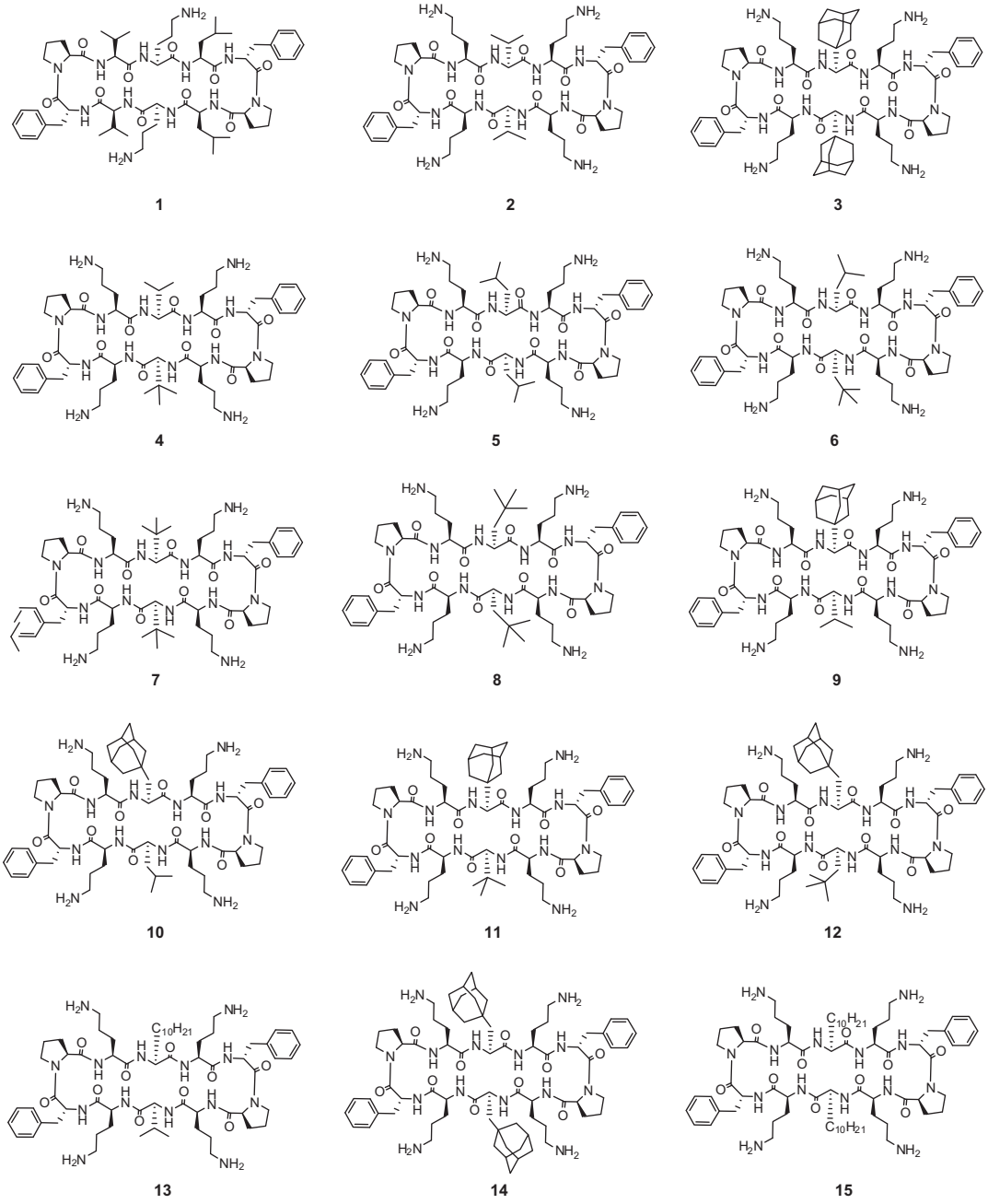

Figure 1. Gramicidin S (**1**), the structures of previously reported compounds **2** and **3**, and their derivatives **4**–**15**.

(*S*)-decylglycine, (*S*)-adamantylglycine[7] and (*S*)-adamantylalanine.[8] These amino acids were incorporated in the central positions of the sequence *cyclo*-(Pro-Orn-(hydrophobic amino acid)-Orn-^{D}Phe)$_2$ and combined in such a way that a series of symmetric and asymmetric cyclic decapeptides with varying hydrophobicity were obtained (peptides **4**–**15**, Fig. 1). The compounds were synthesized using a standard Fmoc peptide synthesis protocol, involving assembly of the linear decapeptides on a solid support, mild acidic cleavage, cyclization in solution, followed by complete deprotection, preparative HPLC purification and characterization.[9] Several of the newly synthesized peptides have multiple conformations on the NMR time scale. One of the peptides (**6**) provided crystals that were suitable for X-ray analysis, its cyclic beta-hairpin structure is depicted in Figure 2.

The reverse phase HPLC retention times of the peptides **4**–**15**, as a measure of peptide hydrophobicity,[10] are listed in Table 1, as well as their hemolytic properties and their growth inhibition of our standard set of bacteria, including four MRSA strains.

Within the peptide series the favorable properties of lead structure **3** that combines highly antibacterial activity with low hemolytic activity are not surpassed. Peptides **13** and **14**, which have a similar hydrophobicity as **3**, as judged by their HPLC retention times under controlled conditions, are less antibacterial and more hemolytic, respectively. There is no correlation between the conformational properties of the peptides as observed by NMR and their biological properties. It seems that the peptide hydrophobicity and molecular requirements for favorable properties in this series of peptides are very strict and the peptides **4**–**11** are simply too

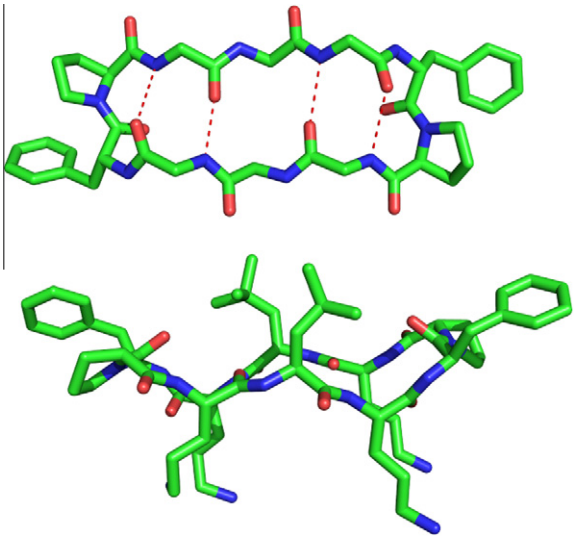

Figure 2. The cyclic beta-hairpin X-ray structure of compound **6**. Intramolecular hydrogen bonding interactions (top, side chains omitted for clarity), amphiphilic characteristics (bottom).

hydrophilic and peptide **15** too hydrophobic to have antibacterial activity.

3. Conclusion

Based on the gramicidin S derivatives **2**[5c] and the **3**,[6] a series of analogs were prepared with varying hydrophobic functionality of the central two amino acid residues. Within this series, the lead compound **3** is not surpassed with respect to high antibacterial- and low hemolytic activity. The peptide hydrophobicity and molecular requirements for favorable properties in this series of molecules are stringent as exemplified by peptide **13** and **14**. Peptide **13** having similar hydrophobic characteristics as **3** is less antibacterial, peptide **14**, the two carbon homolog of **3**, is more hemolytic. In comparing the antibacterial and hemolytic data of the topical antibiotic gramicidin S (**1**, in Table 1) with peptides **13** and **14**, the latter two molecules have a better biological profile. This identifies peptides **13** and **14** as potential biologically interesting novel compounds and forms a good starting point for further studies investigating whether peptides **3**, **13** and **14** can be used to treat topical- and other infections in animal models.

Acknowledgments

We are grateful to Kees Erkelens and Fons Lefeber for assistance in measuring NMR spectra. This work was supported by a grant from the Leiden Institute of Chemistry and BFU2008-01588/BMC of the Spanish Ministry of Science and Innovation. J.M.O. is supported by an 'Angeles Alvariño' contract of the Xunta de Galicia and a grant for working abroad at Leiden University funded by the Xunta de Galicia and the European Social Fund.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.038.

References and notes

1. (a) Prenner, E. J.; Lewis, R. N. A. H.; McElhaney, R. N. *Biochim. Biophys. Acta* **1999**, *1462*, 201; (b) Izumiya, N.; Kato, T.; Aoyaga, H.; Waki, M.; Kondo, M. In *Synthetic Aspects of Biologically Active Cyclic Peptides: Gramicidin S and Tyrocidines*; Izumiya, N., Ed.; Halsted Press: New York, 1979; (c) Gause, G. F.; Brazhnikova, M. G. *Nature* **1944**, *154*, 703.
2. Peschel, A.; Sahl, H.-G. *Nat. Rev. Microbiol.* **2006**, *4*, 529.
3. For recent publications see: (a) Tamaki, M.; Fujinuma, K.; Harada, T.; Takanashi, K.; Shindo, M.; Kimura, M.; Uchida, Y. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 106; (b) Skoda, E. M.; Davis, G. C.; Wipf, P. *Org. Proc. Res. Dev.* **2012**, *16*, 26; (c) Tamaki, M.; Takanashi, K.; Harada, T.; Fujinuma, K.; Shindo, M.; Kimura, M.; Uchida, Y. *Chem. Pharm. Bull.* **2011**, *59*, 1481; (d) Yamada, K.; Kodaira, M.; Shinoda, S.; Komagoe, K.; Oku, H.; Katakai, R.; Katsu, T.; Matsuo, I. *MedChemComm* **2011**, *2*, 644; (e) van der Knaap, M.; Lageveen, L. T.; Busscher, H. J.; Mars-Groenendijk, R.; Noort, D.; Otero, J. M.; Llamas-Saiz, A. L.; van Raaij, M. J.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *ChemMedChem* **2011**, *6*, 840; (f) Knijnenburg, A. D.; Tuin, A. W.; Spalburg, E.; de Neeling, A. J.; Mars-Groenendijk, R. H.; Noort, D.; Otero, J. M.; Llamas-Saiz, A. L.; van Raaij, M. J.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *Chem. Eur. J.* **2011**, *17*, 3995; (g) Kamysz, E.; Mickiewicz, B.; Kamysz, W.; Bielinska, S.; Rodziewicz-Motowidlo, S.; Ciarkowski, J. *J. Pept. Sci.* **2011**, *17*, 211; (h) Derbal, S.; Hensler, M.; Fang, W. Q.; Nizet, V.; Ghedira, K.; Nefzi, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5701; (i) Solanas, C.; de la Torre, B. G.; Fernandez-Reyes, M.; Santiveri, C. M.; Jimenez, M. A.; Rivas, L.; Jimenez, A. I.; Andreu, D.; Cativiela, C. *J. Med. Chem.* **2010**, *53*, 4119.
4. (a) Lee, D. L.; Powers, J. P. S.; Pflegerl, K.; Vasil, M. L.; Hancock, R. E. W.; Hodges, R. S. *J. Pept. Res.* **2004**, *63*, 69; (b) Kondejewski, L. H.; Lee, D. L.; Jelokhani-Niaraki, M.; Farmer, S. W.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **2002**,

Table 1
Antimicrobial properties of peptides **1**–**15**

	Gram+ *S. aureus*	Gram+ *S. epidermidis*	Gram+ *E. faecalis*	Gram+ *B. cereus*	Gram− *E. coli*	Gram− *P. aeruginosa*	MRSA-NT 1110301981H-T034-PVL+	MRSA-NT N133-T034-PVL−	MRSA-NT N229-T034-PVL−	MRSA-Cluster218 USA300-1110301146 PVL+	100% Hemolysis (μM)	HPLC retention time (min)
1	8	8	16	8	32	64	8	8	8	8	31.3	8.38
2	>64	64	>64	>64	>64	>64	>64	>64	>64	>64	>500	4.70
3	8	4	8	8	8	16	8	8	8	8	500	6.39
4	>64	64	>64	>64	>64	>64	>64	>64	>64	>64	>500	4.87
5	>64	64	>64	>64	>64	>64	—	—	—	—	>500	4.97
6	>64	16	>64	>64	>64	>64	>64	>64	>64	>64	>500	5.07
7	>64	64	>64	>64	>64	>64	>64	>64	>64	>64	>500	5.12
8	>64	16	>64	>64	>64	64	>64	>64	>64	>64	>500	5.24
9	64	16	>64	64	>64	64	>64	>64	>64	>64	>500	5.54
10	64	4	64	16	64	16	64	32	32	32	>500	5.77
11	64	4	>64	32	32	32	64	64	64	64	>500	5.85
12	16	4	32	4	16	16	16	16	16	16	500	5.89
13	16	8	16	4	16	16	16	8	8	8	500	6.49
14	8	4	8	8	8	16	8	8	8	8	62.5	6.66
15	64	4	32	8	64	64	64	64	64	64	62.5	7.91

MIC-values (MIC = Minimal Inhibitory Concentration) are given in μg/mL and were measured after 24 h of incubation (—: not tested). Hemolysis values represent the peptide concentration in which 100% of the human erythrocytes are lysed.

277, 67; (c) Kondejewski, L. H.; Jelokhani-Niaraki, M.; Farmer, S. W.; Lix, B.; Kay, C. M.; Sykes, B. D.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1999**, *274*, 13181; (d) Gibbs, A. C.; Kondejewski, L. H.; Gronwald, W.; Nip, A. M.; Hodges, R. S.; Sykes, B. D.; Wishart, D. S. *Nat. Struct. Biol.* **1998**, *5*, 284; (e) Kondejewski, L. H.; Farmer, S. W.; Wishart, D. S.; Kay, C. M.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1996**, *271*, 25261; (f) Ando, S.; Takiguchi, H.; Izumiya, N. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3781.

5. (a) Mihara, H.; Hayashida, J.; Hasegawa, H.; Ogawa, H. I.; Fujimoto, T.; Nishino, N. *J. Chem. Soc., Perkin Trans. 2* **1997**, 517; (b) Soejima, Y.; Hashiguchi, A.; Izumiya, N. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 826; (c) Ando, S.; Nishikawa, H.; Takiguchi, H.; Izumiya, N. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1201.
6. Kapoerchan, V. V.; Knijnenburg, A. D.; Niamat, M.; Spalburg, E.; de Neeling, A. J.; Nibbering, P. H.; Mars-Groenendijk, R. H.; Noort, D.; Otero, J. M.; Llamas-Saiz, A. L.; van Raaij, M. J.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *Chem. Eur. J.* **2010**, *16*, 12174.
7. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcikevicene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025.
8. Kapoerchan, V. V.; Wiesner, M.; Hillaert, U.; Drijfhout, J. W.; Overhand, M.; Alard, P.; Van der Marel, G. A.; Overkleeft, H. S.; Koning, F. *Mol. Immunol.* **2010**, *47*, 1091.
9. Grotenbreg, G. M.; Kronemeijer, M.; Timmer, M. S. M.; El Oualid, F.; van Well, R. M.; Verdoes, M.; Spalburg, E.; van Hooft, P. A. V.; de Neeling, A. J.; Noort, D.; van Boom, J. H.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *J. Org. Chem.* **2004**, *69*, 7851; (b) Tamaki, M.; Akabori, S.; Muramatsu, I. *J. Am. Chem. Soc.* **1993**, *115*, 10492; (c) Minematsu, Y.; Waki, M.; Suwa, K.; Kato, S.; Izumiya, N. *Tetrahedron Lett.* **1980**, *21*, 2179.
10. (a) Kondejewski, L. H.; Lee, D. L.; Jelokhani-Niaraki, M.; Farmer, S. W.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **2002**, *1*, 67; (b) Jelokhani-Niaraki, M.; Kondejewski, L. H.; Farmer, S. W.; Hancock, R. E. W.; Kay, C. M.; Hodges, R. S. *Biochem. J.* **2000**, *349*, 747; (c) McInnes, C.; Kondejewski, L. H.; Hodges, R. S.; Sykes, B. D. *J. Biol. Chem.* **2000**, *275*, 14287.