ACS OMEGA

Article

http://pubs.acs.org/journal/acsodf

Lysine Scanning of Arg_{10}–Teixobactin: Deciphering the Role of Hydrophobic and Hydrophilic Residues

Shimaa A. H. Abdel Monaim,[†] Yahya E. Jad,[†] Estelle J. Ramchuran,[†] Ayman El-Faham,[‡,§] Thavendran Govender,[†] Hendrik G. Kruger,[†] Beatriz G. de la Torre,*[,†] and Fernando Albericio*[,†,‡,⊥,#,∥]

[†]Catalysis and Peptide Research Unit, School of Health Sciences and [∥]School of Chemistry and Physics, University of KwaZulu-Natal, University Road, Durban 4001, South Africa

[‡]Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

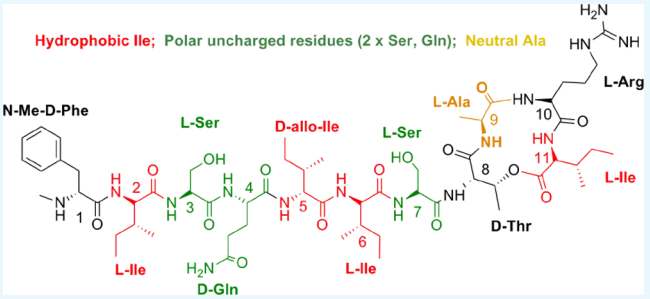
[§]Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt

[⊥]Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1-11, Barcelona 08028, Spain

[#]Networking Centre on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Barcelona Science Park, Baldiri Reixac 10, Barcelona 08028, Spain

Supporting Information

ABSTRACT: Teixobactin is a recently discovered antimicrobial cyclodepsipeptide with good activity against Gram positive bacteria. Taking Arg_{10}–teixobactin as a reference, where the nonproteinogenic residue L-allo-enduracididine was substituted by arginine, a lysine scan was performed to identify the importance of keeping the balance between hydrophilic and hydrophobic amino acids for the antimicrobial activities of this peptide family. Thus, the substitution of four isoleucine residues present in the natural sequence by lysine led to a total loss of activity. On the other hand, the substitution of the polar noncharged residues and alanine by lysine allowed us to keep and in some cases to improve the antimicrobial activity.



1. INTRODUCTION

Infectious diseases, including tuberculosis (TB), cause major global health problems.[1−5] This awareness accompanied by the lobbying of some nongovernmental organizations has fueled the renaissance of the search for new antibiotics.[5] In this scenario, the publication on teixobactin (**1**) (Figure 1) at the beginning of 2015 by Ling et al. raised a pronounced expectation.[6] Teixobactin is a head to side-chain cyclodepsipeptide that exhibits good activity against Gram-positive bacteria and TB.[6−11] At the end of 2015, our group published the first synthesis of an analogue of teixobactin, Arg_{10}–teixobactin (**2**) (Figure 1), where the nonproteinogenic amino acid L-allo-enduracididine was substituted by arginine.[7,12]

Arg_{10}–teixobactin (**2**) exhibits similar biological trends as teixobactin (**1**) by showing better activity against Gram-positive bacteria than against Gram-negative bacteria. However, Arg_{10}–teixobactin (**2**) was shown to be 1 order of magnitude less active than the parent compound. In 2016, two groups, Payne and co-workers and Li and co-workers,[13,14] published the total synthesis of the natural product (**1**).

At a glance, the structure of teixobactin, an 11 amino acid peptide, can be defined as a scaffold formed by a linear segment constituted with seven amino acid residues, which we named tail, followed by a cyclic structure with four residues. In the cycle, a D-Thr is at the bridgehead linked to an L-Ile, which contains a guanidine side-chain amino acid, and the neutral alanine completes the sequence. The tail end is an *N*-Me-D-Phe and contains two more D-amino acids. Another characteristic feature of this peptide is the presence in total of four isoleucine and three polar uncharged residues (two serine and one glutamine).

Although the structure of this peptide was known only relatively recently, there are already a few reports on its structure activity relationship. In this regard, our group and Singh and co-workers independently found that the activity was lost when the three D-amino acids in the tail were substituted by the corresponding L-amino acids (phenylalanine, glutamine, and isoleucine). This was also observed when the *N*-Me-D-Phe at the *N*-terminal was substituted with *N*-Ac-D-Phe, indicating the importance of a positive charge at the *N*-terminal.[8,10]

More recently and during the completion of this study, Nowick and co-workers have reported another bioactivity study, employing Arg_{10}–teixobactin (**2**) as a reference.[15] The main conclusions of their work are that the Lys_{10}–teixobactin (substitution of the arginine by lysine) is more active than (**2**). Furthermore, the absolute stereochemistry is insignificant.

Received: October 31, 2016
Accepted: December 5, 2016
Published: December 19, 2016

DOI: 10.1021/acsomega.6b00354
ACS Omega 2016, 1, 1262−1265

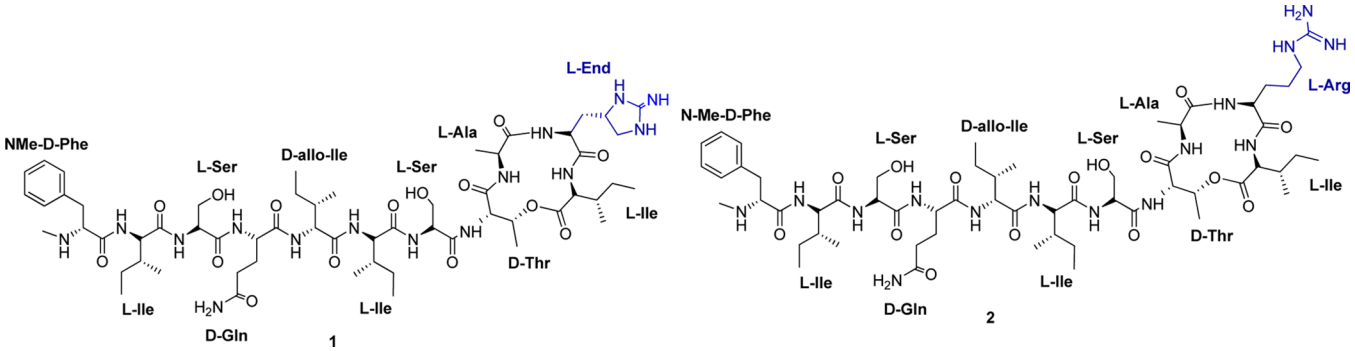


Figure 1. Structure of teixobactin (**1**) and the Arg_{10}–teixobactin analogue (**2**).

Thus, the enantiomer, where L has been substituted by D residues and vice versa, maintains the activity. Finally, the residues 1–5 of the tail could be substituted by a dodecanoyl group.

2. RESULTS AND DISCUSSION

In our current efforts to develop a more potent analogue of teixobactin and later to explore the mode of action of this family of compounds, a lysine scan was carried out. Lysine scanning of a peptide involves the preparation of a set of analogues, where each one contains a lysine residue in a different position of the sequence while keeping the same stereochemistry.[16] In contrast to alanine scanning, which is a more frequently used technique for the identification of amino acids that are not key for biological activities, lysine scanning has not been extensively reported in the literature for antimicrobial peptides (AMPs).[17,18]

Lysine scanning could be extremely important to speed up AMP medicinal chemistry programs because it may render analogues with a better potency and is more easily modifiable through the substitution of lysine by other basic residues.[19]

In this sense, eight analogues containing L-Lys at positions 2, 3, 6, 7, 9, and 11 and D-Lys at positions 4 and 5 were prepared. Because D-Thr is essential for creating the ester bond and to build up the depsipeptide cycle, the corresponding lysine analogue at the bridgehead position 8 was not prepared. Also, neither the analogue at position 1 (importance was previously established by our group)[10] nor the one at position 10 (reported by the group of Nowick and co-workers)[15] was investigated in this study. The eight lysine analogues covered the substitution of the four hydrophobic isoleucine (represented by the red color in Figure 2), the three polar uncharged residues 2× serine, glutamine(represented by the green color in Figure 2), and the neutral alanine (represented by the yellow color in Figure 2) by lysine.

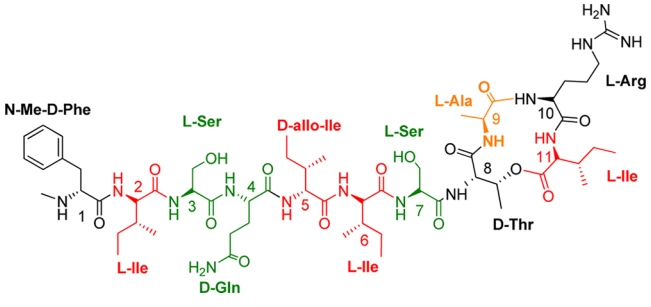


Figure 2. Illustration of lysine scanning on the Arg_{10}–teixobactin analogue.

Table 1 clearly shows that the substitution of four isoleucine (three L- and one D-allo) by lysine led to a total loss of activity.

Table 1. MIC (μg/mL) for Lys_{2-11} Teixobactin Analogues

	MIC μg/mL (ATCC strains)			
	Gram (+)		Gram (−)	
entry	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
Arg_{10}–teixobactin (**2**)[7]	2	0.5	64	NI
Lys_2	NI[a]	NI	NI	NI
Lys_3	4	0.25	32	128
Lys_4	4	1	32	128
Lys_5	NI	NI	NI	NI
Lys_6	NI	NI	NI	NI
Lys_7	32	8	256	256
Lys_9[b]	6	1	50	100
Lys_{11}	NI	NI	NI	NI
meropenem[c]	0.0625	0.0625	0.03125	0.25

[a]NI: no inhibition. [b]The starting concentration is 100 μg/mL. [c]Used as controls.

On the other hand, the substitution of the three polar uncharged amino acids, (Ser_3, Gln_4, and Ser_7) and the neutral Ala_9 maintained or improved the activity in some cases. Thus, these data show that there is a relative balance between the hydrophilic and hydrophobic parts in teixobactin analogues. As shown in Table 1, introducing a polar positively charged residue into the new analogues instead of a polar uncharged residue improved the activity against some strains in comparison with the reference Arg_{10}–teixobactin (**2**). (For example, Lys_3–Arg_{10}–teixobactin shows better activity against the two bacterial strains Bacillus subtilis and Escherichia coli than the reference Arg_{10}–teixobactin. Also, it shows minimal activity against Pseudomonas aeruginosa, whereas the reference shows no activity against the same strains). Furthermore, Lys_4–Arg_{10}–teixobactin and Lys_9–Arg_{10}–teixobactin exhibit activity against Staphylococcus aureus and B. subtilis strains close to Arg_{10}–teixobactin and better activity against E. coli and P. aeruginosa strains than the reference. This will facilitate the possibility of finding analogues with improved activity by substituting lysine by other basic residues such as arginine.

3. EXPERIMENTAL SECTION

3.1. Materials. All reagents and solvents were obtained from commercial suppliers and used without further

DOI: 10.1021/acsomega.6b00354
ACS Omega 2016, 1, 1262–1265

purification. Analytical high-pressure liquid chromatography (HPLC) was performed on an Agilent 1100 system, and the Chemstation software was used for data processing. Buffer A was 0.1% trifluoroacetic acid in H_2O, and buffer B was 0.1% trifluoroacetic acid in CH_3CN. Liquid chromatography−mass spectrometry (LC−MS) was performed on a Shimadzu 2020 ultrafiltration-LC−MS using an YMC-Triart C18 (5 μm, 4.6 × 150 mm) column, and data processing was carried out using the Lab Solution software. Buffer A was 0.1% formic acid in H_2O, and buffer B was 0.1% formic acid in CH_3CN. Crude peptides were purified with a Shimadzu LC-8A preparative HPLC using a Phenomenex Luna C18(2) 100 Å column (10 μm × 250 mm). High-resolution mass spectrometry (HRMS) was acquired using a Bruker ESI-Q-TOF mass spectrometer in the positive mode. Matrix-assisted laser desorption ionization (MALDI) was performed on a Bruker MALDI-TOF Autoflex SmartBeam II system (Bruker Daltonics, Bremen, Germany) using the MALDI matrix (α-cyano-4-hydroxycinnamic acid).

3.2. Methods. The eight analogues were synthesized using the solid-phase peptide synthesis (SPPS) technique as previously described.[7,10] Succinctly, the synthesis was started by anchoring a residue at position 9 [Fmoc-Ala-OH or Fmoc-Lys(Boc)-OH in the case of the Lys_9 analogue] to a chlorotrityl chloride polystyrene resin (CTC-resin), and then the elongation of the peptide chain was achieved by the use of Fmoc-amino acids until residue at position 6 (isoleucine or lysine in the case of the Lys_6 analogue, in all cases except in the synthesis where Lys_{11} analogue). Then, the β-hydroxyl functional group of D-Thr, which had been incorporated without protection, was acylated by Alloc-L-Ile-OH, followed by the incorporation of Alloc-Arg(Pbf)-OH. Finally, the Fmoc-based synthesis was continued until the incorporation of Boc-*N*-Me-D-Phe-OH, whereas in the synthesis of the analogue Lys_{11}, the Fmoc-SPPS was performed until the incorporation of Boc-*N*-Me-D-Phe-OH, and then the esterification of the threonine amino acid was carried out by Fmoc-Lys(Boc)-OH followed by the incorporation of Fmoc-Arg(Pbf)-OH.

The cleavage of the semiprotected peptide, having the free α-amino acid group of arginine and the carboxylic group of alanine or lysine in the case of the Lys_9 analogue, was carried out with 0.1% trifluoroacetic acid (TFA) in dichloromethane followed by cyclization using ((7-azabenzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate/ethyl cyano-(hydroxyimino)acetate/diisopropylethylamine, 3:3:6). The final global deprotection was enabled with TFA/triisopropylsilane/H_2O (95:2.5:2.5), and the peptides were purified using HPLC and characterized using HRMS (see the Supporting Information). The minimum inhibitory concentration (MIC) was determined as previously described, with meropenem as the control (see the Supporting Information).[10]

4. CONCLUSIONS

In conclusion, performing a lysine scan has allowed the identification of the essential residues for maintaining the balance between the hydrophilic and hydrophobic amino acids for the antimicrobial activity of teixobactin analogues. Thus, the substitution of any of the isoleucine residues by lysine rendered the compound with a total loss of activity. Furthermore, the substitution of the three polar uncharged amino acids and alanine by lysine allowed us to sustain the antimicrobial potency and in some cases to improve it. We envisage that the lysine scanning will become a useful tool for the discovery of new AMPs as it has been shown in this study.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00354.

Synthetic methods, HPLC, HRMS, and MALDI (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: albericio@ukzn.ac.za (F.A.).
*E-mail: garciadelatorreb@ukzn.ac.za (B.G.T.).

ORCID

Fernando Albericio: 0000-0002-8946-0462

Author Contributions

All chemistry has been carried out mainly by S.A.H.A.M., with some experiments done by Y.E.J. The biological screening was carried out by E.J.R. The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded in part by the following: the National Research Foundation (NRF) and the University of KwaZulu-Natal (South Africa); the MINECO-FEDER (CTQ2015-67870P), the International Scientific Partnership Program ISPP at King Saud University (ISPP# 0061) (Saudi Arabia); and the Generalitat de Catalunya (2014 SGR 137).

■ ABBREVIATIONS

DIEA, diisopropylethylamine; OxymaPure, ethyl cyano-(hydroxyimino)acetate; PyAOP, (7-azabenzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate; TFA, trifluoroacetic acid; TIS, triisopropylsilane

■ REFERENCES

(1) Corbett, E. L.; Watt, C. J.; Walker, N.; Maher, D.; Williams, B. G.; Raviglione, M. C.; Dye, C. The growing burden of tuberculosis: Global trends and interactions with the HIV epidemic. *Arch. Intern. Med.* **2003**, *163*, 1009−1021.

(2) Mazurek, G. H.; Jereb, J.; LoBue, P.; Iademarco, M. F.; Metchock, B.; Vernon, A. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm. Rep.* **2005**, *54*, 49−55.

(3) Taylor, Z.; Nolan, C. M.; Blumberg, H. M. Controlling Tuberculosis in the United States. Recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. *MMWR Morb. Mortal Wkly. Rep.* **2005**, *54*, 1−81.

(4) Raviglione, M. C.; Harries, A. D.; Msiska, R.; Wilkinson, D.; Nunn, P. Tuberculosis and HIV: Current status in Africa. *AIDS* **1997**, *11* (suppl B), S115−S123.

(5) WHO. *Global Tuberculosis Report 2013*; World Health Organization, 2013.

(6) Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schäberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K. A new antibiotic kills pathogens without detectable resistance. *Nature* **2015**, *517*, 455−459.

(7) Jad, Y. E.; Acosta, G. A.; Naicker, T.; Ramtahal, M.; El-Faham, A.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. Synthesis and biological evaluation of a teixobactin analogue. *Org. Lett.* **2015**, *17*, 6182−6185.

DOI: 10.1021/acsomega.6b00354
ACS Omega 2016, 1, 1262−1265

(8) Parmar, A.; Iyer, A.; Vincent, C. S.; Van Lysebetten, D.; Prior, S. H.; Madder, A.; Taylor, E. J.; Singh, I. Efficient total syntheses and biological activities of two teixobactin analogues. *Chem. Commun.* **2016**, *52*, 6060−6063.
(9) Dhara, S.; Gunjal, V. B.; Handore, K. L.; Reddy, D. S. Solution-Phase Synthesis of the Macrocyclic Core of Teixobactin. *Eur. J. Org. Chem.* **2016**, 4289−4293.
(10) Monaim, S. A. H. A.; Jad, Y. E.; Acosta, G. A.; Naicker, T.; Ramtahal, E. J.; El-Faham, A.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. Re-evaluation of the *N*-terminal substitution and the D-residues of teixobactin. *RSC Adv.* **2016**, *6*, 73827−73829.
(11) Ng, V.; Chan, W. C. New Found Hope for Antibiotic Discovery: Lipid II Inhibitors. *Chem.—Eur. J.* **2016**, *22*, 12606−12616.
(12) Craig, W.; Chen, J.; Richardson, D.; Thorpe, R.; Yuan, Y. A Highly Stereoselective and Scalable Synthesis of l-allo-Enduracididine. *Org. Lett.* **2015**, *17*, 4620−4623.
(13) Jin, K.; Sam, I. H.; Po, K. H. L.; Lin, D.; Zadeh, E. H. G.; Chen, S.; Yuan, Y.; Li, X. Total synthesis of teixobactin. *Nat. Commun.* **2016**, *7*, 12394.
(14) Giltrap, A. M.; Dowman, L. J.; Nagalingam, G.; Ochoa, J. L.; Linington, R. G.; Britton, W. J.; Payne, R. J. Total Synthesis of Teixobactin. *Org. Lett.* **2016**, *18*, 2788−2791.
(15) Yang, H.; Chen, K. H.; Nowick, J. S. Elucidation of the Teixobactin Pharmacophore. *ACS Chem. Biol.* **2016**, *11*, 1823−1826.
(16) Huang, Y.-H.; Colgrave, M. L.; Clark, R. J.; Kotze, A. C.; Craik, D. J. Lysine-scanning mutagenesis reveals an amendable face of the cyclotide kalata B1 for the optimization of nematocidal activity. *J. Biol. Chem.* **2010**, *285*, 10797−10805.
(17) Cunningham, B. C.; Wells, J. A. High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis. *Science* **1989**, *244*, 1081−1085.
(18) Morrison, K. L.; Weiss, G. A. Combinatorial alanine-scanning. *Curr. Opin. Chem. Biol.* **2001**, *5*, 302−307.
(19) Ramesh, S.; Govender, T.; Kruger, H. G.; de la Torre, B. G.; Albericio, F. Short antimicrobial peptides (SAMPs) as a class of extraordinary promising therapeutic agents. *J. Pept. Sci.* **2016**, *22*, 438−451.

DOI: 10.1021/acsomega.6b00354
ACS Omega 2016, 1, 1262−1265