OL Organic Letters

pubs.acs.org/OrgLett Letter

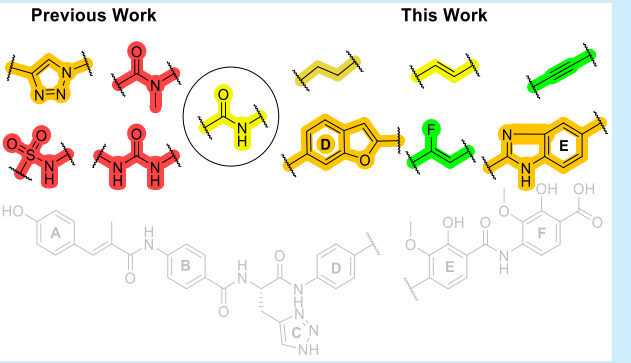
Overcoming AlbD Protease Resistance and Improving Potency: Synthesis and Bioactivity of Antibacterial Albicidin Analogues with Amide Bond Isosteres

Leonardo Kleebauer, Lieby Zborovsky, Kay Hommernick, Maria Seidel, John B. Weston, and Roderich D. Süssmuth*

Cite This: https://doi.org/10.1021/acs.orglett.1c02312 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Albicidin is a potent antibacterial oligoaromatic peptide that is susceptible to the protease AlbD, a resistance factor. This potentially restricts the use of albicidin as a drug. To overcome this obstacle, we synthesized and evaluated six analogues with isosteric replacement of the key amide link. Protease stability was established while maintaining the antibacterial activity, including three analogues with up to eight times higher activity compared with the natural albicidin.



The development of antibacterial resistance toward medically used antibiotics is responsible for the emergence of a life-threatening health crisis. At the same time, big pharmaceutical companies are dropping out of antimicrobial research due to its low profitability.[1] Thus there is an urgent need for novel highly potent, antibiotic therapeutics.[2] The natural product albicidin produced by *Xanthomonas albilineans* was identified but not characterized in 1985.[3] It is an oligoaromatic peptide (Figure 1) that shows remarkable bioactivity against not only G+ve but also G−ve pathogens, with bacterial DNA gyrase as a target. Both the structure elucidation[4] and the first total synthesis were accomplished by our group.[5] This led to the reporting of a number of albicidin structure−activity relationship (SAR) studies describing building block replacements.[6,7]

Albicidin (**1**) is naturally synthesized according to a nonribosomal biosynthesis mechanism and has an unusual structure (Figure 1). It consists of six buildings blocks (A: methylcoumaric acid (MCA), B: *para*-aminobenzoic acid (*p*ABA1), C: L-cyanoalanine (Cya), D: *para*-aminobenzoic acid (*p*ABA2), and E and F: *para*-amino-2-hydroxy-3-methoxybenzoic acid (*p*HMBA1 and *p*HMBA2)) which are linked by five peptide bonds. Our lead structure aza-His albicidin (**2**) with the replacement of Cya by aza-Histidine has an optimized antibacterial profile and a higher chemical stability (Figure 1).

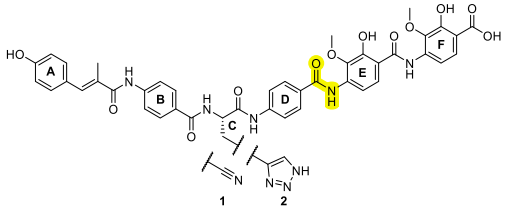


Figure 1. Structure of albicidin (**1**) and aza-His albicidin (**2**) and the assignment of building blocks A−F.

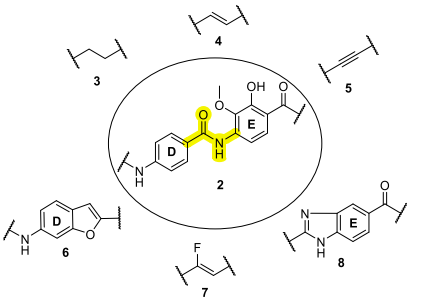
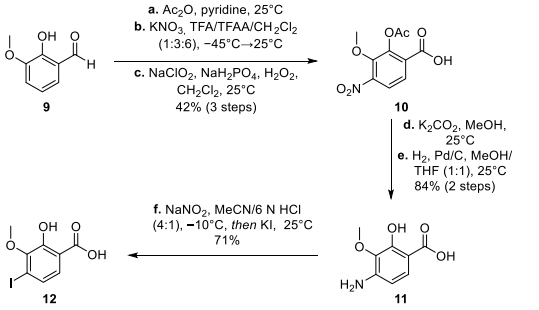


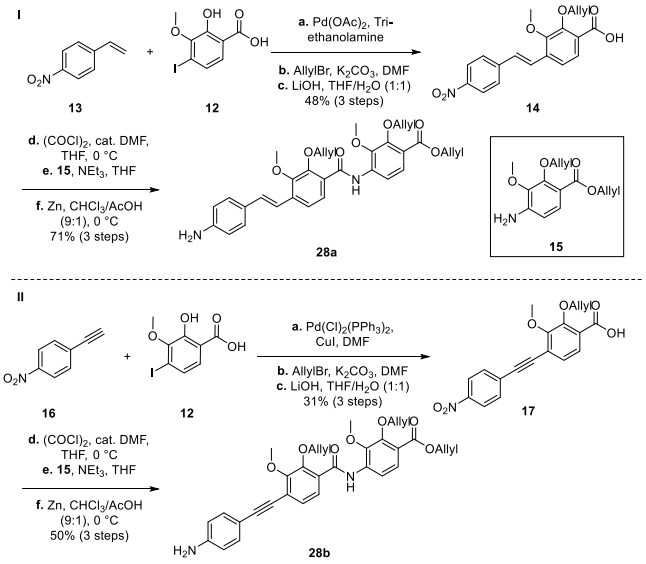
Figure 2. Lead structure (**2**) and targeted D−E isosteres (**3**−**8**).

Received: July 10, 2021

Scheme 1. Synthesis of the Key Building Block 12 for the Synthesis of Albicidin Analogues 3−7



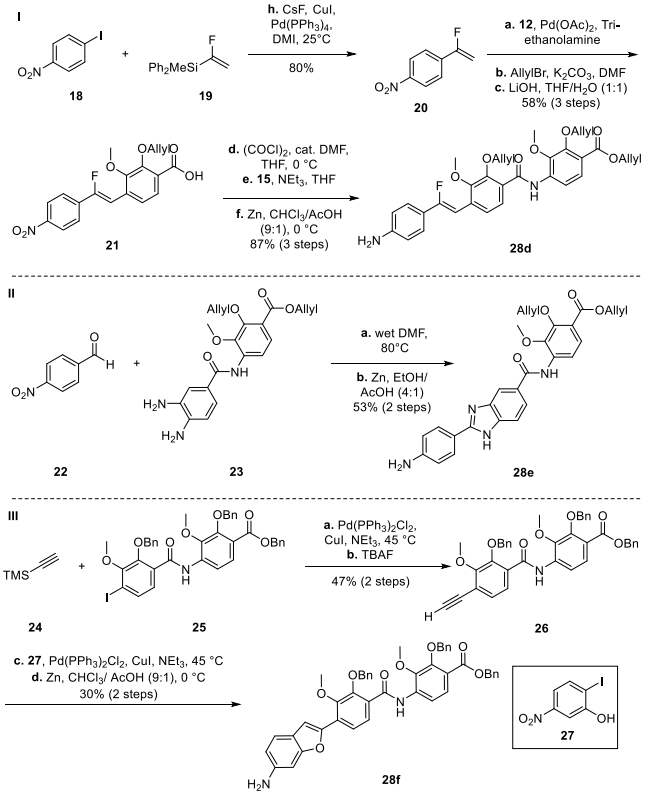
Scheme 2. Palladium-Mediated Cross-Couplings as the Key Steps toward the D−E−F Fragments (28a and 28b)



A number of albicidin resistance factors have been characterized. These include the endopeptidase AlbD from *Pantoea dispersa*.[8] The peptidase specifically cleaves between the D and E building blocks, leading to a complete loss of activity (Figure 1). Structural optimization of albicidin derivatives is one way to overcome these resistance factors. Previously, we synthesized four analogues with isosteric D−E amide replacements (triazole, *N*-methyl amide, urea, and sulfonamide) and tested them against AlbD. Cleavage induced by AlbD could be prevented in all cases (except for the *N*-methylated amide) but mostly at the expense of antibiotic activity measured by minimal inhibitory concentration (MIC).[6] Only the triazole isostere showed an acceptable antibacterial performance (MIC was decreased by approximately two- to four-fold). To prevent cleavage of the amide bond located between building blocks D and E by AlbD and ideally to retain or even to increase the potency, new isosteric replacements were needed. The challenge was to identify new isosteres, either mimicking the amide bond more accurately with regard to steric and electronic properties or abandoning unnecessary structural features while maintaining the antibacterial activity.

We carried out the synthesis of six derivatives with amide bond replacements between building blocks D and E. The critical feature was the stability toward chemical or enzymatic hydrolysis, thus disfavoring esters while maintaining the steric requirement of the peptide motif as much as possible and the

Scheme 3. Synthesis of the D−E−F Fragments 28d−f



overall conformational requirements of the albicidin structure (Figure 1, **1**). Hence, ethane (**3**), ethene (**4**), and ethyne (**5**) linkages connecting building blocks D and E were chosen to investigate the bond flexibility or rotatability. The (*Z*)-fluoroalkene (**7**) seems particularly attractive, as the introduction of the fluorine provides geometrical similarities to an amide link. For example, for an amide, the C═O bond is 1.23 Å and the C−N bond is 1.37 Å, and the C−F bond is 1.38 Å and the C═C bond is 1.33 Å.[9] Furthermore, the fluorine conserves the electron-withdrawing effect of the oxygen of the amide link and polarizes the group to provide weak H-bond acceptor properties similar to those of the amide bond with a dipole moment of 1.4 D (3.6 D in case of an amide).[10] Derivatives (**6**, **8**) (Figure 2) are more unusual analogues that limit the conformational flexibility by ring formation.[11] An additional challenge was that the synthesis strategy had to be individually adapted to the synthesis of each of the analogues. We chose a strategy beginning with the preparation of the individual D−E isosteric fragments.

The best and most flexible synthetic strategy for amide bond isosteres **3**−**5** and **7** (Figure 2) seemed to be the establishment of the isosteric bond from the same preformed E building block followed by coupling to different D building blocks rather than the converse and keeping the D building block constant. Thus the synthesis of 4-iodo-3-methoxy-2-hydroxybenzoic acid (Scheme 1, **12**) for building block E was achieved in six steps, starting from *o*-vanillin (Scheme 1). The starting material *o*-vanillin **9** was acetylated, allowing a more selective nitration at the para position. A Pinnick oxidation followed to give carboxylic acid (**10**). Cleavage of the acetyl group and reduction with palladium on activated charcoal afforded 4-amino-3-methoxy-2-hydroxy benzoic acid (**11**). The key diazotisation step was followed by iodination to provide the tetrasubstituted 4-iodo-3-methoxy-2-hydroxybenzoic acid (**12**) in good yield.

https://doi.org/10.1021/acs.orglett.1c02312
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of Albicidin Isosteres (3−8) from D−E−F Fragments (28a−f)

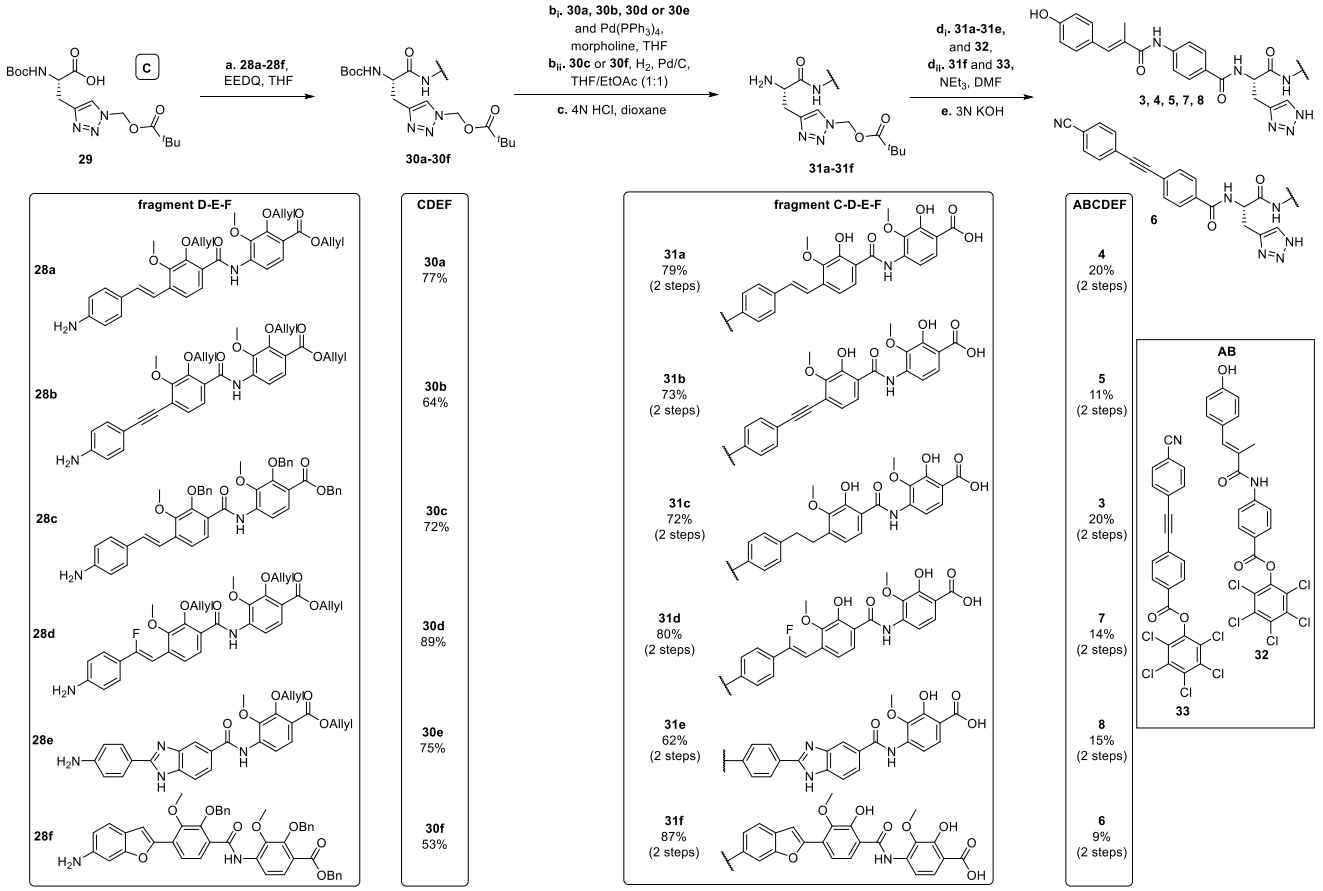
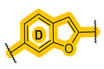
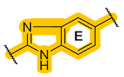


Table 1. Inhibition of *E. coli* DNA Gyrase, Antibacterial Activity, and Activity against *E. coli* DSM 1116 in the Presence of AlbD of Synthetic Isosteres 3−8[a]

bacterial strain									
	2*	**34***	**35***	**3**	**4**	**5**	**6**	**7**	**8**
E. coli BW25113	*0.063*	*0.125*	*8.0*	*0.25*	*0.125*	*0.063*	*1.0*	*0.063*	*0.25*
S. typhimurium TA100	*0.063*	*0.125*	*0.50*	*0.25*	*0.063*	*≤0.016*	*0.125*	*0.031*	*0.063*
B. subtilis DSM10	*0.25*	*4.0*	*8.0*	*0.063*	*0.063*	*0.063*	*0.25*	*0.031*	*2.0*
M. phlei DSM*750*	*2.0*	*8.0*	*8.0*	*1.0*	*0.50*	*0.50*	*1.0*	*0.25*	*8.0*
AlbD assay	✗	√	√	√	√	√	*n.d.*	√	√
E. coli gyrase inhibtion	*n.d.*	↓	↓	≈	↑	↑	↓	↑	↓

[a]Reference compounds **2**, **34**, and **35** are marked with *. MIC values are given in μg/mL: ≤0.031, dark green; 0.063−0.250, light green; 0.5−1, yellow; 2−4, light red; ≥8, red. Activity in the presence of AlbD: ×, inactive; √, active. Gyrase inhibition at a constant concentration of 45 nM of compounds **1** and **2**−**8**: ↑, for stronger inhibition; ≈, for equal inhibition; ↓, for diminished inhibition compared with albicidin **1**.[6]

To prepare albicidins **3** (alkane) and **4** (alkene), respectively, 4-nitro styrene (Scheme 2, **13**) was reacted with the unprotected tetrasubstituted 2-hydroxy-4-iodo-3-methoxybenzoic acid (**12**) in a trans-selective Heck reaction. Protecting groups for the free phenol and carboxylic acid (benzyl, *tert*-butyl, MOM, or *p*-methoxybenzyl (PMB)) were not suitable for reasons of the required stability and orthogonality. Finally, we used allyl protecting groups that could be used only after the Heck reaction to avoid cross-coupling with the allyl groups. After protection of both the hydroxy group and the carboxylic acid, the acid was then deprotected with LiOH in tetrahydrofuran (THF)/water to give **14**.

For the peptide coupling of the D−E fragments with the F fragment **15**, mild reaction conditions were necessary to avoid

https://doi.org/10.1021/acs.orglett.1c02312
Org. Lett. XXXX, XXX, XXX−XXX

isomerization of the C=C double bond. Therefore, coupling was performed with oxalyl chloride, and the resulting pseudotripeptide was reduced with zinc powder to afford aniline **28a**.

Next, we focused on the D−E−F fragment of the alkyne **5** (Scheme 2, II). A Pd−Cu-catalyzed Sonogashira coupling instead of the Heck reaction allowed us to access the D−E alkyne fragment **17**. Using the same protection/deprotection and coupling steps as those for **28a** afforded alkyne **28b**.

For the albicidin alkane isostere **3**, benzyl instead of allyl protecting groups were used. This facilitated a one-step hydrogenation of the alkene (Scheme 4, **30c**) to give the alkane together with concurrent deprotection of the benzyl groups to give the C−D−E−F fragment (Scheme 4, **31c**).

Fluoroalkene **7** was then prepared. Compound **20** (Scheme 3, I) was synthesized from 1-iodo-4-nitrobenzene (**18**) and (1-fluorovinyl)(methyl)diphenylsilane (**19**) by a cesium-fluoride-assisted Cu−Pd-catalyzed reaction.[12]

Next, we synthesized the cyclic isosteres. The benzimidazole tripeptide mimic **28e** was prepared from nitrobenzaldehyde **22** (Scheme 3, II) and dianiline **23** by annulation followed by reduction of the nitro group. For the benzofuran cyclic isostere, a ring fusion to the D fragment (Scheme 3, III, **28f**), we prepared the aryl iodide dipeptide **25** in a nine-step synthesis. (See the SI.) (Trimethylsilanyl)-acetylene (**24**) reacted with dipeptide **25** (Scheme 4) by Sonogashira cross-coupling to provide acetylene **26** after a trimethylsilyl (TMS) deprotection with tetra-*n*-butylammonium fluoride (TBAF). The Cu−Pd-catalyzed reaction of iodophenol **27** with **26** has been previously described.[13] After the reduction, we obtained aminobenzofuran **28f**.

The final five steps were similar for all six D−E−F fragments (Scheme 4, **28a−f**) and were carried out as follows: *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), a coupling agent known for its low rates of racemization[14,15] and successful application in a former albicidin synthesis,[16] was used to couple the D−E−F fragment to the enantiopure C building block (**29**). This was followed by a deprotection of first, the allyl groups (benzyl groups for **30c** and **30f**) and second, the Boc group. Employing a strategy we had used previously, the AB-PCP ester **32** (Scheme 4) was used for the late-stage coupling step. Finally, the pivaloyloxymethyl (POM) protecting group on the C fragment was cleaved under basic conditions. This route worked for five of the six derivatives. When we tried to couple **31f** to the AB-PCP (**32**), no desired product was formed. So, we used AB-PCP ester **33** (see the SI) as an alternative highly potent AB motif.[7] Target compounds were purified by reverse-phase high-performance liquid chromatography (HPLC).

The MIC values, gyrase inhibition, and activity in the presence of AlbD in an agar diffusion assay were determined for all six compounds (Table 1).[8] All six novel derivatives maintained their activity in the presence of AlbD (Figure S1, Supporting Information) and showed overall an improvement in antibacterial activities when compared with previously reported isosteres bearing a triazole (**34**) or a sulfonamide (**35**) (**34**, **35**: Table 1 and Figure S3, Supporting Information)[6] and also when compared with the lead compound aza-His albicidin (**2**). Parent compound **2** is highly active against G−ve *E. coli* and *S. typhimurium*, whereas isosteres **3**, **4**, and **8** are equally or up to 4 times less active and isostere **6** is between 2 and 16 times less active. Interestingly, isosteres **5** and **7** showed an up to four-fold enhancement (compared with **2**) in the activity against G−ve bacteria. Furthermore, almost all isosteres **3**−**7** show an increased activity (up to 4-fold) against G+ve *B. subtilis* and *Mycobacterium phlei*, with only the more polar benzimidazole isostere **8** (HPLC R_T = 6.58 min, Table S1) showing lower activity (up to eight-fold). The inhibition of the *E. coli* gyrase of **4**, **5**, and **7** is stronger than that for albicidin (**1**) and correlates with the observed antibacterial activity (Table S1 Figure S2, Supporting Information).

Regarding the structural features of the isosteres, it appears that fused ring analogs[17] restricting the conformational flexibility are unfavorable. Furthermore, the D−E link consisting of highly conformationally flexible sp^3 carbons (**3**) shows that very high conformational flexibility is not preferable. However, both, the (*Z*)-fluoroalkene (**7**) and the alkyne derivative (**5**) showed an enhanced activity compared with the parent structure (**2**). The (*E*)-alkene (**4**) was less active than the (*Z*)-fluoroalkene (**7**), probably because the (*Z*)-fluoroalkene is more similar to an amide (see previous discussion). Remarkably, alkyne **5** is a good isostere of an amide bond.[18] Apparently, the linearity and stiffness of the D−E moiety due to the sp-hybridized carbons of **5** (180° bond angle) permits good bioactivity, despite a poorer overlay with the parent compound **2**. Another advantage of the alkyne structure could be the impossibility for cis−trans isomerization, observable for alkenes, as shown for coumaric acid (building block A), which is detrimental for antibacterial activity.[6,17]

In summary, we have shown that replacement of the D−E amide bond of albicidin resists protease AlbD cleavage, and at the same time, potency against both G+ve and G−ve bacteria can be increased. Our study on amide bond replacements encourages the application of these isosteres to replace not only other amides of albicidin or related cystobactamide structures[19−21] but also other peptide structures or aromatic oligoamides.[22,23] Whereas the (*Z*)-fluoroalkene (**7**) is a state-of-the-art well-known isostere,[24] to our knowledge, we report one of the very few examples[7] of an alkyne (**5**) considered to act as an amide isostere.[9] This Letter is a further important step in understanding the SARs of albicidin. With the compounds **5** and **7**, we report two albicidin candidates for future preclinical evaluation on the path to resolve the antibiotic resistance crises.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c02312.

General procedures, characterization of new compounds, copies of NMR spectra, AlbD cleavage, and MIC assays (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Roderich D. Süssmuth − *Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany;* orcid.org/0000-0001-7027-2069; Email: Suessmuth@chem.tu-berlin.de

Authors

Leonardo Kleebauer − *Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany*

Lieby Zborovsky − *Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany*

Kay Hommernick − *Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany*

https://doi.org/10.1021/acs.orglett.1c02312
Org. Lett. XXXX, XXX, XXX−XXX

Maria Seidel − *Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany*
John B. Weston − *Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.1c02312

Author Contributions

L.K. designed and synthesized **3**−**7**, analyzed the data, and wrote the manuscript. L.Z. designed and synthesized **8**. M.S. was responsible for biological testing. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge financial support granted by the Deutsche Forschungsgemeinschaft (DFG, SU 239/11-1, PE 2600/1-1, and RTG 2473 "Bioactive Peptides") (L.K. and K.H.) and the Alexander-von-Humboldt-Foundation (L.Z.). We also thank Dr. Andi Mainz (TU Berlin) for valuable discussions, Dr. Simone Kosol (TU Berlin) for AlbD purification, and Marcello Spinczyk (TU Berlin) and Marcel Kulike (TU Berlin) for their contribution in the laboratory. Lastly, we thank Marc Griffel (TU Berlin) and Arnar Sigurdsson (TU Berlin) for HPLC-MS measurements.

■ REFERENCES

(1) Plackett, B. Why big pharma has abandoned antibiotics. *Nature* **2020**, *586*, S50−S52.
(2) Martens, E.; Demain, A. L. The antibiotic resistance crisis, with a focus on the United States. *J. Antibiot.* **2017**, *70*, 520−526.
(3) BIRCH, R. G.; PATIL, S. S. Preliminary Characterization of an Antibiotic Produced by Xanthomonas albilineans Which Inhibits DNA Synthesis in Escherichia coli. *Microbiology* **1985**, *131*, 1069−1075.
(4) Cociancich, S.; Pesic, A.; Petras, D.; Uhlmann, S.; Kretz, J.; Schubert, V.; Vieweg, L.; Duplan, S.; Marguerettaz, M.; Noëll, J.; Pieretti, I.; Hügelland, M.; Kemper, S.; Mainz, A.; Rott, P.; Royer, M.; Süssmuth, R. D. The gyrase inhibitor albicidin consists of p-aminobenzoic acids and cyanoalanine. *Nat. Chem. Biol.* **2015**, *11*, 195−197.
(5) Kretz, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. D. Total Synthesis of Albicidin: A Lead Structure fromXanthomonas albilineansfor Potent Antibacterial Gyrase Inhibitors. *Angew. Chem., Int. Ed.* **2015**, *54*, 1969−1973.
(6) Behroz, I.; Durkin, P.; Gratz, S.; Seidel, M.; Rostock, L.; Spinczyk, M.; Weston, J. B.; Sussmuth, R. D. Extensive Structure-Activity Relationship Study of Albicidin's C-Terminal Dipeptidic p-Aminobenzoic Acid Moiety. *Chem. - Eur. J.* **2019**, *25*, 16538.
(7) Behroz, I.; Kleebauer, L.; Hommernick, K.; Seidel, M.; Grätz, S.; Mainz, A.; Weston, J. B.; Süssmuth, R. Acetylenic Replacement of Albicidin's Methacrylamide Residue Circumvents Detrimental (E)-(Z)-Photoisomerization and Preserves Antibacterial Activity. *Chem. - Eur. J.* **2021**, *27*, 9077.
(8) Vieweg, L.; Kretz, J.; Pesic, A.; Kerwat, D.; Grätz, S.; Royer, M.; Cociancich, S.; Mainz, A.; Süssmuth, R. D. The Albicidin Resistance Factor AlbD Is a Serine Endopeptidase That Hydrolyzes Unusual Oligoaromatic-Type Peptides. *J. Am. Chem. Soc.* **2015**, *137*, 7608−7611.
(9) Kumari, S.; Carmona, A. V.; Tiwari, A. K.; Trippier, P. C. Amide Bond Bioisosteres: Strategies, Synthesis, and Successes. *J. Med. Chem.* **2020**, *63*, 12290−12358.
(10) Meanwell, N. A. Fluorine and Fluorinated Motifs in the Design and Application of Bioisosteres for Drug Design. *J. Med. Chem.* **2018**, *61*, 5822−5880.
(11) Choudhary, A.; Raines, R. T. An Evaluation of Peptide-Bond Isosteres. *ChemBioChem* **2011**, *12*, 1801−1807.
(12) Hanamoto, T.; Kobayashi, T. Cross-Coupling Reactions of (1-Fluorovinyl)methydiphenylsilane1with Aryl Halides and Aryl Triflates. *J. Org. Chem.* **2003**, *68*, 6354−6359.
(13) Sarkar, D.; Melkonyan, F. S.; Gulevich, A. V.; Gevorgyan, V. Twofold Unsymmetrical C-H Functionalization of PyrDipSi-Substituted Arenes: A General Method for the Synthesis of Substituted meta-Halophenols. *Angew. Chem., Int. Ed.* **2013**, *52*, 10800−10804.
(14) Belleau, B.; Malek, G. New convenient reagent for peptide syntheses. *J. Am. Chem. Soc.* **1968**, *90*, 1651−1652.
(15) McDermott, J. R.; Benoiton, N. L. N-Methylamino Acids in Peptide Synthesis. IV. Racemization and Yields in Peptide-bond Formation. *Can. J. Chem.* **1973**, *51*, 2562−2570.
(16) von Eckardstein, L.; Petras, D.; Dang, T.; Cociancich, S.; Sabri, S.; Grätz, S.; Kerwat, D.; Seidel, M.; Pesic, A.; Dorrestein, P. C.; Royer, M.; Weston, J. B.; Süssmuth, R. D. Total Synthesis and Biological Assessment of Novel Albicidins Discovered by Mass Spectrometric Networking. *Chem. - Eur. J.* **2017**, *23*, 15316−15321.
(17) Wipf, P. S.; Erin, M.; Mann, A. Conformational Restriction and Steric Hindrance in Medicinal Chemistry. *The Practice of Medicinal Chemistry* **2015**, 279−297.
(18) Meanwell, N. A. Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54*, 2529−2591.
(19) Elgaher, W. A. M.; Hamed, M. M.; Baumann, S.; Herrmann, J.; Siebenbürger, L.; Krull, J.; Cirnski, K.; Kirschning, A.; Brönstrup, M.; Müller, R.; Hartmann, R. W. Cystobactamid 507: Concise Synthesis, Mode of Action, and Optimization toward More Potent Antibiotics. *Chem. - Eur. J.* **2020**, *26*, 7219−7225.
(20) Hüttel, S.; Testolin, G.; Herrmann, J.; Planke, T.; Gille, F.; Moreno, M.; Stadler, M.; Brönstrup, M.; Kirschning, A.; Müller, R. Discovery and Total Synthesis of Natural Cystobactamid Derivatives with Superior Activity against Gram-Negative Pathogens. *Angew. Chem., Int. Ed.* **2017**, *56*, 12760−12764.
(21) Testolin, G.; Cirnski, K.; Rox, K.; Prochnow, H.; Fetz, V.; Grandclaudon, C.; Mollner, T.; Baiyoumy, A.; Ritter, A.; Leitner, C.; Krull, J.; Van Den Heuvel, J.; Vassort, A.; Sordello, S.; Hamed, M. M.; Elgaher, W. A. M.; Herrmann, J.; Hartmann, R. W.; Müller, R.; Brönstrup, M. Synthetic studies of cystobactamids as antibiotics and bacterial imaging carriers lead to compounds with high in vivo efficacy. *Chemical Science* **2020**, *11*, 1316−1334.
(22) Arrata, I.; Grison, C. M.; Coubrough, H. M.; Prabhakaran, P.; Little, M. A.; Tomlinson, D. C.; Webb, M. E.; Wilson, A. J. Control of conformation in α-helix mimicking aromatic oligoamide foldamers through interactions between adjacent side-chains. *Org. Biomol. Chem.* **2019**, *17*, 3861−3867.
(23) Huc, I. Aromatic Oligoamide Foldamers. *Eur. J. Org. Chem.* **2004**, *2004*, 17−29.
(24) Karad, S. N.; Pal, M.; Crowley, R. S.; Prisinzano, T. E.; Altman, R. A. Synthesis and Opioid Activity of Tyr1-ψ[(Z)CF = CH]-Gly2 and Tyr1-ψ[(S)/(R)-CF3CH-NH]-Gly2Leu-enkephalin Fluorinated Peptidomimetics. *ChemMedChem* **2017**, *12*, 571−576.

https://doi.org/10.1021/acs.orglett.1c02312
Org. Lett. XXXX, XXX, XXX−XXX