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Dihydropyrimidinones—A New Class of Anti-Staphylococcal Antibiotics

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Abstract—We report the synthesis and pharmacological evaluation of new derivatives of natural dipeptide antibiotic TAN-1057 A, B. In the course of this program, we identified novel analogues of the natural product that display similar antibacterial activity and showed improved tolerability.

Introduction

Staphylococcus aureus is the most prominent nosocomial pathogen causing severe infections such as sepsis, pneumonia, or endocarditis. The appearance of multi-resistant staphylococci (MRSA)[1] increases the need for novel antibiotics which effectively combat these resistant pathogens.

For the natural dipeptide antibiotic TAN-1057 A, B (**1**) (Fig. 1) excellent Minimal Inhibitory Concentrations (MIC) against staphylococci including MRSA were reported.[2] However, TAN-1057 A, B suffers from toxic side effects (LD_{50} in the mouse: 50 mg/kg, ip) which in our view is prohibitive for the therapeutic use in humans.

We initiated a chemical optimization program[3] with the goal to identify novel analogues of the natural product which exhibit good anti-staphylococcal activity and possess a favorable toxicological profile.

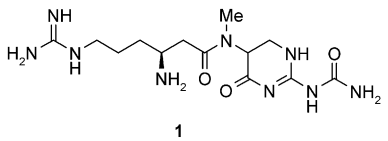

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Figure 1. Natural dipeptide antibiotic TAN-1057 A, B.

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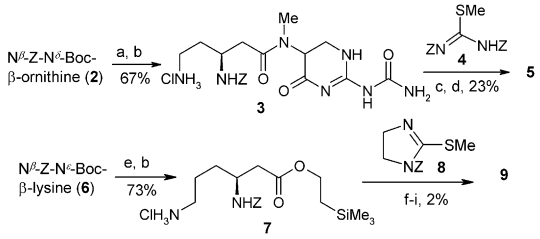
In this communication, we summarize the synthesis and pharmacology of new TAN-1057 A, B analogues focusing on alterations of the β-amino-acid side chain.

Chemistry

At the outset of our program, we studied the influence of the terminal guanidine moiety and its linkage to the β-amino acid on the SAR. Thus, our first set of analogues was structurally closely related to the natural product, bearing functionalized guanidine or amidine moieties differently tethered to the amino acid side chain.

We accessed β-arginine derivative **5** (Scheme 1, Table 1) starting from commercially available BOC-*Z*-β-ornithine



Scheme 1. Synthesis of **5** and **9**, reagents and conditions: (a) EDC, HOBt, DMF, TAN-heterocycle; (b) HCl, dioxane; (c) **4**, $HgCl_2$, DMF, Et_3N, 50 °C; (d) $PdCl_2$, H_2, MeOH; (e) DIC, DMAP, CH_2Cl_2, 2-$SiMe_3$-ethanol; (f) **8**, $HgCl_2$, DMF, DIPEA, 60 °C; (g) TBAF, THF; (h) HATU, DIPEA, DMF, TAN-heterocycle; (i) HBr, acetic acid.

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2[4] which was coupled with the racemic methylamino-dihydropyrimidinone heterocyle (TAN-heterocycle)[5] employing EDC/HOBt. Subsequently, the BOC-group was cleaved off, the resulting primary amine **3** was guanylated using *S*-methylisothiourea **4** as electrophilic component,[6] and the protecting groups were finally removed by hydrogenolysis to yield the β-arginine analogue **5**.

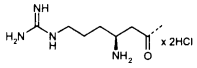
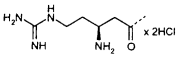
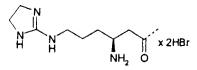
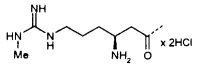
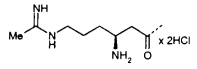
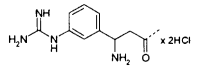
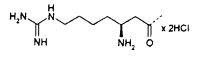
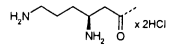
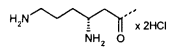
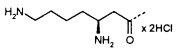
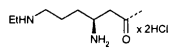
In analogy, the dihydroimidazole derivative **9** (cf. Scheme 1, Table 1) was also obtained via a guanylation protocol. The synthesis of **9** commenced with BOC-Z-β-lysine[4] (**6**) which was transformed into the corresponding trimethylsilylethyl ester followed by BOC-deprotection to give intermediate **7** in 73% yield. The installation of the dihydroindole moiety proved problematic, and optimized conditions led to the *Z*-protected guanidine intermediate in merely 24% yield. The final steps towards **9** included liberation of the carboxylic acid, peptide coupling with the TAN-heterocycle and deprotection to provide **9**, albeit in poor yield.

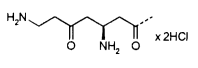
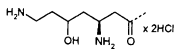
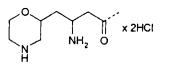
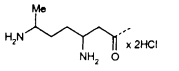
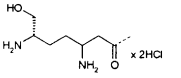
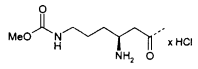
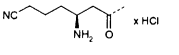
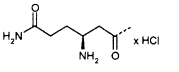
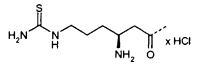
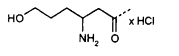
The synthesis of methylated TAN-1057 (**10**) and the amidine analogue **11** as well as phenyl-bridged derivative **12** and homo-TAN-1057 (**13**) (cf. Table 1) have been detailed before.[3d]

The syntheses of β-lysine and β-homolysine analogues **14**–**17** (Table 1) were realized by a convergent synthesis strategy using a peptide coupling to fuse the TAN-heterocycle with the corresponding enantiomerically pure bis-*Z*-protected (*S*)-β-amino acids. Hydrogenolytic cleavage led to the target compounds.[3d]

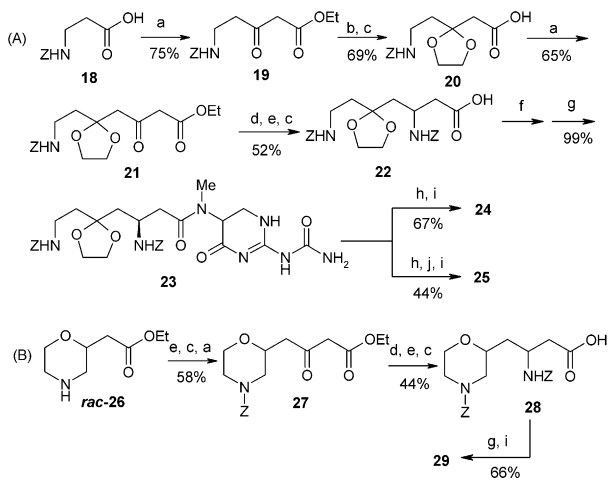
The approach to keto- and hydroxy-substituted β-lysine analogues **24** and **25** is depicted in Scheme 2, path A. Accordingly, *Z*-protected 3-amino propionic acid **18**

Table 1. Pharmacological profile of novel TAN-1057 A, B analogues **1**, **5**, **9**–**17**, **24**, **25**, **29**, **33**, **37**–**40**, **42** and **46**

Compd	Structures	MIC (μg/mL) *S. aureus* 133	IC_{50} (μM), proc. transl.	IC_{50} (μM), euc. transl.	Selectivity index[a]
1[b]		0.10	0.30	0.17	0.57
5[b]		25.0	1.2	1.2	1.0
9[b]		12.5	9.4	9.4	1.0
10[b]		0.80	0.50	4.5	9.1
11[b]		0.40	0.10	0.60	0.17
12[c]		50	0.5	0.26	0.52
13[b]		0.20	0.30	1.6	5.3
14[b]		0.40	2.0	3.7	1.9
15[b]		6.3	6.0	9.4	1.6
16[b]		0.05	0.50	2.8	5.3
17[b]		0.80	4.0	7.6	1.9

Compd	Structures	MIC (μg/mL) *S. aureus* 133	IC_{50} (μM), proc. transl.	IC_{50} (μM), euc. transl.	Selectivity index[a]
24[b]		3.2	3.0	n.d.	n.d.
25[c]		0.40	3.0	3.2	n.d.
29[c]		6.3	1.5	n.d.	n.d.
33[c]		0.40	1.0	1.2	1.2
37[c]		3.2	2.4	2.4	1.0
38[b]		100	>64	n.d.	n.d.
39[b]		50	25	25	1.0
40[b]		12.5	64	n.d.	n.d.
42[b]		0.40	0.20	0.20	1.0
46[c]		100	>64	n.d.	n.d.

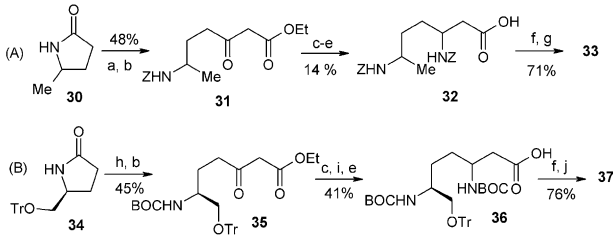
[a]In vitro selectivity index (IC_{50} eucaryotic transl./procaryotic transl.).
[b]Compounds were obtained as a 1:1 mixture of diastereomers.
[c]Compounds were obtained in racemic form as mixtures of diastereomers.



Scheme 2. Synthesis of **24**, **25** and **29**, reagents and conditions: (a) CDI, Mg-ethylmalonate, THF; (b) *p*-toluenesulfonic acid, ethanediol, benzene; (c) CH_2Cl_2, $KOSiMe_3$; (d) NH_3, MeOH, $NaCNBH_3$, HOAc; (e) *Z*-Cl, CH_2Cl_2, Et_3N; (f) separation of enantiomers by chiral HPLC; (g) HATU, DIPEA, DMF, TAN-heterocycle; (h) aq HCl, THF; (i) $PdCl_2$, H_2, MeOH; (j) $NaBH_4$, THF, MeOH, −78 °C, 95%.

was activated with carbonyldiimidazole and reacted with Mg-ethylmalonate to yield ketoester **19** in 75% yield.[7] The keto moiety was protected as a cyclic ketal, the ester was saponified and the resulting carboxylic acid **20** was transferred into the β-ketoester using the above mentioned Mg-malonate method. Subsequently, the amino moiety was installed using a reductive amination protocol followed by Z-protection of the amino group. The ester present in **21** was cleaved and the resulting carboxylic acid **22** was resolved into the single enantiomers employing a chiral HPLC phase. The (*S*)-configurated carboxylic acid was taken further in the sequence. Next, the TAN-heterocycle was introduced by HATU-mediated peptide coupling to yield the fully protected intermediate **23**. Stepwise deprotection led to **24** in 67% yield, while ketal cleavage and $NaBH_4$ reduction followed by removal of the remaining *Z*-groups provided **25** as a mixture of diastereomers in 44% yield.

Racemic morpholine analogue **29** was obtained as follows: **26** was transformed into the β-ketoester **27**, and the amino moiety was introduced by reductive amination as outlined before. Finally, the heterocycle was installed, and the *Z*-groups were removed by hydrogenolysis to give **29** as a mixture of diasteremoers.



Scheme 3. Synthesis of **33** and **37**, reagents and conditions: (a) −78 °C, BuLi, THF, ZCl; (b) −78 °C, LDA, EtOAc; (c) NH_3, MeOH, $NaCNBH_3$, HOAc; (d) K_2CO_3, ZCl, dioxane/H_2O; (e) CH_2Cl_2, $KOSiMe_3$; (f) HATU, DIPEA, DMF, TAN-heterocycle; (g) $PdCl_2$, H_2, MeOH; (h) BOC_2O, DMAP, MeCN; (i) BOC_2O, Na_2CO_3, THF, H_2O; (j) HCl, dioxane.

For the synthesis of branched β-lysine side chains, we elaborated a general route which is depicted in Scheme 3. Accordingly, racemic pyrrolidinone **30** was Z-protected and subsequently treated with the Li-enolate derived from ethyl acetate to furnish **31** in 48% yield (Scheme 4, path A).[8] In close resemblance to the chemistry discussed for Scheme 2, **31** was taken further to the desired product **33**. The synthesis of **37** as outlined in Scheme 3, path B was conducted in close analogy to the synthesis of **33**.

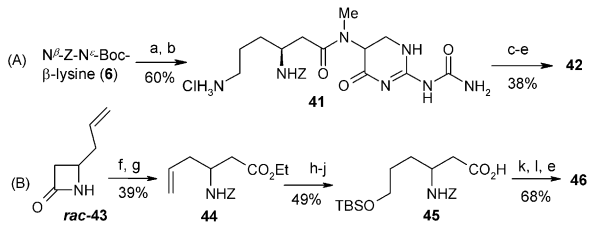
Finally, we synthesized a variety of TAN-1057 derivatives with non-basic end-groups. The constructions of analogues **38**, **39**, and **40** (Table 1) have been reported before,[3d] while the synthesis of thiourea **42** and alcohol **46** is outlined in Scheme 4.

The synthesis of the thiourea derivative **42** commenced with BOC-*Z*-β-lysine (**6**) which was coupled with the TAN-heterocycle followed by removal of the BOC-group to give intermediate **41** in 67% yield for two steps (Scheme 4, path A). The thiourea moiety was installed by reaction of **41** with (FMOC)NCS[9] followed by base-induced cleavage of the FMOC group. Finally, hydrogenolytic deprotection gave rise to the formation of **42**.

For the construction of alcohol **46**, we envisioned a route involving the allyl-substituted β-lactam **43**[10] as starting material (Scheme 4, path B). Thus, racemic azetidinone **43** was opened under acidic conditions, and the resulting β-amino moiety was Z-protected to yield **44** in 39%. Hydroboration, TBS-protection and saponification led to carboxylic acid **45** which was then taken further to **46** as outlined in Scheme 4.

Pharmacology

The antibacterial as well as the cytotoxic properties of TAN-1057 A, B and its derivatives originate from an inhibition of the translation machinery.[2a,3e] Therefore, we determined the in vitro inhibition of the procaryotic- and eucaryotic translation using biochemical translation assays.[11] The therapeutic index was defined as the ratio between the eucaryotic and the procaryotic translation data. We consider this index as a helpful tool for the preliminary assessment of the toxicological profile of the new derivatives. As a whole cell system



Scheme 4. Synthesis of **42** and **46**, reagents and conditions: (a) EDC, HOBt, DMF, TAN-heterocycle; (b) HCl, dioxane; (c) (FMOC)NCS, DIPEA, CH_2Cl_2; (d) piperidine, DMF; (e) $PdCl_2$, H_2, MeOH; (f) HCl, EtOH; (g) ZCl, NEt3, CH_2Cl_2; (h) 9-BBN, THF then H_2O_2, Na_2CO_3; (i) TBSCl, imidazole, DMF; (j) $KOSiMe_3$, CH_2Cl_2; (k) HATU, DIPEA, DMF, TAN-heterocycle; (l) HOAc, THF, H_2O.

we selected a standard MIC assay against *S. aureus 133*. The pharmacological results are summarized in Table 1.

Not surprisingly, for the natural product **1** we found a selectivity towards the eucaryotic translation underscoring the toxic potential of TAN-1057 A, B (selectivity index: 0.57). In turn, some of the closest analogues revealed a more promising pharmacological profile. In particular, methylated guanidine **10**, homo-TAN-1057 **13** and amidine **11** show a significantly improved therapeutic index indicating that these derivatives should be less toxic. At the same time, analogues **10**, **11** and **13** display, compared to the natural product, similar activity in both the procaryotic translation and MIC assay.

In contrast, the antibacterial activity drops dramatically for the β-arginine derivative **5**, for the modified β-homoarginine **9** and for the phenyl-bridged derivative **12**. Additionally, these analogues showed no favorable selectivity index.

As we can further conclude from Table 1, the guanidine present in the natural product can be replaced by primary or secondary amines with retention of the anti-staphylococcal activity (**14**–**37**). The most active molecules in this series contain a β-lysine (**14**) or a β-homolysine (**16**) side chain and small substituents such as methyl (**33**), *N*-ethyl (**17**), or hydroxy (**25**) are well tolerated. More expansive substitutions of the β-lysine moiety as found in **29** or **37** led to less active compounds. Furthermore, the antibacterial activity proved sensitive to the absolute configuration of the β-amino stereocenter since the (*R*)-configurated analogue **15** was by a factor of 15 less active than its (*S*)-counterpart **14**.

Surprisingly, most of the active amino derivatives show a favorable selectivity index. This is especially true for compounds **14**, **16** and **17**. Moreover, these analogues exhibit promising MIC values ranging from 0.04 μg/mL to 0.8 μg/mL.

Finally, we collected pharmacological data for analogues with non-basic end groups. Replacement of the basic guanidine in TAN-1057 A, B by a carbamate (**38**), nitrile (**39**), primary amide (**40**), or alcohol (**46**) resulted in a dramatic loss of activity while thiourea **42** as the only non-basic functionality displayed a promising MIC of 0.4 μg/mL. However, for **42** we observed no selectivity in the translation assays indicating that the thiourea might have a toxic potential.

From the data in Table 1 we can deduce a recipe for the construction of active TAN-1057 A, B analogues which show favorable in vitro selectivity indices: the (*S*)-configurated β-amino-acid fragment has to be linked via a C_3–C_4 spacer to a basic end-group which preferably consists of a methylated guanidine, an amidine or an amino group. Small substitutions such as methyl, oxo or hydroxy are well tolerated in the side chain.

Finally, we selected three of the most promising candidates from Table 1 for further pharmacological studies.

Table 2. Cytotoxicity and in vivo activity for compounds **1**, **10**, **14** and **16**

Compd	EC_{50} (μM) Cytotoxicity	ED_{100} (mg/kg) *S. aureus* 133
1	0.25	0.25
10	5.50	2.0
14	25.0	0.20
16	6.0	0.25

As depicted in Table 2, we collected EC_{50} values for cytotoxicity using a macrophage cell line (J774) which has proven to be predictive for systemic toxicity of inhibitors of the protein biosynthesis. As we can conclude from Table 2, our analogues **10**, **14** and **16** were at least 20-fold less cytotoxic than the natural product. Additionally, all compounds in Table 2 show excellent ED_{100} values (murine sepsis model, route: iv) ranging between 0.2 and 2.0 mg/kg. Thus, compounds **10**, **14** and **16** show similar efficacies as TAN-1057 A, B against *S. aureus 133* combined with a significantly improved tolerability.

Summary

In summary, our chemistry program provided novel analogues of TAN-1057 A,B which showed improved tolerability compared to the natural product with concomitant retention of the excellent anti-staphylococcal activity.

Acknowledgements

We thank F.-U. Geschke, D. Häbich and H. Labischinski for helpful discussions.

References and Notes

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