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Pyrimidinone Antibiotics—Heterocyclic Analogues with Improved Antibacterial Spectrum

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Abstract—We report the synthesis and pharmacological evaluation of new derivatives of the natural dipeptide antibiotic TAN 1057 A,B containing heterocycles either in the β-amino acid side chain or as mimics of the urea function. In the course of this program, we identified novel analogues that display activity towards a broader panel of Gram-positive bacteriae.

Introduction

The appearance of resistant pathogens such as *M*ulti-*R*esistant *S*taphylococcus *A*ureus (MRSA) as well as *V*ancomycin *R*esistant *E*nterococcus Faecium (VRE) constitutes a severe problem particularly in hospital intensive care units. It is generally agreed that the introduction of novel antibiotic classes would contribute to the combat of the ever lasting problem of bacterial resistance.[1] However, despite world wide efforts, only one new antibacterial class (oxazolidinones, namely linezolide) has reached the market within the last 15 years.

In search of promising lead structures for the synthesis of new antibacterials, we have focused our attention on unexplored natural products with reported antibacterial activity. For the natural dipeptide antibiotic TAN 1057 A,B (**1**) (Fig. 1) excellent *M*inimal *I*nhibitory *C*oncentrations (MIC) against staphylococci including MRSA were reported.[2] Two independent total syntheses of the natural compound[3] and some analogues were published recently.[4] In particular the β-lysine and β-homolysine derivatives **2** and **3** were found to exhibit interesting properties such as improved tolerability.[4c]

Further optimisation was aimed towards expanding the limited antibacterial spectrum of TAN 1057 A,B to cover in particular *Staphylococcus pneumoniae* subtypes.

In this communication we summarise the synthesis and antibacterial properties of new TAN 1057 A,B analogues focusing in particular on the introduction of heterocycles into the β-amino acid side chain as well as in the urea part of the TAN 1057 A,B heterocycle.

Chemistry

Some examples of α-amino acids containing constrained basic side chains have been reported,[5] but no precedents are known for β-amino acids. We now report the synthesis of novel enantiomerically pure analogues of β-homolysine ($n = 1$) and β-bishomolysine ($n = 2$) which contain a thiazole ring (**9**, **10** in Scheme 1), as well as the synthesis of a racemic β-homolysine derivative **17** containing an isoxazol ring (Scheme 2).

Key synthetic intermediate for the formation of thiazoles **9**/**10** (Scheme 1) is the α-bromo ketone **6** derived

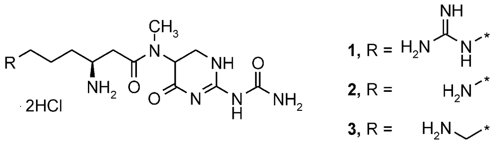

Figure 1. Natural dipeptide antibiotic TAN 1057 A,B (**1**) and synthetic analogues.

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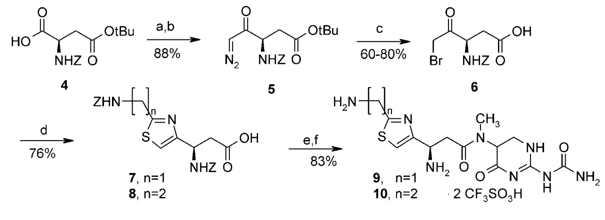
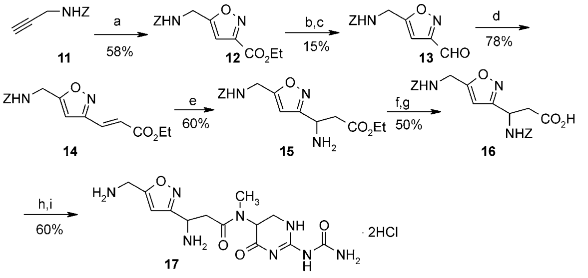
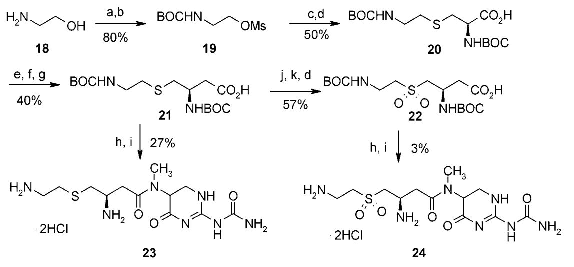


Scheme 1. Synthesis of **9** and **10**, reagents and conditions (yields are comparable for $n=1$ and 2): (a) *i*BuOC(O)Cl, DIPEA, CH_2Cl_2, $-20\,°C$; (b) CH_2N_2; (c) HBr, AcOH; (d) *N*-*Z*-glycine thioamide or *N*-*Z*-β-alanine thioamide, DMF, 100 °C; (e) HATU, DIPEA, DMF, TAN-heterocycle; (f) CF_3SO_3H, anisole, CH_2Cl_2.

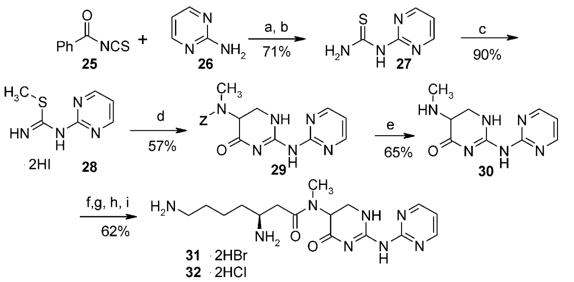


Scheme 2. Synthesis of **17**, reagents and conditions: (a) $NO_2CH_2CO_2Et$, PhNCO, Et_3N, benzene, reflux; (b) DIBAL, CH_2Cl_2, −78 °C; (c) DMSO, $(COCl)_2$, Et_3N, −78 °C; (d) $EtO_2(O)PCH_2CO_2Et$, NaHMDS, THF, 0 °C; (e) NH_3/EtOH (satd.), 100 °C; (f) ZCl, Et_3N, CH_2Cl_2; (g) CH_2Cl_2, $KOSiMe_3$; (h) HATU, DIPEA, DMF, TAN-heterocycle; (i) $PdCl_2$, H_2, MeOH.



Scheme 3. Synthesis of **23** and **24**, reagents and conditions: (a) Boc_2O, $NaHCO_3$, MeOH; (b) MsCl, pyridine; (c) *N*-Boc-cysteine methyl carboxylate, Cs_2CO_3, DMF; (d) CH_2Cl_2, $KOSiMe_3$; (e) *i*BuOC(O)Cl, DIPEA, CH_2Cl_2, −20 °C; (f) CH_2N_2; (g) *hν*, dioxane/water; (h) HATU, DIPEA, DMF, TAN-heterocycle; (i) HCl, dioxane; (j) CH_2N_2; (k) mCPBA, CH_2Cl_2.

from the non-natural enantiomer of aspartic acid. Polyfunctional intermediate **6** already bears the β-amino acid moiety as well as an α-bromo ketone which can function as a thiazole precursor. Diazoketone **5** was obtained from commercially available *N*-(benzyloxycarbonyl) *tert*-butyl aspartate [D-*Z*-Asp(OtBu)-OH, **4**] by a known procedure.[6] The conversion of diazoketone **5** to α-bromo ketone is reported to proceed without cleavage of the *tert*-butyl ester.[7] However in all the cases we obtained the corresponding acid. Condensation of **6** with the required thioamides, prepared from the corresponding carboxamides by treatment with Lawesson's reagent,[8] yielded the desired thiazole β-amino-acids **7/8** which were coupled in the next step with the TAN 1057 heterocycle[3b] under standard conditions. Catalytic



Scheme 4. Synthesis of **31** and **32**, reagents and conditions: (a) acetone, 0 °C; (b) aq NaOH, 90 °C; (c) MeI, CH_3CN, 40 °C; (d) 3-Amino-2-(*N*-*Z*-*N*-methylamino) methyl propionate hydrochloride, NaOAc, CH_3CN, 70 °C; (e) HBr, AcOH; (f) HATU, DIPEA, DMF, N^{β},N^{ω}-bis-*Z*-L-β-homo lysine; (g) HBr, AcOH; (h) aq NH_3; (i) MeOH/HCl.

hydrogenolysis was not applicable for removal of the *Z*-group presumably due to catalyst poisoning caused by sulfur.[9] However, trifluoromethansulfonic acid in the presence of anisole as a scavenger was successfully used for the cleavage.[10]

For the isoxazol containing β-amino-acid **16**, a [3+2] cycloaddition[11] of *Z*-protected propargylamin and the corresponding nitrile oxide was used as a key step followed by introduction of the β-amino group through a Michael addition of ammonia to α,β-unsaturated ester **14** (Scheme 2). This strategy required the transformation of ester **12** via reduction and subsequent Swern oxidation of the corresponding primary alcohol (yields not optimised) followed by a Wittig–Horner olefination. The amino group was protected, the ester was saponified providing the β-amino acid **16** which was coupled with the TAN 1057 heterorocycle. Finally, removal of the *Z*-group by catalytic hydrogenolysis yielded the desired product **17**.

In order to tune physicochemical parameters (e.g., lipophilicity), a sulfide and a sulfone bridge, respectively, were introduced into to the β-amino acid side chain (Scheme 3). For this purpose, S-alkylation of N-Boc cysteine using the mesylate of BOC-ethanolamine **19** was performed. The β-amino acid **21** obtained by standard Arndt–Eistert homologation of the corresponding acid **20** was coupled with the TAN 1057 heterocycle. Finally, removal of the BOC-groups yielded **23**. Compound **24** was obtained following the same synthetical route starting from the oxidised compound **22**, albeit in poor yield.

So far, changes in the periphery of the dihydro pyrimidinone part of TAN 1057 A,B have only rarely been described.[4a] We envisaged to exchange the urea part by amino heterocycles which were believed to appropriately mimic the donor/acceptor properties of the urea group.

For this purpose, different derivatives were synthesised through the route outlined in Scheme 4. Applying known procedures,[12] 2-amino-pyrimidine (**26**) was converted into thiourea **27** which was subsequently treated[13] with methyl iodide to yield the corresponding isothiouronium salt **28**. The coupling reaction between the salt **28** and 3-amino-2-(*N*-*Z*-*N*-methylamino) methyl propionate hydrochloride[3b] provided the protected dihydropyrimidinone **29**. After removal of the *Z*-protecting group, the heterocycle **30** was coupled with appropriately protected β-homolysine. Subsequent removal of the *Z*-protecting group was accomplished using HBr in acetic acid generating dihydrobromide **31**, from which the corresponding dihydrochloride **32** is available by consecutive treatment with ammonia followed by hydrochloric acid. Alternatively, tris-*Z*-protected β-homoarginine can be used for the peptide coupling finally yielding derivative **43** (cf. Table 2).

By analogous procedures, numerous amino or methyl amino heterocycles were introduced yielding novel dihydropyrimidinone antibiotics **32–45** (cf. Table 2).

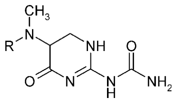
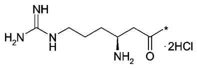
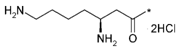
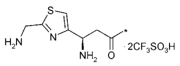
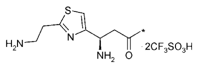
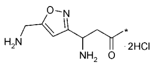
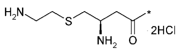
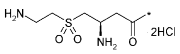
Pharmacology

On a first level of in vitro assays, the novel derivatives were tested for their abilities to inhibit the growth of *Staphylococcus aureus* bacteriae. Since TAN 1057 A,B (**1**) is a known inhibitor of the bacterial translation machinery,[2] respective translation assays[14] were used in parallel to gain in-sight into the level of selectivity of the procaryotic versus the eucaryotic translation. This selectivity delivers a first clue about the toxicity of the samples. As a reference compound, β-homolysine derivative **3** showing markedly improved selectivity in favour of the eucaryotic translation was used.[4c]

As can be deduced from Table 1, introduction of thiazole (**9**/**10**) and isoxazole (**17**) heterocycles into the β-amino acid side chain leads to considerably less active compounds. Also the incorporation of sulfide/sulfone groups leads to loss of activity (compounds **23**/**24**). This is surprising since β-lysine and β-homolysine derivatives are highly active. The loss of activity observed for derivatives **23** and **24** might be explained by changes in lipophilicity influencing for example, up-take by the bacteriae rather than by stereo-electronical reasons.

Variations in the urea part of the TAN 1057 A,B heterocycle led to a completely different picture: derivatives with strong basic nitrogen atoms in *ortho*-position of the amino group show high anti-staphylococcal activity. Examples are the 2-pyridyl derivatives **33** and **44**, the 2-pyrimidyl derivatives **32** and **43**, the 2-pyrazine derivative **37** as well as the 2-quinolyl derivatives **34** and **45**. For some of those examples (e.g., derivatives **34**, **37**, **43**, **45**), an improved target activity compared to TAN 1057 A,B (**1**) is observed. Heterocycles with a less basic nitrogen atom in ortho position to the exocyclic amino group such as thiazole (**35**), benzothiazole (**36**) or benzimidazole (**39**) show considerably weaker effects. Also the shift of the nitrogen atom position from the *ortho* to the *meta* position (**41**) or the transfer of the pyrimidyl residue to tertrahydropyrimidyl (**42**) significantly reduces the activity. Finally, replacement of the heterocycle by a phenyl ring abolishes the activity (**40**). Those observations underline that the nitrogen atom

Table 1. Pharmacological profile of novel TAN 1057 A,B analogues with modified β-amino acid side chain

Compd	Structures[a] 	MIC (μg/mL) *S. aureus 133*	IC_{50} (μM) procaryotic translation	IC_{50} (μM) eucaryotic translation	Selectivity index[b]
1		0.10	0.30	0.17	0.57
3		0.05	0.50	2.8	5.3
9		50	32	n.d.	n.d.
10		100	16	n.d.	n.d.
17[c]		> 100	> 64	n.d.	n.d.
23		100	n.d.	n.d.	n.d.
24		> 100	n.d.	n.d.	n.d.

[a] Obtained as one pair of diastereomers.

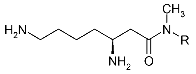
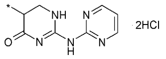
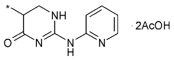
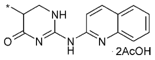
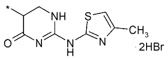
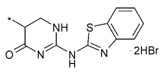
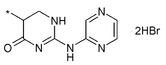
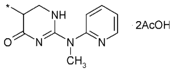
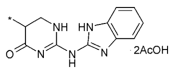
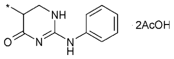
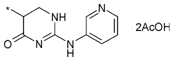
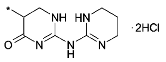
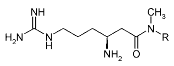
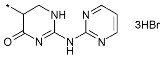
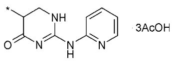
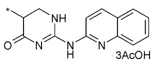
[b] In vitro selectivity index (IC_{50} eucaryotic translation/procaryotic translation).

[c] Obtained as a mixture of diastereomers.

incorporated in the heterocycle functions as a hydrogen bond acceptor and might mimic the carbonyl group of the urea moiety. On the other hand, the bridging amino group most likely displays hydrogen donor properties, since *N*-methyl derivative **38** is inactive.

In-depths investigations were carried out with the most active compounds **44** and **45**. Back to back with the parent compound TAN 1057 A,B (**1**) they were tested on a panel of Gram-positive pathogens (Table 3). Although activity on *S. aureus* and *Enterococcus faecium* remains in the range of the TAN 1057 A,B level, the first considerable activity against *S. pneumoniae* strains was observed for compounds **44** and **45**. *E. faecalis* constitutes the only significant gap in the Gram-positive spectrum.

Table 2. Pharmacological profile of novel TAN 1057 A, B analogues 32–45

Compd	Structures[a] 	MIC (µg/mL) *S. aureus 133*	IC_{50} (µM) procaryotic translation	IC_{50} (µM) eucaryotic translation	Selectivity index[b]
32	 2HCl	1.6	1	0.38	0.38
33	 2AcOH	0.2	0.1–0.5	0.2	0.2
34	 2AcOH	6.3	0.05	0.05	1
35	 2HBr	50	1.0	n.d.	n.d.
36	 2HBr	25	3.2–3.6	n.d.	n.d.
37	 2HBr	<0.1	<0.1	<0.1	n.d.
38	 2AcOH	>100	n.d.	n.d.	n.d.
39	 2AcOH	50	1–2	n.d.	n.d.
40	 2AcOH	100	n.d.	n.d.	n.d.
41	 2AcOH	50	>64	n.d.	n.d.
42	 2HCl	>100	n.d.	n.d.	n.d.
					
43	 3HBr	0.4	<0.05	0.10	>2
44	 3AcOH	0.05	n.d.	0.07	n.d.
45	 3AcOH	0.1	0.1	0.1	1

[a] Obtained as one pair of diastereomers.

[b] In vitro selectivity index (IC_{50} eucaryotic translation/procaryotic translation).

Table 3. Antibacterial activity of derivatives **1**, **44** and **45**

Compd	**1**	**44**	**45**
MIC *S. aureus 133* (μg/mL)	0.25	0.5	0.5
MIC *S. epidermidis 18570* (μg/mL)	0.25	0.25	0.25
MIC *E. faecium L 4001*(μg/mL)	2	2	8
MIC *E. faecalis 18531* (μg/mL)	> 16	> 64	> 16
MIC *S. pneumoniae G9a* (μg/mL)	16	2	n.d.
MIC *S. pneumoniae 1707/4* (μg/mL)	32	2	8
ED_{100} *S. aureus 133* (mg/kg)	0.25	1	n.d.

In an *S. aureus* mouse sepsis model (iv application), derivative **44** shows a slightly lower activity than TAN 1057 A,B. The protective effect remains comparable to penicillin G (ED_{100} 1 mg/kg) which was used as standard.

The cytoxicity potential of **44** and **45** was estimated using macrophage cell lines. Cytotoxic concentrations (CC_{50} values) on those cells are in the same range as observed for TAN 1057 A,B which is known to display a relatively low LD_{50} value in mice of 50 mg/kg iv. The toxicity potential can also be predicted from the selectivity index for the heterocyclic derivatives (Table 2) in comparison to well tolerated derivative **3**. Thus, further optimization of broad spectrum antibacterial activity and tolerability are needed.

Summary

Whereas the incorporation of heterocycles in the β-amino acid side chain cuts down activity significantly, aromatic *ortho*-amino nitrogen heterocycles are suitable replacements of the urea group. In addition, considerable anti-streptococcal activity was observed for amino pyridyl derivative **44** and amino quinolyl derivative **45**. However, the antibacterial spectrum and concomitant tolerability need to be improved further.

Acknowledgements

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