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SAR studies on dihydropyrimidinone antibiotics

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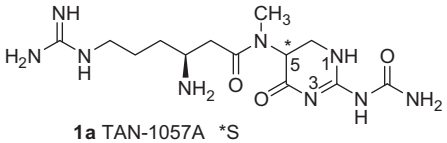
ABSTRACT

There is an urgent need for the development of novel antimicrobial agents that offer effective treatment against MRSA. Using a new class of dipeptide antibiotic TAN-1057A/B as lead, we designed, synthesized and evaluated analogs of TAN-1057A/B. Several novel dihydropyrimidinone antibiotics demonstrating comparable antibiotic efficacy while possessing favorable selectivity were identified.

Many antibacterial products are available on the world market, but successful treatment of bacterial infections is becoming increasingly problematic as resistance to current agents becomes more widespread. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of severe infections in both the hospital and community environments.[1,2] Although, several new classes of antimicrobial agents have reached the market in recent years with world wide efforts, there is still an urgent need for the development of novel agents that offer effective treatment against MRSA.

TAN-1057A/B (**1**) (Fig. 1), isolated from bacteria *Flexibacter* by scientist at Takeda Chemical Co. in 1993, is a dipeptide antibiotic consisting a mixture of two equilibrate epimeric isomer **1a** and **1b**. It represents a novel antibacterial class, containing a unique 2,5-diamino-5,6-1*H*-dihydropyrimidin-4-one scaffold and a β-homoarginine side chain. TAN-1057A/B inhibits the bacterial translation, and displays potent minimal inhibitory concentration (MIC) against staphylococci including MRSA.[3] However, TAN-1057A/B has low selectivity, inhibiting both prokaryotic and eukaryotic translation, it also has significant toxicity in vivo (LD_{50} in the mouse: 50 mg/kg ip and 100 mg/kg iv). In addition, TAN-1057A/B has reduced antibiotic activity against other pathogens, such as enterococci and pneumococci.

Previously, limited structure–activity relationship (SAR) results on TAN-1057A/B have been published.[4] In this Letter, we report systematic SAR studies of TAN-1057A/B series analogs. Inhibitory activity against both prokaryotic and eukaryotic translation using biochemical translation assays[5] and MIC[6] were obtained respectively to evaluate selectivity and potency of the designed analogs.



1a TAN-1057A *S
1b TAN-1057B *R

Figure 1. Dipeptide antibiotics TAN-1057A/B.

Chemistry was developed to enable the detailed studies of each portion of the lead TAN-1057. In general, analogs were assembled according to route depicted in Scheme 1, utilizing the strategy used in the total synthesis of TAN-1057A/B.[7] Dihydropyrimidinone **4** was constructed by treatment of appropriately substituted amine **6** with isothiuronium salt **7**. Coupling of diazoketone **3**, prepared from the corresponding amino acid **5**, with dihydropyrimidinone amine in the presence of $AgClO_4$ followed by necessary deprotection afforded compound **2**.

We initiated our efforts by examining the SAR around the dihydropyrimidinone core, the results are displayed in Table 1. Replacement of methyl (R^2) with ethyl (compound **8**) significantly improved the selectivity but led to a reduction of both MIC and the inhibitory activity against translation. While acetyl amide replacing the urea moiety at the 2-position attaching to the heterocyclic ring (compound **9**) provided mild improvement in both activity against bacterial translation and selectivity, other modifications[8] at R^3 only yielded analogs with reduced potency

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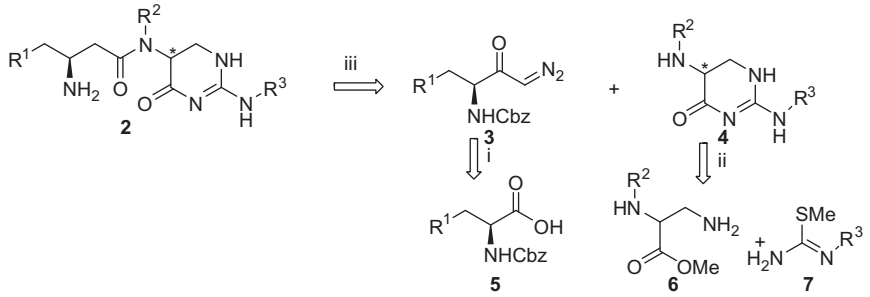
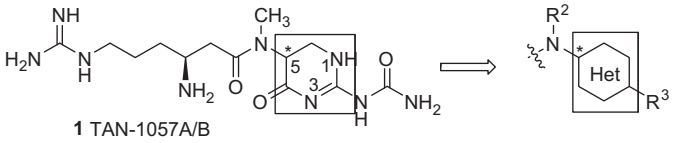
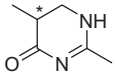
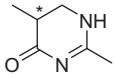
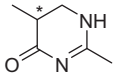
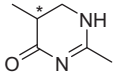
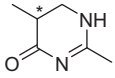
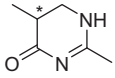
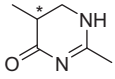
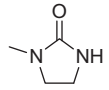
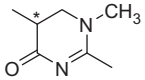
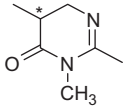
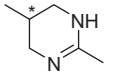
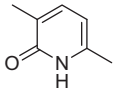
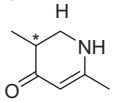
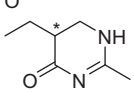


Scheme 1. Synthesis of **2.** Reagents : (i) ClCOOEt, TEA, CH_2Cl_2; CH_2N_2; (ii) NaOAc; (iii) $AgClO_4$ TEA, DMF.

Table 1
SAR of the heterocyclic core region

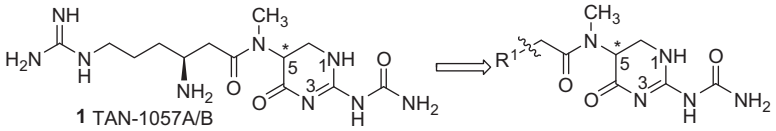


1 TAN-1057A/B

Compound[a]	R^2	Het	R^3	MIC[9] (μg/mL) *S. aureus*	Prokaryotic translation IC_{50} (μM)	Eukaryotic translation IC_{50} (μM)	Selective index[b]
1 (TAN1057 A/B)	CH_3		$-NHCONH_2$	8	0.3	0.2	0.67
8	Et		$-NHCONH_2$	256	1.8	45	25
9	CH_3		$-NHCOCH_3$	8	0.1	0.2	2
10	CH_3		–NHCOEt	16	<5	<5	
11	CH_3		$-N(CH_3)COCH_3$	64	6.8	21	3.1
12	CH_3		$-NHCON(CH_3)_2$	>500	8.8	19.7	3.4
13	CH_3			>500	>500	>500	–
14	CH_3		$-NHCONH_2$	>500	>500	>500	–
15	CH_3		$-NHCONH_2$	>500	>500	>500	–
16[10]	CH_3		$-NHCONH_2$	>256	121	–	–
17[10]	CH_3		$-NHCONH_2$	>500	>500	56	–
18[11]	CH_3		$-NHCOCH_3$	>500	>500	>500	
19	CH_3		$-NHCONH_2$	>500	51	56	1.1

[a] Each compound is an equilibrate epimeric mixture of both *R and *S at 5-position.
[b] In vitro selectivity index (IC_{50} eukaryotic transl/prokaryotic transl).

Table 2
SAR of the side chain



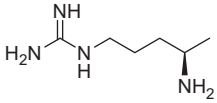
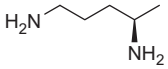
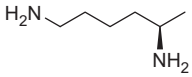
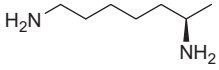
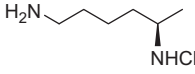
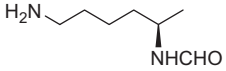
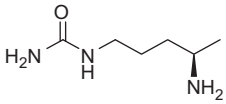
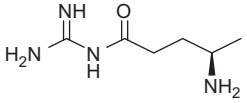
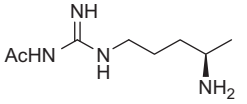
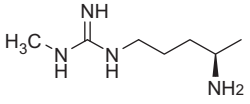
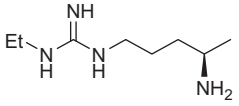
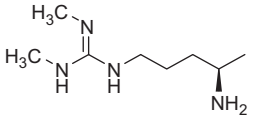
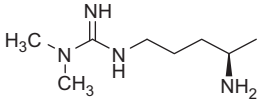
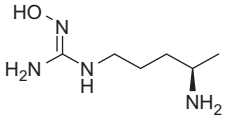
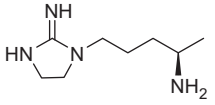
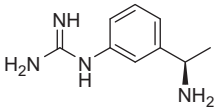
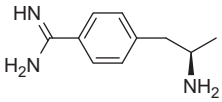
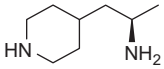
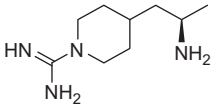
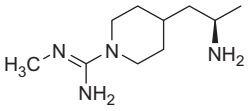
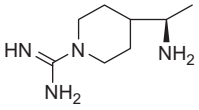
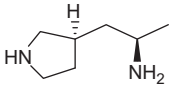
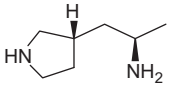
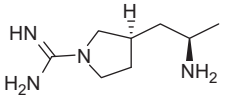
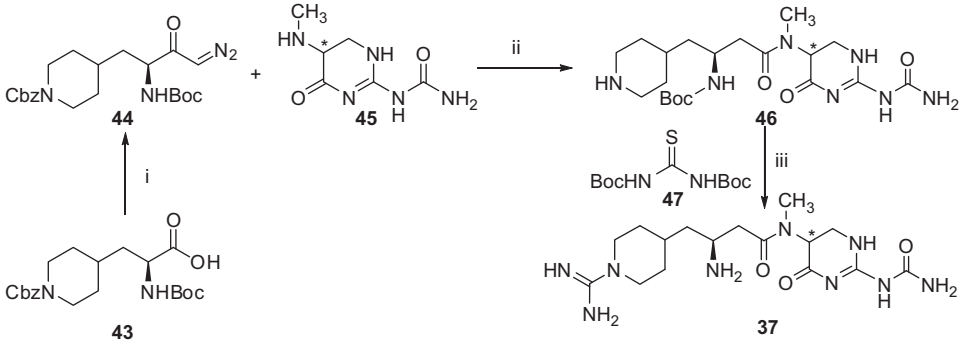
Compound[a]	R^1	MIC[9] (μg/mL) *S. aureus*	Prokaryotic translation IC_{50} (μM)	Eukaryotic translation IC_{50} (μM)	Selective index[b]
1 (TAN1057 A/B)		8	0.3	0.2	0.67
20		64	9	<5	<0.6
21		64	6.5	39.8	6
22		16	5	10	2
23		128	25	5	0.2
24		>500	>500	>500	–
25		>500	>500	>500	–
26		>500	226	7	0.03
27		>256	106	–	–
28		8	0.2	1.3	6.5
29		>256	2.4	27.4	–
30		256	1.3	26.3	20
31		64	4.2	20.6	4.9
32		64	8.2	23.2	2.8
33		128	4.2	35	8.3

Table 2 (*continued*)

Compound[a]	R^1	MIC[9] (μg/mL) *S. aureus*	Prokaryotic translation IC_{50} (μM)	Eukaryotic translation IC_{50} (μM)	Selective index[b]
34		256	0.15	1.9	10
35		512	0.51	12.3	8.2
36		64	6.6	6.6	1
37		16	0.11	0.75	6.8
38		64	3.6	53	14.7
39		16	0.18	0.23	1.3
40		64	2.0	0.6	0.3
41		128	3.9	3.4	0.9
42		8	0.17	0.45	2.6

[a] Each compound is an equilibrate epimeric mixture of both *R and *S at 5-position.
[b] In vitro selectivity index (IC_{50} eukaryotic transl/prokaryotic transl).



Scheme 2. Synthesis of **37**. Reagents : (i) ClCOOEt, TEA, CH_2Cl_2; CH_2N_2; (ii) $AgClO_4$, TEA, DMF; H_2, Pd/C; (iii) $HgCl_2$, DMF; TFA.

(compounds **10**–**13**). Methyl substitution at the heterocyclic ring (**14** and **15**) abolished the antibacterial activity. It has been reported that TAN-1057A/B gradually loses its antibacterial activity in basic aqueous solution due to the hydrolysis of the acetyl amidine and opening of the heterocyclic ring. The heterocyclic core of compounds **16**–**18** was designed[10,11] to be stable toward hydrolysis while causing minimal structure disturbance. Unfortunately, these modifications resulted in a complete loss of antibacterial activity. The intolerability of the core towards any modifications that remove its acetylating ability may indicate that acetylation of the protein with TAN-1057A/B contributes to its mechanism of action. Lastly, inserting a methylene between methyl amine and the core (**19**) also removed the activity.

We next turned our attention to the β-homoarginine side chain, investigating the effect of β-amine, terminal guanidine and linker between them on the SAR. Representative SAR results are summa-

Table 3
Pharmacological profile

Compound	iv PD_{50} (mg/kg)	MIC (μg/mL)	CC_{50} (μg/mL)
1 (TAN1057 A/B)	0.7	12.8	6
Vancomycin	9.6	0.8–2	—
28	3.3	32	131
37	2.4	16	62
42	1.5	16–32	—

rized in Table 2. Replacement of terminal guanidine with primary amines (compounds **20**–**22**, **36, 40** and **41**) cause a significant reduction in inhibitory activity against bacterial translation, yet the reduction of MIC was minor. We speculate that this result may attribute to the better permeability of an amino analog comparing with its corresponding guanidino one. Substitution on the β-amine is detrimental to the potency and selectivity (**23** and **24**). Reduction of the basicity of the terminal guanidine group (compounds **25**–**27**) diminished antibacterial activity, demonstrating the importance of basicity of the terminal moiety for this series of antibacterial in order to interact with the target protein. Moderate selectivity can be achieved through alkylation of the terminal guanidine, yet the potency is sensitively associated with the positions and sizes of the alkyls (**28**–**33**). Compound **28** is the best of this series in terms of selectivity and potency. Modifying the linker also proved to be fruitful. Many analogs incorporated an aromatic ring as a spacer with different orientations and distances were designed and synthesized, two most potent ones (**34** and **35**) were shown in Table 2. Although possessing potent inhibitory activity against the bacterial translation and favorable selectivity, both **34** and **35** failed to provide desirable MIC. However, when using saturated a heterocycle as the spacer, compound **37** and **42** displayed better inhibitory activities against bacterial translation and favorable selectivity, and also showed comparable MIC with TAN-1057A/B.

Synthesis of this group of analogs was outlined in Scheme 2, represented by compound **37**.[12] Protected amino acid **43**, obtained from commercial sources, was converted to diazoketone **44** by treatment with ClCOOEt followed by diazomethane. Dihydropyrimidinone amine **45**, prepared following the procedure disclosed by de Meijere and co-workers,[7] was then reacted with diazoketone **44** in the presence of $AgClO_4$ to give the coupling product. Removal of the Cbz protecting group with hydrogenolysis provided the piperidine **46**. Treatment of the piperidine with Boc-protected thiourea **47** followed by deprotection provided compound **37**.

Compounds **28**, **37** and **42** were further evaluated. Their CC_{50}s was obtained.[13] The data supported that these compounds are more selective than TAN-1057A/B. They were also studied in mouse septicemia model against MRSA, and their PD_{50} was listed in Table 3. In this model, **28**, **37** and **42** are more efficacious than vancomycin.

Previous studies have shown that TAN-1057A/B inhibits the peptidyl transferase reaction which leads to inhibition of translation but does not directly bind to the A or P tRNA binding sites. Analysis of drug resistant mutants shows two classes of mutants depending on selection method. The first class of mutants may be efflux mutants.[14] This finding is supported by MIC studies done with minimal media in the presence or absence of dipeptides, which suggests that TAN-1057A/B is actively transported into bacterial cells via a dipeptide transport mechanism. The second class of mutants is ribosomal mutations of unknown function which confer resistance to TAN-1057A/B.[15]

In summary, novel analogs of dipeptide antibiotics TAN-1057A/B were designed and synthesized. SARs of the dihydropyrimidinone core and side chains were established. Our studies showed that the dihydropyrimidinone core is the phamacophore of this novel class of antibiotics and is highly sensitive to modifications. Optimizations of the side chain provided several analogs with favorable safety while maintain the potency against MRSA. Further work need to be carried out to identify drug candidates that can be used to treat MRSA infections.

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The mixture of above compound (15 mg) and TFA (1 mL) was stayed at room temperature for 1 h and evaporated to dryness. The mixture was then co-evaporated with toluene (×2). The resulting solid was then dissolved in water, pH of the resulting solution was adjusted to 5.5–6.0 with $(NH_4)_2CO_3$ aqueous solution. The mixture was purified with C-18 column to give 7.4 mg of compound after lypholization.
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