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Combinatorial Modification of Natural Products: Synthesis and In Vitro Analysis of Derivatives of Thiazole Peptide Antibiotic GE2270 A: A-Ring Modifications

Jeffrey Clough,[a,†] Shaoqing Chen,[a,‡] Eric M. Gordon,[a,§] Corinne Hackbarth,[a] Stuart Lam,[a,§] Joaquim Trias,[a] Richard J. White,[a] Gianpaolo Candiani,[b] Stefano Donadio,[b] Gabriella Romanò,[b] Romeo Ciabatti[b] and Jeffrey W. Jacobs[a,*,§]

[a]*Vicuron, Inc., 34790 Ardentech Court, Fremont, CA 94555, USA*
[b]*Vicuron, Inc., 21040 Gerenzano (VA), Italy*

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Abstract—Thiazole peptide GE2270 A (**1**) possesses potent antimicrobial activity against many gram-positive pathogens, including methicillin resistant *Staphylococcus aureus* (*S. aureus,* MRSA; $MIC_{90} = 0.06$ μg/mL) and vancomycin resistant *Enterococcus spp.* (VRE; $MIC_{90} = 0.03$ μg/mL); however its poor aqueous solubility has prohibited its development for the clinical treatment of infections. An integrated combinatorial and medicinal chemistry program was employed to identify derivatives of **1** that retain activity but possess greatly enhanced aqueous solubility.

The emergence of multi-drug resistant strains of bacterial pathogens is a significant clinical problem.[1] Of key concern are the Gram positive pathogens, especially *Staphylococcus aureus*, *Staphylococcus pneumoniae*, *Entoerococcus faecium* and *Enterococcus faecalis*.[1] Efforts to improve or expand the utility of existing antibiotics have been only moderately successful; hence, there is an urgent need for novel classes of antibacterial agents that act on previously unexploited biochemical targets essential to the pathogen life-cycle. One such novel class of anti-infective not currently used clinically is the thiazole peptides. We report herein the synthesis and in vitro antibacterial properties of a series of derivatives of thiazole peptide GE2270 A (**1**, Scheme 1) that were engineered to possess greatly enhanced aqueous solubility.[2]

Thiazole peptides comprise a family of non-ribosomally synthesized compounds classified by the architecture of their thiazole heterocycles.[3] These natural products are noted for their potent antibacterial activity, the predominant mechanism of which is inhibition of protein synthesis.[3] Thiazole peptide GE2270 A (**1**) was selected as a starting point for an anti-infective discovery program for the following reasons. First, **1** is a potent inhibitor of elongation factor *Tu* (EF-*Tu*; $IC_{50} = 5$ nM), the molecular chaperone responsible for translocating aminoacylated tRNAs to the bacterial ribosome.[4] EF-*Tu* is essential for bacterial protein biosynthesis, and no antibiotics currently used clinically act via this mechanism. Second, EF-*Tu* from several bacterial species are sensitive to **1**,[5a] whereas the mammalian counterpart to this protein (eukaryotic elongation factor, EF-1 alpha) is structurally distinct from the *Escherichia coli* protein,[5b] and **1** is inactive in a mammalian cell-free protein synthesis system.[6] Third, **1** possesses exquisite antimicrobial activity against many gram-positive pathogens, including MRSA (MIC_{90} 0.06 μg/mL) and VRE (MIC_{90} 0.03 μg/mL),[2d] and has also been shown to be highly efficacious in animal models of infection.[7] The key limitation to the development of this compound has been its very poor solubility in water (<0.0001 mg/mL in PBS pH 7.4 buffer), making formulation for systemic administration to humans difficult. Therefore, the goal of the chemistry program was identification of analogues of **1** which retain potency yet possess greatly enhanced

*Corresponding author at present address: Rigel, Inc., 240 East Grand Avenue, South San Francisco, CA 94080, USA. Tel.: +1-650-266-3628; fax: +1-650-266-3501; e-mail: jjacobs@sunesis.com
†Present address: Rigel, Inc., 240 East Grand Avenue, South San Francisco, CA 94080, USA.
‡Present address: Discovery Chemistry, Hoffmann-La Roche, Inc., 340 Kingsland St., Nutley, NJ 07110, USA.
§Present address: Sunesis Pharmaceuticals, Inc., 341 Oyster Point Boulevard, South San Francisco, CA 94080, USA.

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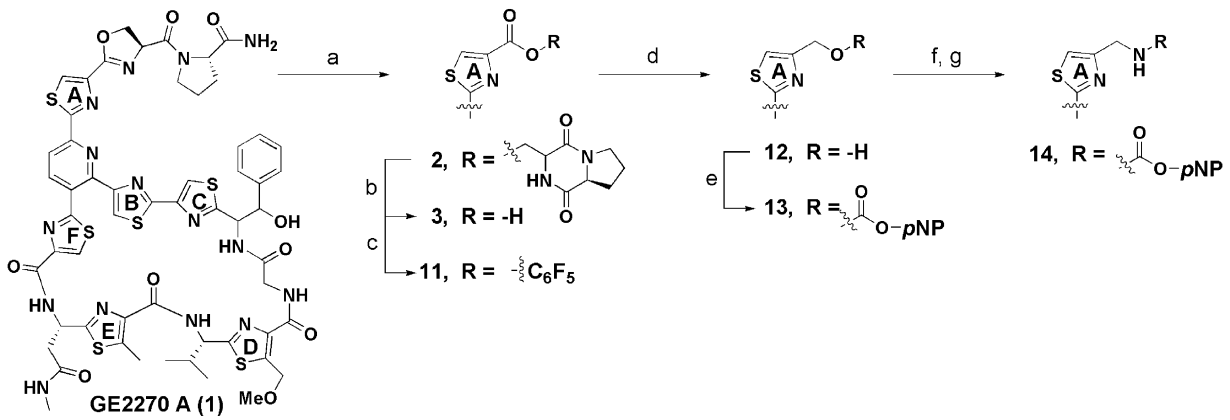
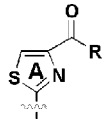


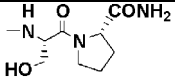
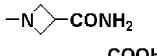
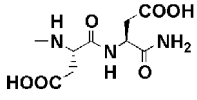
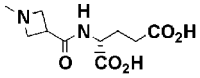
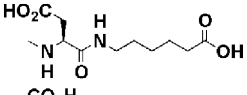
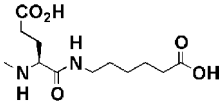
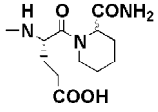
Scheme 1. Hydrolysis of GE2270 A and preparation of intermediates for high-throughput synthesis: (a) H_2SO_4, THF/H_2O 6:4, 60 °C, 4 h, 90%; (b) NaOH, THF/H_2O, 2 h, 90%; (c) 1-[3-(*N*,*N*-dimethylamino)propyl]-3-ethylcarbodiimide HCl, pentafluorophenol, dioxane, 16 h, 85%; (d) sodium borohydride, THF/H_2O 6:4, 73%; (e) 4-nitrophenylchloroformate (*p*NP-Cl), pyridine, THF, 80%; (f) Magtrieve™[a], THF (not isolated); (g) Sieber amide resin:[b] (1) 20% piperidine/DMF; (2) product from 'f', 2% acetic acid/DMF, 12 h; (3) sodiumcyanoborohydride, 1% acetic acid/DMF, 16 h; (4) 5% trifluoroacetic acid (TFA)/DCM, 1 h; (5) *p*NP-Cl, pyridine, DCM; 12 h, 70%. [a]Aldrich Chemical Co., Milwaukee, WI, USA. [b]Calbiochem-Novabiochem Co., San Diego, CA, USA.

aqueous solubility at neutral pH. MIC criteria were set as $\leq$1 μg/mL, which is the MIC of vancomycin obtained against a majority of clinical isolates of MRSA and vancomycin-susceptible enterococci.[8] The aqueous solubility objective was set at a minimum of 0.5 mg/mL in PBS buffer at neutral pH—a modest solubility requirement but a 10^3-10^4 fold improvement over the parent compound.[9] This solubility was estimated to be the lowest acceptable level that would still permit formulation for parenteral administration using pharmaceutically acceptable excipients.[10]

Degradation products of **1** have been described that partially retain antimicrobial activity, including ester **2** and acid **3** (Scheme 1). Acid **3** became the initial focus of the chemistry program because this functionality was hoped to provide a facile entry into analogue synthesis. Since the oxazolino-proline moiety on **1** is the dehydration product of a Ser-Pro dipeptide, our first chemical modifications employed carboxylate **3** for the combinatorial reassembly of variants of this dipeptide motif. Although the chemistry results in peptidic compounds, the diversity of side-chain functionality readily accessible from commercially-available amino acids allows a relatively facile survey of functional groups and was hoped to establish a rough SAR for both the activity and solubility requirements. Acid **3** was prepared as described (Scheme 1),[2e] and was found to cleanly acylate resin-bound mono- and dipeptides using standard techniques (data not shown). Three 88-member libraries of peptidic derivatives were prepared via parallel synthesis, which surveyed a broad range of functionality, including acidic, basic, and neutral side chains, L- and D-configured amino acids, and many unnatural amino acids (data not shown)—264 derivatives in total. In most instances, crude library products exceeded 70% purity while yields were approximately 50% as estimated by reverse-phase (C18) HPLC and MS analysis. Crude library products were initially screened against a panel of four organisms, including three bacterial pathogens, and *Candida albicans* as a eucaryotic surrogate.[8b] Selected compounds were resynthesized and purified to homogeneity, then screened against both an expanded panel of bacteria as well as tested for their aqueous solubility. Representative data are shown in Table 1.

Table 1. Activity and solubility data for selected compounds from A-ring dipeptide libraries



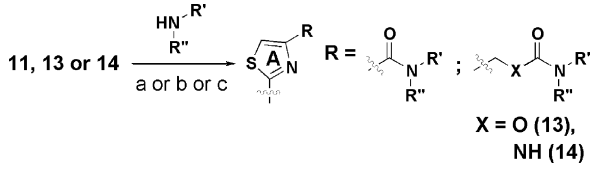
Compd	R	MIC (μg/mL)		Solubility (mg/mL)[c]
		MSSA[a]	MRSA[b]	
4		NT	0.25	0.004
5		NT	0.06	0.00011
6		>32	>32	>1.5
7		>32	>32	>2.8
8		>32	>32	>2
9		>32	>32	2.6
10		NT	4	0.19

[a]ATTC25923.
[b]ATTC33591.
[c]Solubility values are medians of three determinations (see ref 9).

Compound **4** was quite active and is equivalent to the product derived from the hydration of the oxazoline ring of **1**, liberating a serine side chain. Unfortunately, solubility was only marginally improved (4 μg/mL, see Table 1). Compound **5** contains an azetidine 3-carboxamide derivative off the A-ring, and retains potency equivalent to the parent compound, but its aqueous solubility is just barely detectable at 0.1 μg/mL. In contrast, several compounds had excellent solubility characteristics (e.g., examples **6**–**9**) but were devoid of any antibacterial activity in our assay (MIC_{MRSA} >32 μg/mL). These compounds are bis-acids, and perhaps have difficulty crossing the bacterial cell-wall. Interestingly, no basic compounds were identified with aqueous solubilities >10 μg/mL although over 50 were prepared (data not shown). The compound which best approached key criteria in this exercise is *mono*-carboxylic acid **10**.

We next wished to survey a broader range of functionality at the A-ring to more thoroughly establish the MIC and solubility SARs. It was reasoned that an active ester derivative of **3** could permit the rapid incorporation of highly functionalized amines in unprotected form. Thus, the pentafluorophenol (PFP) ester of **3** was prepared (**11**, Scheme 1), and as desired, ester **11** could be used to cleanly acylate a broad cross-section of functionally dense amine nucleophiles (Scheme 2 and Table 2). Acylations were initially performed in DMF in the presence of *N*,*N*-diisopropylethylamine (DIEA); however many hydrophilic amines were insoluble under these conditions. In these instances, most amines were cleanly acylated after first treating them with chlorotrimethylsilane (TMS-Cl) and DIEA to render them soluble in organic solvent (Scheme 2). Desired products were typically isolated in 30–50% yield after semi-preparative reverse-phase (C18) HPLC purification.

Carbamates and ureas could be prepared analogously to amides using appropriate derivatives of GE2270 A. As illustrated in Scheme 1, **2** was further employed as a synthon for the preparation of active intermediates for carbamate and urea synthesis. Thus, reduction of **2** with sodium borohydride ($NaBH_4$) produced alcohol **12**, and acylation of **12** with *p*-nitrophenylchloroformate (pNP-Cl) afforded carbonate **13**—an intermediate for the facile synthesis of carbamates from amine nucleophiles (Scheme 1). Carbamate **14** was prepared as an intermediate for urea synthesis via oxidation of **12** to an aldehyde followed by a solid-phase mediated reductive amination (see Scheme 1).[11] Active intermediates **11**, **13** and **14** were employed to acylate a broad range of functionalized amines and the resulting amides, carbamates and ureas analyzed for their antimicrobial activity and solubility properties (Table 2). Most acylations employing **11** or **13** proceeded in high yield at room temperature in under an hour, while carbamate **14** required heating at 50 °C overnight to obtain the desired ureas (Scheme 2). All products were purified by semi-preparative reverse-phase (C18) HPLC and converted to either their sodium or hydrochloride salt prior to testing. Over 150 diverse GE2270 A analogues were prepared via Scheme 2, and representative derivatives are shown in Table 2.

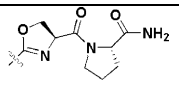
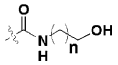
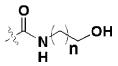
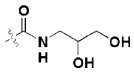
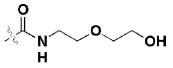
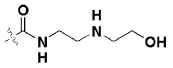
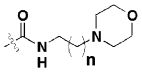
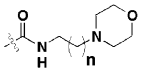
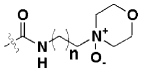
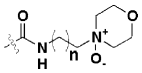
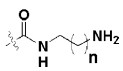
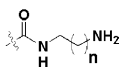
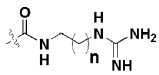
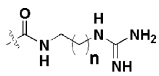
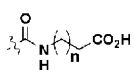
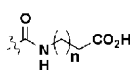
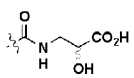
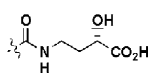
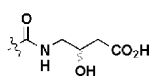
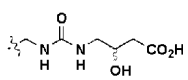
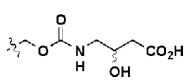


Scheme 2. Acylation of amines with reactive intermediates: (i) Amines soluble in DMF: (a) (**11** or **13**) DIEA, DMF, 1 h; (b) (**14**) DMF, DIEA, 50 °C, 16 h. (ii) Hydrophilic amines not soluble in DMF: (c) amine, chlorotrimethylsilane (TMS-Cl), DIEA, DCM, 40 °C, 1–3 h, concentrate, then: (1) **11** or **13**, DIEA, DMF, 1 h; or (2) **14**, DMF, DIEA, 50 °C, 16 h.

A diverse range of functionality was surveyed at the A-ring, including alcohols (**15**–**19**), amines and amine derivatives (**20**–**27**), and carboxylic acids (**28**–**41**). Including the derivatives made on solid phase, nearly 400 compounds were prepared before the first derivative with an acceptable MIC and solubility profile was identified (**32**, Table 2). An examination of the accumulated SAR suggested that carboxylate derivatives afforded the highest solubility at pH 7.4, and that this functional group must be positioned at least five atoms from the A-ring in order to retain antibacterial potency. Once these criteria were defined, the next 25 compounds we prepared yielded four analogues fulfilling the key objectives (examples **34**–**37**). Acceptable derivatives were identified only in the amide and carbamate series; no urea analogues satisfied the activity goals (cf **33** vs **34**). *N*-Methylation to afford tertiary amides and carbamates resulted in a significant improvement in aqueous solubility (e.g., **37** vs **38**, and **40** vs **41**), perhaps due to conformational changes and/or reduced A-ring conjugation with the tertiary amide. Ultimately, eight compounds were identified that satisfied activity and solubility criteria (**32**, **34**–**39**, **41**), and their potency against an expanded panel of bacteria is illustrated in Table 3. MICs against MRSA and VRE were ≤1 μg/mL and all derivatives were at least equipotent to vancomycin against susceptible strains of these pathogens (Table 3). Most analogues in Table 3 possess a Gram positive spectrum of activity consistant with that of parent compound **1**, but with potency attenuated approximately 10-50-fold. One exception is **38**, which was inactive against the indicated *Streptococcal* strains (MICs >16 μg/mL). Interestingly, some derivatives were active against the Gram negative pathogen *Haemophilus influenzae* (**38**, **39**, and **41**) while **1** is inactive against this strain (MIC >16 μg/mL). In our assay, the aqueous solubility of all compounds in Table 3 was improved approximately 10,000-fold over that of **1** (Table 2).[9]

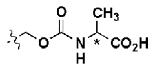
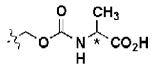
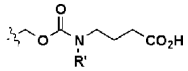
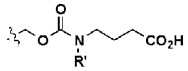
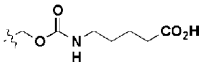
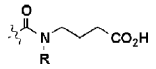
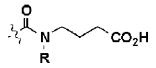
An integrated program of combinatorial solid-phase synthesis and solution-phase medicinal chemistry was employed to synthesize approximately 500 A-ring analogues of thiazole peptide GE2270 A. This exercise rapidly explored the scope and limitations of functionality tolerated at the A-ring and ultimately led to the identification of eight compounds meeting activity and

Table 2. Structure–activity relationship of A-ring amide, carbamate and urea analogues of GE2270 A

Compd	R		MIC (μg/mL)				Solubility (mg/mL)[e]
			MSSA[a]	MRSA[b]	VSE[c]	VRE[d]	
1			0.03	0.03	0.015	0.015	<0.0001
15		$n=1$	0.06	0.125	0.03	0.03	<0.0001
16		$n=3$	0.125	0.125	0.06	0.125	0.0002
17			0.125	0.06	0.015	0.015	<0.0001
18			0.25	0.06	0.125	0.06	<0.0002
19			2	2	2	4	0.0012
20		$n=1$	0.5	0.25	0.06	0.06	0.001
21		$n=2$	0.125	0.06	0.06	0.06	0.0005
22		$n=1$	1	1	0.5	2	0.014
23		$n=2$	2	2	1	2	0.005
24		$n=1$	2	1	2	2	<0.01
25		$n=2$	1	1	2	2	<0.01
26		$n=1$	4	8	8	8	<0.01
27		$n=3$	8	4	8	8	<0.01
28		$n=1$	0.5	0.5	0.06	0.06	0.2
29		$n=4$	0.06	0.015	0.03	0.03	0.05
30			4	4	0.5	1	0.2
31			4	2	0.03	0.25	0.2
32			1	0.25	0.25	0.25	0.5
33			8	8	2	>16	0.66
34			1	0.5	0.5	1	1.2

(continued)

Table 2 (*continued*)

Compd	R		MIC (μg/mL)				Solubility (mg/mL)[e]
			MSSA[a]	MRSA[b]	VSE[c]	VRE[d]	
35		*R*	0.5	0.5	0.25	1.0	0.7
36		*S*	1	0.5	0.25	0.5	0.9
37		R′ = H	0.25	0.125	0.06	0.125	0.5
38		R′ = Me	0.5	0.25	0.5	0.5	0.73
39			0.5	0.25	0.125	0.5	0.6
40		R′ = H	0.25	0.125	0.015	0.03	0.28
41		R′ = Me	0.5	0.25	0.125	0.25	0.91

[a]ATTC25923.
[b]ATTC33591.
[c]BM4147.1.
[d]ATTC51299.
[e]Values are medians of three determinations (see ref 9).

Table 3. MICs of selected compounds against an expanded panel of Gram-positive and Gram-negative pathogens

Bacterial strain	MIC (μg/mL)									
	Van[b]	1	32	34	35	36	37	38	39	41
S. aureus (ATTC 25923)	1	0.03	1	1	0.5	1	0.25	0.5	0.5	0.5
S. aureus (MRSA; ATTC33591)	1	0.03	0.25	0.5	0.5	0.5	0.125	0.25	0.25	0.25
S. epidermis (ATTC 12228)	1	0.03	2	2	2	2	0.25	0.5	0.5	1
E. faecium (BM4147.1)	2	0.015	0.25	0.5	0.25	0.25	0.06	0.5	0.125	0.125
E. faecalis (VRE, ATTC51299)	>16	0.015	0.25	1	1	0.5	0.125	0.5	0.5	0.25
S. pneumoniae (ATTC49619)	0.125	0.06	2	4	2	4	2	>16	2	2
S. pyogenes (ATTC19615)	0.25	0.125	1	8	2	8	8	>16	4	4
Haemophilus influenzae (ATTC31517)	>16	>16	>16	>16	16	16	16	8	8	2
H. influenzae (acr, LS-2)[a]	>16	>16	2	2	1	1	4	2	2	1
Eschericia coli (A G100B)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16

[a]Efflux pump mutant.
[b]Vancomycin.

solubility criteria. Surprisingly, no amine-containing compounds met the solubility requirements although many active derivatives were identified. Acceptable compounds shared several structural features, including a carboxylate moiety to facilitate aqueous solubility, and the placement of this moiety at least five atoms removed from the A-ring thiazole. Selected compounds will next be examined in vivo in animal models of infection, the results from which will be reported elsewhere.

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References and Notes

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8. (a) National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing*. *Informational Supplement M100-S11*; National Committee for Clinical Laboratory Standards, Wayne, PA, 2001 Vol. 21 p90. (b) MICs were determined according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines, but with 0.02% bovine serum albumin (BSA) added to the growth medium.
9. An equilibrium solubility assay was adapted that exploits the chromophore comprised by the A-, B-, C- and F-rings interconnected to the central pyridyl nucleus (see Scheme 1; $\lambda_{max} = 340$ nm, $\varepsilon = 34{,}000$).[2b] Compounds (1–2 mg; sodium or hydrochloride salts) were vigorously shaken in 1 mL PBS pH 7.4 buffer for 3 h, clarified by centrifugation at 10,000*g* for 5 min, filtered (0.2 μm Millipore Millex-LG; PTFE membrane), and then optical densities determined. All compounds were tested in triplicate. The limit of detection of this method was on the order of 0.1 μg/mL. Acid **3** was always included as a control (0.2 ± 0.06 mg/mL); GE2270 A (**1**) was undetectable under these conditions (< 0.1 μg/mL).
10. (a) Estimate based on an approximate clinical dose of 1–2 mg/kg, 80 kg average patient weight, 100 mL maximum volume of diluent for a parenteral antibiotic, and a 2- to 4-fold increase in formulated drug concentration via the addition of excipients. See: Sweetana, S.; Akers, M. J. *PDA J. Pharm. Sci. Tech*. **1996**, *50*, 330. (b) Nema, S.; Washkuhn, R. J.; Brendel, R. J. *PDA J. Pharm. Sci. Tech*. **1997**, *51*, 166.
11. Although this solid-phase route requires several steps, it was superior to repeated attempts at solution-phase reductive amination using ammonia and ammonia equivalents.