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Pyrrolidinedione derivatives as antibacterial agents with a novel mode of action

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Abstract—The pseudopeptide pyrrolidinedione natural products moiramide B and andrimid represent a new class of antibiotics that target bacterial fatty acid biosynthesis. Structure–activity relationship (SAR) studies revealed a high degree of variability for the fatty acid side chain, allowing optimization of physicochemical parameters, and a restricted SAR for the pyrrolidinedione group, indicating major relevance of this subunit for efficient target binding.

Since the introduction of penicillin in the 1940s, antibiotics have a history of success in controlling morbidity and mortality caused by infectious diseases. But, as a consequence of frequent use, bacterial resistance to known classes of antibiotics has become a severe global problem in recent years and presents a continuous clinical challenge.[1–3] Resistance can result from modification of an antibacterial's target or from functional bypassing of that target, or it can be contingent on impermeability, efflux or enzymatic inactivation of the drug.[4] There are serious concerns that untreatable pathogens may develop at an alarming rate in the near future. Strategies to address this challenge include the design of improved versions of antibacterial classes already in clinical use and the use of drug combinations. The application of these strategies can be quite successful, but a high risk of rapid resistance development remains. Thus, an urgent need for new potent classes of antibiotics with novel modes of action persists.

The pseudopeptide pyrrolidinedione natural products moiramide B and andrimid have been described as antibiotics with an unknown mode of action.[5–7] We recently identified the target of this compound class to be the carboxyltransferase subunit of the multimeric bacterial enzyme acetyl-CoA carboxylase (ACC).[8] ACC catalyzes the first committed step in fatty acid biosynthesis and is essential for cell growth. Broad structural conservation among bacteria and a clear distinction from the multifunctional eukaryotic enzyme make ACC a promising target for the design of new broad-spectrum antibacterials with a novel mode of action.

The chemical structure of the natural products moiramide B and andrimid is quite unique, containing four characteristic subunits: an unsaturated fatty acid side chain, the β-amino acid β-(*S*)-phenylalanine, an L-valine derived β-ketoamide moiety and the pyrrolidinedione head group. Since only limited structure–activity relationship data has been published for this compound class,[9] we explored variations of the two terminal subunits, that is, the fatty acid side chain and the pyrrolidinedione group (Fig. 1).

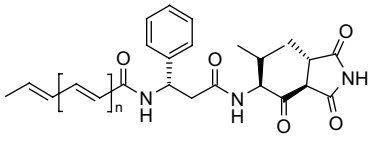

Figure 1. Structures of moiramide B ($n = 1$) and andrimid ($n = 2$).

Keywords: Pyrrolidine diones; Moiramide B; Andrimid; Acetyl-CoA carboxylase; Antibiotic.
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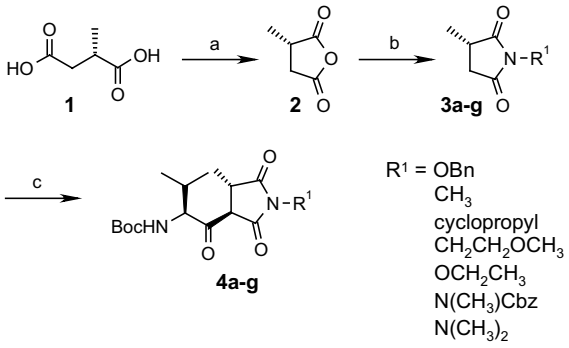
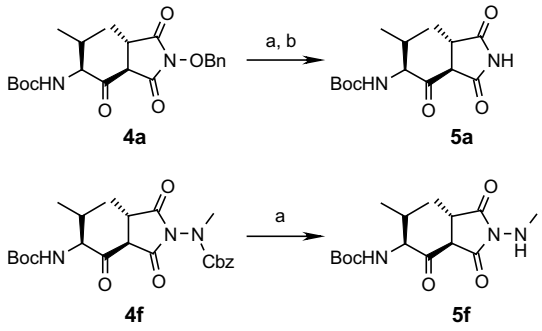


Scheme 1. Reagents and conditions: (a) acetyl chloride, 60 °C; (b) H_2NR^1, CDI, CH_2Cl_2, 0– 45 °C; (c) (1) *N*-Boc-L-valine, CDI, THF; (2) **3a–g**, LiHMDS, THF, −65 °C.

Our chemical synthesis was based on previously published procedures.[9–12] As shown in Scheme 1, treatment of (*S*)-(−)-methylsuccinic acid **1** with acetyl chloride yielded anhydride **2**. The formation of diverse *N*-substituted pyrrolidinediones **3a–g** was achieved by reaction with the appropriate primary amines, hydroxylamines or hydrazines in the presence of *N*,*N*′-carbonyldiimidazole (CDI). The next step involved the diastereoselective formation of the β-ketoamide moiety. For this conversion, *N*-Boc-L-valine was first treated with CDI to form the imidazole derivative. Then, the activated amino acid was mixed with the pyrrolidinedione in tetrahydrofurane and the resulting concentrated solution was slowly added to a lithium hexamethyldisilazide solution at low temperature. This protocol allowed an in situ quench of the labile pyrrolidinedione enolate. Although the β-ketoamide moiety of the products **4a–g** can equilibrate between the *trans*-, *cis*- and enolic-forms, ^{1}H NMR examination revealed the *trans*-isomers to be dominant in the equilibrium mixtures. The other isomers were hardly detectable in most cases.

Removal of the *N*-benzyloxy group in **4a** was achieved by hydrogenation and subsequent treatment of the *N*-hydroxy derivative with 2′-bromoacetophenone and triethylamine.[13] The Cbz-group of hydrazine derivative **4f** was removed by standard hydrogenation (Scheme 2).

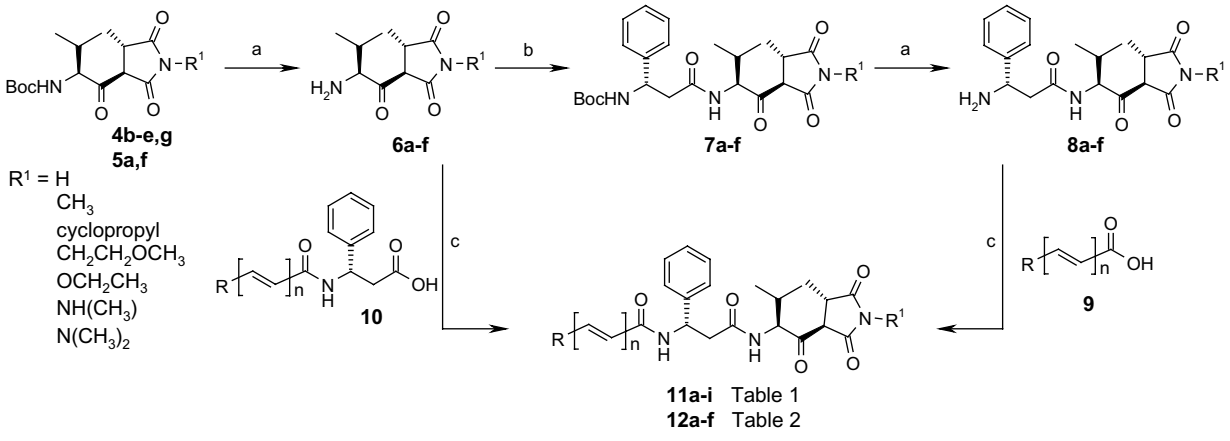


Scheme 2. Reagents and conditions: (a) H_2, Pd/C (10%), EtOH; (b) 2′-bromoacetophenone, Et_3N, CH_3CN.

The introduction of the β-phenylalanine and fatty acid subunits is shown in Scheme 3. Acidic treatment of the Boc-protected β-ketoamide pyrrolidinediones yielded primary amines **6a–f**, ready for amide formation under standard HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) peptide coupling conditions. The final products **11a–i** and **12a–f** were obtained from **6a–f** either by a stepwise procedure consisting of coupling with *N*-Boc-β-(*S*)-phenylalanine, acidic deprotection and final introduction of the fatty acid **9**, or alternatively, by coupling with a preformed β-amino acid–fatty acid building block **10**.

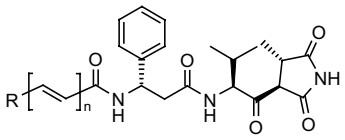
Compounds **13–15** with variations of the pyrrolidinedione 4-methyl group and the piperidine dione derivatives **16a–c** were prepared according to the prescribed procedure, starting from the appropriate dicarboxylic acids or anhydrides.

All compounds were tested for target activity against the carboxyltransferase AccAD subunits derived from *Staphylococcus aureus* and *Escherichia coli*, representing Gram-positive and Gram-negative pathogens, respectively. Antibacterial activity was measured versus *S. aureus* and *Streptococcus pneumoniae* as Gram-positive bacteria, and versus two *E. coli* strains, one of them being an efflux pump deletion strain.[14]



Scheme 3. Reagents and conditions: (a) HCl, 1,4-dioxane; (b) *N*-Boc-β-(*S*)-phenylalanine, HATU, *i*-Pr_2EtN, CH_2Cl_2/DMF; (c) HATU, *i*-Pr_2EtN, CH_2Cl_2/DMF.

Table 1. IC_{50} and MIC values of fatty acid side chain derivatives

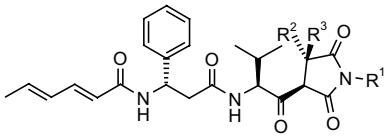


Compound	R	*n*	IC_{50} (nM)		MIC (μg/mL)			
			E. coli	*S. aureus*	*E. coli* HN818	*E. coli* Neumann	*S. aureus* 133	*S. pneumoniae* G9A
11a	CH_3	2	6	96	4	32	8	32
11b	CH_3	3	13	305	32	>64	8	8
11c	CO_2H	1	2	317	>64	>64	>64	>64
11d	4-Imidazolyl	1	6	151	64	>64	>64	>64
11e	Ph	1	9	75	2	32	8	32
11f	3-CH_3O–Ph	1	4	43	2	32	4	16
11g	3,4-Di-Cl–Ph	1	7	288	1	8	2	32
11h	4-Ph–Ph	1	37	540	>64	>64	2	16
11i	6-F-2-pyridyl	0	1	50	1	8	8	16

Variation of the fatty acid side chain revealed that quite diverse substituents were tolerated in this position (Table 1). Natural product moiramide B **11a** displayed IC_{50} values of 6 nM versus *E. coli* AccAD and 96 nM versus the corresponding *S. aureus* enzyme. Synthetic compounds with relatively small, polar residues like carboxylic acid **11c** or imidazole **11d** were similar potent on target level as derivatives with significantly larger, highly lipophilic residues like biphenyl compound **11h**. In general, IC_{50} values were below 10 nM in most cases for the *E. coli* enzyme, whereas higher concentrations of at least 50 nM were usually needed to inhibit *S. aureus* ACC. 3-Methoxycinnamic acid compound **11f** and 5-fluoronicotinic acid derivative **11i** were the most potent compounds with IC_{50} values of 4 and 1 nM versus the *E. coli* enzyme, and 43 and 50 nM versus the *S. aureus* enzyme, respectively. Despite these rather small deviations in in vitro target potency, major differences in antibacterial activity were observed. Carboxylic acid derivative **11c**, for example, did not show any antibacterial activity even at high concentrations, despite being potent on target level, especially against the *E. coli* enzyme (IC_{50} = 2 nM). Biphenyl derivative **11h**, on the other hand, exhibited good antibacterial activity against Gram-positive bacteria although it was the least potent compound on target level. Apparently, the physicochemical properties of test compounds significantly affected antibacterial activity. This was probably due to hampered penetration of polar compounds into the bacterial cell. All compounds showed increased antibacterial activity against the *E. coli* HN818 efflux pump deletion strain compared to the *E. coli* Neumann wild type strain, indicating that pyrrolidinediones were substrates for this efflux mechanism.

In contrast to the broad structural variations tolerated at the fatty acid side chain, a much more concise structure–activity relationship was revealed for the pyrrolidinedione group (Table 2). *N*-Methyl derivative **12a** showed a 2- to 4-fold loss of potency regarding IC_{50} values and MICs compared to moiramide B **11a**. Further decrease in potency was observed with larger carbon linked substituents at the pyrrolidinedione nitrogen atom. It is interesting to note, that oxygen or nitrogen

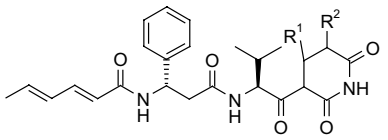
Table 2. IC_{50} and MIC values of pyrrolidinedione group derivatives



Compound	R^1	R^2	R^3	IC_{50} (nM)		MIC (μg/mL)			
				E. coli	*S. aureus*	*E. coli* HN818	*E. coli* Neumann	*S. aureus* 133	*S. pneumoniae* G9A
11a	H	CH_3	H	6	96	4	32	8	32
12a	CH_3	CH_3	H	25	211	16	>64	16	>64
12b	Cyclopropyl	CH_3	H	60	2560	>64	>64	>64	>64
12c	$CH_2CH_2OCH_3$	CH_3	H	150	1303	>64	>64	64	64
12d	OCH_2CH_3	CH_3	H	100	123	64	>64	8	32
12e	$NHCH_3$	CH_3	H	14	105	8	>64	4	16
12f	$N(CH_3)_2$	CH_3	H	33	238	4	>64	4	16
13[a]	CH_3	H	H	>200	>1000	>64	>64	>64	>64
14[a]	CH_3	CH_2CH_3	H	>200	>1000	>64	>64	>64	>64
15[a]	H	CH_3	CH_3	54	900	>64	>64	>64	>64

[a] Mixture of two diastereomers regarding pyrrolidinedione C3/4 stereochemistry.

Table 3. IC_{50} and MIC values of piperidinedione derivatives



Compound	R^1	R^2	IC_{50} (nM)		MIC (μg/mL)			
			E. coli	*S. aureus*	*E. coli* HN818	*E. coli* Neumann	*S. aureus* 133	*S. pneumoniae* G9A
16a	H	H	>300	>1000	>64	>64	>64	>64
16b	CH_3	H	>300	>1000	>64	>64	>64	>64
16c	H	CH_3	295	>1000	>64	>64	>64	>64

linked substituents were better tolerated. Compounds **12d–f** were similar potent against the *S. aureus* enzyme as unsubstituted moiramide B. The 4-(*S*)-methyl group of the pyrrolidinedione moiety seems to be essential for activity. Removal of that methyl group (**13**) or replacement by an ethyl group (**14**) caused total loss of activity. Only dimethyl derivative **15** still showed IC_{50} values at the concentration range tested. However, the potency was 10-fold reduced compared to parent compound moiramide B. Six-membered piperidinedione derivatives were evaluated, as well (Table 3). Neither nonmethylated derivative **16a**, nor 4-methyl compound **16b** showed any activity. Only 5-methyl derivative **16c** was slightly active in the *E. coli* enzyme assay.

In summary, the structure–activity relationship for the fatty acid side chain and the pyrrolidinedione moiety reflect that these two groups play entirely different roles in binding to the target.

A high degree of variability was found for the fatty acid side chain, which apparently is not involved in pivotal binding interactions with the bacterial acetyl-CoA carboxylase. Nevertheless, despite limited influence on enzyme inhibition, a significant impact on antibacterial activity was observed for the side chain variations. Cell penetration issues appear to be responsible for this effect. Consequently, variation of the fatty acid side chain should allow optimization of the physicochemical profile during the drug discovery process. On the other hand, only limited variations were tolerated in the pyrrolidinedione head group. Most derivatives showed a significant decrease or even total loss of target- and antibacterial activity. Only few selected substituents at the pyrrolidinedione nitrogen atom were not detrimental for inhibitory potency. These results indicate major relevance of the 4-(*S*)-methyl pyrrolidinedione group for efficient target binding. Synthetic variation of the β-amino acid and the β-ketoamide moiety, the two subunits that have not been addressed in this study, will show, if a significant improvement of potency compared to the natural products is feasible. A detailed understanding of the structure–activity relationship for all subunits will allow a complete evaluation of the potential of pyrrolidinediones as a new structural class with a novel mode of action for antibacterial therapy.

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