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Rapid synthesis and antimicrobial activity of novel 4-oxazolidinone heterocycles

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ABSTRACT

The synoxazolidinone family of marine natural products bear an unusual 4-oxazolidinone heterocyclic core and promising antimicrobial activity against several strains of pathogenic bacteria. As part of our research program directed at the synthesis and chemical biology of this family of natural products we have developed a one-step method for the generation of variously substituted 4-oxazolidinone scaffolds from readily available materials. These studies revealed the importance of an electron deficient aromatic ring for antimicrobial activity and serve as the basis for future SAR studies around the 4-oxazolidinone core.

The emergence of bacterial pathogens resistant to commonly employed antibiotics represents a significant threat to human and animal health.[1] Numerous approaches to combat resistant bacterial infections exist; however, the development of antibiotics bearing novel scaffolds and mechanisms of action is of significant interest.[2] Natural products have served as a fruitful source of antimicrobial agents and continue to produce exciting lead molecules.[3] The synoxazolidinone family of marine natural products, recently isolated from *Synoicum Pulmonaria* (ascidian found off the Norwegian coast), displays promising antimicrobial activity as well as a unique 4-oxazolidinone heterocyclic core (Fig. 1).[4] 4-Oxazolidinone heterocycles have received little attention from the chemical and medicinal community, likely due to their rarity in nature and perceived chemical instability.[5] From a synthetic standpoint, methods to prepare 4-oxazolidinones bearing exocyclic alkenes are limited and rely on multi-step protocols that are intolerant of diverse functional groups, thereby inhibiting rapid probe generation and structure-activity relationship (SAR) studies.[6]

As part of our program directed toward the discovery of novel scaffolds with antimicrobial activity we have explored several approaches for the preparation of 4-oxazolidinone heterocycles (Fig. 2). During our initial efforts targeted at the natural products themselves, we developed an imine acylation approach to generate 4-oxazolidinones bearing the chloride and guanidine present in **1**,

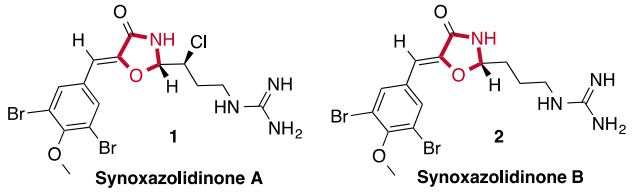

Figure 1. Synoxazolidinone family of marine natural products.

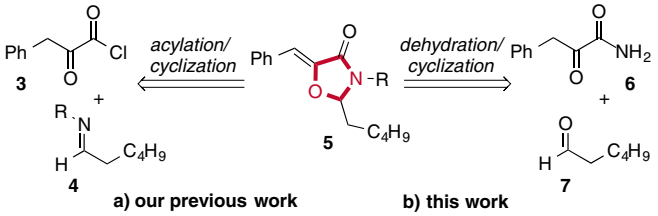

Figure 2. Approaches to 4-oxazolidinones developed in our laboratory.

along with a small set of analogs to define an initial SAR for the synoxazolidinone family of natural products (Fig. 2a).[4d] During these efforts we also optimized an acid-promoted dehydration/cyclization approach to 4-oxazolidinones that couples a phenylpyruvic amide (**6**) with an aldehyde (**7**) in one step (Fig. 2b). Our development of this method and antimicrobial evaluation of the resulting 4-oxazolidinone products is presented herein.

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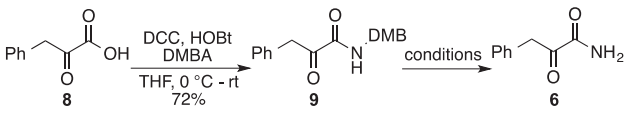
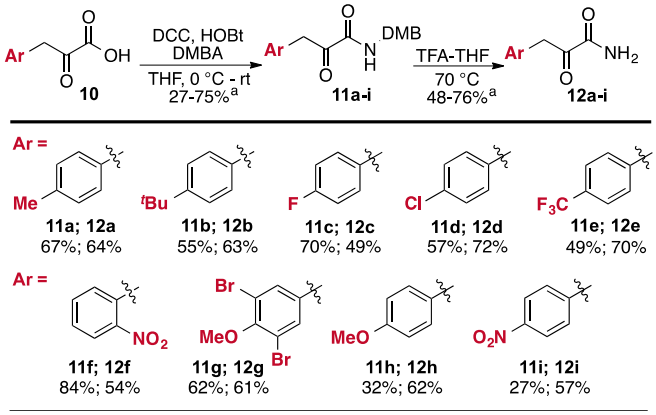
Table 1
Coupling of phenylpyruvic acid **8** to generate **9** and subsequent acid promoted cleavage



Entry	Conditions[a]	Yield (%)[b]
1	TFA-DCM (or THF), 1:1, rt	NR[c]
2	*p*-TsOH, PhMe, rt	NR[c]
3	CAN, MeCN-H_2O, 9:1, rt	NR[c]
4	BF_3•Et_2O or BF_3•AcOH, DCM	NR[c]
5	*p*-TsOH, PhMe, 85 °C	43
6	TFA-THF, 2:1, 70 °C	57
7	TFA-THF, 1:1, 70 °C	65

[a] DCC = dicyclohexyl carbodiimide; HOBt = hydroxy benzotriazole; DMBA = 2,4-dimethoxybenzyl amine; DMB = 2,4-dimethoxybenzyl; TFA = trifluoroacetic acid; DCM = dichloromethane; THF = tetrahydrofuran; *p*-TsOH = *p*-toluene sulfonic acid; CAN = ceric ammonium nitrate.
[b] Isolated yields.
[c] NR = no reaction.
Box in this table signifies the optimized conditions.

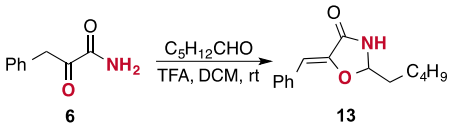
Table 2
Substrate scope for amide coupling/DMB cleavage protocol



[a] Isolated yield.

To begin our development of the dehydration/cyclization approach to **5** we first required access to phenylpyruvic amide **6**. Although the synthesis of many α-keto amides is readily accomplished through standard coupling protocols and nitrile hydrolysis, there are no reports for the preparation of highly enolizable primary phenylpyruvic amides such as **6** from their corresponding acid precursors.[4d,7] We desired an approach that converted phenylpyruvic acids to their primary amide derivatives in one step, and therefore explored the activation of enolizable α-keto acids and coupling with ammonia or ammonia surrogates. Disappointingly, all attempts to prepare **6** via this strategy provided unacceptably low yields due to the reactivity of the enol tautomer of the activated acid predominating under the reaction conditions, leading to dimeric and polymeric material and low yields of desired amide **6** (0–36% yield on 50 mg scale; <10% yield on gram scale). It became clear that a more nucleophilic amine (an easily revealed primary amide synthon) was required for the desired coupling reaction and therefore we

Table 3
Acid concentration screen for 4-oxazolidinone formation



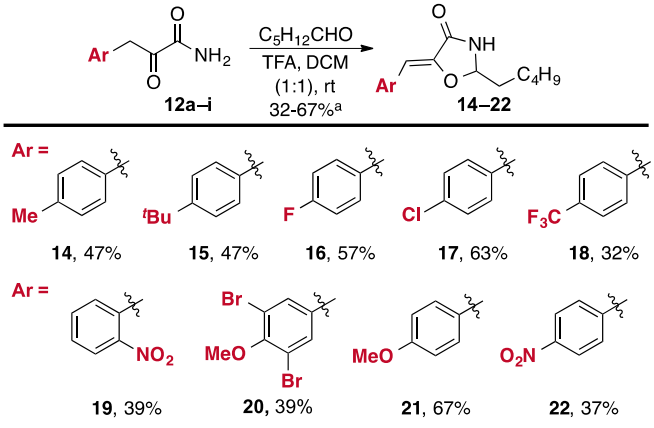
Entry	Conditions	Yield (%)[a]
1	TFA (1 equiv), hexanal (2 equiv)	< 10[b]
2	TFA (4 equiv), hexanal (2 equiv)	< 10[b]
3	TFA (4 equiv), hexanal (10 equiv)	< 10[b,c]
4	TFA (12 equiv), hexanal (2 equiv)	< 10[b,c]
5	TFA (50%, v/v), hexanal (1 equiv)	< 10[b]
6	TFA (50%, v/v), hexanal (2 equiv)	29[b,c]
7	TFA (50%, v/v), hexanal (10 equiv)	< 10[b,c]
8	TFA (50%, v/v), hexanal (2 equiv)	48[b,d]
9	TFA (66%, v/v), hexanal (1 equiv)	< 10[b,e]
10	TFA (66%, v/v), hexanal (2 equiv)	57[b]

[a] Isolated yields.
[b] Incomplete conversion of **6**.
[c] Significant impurities present.
[d] 0.4 mmol scale.
[e] Slow conversion.
Box in this table signifies the optimized conditions.

evaluated 2-step protocols for the preparation of **6** (Table 1). Coupling of acid **8** with 2,4-dimethoxybenzylamine employing DCC/HOBt provided the secondary amide **9** in 72% yield. With **9** in hand we conducted acid-promoted cleavage of the DMB group and found TFA at elevated temperature (70 °C, 1 h) provided efficient conversion to the targeted primary amide **6** (entry 7, Table 1).[8] Although we found other nucleophilic amines to also function well in the coupling reaction, this 2-step protocol afforded the most consistent yields on scale and we were able to apply this approach to a series of substituted aromatic derivatives bearing both electron rich and electron poor rings (**12a–i**, Table 2).

With scalable and rapid access to a wide range of substituted amides in hand we explored the acid catalyzed dehydration/cyclization approach to prepare 4-oxazolidinones (Fig. 2b). At the outset of this work it was unclear whether such reaction conditions would

Table 4
Substrate scope for dehydration/cyclization reaction



[a] Isolated yield.

Table 5
Antimicrobial activity of 4-oxazolidinones

Compound	MIC (μg/mL)		
	S. aureus[a]	MRSA[b]	*A. baumannii*[c]
1	12.5	20	100
2	32	32	>128
13	64	128	>128
14	32	>128	>128
15	>128	>128	>128
16	>128	>128	>128
17	>128	>128	>128
18	16	16	>128
19	>128	>128	>128
20	16	32	>128
21	>128	>128	>128
22	32	32	>128
Vancomycin	1	1	>128
Linezolid	0.5	1	>128
Tetracycline	0.25	128	2

[a]ATCC 29213.
[b]ATCC 33591.
[c]ATCC 19606.
Box in this table signifies the most potent analog.

provide 4-oxazolidinones via the desired *O*-addition or would instead provide 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones via a competing *C*-addition process.[9] We selected to focus our efforts on trifluoroacetic acid for the acid promoter since it was readily removable from our reaction products and proved compatible with the amide starting materials (Table 3). Screening of acid concentration and reactant ratios revealed that 2:1 TFA/THF (v/v) and 2 equiv of aldehyde provided the most efficient conversion, yielding 57% of **13** after purification on silica gel (entry 10, Table 3). We found that there was an ideal range for the acid/reactant ratio and reactions that were too dilute or too concentrated were significantly messier, likely due to the slow initial dehydration reaction and additional decomposition pathways of the starting material or 4-oxazolidinone product if the reaction is allowed to proceed for extended periods. It is important to note that the mass recovery observed in these reactions is high (>80%) and a significant loss of material arises during the purification process (SiO_2).

We then applied the acid promoted dehydration/cyclization reaction to the previously prepared α-keto amides (**12a–i**, Table 2) and hexanal to define the functional group tolerance of the transformation as well as to prepare a series of compounds to explore the SAR of the left hand fragment of the synoxazolidinones. Utilizing the optimized conditions (entry 8, Table 3) a number of electron rich and electron poor arylpyruvic amides were converted to their corresponding 4-oxazolidinone products in moderate to good yields (Table 4). In all cases the 4-oxazolidinone was the only significant product observed in the crude reaction mixtures and no trace of the *C*-addition regioisomer was identified.

The acid promoted dehydration/cyclization method is straightforward for the preparation of 4-oxazolidiones derived from unsubstituted aliphatic aldehydes; however, it is not compatible with more sterically hindered aldehydes such as α-chloro aldehydes, or functionalized aldehydes such as those bearing guanidine groups. Although these limitations have not allowed this approach to be extended to the synoxazolidinone family of natural products, this acid catalyzed method provides an attractive one-step synthesis of analog structures. At the outset of our efforts there was concern regarding the stability of the 4-oxazolidinone heterocycles for use as chemical probes and medicinal lead structures; however, the 4-oxazolidinone products have proven bench stable for months and can be purified by reverse phase chromatography (0.1% TFA). Further, no significant decomposition was observed in biologically relevant aqueous buffers for extended periods of time.

Compounds **13–22** were screened for their ability to inhibit the growth of three pathogenic bacterial strains and compared with natural products **1** and **2** (Table 5).[4d] The synthesized compounds were designed to probe the impact of the benzylidene fragment of the synoxazolidinones on antimicrobial activity. Overall, electron rich aryl groups (**13–15**, **21**) possessed low antimicrobial activity against Gram-positive *Staphylococcus aureus* and MRSA and no activity at the concentrations tested against Gram-negative *Acinetobacter baumannii*. Conversely, electron deficient aromatic rings (**18**, **22**) were significantly more potent with the 4-CF_3 (**18**) displaying the lowest MIC of the series. Trifluoromethyl substituted oxazolidinone **18** has subsequently inspired natural product analogs with improved activity and simplified synthetic access.[4d]

In conclusion, we have developed a one step protocol for the preparation of 4-oxazolidinone heterocycles from α-keto amides and aldehydes. In the course of this work we have also prepared primary α-keto amides bearing enolizable protons via a 2-step amine coupling/deprotection approach. These synthetic efforts have led to a series of 4-oxazolidinone products that allowed for systematic evaluation of the aromatic ring's impact in the antimicrobial activity of the synoxazolidinones. Further exploration into the SAR of this family of marine natural products and efforts to uncover the mechanism of action of our most potent compounds is underway and will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.06.003.

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