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Synthesis and SAR of novel conformationally-restricted oxazolidinones possessing Gram-positive and fastidious Gram-negative antibacterial activity. Part 1: Substituted pyrazoles

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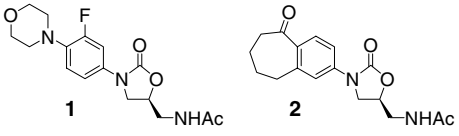
Abstract—A novel series of conformationally-restricted oxazolidinones was synthesized which possess a fused pyrazole ring substituted with various alkyl, aryl and heteroaryl substituents. A number of analogs exhibited potent activity against both Gram-positive and fastidious Gram-negative organisms.

The continual emergence of multi-drug resistant bacteria creates a pressing need for novel antimicrobial agents. Oxazolidinones, as exemplified by linezolid (Zyvox™) **1**, are a novel, completely synthetic class of antibacterial agents that possess potent activity against Gram-positive bacteria.[1] Efforts at our laboratories to identify an oxazolidinone antibacterial agent with an increased spectrum of activity as compared to linezolid led to ketone **2** which displayed activity against Gram-positive (*Staphylococcus aureus* MIC = 1.0 μg/mL, *Streptococcus pneumoniae* MIC = 0.5 μg/mL) but did not display activity against fastidious Gram-negative microorganisms.[2,3]



Genin et al. have reported a series of oxazolidinones possessing 5-membered heterocyclic ring systems (e.g., **3**, Fig. 1), some of which exhibited Gram-negative activity.[4] MIC values for compound **3** against fastidious Gram-negative organisms were in the range of 8–32 μg/mL. Building on these two results, we utilized the principles of 'conformation–restriction' towards the discovery of a novel series of oxazolidinones. Conformation–restriction would provide an entropically

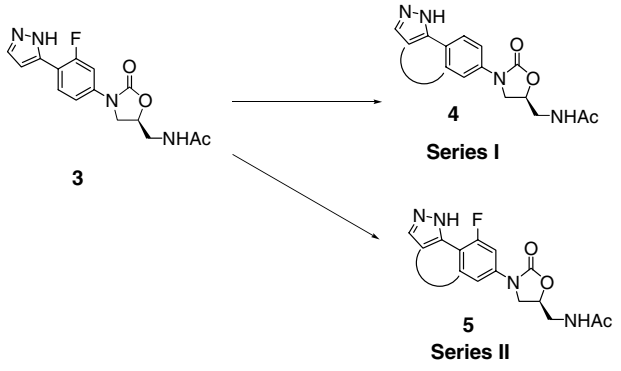


Figure 1.

Keywords: Oxazolidinones; Conformationally-restricted; Gram-positive activity; Fastidious Gram-negative activity.
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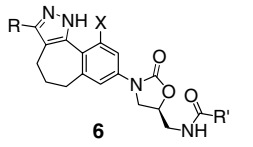
advantageous situation due to the lesser number of degrees of freedom prior to binding with the target ribosome. To design this series of compounds we started with oxazolidinone **3**.[4] Though this compound did not possess fastidious Gram-negative activity, we postulated that conformation–restriction would improve the potencies of the target molecules. Connecting the aromatic ring, either by displacing or retaining the fluorine atom on the phenyl ring, with a pyrazole ring restricts the rotation along the C–C bond. This could lead to two types of structures: one containing a phenyl ring without a fluorine atom (Series I) and the other series with a phenyl ring which possesses a fluorine atom (Series II, Fig. 1). The resulting novel structures would provide entropically favorable states or possibly even mimic 'bioactive conformations'. In order to explore the SAR we synthesized a series of fused pyrazole oxazolidinone analogs which possessed substitution on the 5-position of the pyrazole ring by alkyl, aryl and heteroaryl substituents. In this communication, we describe the synthesis and antibacterial activity of novel conformationally-restricted oxazolidinones of the general structure **6** possessing potent antibacterial activity against Gram-positive as well as Gram-negative and quinolone-resistant microorganisms.[3]



6

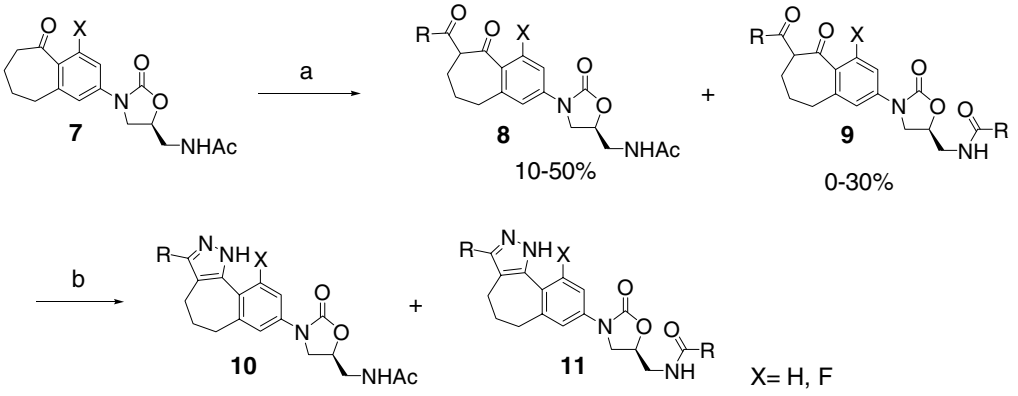
R = H, alkyl, aryl, or heteroaryl
R' = alkyl, aryl, or heteroaryl
X = H, F

The substituted pyrazoles, **10** and **11**, were synthesized from the known ketones **7**[2] as shown in Scheme 1. Treatment of ketone **7** with the appropriate acid chloride or ester in the presence of lithium hexamethyldisilazane, lithium diisopropylamide or lithium *t*-butoxide gave the di-keto compounds **8** in 10–50% yield. In some cases the transamidation products **9** were observed and isolated by flash chromatography. Treatment of **8** or **9** with hydrazine hydrate in ethanol at room temperature gave the desired substituted pyrazoles **10** and **11** (22–75% yield). The unsubstituted pyrazole analogs **10a** and **10w** (R = H) were prepared by treating the appropriate ketone with dimethyl formamide dimethyl acetal in refluxing 1-propanol overnight. The resulting compounds were then subjected to hydrazine hydrate in ethanol at room temperature overnight to yield the desired compounds (38% and 65%, respectively, over two steps).

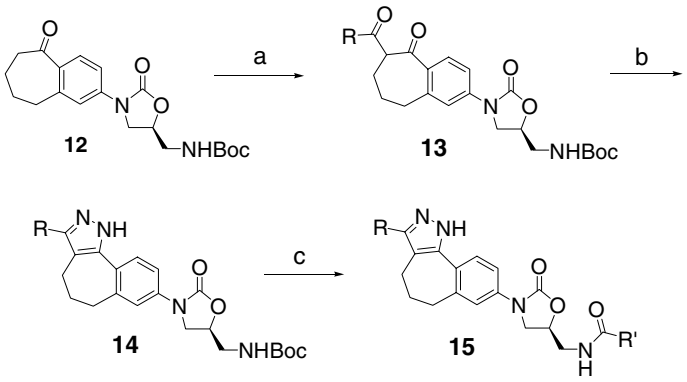
Alternatively, pyrazoles of the general structure **15** in which R does not equal R′ were obtained as shown in Scheme 2. Ketone **12** was treated with the appropriate acid chloride or ester in the presence of lithium hexamethyldisilazane, lithium diisopropylamide or lithium *t*-butoxide to afford the di-keto compounds **8**. Reaction of **13** with hydrazine hydrate in ethanol at room temperature gave the desired pyrazole intermediates **14**. Boc removal with 4 N HCl in 1,4-dioxane followed by treatment with an acid chloride resulted in compounds **15**.

The pyrazole oxazolidinone analogs were tested against a panel of Gram-positive and fastidious Gram-negative bacteria. Minimum inhibitory concentration (MIC, in μg/mL) values were determined by micro broth methodology.[5] The *Escherichia coli* in vitro transcription and translation (TnT) assay was performed in 96-well micro titer plates using a luciferase reporter system.[6] The activities of the pyrazole analogs are summarized in Table 1. MIC data for linezolid **1** is provided for comparison.

The majority of analogs in this series displayed improved activity against both Gram-positive bacteria as well as fastidious Gram-negative bacteria compared to linezolid **1** (Table 1). The unsubstituted pyrazole **10a** exhibited the lowest IC_{50}, (0.45 μM) in the *E. coli* in vitro transcription and translation (TnT) assay of any compound tested in this series of analogs. The in vitro activity of **10a** displayed a fourfold improvement against Gram-positive pathogens *S. aureus* and *S. pneumoniae* and a two and fourfold improvement against the fastidious Gram-negative bacteria *H. influenzae* and *M. catarrhalis* (MICs = 4 and 2 μg/mL, respectively) as compared to linezolid. Substitution on the 5-position of the pyrazole ring with alkyl substituents (**10b**–**10f**) generally led to analogs with one to twofold decrease in activity against Gram-positive pathogens and similar or somewhat less activity against the fastidious Gram-negative bacteria. Only the ethyl-substituted

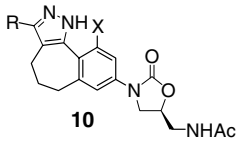


Scheme 1. Reagents and condition: (a) R(C═O)Cl or RCO_2Et, LiHMDs or LDA or *t*-BuOLi; (b) hydrazine hydrate, EtOH, rt.[4]



Scheme 2. Reagents and conditions: (a) R(C═O)Cl or RCO_2Et, LiHMDs or LDA or *t*-BuOLi; (b) hydrazine hydrate, EtOH, rt; (c) i—4 N HCl/1,4-dioxane, rt; ii—R′(C═O)Cl, NEt_3, CH_2Cl_2, 0 °C-rt.

Table 1. Enzymatic activity (IC_{50}, μM) and minimum inhibitory concentrations (MICs, μg/mL) for compounds **1**, **2**, and **10a**–**aa**

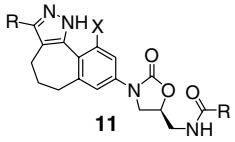


Compound	R	X	EC TnT IC_{50}, μM	S. a. MIC	S. p. MIC	S. py. MIC	E. f. MIC	H. i. MIC	M. c. MIC
1 linezolid	n/a	n/a	0.95	2	1	2	4	8	8
2	n/a	n/a	3.9	1	0.5	0.5	2	—	—
10a	–H	H	0.45	0.50	0.25	0.125	0.25	2	2
10b	$–CH_3$	H	1.2	1	0.5	0.5	1	4	4
10c	$–CF_3$	H	1.3	1	0.25	1	1	4	1
10d	$–CH_2CH_3$	H	1.1	0.5	0.125	0.125	0.25	4	1
10e	$–CH_2CH_2CH_3$	H	3.1	2	0.5	0.5	1	16	4
10f	$–CH(CH_3)_2$	H	4.3	2	0.5	1.0	2	16	8
10g	–Ph	H	2.0	0.5	0.5	0.5	0.5	8	2
10h	–Ph(4-Fl)	H	1.5	0.5	0.25	1	0.5	8	1
10i	–Ph(4-OH)	H	1.7	1	0.25	2	0.5	4	2
10j	–Ph(4-CN)	H	1.4	0.5	0.25	0.125	0.25	4	1
10k	3-Pyridyl-	H	1.0	1	0.125	0.25	0.5	4	2
10l	4-Pyridyl-	H	1.0	2	0.25	0.25	1	4	2
10m	Furan-3-yl-	H	0.70	0.5	0.25	0.125	0.25	4	1
10n	**Isoxazol-5-yl-**	H	**0.76**	**0.25**	**<0.06**	**<0.06**	**0.125**	**2**	**1**
10o	5-Methyl-isoxazol-3-yl-	H	0.90	0.5	0.25	0.25	0.5	4	2
10p	Thiazol-4-yl-	H	1.1	1	0.125	1	0.5	2	1
10q	4-Methyl-[1–3]thiadiazol-5-yl-	H	0.70	0.5	0.25	0.125	0.5	8	2
10r	1,5-Dimethyl-1H-pyrazol-3-yl-	H	1.3	0.5	0.25	0.25	0.5	8	4
10s	2-Phenyl-thiazol-4-yl-	H	>11.4	8	0.5	1	8	>64	>64
10t	5-Phenyl-[1,3,4]oxadiazol-2-yl-	H	—	>64	0.25	0.25	>64	16	>64
10u	Benzofuran-2-yl-	H	>31.2	2	0.5	0.5	0.5	>64	8
10v	Benzothiazol-2-yl-	H	—	>64	>64	>64	>64	>64	>64
10w	–H	F	2.1	2	1	1	2	8	8
10x	Furan-2-yl-	F	5.7	2	1	1	2	64	8
10y	Isoxazol-5-yl-	F	1.4	1	0.5	0.25	0.50	>64	4
10z	5-Methyl-isoxazol-3-yl-	F	2.1	1	0.5	0.5	0.5	>64	4
10aa	1,5-Dimethyl-1H-pyrazol-3-yl-	F	4.2	4	1	1	2	>64	32

Strains: S. a., *Staphylococcus aureus* UC-76 SA-1; S. p., *Streptococcus pneumoniae* SV1 SP-3; S. py., *Streptococcus pyogenes* C-203, SP1-1; E. f., *Enterococcus faecalis* MGH-2 EF1-1; H. i., *Haemophilus influenzae* HI-3542; M. c., *Moraxella catarrhalis* BC-3531.

derivative **10d** exhibited similar activity against all assayed species as the unsubstituted pyrazole analog **10a**. Generally substitution on the pyrazole ring with aryl or heteroaryl substituents (**10g**–**10v**) led to analogs with similar activity as **10a** against Gram-positive pathogens. Analogs with larger heteroaryl substitution (**10s**–**10v**) exhibited a decrease in antibacterial activity against *H. influenzae* and *M. catarrhalis*. One compound in the heteroaryl-substituted pyrazole series stood out. The derivative with isoxazol-5-yl-substitution **10n** exhibited a marked improvement in activity against Gram-positive pathogens with MICs of 8- to >33-fold lower than those exhibited by linezolid **1** against *S. aureus*, *S. pneumoniae* and *S. pyogenes*. Additionally **10n**

Table 2. Enzymatic activity (IC_{50}, μM) and minimum inhibitory concentrations (MICs, μg/mL) for compounds **1**, **10b**, and **11a–h**

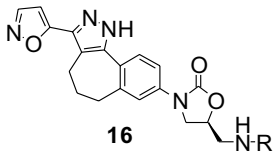


11

Compound	R	X	EC TnT IC_{50}, μM	S. a. MIC	S. p. MIC	S. py. MIC	E. f. MIC	H. i. MIC	M. c. MIC
1 linezolid	n/a	n/a	0.95	2	1	2	4	8	8
10b	$-CH_3$	H	1.2	1	0.5	0.5	1	4	4
11a	$-CH_2CH_2CH_3$	H	5.6	2	1	1	2	>64	8
11b	$-CH(CH_3)_2$	H	7.9	8	4	4	8	64	32
11c	3-Pyridyl-	H	3.7	8	1	2	4	>64	16
11d	Furan-3-yl-	H	3.5	2	1	1	1	8	4
11e	5-Methyl-isoxazol-3-yl-	H	—	>64	1	2	>64	>64	>64
11f	Benzofuran-2-yl-	H	>25	32	8	4	32	>64	>64
11g	5-Methyl-isoxazol-3-yl-	F	11.5	>64	>64	>64	>64	>64	>64
11h	1,5-Dimethyl-1H-pyrazol-3-yl-	F	>25	>64	>64	>64	>64	>64	>64

Strains: S. a., *Staphylococcus aureus* UC-76 SA-1; S. p., *Streptococcus pneumoniae* SV1 SP-3; S. py., *Streptococcus pyogenes* C-203, SP1-1; E. f. *Enterococcus faecalis* MGH-2 EF1-1; H. i., *Haemophilus influenzae* HI-3542; M. c., *Moraxella catarrhalis* BC-3531.

Table 3. Enzymatic activity (IC_{50}, μM) and minimum inhibitory concentrations (MICs, μg/mL) for compounds **1**, **10n**, and **16a–f**



16

Compound	R	EC TnT IC_{50}, μM	S. a. MIC	S. p. MIC	S. py. MIC	E. f. MIC	H. i. MIC	M. c. MIC
1 linezolid	n/a	0.95	2	1	2	4	8	8
10n	–Acetyl	0.76	0.25	<0.06	<0.06	0.125	2	1
16a	–H	8.3	16	4	2	8	>64	64
16b	$-CO_2Et$	1.8	1	0.5	0.5	0.5	>64	4
16c	Difluoroacetyl-	0.21	0.5	0.25	0.125	0.5	4	1
16d	Cyclopropanecarbonyl-	1.3	1	0.25	0.25	0.5	32	1
16e	Isoxazole-5-carbonyl-	16.8	1	0.5	0.5	1	32	4
16f	Pyridine-3-carbonyl-	2.8	2	1	1	1	>64	32

Strains: S. a., *Staphylococcus aureus* UC-76 SA-1; S. p., *Streptococcus pneumoniae* SV1 SP-3; S. py., *Streptococcus pyogenes* C-203, SP1-1; E. f., *Enterococcus faecalis* MGH-2 EF1-1; H. i., *Haemophilus influenzae* HI-3542; M. c., *Moraxella catarrhalis* BC-3531.

displayed 4- and 8-fold increase in activity against the fastidious Gram-negative bacteria *H. influenzae* and *M. catarrhalis* compared to linezolid. The presence of fluorine on the aromatic ring did not prove to be favorable for antibacterial activity. The analogs which possessed fluoro substitution (**10w–10aa**) in general displayed less antibacterial activity than the corresponding compounds that did not possess a fluorine atom. This is particularly evident when comparing the most active analog **10n** with the corresponding compound with aromatic fluorine substitution **10y.** The fluorinated analog was seen to be less active against the target organisms with a complete loss of antibacterial activity against fastidious Gram-negative *H. influenzae*.

Several compounds were assayed in which the C_5-acetamide side chain on the oxazolidinone ring was replaced with other amide substitutions. Compounds where the same R group was substituted on the pyrazole ring as was present on the C_5-amide (analogs of the general structure **11**) were generally less active than the parent C_5-acetamide **10b** against Gram-positive bacteria and for the most part inactive against the fastidious Gram-negative organisms (Table 2). The furan-3-yl analog **11d** did exhibit similar activity against all assayed species as linezolid but was significantly less active than the C_5-acetamide analog with furan-3-yl substitution on the pyrazole **10m**.

To further investigate the effect of C_5-acetamide replacement on antibacterial activity, a series of analogs was prepared in which the R group on the pyrazole ring which displayed the most favorable antibacterial activity, the 5-isoxazole moiety, was held constant and the methylene amido substitution group was varied (general structure **16**).

Although most of the analogs in this series with acyl replacement exhibited better activity against Gram-positive bacteria than linezolid, none exhibited improved activity than the original acetamide derivative **10n** (Table 3). The best acyl replacement analog in this series

is the difluoroacetyl analog **16c** which was the only derivative in this series to maintain favorable activity against *H. influenzae*.

In summary, oxazolidinones of structure **6** were synthesized in which the fused pyrazole ring was substituted with various alkyl, aryl and heteroaryl substituents. Several members of this structural series exhibited potent activity against both Gram-positive and fastidious Gram-negative organisms. The most potent compound in this series, **10n,** was shown to possess broad-spectrum activity with MICs in the range of <0.06–0.25 μg/mL for Gram-positive organisms and 1–2 μg/mL for fastidious Gram-negative organisms. In addition, analogs were generated in which the acetyl group on the methyl amino moiety of the oxazolidinone ring was replaced with various acyl groups (structures **11** and **16**). Acyl replacement was not seen to be beneficial for desired antibacterial activity.

References and notes

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