Antibacterial Oxazolidinones Possessing a Novel C-5 Side Chain. (5*R*)-*trans*-3-[3-Fluoro-4-(1-oxotetrahydrothiopyran-4-yl)phenyl]-2-oxooxazolidine-5-carboxylic Acid Amide (PF-00422602), a New Lead Compound

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Abstract: Oxazolidinones possessing a C-5 carboxamide functionality (reverse amides) represent a new series of compounds that block bacterial protein synthesis. These reverse amides also exhibited less potency against monoamine oxidase (MAO) enzymes and thus possess less potential for the side effects associated with MAO inhibition. The title compound (**14**) showed reduced in vivo myelotoxicity compared to linezolid in a 14-day safety study in rats, potent in vivo efficacy in murine systemic infection models, and excellent pharmacokinetic properties.

Oxazolidinones, as exemplified by (*S*)-*N*-((3-fluoro-4-morpholinophenyl)-2-oxaoxazolidin-5-yl)methyl)acetamide (linezolid, **1**), are a novel, completely synthetic class of antibacterial agents that possess potent activity against Gram-positive bacteria. Herein is reported a series of oxazolidinones bearing a novel C-5 side chain (reverse amide) replacement for the typical substituted aminomethyl side chain that provides a safety advantage over earlier oxazolidinones. Structure−activity studies in this novel series eventually led to the discovery of **14** (PF-00422602), which possesses good antibacterial efficacy in animal models and good pharmacokinetic properties in multiple species.

The continual emergence of multidrug-resistant Gram-positive bacterial pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA[a]), *Staphylococcus epidermidis* (MRSE), and vancomycin-resistant enterococci (VRE) creates a need for novel antimicrobial agents.[1,2] Linezolid **1**, the first member of a new class of synthetic antibacterials (oxazolidinone family) approved by the FDA, possesses Gram-positive activity, including activity versus the above-mentioned resistant strains.[3] This drug was proven to be effective in treating skin and soft tissue infections caused by *S. aureus* or *S. pyogenes*, nosocomial pneumonia caused by *S. aureus* or *Streptococcus pneumoniae*, and VRE infections.

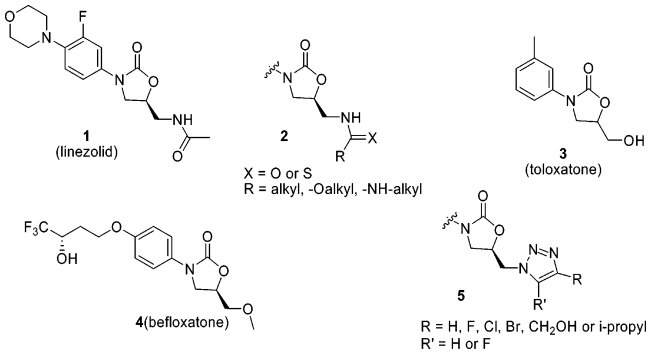

Figure 1. Structures.

Generally, these antibacterial oxazolidinones possess a C-5 aminomethyl functionalized as the amide, urethane, urea, or thiourea (**2**).[4−6] On the basis of the structures of known reversible and competitive inhibitors (e.g., toloxatone **3**, befloxatone **4**) of monoamine oxidase A (MAO-A),[7−9] it is not surprising that linezolid has safety considerations in its label regarding MAO inhibition. In the literature, linezolid is shown to be a weak reversible inhibitor of MAO. In the literature, other side chains such as $-CH_2$-heterocycle (**5**) are reported to address the MAO inhibition issue.[10] Herein we report a novel C-5 side chain for these oxazolidinones, namely, $-CONH_2$ [termed "reverse amide" to differentiate from the orginal methylamide ($-CH_2NHCOR$) functionality]. Analogues bearing a C-5 reverse amide are of lower molecular weight and easily accessible via total synthesis. This structural modification affords analogues with the reduction in MAO inhibition and a reduction in myelotoxicity, which can be a significant side effect with linezolid when used over the long term (>21 days).[11]

As shown in Scheme 1, the reverse amides described herein were synthesized from the corresponding anilines (**6**) or carbamates (**7**) by treating with *n*-butyl (*R*)-glycidate in the presence of a Lewis acid or potassium (*R*)-glycidate.[12] The intermediates **8** and **9** thus obtained were further manipulated to afford the final products **10**. The carbamate used to prepare **11** was described in the synthesis of **1**.[13] Compound **13** was prepared by coupling the 5-(*R*)-3-[4-trimethylstannyl-3-fluorophenyl]-2-oxo-5-oxazolidinonecarboxamide with 3,6-dihyro-2*H*-pyran-4-yltrifluoromethanesulfonic acid ester in the presence of palladium,[14,15] followed by reduction of the double bond. The amine used to prepare **14** was synthesized in a stereodivergent fashion as detailed previously.[16] A similar procedure was adopted to synthesize the aniline precursor to difluoro analogue **15**.

These oxazolidinone analogues were tested against a panel of Gram-positive and fastidious Gram-negative bacteria. Minimum inhibitory concentration (MIC, μg/mL) values were determined by microbroth technology.[17] Selected analogues were also tested against levofloxacin and oxacillin resistant *S. aureus* (LORSA), levofloxacin, pencillin resistant *S. pneumoniae* (LPRSP), and vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* (VREF) strains for the determination of MIC_{90}. The *Escherichia coli* in vitro transcription and translation assay (TnT), providing an assessment of protein synthesis inhibition in a cell-free format, was performed in 96-well microtiter plates using a luciferase reporter system.[18,19] MAO inhibition was measured using recombinant enzymes and a

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[a] Abbreviations: MAO, monoamine oxidase; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; VRE, vancomycin-resistant enterococci; FDA, Food and Drug Administration; MIC, minimum inhibitory concentration; LORSA, levofloxacin and oxacillin resistant *Staphylococcus aureus*; LPRSP, levofloxacin and pencillin resistant *Streptococcus pneumoniae*; VREF, vancomycin resistant *Enterococcus faecalis*; TnT, transcription and translation assay; PEG, polyethylene glycol; PK, pharmacokinetic; CYP, cytochrome P; NOAEL, no adverse effect level.

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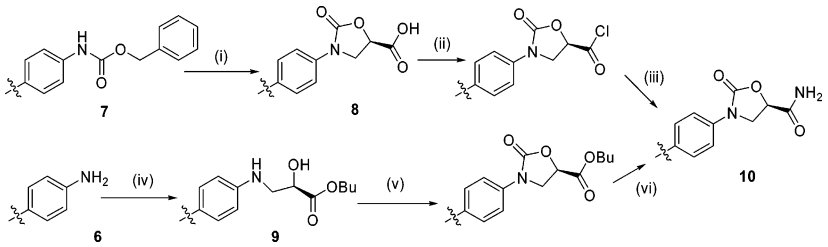
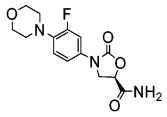
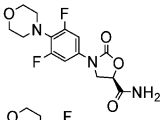
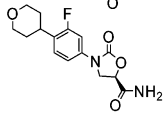
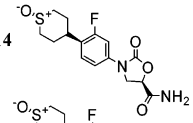
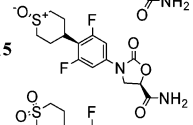
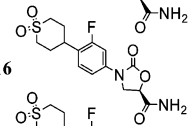
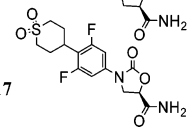
Scheme 1[a]



[a] Reagents and conditions: (i) *n*-butyllithium, −78 °C, potassium (*R*)-glycidate; (ii) oxalylchloride, room temp; (iii) ammonia, 0−25 °C; (iv) *n*-butyl (*R*)-glycidate, lithium triflate, 60 °C; (v) 1,1′-carbonyldimidazole, room temp; (vi) ammonia, room temp.

Table 1. Enzymatic Activity (IC_{50}), Minimum Inhibitory Concentrations (MIC, μg/mL), and Human Monoamine Oxidase Activity (IC_{50}) of Oxazolidinones (MIC Values in Bold Are MIC_{90} Values for the Denoted Organism

Compound		E. *coli* TnT IC_{50} (μM)	S. *aureus*[a] MIC (μg/mL)	S. *pnuemoniae*[b] MIC (μg/mL)	E. *faecalis*[c] MIC (μg/mL)	E. *faecium*[d] MIC (μg/mL)	Human MAO-A Ki (μm)
1	linezolid	3.6	**4**	**1**	**2**	**4**	53
11		23	4	4	32	32	146
12		17	**4**	**4**	**8**	**8**	63
13		6.1	4	2	4	8	58
14		3.4	**2**	**2**	**4**	**4**	546
15		3.6	**2**	**2**	**2**	**2**	355
16		5.89	**2**	**2**	**2**	**4**	105
17		6.28	4	4	4	8	293

[a] *Staphylococcus aureus* UC-76 SA-1. [b] *Streptococcus pneumoniae* SV1 SP-3. [c] *Enterococcus faecalis* MGH-2 EF-1. [d] *Enterococcus faecium.*

chromogenic substrate as described in the literature.[20] The activities of the oxazolidinone analogues are summarized in Table 1, and the values for linezolid are included for comparison.

Reverse amides **13**−**17** demonstrated significant inhibition of TnT activity and had potent Gram-positive antibacterial activities. However, **11** and **12** are less active compared to linezolid (Table 1). In general, the activities of the reverse amides were somewhat similar to or slightly less than those of the corresponding C-5 acetamide analogues. Among the reverse amide analogues, oxazolidinones possessing difluorophenyl or monofluorophenyl groups showed better antibacterial activities than their des-fluoro congeners.[21] Against the fastidious Gram-negative strains *Haemophilus influenzae* and *Moraxella catarrhalis*, these reverse amides (**11**−**17**) showed MIC values of >8 μg/mL. Reverse amides **12** and **14**−**16** were also tested for their MIC_{90} values; the strains include drug-resistant *S. aureus*, *S. pneumoniae*, *E. faecalis*, and *E. faecium* (VREF) as described above, and results are shown in bold in Table 1. More interestingly, reverse amide analogues (**11** and **14**−**17**) showed reduced human MAO-A inhibition compared to linezolid. This suggests that these compounds have much less potential than linezolid to alter neurotransmitter homeostasis and/or cause adverse drug interactions with compounds whose clearance is MAO-mediated (e.g., sympathomimetic amines). Time kill kinetics indicate that oxazolidinone **14** is bacteriostatic versus staphylococci and enterococci at 48 h following continuous drug exposure at (2−16)× the MIC. In contrast, continuous exposure of **14** at (2−16)× the MIC results in eradication of *S. pneumoniae* at 48 h. These results are similar to those of linezolid. Selected carboxamide analogues were tested for in vivo efficacy in mouse systemic *S. aureus* and *S. pyogenes* infection models via oral dosing. These analogues showed

Table 2. Antibacterial Efficacies (PD_{50}) of Selected Reverse Amides in a Mouse Systemic Infection Model[a]

compd	strain	MIC (μg/mL)	PD_{50} (mg/kg)[b]
1	*S. aureus* (UC-76)	4	3.1−8.0
1	*S. aureus* (SA-1417, MRSA)	4	6.25
1	*S. pyogenes* (C-203)	4	5
11	*S. aureus* (UC-76)	4	8.22
12	*S. aureus* (UC-76)	4	6.7
14	*S. aureus* (UC-76)	2	1.25
14	*S. aureus* (SA-1417, MRSA)	4	1.7
14	*S. pyogenes* (C-203)	4	<5
15	*S. aureus* (UC-76)	2	1.1
16	*S. aureus* (UC-76)	2	2.1
17	*S. aureus* (UC-76)	4	15

[a] Female CD-1 mice were challenged with 100 × LD_{50} of bacterial inoculum by intraperitoneal injection, and therapy was initiated immediately by administering the drug by gavage at 2× increasing doses. Mortality of mice was monitored after 7 days of the infection. [b] PD_{50} is the dose at which there is 50% mortality.

Table 3. PK Properties of Selected Oxazolidinones in Sprague-Dawley Male Rats[a]

parameter	**14**	**15**	**16**
C_{max} (μg/mL), po	15.9	4.69	1.88
AUC (μg·h/mL), po	109	15.40	8.62
CL ((mL/min)/kg), iv	15.6	12.8	12.9
V_{dss} (L/kg), iv	6.2	0.83	1.11
$t_{1/2}$ (h), po	5.8	2.24	1.58
F (%)	83	65	40

[a] For po: dose of 20 mg/kg, formulation of 5% PEG 2000/95% methyl cellulose (0.5%). For iv, dose of 10 mg/kg, formulation of 5% DMA/95% 50 mM Tris base.

Table 4. PK Properties of Compound **14** in Mouse and Dogs[a]

parameter	mouse	dog
C_{max} (μg/mL), po	12.3	17.9
AUC (μg*hr/mL), po	48.3	76.2
CL ((mL/min)/kg), iv	25.2	5.7
V_{dss} (L/kg), iv	3.3	0.7
$t_{1/2}$ (h), po	1.5	6.2
F (%)		64

[a] For mouse: po dose of 20 mg/kg, iv dose of 10 mg/kg. For dog: po dose of 40 mg/kg, formulation of 5% PEG 2000/95% methyl cellulose (0.5%); iv dose of 4 mg/kg, formulation of 5% DMA/95% 50 mM Tris base.

efficacies comparable to linezolid (Table 2), demonstrating their potential as antibacterial agents.

Selected oxazolidinones (**14**−**16**) were evaluated for their pharmacokinetic (PK) properties by dosing Sprague-Dawley rats via intravenous and oral administration. The results are shown in Table 3. Since **14** showed better PK properties compared to **15** and **16**, it was evaluated further in mouse and dog, and the results are shown in Table 4. Oxazolidinone **14** showed low plasma clearance and good oral bioavailability in rats and dogs. Pooled human liver microsomes were incubated with CYP isozymes, selective markers of metabolism, in the presence of varying concentrations of the drug to determine the potential of the drug to inhibit their metabolic activity.[22] Compound **14** also had low potential for drug−drug interactions, as this compound showed very little inhibition (IC_{50} > 900 μM) against CYP 3A4, CYP 2C9, CYP 2C19, CYP 1A2, and CYP 2D6.

Because of the excellent profile of oxazolidinone **14**, this compound was evaluated in an exploratory toxicity study in rats to evaluate myelotoxic potential. Toxicology studies of linezolid were previously performed in rats and myelotoxicity was produced, providing an animal model of this clinically relevant toxicity. Female Sprague-Dawley rats (5/group) received vehicle or **14** orally (gavage) at 200, 600, or 900 (mg/kg)/day for 14 days. All animals at 900 (mg/kg)/day were euthanized early in moribund condition. The 600 (mg/kg)/day dose produced toxicity and hematology and bone marrow changes, and 200 (mg/kg)/day was the no-adverse effect level (NOAEL). Hematology changes at 600 (mg/kg)/day included 8% increases in red blood cells, hemoglobin, and hematocrit (likely hemoconcentration) and decreases in reticulocytes (62%). The bone marrow myeloid/erythroid (*M*:*E*) ratio was increased 4.5-fold at 600 (mg/kg)/day. Compound **14** plasma AUC(0−24) values were 223 and 619 μg·h/mL at 200 (NOAEL) and 600 (mg/kg)/day, respectively. These results were compared to a 14-day oral toxicology study of linezolid in rats. Female rats were dosed by gavage with linezolid at 40, 200, or 1000 (mg/kg)/day. Lethality occurred at 1000 (mg/kg)/day, and 40 (mg/kg)/day was the NOAEL. Hematology and bone marrow changes at 200 (mg/kg)/day included decreases of 9−12% in red blood cells, hemoglobin, and hematocrit, a 97% decrease in reticulocytes, and a 8.4-fold increase in *M*:*E* ratio. Linezolid AUC(0−24) values were 105 and 666 μg·h/mL at 40 (NOAEL) and 200 (mg/kg)/day, respectively. On the basis of these in vivo data, it was concluded that **14** was at least 2× less myelotoxic than linezolid because of the higher plasma drug levels at the NOAEL and decreased hematology and bone marrow changes at higher exposures.

In summary, we report the synthesis and in vitro and in vivo activity of novel C-5 carboxamide oxazolidinones. These analogues are low in molecular weight and easy to synthesize, as demonstrated by the scale-up of reverse amide **14** in multigram quantities. This subclass of oxazolidinones showed antibacterial activities similar to those of linezolid but with a decreased monoamine oxidase inhibitory potency. These compounds also showed excellent PK properties and were low risk for drug−drug interactions. Results of in vitro time kill studies demonstrate that, like linezolid, **14** is bacteriostatic for staphylococci and enterococci but is bactericidal for streptococci. Importantly, **14** also showed a reduction in myelotoxicity compared to linezolid.

Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Data not shown.
(22) Incubations were performed in duplicate with 0.03−0.2 mg/mL human liver microsomes, substrate (30 μM phenacetin for CYP 1A2, 7.5 μM diclofenac for CYP 2C9, 50 μM phenacetin for CYP 2C19, 10 μM dextromethorphan for CYP 2D6, 50 μM phenacetin, 50 μM midazolam, 1.5 μM felodipine for CYP 3A4), 1 mM NADPH, and in the presence of a positive control (furafylline for CYP1A2, sulfaphenazole for CYP 2C9, ticlopidine for CYP 2C19, quinidine for CYP 2D6, ketoneconazole and midazolam for CYP 3A4) or drug in 50 mM potassium phosphate buffer at pH 7.4. The concentrations of drug were 0, 1, 3, 7.5, 15, 40, 100, 500, and 1000 μM. The reactions were quenched with 500 μL of 100 ng/mL acetonitrile (ice-cold) after 7−30 min. The samples were analyzed by LC/MS after centrifugation.

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