Communications to the Editor

Nitrogen−Carbon-Linked (Azolylphenyl)oxazolidinones with Potent Antibacterial Activity Against the Fastidious Gram-Negative Organisms *Haemophilus influenzae* and *Moraxella catarrhalis*

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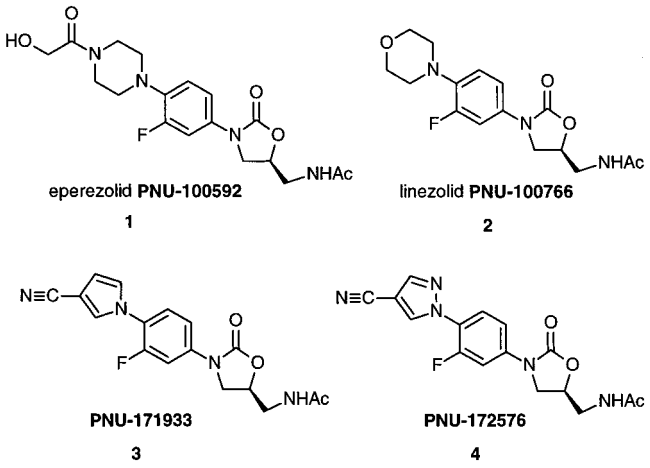
The emergence of multidrug-resistant strains of Gram-positive bacterial pathogens is a problem of ever increasing significance.[1−3] Organisms including methicillin-resistant *Staphylococcus aureus* (MRSA)[4] and *Staphylococcus epidermidis* (MRSE),[4] vancomycin-resistant enterococci (VRE),[5,6] and penicillin- and cephalosporin-resistant streptococci[7] are continually challenging scientists, physicians, and patients. In the case of VRE, most of these isolates are also resistant to other antibiotics as well, and mortality rates of >35% have been reported with VRE infection.[8] In addition, many MRSA isolates are only susceptible to vancomycin which is considered a drug of last resort.[4,9] Of particular concern is the recent emergence of staphylococcal strains with reduced susceptibility to vancomycin, the so-called vancomycin-intermediate strains, in the United States[10] and Japan.[11] Fortunately, vancomycin therapy still effected a cure in these patients. However, the probability that more highly vancomycin-resistant *S. aureus* isolates will be seen in a clinical setting is growing. Since vancomycin is considered a last resort therapy for serious infections, the spread of such an organism could be catastrophic. If such infections are to be controlled, new and powerful antibiotics will be required that act via novel mechanisms.

The oxazolidinones are one such class of totally synthetic antibacterial agents with potent activity against Gram-positive organisms.[12] They have been shown to selectively bind to the 50S ribosomal subunit and to inhibit bacterial translation at the initiation phase of protein synthesis.[13,14] Because of this unique mechanism of action, the oxazolidinones are not cross-resistant with other antibiotics. An extensive structure−activity relationship (SAR) study within Pharmacia & Upjohn led to the discovery of eperezolid (**1**, PNU-100592) and linezolid (**2**, PNU-100766).[15] These compounds are potent antibacterials in vitro and in vivo against numerous important Gram-positive human pathogens including resistant strains of *S. aureus* (MRSA), *S. epidermidis* (MRSE), and the enterococci (VRE).[12] Currently, linezolid is in phase III clinical development for the treatment of Gram-positive infections. Recently, we have been interested in increasing the Gram-positive activity as well as expanding the spectrum of activity of the oxazolidinones to include the fastidious Gram-negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*. In this Communication, we describe (pyrrolylphenyl)- and (pyrazolylphenyl)-oxazolidinones which were discovered during the course of an extensive SAR program which included the replacement of the morpholine moiety of linezolid with various aromatic five-membered nitrogen-containing heterocycles (azoles). The 3-cyanopyrrole and 4-cyanopyrazole moieties were found to impart excellent broad-spectrum antibacterial activity. Both PNU-171933 and PNU-172576 were discovered to have potent antibacterial activity in vitro versus Gram-positive and Gram-negative pathogens. Also, both compounds are very effective in vivo versus *S. aureus* and *S. pneumoniae*, and these compounds are the first class of oxazolidinones reported to demonstrate potent activity versus both Gram-positive and Gram-negative bacteria.



Chemistry. The (azolylphenyl)oxazolidinones of interest were prepared as outlined below. Various synthetic routes were employed depending on the nature of the azole heterocycle. The pyrrole derivative **3**, PNU-171933, was prepared as shown in Scheme 1. Reaction of benzylamine with 3,4-difluoronitrobenzene (**5**) yielded **6** which was hydrogenated to give **7**. Bis-protection of **7** with benzyl chloroformate yielded the fully protected *p*-dianiline **8**. Conversion of **8** to the oxazolidinone **9** was accomplished via the use of standard methods using *n*-butyllithium and (*R*)-(−)-glycidyl butyrate.[15] The oxazolidinone side-chain alcohol of **9** was then converted to the acetamide **10** via an intermediate phthalimide.[15] Hydrogenation of **10** resulted in complete deprotection

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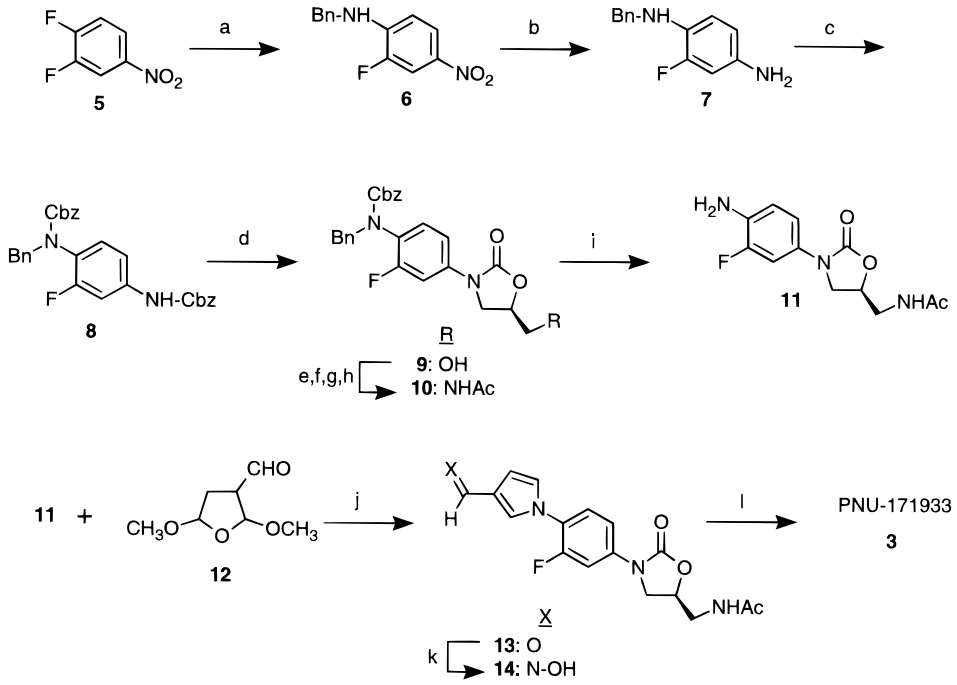
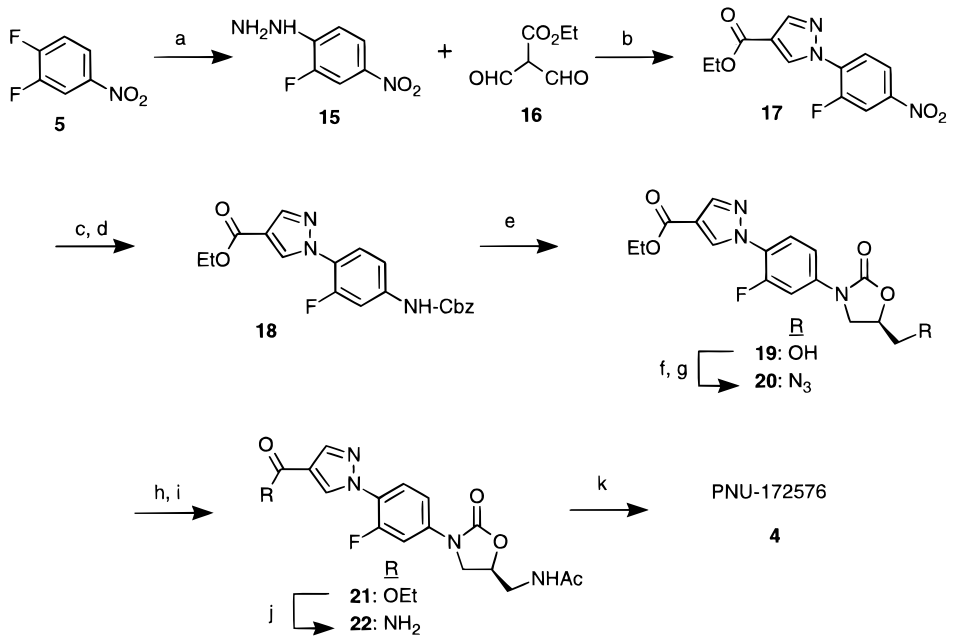
Scheme 1[a]



[a] Reagents: (a) $BnNH_2$, DIEA, CH_3CN, 90 °C, 84%; (b) H_2, 5% Pt/C, THF; (c) *N*,*N*-dimethylaniline, benzyl chloroformate, THF, 0 °C−rt, 72% for steps b and c; (d) (i) *n*-BuLi, THF −78 °C, (ii) (*R*)-(−)-glycidyl butyrate, −78 °C−rt, 77%; (e) MsCl, NEt_3, CH_2Cl_2; (f) potassium phthalimide, CH_3CN, 95 °C, 93% for steps e and f; (g) hydrazine hydrate, MeOH, 80 °C, 99%; (h) Ac_2O, pyridine, 0 °C−rt, 89%; (i) H_2, 10% Pd/C, EtOH, 78%; (j) HOAc, reflux, 85%; (k) NH_2OH, MeOH/CH_2Cl_2, K_2CO_3, 72% *E*/*Z* mixture; (l) PPh_3, CH_3CN/CCl_4, 72%.

Scheme 2[a]



[a] Reagents: (a) hydrazine hydrate, K_2CO_3, CH_3CN, 94%; (b) NaOAc, EtOH, reflux, 65%; (c) H_2, 10% Pd/C, MeOH, 96%; (d) benzyl chloroformate, $NaHCO_3$, THF, 98%; (e) (i) *n*-BuLi, THF, −78 °C, (ii) (*R*)-(−)-glycidyl butyrate, −78 °C−rt, 79%; (f) MsCl, NEt_3, CH_2Cl_2, 98%; (g) NaN_3, DMF, 65 °C, 93%; (h) (i) PPh_3, THF, (ii) H_2O, 65 °C, 77%; (i) Ac_2O, pyridine, CH_2Cl_2, 99%; (j) NH_3/MeOH, KCN, 70 °C, 34%; (k) $SOCl_2$, DMF, 0 °C, 71%.

to give the key intermediate (aminophenyl)oxazolidinone **11**. Condensation of **11** with 2,4-dimethoxy-2,5-dihydrofurancarboxaldehyde (**12**) yielded the 3-formylpyrrolyl intermediate **13**.[16] This material was then converted to the oxime **14** with hydroxylamine in good yields. Dehydration of **14** with triphenylphosphine[17] resulted in the formation of the [(3-cyanopyrrolyl)phenyl]oxazolidinone **3**, PNU-171933.

The regioselective synthesis of the pyrazole analogues was performed as outlined in Scheme 2. Treatment of **5** with hydrazine hydrate in acetonitrile gave an excellent yield of the arylhydrazine **15**. This material was then condensed with (ethoxycarbonyl)malondialdehyde[18] (**16**) in refluxing ethanol to give the 4-carbethoxypyrazole intermediate **17**.[19] Reduction of the nitro group of **17** followed by protection with benzyl chloro-

Table 1. In Vitro Antibacterial Activity, Minimum Inhibitory Concentration (MIC, μg/mL)

compound	*S.a.*[a]	*S.a.*[b]	*S.e.*[c]	*S.p.*[d]	*E.f.*[e]	*H.inf.*[f]	*M.cat.*[g]
PNU-171933	0.5	0.25	≤0.125	≤0.125	0.25	4	1
PNU-172576	0.5	0.5	≤0.125	≤0.125	0.5	4	2
13	0.25	0.25	≤0.125	≤0.125	0.25	2	1
14	1	0.5	0.25	≤0.125	0.5	4	1
21	4	4	2	1	4	>16	>16
22	>16	16	4	0.5	>16	>16	>16
linezolid	4	2	1	1	4	16	8
eperezolid	4	1	0.5	0.5	2	16	8
vancomycin	1	1	2	0.5	4	>32	>32

[a] Methicillin-susceptible *S. aureus* UC 9213. [b] Methicillin-resistant *S. aureus* UC 6685. [c] Methicillin-resistant *S. epidermidis* UC 12084. [d] *S. pneumoniae* UC 9912. [e] *E. faecalis* UC 9217. [f] *H. influenzae* UC 30063. [g] *M. catarrhalis* UC 30610. Minimum inhibitory concentration, lowest concentration of drug (μg/mL) that inhibits visible growth of the organism.

formate yielded **18** which was subjected to the asymmetric oxazolidinone ring formation[15] to give the key intermediate alcohol **19**. The alcohol side chain was converted to the acetamide **21** by previously described methods[15] via the intermediate azide **20**. This material was subjected to ammonolysis to give the carboxamide **22** which was converted to the nitrile **4**, PNU-172576, via dehydration with thionyl chloride.

Biological Results. The oxazolidinones prepared above were tested in vitro versus a panel of Gram-positive and Gram-negative bacterial isolates. Minimum inhibitory concentration (MIC) values were determined using standard agar dilution methods.[15] Also, in vivo efficacy after oral administration was determined via a lethal systemic *S. aureus* and *S. pneumoniae* infection in mice.[15] All of the pyrroles tested had excellent in vitro antibacterial activity (Table 1). The intermediate aldehyde **13** and oxime **14** were very active; however, due to the potential metabolic instability and/or reactivity of the aldehyde and the oxime moieties, they were not evaluated further. The 3-cyanopyrrole analogue **3** (PNU-171933) demonstrated excellent broad-spectrum activity versus several Gram-positive and the fastidious Gram-negative pathogens. The 4-cyanopyrazole congener **4** (PNU-172576) also has excellent broad-spectrum antibacterial activity. Interestingly, the ester analogue **21**, although equivalent to linezolid versus Gram-positive organisms, was not active against the fastidious Gram-negative bacteria. Finally, the carboxamide congener **22** was a poor antibacterial agent. Thus, PNU-171933 and PNU-172576 are very active antibacterial agents with MICs = <0.125−0.5 μg/mL versus Gram-positive bacteria and MICs = 2−4 μg/mL against the fastidious Gram-negative organisms *H. influenzae* and *M. catarrhalis*. These compounds are several times more potent versus all organisms tested than the benchmark vancomycin.

Because of their outstanding in vitro activity, **13**, **14**, PNU-171933, and PNU-172576 were tested in vivo versus *S. aureus* and *S. pneumoniae* in mouse models of human infection.[15] The analogues were dosed orally and compared to either eperezolid, linezolid, or vancomycin as controls (Table 2). When a lethal systemic infection of *S. aureus* was employed, PNU-171933 and PNU-172576 were as effective as the eperezolid control. Against *S. pneumoniae*, PNU-171933 was also as effective as eperezolid. The cyanopyrazole analogue, PNU-172576, was tested against the same infection with linezolid as a control, and it was found to be 1 order of magnitude more effective in this model than linezolid. The intermediate oxime **14** also retained in vivo activity although it was somewhat less potent than eperezolid. The aldehyde **13**, however, was devoid of in vivo activity. This is probably due to problems with bioavailability and/or metabolic stability.

In light of the outstanding in vitro and in vivo activity of PNU-171933 and PNU-172576, the pharmacokinetics of both analogues were evaluated in male Sprague−

Table 2. In Vivo Antibacterial Activity (ED_{50},[a] mg/kg/day)

organism	strain	compound	ED_{50}	control ED_{50}
S. aureus	UC 9213	PNU-171933	1.9 (1.1−2.7)	4.0 (0.1−6.6)
		PNU-172576	1.2 (0.8−2.0)	3.3 (1.8−7.0) eperezolid
		13	>20	1.7 (0.9−2.6) vancomycin
		14	5.5 (3.5−9.1)	2.6 (2.2−2.9) eperezolid
S. pneumoniae	UC 15087	PNU-171933	0.6 (0.1−1.0)	1.4 (0.8−2.0) eperezolid
		PNU-172576	0.35 (0.2−0.6)	3.2 (2.0−4.9) linezolid

[a] Effective $dose_{50}$ is the amount of drug required after oral administration (mg/kg/day) to cure 50% of infected mice subjected to a lethal systemic infection. Numbers in parentheses are 95% confidence ranges. Data shown are from one experiment (n = 36 mice/drug).

Table 3. Single-Dose Pharmacokinetics of PNU-171933, PNU-172576, and Linezolid in Male Sprague−Dawley Rats

compound	route	dose[a] (mg/kg)	C_{max}[b] (μM)	t_{max}[c] (h)	$t_{1/2\beta}$[d] (h)	V_{ss}[e] (L/kg)	CL/F[f] (mL/min/kg)	F[g] (%)
PNU-171933	iv	9.9	26.1 ± 2.8	na	5.15 ± 0.22	0.52 ± 0.07	1.18 ± 0.21	na
	po	42.9	31 ± 1.7	10 ± 1.2	5.14[h]	nc	1.43 ± 0.49	92
PNU-172576	iv	9.0	29.2 ± 3.9	na	5.35 ± 0.81	0.43 ± 0.11	1.04 ± 0.29	na
	po	46.8	29.4 ± 4.0	4.0 ± 0.0	5.35[h]	nc	1.25 ± 0.15	80
linezolid	iv	10	na	na	0.95	0.72	10.5	na
	po	25	15.8	0.3	1.05	nc	na	109

[a] n = 3. [b] Maximum plasma concentration. [c] Time at which C_{max} is achieved. [d] Harmonic mean apparent terminal disposition half-life. [e] Steady-state volume of distribution = dose[(AUMC/(AUC)^2], where AUMC is the area under the first moment curve [$AUMC = {}_0\int^\infty C(t)t\,dt$] and AUC is the area under the concentration−time curve [$AUC = {}_0\int^\infty C(t)\,dt$]. [f] Clearance = dose/AUC. [g] Absolute oral bioavailability assuming linear pharmacokinetics = $[(AUC/dose)_{po}/(AUC/dose)_{iv}] \times 100$. [h] Harmonic mean apparent terminal disposition half-life for iv dose; half-life for po dose could not be calculated because of prolonged absorption or slow clearance.

Dawley rats following single-dose intravenous and oral administration (Table 3). Both analogues demonstrated excellent pharmacokinetic profiles. Absolute oral bioavailability (F) was very good at 92% and 80%, respectively. In addition, blood levels (C_{max}) after oral dosing were high, and the harmonic mean apparent terminal disposition half-lives ($t_{1/2}\beta$) were greater than 5 h for each drug. Also, the volume of distribution (V_{ss}) was moderate, and the mean systemic clearance (CL/F) was low for each analogue (1.18 ± 0.21 mL/min/kg for PNU-171933 and 1.04 ± 0.29 mL/min/kg for PNU-172576).

In conclusion, in an effort to expand the spectrum of antibacterial activity of the oxazolidinones to include Gram-negative organisms, the (azolylphenyl)oxazolidinone subclass has been developed. Certain members of this class have very potent activity versus both Gram-positive and Gram-negative organisms. In particular, the 3-cyanopyrrole and 4-cyanopyrazole congeners **3** and **4** (PNU-171933 and PNU-172576) had *S. aureus* MICs ≤ 0.5 μg/mL and *H. influenzae* and *M. catarrhalis* MICs = 2−4 μg/mL. In addition, both analogues have outstanding pharmacokinetic profiles. Furthermore, these compounds are more effective than linezolid and eperezolid versus *S. aureus* and *S. pneumoniae* in mouse models of human infection. A detailed account of this work including the SAR of other azole analogues (imidazole, triazole, tetrazole) will be reported in due course.

Supporting Information Available: Spectroscopic data for all new compounds (7 pages). Ordering information is given on any current masthead page.

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