Structure-Activity Relationship within a Series of Pyrazolidinone Antibacterial Agents. 2. Effect of Side-Chain Modification on *In Vitro* Activity and Pharmacokinetic Parameters

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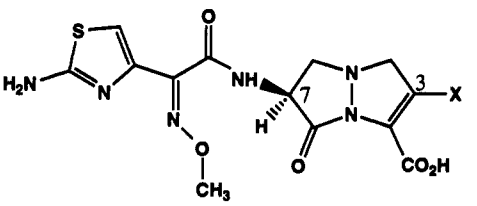
The structure-activity relationship among a series of novel pyrazolidinone antibacterial agents is described. Specifically, the effect of modification of the side chain attached to the nitrogen at C-7 was explored in an attempt to improve the potency and spectrum of activity. This approach was successful in identifying several compounds having good *in vitro* profiles. These top candidates were then evaluated for their activity *in vivo*, and their pharmacokinetic behavior in various animal models was explored. This information proved critical for the identification of candidates for clinical evaluation.

In a previous report,[1] we described the structure-activity relationship among a group of pyrazolidinone-containing antibacterial agents. From this study, two compounds were identified as exhibiting the best activity within the series. These entities, **1** (LY255262) and **2** (LY193239), are characterized by a C-3 cyano and C-3 methyl sulfone [3.3.0] pyrazolidinone nucleus, respectively (Figure 1). Although both compounds demonstrated excellent antibacterial activity *in vitro*, we desired compounds having increased potency against the gram-positive organisms (especially *Staphylococci*). In an attempt to build this activity into the bicyclic pyrazolidinones, we initiated a study of the effect of side-chain modification (i.e., the substituent acylated to the C-7 nitrogen) on *in vitro* antibacterial activity. Once this study was complete, we also examined the pharmacokinetic behavior of the best compounds in order to make an appropriate selection for clinical studies. The results of these investigations are reported herein.



1 (LY 255262) X= -CN

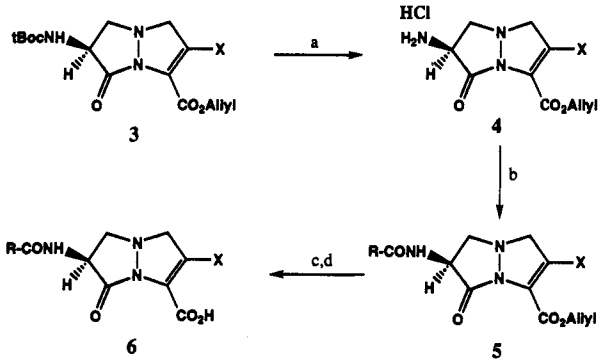
2 (LY193239) X= $-SO_2CH_3$

Figure 1.

Chemistry

The chemistry utilized to prepare the [3.3.0] pyrazolidinone nuclei **3** has been described.[2] The nuclei were deblocked and acylated with the desired side chains in a manner consistent with previously described procedures.[2b] Thus, as shown in Scheme I, the blocking group attached to the nitrogen at C-7 was removed and the resulting amine acylated with a desired acid chloride. Final removal of the blocking groups in one or two steps provided the final products suitable for biological evaluation. The carboxylic acids employed in this study were either commercially available or prepared according to literature procedures.[3] The ready availability of the nuclei **3** along with the efficient attachment of the side chains provided ready access to a large number of novel derivatives.

Scheme I[a]



[a] (a) HCl, HOAc; (b) RCO_2H, $POCl_3$, *N*-methylmorpholine; (c) TFA, Et_3SiH (for tritylated "R"); (d) $Pd(OAc)_2$, PPh_3, nBu_3SnH, Et_3SiH.

Antibacterial Activity

The first goal of the SAR study was to attempt to prepare pyrazolidinones with activity against *Staphylococci*. This important coverage, lacking in the lead compounds **1** (LY255262) and **2** (LY193239), was deemed necessary for further development of this class of antibacterials as broad-spectrum agents. In order to determine if this Gram-positive activity was obtainable with the pyrazolidinone-based antibacterials, we appended some relatively simple side chains to the 3-cyano nucleus. Our selection was based upon the knowledge that these same side chains impart Gram-positive activity to the cephalosporin-based antibacterials. As can be seen from data presented in Table I, we were able to achieve good[4] activity against *Staphylococci* in several cases (**11**, LY211090; **12**, LY193817; and **13**, LY257539). As was anticipated with these side chains, the improved activity against the Gram-positive organisms was accompanied by a corresponding loss in activity against the Gram-negative ones. Nonetheless, this brief study gave us encouragement that the desired activity against Gram-positive pathogens could be realized with the pyrazolidinone antibacterials.

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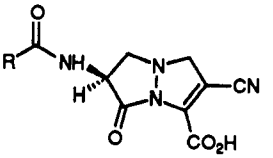
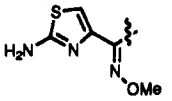
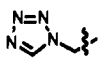
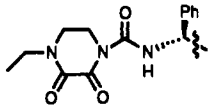
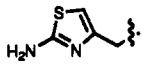
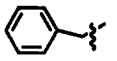
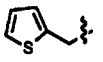
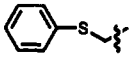
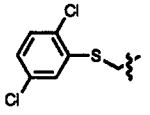
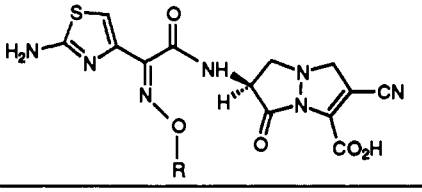
Table I



no.	code LY#	R	MIC (μg/mL)[a] *S. au.* X1.1	*S. ep.* 222	*S. pn.* PARK	*H. in.* C.L.	*E. co.* EC14	*Kleb.* X26	*E. ae.* C32	*Pseu.* PS72
1	LY255262		64	64	0.25	0.5	0.25	0.25	1	>128
7	LY257288		>128	>128	>128	>128	>128	>128	>128	>128
8	LY257105		16	32	4	0.5	1	0.5	2	128
9	LY203212		32	64	2	32	16	16	32	>128
10	LY193375		16	32	8	32	32	32	128	>128
11	LY211090		8	32	2	16	16	32	64	>128
12	LY193817		4	8	0.5	32	32	16	128	>128
13	LY257539		1	4	0.125	8	>128	8	>128	>128

[a] *S. au.* = *Staphylococcus aureus*, *S. ep.* = *Staphylococcus epidermidis*, *S. pn.* = *Streptococcus pneumoniae*, *H. in.* = *Haemophilus influenza*, *E. co.* = *E. coli*, *Kleb.* = *Klebsiella*, *E. ae.* = *Enterobacter aerogenes*, *Pseu.* = *Pseudomonas*.

Table II

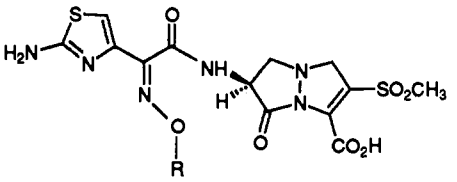


no.	code	R	MIC (μg/mL)[a] *S. au.* X1.1	*S. ep.* 222	*S. pn.* PARK	*H. in.* C.L.	*E. co.* EC14	*Kleb.* X26	*E. ae.* C32	*Pseu.* PS72
1	LY255262	CH_3	64	64	0.25	0.5	0.25	0.25	1	>128
14	LY272447	CH_2CH_2Br	8	4	0.125	0.125	0.125	0.06	0.5	>128
15	LY258818	CH_2Ph	8	8	0.06	0.25	8	0.125	8	>128
16	LY258817	$(CH_2)_3CH_3$	16	16	0.125	0.125	1	0.06	2	>128
17	LY272448	CH_2CH_2F	32	16	0.25	0.25	0.03	0.03	0.125	128
18	LY272567	CH_2CH_2Cl	32	16	0.5	0.5	0.25	0.125	1	>128
19	LY272568	$CH_2CH{=}CH_2$	32	16	0.125	0.125	0.125	0.06	0.5	>128
20	LY222969	$C(CH_3)_2CO_2H$	>128	>128	16	0.06	0.03	0.25	0.125	64

[a] See footnote *a* of Table I.

The challenge at this point was determining how to build in this Gram-positive activity *while good activity against the Gram-negative organisms is maintained.* Our original lead compounds 1 (LY255262) and 2 (LY193239) both demonstrate quite respectable Gram-negative activity. They are similar in that both bear the (*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl ("ATMO") side chain at the C-7 nitrogen. This particular side chain is popular in the cephalosporin area due to its well-known ability to dramatically improve the potency of β-lactam antibacterials.[5] Detailed structure–activity relationship studies have demonstrated that the spectrum of activity of the β-lactams can be changed by altering the group attached to the oximino functionality of the side chain.[3] We chose to adopt a similar approach with the pyrazolidinones and initially prepared a series of 3-cyanopyrazolidinones bearing substituted ATMO side chains. From the data presented in Table II, it was clear that this approach could prove fruitful. Thus, compounds such as the (2-bromoethyl)oximino derivative 14 (LY272447) and the benzyloximino derivative 15 (LY278818) demonstrated improved activity against *Staphylococci* (*Staphylococcus*

Table III



no	code	R	MIC (μg/mL)[a] S. au. X1.1	S. ep. 222	S. pn. PARK	H. in. C.L.	E. co. EC14	Kleb. X26	E. ae. C32	Pseu. PS72
2	LY193239	CH_3	32	64	0.25	0.5	0.06	0.125	0.25	>128
21	LY221820	$(CH_2)_2CH_3$	8	16	0.125	0.125	0.125	0.015	0.25	128
22	LY207631	$(CH_2)_3CH_3$	4	4	0.06	0.125	0.25	0.03	1	>128
23	LY221481	$CH_2CH{=}CH_2$	8	16	0.125	0.125	0.125	0.03	0.25	128
24	LY249767	$CH_2CH{=}C(CH_3)_2$	4	8	0.06	0.25	0.5	0.06	2	>128
25	LY207197	CH_2CCH	32	32	0.25	0.25	0.25	0.125	0.5	>128
26	LY249366	$(CH_2)_2CH{=}CH_2$	4	8	0.06	0.125	0.06	0.015	0.25	128
27	LY222433	$(CH_2)_3CH{=}CH_2$	8	8	0.5	0.25	0.25	0.06	1	128
28	LY214386	CH_2CH_2Cl	16	32	0.25	0.5	0.125	0.03	0.125	>128
29	LY278324	CH_2CH_2Br	8	8	0.125	0.125	0.125	0.03	0.125	>128
30	LY221480	CH_2CH_2F	16	16	0.06	0.25	0.015	0.03	0.06	128
31	LY233630	$(CH_2)_2CH_2F$	8	16	0.06	0.125	0.03	0.03	0.125	128
32	LY278789	$(CH_2)_3CH_2Cl$	32	32	0.25	1	1	0.125	2	>128
33	LY210581	CH_2CN	64	128	0.25	2	0.25	0.25	0.5	>128
34	LY249768	CH_2CO_2H	>128	>128	2	1	0.06	0.125	0.25	128
35	LY249367	$C(CH_3)_2CO_2H$	>128	>128	2	0.06	0.03	0.125	0.06	8

[a] See footnote *a* of Table I.

aureus and *Staphylococcus epidermidis*) while useful activity against gram-negative organisms is maintained. In view of these encouraging results, we shifted our focus to the [3.3.0] pyrazolidinones bearing a sulfone substituent at C-3. This was done primarily due to the fact that the C-3 methyl sulfone **2** (LY193239) was shown to be more potent *in vitro* than its C-3 cyano analogue **1** (LY255262).[1]

The side-chain derivatives bearing a C-3 methyl sulfone were prepared and are listed in Table III along with representative *in vitro* antibacterial activities. Alteration of the oximino substituent was observed to have a dramatic effect on the biological activity. In general, polar substituents were found to result in a decrease of activity against the Gram-positive organisms. Thus, the final three entries in the table (**33**, LY210581; **34**, LY249768; **35**, LY249367) did not achieve our goal of improving activity against *Staphylococci*. These compounds did, however, exhibit potent activity against the Gram-negative organisms. In fact, compound **35** (LY249367), bearing the same side chain as that found on the clinically utilized antibacterial ceftazidime, even exhibited activity against *Pseudomonas*.

The more lipophilic side chains provided better results with respect to improving the Gram-positive activity. Once again, although Gram-negative activity was maintained in these derivatives, there was a trade-off observed for many compounds. Thus, the prenyl derivative **24** (LY249767) exhibited very good Gram-positive activity when compared with the parent **2** (LY193239). Unfortunately, the activity against the Gram-negative organism was observed to decrease (vs *Escherichia coli* and *Enterobacter aerogenes*). Halogenated compounds such as the 2-chloroethyl (**28**, LY214386) and the 2-bromoethyl derivatives (**29**, LY278324) showed slightly improved Gram-positive activity but more importantly were found to maintain exceptional Gram-negative activities. It was felt that side chains such as these may be useful in combination with more lipophilic C-3 groups (*vide post*), which may provide the balance of activities being sought.

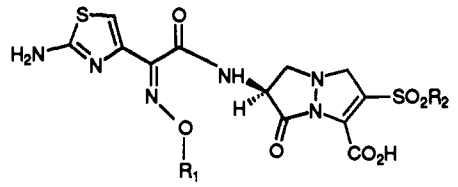
Simple straight-chain alkyl analogues such as the *n*-butyl derivative **22** (LY207631) showed improved *Staphylococcus* activity but once again were somewhat less active against the Gram-negative organisms. It was found that the Gram-negative activity could be gently atenuated by placing a double bond at the terminal end of the oximino substituent. Thus, the 3-butenyl derivative **26** (LY249366) exhibited improved Gram-negative activity as compared to its fully saturated derivative (**22**). Significantly, this modification did not disrupt the potent Gram-positive activity. Thus compound **26** became one of our prime candidates for further preclinical evaluation.

As mentioned previously, we were interested in exploring the effect of combining the derivatized ATMO side chains with different C-3 sulfone moieties on antibacterial activity. In our previous report,[1] it was demonstrated that proceeding from methyl sulfone to larger, more lipophilic sulfones was not necessarily advantageous to *in vitro* antimicrobial activity. Nonetheless, we prepared and evaluated a number of these 'hybrid' structures as outlined in Table IV. Of these compounds, the C-3 *n*-propyl sulfone derivatives functionalized with the (2-chloroethyl)oximino side chain (**38**, LY214912) and the (2-bromoethyl)oximino side chain (**43**, LY221479) were most interesting. At this point in our program, we had in hand several compounds exhibiting good, broad-spectrum activity against a large number of pathogenic bacteria. We next turned our attention to the critical preclinical pharmacokinetic evaluations.

In Vivo Evaluation

The evaluation of the *in vivo* characteristics of the pyrazolidinone antibacterials was viewed as a critical step in the further development of this new class of compounds. Our initial studies directed toward this end were to investigate the *in vivo* efficacy of the lead compounds **1** (LY255262) and **2** (LY193239) against susceptible organisms in well-accepted animal models of infection. The results, shown in Table V, indicate that both compounds

Table IV



no.	code	R_1	R_2	MIC (μg/mL)[a] *S. au.* X1.1	*S. ep.* 222	*S. pn.* PARK	*H. in.* C.L.	*E. co.* EC14	*Kleb.* X26	*E. ae.* C32	*Pseu.* PS72
2	LY193239	CH_3	Me	32	64	0.25	0.5	0.06	0.125	0.25	>128
36	LY207635	$(CH_2)_2CH_3$	*n*-Pr	16	32	0.125	0.5	0.5	0.06	1	>128
37	LY214275	CH_2CH_2Cl	Et	16	32	0.25	1	0.25	0.125	0.5	>128
38	LY214912	CH_2CH_2Cl	*n*-Pr	8	16	0.06	0.125	0.125	0.03	0.5	>128
39	LY278411	CH_2CH_2Cl	*n*-Bu	8	16	0.125	0.5	0.5	0.03	1	>128
40	LY214911	CH_2CH_2Cl	Ph	16	32	0.06	0.25	1	0.06	4	>128
41	LY278790	CH_2CH_2Cl	CH_2Ph	8	16	0.06	0.5	2	0.03	4	>128
42	LY221244	CH_2CH_2Br	Et	8	8	0.125	0.25	0.06	0.015	0.25	>128
43	LY221479	CH_2CH_2Br	*n*-Pr	8	8	0.06	0.125	0.25	0.03	0.5	>128
44	LY207199	CH_2CH_2F	Et	16	32	0.125	0.25	0.06	0.03	0.125	>128
45	LY207632	CH_2CH_2F	*n*-Pr	16	32	0.125	0.125	0.125	0.03	0.25	>128
46	LY207634	CH_2CF_3	*n*-Pr	16	32	0.125	0.25	0.25	0.06	1	>128
47	LY272096	CH_2SCH_3	Et	16	64	0.125	0.5	0.25	0.06	0.5	>128
48	LY207633	$CH_2CH{=}CH_2$	*n*-Pr	16	32	0.06	0.25	0.5	0.06	0.5	>128
49	LY272256	$CH_2CH{=}CH_2$	Allyl	8	32	0.125	0.25	0.125	0.03	0.5	>128
50	LY272097	CH_2-*cyclo*-Pr	Et	16	32	0.06	0.125	0.06	0.015	0.25	>128

[a] See footnote *a* of Table I.

Table V. In Vivo Mouse Protection Study

compound	organism	MIC (μg/mL)	route of admin	ED_{50} (mg/kg) × 2
1 (LY255262)	*S. pyogenes* C203	0.25	sc	0.625
1 (LY255262)	*S. pyogenes* (C203)	0.25	po	6.68
1 (LY255262)	*E. coli* EC14	0.25	sc	0.67
1 (LY255262)	*E. coli* EC14	0.25	po	7.87
2 (LY193239)	*S. pyogenes* C203	0.03	sc	1.80
2 (LY193239)	*S. pyogenes* C203	0.03	po	13.50
2 (LY193239)	*E. coli* EC14	0.06	sc	<0.625
2 (LY193239)	*E. coli* EC14	0.06	po	7.93

Table VI. Pharmacokinetic Parameters of Compounds 1 and 2 in Male CD-1 Mice following Oral or Subcutaneous Administration of a 20 mg/kg Dose

compound	route	C_{max} (μg/mL)	half-life (min)	urinary recovery (% dose)
1 (LY255262)	sc	71.0	12.2	80.0
	po	nd[a]	nd	<2.0
2 (LY193239)	sc	53.8	10.6	100.0
	po	nd	nd	<2.0

[a] nd = not determined.

are effective as antibacterial agents when dosed subcutaneously. When dosed orally, however, their effectiveness was much lower. This provided us with the first indication that the pyrazolidinones would not be orally bioavailable but that they could potentially treat systemic infections under the appropriate administration conditions.

We next turned our attention to detailed pharmacokinetic evaluation of the lead compounds 1 and 2. Confirming our results from the *in vivo* treatment experiments, the oral bioavailability of the compounds as determined in the mouse was extremely low based on urinary recovery after oral dosing (Table VI). In the rat model, low serum binding combined with a moderate half-life produced a pharmacokinetic profile that would not be expected to yield a once or twice daily dosing regimen in man (Table VII).

Table VII. Pharmacokinetic Parameters of Compounds 1, 2, 38, and 26 in Male Sprague–Dawley Rats following Intravenous Administration of a 20 mg/kg Dose

compound	C_{max} (μg/mL)	half-life (min)	urinary recovery (% dose)	% plasma binding[a]
1 (LY255262)	120.2	17.9	87.8	41.5
2 (LY193239)	120.5	24.7	80.4	22.0
38 (LY214912)	74.3	14.7	44.2	nd[b]
26 (LY249366)	70.3	11.3	53.1	nd

[a] Determined at 20 μg/mL via ultrafiltration. [b] nd = not determined.

With the intent of finding some exceptional pharmacokinetic characteristics, we chose to evaluate select compounds from the extended SAR described in this report. Thus, compounds 38 (LY214912) and 26 (LY249366) were examined *in vivo*. Our hope was to find compounds exhibiting a long half-life that could be dosed with once- or twice-a-day scheduling. The results of these additional experiments are shown in Table VII and graphically represented in Figure 2. Unfortunately, an exceptionally long half-life was not found for either of the new analogues. As a final study of the pyrazolidinones' pharmacokinetic behavior, compound 26 (LY249366) was prepared in large quantities and evaluated in the rhesus monkey model. When dosed intraveneously at 30 mg/kg a half-life of 36 min and C_{max} of 167.8 μg/mL was measured. Once again, this confirmed our suspicion that the compound would not be expected to exhibit an unusually long half-life in man (Table VIII).

Although a short half-life is less than desirable for a new antimicrobial agent, if oral bioavailability could be realized, more frequent dosing may be acceptable. In an attempt to render the pyrazolidinones orally bioavailable, the (pivalolyloxy)methyl ester prodrug 53 (LY222622) was prepared as shown in Scheme II. Thus, following initial ester exchange, the side-chain appendage and deblocking followed as in the previous examples. It is known that similar esters of cephalosporins dramatically enhance their oral bioavailability.[6] Unfortunately in the case of this

Table VIII. Interspecies Comparison of Plasm Half-Lives for Cefamandol, Ceftizoxime, Ceftazidime, and Compounds 1, 2, 38, and 26, following Intravenous Administration

compound	half-life (min)		
	rat	monkey	human
1 (LY255262)	18	nd[a]	nd
2 (LY193239)	25	nd	nd
38 (LY214912)	15	nd	nd
26 (LY249366)	11	36	nd
cefamandol	15	34	48
ceftizoxime	15	44	96
ceftazidime	23	49	108

[a] nd = not determined.

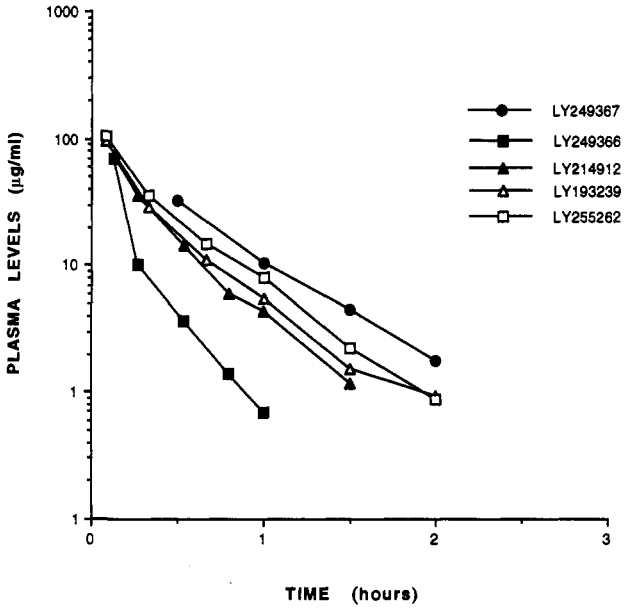
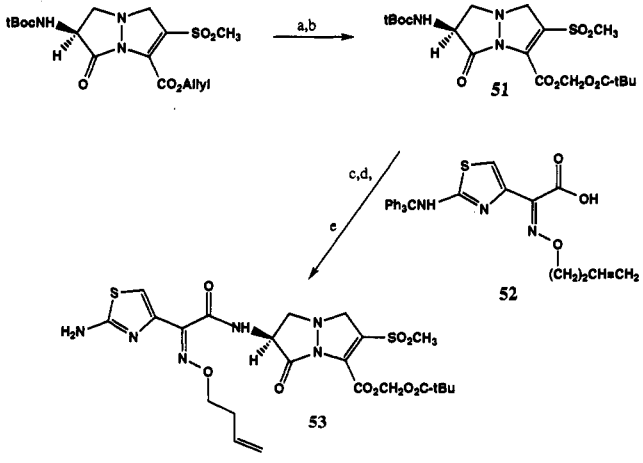


Figure 2. Plasma levels of antibacterial activity in male Sprague-Dawley rats following iv administration of compounds at 20 mg/kg.

Scheme II[a]



[a](a) $Pd(OAc)_2$, Ph_3P, sodium ethyl hexanoate; (b) $ICH_2OCOtBu$, THF, DMF; (c) HCl, HOAc; (d) **52**, $POCl_3$, *N*-methylmorpholine, CH_2Cl_2; (e) TFA, Et_3SiH.

particular pyrazolidinone ester, when dosed to rats (at a dose equivalent to 20 mg/kg of free acid), plasma levels of the parent antimicrobial (**26**; LY249366) were not detected over 4 h (detection limit 0.25 μg/mL of parent). Thus it can be concluded that the pyrazolidinone antibacterials under consideration for clinical evaluation would necessarily be administered at relatively frequent intervals via a nonoral route.

In conclusion, we have shown that the *in vitro* antimicrobial activity of the pyrazolidinone antimicrobials could be effectively modulated utilizing classical medicinal chemical approaches. This has enabled us to prepare several potent, broad-spectrum antibacterials for consideration as clinical candidates.

Experimental Section

All reactions described herein were performed under an inert atmosphere of dry nitrogen in flame-dried glassware unless otherwise noted. All reagents were used as supplied unless stated otherwise. Melting points were recorded on a Thomas-Hoover apparatus and are uncorrected. ^1H NMR spectra were recorded at 300 MHz with a General Electric QE-300 instrument, at 270 MHz with a Brucker W-M instrument and at 90 MHz with a JEOL FX-90 instrument. Chemical shifts are recorded in parts per million (δ) relative to tetramethylsilane. IR spectra were recorded on a Nicolet MX-1 FT-IR, optical rotations were measured on a Perkin-Elmer 241 spectrometer, and UV spectra were obtained on a Cary 219. The mass spectral data were obtained on either a CEC-21-140 or a Varian MAT-731 spectrometer. All MPLC separations were conducted on Merck Lobar columns (LiChroprep RP-18) with the help of a Fluid Metering Inc. pump. Analytical HPLC separations were performed on a Varian chromatographic system utilizing a MicroPak MCH-5N-cap 15 cm × 4 mm column and a variable-wavelength UV detector set to record at 254 nm.

General Procedure for the Preparation of Compounds 7-50 and 53. The following generalized experimental procedures were utilized to prepare all of the new compounds described in this report. Physical data for all compounds is contained in the supplemental material.

Deblocking of Nucleus 3. To a freshly prepared solution of anhydrous HCl in glacial acetic acid (ca. 3 M) was added the solid **3** at 0 °C (concentration of ca. 30 mg of **3**/mL of HCl/HOAc). The cooling bath was removed and the mixture stirred until complete dissolution occurred (ca. 2 min). The reaction was then evaporated to dryness *in vacuo*. The residue was further processed by treating with methylene chloride and evaporating. This sequence was repeated several times. The resulting solid was used directly in the next step without purification.

Acylation (Side-Chain Attachment). The carboxylic acid to be incorporated via acylation to the nitrogen of C-7 was dissolved in CH_2Cl_2 (ca. 1 N) and the stirred solution cooled with an ice bath. $POCl_3$ (1 equiv) was then added to the solution followed by 1 equiv of *N*-methylmorpholine. After stirring for 15 min at 0 °C, the crude deblocked nucleus (from above) was added to the preformed acid chloride as a solution in a volume of CH_2Cl_2 equal to that utilized for the acid chloride preparation. Following the addition, 3 equiv more of *N*-methylmorpholine was introduced to the reaction. Stirring was maintained and the ice bath allowed to melt over 2–3 h. The reaction was poured into water and the aqueous layer extracted twice with CH_2Cl_2. The combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated to yield the crude product, which was purified via chromatography on silica gel (eluting with mixtures of EtOAC/hexane).

Removal of Blocking Groups. In cases where a trityl group was incorporated onto the side chain, the acylated product (from above) was treated with triethylsilane (5 mL/g of starting material) and trifluoroacetic acid (8 mL/g starting material). The resulting mixture was stirred vigorously for 5–10 min and then concentrated. The residue was immediately chromatographed on silica gel (eluting with EtOAC) to provide the partially deblocked material. This crude product was directly used in the following deblocking.

To a solution of $Pd(OAc)_2$ (0.05 equiv based on total material to be deblocked) in acetone (20% of the amount used for dissolution of the material to be deblocked; *vide post*) was added Ph_3P (0.4 equiv). After stirring for 5 min at room temperature, the allyl ester was dissolved in acetone (0.1 M) and added to the catalyst mixture followed by triethylsilane (1.05 equiv). After stirring for several hours, the reaction was concentrated and the residue chromatographed on silica gel (elution with 21:7:7:9

EtOAc/CH_3CN/HOAc/H_2O diluted with an appropriate amount of EtOAc). The material obtained was further purified by C_{18} reverse-phase MPLC to provide the desired final compound.

Preparation of Ester-Exchange Product 51. To a solution of $Pd(OAc)_2$ (52 mg, 0.23 mmol) in 100 mL of EtOAc was added PPh_3 (500 mg, 1.9 mmol). After stirring for 15 min, **3** (X = SO_2-Me) (2.0 g, 5 mmol) was added followed by a solution of sodium 2-ethylhexanoate (920 mg, 5.5 mmol) dissolved in 100 mL of EtOAc. After stirring for 2 h, the solid which had formed was isolated, washed twice with EtOAc, and dried to provide 1.63 g (4.26 mmol, 85%) of the sodium salt of **3** (X = SO_2Me). This product was dissolved in 170 mL of THF and 40 mL of DMF and treated directly with $ICH_2OCOtBu$ (10.3 g, 4.26 mmol). After stirring for 4 h, the reaction was concentrated and purified via chromatography on silica gel (elution with 50% EtOAc/hexane) to provide 972 mg (48%) of **51** as a yellow solid.

Preparation of Oral Ester Prodrug 53. Compound **51** was processed to the ester **53** utilizing the chemistry outlined above and eliminating the final procedure for allyl ester removal.

Pharmacology. Male Sprague–Dawley rats were dosed intravenously with test compounds at 20 mg/kg in 0.9% saline. Dosing and blood sampling were carried out through an indwelling jugular vein cannula, thus permitting serial sampling from individual rats. Plasma levels and cummulative urinary recoveries were determined from samples collected over a 6-h time course. Male CD-1 mice were dosed both orally and subcutaneously with test compound at 20 mg/kg in 0.9% saline. The 0–4 h cumulative urinary recovery was collected. Male rhesus monkeys (n = 3) were dosed intravenously with test compounds at 30 mg/kg in 0.9% saline. Plasma samples were collected from the cephalic vein over an 3 h time course, along with the 0–24 h cumulative urinary recovery.

Urine was collected in 0.1 M sodium citrate buffer, pH 6.5, from animals placed in metabolism cages. Plasma and urine samples were stored at −70 °C prior to analysis.

Plasma half-life was calculated as $0.693/\beta$ where β is the slope for the terminal portion of the plasma vs time curve. The area under the curve was calculated using Simpson's rule. Urinary recovery was calculated as the percent of the administered dose recovered in the urine.

In vitro plasma protein binding was determined in pooled samples of Sprague–Dawley rat plasma at a drug concentration of 20 μg/mL, using an ultrafiltration technique (Centrifree Micropartition System).

Antibiotic concentrations were determined with an agar well diffusion assay (bioassay) employing *E. coli* (ATCC4157) or *Proteus providentia* (XC24) as the bacterial test strain. Standard curves from mouse, rat, monkey, or human plasma spiked with the compound under study were employed for analysis of plasma samples. Urine samples were analyzed by comparison to a standard curve prepared in 0.1 M sodium citrate buffer, pH 6.5. Urine samples were diluted with citrate buffer so that the drug concentration would fall into the range of the standard curve.

Supplementary Material Available: ^1H NMR, IR, UV, and MS for final compounds (10 pages). Ordering information is given on any current masthead page.

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