The Chemistry of Pseudomonic Acid. 17. Dual-Action C-1 Oxazole Derivatives of Pseudomonic Acid Having an Extended Spectrum of Antibacterial Activity†

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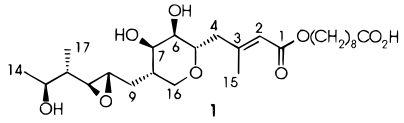
Received December 4, 1995⊗

A series of C-1 oxazole isosteres of pseudomonic acid A (mupirocin) bearing a nitroheterocycle have been synthesized, and significant differences in both spectrum of activity and potency were found between these derivatives and mupirocin. Additionally, the antibacterial potency of two members of this class of compounds against mupirocin-resistant staphylococci could not be accounted for solely by inhibition of the target enzyme isoleucyl-tRNA synthetase (IRS), indicating an additional mode of action. The most potent compound, the nitrofuran **3f** (SB 205952), was the most electron affinic derivative prepared and was transformed by NAD(P)H-dependent bacterial reductases at a rate similar to that for nitrofurantoin. The second mode of action of this compound may therefore arise from its reduction to a species with cellular targets other than IRS. In *in vivo* studies, **3f** was shown to be a very effective agent by both the subcutaneous and oral routes of administration.

Introduction

The discovery of novel chemical entities with unique modes of action is important for the development of antibacterial agents that are not cross-resistant to other classes of antimicrobials. The naturally occurring antibiotic mupirocin (pseudomonic acid A) is a protein synthesis inhibitor[1] that inhibits isoleucyl-tRNA synthetase (IRS).[2] This results in depletion of charged $tRNA^{ile}$ which, through effective amino acid starvation, induces the stringent response.[1] Mupirocin's potent antibacterial activity, particularly against Gram-positive bacteria, has led to its introduction as a topical agent (Bactroban) for the treatment of skin infections and for the prevention of nasal carriage of *Staphyloccocus aureus*, including methicillin-resistant and multiple drug-resistant strains.[3] Although mupirocin exhibits potent activity *in vitro* against the clinically important organisms found in community acquired infections, extending its use to the systemic market is precluded by the metabolic lability of the ester moiety.

Previous papers in the series have described the replacement of the metabolically labile C-1 ester function in mupirocin (**1**) with a variety of bioisosteres.[4,5] During the course of studies on the C-1 oxazoles **2**, a subset of aryl and heteroaryl derivatives containing a nitro group (**3a**−**c**) was identified which displayed improved antibacterial properties.[4,6] In order to unravel and utilize this feature, the series was extended to **3d**−**f**.



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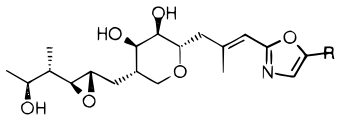

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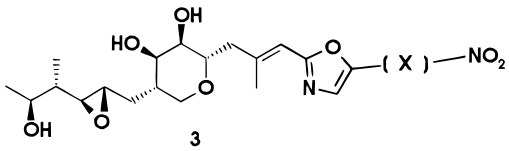

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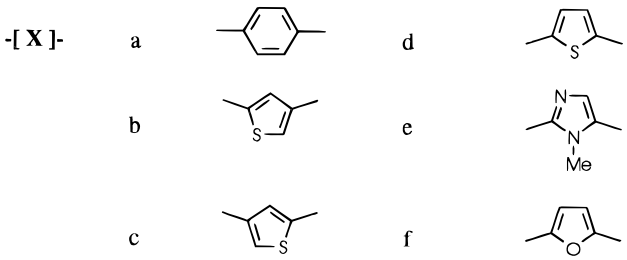

Results and Discussion

As attempts to prepare **3d**−**f** using a dehydrative cyclization procedure[4] were unsuccessful, the recently developed alternative oxazole synthesis[4,6] (Scheme 1), which uses a cycloaddition strategy, was utilized. Dehydration of the tosylmethylmonamide **4** in the presence of an electron deficient aldehyde (**5**) provided the oxazoline **6**. The electron-withdrawing R substituent then promoted conversion to the oxazole **7**, and removal of the trimethylsilyl protecting groups was readily achieved by brief exposure of **7** to dilute acid. Under the original dehydration conditions (PPh_3/CCl_4), these electron deficient aldehydes underwent side reactions to produce 1,1-dichloroethylene derivatives.[7] An alternative method is to use PPh_3/C_2Cl_6 which produces PPh_3Cl_2 as the only reactive species.[8] Using this

† Normonyl, the trivial name for the 3-[(5*S*)-((2*S*,3*S*)-epoxy-(5*S*)-hydroxy-(4*S*)-methylhexyl)-(3*R*,4*R*)-dihydroxytetrahydropyran-(2*S*)-yl]-2-methylprop-1(*E*)-en-1-yl radical, is used throughout for convenience. The approved generic name for pseudomonic acid A is mupirocin. Bactroban is a trademark of SmithKline Beecham.

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⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1996.

S0022-2623(95)00882-X CCC: $12.00

Scheme 1

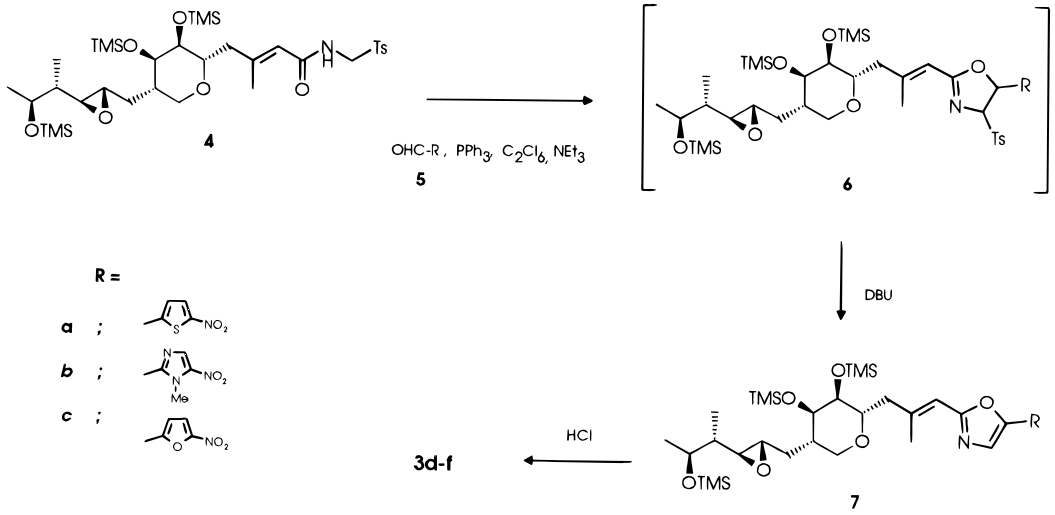

Table 1. Antibacterial Activity and IRS Inhibition Data

	MIC (μg/mL)						
organism	**1**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
B. fragilis NCTC 10581	>64	>64	>64	16	2	0.5	2
E. coli DC0	64	64	32	32	>64	>64	16
E. coli DC2	0.13	<0.06	<0.06	<0.06	<0.06	1	<0.06
Ent. cloacae N1	64	32	16	16	>64	>64	4
Ent. faecalis I	32	32	32	32	>64	64	16
Ps. aeruginosa K799 wt	>64	>64	>64	>64	>64	>64	>64
K. pneumoniae E70	>64	>64	32	64	>64	>64	>64
Pr. mirabilis C889	>64	>64	64	64	>64	>64	>64
H. influenzae Q1	<0.06	0.25	<0.06	<0.06	<0.06	<0.06	<0.06
M. catarrhalis 1502	0.5	1	<0.06	0.125	<0.06	0.25	<0.06
Strep. pneumoniae 1761	<0.06	<0.06	<0.06	<0.06	<0.06	0.5	<0.06
Strep. pyogenes CN 10	<0.06	0.125	<0.06	<0.06	<0.06	0.25	<0.06
S. epidermidis PHLN 20	0.125	0.125	<0.06	0.125	<0.06	<0.06	<0.06
S. aureus NCTC 6571	<0.06	0.125	<0.06	0.125	0.125	2	<0.06
S. aureus C 37	64	>64	32	32	8	>64	4
S. aureus F89	>64	>64	>64	64	8	>64	4
IC_{50} (ng/mL)[a]	0.8	3.9	3.4	2.2	3.3	0.7	2.4

[a] Inhibition of IRS *S. aureus* NCTC 6571.

Table 2. Inhibition of Mupirocin-Sensitive and -Resistant *S. aureus* IRS

	S. aureus NCTC 6571		*S. aureus* 11481		*S. aureus* C 7	
	IC_{50} (ng/mL)	MIC (μg/mL)	IC_{50} (ng/mL)	MIC (μg/mL)	IC_{50} (ng/mL)	MIC (μg/mL)
1	0.8	<0.06	140	64	20000	>2048
3d	3.3	0.13	120	8	<25% at 100000	32
3f	2.4	<0.06	200	4	400000	16

sequence it was possible to exploit the electronic properties of the nitroheterocycles and prepare the oxazoles **3d**−**f** in good yield.

All the compounds shown in Table 1 are extremely effective inhibitors of IRS from *S. aureus* NCTC 6571 (Oxford), having comparable activity to mupirocin (**1**). However as antibacterial agents, the derivatives exhibited important advantages in either potency or spectrum of activity compared to mupirocin. In addition to the good activity against mupirocin-sensitive organisms, e.g., staphylococci, streptococci, *Haemophilus influenzae*, and *Moraxella catarrhalis*, some members of the series showed significant increases in potency against bacterial species and strains that show decreased sensitivity to mupirocin, in particular *Bacteroides fragilis* and the mupirocin-resistant variants of *S. aureus* (C37, F89).

While mupirocin resistance in staphylococci remains rare in the clinic,[9,10] two mechanisms of resistance have been identified. High-level resistance (mupirocin (**1**) MIC > 512 μg/mL) is due to acquisition of a second IRS (often plasmid mediated) with a greatly reduced affinity for mupirocin (**1**),[11,12] while moderate level resistance (mupirocin (**1**) MIC 4−512 μg/mL) is associated with a single mutated chromosomal IRS with reduced affinity for mupirocin (**1**).[13,14] Several compounds displayed significant antibacterial activity against one or both types of strains, but the potency of inhibition of the respective IRS (Table 2) by the most effective agent, the nitrofuran **3f**, was insufficient to account for the low MICs seen particularly against the highly resistant strains. This implies that these nitro compounds have an additional mode of action which is functional in both the mupirocin-resistant and -sensitive organisms but is more significant in the former strains.

The ability of nitro compounds to form highly reactive species due to activation by bacterial NADH- and

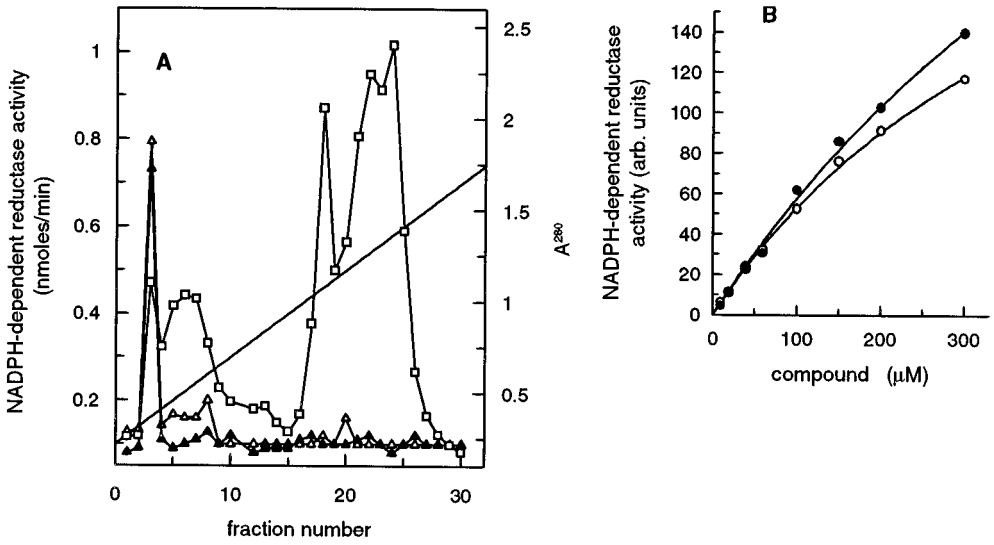

Figure 1. (A) Resource Q chromatography of NADPH-dependent nitrofuran reductase from *E. coli*, using either nitrofurantoin (▲) or **3f** (△) as substrates. The protein elution pattern, measured as A_{280}, is also shown (□). (B) Substrate concentration dependence of NADPH-dependent reductase activity in partially purified material from A.

Table 3. Mouse Blood Level Data for **3f**

	sc	po
C_{max} (μg/mL)	30.5	28.7
T_{max} (min)	5	15
AUC [0−240 min] (μg/mL min)	2407	1734

Table 4. *S. aureus* Smith[a] ip Infection in the Mouse

	CD_{50} (mg/kg) total dose	
compound	sc	po
3f	1.2	3.2
amoxycillin	0.28	0.24
vancomycin	3.8−4.8	nt[b]

[a] *S. aureus* Smith MIC (μg/mL): **3f**, 0.06; amoxycillin, 0.25; vancomycin, 4. Compounds administered 1 + 5 h postinfection. [b] nt: not tested (vancomycin not orally absorbed).

NADPH-dependent reductases is well known.[15] Reduction potential has been used to give a quantitative measure of the relative electron affinities of nitro heterocycles,[16,17] and a relative scale between **3a**−**f** was established from the cyclic voltammetric peak potentials. The ability of the nitro compounds **3** to interact with reductases was examined using the most electron affinic compound, the furan **3f**, as substrate. A crude extract of *Escherichia coli* K12 cells capable of reducing nitrofurantoin (a classical nitrofuran antibiotic) was partially purified, and the NADPH-dependent reductase activity, measured using either compound as substrate, was eluted in a single fraction at around 0.1 M NaCl (Figure 1A). The substrate activation kinetics for both nitrofurantoin and **3f** were similar, with the activity being entirely dependent upon NADPH (Figure 1B).

In pharmacokinetic studies in the mouse following administration at 50 mg/kg, **3f** gave excellent blood levels after both subcutaneous (sc) and oral (po) administration (Table 3). **3f** was also evaluated in a *S. aureus* (penicillin-sensitive strain) intraperitoneal (ip) infection model (Table 4) and, after sc administration, was more effective than the glycopeptide vancomycin. Significant oral efficacy was also obtained.

Conclusion

Structure−activity relationships in the nitro derivatives **3** have been shown to be due to a combination of factors. While the compounds are potent IRS inhibitors, the activity exhibited against the highly mupirocin-resistant variants of *S. aureus* in the absence of significant IRS inhibition indicates that the compounds can inhibit the growth of bacteria through an additional mechanism, and this could account for the improved spectrum of activity. The process is likely to be linked to the electron affinity of the molecules since the nitrofuran **3f** is both the most easily reduced and the most active compound. Furthermore the fact that **3f** is rapidly reduced by NAD(P)H-dependent bacterial reductases, and at a rate similar to nitrofurantoin, strongly suggests that this process underlies the extended antibacterial spectrum of activity. The *in vivo* profile of **3f** is a considerable advance over previously described analogues.[4] High and prolonged blood levels, combined with potency and good bioavailability, have resulted in >30-fold increase in oral efficacy.

Therefore the (nitrofuryl)oxazole **3f** has been identified as a C-1 ester isostere having an extended spectrum of antibacterial activity due to a dual mode of action. A key feature is that both bioactive moieties (nitrofuryl and monic) retain activity against their respective targets without any compromise in affinity, and this has been translated into superior antimicrobial activity. Further studies on the mode of action of this compound, SB 205952, have recently been published.[18] The application of the bifunctional concept to other bioactive molecules is currently being investigated.

Experimental Section

Melting points were determined on a Reichert apparatus and are uncorrected. Infrared spectra were determined in KBr on a Perkin-Elmer PE 983 spectrophotometer. NMR spectra were recorded on a Bruker AC-250F spectrometer. Chemical shifts are expressed in ppm (δ) relative to internal tetramethylsilane. Mass spectra were obtained on a VG ZAB mass spectrometer. All organic phases were dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure with a Buchi rotavapor. Merck Kieselgel 60 (<230 mesh ASTM) was used for column chromatography.

Target compounds were obtained as gums and were pure by ^{1}H NMR, ^{13}C NMR, and HPLC at two wavelengths. Elemental composition was determined by high-resolution mass spectrometry. The HPLC system was developed to detect

acid- or base-catalyzed rearrangement products of the epoxide which would be undetected by elemental analysis. HPLC was performed on a Waters Associates instrument using a C_{18} μ-Bondapak reverse-phase column with pH 4.5 0.05 ammonium acetate buffer−methanol as eluant. Detection was by UV at 240 nm and at the λ_{max} of the test compound.

The synthesis of the oxazoles **3a**[5] and **3b,c**[4] has previously been described. The aldehyde **5e** was prepared using the method of Ross *et al*.[19]

General Method for the Preparation of Oxazoles 3d−f. Tris(trimethylsilyl) tosylmethylmonamide (**4**) (1.0 mmol) and the required aldehyde **5** (1.5 mmol) were dissolved in acetonitrile (6 mL). Triphenylphosphine (4 mmol), triethylamine (9 mmol), and hexachloroethane (4 mmol) were then added sequentially. After 1 min the reaction became exothermic and the mixure turned black. After 20 min 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (10 mmol) was added, and stirring continued for a further 10 min. The mixture was diluted with ethyl acetate, washed with ammonium chloride solution and brine, dried, and evaporated. The crude product was partially purified by flash chromatography, eluting with ethyl acetate/hexane mixtures, to remove polar impurities and give the protected C-1 oxazole **7** as an orange gum. This material was dissolved in tetrahydrofuran (30 mL) and 0.4 N hydrochloric acid (6 mL) added. The mixture was stirred for 2 min, diluted with saturated sodium hydrogen carbonate (6 mL), and extracted with ethyl acetate (×3). The combined organic extracts were washed with brine, dried, and evaporated. The crude product was purified by chromatography, eluting with methanol/dichloromethane mixtures, to give the oxazole **3**.

5-(5-Nitrothien-2-yl)-2-(1-normon-2-yl)oxazole (3d): prepared in 58% yield; IR (KBr) 3442, 1640, 1514, 1429, 1332, 1266, 1118 cm^{-1}; UV (EtOH) 404 (λ_m 20 270), 275 nm (13 940); ^{1}H NMR (CD_3OD) δ 0.95 (3H, d, J = 7.1 Hz, 17-H_3), 1.21 (3H, d, J = 6.4 Hz, 14-H_3), 1.34−1.48 (1H, m, 8-H), 1.65−1.83 (2H, m, 9-H_2), 1.94−2.04 (1H, m, 12-H), 2.31 (3H, s, 15-H_3), 2.36 (1H, dd, J = 9.6, 14.5 Hz, 4-H), 2.69−2.85 (3H, m, 4,10,11-H), 3.41 (1H, dd, J = 3.0, 9.0 Hz, 6-H), 3.60 (1H, d with further fine coupling, J = 11.3 Hz, 16-H), 3.74−3.96 (4H, m, 5,7,13,-16-H), 6.28 (1H, s, 2-H), 7.38 (1H, d, 4.4 Hz, 3″-H), 7.65 (1H, s, 4′-H), 7.98 (1H, d, J = 4.4 Hz, 4″-H); ^{13}C NMR (CD_3OD) δ 12.2 (C-17), 19.9 (C-15), 20.3 (C-14), 33.0 (C-9), 41.7 (C-8), 43.7 (C-12), 44.0 (C-4), 56.9 (C-10), 61.3 (C-11), 66.4 (C-16), 70.0 (C-6), 70.7 (C-13), 71.6 (C-7), 76.4 (C-5), 113.0 (C-2), 124.0 (C-3″), 127.6 (C-4″), 131.1 (C-4′), 137.9 (C-2″), 144.7 (C-5′), 151.7 (C-5″), 152.1 (C-3), 163.7 (C-2′); MS m/z (NH_3, DCI) 495 (MH^+, 100), (EI) 495 (MH^+, 1), 494 (M^+, 2.5), 250 (55), 167 (100). Found: M^+, 494.1721. $C_{23}H_{30}N_2O_8S$ requires M, 494.1723.

5-(1-Methyl-5-nitroimidazol-2-yl)-2-(1-normon-2-yl)oxazole (3e): prepared in 80% yield; IR (KBr) 3449, 1647, 1486, 1366, 1268, 1225 cm^{-1}; UV (EtOH) 361 (λ_m 17 370), 283 nm (15 180); ^{1}H NMR (CD_3OD) δ 0.95 (3H, d, J = 7.1 Hz, 17-H_3), 1.20 (3H, d, J = 6.5 Hz, 14-H_3), 1.35−1.48 (1H, m, 8-H), 1.63−1.79 (2H, m, 9-H_2), 1.91−2.02 (1H, m, 12-H), 2.33 (3H, s, 15-H_3), 2.36 (1H, dd, J = 9.6, 14.6 Hz, 4-H), 2.68−2.86 (3H, m, 4,10,11-H), 3.40 (1H, dd, J = 3.1, 9.0 Hz, 6-H), 3.58 (1H, d with further fine coupling, J = 10.8 Hz, 16-H), 3.74−3.95 (4H, m, 5,7,13,16-H), 4.19 (3H, s, NMe), 6.33 (1H, s, 2-H), 7.82 (1H, s, 4′-H), 8.12 (1H, s, 4″-H); ^{13}C NMR (CD_3OD) δ 12.3 (C-17), 20.0 (C-15), 20.3 (C-14), 33.0 (C-9), 35.3 (NMe), 41.8 (C-8), 43.8 (C-12), 44.1 (C-4), 56.9 (C-10), 61.3 (C-11), 66.4 (C-16), 70.0 (C-6), 70.7 (C-13), 71.5 (C-7), 76.4 (C-5), 113.0 (C-3), 131.7 and 133.7 (C-4′ and C-4″), 140.7, 141.3, 142.3 (C-5′, C-2″, and C-5″), 152.7 (C-3), 164.5 (C-2′); MS m/z 492 (M^+, 4), 248 (100). Found: M^+, 492.2215. $C_{23}H_{32}N_4O_8$ requires M, 492.2220.

5-(5-Nitrofuran-2-yl)-2-(1-normon-2-yl)oxazole (3f). When the reaction was repeated on a large scale (**5c**; 100 mmol), the oxazole **3f** was isolated using the following process, in place of the final chromatographic step. The combined organic extracts containing **3f** were washed with brine (2 × 80 mL), dried, filtered, and reduced to a small volume under vacuum. This oil was stirred vigorously while diisopropyl ether (1 L) was added slowly. This mixture was stirred overnight at room temperature in the dark. The resulting solid was filtered off, washed with diisopropyl ether, and dried under vacuum to give a yellow powder (28.3 g). This material was dissolved in warm acetone (115 mL) and stirred while hexane (80 mL) was added slowly. The product crystallized out and was stirred for 0.5 h before more hexane (220 mL) was added slowly. After a further 0.5 h, the mixture was cooled in an ice bath and stirred for 0.5 h. The product was filtered off, washed with acetone:hexane (1:4, 50 mL) and then hexane, and dried under vacuum to give a yellow microcrystalline powder (26.50 g, 69%): mp 119−120 °C; IR (KBr) 3412,-1507, 1451, 1352, 1275, 1049 cm^{-1}; UV (EtOH) 384 nm (λ_m 18 819); ^{1}H NMR (CD_3OD) δ 0.95 (3H, d, J = 7.0 Hz, 17-H_3), 1.20 (3H, d, J = 6.4 Hz, 14-H_3), 1.34−1.48 (1H, m, 8-H), 1.66−1.76 (2H, m, 9-H_2), 1.91−2.02 (1H, m, 12-H), 2.31 (3H, s, 15-H_3), 2.35 (1H, dd, J = 9.6, 14.5 Hz, 4-H), 2.68−2.87 (3H, m, 4,10,11-H), 3.40 (1H, dd, J = 3.0, 9.0 Hz, 6-H), 3.58 (1H, d with further fine coupling, J = 11.5 Hz, 16-H), 3.74−3.94 (4H, m, 5,7,13-H), 6.28 (1H, s, 2-H), 6.99 (1H, d, J = 3.9 Hz, 3″-H), 7.59 (1H, d, J = 3.9 Hz, 4″-H), 7.69 (1H, s, 4′-H); ^{13}C NMR (CD_3OD) δ 12.3 (C-17), 20.0 (C-15), 20.3 (C-14), 33.0 (C-9), 41.7 (C-8), 43.7 (C-12), 44.0 (C-4), 56.9 (C-10), 61.3 (C-11), 66.4 (C-16), 70.0 (C-6), 70.7 (C-13), 71.6 (C-7), 76.4 (C-5), 110.5, 112.9, and 114.9 (C-2, C-3″, and C-4″), 128.2 (C-4′), 141.1, 146.9 (C-5′ and C-2″), 152.4 and 153.2 (C-3 and C-5″), 164.1 (C-2′); MS m/z 478 (M^+, 15), 234 (100), 121 (74), 75 (75). Found: M^+, 478.1948. $C_{23}H_{30}N_2O_9$ requires M, 478.1951. Anal. ($C_{23}H_{30}N_2O_9$) C, H, N.

Antibacterial Evaluation. Antibacterial activity was determined by a broth microdilution technique in microtiter plates using Hamilton AT+ liquid handling technology and is defined as the minimum inhibitory concentration (MIC in μg/mL) needed to inhibit growth of the micro-organism. Mueller−Hinton broth (Difco) was used as the growth medium; for growth of the more fastidious micro-organisms (*H. influenzae* Q1, *M. catarrhalis* 1502, and the *Streptococcus* sp.), this was supplemented with sterile heat-inactivated donor horse serum (ICN Biomedicals); 5%), hematin (Sigma; 0.02 mg/mL), and NAD (β-nicotinamide adenine dinucleotide, 0.08 mg/mL; Sigma) (all final concentration). Overnight broth cultures were added to give a final concentration of 5 × 10^5 cfu/mL. Plates were incubated at 37 °C for 18 h.

Enzyme Inhibition Studies. The methods used for isolation and assay of IRS from *S. aureus* strains are described in detail elsewhere.[18]

Reductase Activity. *E. coli* K12 cells were harvested at midexponential growth, flash frozen in N_2, and stored at −70 °C. Around 50 g of cells was thawed, suspended in 50 mM phosphate buffer (pH 7.2), and disrupted by sonication (10 × 30 s). Cell debris was then removed by centrifugation (25000*g*, 15 min). The crude extract was dialyzed against 50 mM phosphate buffer (pH 7.2) overnight and then applied to a 6 mL Pharmacia Resource-Q ion-exchange column. Protein was then eluted using a linear gradient of 0−1 M NaCl in phosphate buffer (pH 7.2) at a flow rate of 5 mL/min over 20 column vol; 10 mL fractions were collected. All of the NADPH-dependent nitrofurantoin reductase was found in a single fraction. This material was used for all subsequent experiments. Enzyme-catalyzed reduction of nitrofurans was monitored by following loss of absorbance at 405 nm using a Molecular Devices plate reader. Assays contained an NADPH-regenerating system consisting of 1 IU of glucose 6-phosphate dehydrogenase (from *Torula* sp.), 20 mM glucose, and 1 mM NADPH in 200 μL final volume. Assays also contained 50 mM phosphate buffer (pH 7.2) and substrate (**3f** or nitrofurantoin). Rates of enzyme activity were calculated from calibration curves of substrate in identical media. Spectral data were obtained under identical conditions using a Hewlett Packard diode array spectrophotometer in a total assay volume of 1 mL.

Reduction Potential. The cyclic voltammetry technique for the determination of redox potentials was used[20] measuring voltammetric reduction peak potentials vs the Ag/AgCl reference electrode: cyclic voltammetry at 100 mV/s scan rate; gold/mercury amalgam (small disk) working electrode; pH 7.4 phosphate (0.1 M) buffer saturated with nitrogen; nitroheterocycles were initially dissolved in an appropriate volume of DMF and subsequently diluted into buffer (final concentrations: 1 mM compound and 5% DMF). E(peak), V: **3a**, −0.71;

3b, −0.66; **3c**, −0.58; **3d**, −0.56; **3e**, −0.52; **3f**, −0.46. The voltammetric peak potentials of a series of reference nitroheterocycles were found to be linearly correlated with the literature values[16] (shown in brackets). *E*(peak) [*E*(1/2)], V: nifuroxime, −0.40 [−0.30]; 5-nitrofurantoin, −0.35 [−0.28]; 5-nitro-2-furoic acid, −0.52 [−0.39]; 1,2-dimethyl-5-nitroimidazole, −0.65 [−0.50].

***In Vivo* Studies.** Charles Rivers CD1 male mice (18−22 g), nonfasted, were used in the studies in groups of five. The compounds were dissolved in 10% ethanol and diluted with 1% hydroxypropylmethylcellulose in water or phosphate-buffered saline (pH 7.3) (1:9) for po and sc dosing, respectively. The compounds were administered at 50 mg/kg in a volume of 0.2 mL, and blood samples were collected at 5, 15, 30, 60, 90, 120, and 240 min after dosing. These were assayed using *Bacillus subtilis* bioassay in blood agar base at pH 7.0, with incubation overnight at 30 °C. Standards were prepared in fresh heparinized mouse blood. Mice were infected intraperitoneally with 1.3×10^{10} or 5×10^{9} cfu for po and sc dosing, respectively. The compounds were administered po or sc at 1 + 5 h postinfection in a volume of 0.2 mL/mouse. The CD_{50} was calculated on the second day postinfection as the total dose required to protect 50% of the mice from death.

Acknowledgment. The following are thanked for their input: I. Bennett, for the antibacterial evaluation of the compounds, and T. Farmer, for preliminary enzymology.

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JM950882Q