High-Throughput Catch-and-Release Synthesis of Oxazoline Hydroxamates. Structure−Activity Relationships in Novel Inhibitors of *Escherichia coli* LpxC: In Vitro Enzyme Inhibition and Antibacterial Properties

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Abstract: LpxC is a zinc amidase that catalyses the second step of lipid A biosynthesis in Gram-negative bacteria. Oxazolines incorporating a hydroxamic acid, which is believed to coordinate to the single essential zinc ion, at the 4-position are known inhibitors of this enzyme. Some of these enzyme inhibitors exhibit antibacterial activity through their inhibition of LpxC. We recently developed a method for the synthesis of oxazolines using resin capture and ring-forming release that eliminates traditional purification steps and can be used in high-throughput synthesis. Using our method, oxazoline hydroxamates with diverse 2-substituents were prepared in library form as candidate inhibitors for LpxC. Two conventional methods for oxazoline synthesis were also applied to generate more than 70 compounds. The groups at the 2-position included a wide variety of substituted aromatic rings and a limited selection of alkyl groups. These compounds were screened against wild-type and LpxC inhibitor-sensitive strains of *Escherichia coli*, as well as wild-type *Pseudomonas aeruginosa*. Inhibition of the *E. coli* LpxC enzyme was also investigated. A broad correlation between enzyme inhibitory and antibacterial activity was observed, and novel compounds were discovered that exhibit antibacterial activity but fall outside earlier-known structural classes.

Introduction

Bacterial resistance to clinically available antibiotics is recognized as a major current problem in infectious disease. Such resistance has recently extended to vancomycin, often considered a drug of "last resort" against Gram-positive bacteria.[1] Although there may be creative ways to circumvent resistance,[2,3] the most effective long-term solution to problems of resistance is the development of drugs that act on heretofore unexploited antibacterial targets.[4] One such target is the biosynthesis of lipid A, a key component of the outer membrane of Gram-negative bacteria. The minimal lipid A structure necessary for bacterial cell growth consists of a diphosphorylated tetrasaccharide acylated with six fatty acid chains on the two glucosamine subunits (Figure 1).[5] The biosynthesis of this minimal lipid A structure has been extensively studied; all of the enzymes and corresponding genes responsible for its
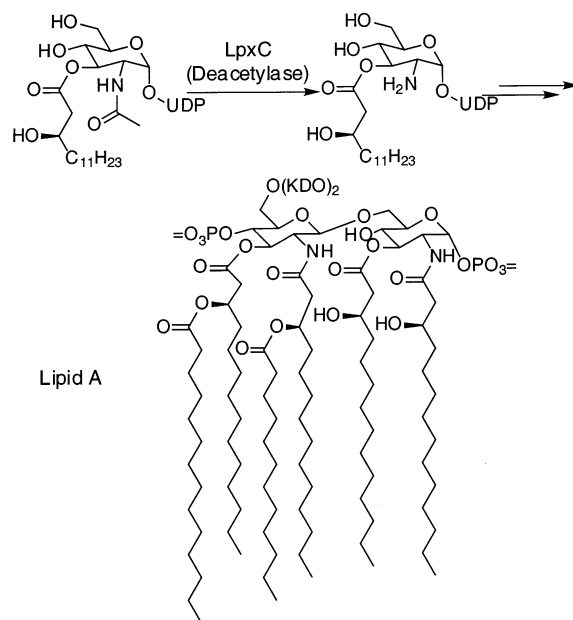

Figure 1. Biosynthesis and structure of lipid A.

synthesis in *Escherichia coli* have been identified.[6] Homologous genes have also been identified from the genomes of most other Gram-negative bacteria.

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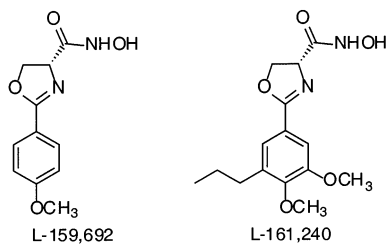
Figure 2. Known inhibitors of LpxC with antibacterial activity against *E. coli.*

Recently, a family of hydroxamic acids that inhibit lipid A biosynthesis and are antibacterial against *E. coli* both in vitro and in animal models have been disclosed.[7] These compounds are aryl oxazoline hydroxamic acids (Figure 2), and the most potent compound of the series, L-161,240, has an MIC (minimum inhibitory concentration) against *E. coli* comparable to those of ampicillin and rifampicin (~2 μg/mL). The target of these small molecules has been shown to be UDP-3-*O*-[*R*-3-hydroxymyristoyl]-GlcNAc deacetylase (LpxC), a zinc amidase encoded by the *lpxC* gene. The antibacterial spectrum of this series of compounds is rather limited although a very wide variety of Gram-negative bacteria have been shown to express homologous LpxCs. Although *Enterobacter cloacae* and *Klebsiella pneumoniae* are sensitive to L-161,240, the growth of *Pseudomonas aeruginosa* and *Serratia marescens* is not affected.[8] This lack of activity against other Gram−negative bacteria is due, at least in part, to subtle differences in enzyme structure between various organisms.[7,8] A second report[9] described sulfonamides of amino acid hydroxamates with nanomolar potency against the *E. coli* LpxC, and MICs of ~1 μg/mL against *E. coli*. Other pathogens are killed with reasonable MICs, including *Klebsiella* and *Serratia*.

LpxC catalyses the first committed step of lipid A biosynthesis, the removal of an *N*-acetyl group from a 3-acyl-*N*-acetylglucosamine moiety (Figure 1). This enzyme contains a single catalytic zinc ion that is coordinated by the nitrogen of two histidine residues, with a third coordinating group that is either a histidine nitrogen or carboxylate of Asp or Glu.[10] No sequence homology with other structurally characterized zinc amidases has been identified.

One requisite feature of these LpxC inhibitors is the hydroxamic acid moiety. As shown in the earlier studies, the conversion of the hydroxamic acid to a carboxylic acid results in the loss of all inhibitory and antibacterial activity. The well-known

Scheme 1. Catch-and-Release Oxazoline Synthesis by Ring-Forming Cleavage
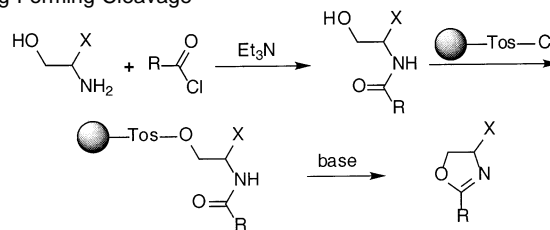

affinity of hydroxamic acids for metals has led to the proposal that the inhibitors act by coordinating to the single catalytic zinc.[11]

Owing to our interest in oxazoline inhibitors of LpxC, we recently developed a "catch-and-release" ring-forming reaction for the parallel synthesis of libraries of oxazolines (Scheme 1).[12] This sequence utilizes β-hydroxyamines and acid chlorides as the diversity inputs. The initial *N*-acylation product can be captured onto polymer-bound tosyl chloride, eliminating the need for an extractive purification in the first step. The resin-bound intermediate is washed extensively to remove non-nucleophilic impurities and then treated with a volatile, non-nucleophilic base to promote ring-forming cleavage from the resin. This sequence results in the formation of oxazoline targets without the need for intermediate or final purification. Oxazolines were obtained in moderate yield (40−60%) and exceptionally high purity (>90%). This sequence has proven to be highly amenable to parallel, semi-automated methods of synthesis.

The key feature of the oxazoline LpxC inhibitors is the zinc binding group at the 4-position of the heterocycle. Although several metal binding groups have shown enzyme inhibitory activity, only hydroxamic acids exhibit the desired antibacterial activity.[13] As such, the ultimate purpose in the development of the high-throughput synthesis outlined above was the generation of a significant collection of diverse oxazoline hydroxamic acids related to the original inhibitors. While two earlier publications disclose several oxazoline hydroxamic acids with antibacterial activity,[7,14] they do not directly address the structure−activity relationships in the aromatic ring. One of the significant questions that also remains to be answered is the reason for the lack of activity of this series of compounds against several strains of Gram-negative bacteria, including *P. aeruginosa*. Originally it was thought that this lack of activity was due to efflux pumps or metabolism.[7] Later reports showed that the lack of antibacterial activity is more likely due to poor inhibition of the *P. aeruginosa* LpxC, which has only recently been purified to homogeneity.[8] A collection of diverse oxazoline hydroxamates may lead to the discovery of a subset of these compounds with improved activity against the *Pseudomonas* enzyme and, consequently, against more important bacterial pathogens.

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Scheme 2. Strategy for High-Throughput Synthesis of Oxazoline Hydroxamates
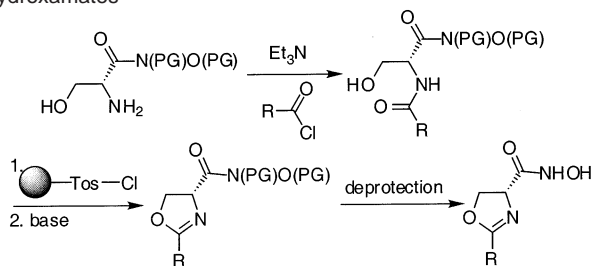
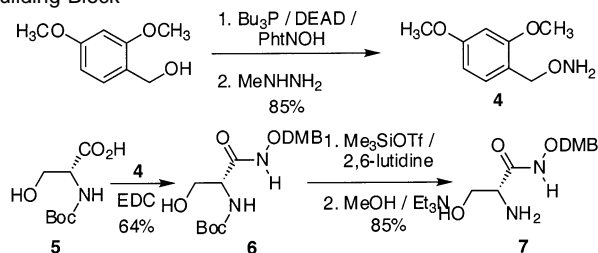**Scheme 3.** Diprotected Oxazoline Hydroxamates Are Not Fully Deprotected

DMB = 2,4-dimethoxybenzyl, TMB = 2,4,6-trimethoxybenzyl, Ar = *p*-methoxyphenyl
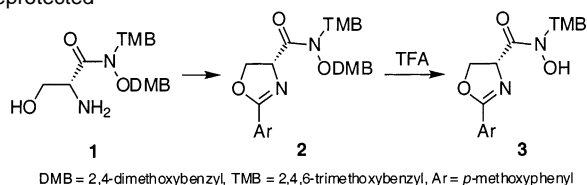
Because heterocyclic hydroxamates related to these oxazoline hydroxamates exhibit activity against phosphodiesterase 4 (PDE4),[15] also a metalloenzyme, this library may also be of value for broader screening against metalloenzyme targets (although not the focus of this work).

Results

The synthesis of this set of oxazoline hydroxamates was planned largely as outlined in Scheme 2. The amino alcohol diversity input is a protected serine hydroxamate, as illustrated in Scheme 3. On the basis of our previous work, we believed that the oxazoline synthesis should proceed smoothly with such a substrate. The complication proved to be the removal of the hydroxamate protecting groups. Both the NH and the OH of the hydroxamic acid are acidic (pK_a 8−10) and could potentially be acylated by acid chlorides. It was therefore initially thought that both the NH and the OH must be protected. Base-cleavable protecting groups are obviously incompatible with the catch-and-release method. The sensitivity of oxazolines to hydrogenolysis precluded the use of benzyl protecting groups. Owing to these constraints, it was clear that acid-cleavable protecting groups would be most appropriate. Barlaam recently described dimethoxybenzyl and trimethoxybenzyl groups as unique hydroxamate protecting groups that can be cleaved with dilute TFA.[16] The byproduct of this deprotection reaction is a polymer derived from the benzyl cations that can be precipitated with methanol and removed by filtration. If the deprotection is done in the presence of a trialkyl silane, the deprotection byproducts are dimethoxytoluene and trimethoxytoluene. In this case, the product hydroxamate is precipitated with ether and filtered. The latter deprotection method proved to be quite reliable in the synthesis of isoxazoline hydroxamates.[13] However, it was quite unsuccessful in the synthesis of oxazoline hydroxamates (Scheme 3). As shown, the diprotected serine hydroxamate **1** successfully underwent the catch-and-release oxazoline formation reaction

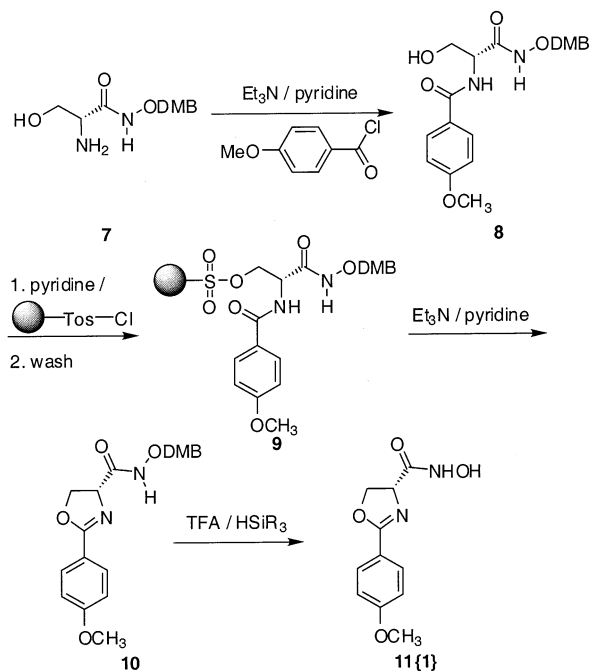**Scheme 4.** Preparation of Monoprotected Serine Hydroxamate Building Block

to form **2** in 48% yield. However, the *N*-trimethoxybenzyl (TMB) group was very resistant to deprotection: even in neat TFA, the *N*-TMB protecting group remained largely intact, giving **3** as the major product, a surprising result based on our previous work with the same protecting group. We believe that the stability of this group may derive from the basicity of *N*-3 of the oxazoline ring: the proximity of a positively charged group may inhibit the protonation of the hydroxamate nitrogen and thus preclude benzyl cation formation.

Rather than drastic modification of the protecting group strategy, we hypothesized that the hydroxamate nitrogen need not be protected at all (Scheme 5). However, it is weakly nucleophilic and therefore might be subject to acylation by the acid chloride. This potential problem was not observed when the acid chloride was used as a slightly limiting reagent (0.9 equiv). In addition to avoiding the deprotection problem outlined above, this strategy also greatly simplified the synthesis of necessary serine hydroxamate **7**.

After showing in pilot experiments that the *O*-protected serine hydroxamate could be used in the desired route, it was necessary to develop a synthesis of **7** that could be used to make significant quantities of material (∼50 g or more). Hydroxylamine **4** was obtained by a considerable modification of Barlaam's procedure. Owing to the acid-sensitive hydroxamate protecting group, the logical starting material for the synthesis of **7** would be Fmoc-protected D-serine. However, given the high cost of this compound and the large amount of material that was needed, it was desirable to develop an approach that instead utilized the significantly less expensive Boc-D-serine (**5**). The procedure outlined in Scheme 4 was eventually developed to suit this purpose. Boc-D-serine was used to acylate the *O*-protected hydroxylamine **4** to give the di-protected serine derivative **6**. The Boc protecting group could be removed using a significant modification of the procedure reported by Sakaitani.[17] Accordingly, **6** was treated with 4 equiv of TMS-triflate in the presence of an excess of 2,6-lutidine to give the corresponding TMS-carbamate. The TMS-carbamate could then be removed by treatment with triethylamine in methanol to give the desired **7**. The final four-step route outlined in Scheme 4 was optimized for large-scale synthesis such that only the final step requires a chromatographic purification—all other steps utilize crystallization techniques. This sequence has been performed on a 30-g scale.

Now having available significant quantities of the protected serine hydroxamate **7**, to optimize the desired synthetic route we studied a model system. As such, compound **7** was acylated with *p*-anisoyl chloride to give **8** (Scheme 5). According to our

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Scheme 5. Synthesis of Oxazoline Hydroxamates **11**{*n*} (Method A)

yield of **10**. After expending a great deal of effort to optimize these reaction conditions, it became apparent that the outstanding purity of **10** warranted further development of this reaction sequence despite the modest yields. Further, compound **10** could be easily deprotected with dilute TFA in the presence of a trialkyl silane to give hydroxamate **11**. Filtration of the crude deprotection mixture through silica gel allowed ready separation of the dimethoxytoluene deprotection byproduct from the desired hydroxamic acid. The overall (three-step) yield of **11**{*1*} was approximately 30% with an HPLC purity of 95%.

Having an adequate procedure in hand, this methodology was adapted through minor modifications to a high-throughput synthesis instrument (Quest 210). The resulting protocol was found to be quite robust and was applied to the synthesis of approximately 50 related oxazolines. A small follow-up set of 16 compounds, based on the biologically active compounds from this series of 50, was synthesized using the same method.

A detailed outline of the high-throughput experimental procedure (Method A) is provided in the Supporting Information. The synthesis begins with the acid chloride dissolved in pyridine. The serine hydroxamate **7** and Et$_3$N are added as a solution in pyridine. After stirring briefly, the crude mixture is added to a Quest 210 reaction vessel containing a 3-fold excess of polymer-bound tosyl chloride. The reaction vessel and its contents are incubated at −15 °C for 24 h, at which time the resin is washed extensively with CH$_2$Cl$_2$/MeOH, DMSO, and then CH$_2$Cl$_2$. As described in our earlier publication, the DMSO wash step is essential to avoid significant amounts of chloride-derived byproducts. The resin is then treated with 49:49:2 THF/pyridine/Et$_3$N and shaken at room temperature for 24 h. The resin is filtered, and the filtrate is evaporated, giving the desired oxazoline in modest yield (20−40%) and exceptional purity.

For reasons that are not entirely clear, the resulting oxazoline products are occasionally contaminated with pyridine salts. Consequently, a neutral aqueous solid-phase extraction (SPE) is performed to remove them.[18] The protected oxazoline is then treated with 1:1:8 TFA/Et$_3$SiH/CH$_2$Cl$_2$ for 20 min to generate the free hydroxamate. Hydroxamates are exceptionally polar functional groups and adhere strongly to silica gel, while the dimethoxy toluene byproduct of deprotection is quickly eluted from silica gel. The hydroxamate can be eluted only with polar solvents such as ethanol, enabling the reaction mixture to be purified by solid-phase extraction. The crude mixture is loaded onto a short bed of silica gel (∼1 g) in a pipet. The silica is washed with 4 mL of 3:2 ethyl acetate/hexanes to elute dimethoxytoluene. The desired product is then eluted with 5 mL of 20% ethanol in CH$_2$Cl$_2$. All compounds are purified using exactly this protocol, and only the second fraction need be collected. Evaporation of the ethanol eluent gives the oxazoline hydroxamic acid product in 20−30% overall yield and 85−99% purity (by HPLC). This procedure was generally performed 10 or 20 reactions at a time on such a scale to yield 5−15 mg of final product for analysis and biological testing. All products were analyzed by NMR and HPLC. A random sampling of approximately 20% of the products was subjected to mass spectrometric analysis. Table 1 provides a listing of the resulting

previously developed procedures, the crude reaction mixture was loaded onto an excess (3 equiv) of tosyl chloride resin at −15 °C to give resin-bound species **9**. The resin was washed extensively and then treated with a mixture of pyridine and Et$_3$N in THF overnight. Filtration and concentration gave compound **10** in 30% yield. It proved to be exceptionally pure as determined by NMR. High purity of crude reaction products, obviating the need for traditional, time-consuming fractionation methods, is an intrinsic property of the solid-phase ring-forming cleavage method and particularly facilitates broad initial biological screening studies.

While the high purity of the oxazoline product **10** was encouraging, the rather low yield was unexpected. Examination of the intermediate washings revealed that a significant amount (∼50%) of **10** was being formed during the loading step. This observation clearly points to a problem that was recognized during the initial development of the catch-and-release method: the rate of the loading step and the rate of the cleavage step are similar. Therefore, a significant quantity of material is being prematurely cleaved from the resin, even at −15 °C. This is in contrast to our earlier results (with a methyl ester) in which low temperatures (<0 °C) greatly retarded the cleavage step. Apparently, the protected hydroxamate somehow facilitates the ring-forming release reaction to the detriment of the overall yield. Interestingly, premature cyclization to give the cognate di-protected derivative **2** did not present the same problem, hence, the higher overall yield in that example. Therefore, it appears that the NH of the hydroxamate somehow facilitates the cyclization reaction. This could be due to an electronic effect but is more likely due to hydrogen bonding.

Whatever the reason for its occurrence, this problem could potentially be solved either by acceleration of the loading step or by deceleration of the cleavage step. To this end, numerous variations of reaction conditions were explored: changes in the temperature, solvent, base, and tosylating agent. However, none of these variations resulted in a significant improvement in the

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Table 1. Synthesis and Antibacterial/Enzyme Inhibitory Activity of Oxazoline Hydroxamates

number	MW	overall yield, % (method)	HPLC purity, %	comments	R477	G17S	PA01	number
11{1}	236.0	19 (A)	88		11	24	<7	61
11{2}	236.0	29 (A)	99		11	22	<7	44
11{3}	266.1	21 (A)	96		10	25	<7	67
11{4}	250.1	12 (A)	73		13	23	<7	46
11{5}	296.0	12 (A)	93	prepared chloride	10	26	<7	72
11{6}	338.0	7 (A)	97	some product lost	9	20	<7	86
11{7}	264.0	26 (A)	98	prepared chloride	14	25	<7	71
11{8}	280.0	9 (A)	56	prepared chloride	7	13	<7	37
11{9}	308.0	22 (A)	89	prepared chloride; same as L-161,240	17	26	<7	96
11{10}	306.2	17 (A)	98		<7	11	<7	86
11{11}	342.0	19 (A)	65	prepared chloride	<7	11	<7	8
11{12}	342.0	18 (A)	86	prepared chloride	<7	12	<7	13
11{13}	340.0	18 (A)	89	prepared chloride	<7	<7	<7	61
11{14}	340.2	16 (A)	80	plus 20 acid	<7	13	<7	35
11{15}	282.2	23 (A)	100		<7	16	<7	69
11{16}	262.2	21 (A)	62	includes *tert*-butyl isomers	13	21	<7	58
11{17}	262.2	21 (A)	87		<7	13	<7	56
11{18}	308.2	20 (A)	97		<7	<7	<7	1
11{19}	256.1	19 (A)	99		<7	12	<7	35
11{20}	256.1	33 (A)	95		<7	20	<7	19
11{21}	196.0	57 (A)	95		<7	<7	<7	0
11{22}	212.1	39 (A)	86		<7	11	<7	19
11{23}	267.2	22 (A)	88		<7	11	<7	18
11{24}	262.2	11 (A)	88		<7	13	<7	10
11{25}	416.1	5 (A)	87		<7	<7	<7	0
11{26}	310.2	6 (A)	55	functional group interference?	<7	14	<7	71
11{27}	284.9	31 (A)	90		<7	10	<7	0
11{28}	251.0	21 (A)	63		11	14	<7	57
11{29}	269.0	30 (C)	97	from the benzoic acid	<7	22	<7	57
11{30}	281.0	31 (C)	99	from the benzoic acid	11	27	<7	78
11{31}	285.5	22 (A)	91		13	27	<7	70
11{32}	265.1	33 (A)	95		13	27	<7	79
11{33}	231.1	25 (A)	96		12	25	<7	54
11{34}	289.0	25 (A)	92		11	21	<7	64
11{35}	290.1	27 (A)	100		12	23	<7	79
11{36}	290.1	26 (A)	100		<7	16	<7	59
11{37}	274.0	36 (A)	97		16	26	<7	65
11{38}	242.1	30 (A)	97		14	27	<7	37
11{39}	275.0	21 (A)	89		11	23	<7	61
11{40}	242.1	35 (A)	97		13	28	<7	60
11{41}	275.0	28 (A)	90		16	25	<7	68
11{42}	275.0	18 (A)	98		<7	21	<7	32
11{43}	342.1	29 (A)	77		<7	13	<7	57
11{44}	292.1	26 (A)	82		19	31	<7	77
11{45}	292.1	28 (A)	70		15	28	<7	55
11{46}	292.1	11 (A)	67		<7	18	<7	12
11{47}	305.0	18 (A)	98		12	18	<7	85
11{48}	258.5	25 (A)	90		13	30	<7	69
11{49}	303.0	54 (C)	89	from the benzoic acid	14	26	<7	66
11{50}	238.0	23 (A)	96		11	26	<7	59
11{51}	260.0	22 (A)	99		13	29	<7	54
11{52}	260.0	25 (A)	95		<7	20	<7	25
11{53}	290.0	18 (A)	93		11	27	<7	55
11{54}	296.0	20 (A)	<50	largely elimination product	<7	<7	<7	0
11{55}	184.1	22 (A)	<50	poor HPLC purity, fair NMR purity	<7	<7	<7	0
11{56}	232.1	43 (A)	87	mixture of cis/trans isomers by NMR	<7	23	<7	39
11{57}	277.1	17 (A)	75	mixture of cis/trans isomers by NMR	<7	8	<7	1
11{58}	300.1	33 (A)	97		<7	14	<7	32
11{59}	266.6	17 (A)	85		<7	14	<7	36
11{60}	250.0	25 (A)	85	prepared chloride	10	19	<7	50
11{61}	262.0	25 (A)	51	prepared chloride	10	18	<7	48
11{62}	220.1	31 (B)	91		<7	<7	<7	34
11{63}	250.1	25 (B)	97		<7	9	<7	0
11{64}	250.1	22 (B)	93		<7	<7	<7	10
11{65}	254.5	10 (B)	97		<7	16	<7	12
11{66}	226.1	13 (B)	80		<7	<7	<7	17
11{67}	246.1	27 (B)	81	mixture of two *trans* diastereomers	<7	15	<7	31
11{68}	234.1	12 (B)	91		<7	11	<7	32
11{69}	230.1	19 (B)	94		<7	<7	<7	18
11{70}	270.2	11 (B)	86		<7	8	<7	60
11{71}	198.1	26 (B)	88		<7	10	<7	7
11{72}	216.1	33 (B)	75	mixture (3:1) of diastereomers	<7	<7	<7	0
11{73}	282.2	23 (B)	94		<7	<7	<7	8
11{74}	265.0	13 (B)	89		15	28	<7	71

Scheme 6. Synthesis of Aliphatic 2-Substituted Oxazoline Hydroxamates (Method B)

Chart 1
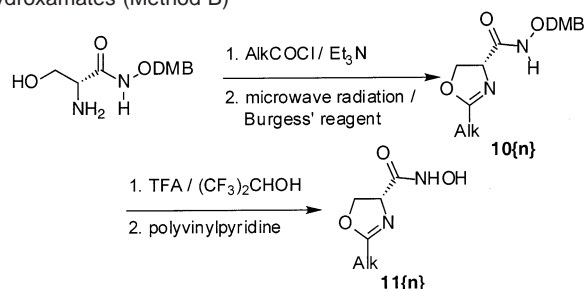
products, whose complete structures are provided in the Supporting Information. Three compounds were prepared from the corresponding carboxylic acids rather than the acid chlorides. The synthesis of these compounds was accomplished by using an alternate method (Method C), as described in the Experimental Section.

The above procedure proved to be very reliable for aromatic and α,β-unsaturated acid chlorides, almost regardless of the steric and electronic nature of the acid chloride. However, significant problems were observed with aliphatic acid chlorides. These reactants were successfully used in our earlier work on oxazoline synthesis by resin capture/ring-forming release,[11] but the same chemistry proved unreliable in the case of these oxazoline hydroxamates. This difficulty was attributed to several problems: (1) the increased reactivity of the aliphatic acid chlorides results in partial acylation of the hydroxamate NH; (2) the DMB-protected oxazoline hydroxamate intermediate **10** is unstable and decomposes to unidentified products over a period of hours; (3) oxazoline hydroxamates with an aliphatic group at the 2-position are unstable to TFA and decompose quite rapidly, especially in the presence of silica gel. While a solution to the last problem was later identified (vide infra), the first two problems seemed to preclude the use of the catch-and-release method. Therefore, an alternate procedure (Method B) was developed which utilized Wipf's method[19] for oxazoline synthesis using Burgess' reagent for the cyclization of the amide (Scheme 6). An enhancement that we made to this procedure involved microwave heating. A microwave synthesizer that accepts small reaction vessels made this step much more convenient to perform. Although this method is not very amenable to high-throughput synthesis, it did allow for the synthesis and investigation of a dozen or so 2-aliphatic oxazoline hydroxamates. Owing to the use of Burgess' reagent and to the formation of the *N*-acylation byproduct, chromatographic purification of the intermediate *O*-protected oxazoline was required in this method. The purified oxazoline was immediately treated with 2% TFA in hexafluoroisopropanol (HFIP). After allowing the solution to stand briefly, the deep-red reaction mixture was quenched by addition to a pipet charged with a layer of polyvinylpyridine over a short pad of silica gel. It is absolutely essential that the TFA be completely quenched by the polyvinylpyridine prior to the solution contacting silica gel. Failure to do so results in nearly complete destruction of the alkyl oxazoline. The decomposition product has not been unambiguously identified. However, NMR evidence strongly indicates opening of the oxazoline ring to form a serine-like derivative.
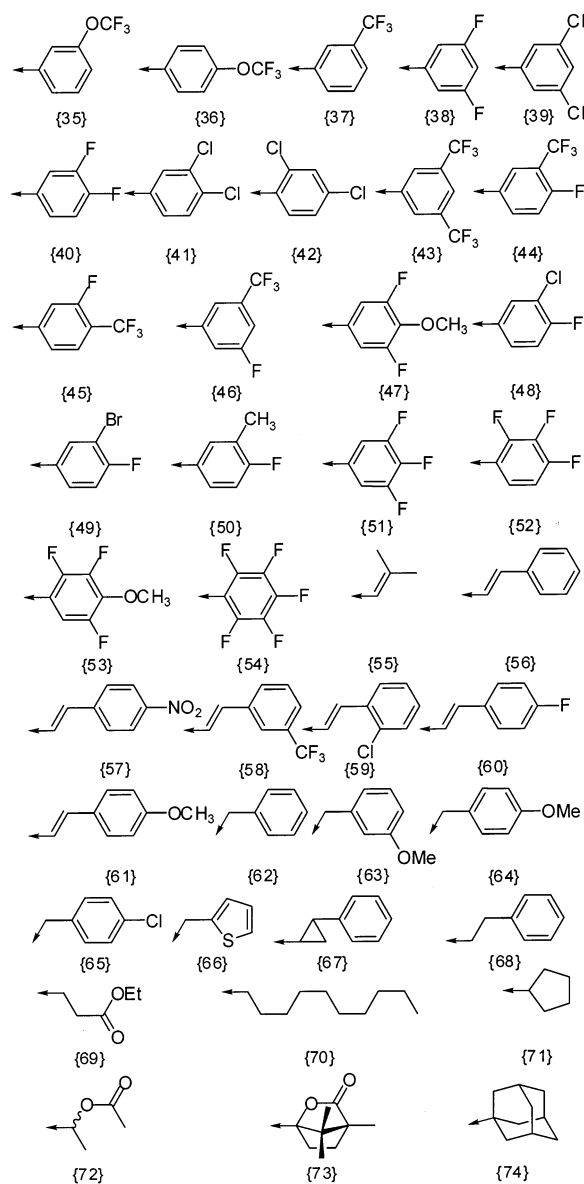
(19) Wipf, P.; Miller, C. P. A Short, Stereospecific Synthesis of Dihydrooxazoles from Serine and Threonine Derivatives. *Tetrahedron Lett.* **1992**, *33*, 907–910.
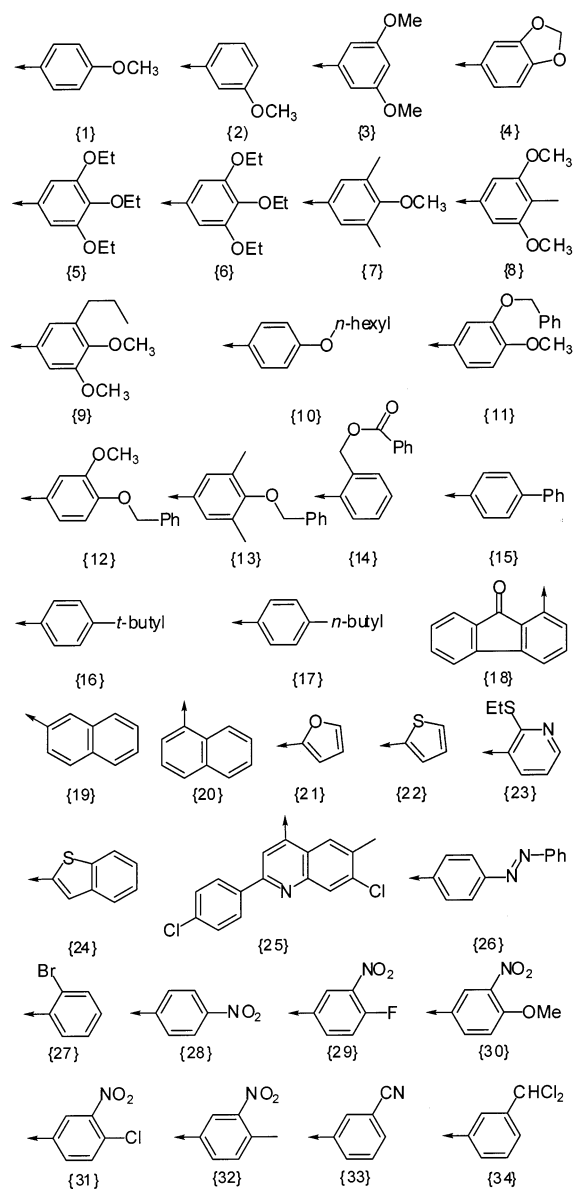
Biological Data. Charts 1 and 2 present the 2-substituents for the oxazoline hydroxamates that were synthesized in this study. Figures S1 and S2 provide a concise listing of the complete structures and compound numbers. Table 1 provides their enzyme inhibitory activity and in vitro biological activities. All compounds were screened for antibacterial activity against wild-type *E. coli* (R477), hypersensitive *LpxC1 E. coli* (G17S),[8] and *P. aeruginosa* (PA01). The antibacterial tests were conducted by treating a 7 mm filter disk with 20 μg of the inhibitor dissolved in 5 μL of DMSO. The disk was placed on a fully plated lawn of bacteria on agar.[8] After overnight incubation, a zone of growth inhibition was clearly visible around the filter disks that contained compounds that exhibited antibacterial activity. Previous studies have shown that the diameter of the zone of inhibition correlates approximately with the MIC of that particular compound.[8]

Owing to the large number of compounds that were synthesized and the operational difficulty of performing the current LpxC assay, it was not practical to determine an IC$_{50}$ for each compound that was prepared. Instead, a simple percent inhibition

Chart 2

{35} {36} {37} {38} {39}

{40} {41} {42} {43} {44}

{45} {46} {47} {48}

{49} {50} {51} {52}

{53} {54} {55} {56}

{57} {58} {59} {60}

{61} {62} {63} {64}

{65} {66} {67} {68}

{69} {70} {71}

{72} {73} {74}

was measured for each of the compounds at a fixed dilution (1 μg/mL). Enzymatic testing was performed only against the *E. coli* LpxC. Although the meaning of the resulting value can be debated, at a minimum it allows a rough correlation between enzyme inhibitory activity and antibacterial activity. For a compound of M_r 250 Da, 50% inhibition of the enzyme at 1 μg/mL roughly correlates to an IC_{50} of 4 μM. To support the validity of percent inhibition as a metric, true IC_{50}'s were determined for a subset of compounds that includes active and inactive agents (Table 2). Correlation of IC_{50} with percent inhibition is quite adequate. Additionally, to support the use of inhibition zones to reflect antibacterial activity, MICs against both wild-type and hypersensitive *E. coli* were determined for the same subset of compounds.

As expected on the basis of the work of Onishi,[7] the addition of electron-donating groups to the aromatic ring of the inhibitors increases both enzyme inhibitory activity and antibacterial activity against *E. coli*. This is illustrated by the series of compounds **11**{*2*}, **11**{*3*}, and **11**{*5*} in which the sequential addition of methoxy groups increases the inhibition of LpxC

from 44% to 67% to 72%. Despite the increase in enzyme inhibitory activity, the antibacterial activity is only marginally increased. More hydrophobic alkoxy groups are also beneficial, as exemplified by the *p*-hexyloxy **11**{*10*} (86%) and triethoxy **11**{*6*} (86%). These two compounds were among the best LpxC inhibitors in the library, but for reasons that are not entirely clear, they displayed little or no antibacterial activity. One possibility is that very hydrophobic compounds may become trapped in the cell membrane, resulting in low concentrations of inhibitor in the cytoplasm. Interestingly, large alkoxy groups at the meta position seem to be quite unfavorable for LpxC inhibition as illustrated by *m*-benzyloxy **11**{*11*} (8%). Other electron-donating groups are also favorable for enzyme inhibitory activity. This is evident in *p*-phenyl **11**{*15*} (69%), *p*-*tert*-butyl **11**{*16*} (58%), and dimethyl **11**{*7*} (71%). Moreover, the combination of electron-donating alkoxy and alkyl groups can result in compounds with excellent LpxC activity, as in the known L-161,240 (**11**{*9*}). However, **11**{*13*}, **11**{*7*}, and **11**{*8*} also incorporate a combination of alkoxy and alkyl groups yet have only weak-to-moderate enzyme inhibitory and antibacterial activity.

The above generalizations hold true only at the meta and para positions on the aromatic ring. Substitution at the ortho positions by either electron-donating groups (**11**{*14*}) or electron-withdrawing groups (**11**{*27*}) results in a nearly complete loss of activity. Bicyclic oxazoline hydroxamates such as naphthalenes (**11**{*19*} and **11**{*20*}) as well as fluorenone **11**{*18*} were also found to have quite poor activity. Similarly, heterocyclic oxazoline hydroxamates such as furan **11**{*21*}, thiophene **11**{*22*}, pyridine **11**{*23*}, and quinoline **11**{*25*} were found to have little-to-no enzyme inhibitory or antibacterial activity.

An unexpected discovery from this set of compounds was that electron-withdrawing groups can enhance both enzyme inhibitory and antibacterial activity. For example, the electronically very different *p*-nitro **11**{*28*} and *p*-methoxy **11**{*1*} have nearly the same enzyme inhibitory and antibacterial activity. This result strongly suggests that sterics, not electronics, play the most important role in the binding of these compounds into the LpxC active site. Nitro groups at the meta position are also quite beneficial as illustrated by **11**{*29*}−**11**{*32*}. Other electron-withdrawing groups were also found to enhance enzyme inhibitory activity. These include *m*-cyano (**11**{*33*}), *m*-CHCl_2 (**11**{*34*}), and *p*-OCF_3 (**11**{*36*}).

The meta CF_3 analogue (**11**{*37*}) showed excellent enzyme inhibitory activity and proved to be one of the most potent antibacterial agents of this series. Interestingly, the addition of a second meta electron-withdrawing group (CF_3 or F) resulted in a drastic loss of enzyme inhibitory and antibacterial activity (**11**{*43*} and **11**{*46*}). On the contrary, the addition of a fluorine to the para position (**11**{*44*}) resulted in the most active antibacterial agent of the series—even more active than the heretofore most potent compound, L-161,240 (**11**{*9*}). Despite its superior antibacterial activity, this compound is a significantly less potent enzyme inhibitor. An analogue of this compound in which the CF_3 and the F have traded places (**11**{*45*}) has only slightly lower enzyme inhibitory and antibacterial activity.

Another unexpected finding was that polyhalogenated aromatics frequently showed excellent biological activity. Moderate activity was observed from 3,5-dihalogenated compounds such as **11**{*39*} and **11**{*38*}. More interestingly, 3,4-dihalogenated

Table 2. Enzyme Inhibitory Activity and Antibacterial Activity of Select Oxazoline Hydroxamates

cmpd	IC_{50} (μM)	LpxC inhibition (%)	MIC R477 (μg/mL)	inhibition zone R477 (mm)	MIC G17S (μg/mL)	inhibition zone G17S (mm)
11{9}	0.097	96	4	17	0.16	26
11{47}	0.33	85	20	12	0.16	18
11{10}	0.75	86	>100	<7	4	11
11{53}	1.6	53	100	11	4	27
11{38}	6.8	37	100	14	4	27

compounds tended to show excellent biological activity. These include 3,4-difluoro **11**{*40*}, 3-chloro-4-fluro **11**{*48*}, and 3-bromo-4-fluoro **11**{*49*}. The 3,4-dichloro **11**{*41*} showed nearly the same antibacterial activity as L-161,240 (**11**{*9*})! The combination of halogens and an electron-donating group also produced compounds with good activity (**11**{*47*}, **11**{*50*}, and **11**{*53*}).

Oxazoline hydroxamates with nonaromatic substituents at the 2-position (**11**{*55*}−**11**{*74*}) were generally observed to have quite poor enzyme inhibitory and antibacterial activity. While a few of the cinnamate derivatives (**11**{*55*}−**11**{*61*}) had moderate enzyme inhibitory activity, only **11**{*60*} and **11**{*61*} showed any antibacterial activity at all against wild-type *E. coli*. Very few of the aliphatic substituted oxazolines showed significant activity against either the enzyme or against bacteria. Only very hydrophobic 2-substituents such as dodecyl **11**{*70*} and adamantyl **11**{*74*} resulted in compounds with high enzyme inhibitory activity. Of these, only adamantyl **11**{*74*} showed significant antibacterial activity.

The data in Table 2 provide an interesting comparison of rough and more rigorous assessments of the biological activity in this set of compounds. They include the structurally homologous series **11**{*38*}, **11**{*47*}, **11**{*53*}, which are the difluoro, methoxy-difluoro, and methoxy-trifluoro compounds. Correlation of IC_{50} with MIC fails most obviously with the hexyloxy compound **11**{*10*}, which as presented below is thought to be too hydrophobic. Correlation of inhibition zone with MIC seems problematic with the hypersensitive *E. coli* strain.

None of the 74 compounds in this series showed any antibacterial activity against *P. aeruginosa*, making their testing against *Pseudomonas* LpxC a high priority. Previous studies have shown that L-161,240 binds about 20 times less effectively to *Pseudomonas* LpxC than to *E. coli* LpxC.[8] It is therefore likely that the lack of *Pseudomonas* antibacterial activity in this series of compounds is due to weak inhibition of the *P. aeruginosa* enzyme. The development of potent anti-*Pseudomonas* agents will require the synthesis and testing of larger LpxC inhibitor collections against the *P. aeruginosa* enzyme. Kline recently reported the in vitro enzyme inhibitory properties of a family of aryl heterocyclic hydroxamates similar to L-161,240 against LpxC from *Pseudomonas aeruginosa*.[20]

Discussion

It is interesting but not unexpected that the antibacterial activity as measured by disk diffusion and the enzyme inhibitory activity of these compounds are not strictly correlated. For
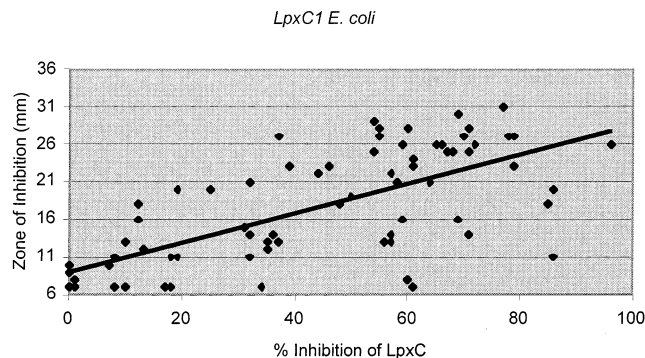
(20) Kline, T.; Andersen, N. H.; Harwood, E. A.; Bowman, J.; Malanda, A.; Endsley, S.; Erwin, A. L.; Doyle, M.; Fong, S.; Harris, A. L.; Mendelsohn, B.; Mdluli, K.; Raetz, C. R. H.; Stover, C. K.; Witte, P. R.; Yabannavar, A.; Zhu, S. Potent, Novel in Vitro Inhibitors of the *Pseudomonas aeruginosa* Deacetylase LpxC. *J. Med. Chem.* **2002**, *45*, 3112−3129.

LpxC1 E. coli

Figure 3. Correlation of antibacterial activity against the hypersensitive *E. coli* strain and enzyme inhibitory activity. The line is drawn arbitrarily.

example, the triethoxybenzene analogue **11**{*6*} inhibits LpxC activity by 86% but is a rather poor antibacterial agent. On the other hand, piperonyl **11**{*4*} inhibits LpxC activity by 46% but is a very potent antibacterial agent. Therefore, it is clear that factors other than enzyme inhibitory potency play a role in determining the antibacterial activity of a particular inhibitor. These factors could include the following: membrane permeability, nonspecific binding to various bacterial proteins, interactions with unintended enzymes or receptors, metabolism, and efflux by membrane pumps or channels. Moreover, it is also plausible that specific structural features of certain compounds will allow them to be selectively transported *into* the bacterial cells through specific channels or pumps. This situation would result in antibacterial activity that is greater than might be guessed on the basis of enzyme inhibitory potency. The identification of structural features that either inhibit or promote compound transport into bacterial cells is of high importance in the design of antibacterial compounds based upon the oxazoline hydroxamate scaffold.

These structural features may be identified by a careful comparison of enzyme inhibitory activity and antibacterial activity. In a situation where net membrane transport has no influence, enzyme inhibitory activity and antibacterial activity should be directly correlated. The expectation of such a correlation is expressed graphically as the arbitrarily drawn lines in Figures 3 and 4. However, in reality, factors such as those described above can cause significant deviations from this correlation. Figures 3 and 4 depict a rough correlation of enzyme inhibitory and antibacterial activity. There is no particular theoretical basis for such a correlation, despite the fact that it exists here and seems logical. As may be expected, the correlation is more pronounced in the case of the hypersensitive *LpxC1* strain of *E. coli*, which has a defective outer membrane because of a point mutation that causes partial inhibition of lipid A synthesis. Most importantly, there are no compounds that exhibit significant antibacterial activity but lack enzyme inhibitory activity. This observation strongly suggests that the
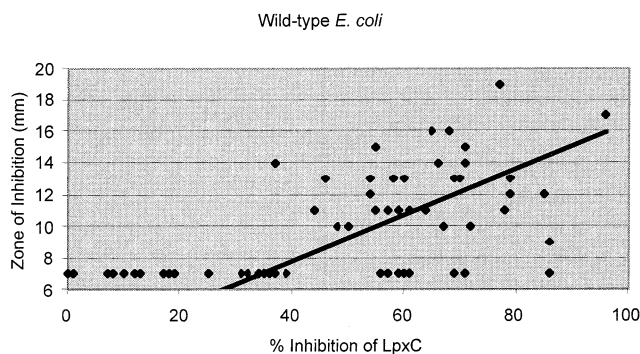
Figure 4. Correlation of antibacterial activity against the wild-type *E. coli* strain and enzyme inhibitory activity can be used to group compounds into three sets.

observed antibacterial activity is due to the inhibition of the targeted enzyme. The correlation between the enzymatic IC_{50} and the MIC against wild-type *E. coli* given in Table 2 also supports the hypothesis (with the proviso that the data set is small) that LpxC is the target of antibacterial activity of these oxazoline hydroxamates. In the case of wild-type *E. coli*, it appears (on the basis of Figure 4) that a "minimum" threshold of enzyme inhibitory activity must be reached before antibacterial activity is observed. This threshold is approximately 45% inhibition, which roughly translates to an IC_{50} of $2-4$ μM. On the other hand, hypersensitive *LpxC1 E. coli* are sensitive to even quite weak enzyme inhibitors. In a few cases, weak antibacterial activity is observed even with inhibitors that only block 15% or less of enzyme inhibitory activity at 1 μg/mL. Interestingly, compounds **11**{*27*} and **11**{*63*} have weak antibacterial activity against *LpxC1 E. coli* and yet exhibit no enzyme inhibitory activity. This is not surprising given that the hypersensitive strain presents a much less formidable membrane barrier and barely has sufficient lipid A for cell growth. It might also be due to binding to an unidentified bacterial target.

Compounds with a disproportionately high or disproportionately low antibacterial activity for a given enzyme inhibitory activity should be carefully analyzed for structural features that may inhibit or facilitate the desired activity. In other words, compounds that are significantly above or below the line in Figure 4 are worthy of study. The active compounds in Figure 4 can be grouped into three somewhat arbitrary sets: set A includes compounds with greater than expected antibacterial activity, set B includes those with roughly the expected antibacterial activity, and set C includes those with less than expected antibacterial activity. Analysis of the structures in these three sets may reveal unique features that predispose a given compound to have either greater than expected or less than expected activity. The structures of the compounds in the sets A and C are presented in Figures 5 and 6, respectively. Interestingly, three of the six compounds in set A contain a meta or para-CF_3 group (**11**{*37*}, **11**{*44*}, and **11**{*45*}). Compounds with other electron-withdrawing groups at these positions such as nitro and OCF_3 fall into set B, strongly suggesting that the aryl CF_3 group may be a privileged structural feature. However, set C contains a compound with two CF_3 groups (**11**{*43*}). The addition of the second CF_3 somehow results in compounds that exhibit lower than anticipated antibacterial activity. While set A also contains two compounds with multiple aromatic halogens, the significance of this is
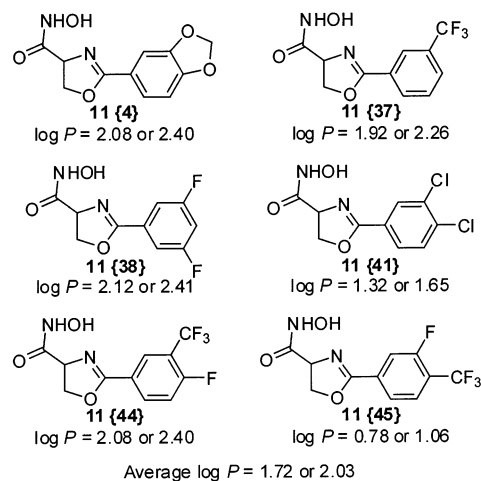

11 {4}
log *P* = 2.08 or 2.40

11 {37}
log *P* = 1.92 or 2.26

11 {38}
log *P* = 2.12 or 2.41

11 {41}
log *P* = 1.32 or 1.65

11 {44}
log *P* = 2.08 or 2.40

11 {45}
log *P* = 0.78 or 1.06

Average log *P* = 1.72 or 2.03

Figure 5. Structures of the compounds in set A with calculated log *P* values for each. The two log *P* values were calculated using ChemDraw Ultra 6.0 by Crippen's fragmentation method and Viswanadhan's fragmentation method, respectively.

11 {6}
log *P* = 1.64 or 1.64

11 {10}
log *P* = 2.95 or 3.12

11 {13}
log *P* = 3.58 or 3.83

11 {17}
log *P* = 2.74 or 3.03

11 {26}
log *P* = 3.44 or 3.68

11 {36}
log *P* = 2.53 or 2.87

11 {43}
log *P* = 2.85 or 3.14

11 {70}
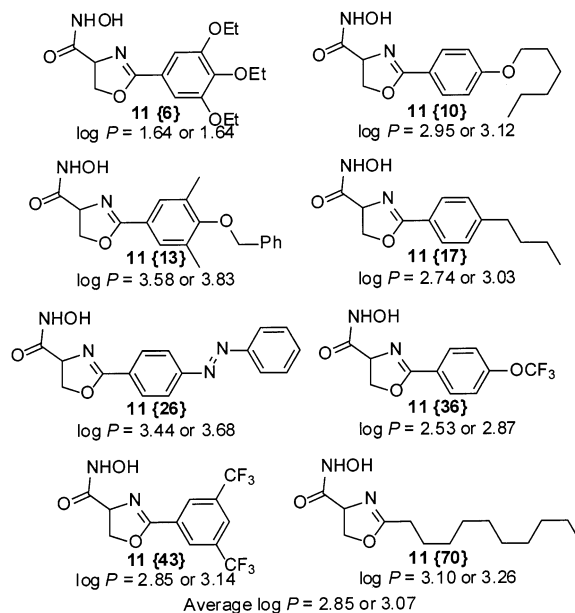log *P* = 3.10 or 3.26

Average log *P* = 2.85 or 3.07

Figure 6. Structures of the compounds in set C along with the calculated log *P* values.

unclear because set B also contains several similar compounds. Trimethoxy **11**{*5*} inhibits LpxC activity by 72% and has an antibacterial halo of 10 mm. On the other hand, piperonyl **11**{*4*} is a much weaker inhibitor (46%), but shows a much broader antibacterial halo (13 mm). Thus, the piperonyl group (also in set A) may be a privileged structural feature, since piperonyl is also present in potent isoxazoline hydroxamates (Pirrung, M. C.; Zhu, J., unpublished).

Certainly the most obvious generalization that can be made from these three sets of compounds is that very hydrophobic oxazoline hydroxamates tend to have quite poor antibacterial activity. Set C contains several compounds with quite hydrophobic substituents at the 2-position of the oxazoline. Some of these compounds such as **11**{*6*}, **11**{*17*}, and **11**{*10*} are quite potent enzyme inhibitors despite exhibiting little to no antibacterial activity. It has been previously suggested that very hydrophobic inhibitors may become trapped in the cell membrane and be unavailable for the cytosolic inhibition of LpxC.[7]

In fact a comparison of the log P values for each of these compounds reveals that, on average, the compounds in set A have an octanol/water partition coefficient about one log unit lower than those in set C (Figures 5 and 6). In other words, the compounds in set C are significantly more lipophilic and may associate nonspecifically with bacterial lipid bilayers. Regardless of the reason, it is clear that large hydrocarbon substituents predispose a compound toward poor antibacterial activity. It is important to emphasize that the differences in antibacterial activity between compounds in set A and set C are not based on enzyme inhibitory potency—all of the compounds exhibit moderate-to-good activity against LpxC. Rather, the structural features in these two sets may enhance or diminish other factors that influence antibacterial activity. These factors could include the following: membrane permeability, nonspecific binding to bacterial proteins, interactions with unintended enzymes or receptors, metabolism, and influx or efflux by membrane pumps or channels.

Conclusions

In summary, a diverse set of oxazoline hydroxamates has been synthesized using a unique solid-phase catch-and-release ring-forming strategy. This method proved to be quite reliable and resulted in the formation of very high purity products that could be directly used for biological testing. The resulting set of 74 compounds was screened for antibacterial activity against wild-type *E. coli*, hypersensitive *E. coli*, and *P. aeruginosa*. Although no compounds were found to inhibit the growth of *P. aeruginosa*, numerous compounds were found to be very effective antibacterial agents against the two strains of *E. coli*. In particular, one compound was found to have greater antibacterial activity than the most potent growth inhibitor from earlier work. The library of compounds was also screened for enzyme inhibitory activity against *E. coli* LpxC. In contrast to previous studies of this class of inhibitors, electron-withdrawing groups on the aromatic ring were found to be beneficial for enzyme inhibitory and antibacterial activities. Several of the most potent compounds from this series incorporate electron-withdrawing groups. Careful correlation of enzyme inhibitory and antibacterial activities allowed the identification of at least two structural features (CF$_3$ and piperonyl groups) that play an important *nonenzymatic* role in determining the antibacterial activity of oxazoline hydroxamates. Very hydrophobic oxazoline hydroxamates often exhibit excellent enzyme inhibitory activity while showing little-to-no antibacterial activity. In addition to identifying several compounds nearly equipotent with L-161,240, these studies have allowed the identification of several important structural features that facilitate enzyme binding. This knowledge may aid in the design of related classes of LpxC inhibitors.

Experimental Section

Enzyme Assays and Antibacterial Testing: Buffers and Reagents. [α-^{32}P]-UTP was purchased from NEN Dupont. PEI-Cellulose TLC plates and sodium phosphate (dibasic and monobasic) buffer were obtained from E. Merck, Darmstadt, Germany. Bovine serum albumin (BSA) was purchased from Sigma, and DMSO was purchased from Mallinckrodt.

LpxC Activity Assay. The *E. coli* LpxC substrate, [α-^{32}P]-UDP-3-*O*-(*R*-3-hydroxymyristoyl)-GlcNAc was prepared and purified as described previously by acylation of [α-^{32}P]-UDP-GlcNAc using purified *E. coli* LpxA (provided by T. J. O. Wyckoff, Duke University). These assays were performed at 30 °C, and contained 4 μM UDP-3-*O*-(*R*-3-hydroxymyristoyl)GlcNAc, 1 mg/mL BSA in 25 mM sodium phosphate, pH 7.4. The activity assays were performed in plastic microcentrifuge tubes in a reaction volume of 20 μL. At each time point (chosen so that the total conversion to product was less than 10%), 5 μL portions of each reaction mixture were removed and added to 1 μL of 1.25 M NaOH to stop the reaction. The alkaline samples were incubated for an additional 10 min at 30 °C to ensure complete hydrolysis of the ester-linked acyl chains from the LpxC substrate and product and then were neutralized by the addition of 1 μL 1.25 M acetic acid and 1 μL 5% trichloroacetic acid. The neutralized samples were incubated on ice for 5 min and centrifuged for 2 min in a microcentrifuge. Portions of the supernatants (1 μL) were spotted onto PEI-cellulose TLC plates for separation of the remaining substrate (detected as [α-^{32}P]-UDP-GlcNAc) from the product (detected as [α-^{32}P]-UDP-GlcN). After air-drying, the plates were soaked for 10 min in methanol to improve resolution before chromatography. The plates were developed with 0.2 M aqueous guanidine—HCl as the solvent system. The radioactive spots on the plates were analyzed using a PhosphorImager equipped with ImageQuant software (Molecular Dynamics, Inc.) to determine the yields of product produced in each reaction mixture.

Inhibition of LpxC Activity. Stock solutions (10 mg/mL) and any further dilutions of each inhibitor were made in 100% dimethyl sulfoxide (DMSO). Compounds were added to a final concentration of 1 μg/mL to an assay mixture containing 4 μM UDP-3-*O*-acyl-GlcNAc in 25 Mm sodium phosphate buffer, pH 7.4. Additional DMSO was added to maintain compound solubility at a final assay concentration of 10% in DMSO. Purified *E. coli* LpxC was incubated with 1 mg/mL BSA in buffer and diluted into the final assay mixture of 0.1 nM at 30 °C. The initial velocities were plotted as a function of inhibitor concentration for each compound. The resulting data were fit to eq 1, where v_i = the initial velocity at a given concentration of inhibitor and v_c = the initial velocity of a control reaction containing no inhibitor, to yield the IC$_{50}$ for inhibition at 30 °C by each compound.

$$v_i/v_c = \text{IC}_{50}/([\text{I}] + \text{IC}_{50}) \tag{1}$$

Disk Diffusion Test for Antibacterial Activity of LpxC Inhibitors. A 5 mL culture of *E. coli* strain R477 (wild type), G17S (*LpxC1* mutant), or PA01 (wild-type *Pseudomonas*) was grown to stationary phase at 37 °C in LB broth. Media for *E. coli* growth also contained 30 μg/mL streptomycin.[8] This culture was diluted 1:100 with LB broth and grown at 37 °C to A_{600} = 0.2. A sterile cotton swab was used to spread an even lawn of the early log phase culture onto an LB agar plate. Filter paper disks (7-mm diameter) were saturated with 20 μg of the compound (in 100% DMSO) to be tested for antibacterial activity. The disks were placed on the lawn of freshly plated cells, and the plates were then incubated at 37 °C. Inhibition of bacterial growth was detected as a zone of clearing around each disk. The diameter of the zone of growth inhibition was measured for each compound after overnight incubation. A diameter of 7 mm indicated that there was no visible inhibition of growth beyond the edges of the disk.

MIC. Antibacterial activity of specific LpxC inhibitors was determined using two strains of *E. coli*, the wild-type strain (R477) and the hypersensitive strain (G17S), which contains a point mutation in LpxC. Overnight cultures of each strain were grown at 37 °C and then diluted to OD$_{660}$ = 0.1. The diluted culture was further diluted 1:100 into 1 mL of LB containing varied concentrations of inhibitor or DMSO as a control. Growths were performed in test tubes such that each tube contained a different amount of inhibitor (0.00005−100 μg/mL) so that each concentration was varied by a factor of 5. The cultures were allowed to grow for 7 h at 37 °C with shaking. The MIC was defined as the lowest inhibitor concentration that inhibited growth as measured by no increase in A_{660} during the time of the assay.

General. (Polystyrene)tosyl chloride resin was obtained from Argonaut Technology. HPLC analysis was performed with a Hewlett-Packard series 1100 HPLC using an Econosphere C18 5U column (4.6 mm × 250 mm). HPLC conditions were as follows: 25 °C, flow rate = 1 mL/min, 10 μL injection (as a solution in MeOH and CH$_3$CN), UV detection (220 nM). Gradient elution A: CH$_3$CN (+0.1% TFA); B: H$_2$O (+0.1% TFA). $T = 0$ min: 25% A, 75% B. $T = 10$ min: 65% A, 35% B. $T = 13$ min: 25% A, 75% B. NMR analysis was performed using a Varian INOVA 300 MHz instrument. Direct probe ionization (DIP) MS analysis was performed on an HP 5988A. FAB-MS was performed using a JEOL SX102 instrument. Pyridine and CH$_2$-Cl$_2$ were distilled from CaH$_2$ immediately prior to use.

***O*-(2,4-Dimethoxybenzyl)hydroxylamine (4).** *N*-Hydroxyphthal-imide (17.5 g, 107 mmol) and 2,4-dimethoxybenzyl alcohol (17.5 g, 107 mmol) were stirred in 700 mL of CH$_2$Cl$_2$ at 0 °C. Tributyl phosphine (40.0 mL, 161 mmol) was added followed (slowly) by diisopropyl azodicarboxylate (31.9 mL, 161 mmol). The solution was stirred at room temperature for 24 h. The reaction was concentrated and recrystallized from 1.5 L of boiling ethanol to give 28.0 g (85%) of the desired intermediate as white crystals. This white solid was stirred as a suspension in 800 mL of refluxing ethanol. *N*-Methylhydrazine (5.20 mL, 100 mmol) was added, and the mixture was stirred at reflux for 1 h. The solution was concentrated to remove the ethanol. Ether (800 mL) was added, and the reaction mixture was allowed to stand at room temperature for 30 min. The resulting solid was filtered. The organic solution was concentrated to give 17 g of the desired product as an oil (100% plus minor amounts of the phthalamide byproduct). The product could be further purified by repeating the ether treatment. The product thus obtained was identical to the previously reported compound.

[1-(2,4-Dimethoxybenzyloxycarbamoyl)-2-hydroxy-ethyl]carbam-ic Acid *tert*-Butyl Ester (6). Boc-D-Ser-OH (1.2 g, 6.0 mmol) and **4** (1.0 g, 6.0 mmol) were dissolved in 50 mL of CH$_2$Cl$_2$. After stirring briefly, EDC (ethyldimethylaminopropyl carbodiimide, 1.5 g, 7.8 mmol) was added, and the solution was stirred overnight. The solution was washed once with water, dried over MgSO$_4$, filtered, and concentrated. The desired product could be purified by chromatography (3:2 EtOAc/Hex, $R_f \approx 0.17$) to give 1.3 g (64%) to give **6** as a white solid. Alternatively, recrystallization of the crude product from ethanol/water gave 58% of **6**; mp 115−116 °C. IR (thin film) 3305, 2982, 1668, 1616, 1511, 1292, 1162 cm^{-1}. ^{1}H NMR (CD$_3$CN) δ 9.17 (1 H, bs), 7.24 (1 H, d, $J = 8.7$ Hz), 6.48−6.45 (2 H, m), 5.53 (1 H, bs), 4.91 (2 H, s), 4.11−3.98 (2 H, m), 3.84 (3 H, s), 3.81 (3 H, s), 3.66−3.58 (1 H, m), 3.02 (1 H, bs), 1.43 (9 H, s). ^{13}C NMR (CDCl$_3$) δ 168.66, 161.83, 159.55, 156.12, 132.85, 116.04, 104.34, 98.77, 80.80, 73.53, 62.93, 55.82, 55.65, 53.55, 28.64. FAB MS (M): 370. GCMS (M + H): 371. HRMS m/z calcd for C$_{13}$H$_{17}$N$_2$O$_6$ (M-*tert*-butanoate): 297.1087, found 297.1085.

2-Amino-*N*-(2,4-dimethoxybenzyloxy)-3-hydroxypropionamide (7). Serine derivative **6** (0.80 g, 2.2 mmol) was stirred in 16 mL of CH$_2$Cl$_2$ under N$_2$. After cooling to 0 °C, 2,6-lutidine (1.2 mL, 11 mmol) was added followed by TMS-triflate (1.6 mL, 8.6 mmol). The solution was warmed to room temperature and stirred for 1.5 h. Methanol (40 mL) was added followed by Et$_3$N (8 mL), and the stirring was continued overnight. The solution was concentrated in a vacuum under gentle heat. The desired product was isolated by chromatography (10% ◇ 20% EtOH in CH$_2$Cl$_2$, $R_f \approx 0.05$, stained with PMA) to give 0.44 g (76% from **6**) of **7** as a thick oil that solidified overnight; mp 122−124 °C. IR (Nujol) 3444, 3217, 1614, 1593, 1509, 1464, 1290 cm^{-1}. ^{1}H NMR (CD$_3$CN) δ 7.22 (1 H, d, $J = 8.4$ Hz), 6.54 (1 H, d, $J = 2.4$ Hz), 6.49 (1 H, dd, $J = 2.4$, 8.4 Hz), 4.80 (2 H, s), 4.48 (3 H, bs), 3.81 (3 H, s), 3.79 (3 H, s), 3.70−3.58 (2 H, m), 3.52 (1 H, t, $J = 4.6$ Hz). ^{13}C NMR (CD$_3$OD) δ 169.43, 160.74, 158.43, 131.72, 115.96, 104.38, 98.16, 71.28, 64.00, 55.43, 55.21, 54.83. FAB MS (M + H): 271. Anal. Calcd

for C$_{12}$H$_{18}$N$_2$O$_5$ + 0.5H$_2$O: C, 51.62; H, 6.86; N, 10.03. Found: C, 52.02; H, 6.54; N, 9.79.

General Procedure (A) for the Synthesis of 11{*n*} (*n* = 1−28, 31−48, 50−61). The acid chloride (0.17 mmol) was dissolved in 0.5 mL of freshly distilled pyridine in a 10-mL scintillation vial. To this was added a solution of **7** (50 mg, 0.18 mmol) and Et$_3$N (0.17 mmol, 23 μL) in 1.5 mL freshly distilled pyridine. After stirring for 10 min, the resulting solution was added to a 5-mL Quest 210 reaction vessel containing 0.27 g (∼0.67 mmol) of polystyrene tosyl chloride resin. The vessel was capped and placed in a freezer (−15 °C) for 24 h. The vessel was then inserted into the Quest 210 synthesizer, and the resin was washed with CH$_2$Cl$_2$ (2×), DMSO (2×), and CH$_2$Cl$_2$/MeOH (1:1, 7×). The resin was then treated with 3 mL of 49:49:2 pyridine:THF: Et$_3$N and agitated for 24 h at room temperature. The resin was filtered and washed with CH$_2$Cl$_2$ (2×). The combined organic filtrates were concentrated overnight on a speedvac (37 °C). The residue was dissolved in 1 mL of CH$_2$Cl$_2$ and added to the top of a Celite cartridge saturated with water. The Celite pad was washed with CH$_2$Cl$_2$ (2 × 2 mL), and the combined organic washings were concentrated on a speedvac. The residue was dissolved in 800 μL CH$_2$Cl$_2$ and treated with Me$_2$EtSiH (100 μL) and TFA (100 μL). After allowing the reaction to stand for 1 h, the solution was added to the top of a 1 in. silica pad in a Pasteur pipet. After allowing the mixture to drain into the silica, the pipet column was washed twice with 1.5 mL of 3:1 Hex/EtOAc. The washings were discarded. The hydroxamate product was eluted into a preweighed vial with 4 × 1.5 mL 2:1 CH$_2$Cl$_2$/EtOH. Concentration in a speedvac (37 °C) gave the desired oxazoline hydroxamates **11{*n*}**.

General Procedure (B) for the Synthesis of 11{*n*} (*n* = 62−74). Serine derivative **7** (50 mg, 0.19 mmol) and Et$_3$N (26 μL, 0.185 mmol) were stirred in 2 mL of dry DMF in a 10-mL vial. The acid chloride (0.185 mmol) was added as a solution in 1 mL of CH$_2$Cl$_2$. After stirring for 20 min, Burgess' reagent (57 mg, 0.24 mmol) was added, and the vial was sealed. The solution was heated in a Discover (CEM) microwave at 200 W for 10 min (maximum temperature, 85 °C). After cooling, the solution was poured over EtOAc (10 mL) and washed twice with water. After drying over MgSO$_4$, the mixture was purified on a preparative TLC plate (3:1 EtOAc/Hex, UV or PMA stain; see specific examples for R_f values). The resulting intermediate was immediately treated with 1.5 mL of hexafluoro2-propanol and 30 μL of anisole. TFA (50 μL) was added, and the deep red solution was allowed to stand for 20 min. The resulting reaction mixture was added slowly to the top of a 10-mL syringe containing 2 mL of polyvinylpy-ridine above 1.5 mL of silica gel (prewet with CH$_2$Cl$_2$). Slow addition of the solution was essential because silica gel in the presence of trace amounts of TFA quickly decomposes the desired product. The column was washed with 10 mL of CH$_2$Cl$_2$ and 4 mL of 1% EtOH in CH$_2$Cl$_2$. The washings were discarded. The product was eluted into a preweighed vial with 10 mL of 20% EtOH in CH$_2$Cl$_2$.

General Procedure (C) for the Synthesis of 11{*n*} (*n* = 29, 30, 49). Serine derivative **7** (0.050 g, 0.19 mmol) was dissolved in 2 mL of pyridine. The corresponding benzoic acid (0.19 mmol) was added followed by EDC (46 mg, 0.24 mmol). The solution was stirred for 10 min and then evaporated under gentle heat (∼55 °C). The residue was partitioned between EtOAc and H$_2$O (15 mL each), and the organic layer was washed twice with water. The solution was dried over MgSO$_4$, filtered, and evaporated. The residue was dissolved in 2 mL of THF and treated with Burgess' reagent (57 mg, 0.24 mmol) and then heated in a microwave reactor (Discover, CEM) to 100 °C at 80 W for 15 min. The residue was concentrated and purified by prep-TLC (3:1 EtOAc/Hex; see specific examples for R_f values.) The resulting product was dissolved in 1 mL of CH$_2$Cl$_2$ and treated with 100 μL each of Et$_3$SiH and TFA. The solution was evaporated after standing for 10 min. A few drops of MeOH were added followed (slowly) by 2 mL of ether. The desired product precipitated as a white or pink solid.

Supporting Information Available: Structures of compounds **11**{*n*}, characterization of compounds **11**{*n*}, Schemes for high-throughput oxazoline synthesis and high-throughput deprotection/solid-phase extraction (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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