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CARBOHYDROXAMIDO-OXAZOLIDINES: ANTIBACTERIAL AGENTS THAT TARGET LIPID A BIOSYNTHESIS

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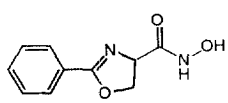
Abstract: A series of carbohydroxamido-oxazolidine inhibitors of UDP-3-O-[R-3-hydroxymyristoyl]-GlcNAc deacetylase, the enzyme responsible for the second step in lipid A biosynthesis, was identified. The most potent analog L-161,240 showed an IC_{50} = 30 nM in the DEACET assay and displayed an MIC of 1~3 μg/mL against wild-type *E. coli*.

The widespread use of antibiotics has inevitably resulted in an increased bacterial resistance to existing drugs which threatens public health. Although new antibiotics are being developed, most of them are improved versions of existing drugs. Recently, we reported[1] novel lipid A biosynthesis inhibitors possessing antibacterial efficacy, thereby validating the early steps of this pathway as a suitable target for antibiotic development. Lipopolysaccharide (LPS) is located in the outer leaflet of the outer membrane and consists of four domains: lipid A, the inner core oligosaccharide, the outer core oligosaccharide, and the O-antigen. Lipid A is an essential component of the outer membranes of Gram-negative bacteria. Genetic evidence has established that inhibition of its biosynthesis is lethal to Gram-negative bacteria.[2a,b] Furthermore, blocking lipid A biosynthesis renders bacteria sensitive to other antibiotics that poorly penetrate Gram-negative organisms with an intact outer membrane.[3] Earlier attempts to inhibit LPS biosynthesis by blocking the formation of 3-deoxy-D-manno-octulosonic acid (KDO), a major component of the inner core, did not lead to useful therapeutics.[4]

The second step in lipid A biosynthesis involves the deacetylation of uridine 5'-diphosphate-3-O-[R-3-hydroxymyristoyl]-N-acetylglucosamine by UDP-3-O-[R-3-hydroxymyristoyl]-GlcNAc deacetylase. Broad screening identified the lead compound, L-573,655, as an inhibitor of this enzyme. This lead compound is a reversible inhibitor with an IC_{50} of 8.5 μM. It possesses modest antibacterial activity against a wild-type strain of *E. coli* with a minimal inhibitory concentration (MIC) of 200~400 μg/mL.



L-573,655

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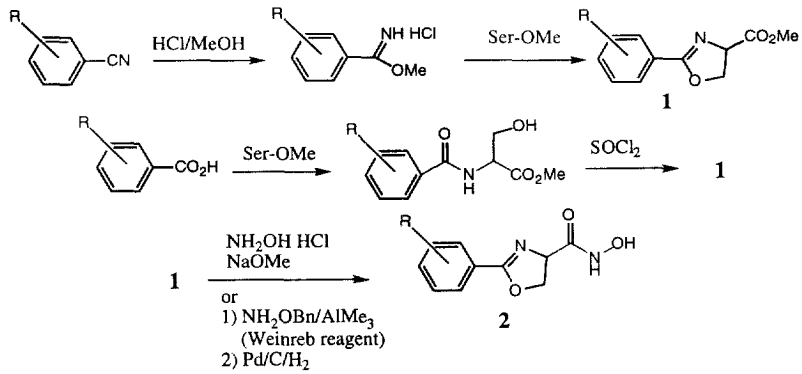
An extensive program was undertaken to modify the phenyl ring, the oxazolidine ring and the hydroxamic acid functional group of the lead in order to increase the inhibition of lipid A biosynthesis and improve antibacterial activity. In this paper some aspects of these SAR studies are presented.

Chemistry

The synthetic methods used for the preparation of analogs of L-573,655 are illustrated in Scheme 1. Polysubstituted benzonitriles were converted to imino esters by standard MeOH/HCl conditions.[5] The resulting imino esters were reacted with serine methyl ester to afford the oxazolidine methyl esters **1**.[6] An alternative way to synthesize **1** was to couple a polysubstituted benzoic acid and serine methyl ester to give an intermediate which was cyclized to the oxazolidine derivative **1** by thionyl chloride.[7] The racemic oxazolidine methyl esters **1** were converted to LPS inhibitors **2** by reaction with hydroxyamine hydrochloride in the presence of sodium methoxide.[8] When chiral hydroxamic acids **2** were desired, the chiral intermediate **1**, obtained from optically pure serine methyl ester, was reacted with the Weinreb reagent[9] and then the benzyl group was removed by hydrogenation to give the chiral inhibitor **2**.

Scheme 1



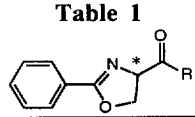
Biology

Initially, inhibitors were identified by inhibition of UDP-3-O-[R-3-hydroxymyristoyl]-GlcNAc deacetylase using a coupled radiochemical assay (WAVE).[2b] The enzyme souce was a membrane-free extract of *E. coli* strain JB 1104.[10] As the project progressed, the deacetylase enzyme was purified[11] and the compounds were screened by direct Deacetylase Assay (DEACET).[12] Antibacterial activities of this series of deacetylase inhibitors expressed as the Minimal Inhibitory Concentrations (MICs) were compared with clinically relevant antibiotics. A wild-type strain of *E. coli* and an *E. coli* mutant (*env*A1)[13] in the deacetylase gene were used.

Results and Discussion

Table 1 highlights the findings from SAR studies that were carried out at the 4-position of the oxazolidine nucleus of L-573,655. Since the lead compound, L-573,655, is a racemic mixture both the (*R*)- and the (*S*)-antipodes, **3** and **4** were prepared as single stereoisomers. The higher inhibition of **3** established the importance of the (*R*)-stereochemistry in the 4-position of the oxazolidine ring. Analogs in which the hydroxamic acid was replaced with carboxylic acid **5**, carboxylic ester **6**, and its derivatives such as N-methyl analog **7** or N-methyl O-benzyl analog **8** led to complete loss of potency. Given the well-known chelating properties of hydroxamic acids, the requirement for this functionality suggests that the active site of the deacetylase contains a mechanistically important metal atom.[12] The inhibition of metalloproteases by chelating to the metal in their active sites is well documented.[14]

Table 1



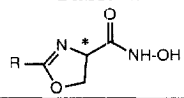
COMPOUND	R	*	WAVE IC_{50} μM	DEACET IC_{50} μM
L-573,655	NH-OH	R,S	3	8.5
3	NH-OH	R	1.5	6
4	NH-OH	S	360	NA
5	OH	R,S	>400	NA
6	OMe	R,S	>400	NA
7	NMe-OH	R	>400	NA
8	NMe-OBn	R	>400	NA

NA=not available.

Table 2 illustrates the effect of modifying the oxazolidine nucleus at the 2-position of L-573,655. Replacement of the phenyl ring by a heterocycle such as 2-furyl (**9**), 3- or 4-pyrido (**10** and **11**) resulted in loss of deacetylase inhibitory activity. Attention was focused on a series of substituted phenyl analogs. The 2-tolyl analog **12** was essentially equivalent in potency to the lead, however a modest increase in inhibition was observed with a methyl substitution on the phenyl ring at the 3- and the 4- positions (**13**, and **14**). The 2-chlorophenyl analog **15** and the 2,6-dichlorophenyl analog **16** were considerably less potent than the lead. This may be due to electron deficiency of the phenyl ring. Attachment of methoxy and other electron-donating hydrophobic groups at either the 3- or the 4- position of the phenyl ring gave more potent inhibitors. For example, the 4-ethylphenyl, **17**, the 4-*n*-propylphenyl, **18**, the 4-methoxyphenyl, **19**, and the 3-*n*-propylphenyl, **20**, analogs displayed increased deacetylase inhibition activity by more than five fold. The 3,4-disubstituted analogs led to a further increase in

deacetylase inhibition. The 4-methoxy-3-n-propylphenyl analog **21** was 30-fold more potent than L-573,655. In fact, incorporation of a 3,4,5-trisubstituted phenyl moiety onto the oxazolidine hydroxamic acid increased inhibition activity significantly and was found to afford the most potent inhibitors in the series. The 3,4,5-trimethoxylphenyl analog **22** increased potency by an order of magnitude over the lead. The 3,4-dimethoxy-5-*n*-propyl analog **23** as a racemate and as a single stereoisomer, L-161,240, had significantly increased potency by 200-fold over L-573,655. A larger hydrophobic group at the 3-position of compound, **23**, improved inhibition of the deacetylase enzyme further as illustrated by the 3-benzoxy-4-methoxy-5-*n*-propyl analog **24** (IC_{50} = 20 nM) as a racemic mixture. In contrast, hydroxy substitution on the phenyl ring was found to reduce intrinsic inhibition, as illustrated by analogs **25** and **26**. These results suggest that a large hydrophobic space exists in the enzyme active site adjacent to the oxazolidine binding region.

Table 2



COMPOUND	R	*	WAVE IC_{50} μM	DEACET IC_{50} μM	MIC μg/mL *E. coli*	MIC μg/mL *env*A1
L-573,655	Ph	R,S	3	8.5	200-400	3.6
9		R	9	NA	NA	NA
10		R,S	NA	100	NA	NA
11		R,S	NA	100	NA	NA
12	2-tolyl	R,S	2.6	NA	NA	NA
13	3-tolyl	R,S	1.1	NA	NA	NA
14	4-tolyl	R,S	1.7	NA	NA	NA
15	2-(Cl)-Ph	R,S	NA	30	NA	NA
16	2,6-di(Cl)-Ph	R,S	NA	>200	NA	NA
17	4-(Et)-Ph	R	0.2	0.6	NA	NA
18	4-(*n*-Pr)-Ph	R	0.1	NA	NA	NA
19	4-(OMe)-Ph	R	0.3	1.4	25-50	0.25
20	3-(*n*-Pr)-Ph	R	0.05	1.6	12.5	0.1
21	4-(OMe)-3-(*n*-Pr)-Ph	R,S	NA	0.3	12.5	0.06
22	3,4,5-tri(OMe)-Ph	R	NA	0.3	NA	NA

23	3,4-di(OMe)-5-(*n*-Pr)-Ph	R,S	NA	0.05	3-6	<0.05
L-161,240	3,4-di(OMe)-5-(*n*-Pr)-Ph	R	NA	0.03	1-3	0.008-0.016
24	3-(OBn)-4-(OMe)-5-(*n*-Pr)-Ph	R,S	NA	0.02	25	<0.008
25	3-(OH)-4-(OMe)-5-(*n*-Pr)-Ph	R,S	NA	0.1	12.5	0.06
26	3-(OMe)-4-(OH) 5-(*n*-Pr)-Ph	R,S	NA	1.4	>100	0.39

NA=not available.

Improvements in deacetylase inhibition are expected to lead to higher antibacterial activities if the inhibitors are able to penetrate the outer membrane of bacteria. L-573,655 had modest activity against wild-type *E. coli* at 200~400 μg/mL and had better activity (MIC = 3~6 μg/mL) against the mutant *env*A1 cells which have weakened outer membranes. Mono-substitution on the phenyl ring of L-573,655 enhanced antibacterial potency against both wild-type *E. coli* and *env*A1 strains. Both the 4-methoxy analog **19** and the 3-*n*-propyl analog **20** had greater than an order of magnitude more antibacterial activity than the lead. The more potent deacetylase inhibitors such as the 4-methoxy-3-*n*-propyl analog, **21**, and L-161,240 were found to be the better antibacterial agents. Indeed, L-161,240 is one of the most potent inhibitors and antibacterial agents in this series. Although analog **24** and L-161,240 had comparable potency in both deacetylase enzyme inhibition and in *env*A1 strains, **24** was a much poorer antibacterial agent against wild-type *E. coli* . This may be due to a reduced ability to penetrate the bacterial cell wall. Although L-161,240 showed good antibacterial activity toward wild-type *E. coli* bacteria, no antibacterial activity was observed with *Pseudomonas* and *Serratia* up to 100 μg/mL. Since L-161,240 inhibits the deacetylase of *Pseudomonas* although at higher levels (data not shown), we suspect that the compound may not penetrate well or may be actively extruded by these bacteria.

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