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Elimination of antibacterial activities of non-peptide luteinizing hormone-releasing hormone (LHRH) antagonists derived from erythromycin A

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Abstract—Antibacterial SAR for a series of macrolides derived from erythromycin A that are potent LHRH antagonists was developed in an attempt to eliminate the antibiotic activities of these compounds. Increasing the size of the alkyl substituents on the desosamine 3′-amine resulted in potent LHRH antagonists that were inactive against staphylococcal bacteria strains, and were significantly (>10-fold) less active against streptococcal bacteria strains. Complete elimination of antibacterial activities could be achieved by replacement of one or both methyl groups on the 3′-amine with a large alkyl substituent.

We recently reported a series of potent, non-peptide antagonists of the luteinizing hormone-releasing hormone (LHRH) receptor derived from erythromycin A.[1] These compounds are structurally related to a series of 11,12-cyclic carbamate erythromycin antibiotics, also discovered in our laboratories.[2] In order to identify macrolides that are therapeutically useful for the treatment of physical disorders dependent on the release of the gonadal steroid hormones (including prostate cancer, endometriosis, and precocious puberty), we focused our medicinal chemistry efforts on both optimizing LHRH receptor affinity and eliminating the antibacterial activity of these compounds. As a goal in the latter effort, we hoped to find compounds having minimum inhibitory concentrations (MICs) no less than 10 μg/mL for all test strains of bacteria. It was believed that this low level of antibacterial activity would provide a safe therapeutic index for the clinical study of these compounds as LHRH antagonists.[3]

Compounds were screened against a standard battery of bacteria, with erythromycin A (Ery A) used as a positive control.[4] Table 1 presents a summary of results for macrolide LHRH antagonists against selected Gram-positive bacteria that are highly sensitive to Ery A. The otherwise unmodified 4-chlorophenethyl-11,12-cyclic carbamate of erythromycin A (**1A**), which has submicromolar affinity for the human LHRH receptor ($pK_I = 6.72$), is a potent antibiotic maintaining activity within 2-fold of Ery A. Removal of cladinose to give **1C** resulted in a dramatic decline in activity against staphylococci (>60-fold less active than Ery A), but had a marginal effect on other strains, including *Streptococcus agalactiae*. However, this modification also resulted in a large loss in affinity for the LHRH receptor, indicating the importance of the cladinose substituent to binding. Removal of one of the methyl groups at the 3′-amino position of desosamine gave **2A**,[6] which was also significantly less potent (~10-fold) than the control against staphylococci, but was well tolerated in the LHRH receptor binding assay. It was found that replacement of the methyl group on the 3′-amine with a larger alkyl group, such as isopropyl (**3A**), resulted in more potent LHRH receptor antagonists with reduced antibiotic activity (relative to **1A**). Furthermore, replacement of cladinose with a 4-*S*-methyloxazolidinone carbamate (**3B**), resulted in a compound having similar LHRH activity to **3A**, but considerably less antibiotic activity. Thus, compound **3B** meets the goals we set

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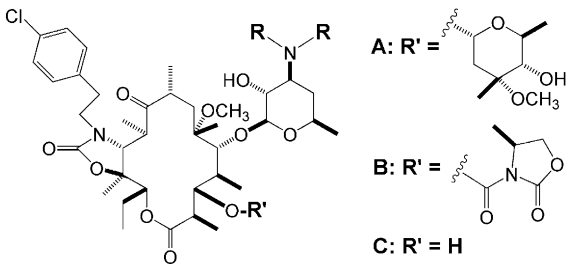
Table 1. In vitro activities of macrolide LHRH antagonists



Compd	Ery A	**1A** R = 2×Me	**1C** R = 2×Me	**2A** R = H,Me	**3A** R = Me,*i*Pr	**3B** R = Me,*i*Pr
LHRH receptor affinity (pK_i)[a]	—	6.72	~5	7.10	7.65	7.73
Bacterial strain			MIC (μg/mL)			
Staphylococcus aureus ATCC 6538P	0.2	0.39	12.5	1.56	1.56	25
Staphylococcus epidermidis 3519	0.2	0.39	12.5	1.56	1.56	25
Micrococcus luteus ATCC 9341	0.02	0.05	0.78	0.2	—	0.78
Enterococcus faecium ATCC 8043	0.05	0.1	1.56	0.39	0.2	0.78
Streptococcus bovis A-5169	0.05	0.02	—	0.2	0.2	0.1
Streptococcus agalactiae CMX 508	0.05	0.02	0.02	0.05	0.02	—
Streptococcus pyogenes EES61	0.05	—	—	0.1	—	0.02

Compd	**4A** R = Me,cpm[b]	**4B** R = Me,cpm[b]	**5A** R = Me,cPent	**5B** R = Me,cPent	**6A** R = Me,cHex	**7A** R = *i*Bu,cpm[b]
LHRH receptor affinity (pK_i)[a]	7.88	7.73	8.12	8.41	6.69	7.56
Bacterial strain			MIC (μg/mL)			
S. aureus ATCC 6538P	>100	100	25	>100	>100	>100
S. epidermidis 3519	>100	>100	50	>100	>100	>100
M. luteus ATCC 9341	—	12.5	1.56	12.5	12.5	>100
E. faecium ATCC 8043	1.56	3.1	1.56	6.2	25	>100
S. bovis A-5169	—	0.78	0.78	3.1	3.1	>100
S. agalactiae CMX 508	1.56	1.56	0.78	3.1	12.5	>100
S. pyogenes EES61	—	0.78	0.78	3.1	0.78	>100

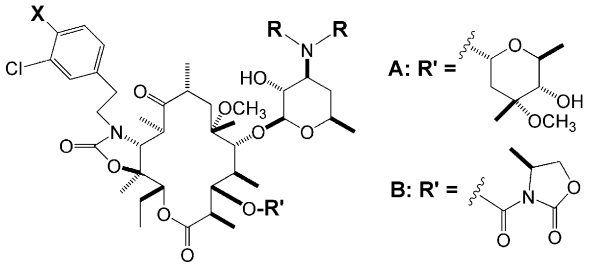
[a] In vitro receptor binding results for LHRH antagonists were conducted using human receptors cloned in CHO cells.[7]
[b] cpm = Cyclopropylmethyl.

for little activity relative to staphylococci, but maintains good activity against other strains, including streptococci.

Increasing the size of the 3′-amine alkyl substituent further decreased the susceptibilities of the bacterial test strains toward these compounds. The cyclopropylmethyl (cpm) derivatives **4A** and **B** and cyclopentyl derivatives **5A** and **B** demonstrated optimal affinity for the LHRH receptor in the 4-chlorophenethyl cyclic carbamate series. These compounds were inactive against staphylococci, and were >10-fold less active against streptococci. With the exception of *M. luteus*, where the cladinose-replacement derivatives were significantly less active than the cladinose-containing analogues, there were only marginal differences (<5-fold) in the overall antibiotic activities in the corresponding analogues of compounds having these larger 3′-amino alkyl groups (compare **4A** with **4B** and **5A** with **5B**). Increasing the size of the 3′-amino substituent to a cyclohexyl group (**6A**) or replacing the second methyl substituent on the 3′-amine (**7A**) resulted in further attenuation of the antibacterial activities of these compounds. The 3′-*N*-cyclopropylmethyl-*N*-isobutyl derivative **7A** was completely inactive against all bacterial test strains. However, it should be noted that compounds having a cyclohexyl or larger alkyl substituent were less effective as LHRH antagonists relative to the cyclopentyl and cyclopropylmethyl analogues.

The most potent LHRH antagonists are those bearing a 3,4-dihalo substituent on the 11,12-cyclic carbamate (see Table 2). The 3,4-dichlorophenethyl derivatives **8A** and **9B** represent two of the most effective compounds for suppressing LH release, both in vitro and in vivo.[1] While the second halogen substituent on the phenyl ring was important in optimizing affinity for the LHRH receptor, it did not have a significant effect on antibacterial activity (compare **8A** with **5A** and **9B** with **4B**). The cladinose-replacement analogue **9B** was of particular interest for further study owing to the ability of this compound to suppress LH in castrate male rats upon oral administration.[1a,7] While this compound was inactive against staphylococci, the residual activity, especially against several streptococci strains, caused some concern for further development. Structural modification of the desosamine substituents was therefore conducted in an effort to eliminate residual antibacterial activity. Replacement of the 3′-*N*-cyclopropylmethyl substituent in **9B** with a larger alkyl substituent such as cyclohexyl (**10B**) significantly reduced antibacterial activities, but also resulted in

Table 2. In vitro activities of macrolide LHRH antagonists.



Compd	**8A** R = Me,cPent X = Cl	**9B** R = Me,cpm[b] X = Cl	**10B** R = Me, cHex X = Cl	**11B** R = 2×cpm[b] X = Cl	**12B** R = Me,cpm[b] X = F	**13A** R = 2×cpm[b] X = F
LHRH receptor affinity (pK_i)[a]	8.82	8.73	8.14	8.89	9.48	9.07
Bacterial strain	MIC (μg/mL)					
S. aureus ATCC 6538P	>100	>100	>100	>100	100	>100
S. epidermidis 3519	>100	100	>100	>100	50	>100
M. luteus ATCC 9341	3.1	3.1	>100	100	1.56	25
E. faecium ATCC 8043	3.1	3.1	>100	>100	1.56	25
S. bovis A-5169	3.1	0.78	6.2	50	0.39	25
S. agalactiae CMX 508	3.1	0.78	6.2	100	0.39	50
S. pyogenes EES61	3.1	0.78	6.2	50	0.39	25

[a] In vitro receptor binding results for LHRH antagonists were conducted using human receptors cloned in CHO cells.[7]
[b] cpm = Cyclopropylmethyl.

reduced affinity for the LHRH receptor. However, replacement of the 3′-methyl group of **9B** with a second cyclopropylmethyl group (**11B**), effectively eliminated antibacterial activity without significantly reducing LHRH receptor affinity.

The 4-fluoro analogues **12B** and **13A** are some of the most potent LHRH antagonists in vitro. A comparison of **12B** with the dichloro analogue **9B** further illustrates that the halogen substitution pattern on the phenethylcarbamate side-chain does not significantly affect antibacterial activities. The 3′-bis-*N*-cyclopropylmethyl analogue **13A** is a subnanomolar antagonist of the LHRH receptor in vitro, that has no significant antibacterial activity.

In summary, antibacterial activities have been determined for a series of macrolide LHRH antagonists. In an effort to eliminate the antibacterial activities of these compounds, SAR was developed using bacterial strains highly sensitive to erythromycin A. Chemical modification of the 3′-amino alkyl substituents and/or replacement of the cladinose group at position 3 of the erythronolide core were found to be useful strategies for eliminating antibacterial activities, as well as optimizing affinity for the LHRH receptor.[8,9] In general, as the size of the alkyl substituent on the 3′-amine was increased, the antibacterial activities for these compounds decreased. The cyclopentyl and cyclopropylmethyl groups, which were found to be optimal 3′-amine substituents for LHRH activity, gave compounds in which activities against staphylococci were eliminated. Replacement of both methyl groups on the 3′-amine with a 3-carbon or larger alkyl group resulted in compounds in which all antibacterial activities were effectively eliminated.

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