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NOVEL 3-DEOXY-3-DESCLADINOSYL-6-*O*-METHYL ERYTHROMYCIN A ANALOGUES. SYNTHESIS AND IN VITRO ACTIVITY.

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Abstract: A series of novel 3-deoxy-3-des-cladinosyl-6-*O*-methyl erythromycin A analogues has been synthesized and evaluated in vitro for antibacterial activity. These analogues were readily synthesized by tributyltin hydride-mediated radical reduction of a 3-*O*-xanthyl intermediate to afford the 3-deoxy macrolide. A number of oxime, carbonate, and carbamate derivatives were synthesized and evaluated for antibacterial activity. Overall, these analogues had fairly good antibacterial activity against gram-positive bacteria, although they were generally less potent than the corresponding 3-*O*-cladinosyl or 3-keto analogues.

The emergence of bacteria resistant to existing antibacterial agents, including macrolide antibiotics such as erythromycin A (Ery A, **1**), has accelerated the search for newer and more effective antibacterial agents.[1-3] Second-generation macrolide antibiotics, such as Biaxin® (**2**), have enjoyed great clinical and commercial success due to their improved antibacterial activity and pharmacokinetic properties, expanded spectrum of activity, and attenuation of side effects compared to erythromycin, making the macrolide class of antibiotics an integral part of the arsenal against infectious diseases.[4-7] The macrolides are also of considerable interest due to a number of potential non-traditional therapeutic utilities,[8] including utility as prokinetic agents,[9] eradication of *H. pylori*,[10] and potential use in the treatment of inflammatory etiologies such as asthma.[11-13]

Recent breakthroughs in the development of the structure–activity relationships are driving the resurgence of interest in macrolides. For the greater part of the forty-four years since the discovery of Ery A the cladinose sugar was considered an essential component for antibacterial activity, as all activity was lost upon hydrolytic removal of this group. However, in the late 1980s a series of 3-keto-6-*O*-methyl erythromycin A analogues **3**, which lacked the cladinose sugar residue but still maintained potent antibacterial activity against susceptible organisms, was developed by Rousell–Uclaf. In addition, this class of macrolides, termed "ketolides", demonstrated significantly improved activity against MLS (macrolide, lincosamide, and streptogramin B) inducibly-resistant organisms.[14,15] This important discovery led to a large number of 3-keto-6-*O*-methyl Ery A analogues, many of them modified at the C10-C12 position, as originally described by Baker et al. on Clarithromycin itself[16] to include cyclic carbonates, carbamates, and the newly disclosed cyclic carbazates.[17,18] In a separate patent Taisho Pharmaceutical described a series of substituted 3-*O*-phenyl des-cladinosyl macrolides **4** that also demonstrated good antibacterial activity, representing a second class of 14-membered macrolides in which the cladinose residue is not essential for activity.[19]

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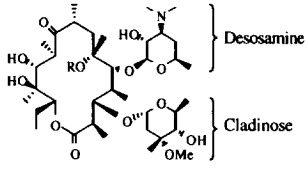
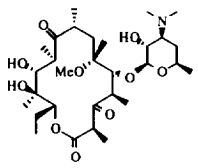
These findings prompted us to explore other C-3 modified derivatives of 6-*O*-methyl erythromycin and to investigate whether it was possible to altogether remove the C-3 oxygen and maintain good antibacterial activity.[20] We herein report on the synthesis and antibacterial activity of novel analogues of 3-deoxy-6-*O*-methyl erythromycin A **6**.[21]

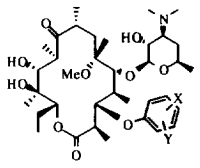
Figure 1. Structures of Erythromycin A and C-3 Modified Analogues.



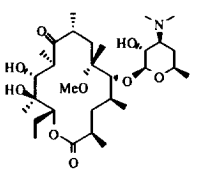
1 Erythromycin A (R = H)
2 Clarithromycin (R = Me)



3
3-Keto Clarithromycin



4
3-*O*-Phenyl ethers



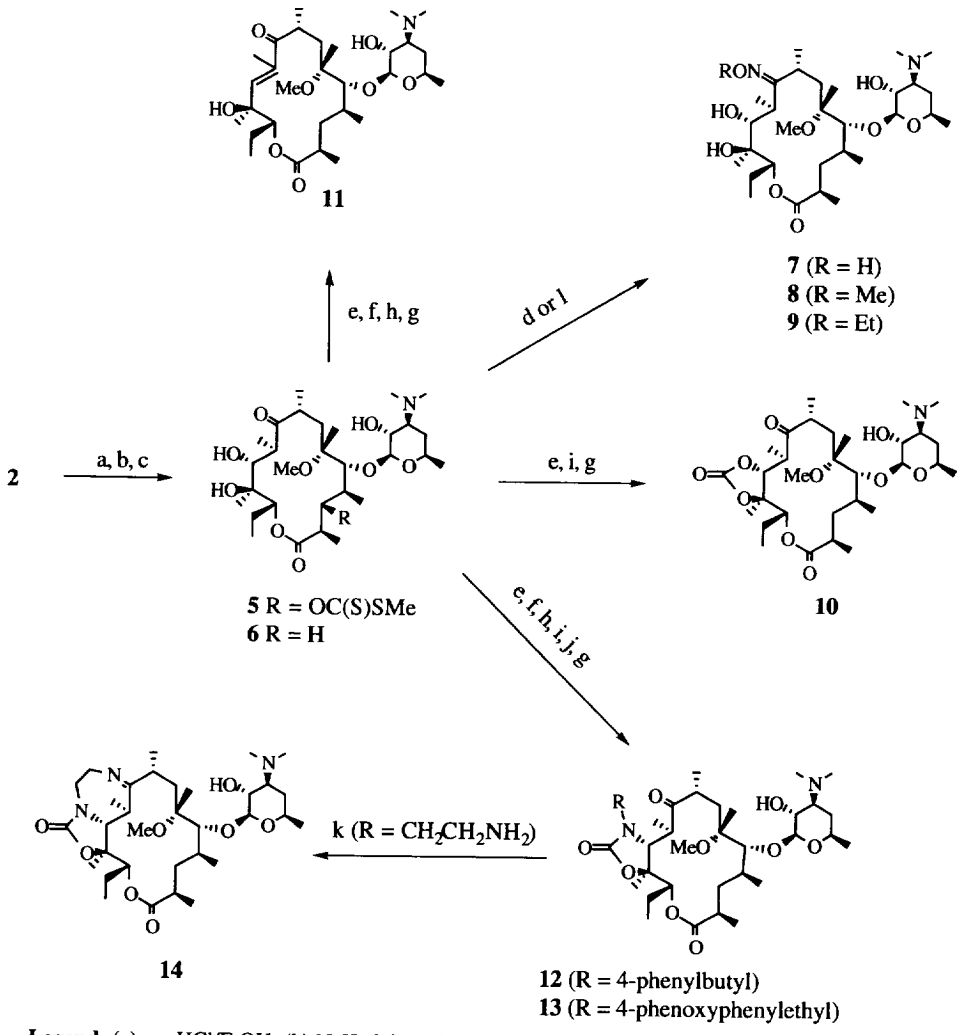
6
3-Deoxy Clarithromycin

Chemistry

The synthetic strategy we chose for the synthesis of 3-deoxy macrolides necessitated selective transformation of the C-3 hydroxyl group of descladinosyl 6-*O*-methyl erythromycin A to the C-3 xanthate, followed by Bu_3SnH/AIBN mediated radical deoxygenation. Although it was not initially obvious how to selectively functionalize the C-3 hydroxy group in the presence of the C-11, C-12, and C-2′ hydroxy groups, it was discovered that direct conversion of des-cladinosyl 6-*O*-methyl Ery A to the C-3 xanthate could be achieved using conditions similar to those developed by Taisho[19] to synthesize C-3 *O*-phenyl analogues, that is, 3 equiv of sodium hydride in THF at −10 °C, followed by sequential addition of CS_2 and methyl iodide (Scheme 1). Using these conditions the des-cladinosyl macrolide was cleanly converted to the desired 3-*O*-xanthyl macrolide **5** in 56% yield (Scheme 1). Interestingly, under these conditions only a small amount of the 2′,3-*O*-bis xanthate macrolide was observed, which was readily removed via column chromatography. Subsequent Bu_3SnH-mediated radical reduction of **5** proceeded smoothly to afford the desired 3-deoxy macrolide **6** in good yield. A crystal suitable for X-ray analysis was obtained via recrystallization from hexane, confirming the structure. This three-step route from 6-*O*-methyl Ery A allowed for the synthesis of multigram quantities of 3-deoxy macrolide **6**, which was a versatile intermediate for many analogues.

Following procedures that had been developed for erythromycin,[16] **6** was transformed into a number of 3-deoxy analogues, including 9-oximes (**7**) and *O*-alkyl oximes (**8** and **9**), the C11-C12 carbonate (**10**), and C11-C12 carbamates (**12-14**) as outlined in Scheme 1.

Scheme 1. Synthesis of 3-deoxy-6-*O*-Methyl Erythromycin A Analogues.



Legend: (a) aq. HCl/EtOH; (b) NaH (3.0 equiv)/THF/-20 °C; then CS_2 (1.0 equiv); then MeI (1.0 equiv); then let gradually warm; (c) Bu_3SnH/AIBN/C_6H_6 (reflux); (d) H_2NOH-HCl/pyridine/80 °C/2 days; (e) Ac_2O/TEA/CH_2Cl_2; (f) methanesulfonyl anhydride/pyridine/re; (g) MeOH/rt/overnight; (h) DBU/acetone; (i) NaN(TMS)$_2$/THF/-40 °C; then CDI/DMF, rt; (j) MeCH/H_2O/RNH_2; (k) AcOH/EtOH/rt; (l) $RONH_2$/solvent (e.g., toluene)/reflux.

Results and Discussion

The in vitro antibacterial activity of 3-deoxy-6-*O*-methyl Ery A analogues is shown in Table 1. As is typical for macrolide antibiotics, all of the analogues tested had poor MIC values (>100 μg/mL) against gram-negative organisms (data not shown). The 3-*O*-xanthyl macrolide **5**, which was a synthetic intermediate to the 3-deoxy analogues, had only moderate activity against most of the test organisms, with MIC values generally

in the 1–25 μg/mL range. The parent compound, 3-deoxy-6-*O*-Me erythromycin (**6**), also had moderate antibacterial activity against the gram positive organisms, and was generally 10- to 20-fold less active than the erythromycin, clarithromycin (**2**), or the ketolide **3**. The unsubstituted oxime **7** was generally one dilution factor more potent than **6**, although the *O*Me and *O*Et oximes **8** and **9** had poor antibacterial activity. Further improvement in antibacterial activity was seen with the carbonate **10**, with antibacterial activity generally 5–10 times better than **6**. Interestingly, **10** had substantially improved activity against the resistant *S. pyogenes* PIU 2548, having an MIC value of 0.78 μg/mL compared to 8 μg/mL for **2**. The hydroxy enone macrolide **11**, a synthetic intermediate to 11,12-cyclic carbamates, was inactive with MIC values >100 μg/mL. The three C11,C12-cyclic carbamates **12-14** all had superior MIC values compared to the parent 3-deoxy macrolide **6**. The *p*-phenoxyphenylethyl carbamate **13** and the bicyclic iminocarbamate **14** had MIC values similar to the cyclic carbamate **10**. The phenylbutylcarbamate **12** was the most potent compound evaluated, having MIC values of 1.56 μg/mL against all the *S. aureus* organisms (except for the constitutively resistant *S. aureus* A-5278 and 1775), and MIC values of 1.56 μg/mL or lower for the majority of the other gram-positive organisms screened. Compound **12** was particularly potent against *S. pyogenes* PIU 2548 with an MIC value of 0.2 μg/mL. Interestingly, both arylalkyl carbamates **12** and **13** demonstrated improved antibacterial activity (albeit still poor) compared to **1** against the constitutively resistant *S. aureus* A-5278, *S. aureus* 1775, and *S. pyogenes* 930.

Despite the improvement in antibacterial activity of **12** over the parent compound **6**, **12** was still generally one order of magnitude less potent than either **1**, **2**, or **3** against macrolide-susceptible organisms. It is unclear what factor(s) are responsible for the reduced activity of this series compared to cladinose-containing compounds (e.g., **1** or **2**) or the ketolides. The conformation of the macrolide ring observed in the X-ray structure of **6** overlaps well with X-ray structures and computationally minimized structures of **1-3**, so it seems unlikely to be solely an issue of an unfavorable conformational bias. It may be that a polar group (such as the ketone) or a hydrogen-bond acceptor may be needed at the C-3 position for maximal antibacterial activity, or perhaps removal of the C-3 oxygen imparts an unfavorable increased flexibility on these 3-deoxy analogues. However, other factors such as cell-wall penetration may also be important in determining the overall antibacterial activity of these compounds. Interestingly, the observed SAR trends in this 3-deoxy series appears to be fairly similar to that found in the ketolide series (data not shown), which may be indicative of similar binding modes with the bacterial ribosomal RNA.

In summary, a series of 3-deoxy-6-*O*-methyl erythromycin analogues were synthesized and evaluated in vitro for antibacterial activity. Exploration of the SAR of this series led to a 10- to 20-fold improvement against many of the organisms tested, although the most potent compound (**12**) was still more than one order of magnitude less potent than erythromycin and related compounds.

Table 1. In Vitro Antibacterial Activity of 3-Deoxy-6-*O*-Methyl Erythronolide A Analogues.[a,b,c]

Organism	Compound											
	2	**3**	**5**[d]	**6**	**7**[e]	**8**	**9**	**10**	**11**[f]	**12**	**13**	**14**[g]
S. aureus ATCC 6538P	0.2 *0.2*	0.2 *0.2*	25 *0.2*	25 *0.1*	12.5 *0.2*	100 *0.2*	25 *0.39*	6 .2 *0.2*	>100 *0.39*	1.56 *0.39*	12.5 *0.39*	6.2 *0.39*
S. aureus A5177	1.56 *3.1*	0.2 *1.56*	25 *3.1*	25 *1.56*	25 *3.1*	100 *6.2*	25 *3.1*	3.1 *3.1*	>100 *3.1*	1.56 *25*	6.2 *12.5*	6.2 *12.5*
S. aureus A-5278	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	50 *>100*	25 *>100*	>100 *>100*
S. aureus CMX 642A	0.03 *0.03*	0.39 *0.2*	25 *0.2*	25 *0.2*	12.5 *0.2*	100 *0.39*	50 *0.39*	3.1 *0.2*	>100 *0.39*	1.56 *0.39*	12.5 *0.39*	6.2 *0.39*
S. aureus NCTC10649M	0.03 *0.03*	0.1 *0.2*	12.5 *0.2*	6.2 *0.2*	12.5 *0.2*	100 *0.39*	12.5 *0.39*	6.2 *0.2*	>100 *0.39*	1.56 *0.39*	6.2 *0.39*	6.2 *0.39*
S. aureus CMX 553	0.12 *0.12*	0.2 *0.2*	25 *0.2*	25 *0.2*	12 .5 *0.2*	100 *0.39*	50 *0.39*	3 .1 *0.2*	>100 *0.39*	1.56 *0.39*	6.2 *0.39*	6.2 *0.39*
S. aureus 1775	>100 >100	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	>100 *>100*	50 *>100*	25 *>100*	>100 *>100*
S. epidermis 3519	0.12 *0.03*	0.39 *0.2*	25 *0.2*	50 *0.1*	12 .5 *0.2*	100 *0.2*	25 *0.39*	6 .2 *0.2*	>100 *0.39*	1.56 *0.39*	12.5 *0.39*	12.5 *0.39*
E. faecium ATCC 8043	0.05 *0.05*	0.2 *0.05*	6.2 *0.05*	25 *0.05*	3 .1 *0.05*	100 *0.1*	25 *0.05*	0 .78 *0.05*	>100 *0.2*	0.39 *0.1*	1.56 *0.05*	0.78 *0.05*
S. bovis A-5169	0.01 *0.01*	0.05 *0.02*	0.39 *0.02*	0.78 *0.02*	0.78 *0.05*	3.1 *0.01*	3.1 *0.01*	0 .2 *0.05*	>100 *0.2*	0.02 *0.02*	1.56 *0.05*	0.39 *0.05*
S. agalactiae CMX 508	0.02 *0.02*	0.2 *0.05*	0 .78 *0.02*	3.1 *0.02*	0 .78 *0.01*	25 *0.02*	3.1 *0.1*	0 .2 *0.01*	25 *0.02*	0.1 *0.02*	1.56 *0.05*	0.39 *0.05*
S. pyogenes EES61	0.01 *0.01*	0.1 *0.05*	0 .78 *0.02*	1.56 *0.02*	0 .78 *0.01*	6.2 *0.01*	3.1 *0.02*	0 .2 *0.01*	12.5 *0.01*	0.05 *0.02*	1.56 *0.02*	0.39 *0.02*
S. pyogenes 930	>100 *>100*	>100 *>100*	100 *>100*	>100 *>100*	>100 *>100*	100 *>100*	>100 *>100*	>100 *>100*	>100 *12.5*	NT[c]	6.2 *>100*	>100 *>100*
S. pyogenes PIU 2548	8 *8*	0.3 *3.1*	1.56 *6.2*	3.1 *6.2*	1.56 *6.2*	NT[c]	25 *3.1*	0 .78 *6.2*	>100 *12.5*	0.2 *6.2*	3.1 *6.2*	0.78 *6.2*
E. coli JUHL	100 *50*	>100 *50*	>100 *50*	>100 *50*	>100 *50*	>100 *50*	>100 *25*	>100 *50*	>100 *50*	>100 *100*	NT[c]	NT[c]
E. coli SS	0.39 *0.39*	0.39 *0.2*	0.39 *0.2*	0.78 *0.1*	0.78 *0.2*	6.2 *0.39*	3.1 *0.2*	0 .78 *0.2*	>100 *0.78*	1.56 *0.39*	NT[c]	NT[c]

[a]All compounds were fully characterized by mass spec, ^1H and ^{13}C NMR, and had elemental analyses within ±0.4% of theoretical values unless noted otherwise. Compounds were tested using standard agar dilution methods. Minimum inhibitory concentrations (MIC) values are in μg/mL. [b]Ery A MIC values (μg/mL) in italics [c]NT = not tested. [d]Anal. calcd for $C_{32}H_{57}NO_{10}S_2$: C, 56.52, H, 8.45, N, 2.06. Found: C, 56.96, H, 8.65, N, 1.92. [e]High-resolution MS calcd for $C_{30}H_{57}N_2O_9$: 589.4064; Found: 589.4046. [f]High-resolution MS calcd for $C_{30}H_{53}NO_8$: 556.3849; Found: 556.3835 [g]High-resolution MS calcd for $C_{33}H_{57}N_3O_8$: 624.4224; Found: 624.4227.

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