Letters

Synthesis and Antibacterial Activity of a Novel Class of 4′-Substituted 16-Membered Ring Macrolides Derived from Tylosin

Ly T. Phan, Tianying Jian, Zhigang Chen, Yao-Ling Qiu, Zhaolin Wang,* Theresa Beach, Alex Polemeropoulos, and Yat Sun Or

Enanta Pharmaceuticals, Inc., 500 Arsenal Street, Watertown, Massachusetts 02472

Received November 11, 2003

Abstract: Novel 4′-substituted 16-membered ring macrolides were synthesized by the cleavage of the mycarose sugar of tylosin and subsequent modification of 4′-hydroxyl group. This new class of macrolide antibiotics exhibited potent activity against some key erythromycin-resistant pathogens.

Macrolide antibiotics have been widely used to treat bacterial infections for the past 50 years.[1] They are considered as the preferred therapeutic agents for the treatment of upper and lower respiratory tract infections because of their safety and efficacy.[2] Macrolides exert their potent antibacterial effect by selectively binding to the 50S subunit of the bacterial ribosome and inhibit protein synthesis.[3] As a large family of both natural and semisynthetic antibiotics, macrolides are classified according to the size of the lactone ring consisting of 12−16 atoms. Erythromycin A (**1**) (Figure 1) and its derivatives, such as clarithromycin (**2**), which have a 14-membered lactone ring, are among the most commonly prescribed macrolides. However, erythromycin-resistant pathogens have become increasingly prevalent over the past decade because of extensive usage.[4] The two most common mechanisms of macrolide resistances arise from ribosome methylation and efflux pumps encoded by erm genes and mef genes, respectively. To address the problem of bacterial resistance, a new generation of 14-membered macrolides known as ketolides, such as telithromycin (**3**)[5] and cethromycin (ABT-773),[6] was developed.

The 16-membered ring macrolides, such as leucomycins and tylosin (**4**) (Figure 1), for example, constitute another important class of useful antibiotics of the macrolide family.[7] They have some advantages over the 14-membered erythromycin A derivatives such as gastrointestinal tolerance and activity against resistance-expressing strains.[8] During our efforts to develop novel macrolides active against resistant bacteria, we became interested in tylosin primarily because its activities are not affected by efflux resistance. Although substantial work to improve antibacterial activity has been carried out on tylosin and its derivatives over many years, not much success was observed with this subclass.[9] Especially little research has been done to improve the acid stability. We felt that removal of the acid labile mycarose sugar from tylosin could be an innovative starting point for further modifications to enhance its antibacterial activity and acid stability. Herein we report the synthesis of a series of novel 4′-substituted 16-membered ring macrolides and their activity against constitutively resistant bacteria.

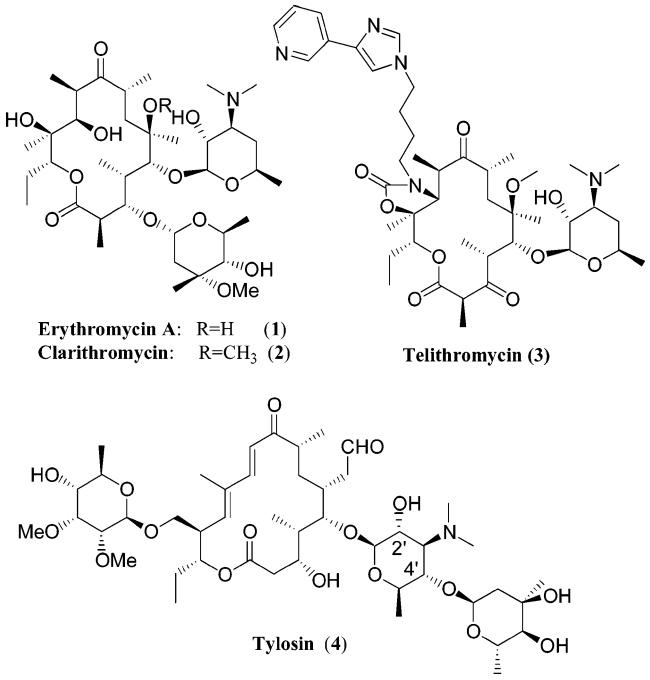


Figure 1. Structure of erythromycin A (**1**), clarithromycin (**2**), telithromycin (**3**), and tylosin (**4**).

Our approach to 4′-substituted tylosin derivatives is to remove the mycarose sugar and to modify the 4′-hydroxyl group. Scheme 1 outlines the synthesis of the 4′-carbamate **6**. The 2′-hydroxyl group of tylosin (**4**) was first protected as an acetyl ester followed by protetion of the aldehyde group with ethylene glycol and selective cleavage of the mycarose sugar under acidic conditions. Protection of the 4‴-hydroxyl group on the mycinose sugar with TBDPSCl generated **5** in 64% yield over four steps. Subsequent reaction of **5** with phenethyl isocyanate followed by the deprotections proceeded smoothly to afford the desired product 4′-carbamate **6** in 43% yield over four steps.

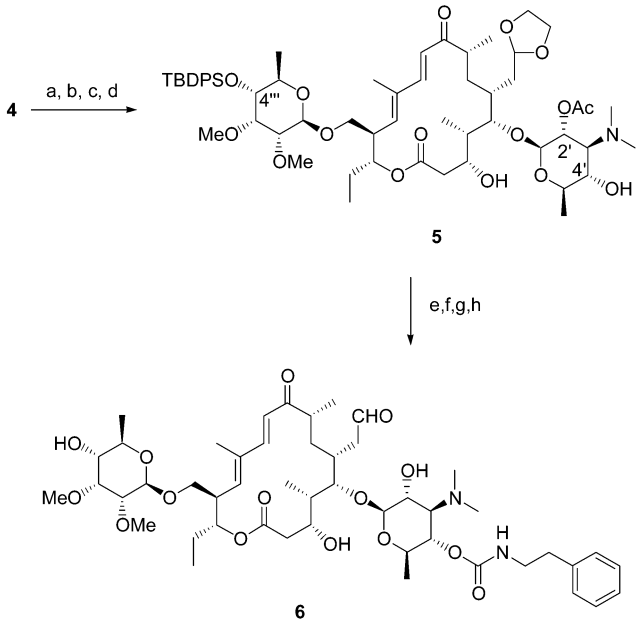
The synthesis of an allyl ether is described in Scheme 2. Palladium-catalyzed reaction of compound **5** with allyl-*tert*-butyl carbonate generated 4′-substituted allyl ether **7** as the major product in 60% yield and a small amount of the 3-position allylation compound.[10] Deprotection of **7** gave the final product **9** in 61% yield over three steps.

Introduction of a quinoline group[6] to the allyl side chain of **8** was achieved by utilizing Heck reaction.[11] Under optimized conditions ($Pd(OAc)_2/P(o\text{-}Tol)_3/Et_3N$/

* To whom correspondence should be addressed. Phone: 781-235-0842. E-mail: zhaolinwang@comcast.net. Present address: 88 Kingsbury Street, Wellesley, MA 02481.

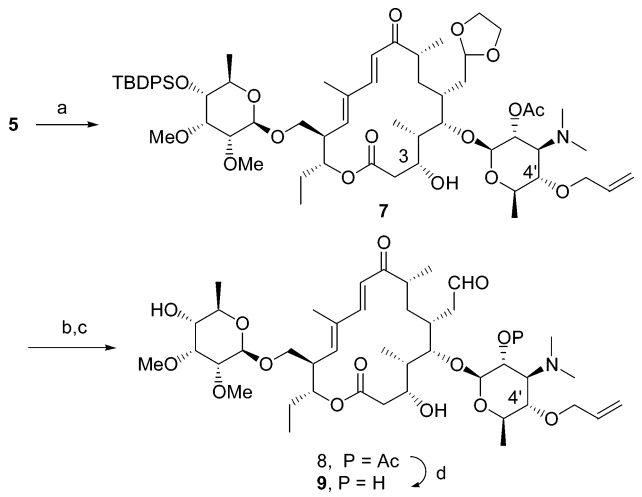
10.1021/jm034233n CCC: $27.50
Published on Web 05/04/2004

Scheme 1[a]



[a] Conditions: (a) Ac_2O (5.3 equiv), acetone, room temp, 2 h, 100%; (b) *p*-TsOH, ethylene glycol, benzene, reflux, 4 h; (c) Ch_3CN, room temp, 16 h; (d) TBDPSCl (1.1 equiv), imidazole (1.2 equiv), DMF, room temp, 16 h, 64% in three steps; (e) $PhCH_2CH_2-N{=}C{=}O$, Et_3N, CH_2Cl_2, room temp, 16 h; (f) TBAF (5 equiv), THF, room temp, 1 h; (g) 0.3 N HCl, CH_3CN, room temp, 1 h; (h) MeOH, room temp, 16h, 43% in four steps.

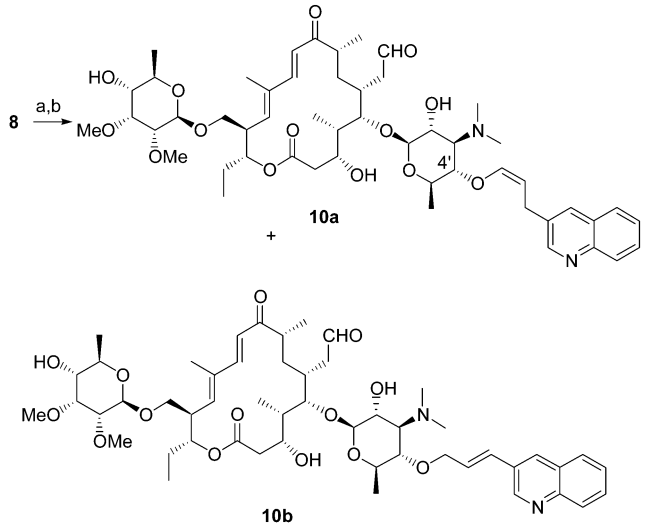
Scheme 2[a]



[a] Conditions: (a) allyl-*tert*-butyl carbonate (3 equiv), $Pd_2(dba)_3$ (0.1 equiv), dppb (0.2 equiv), THF, reflux, 4 h, 60%; (b) TBAF (5 equiv), THF, room temp, 1 h; (c) 0.3 N HCl, CH_3CN, room temp, 1 h; (d) MeOH, room temp, 16 h, 61% in three steps.

CH_3CN), the reaction of **8** with 3-bromoquinoline followed by deprotection in methanol gave *cis*-enol ether **10a** as the major product together with the conjugated quinolyl **10b** as the minor product in a 8:1 ratio (Scheme 3). The structure of **10a** was confirmed by detailed NMR analysis of the compound (see Supporting Information).

Scheme 3[a]



[a] Conditions: (a) 3-bromoquinoline (2 equiv), $Pd(OAc)_2$ (0.16 equiv), $P(o\text{-}Tol)_3$ (0.31 equiv), Et_3N (2.5 equiv), CH_3CN, 80 °C, 16 h, 50%; (b) MeOH, room temp, 16 h.

The 4′-substituted 16-membered macrolides were tested against a panel of representative respiratory tract pathogens together with erythromycin A and tylosin as references. Various macrolide-resistant strains were also included in order to evaluate the activities of this novel series of macrolide to overcome bacterial resistance. *Staphylococcus aureus* 25923 and *Streptococcus pneumoniae* 49619 are erythromycin-susceptible strains. *Streptococcus pneumoniae* 700904 and *Streptococcus pyogenes* 2912 are macrolide−lincosamide−streptogramin B (MLS_B) resistant strains encoded by the erm gene. *Streptococcus pneumoniae* 7701 and *Streptococcus pyogenes* 1323 are efflux-resistant strains encoded by the mef gene. *Haemophilus influenzae* 33929 is an ampicillin-resistant strain. The in vitro antibacterial activities are reported as minimum inhibitory concentrations (MICs) that were determined by the agar dilution method as recommended by the NCCLS (National Committee for Clinical Laboratory Standards).[12]

The results in Table 1 show the antibacterial activity of 4′-substituted tylosin derivatives with the reference compounds (tylosin and erythromycin A). Erythromycin A is active against the sensitive strains but weakly active against the efflux-resistant strains and inactive against the constitutively MLS_B-resistant strains. Tylosin is active against the efflux-resistant strains but not active against the erm-resistance strains.[8]

The carbamate compound **6** showed weak antibacterial activity. However, the 4′-substituted ally ether compound **9** exhibited improved activity compared to

Table 1. In Vitro Antibacterial Activity of Selected Compounds (MIC, μg/mL)

strain		**6**	**9**	**10a**	**10b**	tylosin	Ery A
S. aureus 25923	Ery S	2	1	0.5	0.5	2	0.5
S. pneumoniae 49619	Ery S	2	0.5	0.0625	0.0625	0.25	0.06
S. pneumoniae 700904	Ery R-erm	>64	>64	0.5	1	64	>64
S. pneumoniae 7701	Ery R-mef	2	0.5	0.0625	0.0625	0.25	8
S. pyogenes 2912	Ery R-erm	>64	32	16	8	>64	>64
*S. pyogenes*1323	Ery R-mef	0.25	0.125	0.0625	0.0625	0.25	16
H. influenzae 33929	Amp R	16	8	4	4	8	4

carbamate **6** against most of the pathogens tested (Table 1). Furthermore, compound **9** is slightly more active than tylosin against erythromycin-resistant strains of *S. pyogenes* 2912 and 1323 encoded by the erm and mef genes, respectively. Compounds **10a** and **10b** showed much improved overall antibacterial activities against susceptible and resistant organisms, indicating that the addition of a 3-quinolyl group to **9** has a significant effect on its activity. Compared to erythromycin A, **10a** and **10b** exhibited excellent activity against erythromycin-resistant strains. For example, **10a** demonstrated 128-fold greater activity than erythromycin against resistant *S. pneumoniae* 700904 and *S. pneumoniae* 7701 strains. In addition, **10b** showed a 256-fold improved activity against efflux-resistant strain of *S. pyogenes* 1323 than erythromycin A.

The recent high-resolution X-ray cocrystal structures of the bacterial ribosome with the macrolides have revealed their detailed interactions at the atomic level.[13] These structures demonstrate that macrolides inhibit bacterial protein synthesis by sterically blocking the passage of the nascent polypeptides through the exit tunnel of the ribosome. From the cocrystal structures of the 50S ribosomal subunit of *Haloarcula marismortui* complexing with tylosin, it is shown that the disaccharide of the tylosin extends toward the peptidyl transferase center (PTC) in the exit tunnel.[14] The PTC is the site of catalysis for the formation of bacterial polypeptide. According to this structure, the mycarose group of tylosin is so close to PTC that it allows only one dipeptide formation. In contrast, erythromycin A, which has only one monosaccharide extension from the 5-position, allows the synthesis of longer peptides up to four amino acids. This structural information implies that tylosin interferes more directly with the activity of the PTC than erythromycin A. Therefore, replacing the acid labile mycarose sugar of tylosin to make an even longer extension at the 4′-position with aromatic substituents should improve acid stability and may also increase its binding interactions with the ribosome as well.

The most active compounds (**10a** and **10b**) were obtained when a 3-quinolyl group was attached to the allyl ether at the 4′-position. We believe that the improved antibacterial activity against MLS_B-resistant bacteria achieved by these novel macrolides compared to tylosin is the result of possible interactions of the aryl group with a secondary ribosomal binding site. The heteroaromatic quinolyl group may have $\pi-\pi$ stacking and hydrophobic interactions with ribosome RNA bases in the exit tunnel because these interactions are much favored. In addition, the quinolyl group may reach into the PTC and strongly prevent the formation of even the first peptide bond. It is presumably these tight binding interactions with the ribosome that overcome the bacterial resistance.

The introduction of a versatile allyl group at the 4′-position of tylosin paves the way for the further diverse modifications of tylosin.[15]

Novel 4′-substituted 16-membered ring macrolides were synthesized and evaluated for antibacterial activity. Compounds containing a quinolyl group at the 4′-position were the most active against both erythromycin-susceptible and erythromycin-resistant bacteria. Compound **10a** exhibited highly improved activities against some key erythromycin-resistant pathogens compared to tylosin and erythromycin A. The 4′-substitution presents promising opportunities for the development of new macrolide antibiotics to overcome bacterial resistance.

Acknowledgment. The authors thank Dr. Thoai Lien for help in getting the HRMS data.

Supporting Information Available: Experimental procedures and spectral data for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) *Macrolides: Chemistry, Pharmacology and Clinical Uses*; Bryskier, A. J., Butzler, J.-P., Neu, H. C., Tulkens, P. M., Eds.; Arnette Blackwell: Paris, 1993. (b) Henninger, T. C. Recent Progress in the Field of Macrolide Antibiotics. *Expert Opin. Ther. Pat.* **2003**, *13*, 787−805.

(2) Zhanel, G. G.; Dueck, M.; Hoba, D. J.; Vercaigene, L. M.; Embil, J. M.; Gin, A. S.; Karlowsky, J. A. Review of Macrolides and Ketolides: Focus on Respiratory Tract Infections. *Drugs* **2001**, *61*, 443−498.

(3) Yonath, A.; Lonard, K. R.; Wittmann, H. G. A Tunnel in the Large Ribosmal-Subunit Revealed by 3-Dimensional Image-Reconstruction. *Science* **1987**, *236*, 813−816.

(4) Doern, G. V.; Heilmmann, K. P.; Huynh, H. K.; Rhomberg, P. R.; Coffman, S. L.; Burueggemann, A. B. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in the United States during 1999−2000, including a comparison of resistance rates since 1994−1995. *Antimicrob. Agents Chemother.* **2001**, *45*, 1721−1729.

(5) Agouridas, C.; Denis, A.; Augar, J.-M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J.-F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N. Synthesis and Antibacterial Activity of Ketolides (6-*O*-Methyl-3-oxoerythromycin Derivatives): A New Class of Antibacterials Highly Potent against Macrolide-Resistant and -Susceptible Respiratory Pathogens. *J. Med. Chem.* **1998**, *41*, 4080−4100.

(6) Ma, Z.; Clark, R. F.; Brazzale, A.; Wang, S.; Rupp, M. J.; Li, L.; Griesgraber, G.; Zhang, S.; Yong, H.; Phan, L. T.; Nemoto, P. A.; Chu, D. T. W.; Plattner, J. J.; Zhang, X.; Zhong, P.; Cao, Z.; Nilius, A. M.; Shortridge, V. D.; Flamm, R.; Mitten, M.; Meulbroek, J.; Ewing, P.; Alder, J.; Or, Y. S. Novel Erythromycin Derivatives with Aryl Groups Tethered to the C-6 Position Are Potent Protein Synthesis Inhibitors and Active against Multidrug-Resistant Respiratory Pathogens. *J. Med. Chem.* **2001**, *44*, 4137−4156.

(7) Kirst, H. A. Semi-Synthetic Derivatives of 16-Membered Macrolide Antibiotics. *Prog. Med. Chem.* **1994**, *31*, 265−295.

(8) Kirst, H. A.; Sides, G. D. New Directions for Macrolide Antibiotics−Structural Modifications and Invitro Activity. *Antimicrob. Agents Chemother.* **1989**, *33*, 1413−1418.

(9) (a) Kirst, H. A. Recent Developments with Macrolide Antibiotics. *Expert Opin. Ther. Pat.* **1998**, *8*, 111−120. (b) Mandic, Z.; Naranda, A.; Novak, P.; Brajsa, K.; Derek, M. New Derivatives of Tylosin: Chemical and Electrochemical Oxidation Products of Desmycosin. *J. Antibiot.* **2002**, *55*, 807−813. (c) Hranjec, M.; Starcevic, K.; Zamola, B.; Mutak, S.; Derek, M.; Karminski-Zamola, G. New Amidino-benzimidazolyl Derivatives of Tylosin and Desmycosin. *J. Antibiot.* **2002**, *55*, 308−314. (d) Creemer, L. C.; Toth, J.; Kirst, H. A. Synthesis and in Vitro Antimicrobial Activity of 3-Keto 16-Membered Macrolides Derived from Tylosin. *J. Antibiot.* **2002**, *55*, 427−436. (e) Mutak, S.; Marsic, N.; Kramaric, M. D.; Pavlovic, D. Semisynthetic Macrolide Antibacterials Derived from Tylosin. Synthesis and Structure−Activity Relationships of Novel Desmycosin Analogues. *J. Med. Chem.* **2004**, *47*, 411−431.

(10) Stoner, E. J.; Peterson, M. J.; Allen, M. S.; DeMattei, J. A.; Haight, A. R.; Leanna, M. R.; Patel, S. R.; Plata, D. J.; Premchandran, R. H.; Rasmussen, M. Allylation of Erythromycin Derivatives: Introduction of Allyl Substituents into Highly Hindered Alcohols. *J. Org. Chem.* **2003**, *68*, 8847−8852.

(11) Cabri, W.; Candiani, I. Recent Developments and New Perspectives in the Heck Reaction. *Acc. Chem. Res.* **1995**, *28*, 2−7.

(12) The MIC assays were performed in accordance with the National Committee of Clinical Laboratory Standards (NCCLS) guidelines: (a) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 5th ed.; NCCLS Document M7-A5; NCCLS, January 2000; Vol. 20, No. 2. (b) *Performance Standards for Antimicrobial Susceptibility Testing: Eleventh Informational Supplement*; NCCLS Document M100-S11; NC-

CLS, January 2001; Vol. 21, No. 1. (c) Amsterdam, D. Susceptibility Testing of Antimicrobials in Liquid Media. In *Antibiotics in Laboratory Medicine*, 4th ed.; Lorain, V., Ed.; Williams & Wilkins: Baltimore, MD, 1996; pp 52−111.

(13) (a) Schlunzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. Structural Basis for the Interaction of Antibiotics with the Peptidyl Transferase Centre in Eubacteria. *Nature* **2001**, *413*, 814−821. (b) Schlunzen, F.; Harms, J. M.; Franceschi, F.; Hansen, H. A. S.; Bartels, H.; Zarivach, R.; Yonath, A. Structural Basis for the Antibiotic Activity of Ketolides and Azalides. *Structure* **2003**, *11*, 329−338. (c) Berisio, R.; Harms, J.; Schluenzen, F.; Zarivach, R.; Hansen, H. A. S.; Fucini, P.; Yonath, A. Structural Insight into the Antibiotic Action of Telithromycin against Resistant Mutants. *J. Bacteriol.* **2003**, *185*, 4276−4279.

(14) Hansen, J. L.; Ippolito, J. A.; Ban, N.; Nissen, P.; Moore, P. B.; Steitz, T. A. The Structures of Four Macrolide Antibiotics Bound to the Large Ribosomal Subunit. *Mol. Cell* **2002**, *10*, 117−128.

(15) (a) Clark, R. F.; Ma, Z.; Wang, S.; Griesgraber, G.; Tufano, M.; Yong, H.; Li, L.; Zhang, X.; Nilius, A. M.; Chu, D. T. W.; Or, Y. S. Synthesis and Antibacterial Activity of Novel 6-O-Substituted Erythromycin A Derivatives. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 815−819. (b) Or, Y. S.; Clark, R. F.; Wang, S.; Chu, D. T. W.; Nilius, A. M.; Flamm, R.; Mitten, M.; Ewing, P.; Alder, J.; Ma, Z. Design, Synthesis, and Antimicrobial Activity of 6-O-Substituted Ketolides Active against Resistant Respiratory Tract Pathogens. *J. Med. Chem.* **2000**, *43*, 1045−1049.

JM034233N