Bioorganic & Medicinal Chemistry Letters 23 (2013) 5057–5060

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and biological evaluation of azithromycin glycosyl derivatives as potential antibacterial agents

Lei Zhang [a,b,†], Xiaoyun Chai [a,†], Baogang Wang [a], Shichong Yu [a], Honggang Hu [a], Yan Zou [a], Qingjie Zhao [a], Qingguo Meng [b,*], Qiuye Wu [a,*]

[a] Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China
[b] Department of Medicinal Chemistry, School of Pharmacy, Yantai University, 30 Qingquan Road, Yantai 264005, China

ARTICLE INFO

Article history:
Received 13 June 2013
Revised 15 July 2013
Accepted 17 July 2013
Available online 25 July 2013

Keywords:
Azithromycin
Antibacterial activity
Glycosyl
Synthesis

ABSTRACT

A series of 11,12-cyclic carbonate azithromycin-4″-*O*-carbamoyl glycosyl derivatives were designed, synthesized, and evaluated as antibacterial agents to search for target compounds with excellent activity. The results of preliminary antibacterial tests against eight strains in vitro revealed that all of the title compounds exhibited improved activities with broad spectrum compared with the parent compound. The glycosylated side chains may be the pharmacophores responsible for the improved activity.

Over the past decade, macrolide antibiotics have played an important role in the clinical treatment of upper and lower respiratory tract infections. These antibiotics act by binding to the ribosomal RNA of bacteria. The previous studies provided strong, detailed evidence of this aforementioned interaction at the molecular level via high-resolution X-ray cocrystal structures.[1–4] These X-ray studies demonstrated that macrolides inhibit bacterial protein synthesis by sterically blocking the passage of nascent polypeptides through the exit tunnel of the ribosome.[5]

Macrolide antibiotics, such as erythromycin A (ERY), clarithromycin (CLA), and azithromycin (AZI) (Fig. 1), are widely prescribed clinically. However, these antibiotics have various disadvantages. ERY, a first-generation macrolide, is readily degraded under acidic conditions, thus losing its antibacterial activity;[6] these degraded products are responsible for undesirable gastrointestinal side effects.[7,8] Second-generation macrolides, such as CLA and AZI, have been widely used for respiratory tract infection due to their superior antibacterial activity, pharmacokinetic properties and fewer gastrointestinal side effects compared to ERY.[9] However, their clinical uses have been limited by the emergence of drug resistance.[10] The increasing incidence of bacterial resistance is becoming a major threat to the successful treatment of infectious diseases. The most serious threat is the increasing resistance of community-acquired respiratory tract infections to various antimicrobials, which is a pandemic phenomenon.[11] Third-generation macrolide ketolides, such as telithromycin, can effectively address bacterial resistance and other issues associated with current macrolide regimens.[12,13] However, the use of telithromycin suffers from limitations such as hepatotoxicity.[14]

With the widespread emergence of antibacterial resistance against macrolides, there is still a need to develop and extend safe chemotherapeutic agents with potent antibacterial activity. Recently, considerable efforts have been focused on discovering novel macrolides to combat the resistance. Research has indicated that compounds containing particular groups in the 4″-position of the cladinose sugar were effective against macrolide resistant strains.[15] Additionally, the C-11,12 carbamate side chain of macrolides may aid in interactions with the target enzyme.[16] So, we have chosen the 4″-position of the cladinose sugar as the modification position.

Glycosyl moieties exist extensively in organisms and exhibit a variety of biological functions, such as diagnostic and therapeutic potential. D-desosamine and L-cladinose, the two monosaccharides in macrolide structures, are important for the binding of macrolides to ribosomes. X-ray analyses of macrolides have revealed that the hydroxy group of a glycosyl moiety was important for hydrogen bond formation.[2] However, there have been few reports about glycosylation modification of macrolide antibiotics.[17,18] As we believe that the glycosylation may help for the hydrogen bond formation, we therefore introduced glycosyl moieties to AZI through a carbamoyl group and examined the antibacterial activities of the resultant products.

* Corresponding authors. Tel./fax: +86 21 81871225. (Q.W.).
E-mail addresses: qinggmeng@163.com (Q. Meng), wuqy640319@163.com (Q. Wu).
† These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.bmcl.2013.07.042

By substitution of the 4″-position with various carbamoyl-groups, a series of novel 4″-substituted azithromycin derivatives were obtained. Further modification at 11,12-cyclic carbonate azithromycin was performed. We hypothesized that the 11,12-cyclic carbonate azithromycin-4″-*O*-carbamoyl glycosyl derivatives might enhance the antibacterial activity against resistant strains.

Eight glycosylated intermediates **1f**–**8f** were prepared from the corresponding commercially available saccharides by following a series of established transformations. We have chosen **1f** as an example (Scheme 1). First, all the hydroxyl groups of glucose (**1a**) were protected with acetyl groups to give the acetylated saccharide (**1b**) in an excellent yield. The treatment of **1b** with 2-azidoethanol in the presence of $BF_3{\cdot}Et_2O$ afforded a series of protected azidoethyl-glycosides (**1c**) in the β-configuration with a very good yield. After deprotection of **1c**, we obtained the azidoethyl-glycosides (**1d**). Compound **1d** was protected with benzyl groups to give the benzylated saccharide (**1e**) in an excellent yield. Lastly, **1f** was reduced by $Pd/CaCO_3$ under a hydrogen atmosphere. The other seven glycosylated intermediates (**2f**–**8f**) were synthesized in the same manner.

The general synthetic methodology for preparing the compounds of interest (**F1**–**F8**) is outlined in Scheme 2. Protection of the 2′-hydroxyl group of azithromycin with acetic anhydride provided 2′-acetyl azithromycin (**B**) in 82% yield. 11,12-Cyclic carbonate azithromycin 4″-*O*-acylimidazolide (**C**) was obtained in 62% yield by treatment of compound **B** with NaH and *N*,*N*′-carbonyldiimidazole (CDI) in DMF. Then, the intermediates (**D1**–**D8**) were prepared by coupling compound **C**, followed by benzyl protection of the glycosylated group in the presence of 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU). Lastly, intermediates **D1**–**D8** were reduced by 10% Pd-C under a hydrogen atmosphere. After methanolysis, we obtained compounds **F1**–**F8**. The yields were within the range of 70–80%.

The in vitro antibacterial activities were reported as minimum inhibitory concentrations (MICs), which were determined using astandard dilution assay as recommended by the NCCLS (National Committee of Clinical Laboratory Standard).[19] The MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well. For the assays, the title compounds to be tested were dissolved in dimethyl sulfoxide (DMSO), serially diluted in growth medium, inoculated and incubated at 35 °C.

The selected strains evaluated were methicillin-susceptible *Staphylococcus aureus* MSSA-1 (*S. aureus MSSA-1*), methicillin-resistant *Staphylococcus aureus* MRSA-1 (*S. aureus MRSA-1*), *Staphylococcus aureus* ATCC25923 (*S. aureus ATCC25923*), *Streptococcus pneumoniae* 943 (*S. pneumoniae943)*, *Staphylococcus pneumonia* 746 (*S. pneumoniae 746)*, *Streptococcus pyogenes* 447 (*S. pyogenes 447)*, *Escherichia coli* 236 (*E. coli 236*), and *Escherichia coli* ATCC25922 (*E. coli ATCC25922)*. ERY and AZI served as the positive control and were obtained from their respective manufacturers. The results of the assays are summarized in Table 1. The data points express the mean of replicate experiments. All of our susceptibility tests were performed three times using each antibacterial agent.

The results indicated that nearly all of the 11,12-cyclic carbonate azithromycin-4″-*O*-carbamoyl glycosyl derivatives showed moderate activity against all the strains, and someof the derivatives exhibited improved activity compared with AZI and ERY. Notably, the MIC_{80} values indicate that compounds **F1** and **F2** showed improved activity against all of the bacterial strains relative to the other compounds, expressing the same or higher antibacterial activities as AZI. Compounds **F7** and **F8** with the disaccharide side chain showed the least activity; this observation could be due to increasing side chain length. Among the compounds tested, Compounds **F1** and **F6** showed the same activity against the *S. aureus MSSA-1* as AZI, and they exhibited eightfold higher activity than ERY. Particularly, compounds **F1** and **F2** showed fourfold and eightfold higher activity against the *S. pneumoniae 943* than AZI and ERY, respectively. In addition, the activity of compounds**F1** and **F2** (MIC_{80} 2 μg/mL) increased significantly against *S. pneumoniae 746*, showing eightfold higher activity than ERY. The activity of compounds

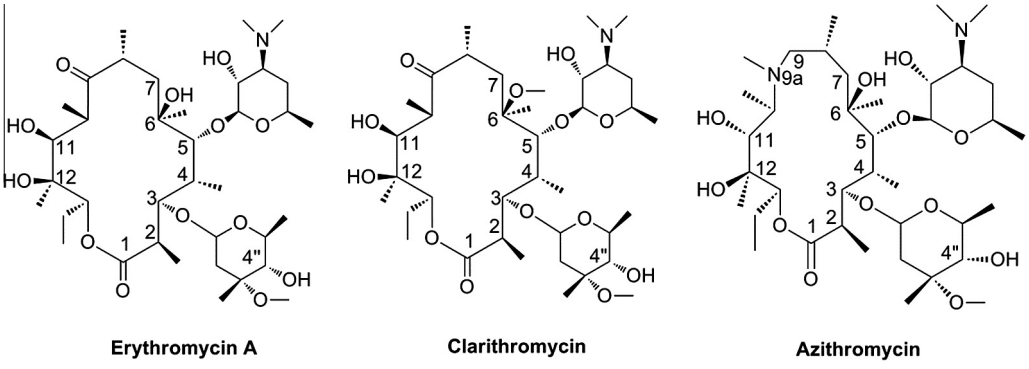
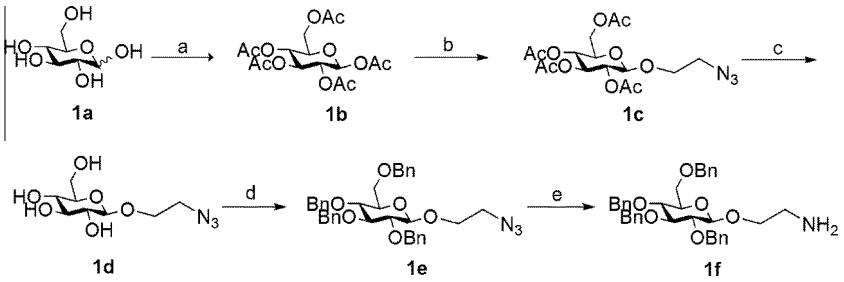


Figure 1. The structures of macrolide antibiotics.



Scheme 1. The synthesis of the saccharide intermediates. Reactions and conditions: (a) Ac_2O, CH_3COONa, reflux, 5 h, in 90% yield; (b) $HOCH_2CH_2N_3$, $BF_3{\cdot}Et_2O$, DCM, Ar, 0 °C, 18 h, in 80% yield; (c) CH_3ONa, CH_3OH, 4 h, in 93% yield; (d) BnBr, NaH, TBAI, DMF, in 52% yield; and (e) $Pd/CaCO_3$, H_2, CH_3OH, in 98% yield.