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Synthesis, activity and pharmacokinetics of novel antibacterial 15-membered ring macrolones

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ABSTRACT

Synthesis, antibacterial activity and pharmacokinetic properties of a novel class of macrolide antibiotics—macrolones—derived from azithromycin, comprising oxygen atom(s) in the linker and either free or esterified quinolone 3-carboxylic group, are reported. Selected compounds showed excellent antibacterial potency towards key erythromycin resistant respiratory pathogens. However, the majority of compounds lacked good bioavailability. The isopropyl ester, compound **35**, and a macrolone derivative with an elongated linker **29** showed the best oral bioavailability in rats, both accompanied with an excellent overall microbiology profile addressing inducible and constitutive MLSb as well as efflux mediated macrolide resistance in streptococci, while compound **29** is more potent against staphylococci.

1. Introduction

A continuous increase in the number of infections caused by bacteria resistant to one or multiple antibiotic classes poses a significant threat [1,2] as it may lead to treatment failures and complication. A spread of resistance among common respiratory pathogens, including *Streptococcus pneumoniae*, is recognized by Infectious Diseases Society of America as one of the three major areas of concern that creates a need for new antibiotics [2].

One of the main driving forces for development and spread of resistance is high antibiotic consumption, reflected in resistance rates directly correlating with the prescription of antimicrobial drugs [3].

Therefore, considerable differences are reported throughout the world and levels of *S. pneumoniae* resistance to the two most frequently used antibiotic classes—beta-lactams and macrolides—vary substantially in Europe, ranging from <10% penicillin non-susceptible *S. pneumoniae* (PNSP) and erythromycin resistant pneumococci (ERP) isolates in northern countries to >25% in the south [4]. Levels of macrolide resistance and underlying resistance mechanisms in different parts of the world differ, with efflux tending to be more prevalent in North America while *erm*(B) is most common in many parts of Europe (South-eastern in particular) [4,5] and Asia [6–8]. Among macrolide resistant strains in Asia, there is a high proportion of isolates having both *erm*(B) and *mef*(E) [6,7]. Generally, demographics play an important role in incidence of both penicillin and macrolide resistance, with a higher frequency of resistance consistently found among children [9].

To overcome resistance, in addition to prudent use of available drugs, a constant effort to discover and develop new agents with an

Abbreviations: EDCxHCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DBU, 1,8-Diazabicyclo[5.4.0] undec-7-ene; AZM, azithromycin; TEL, telithromycin; eryS, erythromycin sensitive; MLSb, macrolide, licosamide and streptogramin (MLSb) antibiotics; iMLSb, inducible resistance to macrolide, lincosamide and streptogramin antibiotics; iMcLSb, inducible resistance to macrolide and constitutive resistance to lincosamide antibiotics; cMLSb, constitutive MLSb resistance; M, efflux mediated macrolide resistance; PK, pharmacokinetics; IV, intravenous; PO, per os; CL, systemic clearance; CLi, intrinsic clearance; Vd, volume of distribution; LBF, liver blood flow; oral F, oral bioavailability.

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improved spectrum of activity is required. A variety of chemical modifications were carried out so far around the macrolide backbone (core). Among 14-membered derivatives, cethromycin [10–13], a 3-keto derivative, seems to exhibit antibacterial potency against resistant pathogens comparable/exceeding to telithromycin. In the past decade, we have explored 15-membered macrolide cores, including modifications on the N-9 position of aglycone ring [14–16], 3-decladinosyl derivatives [17,18] and acyl derivatives on 4″-position of cladinose [19] without achieving desirable antimicrobial profile.

Macrolide compounds modified at the 4″-position present a considerable opportunity for the development of novel antibiotics to effectively address the growing problem of macrolide resistance. We reported on the first 4″-*O*-quinolone derivative on 6-*O*-methyl-8a-aza-8a-homoerythromycin, with improved activity against macrolide resistant *S. pneumoniae* (with both inducible and constitutive *erm*(B) expression), iMLSb *Streptococcus pyogenes* and *Haemophilus influenzae*, compared to golden standard of macrolide therapy, azithromycin [20]. Subsequently we explored 4″-*O*-(quinolylamino-alkylamino) propionyl derivatives on various macrolide scaffolds, 8a-aza-8a-homoerythromycin, clarithromycin and azithromycin [21]. From SAR trends observed in this series, azithromycin compounds with quinolone containing cyclopropyl on N(1) position had the most favorable antibacterial profiles. Here we describe a derivatization stream followed in order to investigate the influence of linker features on biological properties, both antibacterial and pharmacological. Therefore, we introduced one or two oxygen atoms in the linker, retaining similar length or elongating it. Additional modification of quinolone carboxylic acid by esterification was performed in order to optimize pharmacokinetic (PK) properties.

2. Results and discussion

2.1. Chemistry

Synthesis of quionolone-3-carboxylic intermediates **18**–**23** can be approached by route outlined in Scheme 1.

Synthesis is based on the commercially available 6,7-disubstituted quinolone precursors **2**–**5**. The electronic effects of the other two groups on the benzene unit in quinolones **2**–**5** tend to diminish the inherent difference in the reactivity of these two halogen atoms in nucleophilic substitution. The vinilogous-amide type nitrogen, present in the *para* position to fluorine, lowers its nucleofugal aptitude, whereas carbonyl group, *para*-situated to chlorine enhances its nucleofugal property [22]. The net synthetic result of this leveling is the formation of mixtures of 6- and 7-substituted quinolones **6**–**11** (Scheme 1). On varying the solvent and temperature, the ratio of the two substitution products **6a**–**11a**/**6b**–**11b** remained in the range 45:55 ± 5%. In spite of this undesired ratio, this reaction step became workable when an effective, non-chromatographic separation process for **6a**–**11a** and **6b**–**11b** was developed (see Experimental). This protocol has allowed isolation of pure 6-chloro-7-substituted derivatives **6a**–**11a** and their transformation to the target structures **18**–**23**.

Synthesis of final macrolones **24**–**46** is outlined in Scheme 2.

In the first step, site-selective acylation at 4″–OH of 2′-*O*-acetyl azithromycin **1** was performed with an excess of quinolone diacids **18**–**23**. Several condensation procedures were investigated, e.g. mixed anhydride of diacids with pivaloyl chloride, DCC, EDC, and the most suitable reagent was EDCxHCl/DMAP. Determination of pK_a values of quinolone diacid **18** has shown remarkable differences in acid strengths of two carboxylic groups in the molecule (Fig. 1) which explains regioselectivity in the esterification reaction [23,24].

Subsequent deprotection in methanol and purification either by column chromatography or precipitation from ethyl acetate with hexane yielded final quinolone-macrolide derivatives **24**–**29**.

In order to determine the influence of halogen atom on antimicrobial activity, a series of C(7)-dechloro derivatives **30**–**33** have been synthesized in high yield and purity by catalytic hydrogenolysis of **24**–**26** and **29** (Scheme 2).

In order to improve *in vivo* biological properties, ester derivatives on quinolone-3-carboxylic acid **34**–**46** have been prepared by alkylation of macrolone acids **24**, **25**, **30** and **31** with alkyl iodide in presence of K_2CO_3 as base (Scheme 2).

Structures of new molecules were confirmed by the high-resolution mass spectra (HRMS) and NMR. NMR spectra showed two sets of signals, one corresponding to the macrolide, the other to the quinolone part of the molecule, as well as NMR signals of methylene groups of the linker. The proton on the 4″-position of the cladinose sugar revealed a shift from around 2.90 ppm in azithromycin to around 4.70 ppm in final macrolone compounds. The long-range coupling of 4″-proton with new carbonyl signal at around 171 ppm provided additional evidence that a new ester function was introduced at C(4″)-position of macrolide. ^{1}H NMR spectra of C(7)-dechloro compounds **30**–**33** showed a new proton in aromatic part of spectra at around 7.20 ppm and in ^{13}C NMR spectra an upfield shift of C(7) signal for 5 ppm is noticed.

Although C-6, C-7, and N-1 positions in the quinolone part of target molecules should correctly be C-6‴, C-7‴, and N-1‴, the designation used in this article are C(6), C(7), and N(1) because of the clearness.

2.2. X-ray crystal structure

The described synthetic procedures provided compound **29** as an amorphous solid. Significant efforts were made to obtain crystals of **29** from some organic solvents as solvates. High quality crystals were obtained from solvent mixture cyclohexane:*sec*-butyl acetate = 1:1. Single crystal analyses reveal that the compound crystallizes with two independent molecules in asymmetric unit (Fig. 2) with significant conformational differences in orientation of cladinose substituent (Fig. 3). Orientation of C30 and C30B methyl groups into region between desosamine and cladinose sugars indicates typical “folded-in” conformation of aglycone rings. Compound crystallizes as solvate with unidentified numbers of *sec*-butyl acetate and water molecules. Molecules of compound **29** occupy about 85% of unit cell volume while the rest is filled with solvent molecules of which positions cannot be clearly defined (Fig. 4). This assumption is confirmed also with low value of calculated crystal density (1.01 mg/m^3).

Molecules of compound **29** are connected by hydrogen bonds making layers within unit cell, while solvent molecules are situated in holes between the layers and do not participate in hydrogen bonding.

2.3. In vitro antibacterial activity

The antibacterial activity of all novel compounds was determined by a standard broth microdilution method [25] and the data is expressed as minimum inhibitory concentrations (MICs) in units of μg/mL. The organisms studied represent relevant Gram-positive (*Streptococcus pneumoniae*, *S. pyogenes* and *Staphylococcus aureus*) and Gram-negative (*H. influenzae* and *Moraxella catarrhalis*) respiratory tract pathogens, and are either sensitive or resistant to macrolide antibiotics. Underlying resistant phenotypes are two major mechanisms – production of efflux pumps (M), or ribosome modification by methylation, where methylase expression is inducible (iMLSb) or constitutive (cMLSb).