



Synthesis of novel macrolide derivatives with imidazo[4,5-*b*]pyridinyl sulfur contained alkyl side chains and their antibacterial activity

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ABSTRACT

In an effort to find new antibiotics, a novel series of 14-membered macrolides with imidazo[4,5-*b*]pyridinyl sulfur contained alkyl side chains has been synthesized based on commercially available clarithromycin. Chemical transformation of hydroxy group at position C-3 afforded range of ketolides and acylides. Compared to telithromycin, compound **15a** demonstrated improved in vitro activity against erythromycin-susceptible and -resistant strains.

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During the last years, great efforts have been making to discover novel macrolides to combat the growing problems of MLS-resistance. Industrial researchers from Aventis and Abbott Laboratories reported two novel semisynthetic macrolides, telithromycin **1**¹ and cethromycin **2**² (Fig. 1). These two compounds showed significantly improved activities against major macrolide-resistant strains such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. Both the compounds comprise same key structure features: a 3-keto group and an 11,12-carbamate functionality. In addition, they have a proper arylalkyl side chain which can interact with nucleotide A752 in domain II of the 23S rRNA.³

Chemical modification of macrolides has been pursued by numerous investigators attempting to prepare chemical derivatives with improved profiles and biological activity. Many reviews about the recent semisynthetic macrolide antibiotics have been published by various authors.^{4a–c} These investigations have lead to the discovery of 3-acylide,⁵ 3-ether,⁶ 3-carbonate,⁷ and 3-carbamate.⁸ Some of the leading 3-acylides demonstrated excellent activities against macrolide-susceptible and -resistant organism in vivo and in vitro.⁵ Although much effort had been invested for the new derivatives of ketolides and acylides, the innovation in this field is still possible especially in the part of (hetero)arylalkyl side chain and 11,12-carbamate functionality. For example, Lin, X., Chu, D. T. and Plattner, J. J. developed a series of novel C-12 des-methyl ketolide analogs with different heterocyclic moieties at 11-N,12-O

carbamate.⁹ Hunziker et al. published their work about synthesis of a series of novel ketolide antibiotics with a fused five-membered lactone ring and S-alkyl or aryl substituted α -thio acetic acid side chains.¹⁰ These sulfur contained ketolide products exhibited potent antimicrobial activity against both erythromycin sensitive and resistant strains of Gram-positive pathogens. In vivo activities of some compounds obtained, **3** for example (Fig. 1), were in the range as the reference ketolide telithromycin. This evokes us to publish our work about synthesis of ketolides with imidazo[4,5-*b*]pyridinyl sulfur contained alkyl-substituted 11,12-carbamate group (Fig. 2).

The use of erythromycin, as well as the third generation macrolide telithromycin, suffered from a few limitations such as hepatotoxicity.^{4c} The hepatotoxicity can be decreased by increasing protonation or diminishing the hydrophobicity of the erythromycin structure.¹¹ We presume that it is possible to increase protonation of compound **4** because of the existence of proton in the structure of (5-methoxy-3H-imidazo[4,5-*b*]pyridin-2-yl)thiyl group.

To obtain the best antibacterial activity, it was generally accepted that the optimal length of the linker between 11-N and aryl ring is 4 atoms.^{4b} In our research, both 3 and 4 carbon amines were used for lengthening the side chains. With an additional sulfur atom, the side chains will be 4 or 5 atoms of length. Because the radius of carbon and sulfur atom is different, the length of the linker could be slightly adjusted in our strategy. This may help us finding a more suitable side chain on 11,12-carbamate.

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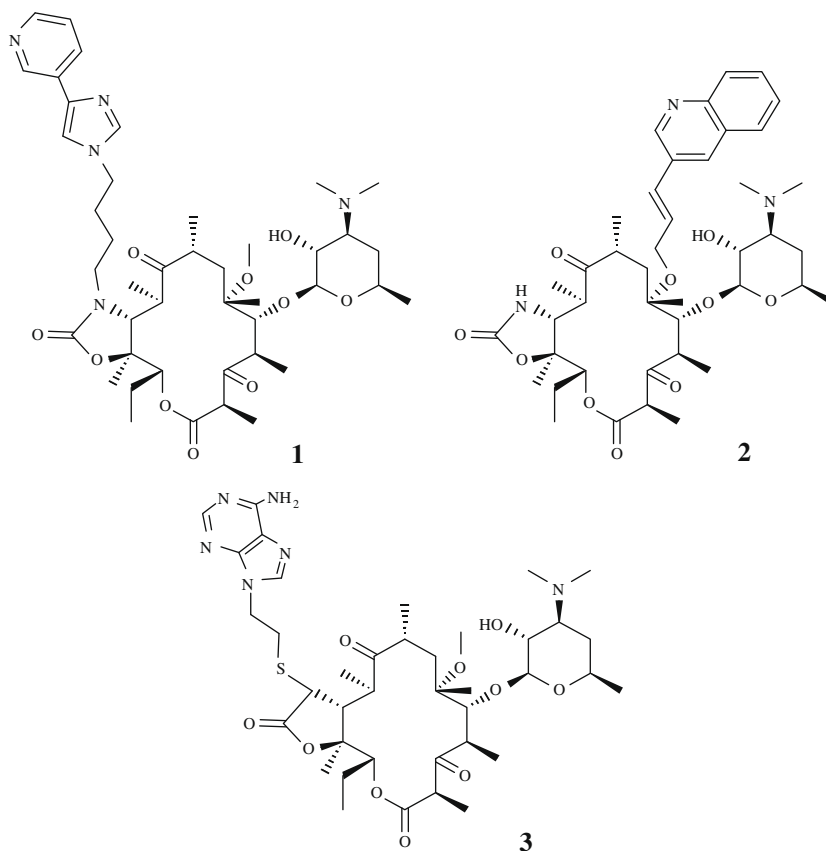


Figure 1.

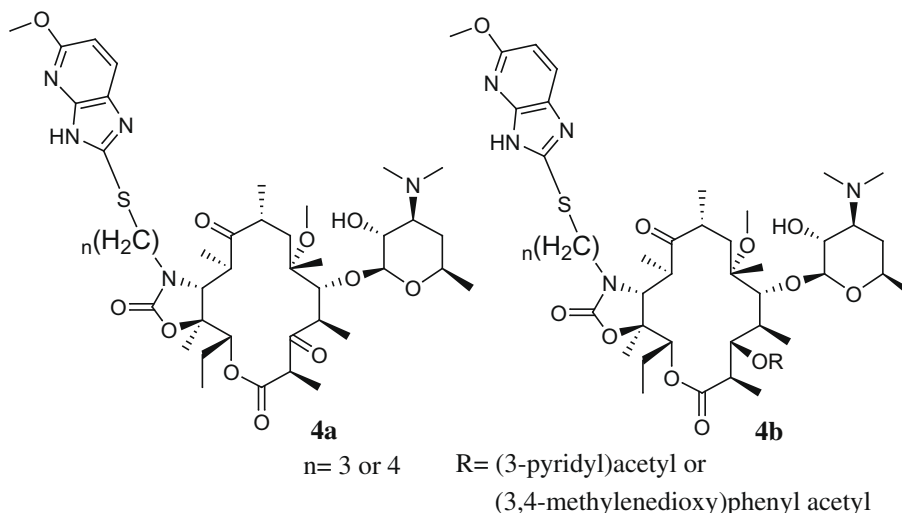


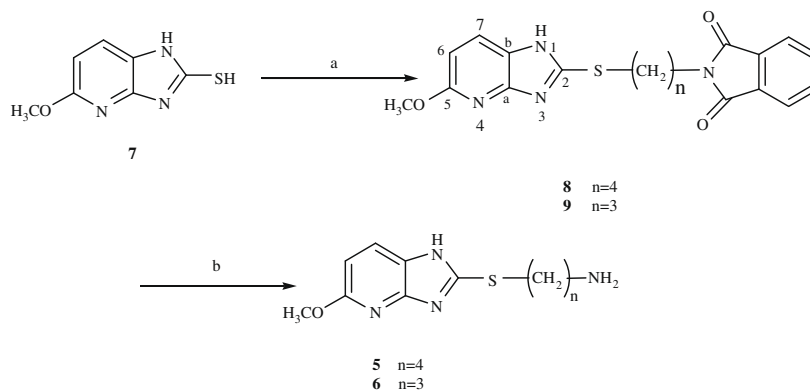
Figure 2.

For comparison with ketolide, we also synthesized 3-acylide derivatives **4b** with the same side chains at 11-N,12-O carbamate group. The acyl groups we applied at 3-O position, including 3-O-pyridyl acetyl and (3,4-methylenedioxy)phenyl acetyl groups, were proved having significant effect for enhancement of antibacterial activity.¹²

A novel class of sulfur contained side chains, **5** and **6**, were synthesized in order to research the SAR of antibiotics and to find better leading compounds. The synthesis of **5** and **6**, depicted in scheme 1, was used the method we had published previously.¹³ Treatment of **7** with one equivalent of *N*-(4-bromobutyl)phthalimide in DMF at 80 °C, product **8**

was achieved with a yield of 68%. To confirm the reaction was taken place on the sulfur atom, we did the HMBC experiment. In the HMBC spectrum of **8**, a long-range correlation exist within H(S-CH₂-, δ 3.37) and C-2(δ 148.6), but long-range correlations between this hydrogen with C-a or C-b were not detected.

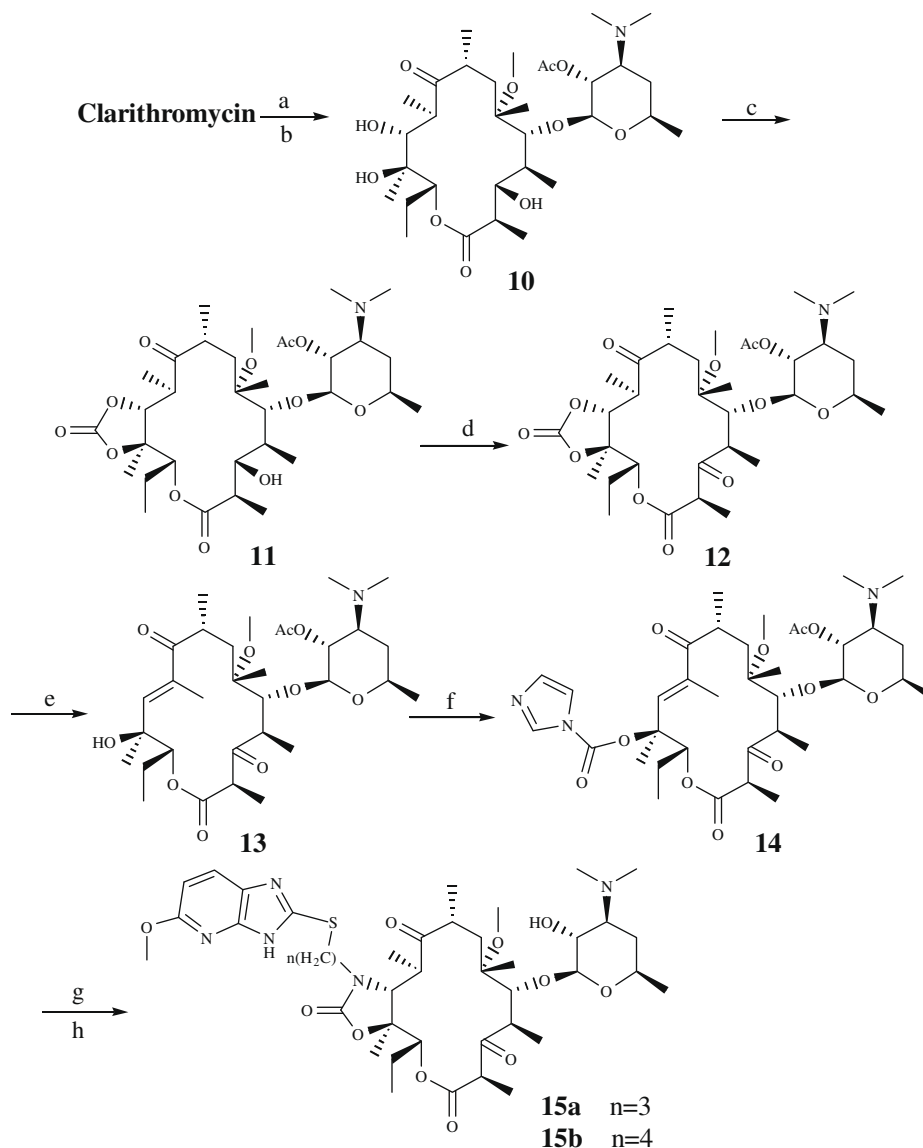
For synthesis of compound **9**, the analog of compound **8**, treatment of **7** with one equivalent amount of *N*-(3-bromopropyl)phthalimide, the similar result could be observed. After removing the phthalimide groups of **8** and **9** in a solution of 85% aqueous hydrazine in EtOH, two free amine products **5** and **6** were obtained.



Scheme 1. Reagents and conditions: (a) 1 equiv *N*-(4-bromobutyl)phthalimide or *N*-(3-bromopropyl)phthalimide, K_2CO_3 , DMF, 45–68°C; (b) 85% aqueous hydrazine, EtOH, 69–76%.

Scheme 2 indicates the actual synthesis process that started from clarithromycin via intermediate 2'-*O*-acetyl-3-*O*-descladinosyl-5-*O*-desosaminyl-6-*O*-methyl erythromycin **A10**.⁵ The synthesis of **11** was followed the similar manner of Tanikawa et al.⁵

Formation of 11,12-carbonate **11** was carried out with trichloromethyl chloroformate in a mixture of CH_2Cl_2 and pyridine at 0 °C in 65% yield.



Scheme 2. Reagents and conditions: (a) 12 N HCl, H_2O , rt; (b) Ac_2O , K_2CO_3 , acetone or Ac_2O , Et_3N , CH_2Cl_2 ; (c) pyridine, trichloromethyl chloroformate, CH_2Cl_2 , rt; (d) $(\text{COCl})_2$, DMSO, Et_3N , CH_2Cl_2 , –60 °C; (e) DBU, acetone, rt; (f) NaH, CDI, DMF, –25 °C; (g) $\text{CH}_3\text{CN}:\text{H}_2\text{O}/10:1$, side chain, 50 °C; (h) MeOH, reflux.

Oxidation of 3-OH could be carried out by different methods. Agouridas et al. prepared their ketolide analogues, for example telithromycin, by Jones oxidation.¹⁴ Researchers from Abbott synthesized cethromycin by NCS and Me₂S in presence of Et₃N. In our case, **11** was converted to ketolide **12** by reaction with (COCl)₂ and DMSO in CH₂Cl₂ in presence of Et₃N at –60 °C in a yield of 55%.¹⁵ The synthesis of the 12-acylimidazolyl ketolide **14** was carried out in two steps.

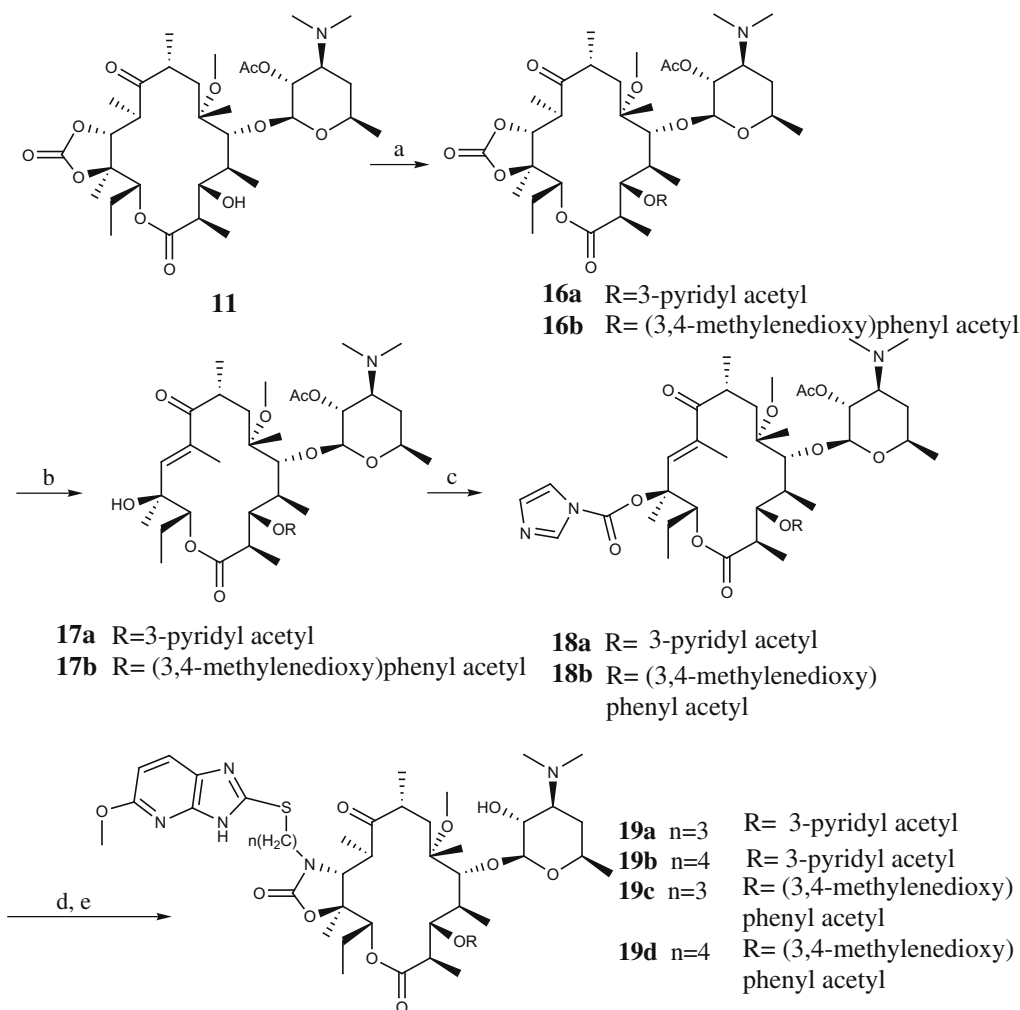
Compound **12** was underwent smooth elimination by treatment with DBU in acetone to give **13** in 50% yield. Acylation of **13** by treatment with NaH in DMF at –25 °C followed by addition of carbonyldiimidazole (CDI). Compound **14** was obtained in a yield of 65%. Stirring **14** with 4-((5-methoxy-3H-imidazol-[4,5-*b*]pyridin-2-yl)thio)propyl amine **9** or 4-((5-methoxy-3H-imidazol-[4,5-*b*]pyridin-2-yl)thio)butyl amine **8** at 60 °C, the desired ketolides were obtained. Refluxing of the ketolides in MeOH gave corresponding **15a** and **15b** in 22% and 15% in two steps.

The 11,12-carbamate acylide analogs **19a–d** were prepared following the method of Tanikawa et al.⁵ (Scheme 3). The acyl groups applied at 3-position were 3-pyridyl acetyl and (3,4-methylenedioxy)phenyl acetyl group. 3-Acylides **16a**, **16b** were prepared from 11,12-carbonate **11** with corresponding carboxylic acid in presence of 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 4-(dimethyl-amino)pyridine (DMAP) in CH₂Cl₂ at room temperature.

Treatment of **16a**, **16b** with DBU in acetone allowed formation of **17a**, **17b**. The resulting allyl alcohol **17a**, **17b** was transformed to C-12 imidazole carbamate which upon reaction with an excess of free amine **9** or **8** and deprotection of the 2'-O-acetate by heating in methanol yielded final acylide analogs **19a–d**.

The newly prepared macrolide derivatives **15a**, **15b** and **19a–d**, together with telithromycin, azithromycin and clarithromycin as references, were tested against a panel of representative respiratory tract pathogens (Table 1).

The strains were *S. aureus* ATCC 29213 (methicillin-sensitivity staphylococcus aureus (MSSA)), *S. aureus* 07H260, BK2464, ANS46 (methicillin-resistant staphylococcus aureus (MRSA)), *S. epidermidis* was 07R066 MSSE (methicillin-sensitivity staphylococcus epidermidis). *S. epidermidis* 07G235, 07T202, 07C068 were MRSE (methicillin-resistant staphylococcus epidermidis). *S. pneumoniae* ATCC 49619, 07H252 were erythromycin-susceptible strain (Ery-S), *S. pneumoniae* 07Z001, 05Z006 were erythromycin-resistant strain (Ery-R), *Streptococcus pyogenes* 07U084, 07U086 were erythromycin-susceptible strain (Ery-S), *S. pyogenes* 07Z011, 07Z014, 07Z016 (erythromycin-resistant strain (Ery-R)), *Enterococcus faecalis* ATCC 29212, 07B116 were vancomycin-sensitivity strain (VSE), *E. faecalis* WHO14, ZB17 were vancomycin-resistant strain (VRE). All the MRSA and Ery-R strains chosen in this test were constitutively resistant strains supplied by the Ministry of Health National Antimicrobial Resistance Investigation Net (MOHNARIN, China). The in vitro antibacterial activity was reported as minimum inhibitory



Scheme 3. Reagents and conditions: (a) pyridine-3-acetic acid or 3,4-(methylenedioxy)phenylacetic acid, EDC·HCl, DMAP, CH₂Cl₂, rt; (b) DBU, acetone, rt; (c) NaH, CDI, DMF, –25 °C; (d) CH₃CN:H₂O/10:1, side chain, 50 °C; (e) MeOH, reflux.

Table 1
Antibacterial activity of ketolides **15a**, **15b** and acylides **19a–d**

	Pathogens	19a	19b	19c	19d	15a	15b	Teli	Azi	clar
ATCC 29213	MSSA	0.25	0.5	0.25	0.5	0.125	0.125	0.125	1	0.25
07H260	MRSA	>16	>16	>16	>16	>16	>16	>16	>256	>256
BK2464	MRSA (SCCmecII)	>16	>16	>16	>16	>16	>16	>16	>256	>256
ANS46	MRSA (SCCmecIII)	>16	>16	>16	>16	>16	>16	>16	>256	>256
07R066	MSSE	0.25	0.25	0.125	0.25	0.062	0.125	0.125	0.25	0.25
07G235	MRSE	16	>16	>16	16	>16	>16	16	>256	>256
07T202	MRSE	>16	>16	>16	>16	>16	>16	>16	>256	>256
07C068	MRSE	16	>16	>16	>16	>16	>16	8	>256	>256
ATCC 49619	<i>S. pneumoniae</i> (Ery-S)	0.062	0.125	0.125	0.125	0.016	0.062	0.031	0.125	0.031
07H252	<i>S. pneumoniae</i> (Ery-S)	0.031	0.016	0.016	0.016	0.016	0.016	0.016	0.031	0.016
07Z001	<i>S. pneumoniae</i> (Ery-R)	0.031	0.125	0.062	NA	0.016	NA	0.016	NA	128
05Z006	<i>S. pneumoniae</i> (Ery-R)	0.031	0.125	0.061	NA	0.016	NA	0.016	NA	128
07U084	<i>S. pyogenes</i> (Ery-S)	0.031	0.031	0.062	0.125	0.016	0.031	0.031	0.125	0.031
07U086	<i>S. pyogenes</i> (Ery-S)	0.062	0.062	0.062	0.25	0.031	0.031	0.25	0.062	1
07Z011	<i>S. pyogenes</i> (Ery-R)	0.125	0.5	0.5	NA	0.062	NA	0.062	NA	256
07Z014	<i>S. pyogenes</i> (Ery-R)	0.25	1	1	NA	0.062	NA	0.125	NA	256
07Z016	<i>S. pyogenes</i> (Ery-R)	0.25	1	0.5	NA	0.125	NA	0.125	NA	256
ATCC 29212	<i>Enterococcus faecalis</i> (VSE)	0.25	0.5	0.25	0.5	0.031	0.062	0.062	2	1
07B116	<i>Enterococcus faecalis</i> (VSE)	0.25	0.25	0.125	0.125	0.062	0.062	0.062	4	1
WHO14	<i>Enterococcus faecalis</i> (VRE, VanB)	4	8	16	16	16	16	4	128	16
ZB17	<i>Enterococcus faecalis</i> (VRE, VanB)	16	16	>16	16	>16	>16	8	>256	>256

concentrations (MICs), which was determined by the broth micro-dilution method as recommended by the NCCLS.¹⁶

The tabulated results exhibited that azithromycin and clarithromycin were potent against erythromycin-susceptible strains and inactive against all the resistant strains. Telithromycin was potent against both erythromycin-susceptible strains and the resistant strains.

Compounds **19a–d** showed improved activity against all the erythromycin-resistant strains, for example *S. pneumoniae* 07H252, 07Z001, 05Z006; *S. pyogenes* 07U011, 07Z014, 07Z016; the activity against erythromycin-susceptible strains of **19a–d** kept the same as telithromycin, azithromycin, clarithromycin.

The ketolides **15a** and **15b** proved to be very effective antimicrobial agents and both of them were highly activity against the erythromycin-resistant strains tested. To the erythromycin-susceptible strains, they also showed the potent antibacterial activity. Meanwhile, **15a** demonstrated slightly more active against erythromycin-susceptible strains and erythromycin-resistant strains comparable to telithromycin.

Compared **15a** with **15b**, the former showed more potent against both erythromycin-susceptible strains and erythromycin-resistant strains. The influence of chain length of the thio-contained linker was investigated with these two compounds. Generally, the ketolides **15a**, **15b** seemed to be more active than acylides **19a–d** against *S. pneumoniae* (Ery-R) and *S. pyogenes* (Ery-R).

All the compounds synthesized showed improved activity against methicillin-resistant staphylococcus aureus and methicillin-resistant staphylococcus epidermidis. These compounds exhibited similar activity against methicillin-susceptible strains as telithromycin, azithromycin and clarithromycin.

A novel series of ketolides and acylides were synthesized and evaluated for antibacterial activity against erythromycin-susceptible strains and erythromycin-resistant strains, as well as the activities against MSSA, MSSE and constitutively MRSA, MRSE strains. Using **15a**, **15b** as leading compounds, other novel derivatives could be worked out. For example, 5-methoxy group on the (3-((5-substituted-3H-imidazo [4,5-b]pyridin-2-yl)thio)alkyl) amine side chains could be replaced with different substitutive groups. Also, the sulfur atom may be oxidized to the corresponding sulfone that could alter the lipophilicity of the anchor group and affect the entire molecule while activity is retained. This study presents a considerable opportunity for the development of new macrolide antibiotics to combat the growing problem of MLS-resistance.

Acknowledgments

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Supplementary data

Supplementary data (¹H NMR, ¹³C NMR, HRMS data of compounds **15a**, **15b**, **19a–d** and experimental procedures of these compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.023.

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