

Synthesis and antibacterial activities of acylide derivatives bearing an aryl-tetrazolyl chain

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Abstract: Seventeen acylides bearing an aryl-tetrazolyl alkyl-substituted side chain were synthesized, starting from clarithromycin, via several reactions including hydrolysis, acetylating, esterification, carbamylation, and Michael addition. The structures of all new compounds were confirmed by ¹H nuclear magnetic resonance spectroscopy, ¹³C nuclear magnetic resonance spectroscopy, and mass spectrometry. All these synthesized acylides were evaluated for in vitro antimicrobial activities against gram-positive pathogens (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and gram-negative pathogens (*Pseudomonas aeruginosa*, *Escherichia coli*), using the broth microdilution method. Results showed that compounds 10e, 10f, 10g, 10h, 10o have good antibacterial activities.

Keywords: acylide, clarithromycin, synthesis, antibacterial activity

Introduction

Since the 1950s, macrolide antibiotics have been widely used for the clinical treatment of respiratory tract or soft tissue infections, but because of the extensive use of antibiotics, bacterial resistance has increased year by year.¹⁻⁴ Allen proved that cladinose is the key group for bacterial resistance, and deglycosylation or modification of its cladinose is a feasible way to change a drug's resistance.⁵ Modification at C-3 could enhance activity against efflux resistance. Tanikawa et al synthesized a series of clarithromycin derivatives in which hydrolysis and acylation occur at the C-3 position.⁶ It has been proven that compounds TEA-0769 and FMA-481 have better antibacterial activity than clarithromycin for macrolide-susceptible strains. They also have antibacterial activity against MLS_B-resistant *Staphylococcus aureus* and efflux-resistant *Streptococcus pneumoniae*. After ketolide, acylide is another class of erythromycin derivatives that has antibacterial activity against macrolide resistance strains and has the potential to become a new generation of macrolide antibiotics.

Tetrazole, a bioisostere of carboxyl group or a peptide bond,^{7,8} is an important pharmacophore widely used in drugs such as matrix metalloproteinase inhibitors,⁹ monoamine oxidase B inhibitors,¹⁰ and antibacterial, antifungal, and antiproliferative agents.¹¹ Water solubility can be improved by introducing tetrazole, because of its hydrophilicity, and its basicity will be also increased. Therefore, the introduction of tetrazole into acylide may not only enhance antibacterial activity but also improve its pharmacokinetic properties. On the basis of the previous study about ketolides, performed in our laboratory,¹² the cladinoyl of the C-3 position in macrolide was substituted by aroyl, and the carbamate ring was connected with some different alkyl side chains bearing aryl-tetrazolyl. As a consequence, 17 new acylide derivatives

were synthesized and evaluated for in vitro antimicrobial activities.

Materials and methods

Chemistry

Unless otherwise noted, all materials were commercially available and used without further purification. Dichloromethane and triethylamine were distilled from calcium hydride and sodium, respectively. The reactions were monitored by thin-layer chromatography (silica gel 60 GF254; Qingdao Haiyang Chemical Co, Ltd, Qingdao, Shandong, People's Republic of China) at 254 nm. ^1H nuclear magnetic resonance spectroscopy (NMR) and ^{13}C NMR spectra were recorded on Bruker-AV300MHz nuclear magnetic resonance spectrometers (Bruker Biosciences Corporation, Billerica, MA, USA). Chemical shifts are provided in parts per million downfield from tetramethylsilane (internal standard) with a coupling constant in hertz. Mass spectra and high-resolution mass spectrometry (HRMS) results were recorded on Thermo Finnigan LCQ Advantage MAX (Thermo Fisher Scientific, Waltham, MA, USA).

The compounds 2-(2H-tetrazol-5-yl) pyridine (3a), 3-(2H-tetrazol-5-yl) pyridine (3b), 4-(2H-tetrazol-5-yl) pyridine (3c), 5-phenyl-2H-tetrazole (3d), and 5-(thiophen-2-yl)-2H-tetrazole (3e) were prepared by a known procedure,¹³ and 1-methyl-1H-tetrazole-5-thiol (3f) was commercially available. The compounds 4a–4q and 5a–5q have been synthesized via a method we previously published.¹²

General procedure for preparation of compounds 4a–4q

To a solution of compounds 3a–3f (1 mmol) in anhydrous N, N-dimethyl formamide (5 mL) were added, successively, N-bromo-phthalimide (1 mmol) and anhydrous potassium carbonate (1 mmol), and the mixture was stirred for 10 hours at 80°C under nitrogen. The reaction mixture was quenched with ice water (10 mL). A crystalline solid was precipitated, and the filtered product was purified by silica-gel column chromatography (ether/ethyl acetate acetate =1:1 to petroleum ether/ethyl acetate/dichloromethane =1:1:1) to afford 4a–4q as white solid.

General procedure for the synthesis of compounds 5a–5q

To a solution of compounds 4a–4q (1 mmol) in the mixture of anhydrous ethanol (7 mL) and acetonitrile (5 mL) was added hydrazine monohydrate (2 mmol), and the mixture was then refluxed for 6 hours. After cooling, the mixture was

filtered and the filtrate was evaporated in vacuo. Aqueous NaOH (20 mmol) was subsequently added to the residue and then extracted with dichloromethane (10 mL three times), washed with a saturated brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The purification of the crude product by silica gel column chromatography (dichloromethane/methanol/triethylamine =9:1:0.1) afforded the desired compounds 5a–5q.

3-O-descladinosyl-10,11-anhydro-6-O-methyl-erythromycin A (compound 6)

To a solution of ethylene carbonate (51.6 mmol) and triethylamine (40 mL), clarithromycin (13.4 mmol) was added, and the reaction mixture was refluxed for 24 hours. Then additional ethylene carbonate (34.4 mmol) was added and the mixture was refluxed for 18 more hours. The solvent was removed under reduced pressure, and 23 mmol HCl aqueous solution, 40 mL ethanol, and 100 mL water were added. The reaction mixture was stirred for 24 hours at room temperature and then basified with NaOH aqueous solution (1 mol/L) to Ph 10–11. The precipitated, crude product was filtered off and recrystallized from the mixture of petroleum ether and acetone to afford compound **6**, a white solid (7.2 g, 94.1%). ^1H NMR (300 MHz, CDCl_3) δ 6.39 (s, 1H), 5.00 (d, J=9.1 Hz, 1H), 4.69 (d, J=5.0 Hz, 2H), 3.85 (s, 1H), 3.74 (t, J=9.3 Hz, 1H), 3.49 (dd, J=10.8, 5.5 Hz, 1H), 3.15 (dd, J=27.9, 12.8 Hz, 1H), 3.08 (s, 3H), 2.74–2.56 (m, 3H), 2.44 (s, 3H), 2.22 (s, 6H), 2.09 (s, 3H), 2.02 (s, 3H), 1.94 (dd, J=9.2, 4.9 Hz, 1H), 1.88–1.77 (m, 1H), 1.72 (dd, J=12.9, 2.6 Hz, 1H), 1.52 (dd, J=12.7, 6.6 Hz, 3H), 1.37 (s, 3H), 1.27 (d, J=3.9 Hz, 6H), 1.16 (d, J=6.3 Hz, 3H), 0.91 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 207.59, 176.68, 141.29, 138.77, 106.77, 91.96, 80.90, 79.17, 77.65, 77.22, 73.63, 70.50, 69.69, 65.58, 48.26, 44.33, 40.29, 38.32, 36.93, 36.40, 30.93, 21.34, 20.86, 20.37, 20.22, 16.13, 15.88, 12.94, 10.50, 7.62.

2'-Acetyl-3-O-descladinosyl-10,11-anhydro-6-O-methyl-erythromycin A (compound 7)

To a solution of acetic anhydride (16.8 mmol) and anhydrous triethylamine (16.8 mmol) in dichloromethane (30 mL), compound **6** (8.4 mmol) was added and stirred for 24 hours at room temperature. The reaction mixture was poured into saturated aqueous NaHCO_3 (30 mL) and extracted with CH_2Cl_2 (20 mL \times 3). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 , and filtered, and the solvent was removed under reduced pressure to yield white solid **7** (4.68 g, 90%). ^1H NMR (300 MHz, CDCl_3) δ 6.40 (s, 1H), 5.28 (s, 1H),

5.07–4.93 (m, 1H), 4.68 (dd, $J=9.5, 5.3$ Hz, 2H), 3.86 (d, $J=2.7$ Hz, 1H), 3.76 (d, $J=10.4$ Hz, 1H), 3.49 (dd, $J=9.4, 5.9$ Hz, 1H), 3.12 (d, $J=7.0$ Hz, 1H), 3.08 (s, 2H), 2.63 (dd, $J=10.4, 6.8$ Hz, 2H), 2.18 (d, $J=17.9$ Hz, 6H), 2.08 (s, 3H), 2.02 (s, 2H), 1.92 (dd, $J=14.7, 7.1$ Hz, 1H), 1.84–1.65 (m, 2H), 1.64–1.41 (m, 3H), 1.37 (d, $J=7.7$ Hz, 3H), 1.33–1.18 (m, 12H), 1.16 (d, $J=6.5$ Hz, 3H), 0.93–0.82 (m, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 207.80, 175.83, 170.25, 140.93, 139.43, 102.53, 79.97, 79.38, 77.15, 73.29, 71.80, 69.18, 64.09, 48.84, 44.26, 40.69, 37.69, 37.43, 37.18, 30.12, 29.69, 21.45, 21.29, 21.19, 20.66, 20.27, 17.00, 15.55, 13.51, 10.58, 7.94.

2'-Acetyl-3-O-descladinosyl-3-O-(3-pyridyl)acetyl-10, 11-anhydro-6-O-methyl-erythromycin A (compound 8)

Triethylamine (10.8 mmol) was dissolved in a solution of 3-pyridine acetic acid hydrochloride (10.8 mmol) in anhydrous dichloromethane (20 mL). The mixture was cooled to -15°C , and pivaloyl chloride (21.6 mmol) was added drop wise, with intensive stirring under nitrogen. After completion of dripping, the resulting reaction mixture was stirred for 1 hour at -15°C , followed by the drop-wise addition of a solution of compound 7 (3.2 mmol) in anhydrous dichloromethane (10 mL) over a period of 15 minutes. 4-Dimethylaminopyridine (0.32 mmol) was then added and stirred for 5 hours at room temperature. The reaction mixture was finally poured into saturated aqueous NaHCO_3 (30 mL) and extracted with dichloromethane. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The crude product was purified by column chromatography (petroleum ether/acetone/triethylamine =6:1:1 to 4:1:1) to afford the desired product 8 as white solid (1.6 g, 66.8%). NMR (300 MHz, CDCl_3) δ 8.55–8.44 (m, 2H), 7.66 (dt, $J=7.9, 1.9$ Hz, 1H), 7.32–7.21 (m, 2H), 6.55 (d, $J=0.9$ Hz, 1H), 5.58 (dd, $J=5.3, 1.8$ Hz, 1H), 5.05 (dd, $J=10.8, 2.5$ Hz, 1H), 4.66 (dd, $J=10.5, 7.5$ Hz, 1H), 4.17 (d, $J=7.4$ Hz, 1H), 3.65 (s, 2H), 3.43 (d, $J=7.6$ Hz, 1H), 3.31 (dd, $J=10.1, 6.2$ Hz, 1H), 3.03 (s, 2H), 2.86 (s, 3H), 2.66–2.55 (m, 3H), 2.23 (s, 6H), 2.10 (d, $J=3.9$ Hz, 1H), 2.04 (s, 3H), 1.92 (d, $J=0.6$ Hz, 3H), 1.83 (ddd, $J=14.1, 7.5, 2.6$ Hz, 1H), 1.69 (dd, $J=13.0, 2.5$ Hz, 1H), 1.47 (ddd, $J=14.1, 10.7, 7.3$ Hz, 2H), 1.34 (s, 3H), 1.23 (s, 3H), 1.20–1.17 (m, 6H), 0.99 (d, $J=7.1$ Hz, 3H), 0.94 (d, $J=7.0$ Hz, 3H), 0.88 (t, $J=7.4$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 206.94, 173.49, 172.39, 169.82, 150.25, 148.64, 139.73, 137.12, 129.28, 123.50, 101.78, 93.47, 82.62, 79.39, 77.23, 75.96, 73.99, 71.38, 70.43, 69.12, 63.12, 49.97, 42.41, 40.81, 40.42, 40.16,

38.37, 31.78, 30.50, 27.31, 23.09, 22.17, 21.39, 21.17, 20.99, 20.41, 19.72, 13.52, 12.76, 10.70, 10.14.

2'-Acetyl-3-O-descladinosyl-3-O-(3-pyridyl)acetyl-10, 11-anhydro-11-deoxy-12-O-(1H-1-imidazolylcarbonyl)-6-O-methyl-erythromycin A (compound 9)

Compound 8 (1.4 mmol), carbonyl diimidazole (5.6 mmol), and 4-dimethylamino pyridine (0.14 mmol) were dissolved in anhydrous dichloromethane (20 mL). The solution was stirred for 48 hours at room temperature. The reaction mixture was quenched with saturated aqueous NaHCO_3 (20 mL) and extracted with dichloromethane. The organic layer was washed with saturated brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was recrystallized from the mixture of petroleum ether and acetone (4:1) to afford white solid 9 (0.82 g, 80%). ^1H NMR (300 MHz, CDCl_3) δ 8.60–8.53 (m, 2H), 8.06 (s, 1H), 7.77–7.70 (m, 1H), 7.38–7.27 (m, 2H), 7.05 (dd, $J=1.6, 0.8$ Hz, 1H), 6.64 (s, 1H), 5.84 (dd, $J=10.2, 3.0$ Hz, 1H), 5.10 (d, $J=10.3$ Hz, 1H), 4.65 (dd, $J=10.5, 7.4$ Hz, 1H), 3.90 (s, 1H), 3.70 (s, 2H), 3.60 (d, $J=4.8$ Hz, 1H), 3.11 (s, 3H), 3.08–3.02 (m, 1H), 2.86 (d, $J=5.9$ Hz, 1H), 2.65–2.52 (m, 1H), 2.24 (s, 6H), 2.15 (s, 1H), 2.04 (s, 3H), 1.85 (s, 3H), 1.77 (s, 3H), 1.70–1.61 (m, 2H), 1.35 (dd, $J=9.8, 4.8$ Hz, 2H), 1.23 (s, 3H), 1.20 (d, $J=4.1$ Hz, 3H), 1.13 (dd, $J=6.1, 3.9$ Hz, 6H), 1.01 (d, $J=6.7$ Hz, 3H), 0.89 (d, $J=1.5$ Hz, 3H), 0.86 (d, $J=5.3$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 170.19, 169.80, 150.24, 148.85, 145.84, 137.63, 137.08, 130.82, 129.34, 123.60, 117.13, 100.99, 84.37, 78.35, 75.82, 71.16, 69.13, 63.21, 50.60, 42.90, 40.52, 38.50, 30.28, 29.69, 27.42, 21.44, 20.89, 19.92, 15.11, 13.57, 10.26.

General procedure for the synthesis of compounds 10a–10q

To a solution of compound 9 (0.30 mmol) in acetonitrile (1 mL), compounds 5a–5q (0.60 mmol) were added, and the resulting mixture was stirred for 16–25 hours at 55°C . After cooling, 5% KH_2PO_4 (2 mL) was added. The mixture was extracted with ethyl acetate ester. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was dissolved in methanol (1 mL), and the solution was refluxed for 25 hours. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (petroleum ether/acetone/triethylamine =5:1:1) to afford compounds 10a–10q as white foam.

Characterization of compounds 10a–10q, of which some solid or powder compounds like 10c, 10d, 10g, 10k, 10n, 10o, and 10q give melting point data
3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(3-[5-(pyridin-2-yl)-1H-tetrazol-1-yl])propyl]imino) erythromycin A (compound 10a)

Yield 53.7%. ¹H NMR (300 MHz, CDCl₃) δ 8.69 (dd, J=4.8, 0.7 Hz, 1H), 8.55–8.42 (m, 2H), 8.27 (d, J=7.9 Hz, 1H), 7.82 (td, J=7.8, 1.7 Hz, 1H), 7.70 (d, J=7.9 Hz, 1H), 7.37 (ddd, J=7.6, 4.9, 1.0 Hz, 1H), 7.25 (dd, J=7.9, 4.7 Hz, 1H), 5.26 (s, 3H), 5.10–4.97 (m, 2H), 4.96–4.88 (m, 2H), 3.81 (d, J=7.2 Hz, 1H), 3.67–3.61 (m, 3H), 3.13 (dd, J=10.1, 7.2 Hz, 1H), 3.05–2.94 (m, 2H), 2.85 (s, 3H), 2.82–2.74 (m, 1H), 2.41 (ddd, J=20.6, 12.6, 5.9 Hz, 3H), 2.28 (s, 6H), 2.10 (s, 3H), 1.90–1.76 (m, 1H), 1.58–1.47 (m, 3H), 1.33 (s, 3H), 1.19 (d, J=5.2 Hz, 3H), 1.12 (s, 6H), 1.08 (s, 3H), 1.07 (d, J=6.4 Hz, 4H), 1.03 (d, J=7.1 Hz, 3H), 0.93 (d, J=6.8 Hz, 3H), 0.83 (d, J=6.7 Hz, 3H), 0.71 (t, J=7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 215.55, 174.06, 170.49, 157.22, 151.75, 150.06, 149.55, 148.51, 144.89, 137.37, 129.52, 125.22, 124.46, 123.59, 103.68, 82.84, 81.07, 78.34, 77.58, 77.16, 76.74, 76.38, 70.12, 69.22, 65.80, 60.73, 49.97, 47.62, 45.47, 42.87, 41.54, 39.97, 38.71, 38.32, 36.32, 30.93, 29.64, 28.51, 27.57, 21.94, 20.98, 19.52, 18.74, 15.01, 14.15, 10.11, 8.87. HRMS (ESI) m/z calcd for C₄₇H₆₈N₈O₁₁ [M + H]⁺: 921.5080, found: 921.5081.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(3-[5-(pyridin-2-yl)-2H-tetrazol-2-yl])propyl]imino) erythromycin A (compound 10b)

Yield 54.6%. ¹H NMR (300 MHz, CDCl₃) δ 8.70 (d, J=4.2 Hz, 1H), 8.53–8.41 (m, 2H), 8.17 (d, J=7.9 Hz, 1H), 7.77 (td, J=7.8, 1.7 Hz, 1H), 7.70 (d, J=7.9 Hz, 1H), 7.36–7.21 (m, 2H), 5.48 (s, 3H), 5.04–4.87 (m, 2H), 4.84–4.67 (m, 2H), 3.81 (d, J=7.1 Hz, 1H), 3.66 (dd, J=8.0, 3.9 Hz, 3H), 3.14 (dd, J=9.7, 6.9 Hz, 1H), 3.06–2.92 (m, 2H), 2.91 (s, 3H), 2.48–2.38 (m, 3H), 2.29 (s, 6H), 2.09 (s, 3H), 1.86 (dd, J=11.1, 6.2 Hz, 1H), 1.56–1.46 (m, 3H), 1.35 (s, 3H), 1.17 (d, J=2.9 Hz, 3H), 1.12 (s, 6H), 1.08 (d, J=5.3 Hz, 3H), 1.02 (s, 3H), 0.94 (d, J=6.7 Hz, 3H), 0.85 (d, J=6.7 Hz, 3H), 0.77 (t, J=7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 215.68, 174.15, 170.52, 164.66, 157.28, 150.08, 148.44, 146.85, 137.47, 137.05, 129.55, 124.69, 123.60, 122.46, 103.65, 82.90, 81.07, 78.34, 76.53, 70.08, 69.12, 65.79, 60.56, 51.20, 50.18, 45.47, 42.88, 41.36, 39.91, 38.73, 38.29, 36.37, 30.92, 29.64, 28.58, 27.52, 27.04, 21.96, 20.95,

19.44, 18.75, 15.02, 14.19, 10.15, 8.89. HRMS (ESI) m/z calcd for C₄₇H₆₈N₈O₁₁ [M + H]⁺: 921.5080, found: 921.5083.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(3-[5-(pyridin-3-yl)-2H-tetrazol-2-yl])propyl]imino) erythromycin A (compound 10c)

Yield 55.8%. melting point (Mp) = 173°C–175°C; ¹H NMR (300 MHz, CDCl₃) δ 9.33 (s, 1H), 8.57 (d, J=40.9 Hz, 3H), 8.39 (d, J=7.9 Hz, 1H), 7.69 (d, J=7.7 Hz, 1H), 7.43–7.32 (m, 1H), 7.26 (s, 1H), 4.98 (t, J=11.6 Hz, 2H), 4.76 (dd, J=10.1, 6.8 Hz, 2H), 3.82 (d, J=7.0 Hz, 1H), 3.67 (d, J=4.0 Hz, 4H), 3.13–2.98 (m, 3H), 2.91 (s, 3H), 2.85 (dd, J=11.1, 6.8 Hz, 1H), 2.49–2.36 (m, 3H), 2.23 (s, 6H), 2.13–2.05 (m, 2H), 1.90 (dd, J=13.6, 6.9 Hz, 1H), 1.67–1.60 (m, 1H), 1.58–1.46 (m, 4H), 1.38 (s, 3H), 1.20 (d, J=4.7 Hz, 5H), 1.09 (dd, J=12.4, 6.6 Hz, 10H), 0.98 (d, J=6.7 Hz, 3H), 0.88 (d, J=6.6 Hz, 3H), 0.79 (t, J=7.2 Hz, 3H). HRMS (ESI) m/z calcd for C₄₇H₆₈N₈O₁₁ [M + H]⁺: 921.5080, found: 921.5082.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(3-[5-(pyridin-4-yl)-2H-tetrazol-2-yl])propyl]imino) erythromycin A (compound 10d)

Yield 47.9%. Mp = 170°C–172°C; ¹H NMR (300 MHz, CDCl₃) δ 9.34 (s, 1H), 8.66 (s, 1H), 8.52 (s, 2H), 8.40 (d, J=7.9 Hz, 1H), 7.70 (d, J=7.9 Hz, 1H), 7.38 (dd, J=7.8, 4.8 Hz, 1H), 7.29 (s, 1H), 4.99 (t, J=11.3 Hz, 2H), 4.84–4.68 (m, 2H), 3.83 (d, J=7.0 Hz, 2H), 3.68 (d, J=3.9 Hz, 3H), 3.12–3.03 (m, 2H), 2.92 (s, 3H), 2.88–2.82 (m, 1H), 2.47–2.38 (m, 2H), 2.25 (s, 6H), 2.13 (s, 2H), 1.95–1.86 (m, 1H), 1.56 (d, J=7.0 Hz, 2H), 1.39 (s, 3H), 1.20 (s, 3H), 1.14–1.09 (m, 6H), 1.06 (s, 1H), 0.99 (d, J=6.7 Hz, 3H), 0.89 (d, J=6.6 Hz, 3H), 0.80 (t, J=7.2 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 215.68, 174.25, 170.43, 162.77, 157.32, 151.00, 150.55, 150.29, 148.84, 148.13, 137.07, 134.22, 123.64, 103.78, 82.92, 80.91, 78.42, 78.35, 70.28, 69.63, 65.96, 60.56, 51.14, 50.06, 45.54, 42.93, 41.32, 40.29, 38.76, 38.45, 38.38, 36.37, 30.92, 28.17, 27.13, 22.01, 19.44, 18.78, 21.05, 14.22, 14.16, 10.20, 8.85. HRMS (ESI) m/z calcd for C₄₇H₆₈N₈O₁₁ [M + H]⁺: 921.5080, found: 921.5083.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(4-[5-(pyridin-2-yl)-1H-tetrazol-1-yl])butyl]imino) erythromycin A (compound 10e)

Yield of 52.3%. ¹³C NMR (75 MHz, CDCl₃) δ 215.74, 182.80, 176.89, 174.13, 170.75, 157.31, 151.58, 149.73, 148.14, 144.82, 137.83, 137.32, 129.84, 125.24, 124.39, 123.69,

103.32, 82.75, 81.21, 78.34, 76.47, 69.65, 68.41, 65.82, 60.29, 49.89, 49.18, 45.50, 44.93, 42.88, 39.47, 38.66, 38.14, 36.37, 29.37, 27.52, 22.65, 20.80, 19.48, 18.73, 15.05, 14.12, 10.15, 8.86, 8.41. HRMS (ESI) m/z calcd for $C_{48}H_{70}N_8O_{11}$ $[M + H]^+$: 935.5237, found: 935.5232.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(4-[5-(pyridin-2-yl)-2H-tetrazol-2-yl])butyl]imino) erythromycin A (compound 10f)

Yield 55.4%. 1H NMR (300 MHz, $CDCl_3$) δ 8.72 (d, $J=4.0$ Hz, 1H), 8.57 (s, 1H), 8.51 (d, $J=3.6$ Hz, 1H), 8.18 (d, $J=7.9$ Hz, 1H), 7.79 (ddd, $J=10.1, 5.9, 2.0$ Hz, 2H), 7.39–7.28 (m, 2H), 6.27 (s, 3H), 5.06–4.86 (m, 2H), 4.73 (t, $J=7.1$ Hz, 2H), 3.86 (d, $J=7.1$ Hz, 1H), 3.75 (d, $J=2.3$ Hz, 1H), 3.70–3.62 (m, 3H), 3.28–3.15 (m, 1H), 3.05 (dd, $J=14.9, 7.3$ Hz, 2H), 2.95 (s, 2H), 2.89–2.73 (m, 2H), 2.45 (s, 3H), 2.18–2.07 (m, 3H), 1.95–1.82 (m, 1H), 1.77–1.45 (m, 6H), 1.37 (s, 3H), 1.22 (d, $J=5.7$ Hz, 3H), 1.16 (s, 9H), 1.08 (t, $J=6.8$ Hz, 6H), 0.98 (d, $J=6.7$ Hz, 3H), 0.86 (d, $J=6.8$ Hz, 3H), 0.77 (t, $J=7.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.79, 183.82, 174.10, 170.63, 164.60, 157.42, 150.17, 149.74, 148.07, 146.84, 137.96, 137.09, 124.71, 123.74, 122.42, 103.47, 82.80, 81.38, 78.48, 78.42, 69.87, 68.62, 65.81, 60.36, 53.01, 50.08, 45.53, 42.89, 39.61, 38.72, 38.07, 36.37, 29.66, 27.35, 26.93, 24.13, 21.98, 20.87, 19.53, 18.81, 15.02, 14.21, 10.22, 8.93. HRMS (ESI) m/z calcd for $C_{48}H_{70}N_8O_{11}$ $[M + H]^+$: 935.5237, found: 935.5231.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(4-[5-(pyridin-3-yl)-2H-tetrazol-2-yl])butyl]imino) erythromycin A (compound 10g)

Yield 51.2%. Mp = 133°C–135°C; 1H NMR (300 MHz, $CDCl_3$) δ 9.34 (d, $J=1.5$ Hz, 1H), 8.67 (dd, $J=4.9, 1.6$ Hz, 1H), 8.62–8.49 (m, 2H), 8.41 (dt, $J=8.0, 1.9$ Hz, 1H), 7.76 (d, $J=7.9$ Hz, 1H), 7.40 (dd, $J=8.0, 4.8$ Hz, 1H), 7.31 (dd, $J=7.7, 4.8$ Hz, 1H), 4.97 (dd, $J=10.0, 3.7$ Hz, 2H), 4.75 (dd, $J=14.0, 6.3$ Hz, 3H), 3.85 (d, $J=7.2$ Hz, 1H), 3.80–3.65 (m, 6H), 3.48 (s, 3H), 3.19 (dd, $J=9.9, 7.3$ Hz, 1H), 3.06 (d, $J=6.8$ Hz, 2H), 2.99 (s, 3H), 2.87 (dd, $J=11.1, 6.9$ Hz, 1H), 2.65–2.41 (m, 2H), 2.37 (s, 6H), 2.12 (dd, $J=14.7, 7.3$ Hz, 3H), 1.88 (s, 4H), 1.73 (d, $J=7.6$ Hz, 2H), 1.65–1.55 (m, 3H), 1.40 (s, 3H), 1.26 (d, $J=6.3$ Hz, 3H), 1.14 (d, $J=2.3$ Hz, 3H), 1.09 (s, 3H), 1.01 (d, $J=6.8$ Hz, 3H), 0.87 (d, $J=6.8$ Hz, 3H), 0.81 (t, $J=7.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.82, 174.13, 170.46, 162.60, 157.40, 150.92, 150.25, 148.72, 147.96, 137.22, 134.15, 129.40, 123.83, 123.68, 123.54,

103.57, 82.77, 80.86, 78.38, 78.33, 76.57, 70.16, 69.36, 65.90, 60.29, 52.91, 50.04, 45.56, 42.77, 40.21, 38.75, 38.31, 36.30, 28.47, 26.98, 24.14, 21.95, 20.99, 19.53, 18.79, 14.93, 14.20, 10.23, 8.86. HRMS (ESI) m/z calcd for $C_{48}H_{70}N_8O_{11}$ $[M + H]^+$: 935.5237, found: 935.5234.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(4-[5-(pyridin-4-yl)-2H-tetrazol-2-yl])butyl]imino) erythromycin A (compound 10h)

Yield 47.9%. 1H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, $J=5.5$ Hz, 2H), 8.60–8.37 (m, 2H), 7.97 (d, $J=6.0$ Hz, 2H), 7.73 (d, $J=7.9$ Hz, 1H), 7.36–7.21 (m, 1H), 4.91 (d, $J=11.0$ Hz, 2H), 4.70 (t, $J=7.1$ Hz, 2H), 3.83 (d, $J=7.1$ Hz, 1H), 3.77–3.57 (m, 6H), 3.15 (dd, $J=10.0, 7.3$ Hz, 1H), 3.06–3.00 (m, 1H), 2.93 (d, $J=4.3$ Hz, 3H), 2.82 (dd, $J=11.1, 6.8$ Hz, 1H), 2.55–2.39 (m, 2H), 2.29 (s, 3H), 2.10 (s, 3H), 2.05 (dd, $J=9.0, 5.7$ Hz, 3H), 1.94–1.78 (m, 1H), 1.70 (dd, $J=15.6, 7.7$ Hz, 2H), 1.58 (d, $J=14.9$ Hz, 3H), 1.36 (s, 3H), 1.22 (s, 3H), 1.19 (s, 1H), 1.14 (s, 6H), 1.07 (dd, $J=8.3, 5.4$ Hz, 6H), 0.96 (d, $J=6.7$ Hz, 3H), 0.81–0.78 (m, 3H), 0.73 (d, $J=7.2$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.84, 174.12, 170.51, 162.78, 157.37, 150.11, 148.44, 137.37, 135.13, 129.53, 123.59, 120.87, 103.66, 82.72, 80.96, 78.3, 76.48, 70.08, 69.14, 65.74, 60.23, 53.01, 49.99, 45.54, 44.81, 42.72, 39.86, 38.52, 38.18, 36.31, 30.90, 30.10, 29.62, 28.51, 27.52, 26.93, 24.10, 22.62, 21.91, 20.96, 19.50, 18.76, 14.87, 14.16, 10.21, 8.77. HRMS (ESI) m/z calcd for $C_{48}H_{70}N_8O_{11}$ $[M+H]^+$: 935.5237, found: 935.5236.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(5-[5-(pyridin-2-yl)-1H-tetrazol-1-yl])pentyl]imino) erythromycin A (compound 10i)

Yield 47.8%. 1H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, $J=4.8$ Hz, 1H), 8.55–8.45 (m, 2H), 8.28 (d, $J=7.9$ Hz, 1H), 7.84 (td, $J=7.8, 1.7$ Hz, 1H), 7.69 (d, $J=7.9$ Hz, 1H), 7.38 (ddd, $J=7.6, 4.9, 1.0$ Hz, 1H), 7.25 (dd, $J=8.0, 4.7$ Hz, 1H), 4.99–4.88 (m, 4H), 3.83 (d, $J=7.2$ Hz, 1H), 3.69 (d, $J=2.4$ Hz, 3H), 3.63 (s, 1H), 3.59–3.50 (m, 2H), 3.07 (ddd, $J=14.4, 8.7, 4.6$ Hz, 3H), 2.93 (s, 3H), 2.84 (dd, $J=11.1, 6.7$ Hz, 1H), 2.24 (s, 6H), 2.12 (s, 6H), 2.03–1.91 (m, 2H), 1.64 (d, $J=9.2$ Hz, 2H), 1.55 (dd, $J=12.9, 4.4$ Hz, 3H), 1.35 (d, $J=4.0$ Hz, 3H), 1.23 (s, 3H), 1.20–1.13 (m, 3H), 1.13–1.08 (m, 6H), 1.06 (d, $J=7.1$ Hz, 3H), 0.96 (d, $J=6.8$ Hz, 3H), 0.86 (d, $J=6.7$ Hz, 3H), 0.74 (t, $J=7.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.73, 207.12, 174.08, 170.47, 157.29, 151.62, 150.26, 149.60, 148.78, 144.89, 137.36, 137.13,

129.33, 125.24, 124.45, 123.52, 103.71, 82.62, 80.94, 78.39, 76.55, 70.25, 69.57, 65.91, 60.37, 50.00, 49.61, 21.99, 21.05, 19.55, 18.82, 15.00, 14.25, 14.19, 10.24, 8.87, 8.83. HRMS (ESI) m/z calcd for $C_{49}H_{72}N_8O_{11}$ $[M + H]^+$: 949.5393, found: 949.5395.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(5-[5-(pyridin-2-yl)-2H-tetrazol-2-yl]pentyl)imino])erythromycin A (compound 10j)

Yield 62.7%. 1H NMR (300 MHz, $CDCl_3$) δ 8.76 (d, $J=4.1$ Hz, 1H), 8.58 (s, 1H), 8.56–8.47 (m, 1H), 8.22 (d, $J=7.9$ Hz, 1H), 7.90–7.74 (m, 2H), 7.41–7.28 (m, 2H), 4.99 (d, $J=11.1$ Hz, 2H), 4.68 (t, $J=7.2$ Hz, 2H), 3.88 (d, $J=7.1$ Hz, 1H), 3.80–3.69 (m, 3H), 3.66 (s, 1H), 3.65–3.52 (m, 2H), 3.25 (dd, $J=10.0, 7.2$ Hz, 1H), 3.02 (dd, $J=14.1, 6.8$ Hz, 6H), 2.91–2.81 (m, 1H), 2.77–2.64 (m, 1H), 2.58–2.48 (m, 1H), 2.42 (s, 3H), 2.21 (t, $J=5.9$ Hz, 3H), 2.17–2.11 (m, 3H), 1.70 (dd, $J=18.7, 11.9$ Hz, 3H), 1.39 (s, 3H), 1.26 (d, $J=1.7$ Hz, 3H), 1.24 (s, 3H), 1.21 (s, 3H), 1.18–1.17 (m, 3H), 1.12–1.07 (m, 6H), 0.99 (d, $J=6.8$ Hz, 3H), 0.92 (d, $J=6.7$ Hz, 3H), 0.79 (t, $J=7.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.90, 184.13, 174.19, 170.71, 164.70, 157.45, 150.30, 149.83, 148.17, 146.96, 138.02, 137.19, 129.95, 128.38, 127.94, 125.88, 124.81, 123.81, 122.51, 103.66, 82.79, 81.49, 78.64, 78.55, 77.37, 70.08, 68.85, 65.85, 60.52, 53.49, 50.18, 45.65, 45.06, 43.43, 43.02, 40.73–40.56 (m), 39.71, 38.72, 38.51, 38.19, 36.45, 31.99, 31.50, 30.25, 29.77, 29.09, 27.55, 26.42–26.15 (m), 23.96, 22.76, 22.11, 20.99, 19.64, 18.93, 15.14, 14.49–14.07 (m), 10.35, 9.03, 8.63. HRMS (ESI) m/z calcd for $C_{49}H_{72}N_8O_{11}$ $[M + H]^+$: 949.5393, found: 949.5389.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(5-[5-(pyridin-3-yl)-2H-tetrazol-2-yl]pentyl)imino])erythromycin A (compound 10k)

Yield 56.3%. Mp =139°C–141°C; 1H NMR (300 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.63 (d, $J=33.2$ Hz, 2H), 8.55–8.34 (m, 2H), 7.77 (d, $J=7.8$ Hz, 2H), 7.45–7.40 (m, 1H), 7.37–7.27 (m, 1H), 5.05 (d, $J=37.1$ Hz, 3H), 4.97 (d, $J=11.0$ Hz, 2H), 4.65 (t, $J=7.0$ Hz, 2H), 3.88 (d, $J=7.0$ Hz, 1H), 3.84–3.69 (m, 3H), 3.65–3.54 (m, 3H), 3.39–3.20 (m, 1H), 3.07 (dd, $J=14.5, 7.2$ Hz, 6H), 2.97 (s, 3H), 2.85 (d, $J=11.0$ Hz, 2H), 2.49 (s, 6H), 2.26–2.03 (m, 3H), 1.88 (dd, $J=13.4, 7.2$ Hz, 1H), 1.68 (s, 3H), 1.57 (d, $J=8.3$ Hz, 2H), 1.38–1.32 (m, 6H), 1.23 (s, 3H), 1.17 (s, 6H), 1.10 (s, 3H), 0.98 (d, $J=6.4$ Hz, 3H), 0.91 (d, $J=6.4$ Hz, 3H), 0.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.29, 183.33, 173.95, 170.73, 162.55, 157.35,

150.69, 149.77, 148.13, 147.71, 137.95, 134.47, 123.84, 103.36, 82.70, 78.41, 69.87, 68.60, 65.91, 60.39, 53.33, 50.08, 45.61, 42.91, 39.78, 38.72, 38.21, 29.32, 28.97, 27.33, 26.43, 23.82, 20.87, 19.56, 18.84, 15.08, 14.25, 10.27, 8.95, 8.61. HRMS (ESI) m/z calcd for $C_{49}H_{72}N_8O_{11}$ $[M + H]^+$: 949.5393, found: 949.5392.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(5-[5-(pyridin-4-yl)-2H-tetrazol-2-yl]pentyl)imino])erythromycin A (compound 10l)

Yield 54.2%. 1H NMR (300 MHz, $CDCl_3$) δ 8.71 (dd, $J=4.6, 1.5$ Hz, 2H), 8.54 (d, $J=1.7$ Hz, 1H), 8.49 (dd, $J=4.8, 1.4$ Hz, 1H), 7.99 (dd, $J=4.6, 1.5$ Hz, 2H), 7.74 (d, $J=7.9$ Hz, 1H), 7.28 (d, $J=2.9$ Hz, 1H), 4.95 (d, $J=11.1$ Hz, 2H), 4.64 (t, $J=7.1$ Hz, 2H), 3.85 (d, $J=7.1$ Hz, 1H), 3.73 (s, 2H), 3.66 (dd, $J=11.6, 7.4$ Hz, 2H), 3.56 (dd, $J=9.2, 6.4$ Hz, 2H), 3.22 (dd, $J=10.1, 7.2$ Hz, 1H), 3.01 (q, $J=7.3$ Hz, 5H), 2.95 (s, 3H), 2.39 (s, 3H), 2.15–2.03 (m, 3H), 1.92–1.80 (m, 1H), 1.60 (s, 3H), 1.51 (d, $J=12.7$ Hz, 3H), 1.35 (s, 3H), 1.26 (d, $J=7.3$ Hz, 3H), 1.21 (d, $J=4.0$ Hz, 3H), 1.14 (s, 6H), 1.09–1.04 (m, 6H), 0.95 (d, $J=6.8$ Hz, 3H), 0.87 (d, $J=6.7$ Hz, 3H), 0.74 (t, $J=7.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.91, 183.67, 174.21, 170.72, 162.77, 157.34, 150.07, 149.79, 148.17, 137.82, 135.27, 129.76, 123.70, 120.98, 103.53, 82.70, 81.21, 78.37, 69.92, 68.75, 65.76, 60.35, 53.40, 50.04, 45.56, 45.17, 43.26, 42.87, 39.65, 38.72, 38.22, 36.35, 28.95, 27.48, 26.38, 23.75, 21.98, 20.90, 19.53, 18.82, 15.03, 14.21, 10.25, 8.92, 8.56. HRMS (ESI) m/z calcd for $C_{49}H_{72}N_8O_{11}$ $[M + H]^+$: 949.5393, found: 949.5392.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(4-[5-phenyl-2H-tetrazol-2-yl]pentyl)imino])erythromycin A (compound 10m)

Yield 56.4%. 1H NMR (300 MHz, $CDCl_3$) δ 8.60–8.47 (m, 2H), 8.18–8.08 (m, 2H), 7.75 (d, $J=8.0$ Hz, 1H), 7.46 (d, $J=6.6$ Hz, 3H), 7.30 (dd, $J=7.8, 4.9$ Hz, 1H), 5.01 (d, $J=11.0$ Hz, 2H), 4.65 (t, $J=7.2$ Hz, 2H), 3.98–3.81 (m, 2H), 3.74 (s, 3H), 3.66–3.54 (m, 2H), 3.26 (ddd, $J=24.7, 16.2, 7.2$ Hz, 3H), 3.01 (s, 3H), 2.91–2.84 (m, 3H), 2.60–2.45 (m, 2H), 2.37 (s, 3H), 2.26 (d, $J=5.0$ Hz, 1H), 2.18–2.10 (m, 3H), 1.96–1.78 (m, 2H), 1.72 (d, $J=7.1$ Hz, 2H), 1.67 (s, 1H), 1.60 (d, $J=10.3$ Hz, 2H), 1.46 (s, 1H), 1.40 (s, 3H), 1.27 (d, $J=2.7$ Hz, 3H), 1.20 (s, 3H), 1.18 (s, 3H), 1.15 (d, $J=2.8$ Hz, 3H), 1.09 (s, 3H), 1.01 (d, $J=6.7$ Hz, 3H), 0.93 (d, $J=6.8$ Hz, 3H), 0.80 (t, $J=7.2$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.77, 174.13, 170.56, 166.98, 157.33, 150.23, 148.72,

137.35, 130.14, 129.49, 126.85, 123.61, 102.62, 82.67, 81.20, 78.36, 70.12, 69.04, 66.06, 60.42, 53.05, 50.11, 45.60, 43.37, 40.21, 38.82, 36.32, 31.44, 30.17, 29.04, 27.52, 26.50, 23.88, 22.03, 21.01, 19.58, 18.86, 15.08, 14.30, 10.28, 8.95. HRMS (ESI) m/z calcd for $C_{50}H_{73}N_7O_{11}$ [M + H]⁺: 948.5441, found: 948.5444.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(3-[5-(thiophen-2-yl)-2H-tetrazol-2-yl])pentyl]imino) erythromycin A (compound 10n)

Yield 60.6%. Mp =99°C–101°C; ¹H NMR (300 MHz, CDCl₃) δ 8.51 (dd, J=4.9, 1.4 Hz, 2H), 7.76 (dd, J=3.6, 1.2 Hz, 1H), 7.70 (dt, J=7.8, 1.8 Hz, 1H), 7.40 (dd, J=5.0, 1.2 Hz, 1H), 7.27 (dd, J=7.6, 4.7 Hz, 1H), 7.10 (dd, J=5.0, 3.7 Hz, 1H), 5.06–4.91 (m, 2H), 4.59 (t, J=7.2 Hz, 2H), 3.85 (d, J=7.2 Hz, 1H), 3.74–3.68 (m, 3H), 3.66 (s, 1H), 3.58 (dd, J=8.4, 5.9 Hz, 2H), 3.19–3.01 (m, 3H), 2.98 (d, J=6.2 Hz, 3H), 2.93–2.80 (m, 2H), 2.68–2.59 (m, 1H), 2.23 (d, J=4.4 Hz, 6H), 2.16–2.05 (m, 3H), 1.78–1.66 (m, 2H), 1.57 (t, J=10.9 Hz, 3H), 1.38 (s, 3H), 1.26 (s, 3H), 1.16–1.04 (m, 12H), 0.98 (d, J=6.8 Hz, 3H), 0.90 (d, J=6.8 Hz, 3H), 0.80 (ddd, J=15.0, 5.9, 2.6 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 215.75, 174.10, 170.43, 161.04, 157.32, 150.27, 148.80, 137.11, 129.27, 127.76, 123.52, 103.79, 82.67, 81.01, 78.43, 70.32, 69.66, 65.92, 60.42, 53.12, 50.09, 45.84, 45.59, 43.34, 42.94, 40.29, 38.6, 36.28, 28.97, 28.11, 26.45, 23.82, 22.02, 21.07, 19.56, 18.84, 15.01, 14.24, 10.26, 8.89. HRMS (ESI) m/z calcd for $C_{48}H_{71}N_7O_{11}S$ [M + H]⁺: 954.5005, found: 954.5003.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(3-[4-(1-methyl-1H-tetrazol-5-thio)]propyl]imino) erythromycin A (compound 10o)

Yield 53.2%. Mp =202°C–204°C; ¹H NMR (300 MHz, CDCl₃) δ 8.52 (dd, J=4.9, 1.4 Hz, 2H), 7.78–7.65 (m, 1H), 7.32–7.26 (m, 1H), 4.96 (dd, J=13.5, 6.5 Hz, 2H), 3.90 (s, 3H), 3.85 (d, J=7.2 Hz, 1H), 3.75 (t, J=7.2 Hz, 2H), 3.71 (d, J=1.9 Hz, 3H), 3.65 (s, 1H), 3.51–3.29 (m, 3H), 3.18–3.10 (m, 1H), 3.05 (d, J=7.0 Hz, 2H), 2.99 (s, 3H), 2.86 (dd, J=11.1, 6.7 Hz, 1H), 2.51 (dd, J=9.1, 5.4 Hz, 1H), 2.28 (s, 6H), 2.17–2.04 (m, 3H), 1.96–1.82 (m, 1H), 1.64–1.52 (m, 3H), 1.38 (s, 3H), 1.26 (dd, J=13.7, 10.6 Hz, 6H), 1.10 (dd, J=10.2, 6.6 Hz, 9H), 0.98 (d, J=6.8 Hz, 3H), 0.90 (d, J=6.7 Hz, 3H), 0.79 (t, J=7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 215.71, 174.17, 170.51, 157.37, 154.17, 150.31, 148.86, 137.13, 129.31, 123.55, 103.73, 82.83, 80.94, 78.40, 76.58, 70.24,

69.58, 65.97, 60.41, 50.23, 45.58, 42.92, 42.29, 40.31, 38.79, 38.40, 36.32, 33.43, 31.07, 28.24, 26.80, 22.00, 21.06, 19.58, 18.85, 15.05, 14.25, 10.22, 8.88. HRMS (ESI) m/z calcd for $C_{43}H_{67}N_7O_{11}S$ [M + H]⁺: 890.4692, found: 890.4691.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(4-[4-(1-methyl-1H-tetrazol-5-thio)]butyl]imino) erythromycin A (compound 10p)

Yield 55.9%. ¹H NMR (300 MHz, CDCl₃) δ 8.53 (d, J=6.0 Hz, 2H), 7.74 (d, J=7.9 Hz, 1H), 7.29 (dd, J=7.8, 4.9 Hz, 1H), 4.97 (t, J=10.2 Hz, 2H), 3.88 (s, 3H), 3.79–3.69 (m, 3H), 3.67–3.58 (m, 3H), 3.47 (s, 2H), 3.39 (dd, J=8.2, 5.2 Hz, 2H), 3.15 (dd, J=10.1, 7.3 Hz, 1H), 3.04 (d, J=6.6 Hz, 2H), 2.96 (d, J=3.8 Hz, 3H), 2.91–2.77 (m, 1H), 2.59–2.48 (m, 1H), 2.46–2.33 (m, 1H), 2.29 (s, 5H), 2.23 (d, J=4.8 Hz, 2H), 2.19–2.14 (m, 2H), 2.09 (dd, J=12.2, 5.1 Hz, 2H), 1.81 (s, 3H), 1.59 (dd, J=18.7, 11.5 Hz, 3H), 1.38 (s, 3H), 1.27 (s, 3H), 1.17 (d, J=3.9 Hz, 3H), 1.14 (s, 3H), 1.09 (d, J=7.2 Hz, 3H), 0.99 (d, J=6.7 Hz, 3H), 0.91 (d, J=6.7 Hz, 3H), 0.78 (t, J=7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 215.76, 174.12, 170.56, 157.32, 154.21, 150.26, 148.75, 137.26, 103.54, 82.70, 81.04, 78.46, 78.39, 77.49, 77.07, 76.64, 70.15, 69.29, 66.04, 60.45, 50.00, 45.58, 42.96, 40.25, 38.80, 38.43, 38.34, 36.31, 33.33, 32.87, 28.66, 26.72, 26.10, 22.03, 21.00, 19.57, 18.81, 15.06, 14.27, 14.21, 10.26, 8.91. HRMS (ESI) m/z calcd for $C_{44}H_{69}N_7O_{11}S$ [M + H]⁺: 904.4849, found: 904.4844.

3-O-descladinosyl-3-O-(3-pyridyl)acetyl-6-O-methyl-11,12-dideoxy-12,11-(oxycarbonyl[(5-[4-(1-methyl-1H-tetrazol-5-thio)]pentyl]imino) erythromycin A (compound 10q)

Yield 52.9%. Mp =101°C–104°C; ¹H NMR (300 MHz, CDCl₃) δ 8.60–8.46 (m, 2H), 7.74 (d, J=7.9 Hz, 1H), 7.35–7.27 (m, 1H), 4.98 (d, J=11.0 Hz, 2H), 3.88 (s, 3H), 3.74–3.67 (m, 3H), 3.65 (s, 1H), 3.61–3.51 (m, 2H), 3.33 (t, J=7.1 Hz, 2H), 3.17 (dd, J=10.0, 7.3 Hz, 1H), 3.08–3.02 (m, 1H), 2.98 (s, 2H), 2.92–2.81 (m, 1H), 2.59–2.40 (m, 2H), 2.33 (s, 5H), 2.19–2.06 (m, 2H), 1.84 (dd, J=15.1, 7.6 Hz, 3H), 1.62 (d, J=11.9 Hz, 3H), 1.53–1.43 (m, 3H), 1.38 (s, 3H), 1.26 (dd, J=13.3, 10.7 Hz, 6H), 1.17 (s, 2H), 1.14–1.06 (m, 9H), 0.99 (d, J=6.8 Hz, 3H), 0.91 (d, J=6.7 Hz, 3H), 0.78 (t, J=7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 215.77, 174.11, 170.57, 157.33, 154.38, 150.17, 148.65, 137.34, 129.48, 123.60, 103.63, 82.65, 81.09, 78.50, 78.39, 70.19, 69.31, 65.94, 60.42, 50.07, 45.58, 43.40, 42.94, 40.14, 38.81, 38.42, 38.32, 36.30, 33.33, 33.28, 28.76, 27.48, 26.52, 25.81, 22.04, 21.00,

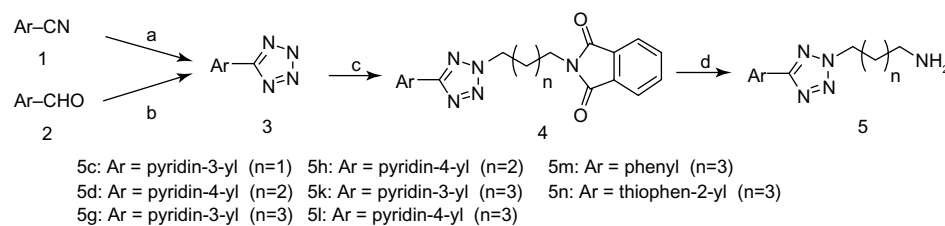


Figure 1 Synthesis of N2-substituted 5-aryl-tetrazolyl alkylamines.

Notes: Reagents and condition: (a) NaN_3 , NH_4Cl , DMF, 130°C and (b) i) I_2 , aq NH_3 , rt; ii) NaN_3 , ZnBr ; reflux; (c) N-(3-bromopropyl)phthalimide, N-(4-bromobutyl)phthalimide or N-(5-bromopentyl)phthalimide, K_2CO_3 , DMF, 80°C ; (d) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, $\text{EtOH}/\text{CH}_3\text{CN}$, reflux.

Abbreviations: Ar, aromatic nucleus; DMF, N, N-dimethyl formamide; aq, aqueous; rt, room temperature; EtOH, ethanol.

19.57, 18.85, 15.04, 14.27, 10.27, 8.91. HRMS (ESI) m/z calcd for $\text{C}_{45}\text{H}_{71}\text{N}_7\text{O}_{11}\text{S}$ $[\text{M} + \text{H}]^+$: 918.5005, found: 918.4993.

Minimum inhibitory concentration assay

The standard antibiotics erythromycin A and clarithromycin were obtained from commercial sources. The antimicrobial susceptibility test in vitro against acylide derivatives and standard antibiotics was performed using broth microdilution method, according to National Committee for Clinical Laboratory Standards guidelines. Acylides and the reference drugs were dissolved in glacial acetic acid as mother liquor; the amount of acetic acid must be less than $2.5 \mu\text{L}/\text{mL}$ to exclude glacial acetic acid effect on antibacterial activity in vitro. Minimum inhibitory concentration ($\mu\text{g}/\text{mL}$) is used to report the antibacterial activities of the compounds in vitro.

All of the synthesized acylides and standard antibiotics were tested for in vitro antibacterial activity

against two erythromycin-susceptible strains of *S. aureus* (American Type Culture Collection [ATCC] 25923, ATCC 6538), one strain of *Staphylococcus epidermidis* (ATCC 12228), two strains of *Pseudomonas aeruginosa* (ATCC 9027 and a clinical isolate of resistant PA 1317), and two strains of *Escherichia coli* (ATCC 8739, ATCC 25922).

Results and discussion

Chemical syntheses

The side chain of alkyl amines 5a–5g (Figures 1 and 2) was synthesized using the method we published previously.¹² Starting from aromatic nitriles or aromatic aldehydes as raw material, aromatic tetrazoles **3** were synthesized by (2+3) cycloaddition reaction with sodium azide, followed by reaction with phthalimide N-alkyl bromides N-(3-bromopropyl) phthalimide, N-(4-bromo-butyl) phthalimide, or N-(5-bromopentyl) phthalimide to get 4a–4g, which

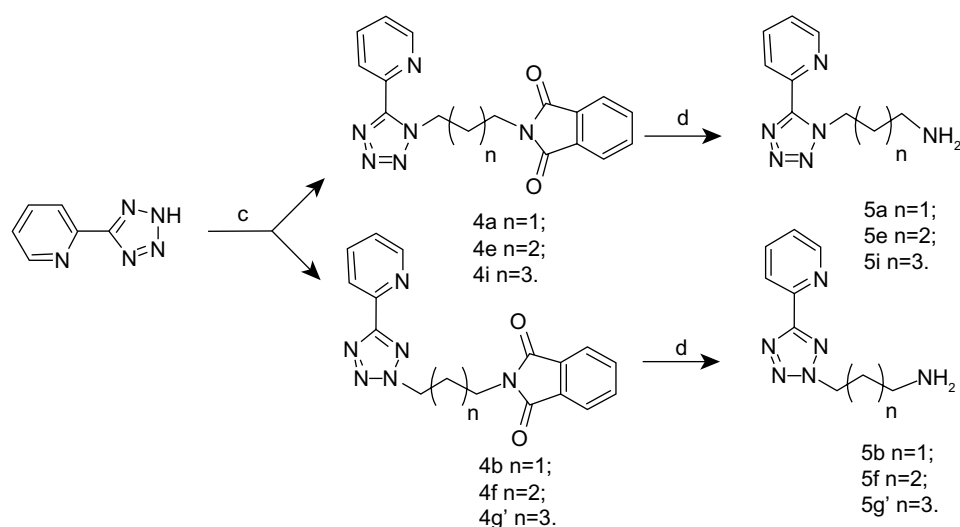


Figure 2 Synthesis of N1-substituted and N2-substituted 5-aryl-tetrazolyl alkylamines.

Notes: Reagents and condition: (c) N-(3-bromopropyl)phthalimide, N-(4-bromobutyl)phthalimide or N-(5-bromopentyl)phthalimide, K_2CO_3 , DMF, 80°C ; (d) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, $\text{EtOH}/\text{CH}_3\text{CN}$, reflux.

Abbreviations: DMF, N, N-dimethyl formamide; EtOH, ethanol.

were then hydrazinolized to the desired tetrazole alkyl amine side chain.

Tanikawa et al¹⁴ synthesized similar acylide derivatives from clarithromycin by seven-step reactions in which selective removal of the C-3 cladinose, protection of 2'-OH with acetyl, modification of the C-11,12 hydroxyl group by trichloromethyl chloroformate, acylation of the C-3 hydroxyl group, dehydrogenation of C-10,11, addition of carbonyl imidazole to C-12, and then reaction with side chain amines were performed successively to get the C-11,12 cyclic carbamates clarithromycin derivatives. Trichloromethyl chloroformate used in this routine has high toxicity and can cause serious health damage. Elliott et al¹⁵ improved the routine via six steps to synthesize a series of C-11,12 cyclic carbamate acylide derivatives from clarithromycin. On the basis of the two classic methods, we have designed a new approach (Figure 3), less the reaction steps, in which the use of toxic trichloromethyl chloroformate could be avoided.

Treatment of clarithromycin with ethylene carbonate in refluxing triethylamine and vigorously stirring, triethylamine was distilled off, and then the cladinose was selectively removed under dilute aqueous acid to prepare compound 6; the 2'-hydroxyl group was protected with acetic anhydride, using dichloromethane as solvent to obtain compound 7,

followed by the esterification of 3-hydroxyl group with 3-pyridylacetic acid hydrochloride to yield compound 8, in which the reaction must be carried out in real-time monitoring to prevent excessive reaction of esterification of the C-12 hydroxyl group. Compound 8, dissolved in anhydrous dichloromethane reacted with carbonyl diimidazole at room temperature, yielded the C-12 imidazolyl carbamate 9, followed by the reaction with excess side chain amines and then deprotection of the acetyl group by refluxing in methanol to yield the desired acylides (10a–10q) shown in Figure 4.

Antibacterial activity

The in vitro antimicrobial activities of acylides 10a–10q and standard antibiotics are shown in Table 1. The tabulated results show that all of the acylide derivatives 10a–10q exhibited potent antibacterial activity against the erythromycin-susceptible strains *S. aureus* ATCC 25923 and ATCC 6538, and most of them displayed excellent minimum inhibitory concentration values in the range of 0.06–0.5 µg/mL, which is better than or comparable to erythromycin and clarithromycin, among which compounds 10g and 10o were found to have the most potent activity against the erythromycin-susceptible strains tested. Compounds 10f and 10g also exhibited excellent activity against *S. epidermidis* ATCC 12228.

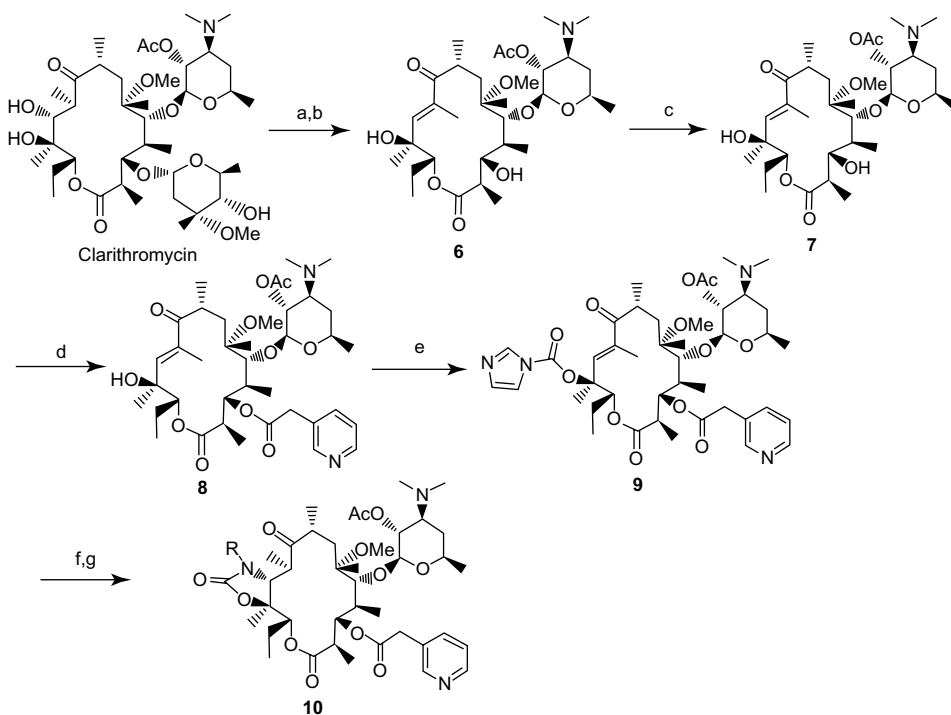


Figure 3 Synthesis of a set of acylide derivatives for compounds 10a–10q.

Notes: Reagents and conditions: (a) Ethylene carbonate, TEA, reflux; (b) HCl, EtOH, H₂O; (c) Ac₂O, TEA, CH₂Cl₂; (d) PivCl, DMAP, CH₂Cl₂, –15°C to rt; (e) CDI, DMAP, CH₂Cl₂, rt; (f) RNH₂, CH₃CN/H₂O, 55°C; (g) CH₃OH, reflux.

Abbreviations: TEA, triethylamine; PivCl, pivaloyl chloride; EtOH, ethanol; CDI, carbonyl diimidazole; DMAP, 4-Dimethylaminopyridine; Ac, acetoxy; rt, room temperature.

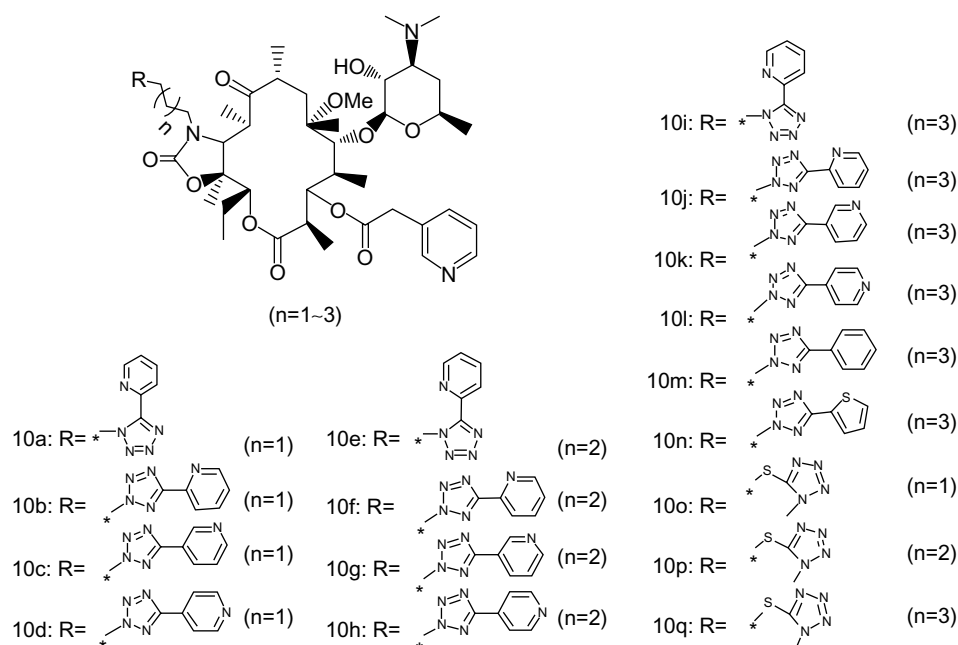


Figure 4 Structure synthesized acylides.

Note: *Connection position of R group to the parent compound.

Abbreviations: R, substituted group; Me, methyl.

Table I The antibacterial activities of novel acylides in vitro

Compound	Minimum inhibitory concentration, $\mu\text{g/mL}$						
	<i>Staphylococcus aureus</i>		<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>		<i>Escherichia coli</i>	
	ATCC 25923	ATCC 6538	ATCC 12228	ATCC 1317	ATCC 9027	ATCC 8739	ATCC 25922
ERY	0.5	0.5	0.5	>128	128	32	8
CLA	0.25	0.5	0.25	128	64	16	4
10a	0.25	1	0.125	32	64	16	16
10b	0.25	0.25	0.25	128	32	32	64
10c	1	0.5	0.25	128	128	64	64
10d	0.5	1	0.5	>128	128	64	64
10e	0.125	0.25	0.125	16	8	8	2
10f	0.125	0.125	<0.0625	64	64	16	4
10g	0.125	0.063	<0.0625	64	32	32	16
10h	0.125	0.25	0.125	128	64	32	16
10i	0.25	0.5	0.125	128	64	64	32
10j	0.25	0.5	0.125	64	128	64	64
10k	0.5	0.5	0.25	128	64	64	64
10l	0.5	0.25	0.5	128	128	128	64
10m	0.125	0.5	0.5	128	64	64	64
10n	0.125	0.5	0.5	64	32	16	16
10o	<0.0625	0.125	0.25	16	16	8	4
10p	0.125	0.125	0.25	64	32	32	32
10q	0.25	0.25	0.25	128	128	128	64

Abbreviations: ATCC, American Type Culture Collection; ERY, erythromycin; CLA, clarithromycin.

Some of the acylide derivatives showed slightly more potent activity against *P. aeruginosa* and *E. coli*. Compounds 10e and 10o seemed to be more potent than other tetrazole-containing acylide derivatives against *P. aeruginosa* ATCC 9027 and ATCC 1317. Compared with erythromycin, compound 10e and

compound 10o also exhibited improved potencies against *E. coli* strains.

Conclusion

Seventeen acylide derivatives have been synthesized and evaluated for in vitro antibacterial activities against

Gram-positive and Gram-negative pathogens. All of them were found to be potent against the strains. In particular, the compounds 10e–10h, with a long side-chain alkyl having four carbon atoms, exhibited better antibacterial activities against erythromycin-susceptible strains. Compound 10o with a side chain of 3 carbon atoms and a sulfur atom also showed better antibacterial activity against the five strains.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No 81072554, 81373285) and by the 111 Project (No B13038).

Disclosure

The authors report no conflicts of interest in this work.

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