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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6340–6344
Bioorganic & Medicinal Chemistry Letters

Design and synthesis via click chemistry of 8,9-anhydroerythromycin A 6,9-hemiketal analogues with anti-MRSA and -VRE activity

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Received 23 June 2007; revised 27 August 2007; accepted 29 August 2007
Available online 1 September 2007

Abstract—An erythromycin analogue, 11,12-di-*O*-*iso*-butyryl-8,9-anhydroerythromycin A 6,9-hemiketal (**1b**), was found to be a potential anti-MRSA and anti-VRE agent. The use of copper catalyzed azide–acetylene cycloaddition, and click chemistry, readily provided 10 types of triazole analogues of **1b** in good to nearly quantitative yield. Among the library, **5b** exhibited activity against MRSA and VRE bacterial strains, representing more than twice the potency of **1b**.

Although macrolides, including erythromycin A (EMA), have been widely prescribed for more than 50 years,[1] the emergence of widespread bacterial resistance is a serious and expanding problem.[2] There is a great medical need for new macrolide antibiotics to specifically cope with the problems of antibiotic resistant, against not only erythromycin-resistant *Streptococcus pneumoniae* (ERSP) but also methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) strains of bacteria. More recently, third generation macrolides, such as telithromycin,[3] have been developed as effective means to overcome resistant strains. Despite tremendous efforts, however, only a few macrolide candidates with activity against MRSA have been identified to date.[4] Consequently, we have re-examined various derivatives of EMA, which have been previously synthesized at The Kitasato Institute, evaluating them against 12 types of Gram-positive bacteria, including macrolide-resistant strains, and one Gram-negative organism.[5] We found that 11,12-di-*O*-butyryl-8,9-anhydroerythromycin A 6,9-hemiketal (**1a**)[6] showed moderate minimum inhibitory concentration (MIC) against four types of MRSA strains and two types of VRE strains.

We envisioned that 11,12-di-*O*-acyl groups are essential for anti-MRSA and -VRE activity. We thus began our investigations by looking into several 11,12-di-*O*-acyl-8,9-anhydroerythromycin A 6,9-hemiketal derivatives to elucidate the structure–activity relationships against anti-MRSA and -VRE strains. After screening various diacyl compounds, 11,12-di-*O*-*iso*-butyryl-8,9-anhydroerythromycin A 6,9-hemiketal (**1b**)[7] was found to be a potential agent against MRSA and VRE strains (Fig. 1).

To obtain more potent derivatives, we decided to synthesize new 8,9-anhydroerythromycin A 6,9-hemiketal

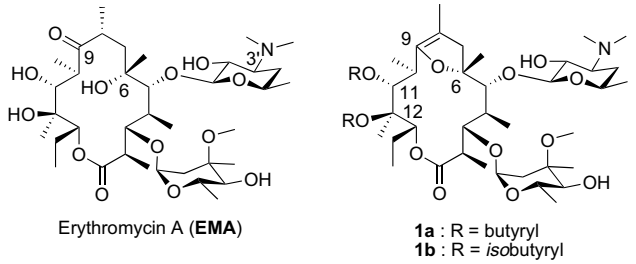

Figure 1. Structures of erythromycin A, **1a**, and **1b**.

Keywords: Erythromycin A; Macrolides; Click chemistry; Anti-MRSA and -VRE agent.
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doi:10.1016/j.bmcl.2007.08.068

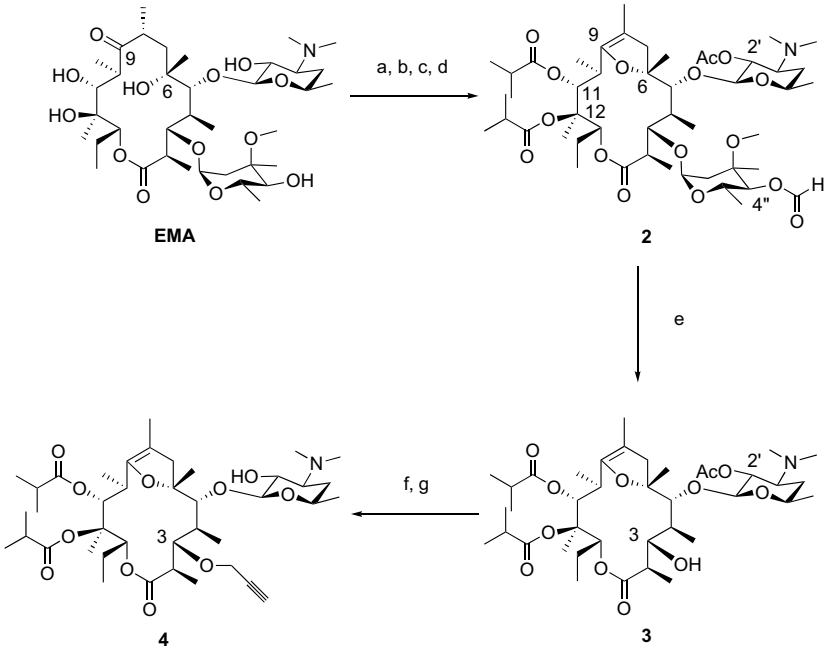
derivatives by using a copper catalyzed azide–alkyne cyclization reaction,[8] which is one of the most reliable methodologies for ideal 'click chemistry'. The methodology of click chemistry has found applications in drug discovery, bioconjugations, and materials science.[9] Since EMA and its derivatives have many reactive functional groups, containing four kinds of hydroxy groups at the 11, 12, 2′, 4″ positions and 3′-dimethyl amino group on desosamine, click chemistry application would easily introduce a specific triazole group without the need for any protection and deprotection procedures to synthesize a triazole derivative. Thus we replaced the cladinose moiety on the C3 hydroxy with a $–CH_2–C{\equiv}CH$ group, to enable a fast SAR in this sector using click chemistry. This was done because the cladinose of EMA is not essential for its anti-bacterial activity[10] but also because this moiety induces macrolide-drug resistance. We herein report a quick and simple route for the preparation of 3-*O*-alkyl derivatives from one common alkyne precursor **4**, with 10 kinds of azide blocks, together with their anti-bacterial activities.

We designed 3-de-*O*-cladinosyl-11,12-di-*O*-*iso*-butyryl-3-*O*-propargyl-8,9-anhydroerythromycin A 6,9-hemiketal (**4**) as a precursor of a 1,2,3-triazole reaction in the presence of copper (I), and synthesis of **4** is outlined in Scheme 1. Treatment of EMA with acetic acid provided the 8,9-anhydroerythromycin A 6,9-hemiketal in 73% yield by using the known procedure,[11] followed by selective protection at 2′, 4″-hydroxy groups with acetic anhydride[12] and acetic formic anhydride,[13] respectively, in 86% yield for 2 steps. *O*-Diacylation at the 11,12-hydroxy groups afforded the required diacylated product (**2**) in quantitative yield. Removal of cladinose with 10-camphorsulfonic acid in MeOH at room temperature gave decladinosyl (**3**) in 97% yield. Finally, to synthesize the triazole reaction precursor (**4**), *O*-alkylation at the 3-hydroxy group with propargyl bromide in the presence of sodium hydride without *N*-alkylated product, followed by methanolysis, furnished **4** in 72% yield for 2 steps.

Table 1. Minimum inhibitory concentrations (MICs) value of EMA and **1b**, and **2–4**

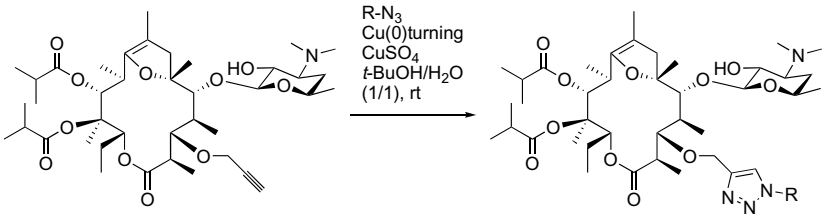
Strain/compound	MICs (μg/mL)				
	EMA	**1b**	**2**	**3**	**4**
S. aureus FDA209P[a]	⩽0.5	16	16	64	32
S. aureus Smith[a]	⩽0.5	16	16	>128	32
MRSA N315 IR94[b]	>256	16	16	64	16
MRSA N315 IR94 HR-1[b]	>256	16	16	64	16
MRSA 70[b]	>256	32	16	64	16
MRSA 92-1191[b]	>128	32	NT[j]	64	16
S. aureus ISP447[c]	>256	32	16	128	32
S. aureus ISP217[d]	>256	16	16	64	16
S. aureus 8325 (pEP2104)[e]	64	32	32	128	32
S. aureus 8325 (pMS97)[f]	>256	16	16	64	16
E. faecalis NCTC12201[g]	>256	64	32	64	16
E. faecium NCTC12203[h]	>256	32	32	64	16
E. coli NIHJ JC-2[i]	>256	>128	>128	>128	>128

NT, not tested.
[a] *Staphylococcus aureus* FDA209P and Smith: susceptible strains.
[b] *S. aureus* MRSA N315 IR94, MRSA N315 IR94 HR-1, MRSA 70, and MRSA 92-1191: MRSA strains isolated from clinical patients.
[c] *S. aureus* ISP447: inducibly resistant strain.
[d] *S. aureus* ISP217: constitutively resistant strain.
[e] *S. aureus* 8325 (pEP2104): encoded by *erm* gene.
[f] *S. aureus* 8325 (pMS97): encoded by *erm* and *mef* gene.
[g] *Enterococcus faecalis* NCTC12201: encoded by *van* A gene.
[h] *E. faecium* NCTC12203: encoded by *van* A gene.
[i] *E. coli* NIHJ JC-2: susceptible Gram-negative strain.

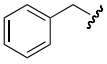
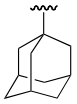
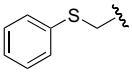
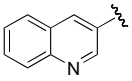
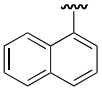
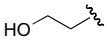
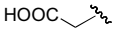
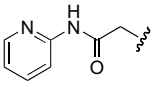
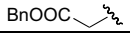


Scheme 1. Synthesis of **4** as a precursor of click reaction. Reagents and conditions: (a) acetic acid, rt, 0.5 h, 73%; (b) acetic anhydride, acetone, rt, 1 h; (c) aceticformic anhydride, pyridine, 4-dimethylaminopyridine, THF, 0 °C, 1 h, 86% (for 2 steps); (d) *iso*-butyric anhydride, 4-dimethylaminopyridine (excess), pyridine, rt, 48 h, quant; (e) 10-camphorsulfonic acid, MeOH, rt, 6 h, 97%; (f) propargyl bromide, NaH, THF, rt, 3.5 h, 72%; (g) MeOH, 50 °C, 12 h, quant.

Table 2. Copper-catalyzed 1,2,3-triazole formation



4 **5a-j**

Compound	R	Time	Yields[a]
5a		1 d	92%
5b		1.5 d	97%
5c		1 d	quant.
5d		17 h	quant.
5e		2.5 d	quant.
5f		2.5 d	quant.
5g		1.5 d	85%
5h		1.5 d	96%
5i		12 h	92%
5j		1 d	90%

[a] Isolated yield.

We subsequently evaluated the anti-bacterial activity of **1b** and **2**–**4** against twelve Gram-positive strains (*S. aureus* FDA209P; *S. aureus* MRSA N315 IR94, MRSA N315 IR94 HR-1, MRSA 70, and MRSA 92-1191; *S. aureus* ISP447; *S. aureus* ISP217; *S. aureus* 8325 (pEP2104); *S. aureus* 8325 (pMS97); *E. faecalis* NCTC12201; *E. faecium* NCTC12203) and one Gram-negative (*E. coli* NIHJ JC-2) strain using standard serial-dilution techniques[5] (Table 1). The 2′, 4″-protected product (**2**) showed similar activity compared with **1b**. In contrast, the alcohol (**3**) exhibited 2- to 4-fold less activity against MRSA and VRE strains than the lead derivative (**1b**). Interestingly, the propargyl compound (**4**) possessed better anti-bacterial activity than **3**, suggesting that the propargyl group and/or its derivatives could be substituted instead of cladinose without decreasing either anti-MRSA or anti-VRE activity.

The acetylene compound, **4**, was readily converted to the 1,4-disubstituted triazole products **5a–j** with 10 azide compounds in the presence of copper catalyzed conditions in good-to-quantitative yield without 1,5-disubstituted triazole products[14] (Table 2). This result also suggested that click chemistry methodology allowed apparently useful selective reactions for EMA, including many reactive functional groups, without difficulties.

As a consequence, 10 kinds of triazole compounds were efficiently prepared for in vitro anti-bacterial testing (vide ante), using standard serial-dilution techniques[5] (Table 3). Although EMA demonstrates no activity against clinically isolated MRSA strains and VRE strains (MICs >256 μg/mL, Table 3), di-*iso*-butyryl **4** is slightly effective against MRSA and VRE strains (MICs 16 μg/mL, Table 2). Notably, the adamantyl-triazole derivative (**5b**) showed 2-fold greater activity against MRSA and VRE strains (MICs 8 μg/mL, Table 3) than the lead derivative (**1b**) and **4**. In contrast, hydrophilic triazole derivatives (**5g** and **5h**) show no activity (Table 3).

In conclusion, we found that the propargyl (**4**) and some triazole groups (**5a–5f**, **5i–j**) can be substituted instead of

Table 3. Minimum inhibitory concentrations (MICs) value of EMA and analogues

Strain/compound	MICs (μg/mL)											
	EMA	**1b**	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**
S. aureus FDA209P[a]	⩽0.5	16	16	8	16	16	32	32	64	>128	32	32
S. aureus Smith[a]	⩽0.5	16	32	16	32	32	64	64	>128	>128	32	128
MRSA N315 IR94[b]	>256	16	8	8	8	8	16	16	64	>128	16	16
MRSA N315 IR94 HR-1[b]	>256	16	8	8	8	16	16	16	64	>128	16	16
MRSA 70[b]	>256	32	16	8	16	16	16	16	64	>128	32	16
MRSA 92–1191[b]	>128	32	16	8	16	16	32	32	128	>128	32	32
S. aureus ISP447[c]	>256	32	16	16	16	32	64	32	>128	>128	32	64
S. aureus ISP217[d]	>256	16	16	8	16	16	16	16	128	>128	16	16
S. aureus 8325 (pEP2104)[e]	64	32	16	16	16	32	128	64	128	>128	32	64
S. aureus 8325 (pMS97)[f]	>256	16	8	8	16	8	16	16	64	>128	16	16
E. faecalis NCTC12201[g]	>256	64	16	8	16	32	32	32	128	>128	>128	32
E. faecium NCTC12203[h]	>256	32	16	8	16	16	16	16	64	>128	32	32
E. coli NIHJ JC-2[i]	>256	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

[a] *Staphylococcus aureus* FDA209P and Smith: susceptible strains.
[b] *S. aureus* MRSA N315 IR94, MRSA N315 IR94 HR-1, MRSA 70, and MRSA 92-1191: MRSA strains isolated from clinical patients.
[c] *S. aureus* ISP447: inducibly resistant strain.
[d] *S. aureus* ISP217: constitutively resistant strain.
[e] *S. aureus* 8325 (pEP2104): encoded by *erm* gene.
[f] *S. aureus* 8325 (pMS97): encoded by *erm* and *mef* gene.
[g] *Enterococcus faecalis* NCTC12201: encoded by *van* A gene.
[h] *E. faecium* NCTC12203: encoded by *van* A gene.
[i] *E. coli* NIHJ JC-2: susceptible Gram-negative strain.

cladinose, to produce anti-MRSA and anti-VRE activity. Moreover, the use of copper catalyzed azide–acetylene cycloaddition efficiently provided various triazole libraries with azide compounds, avoiding complicated production mechanisms. Additionally, one of 10 kinds of triazole compounds, the adamantyl-triazole product **5b**, was produced as a potential lead of new antibiotic for use against MRSA and VRE strains, using click methodology. We believe this click methodology could be widely applied in the preparation of complicated natural-product analogues. Further in vitro and in vivo studies on these erythromycin analogues are in progress.

Acknowledgments

This work was supported by a grant from the 21st Century COE Program, Ministry of Education, Culture, Sports, Science and Technology. We also thank Ms. A. Nakagawa, Ms. C. Sakabe, and Ms. N. Sato for the various instrumental analysis.

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7. Mp 219–222 °C, ^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm): 5.30 (d, J = 9.4 Hz, 1H), 4.89 (d, J = 4.7 Hz, 1H), 4.77 (m, 1H), 4.42 (d, J = 7.2 Hz, 1H), 4.39 (bs, 1H), 4.03 (dq, J = 15.4, 12.7, 6.3 Hz 1H), 3.96 (d, J = 8.0 Hz, 1H), 3.59 (m, 1H), 3.52 (m, 1H), 3.23 (s, 3H), 3.21 (dd, J = 9.9, 7.4 Hz, 1H), 3.05 (appear, t, J = 9.6 Hz, 1H), 2.70 (d, J = 16.0 Hz, 1H), 2.68 (m, 1H), 2.56–2.44 (complex mixture, 3H), 2.37 (d, J = 15.4 Hz, 1H), 2.30 (s, 6H), 2.14 (d, J = 10.5 Hz, 1H), 2.05 (appear, t, J = 6.1 Hz, 1H), 1.91 (d, J = 15.4 Hz, 1H), 1.78 (m, 1H), 1.69 (bd, J = 11.8 Hz, 1H), 1.62 (d, J = 5.0 Hz, 1H), 1.60 (s, 3H), 1.49 (s, 3H), 1.46 (m, 1H), 1.33 (s, 3H), 1.28 (d, J = 6.3 Hz, 3H), 1.23–1.22 (complex mixture, 4H), 1.17 (d, J = 7.2 Hz, 3H), 1.15–1.13 (complex mixture, 15H), 1.10 (d, J = 7.4 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H), ^{13}C NMR (150 MHz, $CDCl_3$), δ (ppm): 175.4, 175.2, 174.6, 151.0, 103.2, 102.3, 95.8, 86.6, 85.5, 80.4, 78.2, 76.8, 76.2, 73.1, 70.9, 70.5, 68.8, 65.8, 65.3, 49.4, 44.0, 42.9, 42.6, 40.3 (C × 2), 35.0, 34.7, 34.3, 32.0, 29.0, 25.8, 23.1, 21.7, 21.2, 19.2 (C × 2), 19.0, 18.8, 18.1, 15.8, 14.3, 13.5, 12.0, 11.4, 8.9, HR-MS (FAB) (matrix; m-NBA, PEG600+NaI), *m/z*: 856.5432 $[M+H]^+$, calcd for $C_{45}H_{78}NO_{14}$: 856.5422 [M+H].
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14. Typical experimental procedure; 3-*O*-[(1-adamantyl)-1,2,3-triazol-4-yl]methyl-3-de-*O*-cladinosyl-11,12-di-*O*-*iso*-butyryl 8,9-anhydroerythromycin A 6,9-hemiketal (**5b**); **4** (9.8 mg, 0.013 mmol) and 1-azidoadamantane (2.4 mg, 0.013 mmol) were suspended in a 1:1 mixture of water and *t*-BuOH (1.3 mL). To this mixture were added copper turning and copper sulfate solution (0.0013 mmol). The mixture was stirred at room temperature for 1.5 days. After completion of the reaction, the copper turning was removed and the mixture was extracted with $CHCl_3$ (10 mL ×1), then the organic layer washed with satd NaCl aq solution (5 mL ×1), dried over Na_2SO_4, and concentrated. Flash chromatography ($CHCl_3$:MeOH:30% aq NH_4OH = 50:1:0.1) afforded **5b** in 97% yield as a white amorphous. ^1H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.21 (s, 1H), 5.43 (d, J = 8.8 Hz, 1H), 5.22 (d, J = 12.1 Hz, 1H), 4.73 (d, J = 12.7 Hz, 1H), 4.69 (m, 1H), 4.18 (d, J = 6.3 Hz, 1H), 3.99 (s, 1H), 3.90 (bs, 1H), 3.43–3.41 (complex mixture, m, 2H), 3.17 (dd, J = 9.9, 7.2 Hz, 1H), 2.88–2.84 (complex mixture, m, 2H), 2.56–246 (complex mixture, m, 5H), 2.25–2.24 (complex mixture, m, 9H), 2.17 (bs, 3H), 2.06 (d, J = 15.7 Hz, 1H), 1.85 (m, 1H), 1.76–1.70 (complex mixture, m, 6H), 1.65–1.59 (complex mixture, m, 4H), 1.52 (s, 3H), 1.43 (m, 1H), 1.34–1.33 (d, J = 6.9 Hz, 1H), 1.18–1.16 (complex mixture, m, 15H), 1.14 (d, J = 7.2 Hz, 1H), 1.08 (d, J = 6.1 Hz, 1H), 1.10 (d, J = 7.2 Hz, 1H), 0.94–0.91 (complex mixture, m, 6H), ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 175.4, 174.9, 171.7, 150.1, 145.9, 120.5, 105.3, 102.5, 89.7, 85.5, 83.6, 80.3, 76.8, 71.0, 70.5, 69.6, 65.8, 58.9, 43.0, 42.8 (C × 3), 42.0, 40.3 (C × 2), 36.0 (C × 3), 35.8, 34.9, 34.2, 31.8, 29.5 (C × 3), 28.3, 28.2, 23.9, 21.1, 19.3 (C × 2), 49.2, 19.0, 18.6, 16.9, 15.0, 14.3, 12.3, 11.8, HR-MS (FAB) (matrix; m-NBA, PEG600+NaI) *m/z*: 913.5941 $[M+H]^+$, calcd for $C_{50}H_{81}N_4O_{11}$: 913.5902 [M+H].