The Journal of Antibiotics (2013) 66, 195–198

www.nature.com/ja

NOTE

Synthesis of novel lincomycin derivatives and their *in vitro* antibacterial activities

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The Journal of Antibiotics (2013) **66,** 195–198; doi:10.1038/ja.2012.107; published online 12 December 2012

Keywords: antibiotic; clindamycin; lincomycin; resistant bacteria; *S. pneumoniae*

INTRODUCTION

Macrolide antibiotics are active against Gram-positive bacteria, especially *Streptococcus pneumoniae*, and their safety as an oral agent has already been proved. Therefore, macrolide antibiotics are regarded as very important chemotherapeutic agents against bacterial respiratory infections as in the case of β-lactam antibiotics or new quinolones. Although clarithromycin and azithromycin, which are representatives of widely used macrolides, exhibit enhanced antibacterial activities and characteristic pharmacokinetics compared with those of erythromycin, they are not active enough against resistant bacteria of *S. pneumoniae* with *erm* gene. Recently these resistant bacteria have widely spread, especially in European and Asian countries, and caused severe social problems. Chemical modifications of erythromycin provided the clinical site with two novel ketolides, telithromycin and cethromycin, which are effective against resistant bacteria of *S. pneumoniae* with *erm* gene. No oral antibiotic, however, has been launched so far, which is effective against the resistant bacteria of *S. pneumoniae* and does not have any problems in safety or taste.

Lincomycin (LCM) isolated as a secondary metabolite from fermentation broth of *Streptomyces lincolnensis* was chemically transformed to a useful oral antibiotic, clindamycin (CLDM) (Figure 1), which inhibits bacterial protein synthesis in a similar manner to macrolides. Although telithromycin is still influenced by efflux pumps of resistant *S. pneumoniae* with *mef* gene, CLDM is not influenced by efflux pumps. We focused not only on its safety and effectiveness against efflux pumps but its possibility of switch therapy by CLDM analogs, and planned to generate a novel oral antibiotic, which is effective against resistant bacteria of *S. pneumoniae* with *erm* gene or *mef* gene by chemical modifications of LCM.

BACKGROUND AND MOLECULAR DESIGN

Regarding chemical modification of LCM, Argoudelis *et al.*[1] investigated fundamental SAR analysis focused on the C-6 (N-1′ and C-4′) and C-7 positions in 1960s and Magerlein *et al.*[2,3] generated U-24,279 A, (Figure 1) which exhibited much stronger *in vitro* and *in vivo* activities compared with LCM. On the other hand, Lewis *et al.*[4,5] disclosed VIC-105555 (Figure 1) and proved that its *in vitro* activity against *Enterococcus faecalis* was much stronger than that of CLDM and its half-life in plasma was greatly improved compared with that of CLDM in a variety of animal species. As a pioneer work, Sztaricskai and Ōmura *et al.*[6] reported introduction of a heterocycle *via* sulfur atom onto the LCM carbon framework (**1** and **2** shown in Figure 1) with (7*R*)-configuration, by means of S_N2 reaction analogous to the synthesis of CLDM. All these derivatives introduced as shown in the above exhibited substantially same antibacterial spectra as those of LCM or CLDM, and there was no description about their antibacterial activities against resistant bacteria of *S. pneumoniae* with *erm* gene. Because 3D structural interaction of CLDM-*r*RNA complex by X-ray single crystallographic analysis[7] indicated that there were several interactions, mainly a hydrogen bonding, between the peptidyl transferase cavity (A2058Ec, G2520Ec and A2059Ec) and hydroxyl groups at the sugar moiety of CLDM, chemical modification at the sugar part was supposed to be rather difficult for improving activity. As a matter of fact, 2-deoxy-LCM[8] shows only 1% activity of LCM, and this result can be explained by lack of the hydrogen bonding between A2058Ec and 2-OH of LCM. We consequently pursued modification at C-7 and then C-6 (N-1′ and C-4′), focusing on hydrophilic interaction in extra 3D spaces in the above analysis.

RESULTS AND DISCUSSION

We paid attention to the fact that stereochemistry at the C-7 position affected antibacterial activity, and synthesized a variety of (7*S*)-7-sulfur-aromatic derivatives of LCM firstly, in application of 2,3,4-tris-(trimethylsilyl)-LCM[9] as a key intermediate prepared *via* 2,3,4,7-tetrakis-(trimethylsilyl)-LCM. Precise molecular design, practical synthetic procedures and full package of physicochemical properties of each derivative will be reported elsewhere. Although every

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As a special article for Professor Tatsuta's memorial issue.
Received 28 September 2011; revised 28 October 2012; accepted 30 October 2012; published online 12 December 2012

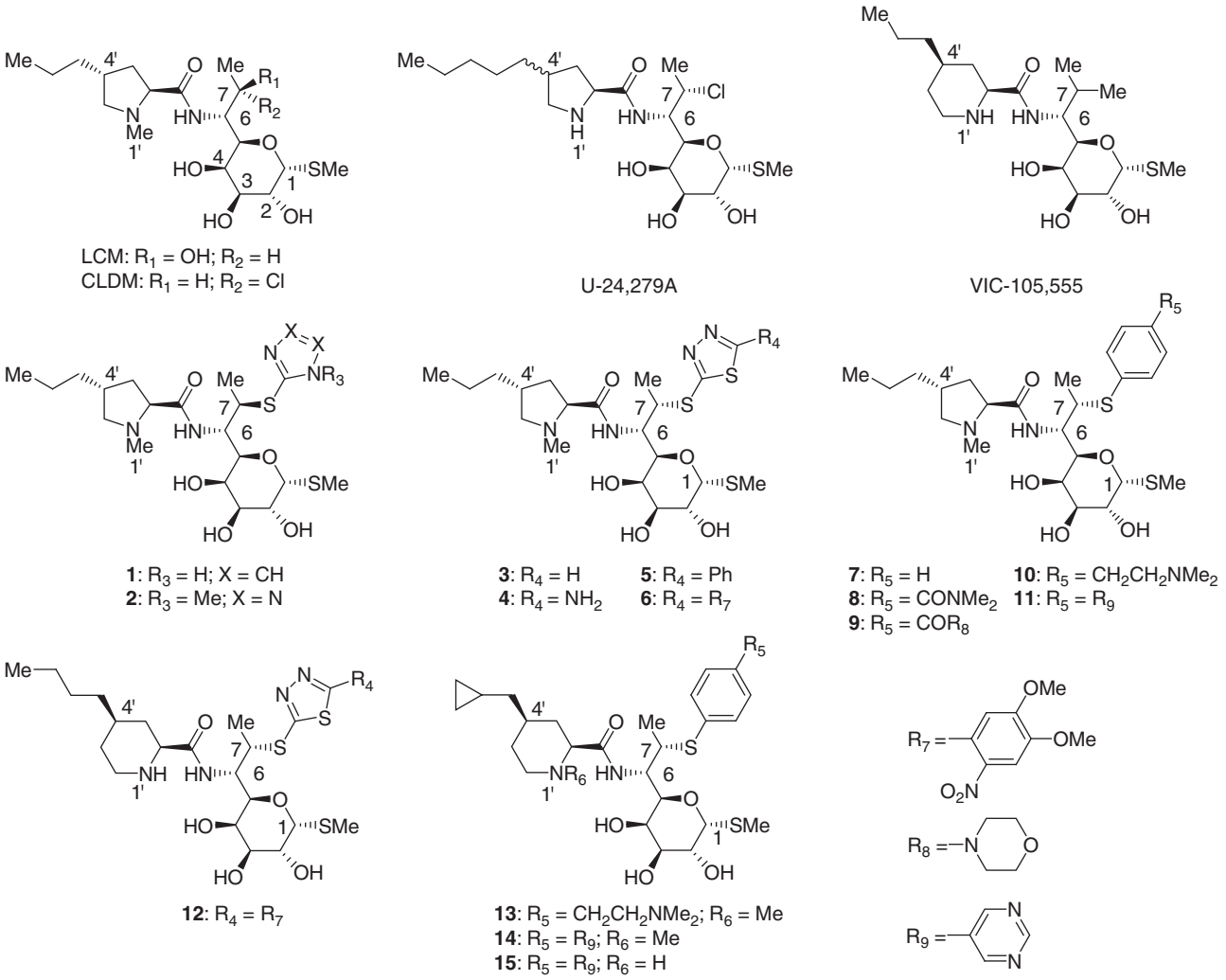

Figure 1 Chemical structures of LCM, CLDM, their reported analogs and novel LCM derivatives.

derivative exhibited almost the same *in vitro* activities, introducing a substituent to an aromatic ring (1,3,4-thiadiazole or a benzene ring) enhanced antibacterial activity (**3** to **4** and **7** to **8**) (Table 1, all antibacterial evaluations were performed as hydrochloride). Representative ^{1}H NMR data and MASS data of each analog are shown in Table 2. Then, we selected compounds **3** and **7** as lead compounds and started systematic synthesis of derivatives of **3** and **7** through introducing a substituent onto 1,3,4-thiadiazole or a benzene ring.

In the thiadiazole analogs synthesis, 5-phenyl-1,3,4-thiadiazol-2-yl analog (**5**) exhibited moderate activities against resistant bacteria of *S. pneumoniae* and *Streptococcus pyogenes* with *erm* gene. Then, optimization of 7-sulfur-substituent by the introduction of an additional substituent onto the phenyl group of **5** gave key compound **6**. In the benzene analogs synthesis, locally optimized compound **9** showed stronger activity against the target compared with **8**, but there seemed to be limitation for further enhancement of *in vitro* activity. Then, basic functionality or one more aromatic ring (by SAR of compound **5**) was introduced to the *para*-position of the benzene ring of compound **7**, and partially optimized compounds **10** and **11** were prepared. Because compounds **6**, **10** and **11** exhibited strong antibacterial activities against clinically important pathogens for respiratory infections (Table 1), we performed final optimization of the whole molecule, especially by handling the proline part.

Ring expansion strategies (from a proline framework to a pipecolic acid framework, see VIC-105,555) have already been reported by Birkenmeyer *et al.*,[10,11] and we expanded a five-membered ring to six- or seven-membered ring and simultaneously optimized structure of a side chain at the C-4′ position. As a conclusion,[12–14] compounds **12** and **13** to **15** were generated as candidates for further biological evaluations. Introduction of a cyclopropylmethyl group to the pipecolic acid moiety in the LCM framework has already been reported by Lewis *et al.*[15,16] As shown in Table 1, these derivatives possessed very strong antibacterial activities against not only resistant bacteria of *S. pneumoniae* with *erm* or *mef* gene, but other clinically important pathogens for respiratory infections such as *S. pyogenes*. Among them, compounds **12**, **14** and **15** unexpectedly exhibited extremely potent antibacterial activities against resistant *S. pneumoniae* with *erm* gene even when compared with telithromycin and these derivatives were recognized as the first examples which were effective enough *in vitro* against the target resistant *S. pneumoniae* apart from ketolides.

EXPERIMENTAL PROCEDURE

In vitro antibacterial activity

Minimum inhibitory concentration was determined by the agar dilution method. Test strains were subjected to seed culture using sensitivity test broth (STB, Nissui Pharmaceutical, Tokyo, Japan)

Table 1 Antibacterial activities of novel LCM derivatives against *S. pneumoniae*, *S. pyogenes* and *H. influenzae*. Minimum inhibitory concentration ($\mu g ml^{-1}$)[a]

No.		Characteristics	LCM	CLDM	3	4	5	6	7	8	9	10	11	12	13	14	15	TEL
1	*Streptococcus pneumoniae* DP1 Type I	Susceptible	1	0.13	0.13	0.03	0.06	0.015	0.25	0.03	0.06	⩽0.008	⩽0.008	0.03	⩽0.008	⩽0.008	⩽0.008	⩽0.008
2	*S. pneumoniae* No.2	Susceptible	1	0.13	0.25	0.03	0.13	0.015	0.25	0.03	0.06	0.015	⩽0.008	0.015	⩽0.008	⩽0.008	⩽0.008	⩽0.008
3	*S. pneumoniae* No.3	Susceptible	1	0.13	0.13	0.03	0.03	0.015	0.13	0.03	0.06	0.015	⩽0.008	0.03	0.015	⩽0.008	⩽0.008	⩽0.008
4	*S. pneumoniae* No.4	*erm B* methylase (c)	>128	>128	128	8	8	0.5	128	8	8	4	0.5	0.03	0.5	0.13	0.03	0.5
5	*S. pneumoniae* No.5	*erm B* methylase (c)	>128	>128	NT	NT	NT	0.25	NT	2	1	2	0.5	0.03	0.25	0.06	0.015	0.03
6	*S. pneumoniae* No.6	*erm B* methylase (c)	>128	>128	128	64	8	0.25	128	8	2	4	1	0.06	0.5	0.13	0.03	1
7	*S. pneumoniae* No.7	*erm B* methylase (i)	>128	128	128	16	8	0.25	128	4	2	1	0.25	0.015	0.03	0.06	0.015	0.03
8	*S. pneumoniae* No.8	*erm B* methylase (i)	>128	128	128	16	8	0.25	128	4	1	NT	0.25	0.015	NT	0.03	0.015	0.03
9	*S. pneumoniae* No.9	*mef E* efflux	1	0.13	0.13	0.03	0.06	⩽0.008	0.13	0.03	0.03	⩽0.008	⩽0.008	0.015	⩽0.008	⩽0.008	⩽0.008	0.06
10	*S. pneumoniae* No.10	*mef E* efflux	1	0.13	0.13	0.03	0.06	0.015	0.13	0.03	0.03	NT	⩽0.008	0.015	NT	⩽0.008	⩽0.008	0.06
11	*Streptococcus pyogenes* Cook	Susceptible	0.13	0.13	0.06	0.03	0.06	0.03	0.13	0.03	0.06	0.015	⩽0.008	0.03	⩽0.008	⩽0.008	⩽0.008	⩽0.008
12	*S. pyogenes* No.2	*erm B* methylase (c)	>128	>128	128	16	2	0.25	128	4	4	NT	0.5	0.06	0.25	0.06	0.03	16
13	*S. pyogenes* No.3	*mef E* efflux	0.25	0.13	0.25	0.03	0.06	0.015	0.13	0.03	0.06	0.015	0.015	0.06	0.015	⩽0.008	⩽0.008	0.25
14	*Haemophilus influenzae* No.1	Susceptible	8	8	16	4	16	2	16	32	4	2	4	2	1	2	1	0.5
15	*H. influenzae* No.2	Susceptible	16	8	16	4	8	2	8	16	4	2	2	2	1	1	1	2
16	*H. influenzae* No.3	Susceptible	16	32	>128	16	64	2	64	32	8	4	8	2	2	2	1	1

Abbreviations: c, constitutive; i, inducible; NT, not tested; TEL, telithromycin.
[a]All anticacterial evaluations were performed as hydrochloride.

Table 2 Representative ^{1}H NMR data and MASS data of each analog.[a]

			Selective ^{1}H NMR data (δ in p.p.m.)[b]				
	1-H 1H	$J_{1,2}$(Hz)	1-SMe 3H	1'-NMe 3H	Other characteristic signals	FAB-MS (m/z) as ($M^+ + 1$)	Molecular formula
3	5.27 d	5.7	1.93 s	2.40 s	9.38 (1H, s, thiadiazole)	507	$C_{20}H_{34}N_4O_5S_3$
4[c]	5.35 d	5.7	2.10 s	2.43 s		522	$C_{20}H_{35}N_5O_5S_3$
5[c]	5.34 d	5.4	2.12 s	2.39 s		583	$C_{26}H_{38}N_4O_5S_3$
6	5.36 d	5.6	2.18 s	2.40 s	4.00, 4.03 (6H, 2 x s, OMe)	688	$C_{28}H_{41}N_5O_9S_3$
7	5.27 d	5.4	1.98 s	2.41 s		499	$C_{24}H_{38}N_2O_5S_2$
8	5.26 d	5.6	1.90 s	2.64 s	3.00, 3.08 (6H, 2 x s, NMe_2)	570	$C_{27}H_{43}N_3O_6S_2$
9	5.26 d	5.6	1.91 s	2.45 s		612	$C_{29}H_{45}N_3O_7S_2$
10	5.26 d	5.6	2.01 s	2.40 s	2.50 (6H, s, NMe_2)	570	$C_{28}H_{47}N_3O_5S_2$
11	5.27 d	5.4	1.96 s	2.43 s	9.07 (2H, s, pyrimidine) 9.13 (1H, s, pyrimidine)	577	$C_{28}H_{40}N_4O_5S_2$
12	5.28 d	5.8	2.01 s	N/A	3.95, 3.98 (6H, 2 x s, OMe)	702	$C_{29}H_{43}N_5O_9S_3$
13	5.24 d	5.6	1.97 s	2.24 s	2.35 (6H, s, NMe_2)	596	$C_{30}H_{49}N_3O_5S_2$
14	5.27 d	5.6	1.95 s	2.27 s	9.07 (2H, s, pyrimidine) 9.12 (1H, s, pyrimidine)	603	$C_{30}H_{42}N_4O_5S_2$
15	5.27 d	5.6	1.93 s	N/A	9.07 (2H, s, pyrimidine) 9.12 (1H, s, pyrimidine)	589	$C_{29}H_{40}N_4O_5S_2$

[a]All ^{1}H NMR spectra and FAB-MS were measured as free base.
[b]400 MHz in CD_3OD.
[c]300 MHz in CD_3OD.

cultured on blood agar plate for *S. pneumoniae*, *S. pyogenes* and *H. influenzae*. A 5 µl portion of cell suspension of the test strains having about 10^6 CFU per ml was inoculated into sensitivity disk agar (SDA, Nissui Pharmaceutical) supplemented with 5% horse blood and incubated at 37 °C for 20 h. Then, minimum inhibitory concentration was measured.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors wish to greatly thank Professor Emeritus Masatoshi Konno for his excellent supervision.

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