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ORIGINAL ARTICLE

Synthesis and antibacterial activity of novel lincomycin derivatives. II. Exploring (7*S*)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-7-deoxylincomycin derivatives

Ko Kumura, Yoshinari Wakiyama, Kazutaka Ueda, Eijiro Umemura, Takashi Watanabe, Megumi Kumura, Takuji Yoshida and Keiichi Ajito

The synthesis and antibacterial activity of (7*S*)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-7-deoxylincomycin derivatives are described. These derivatives were mainly prepared by the Mitsunobu reaction of 2,3,4-tris-*O*-(trimethylsilyl)lincomycin and the corresponding thiols. Exploring structure–activity relationships of the substituent at the 5 position of a thiadiazole ring revealed that compounds with the *ortho* substituted phenyl group showed improved antibacterial activities against *Streptococcus pneumoniae* and *Streptococcus pyogenes* with *erm* gene compared with the reported compound (1) that had an unsubstituted benzene ring.
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INTRODUCTION

Lincomycin[1] is a secondary metabolite of *Streptomyces lincolnensis* and active mainly against Gram positive bacteria. Clindamycin[2] (CLDM) derived from lincomycin is a useful semisynthetic antibiotic that is most widely used in the lincosamide class (Figure 1). Lincosamide antibiotics are protein synthesis inhibitors[3] that act on 50S ribosome in a similar way to macrolide antibiotics such as clarithromycin.[4] However, CLDM shows almost no antibacterial activity against resistant pathogens such as *Streptococcus pneumoniae* and *Streptococcus pyogenes* with *erm* gene as shown in Table 1. Moreover, major macrolides, clarithromycin and azithromycin,[5] are also not active against those pathogens with *erm* gene. Erm methyltransferases methylate A2058Ec of rRNA and diminish the affinity of clinically important macrolides, lincosamides and streptogramin B[3], and this mode of resistance is referred to as MLS resistance.[6] Increased emergence of resistant bacteria has been causing serious problems at clinical sites.[7] CLDM is attractive because of its safety and effectiveness against resistant pathogens with efflux pump. It is known that the antibacterial activities of macrolide antibiotics are influenced by efflux pumps of resistant *S. pneumoniae* and *S. pyogenes* with *mef* gene (Figure 1; Table 1). Furthermore, CLDM can be administered as oral and injectable agents. As a rare case, moreover, it has been reported that CLDM is effective for invasive group A streptococcal infections caused by *S. pyogenes*.[8] According to these reasons, we selected lincosamide (not macrolide) as a starting material for medicinal chemistry. In order to generate a novel chemotherapeutic agent that is effective against resistant *S. pneumoniae* and *S. pyogenes* with *erm* and *mef* genes, we started chemical modification of lincomycin and clarified that (7*S*)-7-arylthio-7-deoxylincomycin derivatives[9–11] and (7*S*)-7-(azetidin-3-yl-thio)-7-deoxylincomycin derivatives[12] exhibited moderate to strong antibacterial activities against *S. pneumoniae* and *S. pyogenes* with *erm* gene. In this article, we report optimization of previously reported (7*S*)-7-deoxy-7-(5-phenyl-1,3,4-thiadiazol-2-yl-thio)lincomycin (**1**). On the other hand, telithromycin[13] is effective enough against *S. pneumoniae* with *erm* gene, but it has been reported to have potential to cause side effects in clinical use.[7] Novel azalides[14] were generated starting from 16-membered macrolides, and several optimized 16-membered azalides[15] are effective against resistant *S. pneumoniae* and *S. pyogenes* with *erm* gene. These analogs, however, are still under research process and have not been developed yet. Currently available oral drugs are not effective enough against resistant bacteria with *erm* and *mef* genes causing respiratory infections and have some problems in safety or taste in clinical site.

Chemistry

Schemes 1 and 2 show the synthetic routes for novel (7*S*)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-7-deoxylincomycin derivatives. We utilized reported 2, 3, 4-tris-*O*-(trimethylsilyl)lincomycin (**2**)[16] as a substrate for the Mitsunobu reaction with various thiols as we reported earlier.[10] After the Mitsunobu reaction, trimethylsilyl groups were removed by acid treatment to give **3**–**22** (Scheme 1). Although

Pharmaceutical Research Center, Meiji Seika Pharma Co., Ltd., Yokohama, Japan
Correspondence: Dr K Ajito, Pharmaceutical Research Center, Meiji Seika Pharma Co., Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan.
E-mail: keiichi.ajito@meiji.com
This paper is dedicated to Professor Dr. Satoshi Ōmura for his Nobel Prize in Physiology or Medicine 2015.
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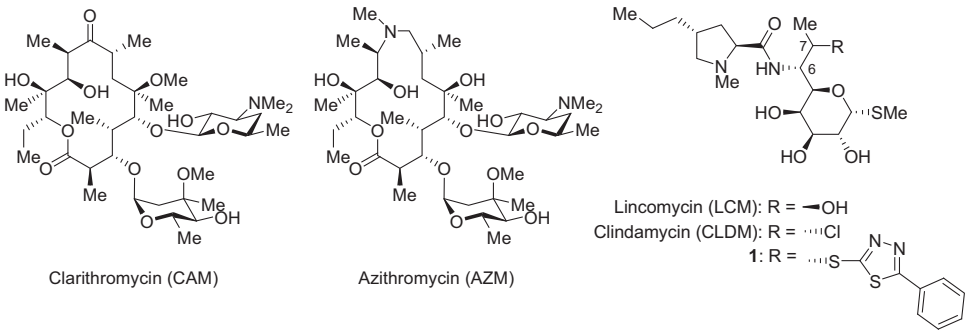
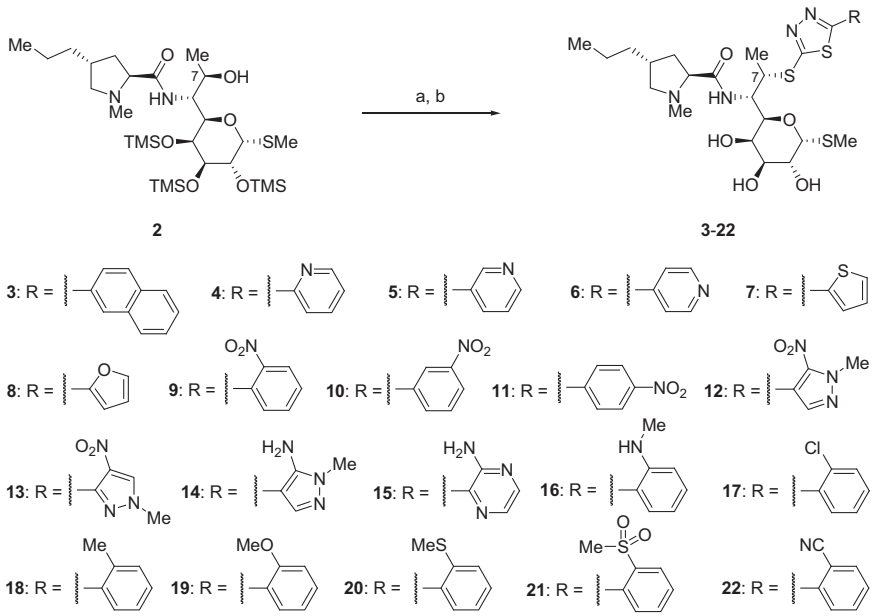


Figure 1 Structures of clinically important macrolides, lincomycin, clindamycin and compound **1**.

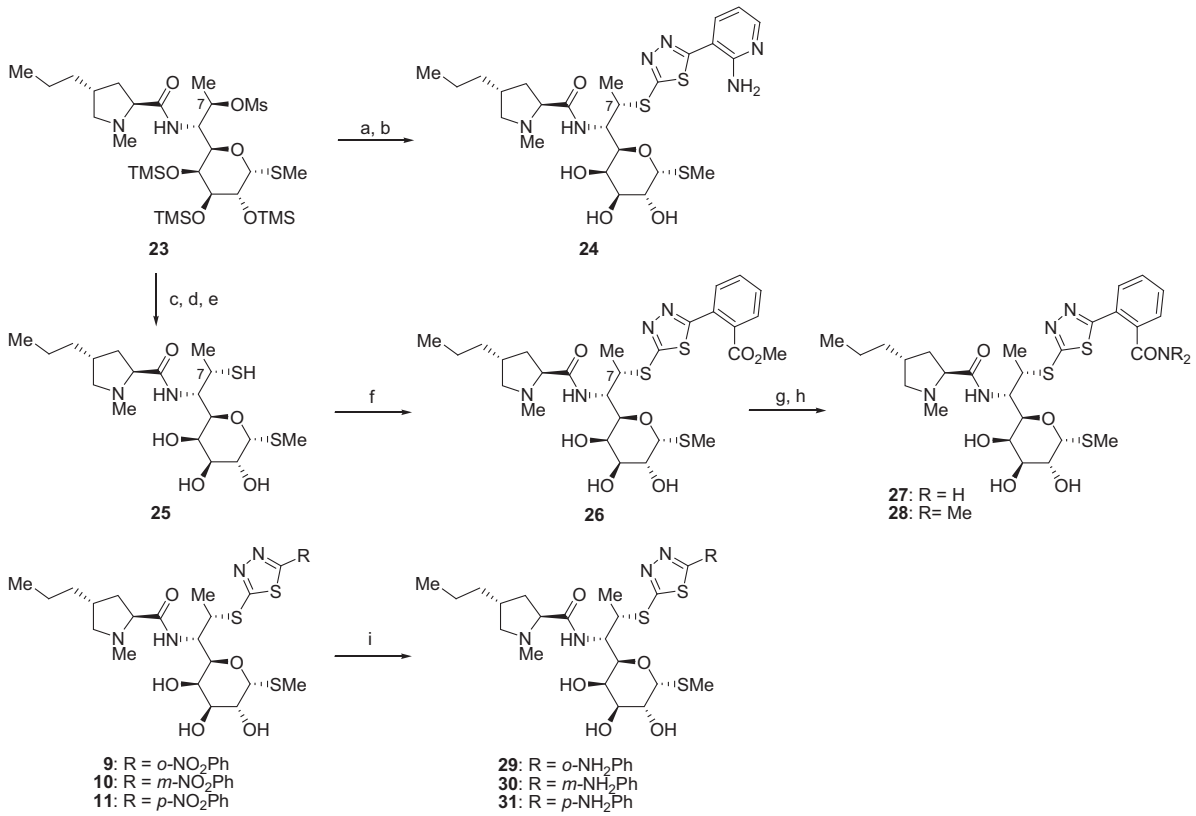
Table 1 Antibacterial activities of CAM, AZM, LCM, CLDM and compound 1 (MIC; μg ml^{-1})[a]

No.	*Test organism*[b]	*Characteristics*	*CAM*	*AZM*	*LCM*	*CLDM*	***1***
1	*Streptococcus pneumoniae* DP1 type I	Susceptible	0.03	0.06	1	0.13	0.13
2	*S. pneumoniae* #2	Susceptible	0.03	0.03	1	0.13	0.06
3	*S. pneumoniae* #3	Susceptible	0.015	0.03	0.25	0.13	0.06
4	*S. pneumoniae* #4	*ermB methylase* (c)	>128	>128	>128	>128	64
5	*S. pneumoniae* #5	*ermB methylase* (c)	>128	>128	>128	>128	32
6	*S. pneumoniae* #6	*ermB methylase* (c)+*mefE*	>128	>128	>128	>128	128
7	*S. pneumoniae* #7	*ermB methylase* (i)	>128	>128	128	>128	16
8	*S. pneumoniae* #8	*ermB methylase* (i)	>128	>128	128	>128	16
9	*S. pneumoniae* #9	*mefE efflux*	0.5	0.5	1	0.13	0.06
10	*S. pneumoniae* #10	*mefE efflux*	0.5	0.5	1	0.13	0.06
11	*Streptococcus pyogenes* Cook	Susceptible	0.015	0.06	0.13	0.13	0.06
12	*S. pyogenes* #2	*ermB methylase* (c)	>128	>128	>128	>128	4
13	*S. pyogenes* #3	*mefE efflux*	8	8	0.25	0.13	0.13
14	*Haemophilus influenzae* #1	Susceptible	2	0.25	8	8	16
15	*H. influenzae* #2	Susceptible	4	1	16	8	16
16	*H. influenzae* #3	Susceptible	8	2	16	32	64

Abbreviations: AZM, azithromycin; c, constitutive; CAM, clarithromycin; CLDM, clindamycin; i, inducible; LCM, lincomycin; MIC, minimum inhibitory concentration.
[a]Gray shading strains are target strains.
[b]All strains except standard organisms were clinically isolated.



Scheme 1 Synthesis of (7*S*)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-7-deoxylincomycin derivatives. Reagents: (a) ArSH, diethyl azodicarboxylate (DEAD), PPh_3 and tetrahydrofuran; (b) 1 N HCl and MeOH.



Scheme 2 Synthesis of (7*S*)-7-(5-aryl-1,3,4-thiadiazol-2-yl-thio)-7-deoxylincomycin derivatives. Reagents: (a) 5-(2-aminopyridin-3-yl)-1,3,4-thiadiazole-2-thiol, K_2CO_3 and DMF; (b) 1 N HCl and MeOH; (c) KSAc and DMF; (d) 2 N HCl and MeOH; (e) NaOMe and MeOH; (f) methyl 2-(5-chloro-1,3,4-thiadiazol-2-yl) benzoate, NaHMDS and DMF; (g) 2 N NaOH and MeOH; (h) NH_3 for **27**, $HNMe_2$ for **28**, WSC, HOBt and DMF; (i) $SnCl_2$, $NaBH_4$ and EtOH.

the Mitsunobu reaction is robust, 5-(2-aminopyridin-3-yl)-1,3,4-thiadiazole-2-thiol (a side chain thiol of **24**) did not give a desired condensation product. In this case, the thiol was reacted with (7*R*)-7-*O*-methanesulfonyllincomycin (**23**)[10–12] in a basic condition to give **24** after an acid treatment (Scheme 2). Compounds **27** and **28** were synthesized by an S_NAr reaction of (7*S*)-7-deoxy-7-mercaptolincomyicn (**25**)[11] and methyl 2-(5-chloro-1,3,4-thiadiazol-2-yl)benzoate followed by hydrolysis of methyl ester (**26**) and condensation of the corresponding amines. A nitro group of compounds **9**, **10** and **11** were converted to an amino group by stannous chloride and sodium borohydride to give compounds **29**, **30** and **31**, respectively.

RESULTS AND DISCUSSION

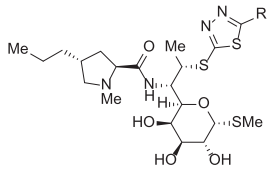
We reported that compound **1** exhibited weak antibacterial activities against *S. pneumoniae* and *S. pyogenes* with *erm* gene, although CLDM did not show any activities against those pathogens.[10] To enhance the antibacterial activities of compound **1**, we first changed the benzene ring of compound **1** to other aryl and hetero aryl groups as shown in Table 2. Compound **3** having a 2-naphtyl group showed weak antibacterial activities against most of tested pathogens probably due to bulkiness of the substituent based on our three-dimensional analysis.[11] As for pyridine analogs, antibacterial activities of compounds **4** and **5** against *erm*-resistant *S. pneumoniae* were comparable to those of compound **1**, but compound **6** having a 4-pyridyl group showed decreased activities against those pathogens. Compounds **7** and **8** possessing a thienyl group or a furanyl group showed comparable antibacterial activities against *S. pneumoniae* to compound **1**, but decreased activities against *S. pyogenes* with *erm* gene. On the basis of the results obtained in the above, we performed further optimization focusing on substituents on the benzene ring.

To determine the optimal site of a substituent on the phenyl group, we investigated compounds having a nitro group or an amino group as shown in Table 3. As a matter of fact, compounds having a nitro or an amino group at the *ortho* position (compounds **9** and **29**) exhibited clearly enhanced antibacterial activities against *S. pneumoniae* with *erm* gene. Similarly, compounds with those groups at the *meta* position improved the activities (compounds **10** and **30**) but the enhancement effect of the *meta* substitution seemed to be less than that of the *ortho* substitution. Antibacterial activities of compounds with a *para* substituted phenyl group were comparable to those of compound **1** against *S. pneumoniae* with *erm* gene but stronger than those of compound **1** against *S. pneumoniae* with *mef* gene. On the other hand, the position of a substituent did not significantly affect antibacterial activities against *S. pyogenes*.

Our finding concerning the *ortho* substitution at the benzene ring encouraged us to replace the benzene ring with other hetero aromatic rings. Antibacterial activities of compounds having a pyrazole, a pyridine or a pyrazine ring with a nitro or an amino group are shown in Table 4. Although compound **14** showed improved antibacterial activities against *S. pneumoniae* with *erm* gene, its antibacterial activities were limited.

We finally examined other substituents on the benzene ring instead of a nitro or an amino group as shown in Table 5. Compounds **16**, **18** and **19** have an electron donating group at the *ortho* position of the benzene ring. Among them, compound **16** exhibited comparable antibacterial activities to compounds **9** and **29** against *S. pneumoniae*

Table 2 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



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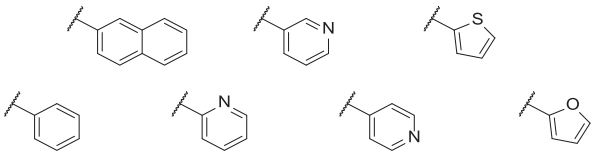


No.	Test organism[b]	Characteristics	1	3	4	5	6	7	8	CLDM
1	*Streptococcus pneumoniae* DP1 TypeI	susceptible	0.13	0.5	0.06	0.06	0.06	0.06	0.03	0.13
2	*S. pneumoniae* #2	susceptible	0.06	0.5	0.06	0.06	0.13	0.06	0.03	0.13
3	*S. pneumoniae* #3	susceptible	0.06	0.5	0.06	0.03	0.06	0.06	0.03	0.13
4	*S. pneumoniae* #4	*ermB methylase* (c)	64	64	8	32	>64	16	32	>128
5	*S. pneumoniae* #5	*ermB methylase* (c)	32	64	16	32	>64	64	128	>128
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	128	64	128	64	>64	128	128	>128
7	*S. pneumoniae* #7	*ermB methylase* (i)	16	64	32	32	64	32	32	>128
8	*S. pneumoniae* #8	*ermB methylase* (i)	16	64	32	32	64	64	32	>128
9	*S. pneumoniae* #9	*mefE efflux*	0.06	0.25	0.06	0.03	0.06	0.06	0.03	0.13
10	*S. pneumoniae* #10	*mefE efflux*	0.06	0.5	0.06	0.03	0.06	0.06	0.03	0.13
11	*Streptococcus pyogenes* Cook	susceptible	0.06	0.5	0.03	0.03	0.03	0.06	0.03	0.13
12	*S. pyogenes* #2	*ermB methylase* (c)	4	32	4	16	16	16	16	>128
13	*S. pyogenes* #3	*mefE efflux*	0.13	0.5	0.13	4	0.13	0.06	0.06	0.13
14	*Haemophilus influenzae* #1	susceptible	16	128	16	16	16	32	32	8
15	*H. influenzae* #2	susceptible	16	32	8	16	8	16	16	8
16	*H. influenzae* #3	susceptible	64	128	64	64	64	128	>128	32

Abbreviations: c, constitutive; CLDM, clindamycin; i, inducible; MIC, minimum inhibitory concentration.
[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.
[b]All strains except standard organisms were clinically isolated.

and *S. pyogenes* with *erm* gene. Among the compounds with an electron withdrawing group, compounds **27** and **22** showed comparable antibacterial activities against *S. pneumoniae* and *S. pyogenes* with *erm* gene to compounds **9** and **29**.

CONCLUSIONS

In summary, we identified compounds **9**, **16**, **22**, **27** and **29**, which exhibited improved antibacterial activities against *S. pneumoniae* and *S. pyogenes* with *erm* gene by chemical modification of (7*S*)-7-deoxy-7-(5-phenyl-1,3,4-thiadiazol-2-yl-thio)lincomycin (**1**). These results indicate that a (7*S*)-7-deoxy-7-[5-(*ortho*-substituted-phenyl)-1,3,4-thiadiazol-2-yl-thio]lincomycin analog is a promising framework to overcome resistant *S. pneumoniae* and *S. pyogenes*. Further structural optimizations are in progress.

EXPERIMENTAL PROCEDURE

General

^{1}H nuclear magnetic resonance (NMR) spectra were measured with Varian Gemini-300 (Varian, Palo Alto, CA, USA) for 300 MHz, JEOL JNM-GSX 400 (JEOL, Tokyo, Japan) for 400 MHz or BRUKER Ascend 400 NMR spectrometer (BRUKER Corporation, Coventry, UK) for 400 MHz in $CDCl_3$ or CD_3OD with 0.03% tetramethylsilane as an internal standard. ^{13}C NMR spectra were measured with BRUKER Ascend 400 NMR spectrometer (BRUKER Corporation) for 100 MHz. Mass spectra were obtained on a JEOL JMS-FABmate spectrometer or JEOL JMS-700 mass spectrometer or Agilent Technologies 6530-Q-TOF LC/MS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The optical rotations were recorded with Jasco P-2300 digital polarimeter (Jasco, Tokyo, Japan). The melting points were measured with Yanaco MP-S (Yanaco, Tokyo, Japan). The infrared (IR) spectra were measured with Jasco FT/IR-410 (Jasco, Tokyo, Japan). Column chromatography was performed with silica gel 60N (Kanto Chemical, Tokyo, Japan; spherical, neutral).

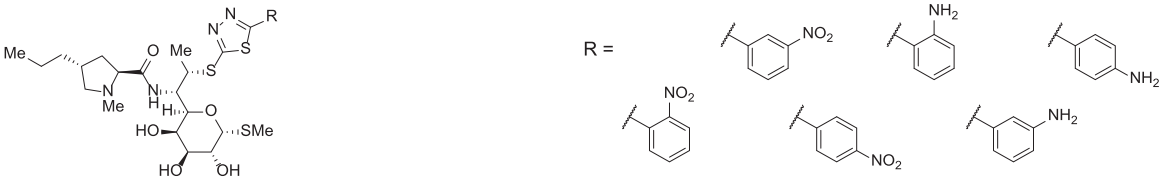
(7*S*)-7-Deoxy-7-[5-(2-naphtyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (3)

To a solution of compound **2** (240 mg, 0.39 mmol) in tetrahydrofuran (5 ml) at 0 °C were added triphenylphosphine (160 mg, 0.61 mmol) and diethylazodicarboxylate (0.1 ml, 0.55 mmol) and stirred at 0 °C for 30 min, and 5-(naphthalen-2-yl)-1,3,4-thiadiazole-2-thiol (130 mg, 0.53 mmol) was added and stirred overnight at room temperature. The mixture was concentrated *in vacuo* and added MeOH (5 ml), 1 N HCl (0.5 ml) and stirred at room temperature for 30 min and concentrated *in vacuo*. The resulting residue was dissolved in water and washed with diethyl ether. To the mixture was added $NaHCO_3$, and the mixture was extracted with ethyl acetate. The organic phase was washed with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was purified by preparative thin-layer chromatography ($CHCl_3$/CH_3OH/28% aq NH_4OH = 20/1/0.1) to afford **3** (22.3 mg, 9%) as colorless solid. $[\alpha]_D^{27}$ +64° (*c* 0.79, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (d, J = 8.8 Hz, 1H), 8.31 (s, 1H), 8.04–8.08 (m, 1H), 7.87–8.02 (m, 3H), 7.56–7.64 (m, 2H), 5.37 (d, J = 5.8 Hz, 1H), 3.99–4.48 (m, 1H), 4.31–4.37 (m, 1H), 4.28 (d, J = 10.4 Hz, 1H), 4.16 (dd, J = 9.9, 6.0 Hz, 1H), 3.68–3.74 (m, 1H), 3.54–3.64 (m, 1H), 3.42 (dd, J = 7.7, 5.5 Hz, 1H), 3.11 (dd, J = 9.9, 4.7 Hz, 1H), 2.41 (s, 3H), 2.19 (s, 3H), 2.06–2.18 (m, 2H), 1.86–2.02 (m, 2H), 1.58 (d, J = 6.9 Hz, 1H), 1.24–1.42 (m, 4H) and 0.86–0.99 (m, 3H); MS (FAB) *m/z* 633 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{30}H_{41}N_4O_5S_3$ 633.2234, found 633.2235 $(M+H)^+$.

(7*S*)-7-Deoxy-7-[5-(2-pyridyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (4)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(pyridin-2-yl)-1,3,4-thiadiazole-2-thiol (100 mg, 0.51 mmol) gave **4** as a colorless solid in 11% yield by a similar procedure to **3**. $[\alpha]_D^{27}$ +140° (*c* 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (d, J = 9.1 Hz, 1H), 8.63–8.67 (m, 1H), 8.28 (d, J = 8.0 Hz, 1H),

Table 3 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



No.	Test organism[b]	Characteristics	9	10	11	29	30	31
1	*Streptococcus pneumoniae* DP1 TypeI	susceptible	0.06	0.06	0.13	0.13	0.06	0.03
2	*S. pneumoniae* #2	susceptible	0.06	0.13	0.13	0.13	0.06	0.06
3	*S. pneumoniae* #3	susceptible	0.13	0.06	0.03	0.03	0.03	0.03
4	*S. pneumoniae* #4	*ermB methylase* (c)	4	16	32	8	16	16
5	*S. pneumoniae* #5	*ermB methylase* (c)	8	16	64	8	16	64
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	64	64	128	32	128	128
7	*S. pneumoniae* #7	*ermB methylase* (i)	4	8	16	4	16	16
8	*S. pneumoniae* #8	*ermB methylase* (i)	4	8	16	4	16	32
9	*S. pneumoniae* #9	*mefE efflux*	0.06	0.13	0.015	0.03	0.06	0.015
10	*S. pneumoniae* #10	*mefE efflux*	0.06	0.13	0.06	0.13	0.06	0.008
11	*Streptococcus pyogenes* Cook	susceptible	0.06	0.13	0.13	0.13	0.06	0.03
12	*S. pyogenes* #2	*ermB methylase* (c)	4	8	8	4	8	8
13	*S. pyogenes* #3	*mefE efflux*	0.13	0.13	0.06	0.13	0.06	0.03
14	*Haemophilus influenzae* #1	susceptible	16	32	8	16	32	16
15	*H. influenzae* #2	susceptible	8	8	4	8	8	8
16	*H. influenzae* #3	susceptible	64	64	32	32	64	64

Abbreviations: c, constitutive; i, inducible; MIC, minimum inhibitory concentration.
[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.
[b]All strains except standard organisms were clinically isolated.

7.84–7.91 (m, 1H), 7.38–7.44 (m, 1H), 5.36 (d, J=5.5 Hz, 1H), 5.29 (m, 1H), 4.32–4.47 (m, 2H), 4.25 (d, J=9.9 Hz, 1H), 4.10–4.19 (m, 1H), 3.69–3.74 (m, 1H), 3.55–3.64 (m, 1H), 3.34–3.41 (m, 1H), 3.10 (dd, J=10.3, 4.8 Hz, 1H), 2.71–2.79 (m, 1H), 2.41 (s, 3H), 2.15 (s, 3H), 2.06–2.14 (m, 2H), 1.88–2.00 (m, 2H), 1.57 (d, J=6.9 Hz, 3H), 1.28–1.41 (m, 4H) and 0.88–0.97 (m, 3H); MS (FAB) *m/z* 584 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{38}N_5O_5S_3$ 584.2030, found 584.2032 (M+H)$^+$.

(7*S*)-7-Deoxy-7-[5-(3-pyridyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (5)
Reaction of **2** (240 mg, 0.39 mmol) with 5-(pyridin-3-yl)-1,3,4-thiadiazole-2-thiol (100 mg, 0.51 mmol) gave **5** as a colorless solid in 37% yield by a similar procedure to **3**. $[\alpha]_D^{27}$ +105° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.09 (s, 1H), 9.05 (d, J=8.0 Hz, 1H), 8.72–8.78 (m, 1H), 8.24 (d, J=8.2 Hz, 1H), 7.42–7.51 (m, 1H), 5.36 (d, J=5.8 Hz, 1H), 5.31 (br s, 1H), 4.31–4.50 (m, 2H), 4.24 (d, J=9.9 Hz, 1H), 4.09–4.19 (m, 2H), 3.69–3.75 (m, 1H), 3.51–3.61 (m, 1H), 3.36–3.44 (m, 1H), 3.07–3.14 (m, 1H), 2.41 (s, 3H), 2.18 (s, 3H), 2.04–2.16 (m, 2H), 1.84–2.02 (m, 2H), 1.57 (d, J=6.9 Hz, 3H), 1.28–1.38 (m, 4H) and 0.87–0.96 (m, 3H); MS (FAB) *m/z* 584 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{38}N_5O_5S_3$ 584.2030, found 584.2027 (M+H)$^+$.

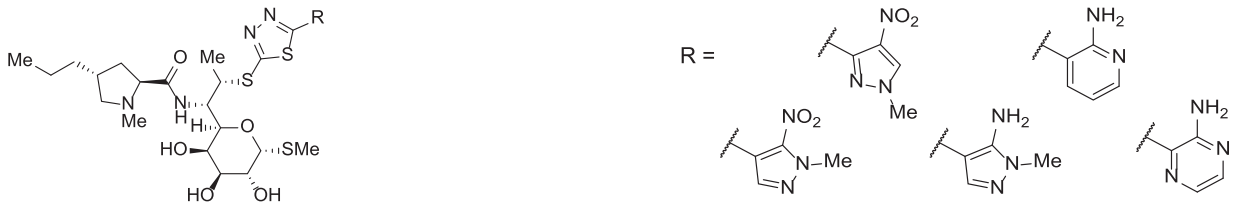
(7*S*)-7-Deoxy-7-[5-(4-pyridyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (6)
Reaction of **2** (240 mg, 0.39 mmol) with 5-(pyridin-4-yl)-1,3,4-thiadiazole-2-thiol (100 mg, 0.51 mmol) gave **6** as a colorless solid in 48% yield by a similar procedure to **3**. $[\alpha]_D^{27}$ +124° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (d, J=8.8 Hz, 1H), 8.75–8.82 (m, 2H), 7.73–7.79 (m, 2H), 5.36 (d, J=5.5 Hz, 1H), 5.27 (br s, 1H), 4.44–4.54 (m, 1H), 4.39 (qd, J=6.9, 3.3 Hz, 1H), 4.25 (d, J=10.2 Hz, 1H), 4.17 (dd, J=10.2, 5.5 Hz, 1H), 3.77 (br s, 1H), 3.59 (dd, J=10.2, 3.0 Hz, 1H), 3.43–3.50 (br s 1H), 2.51 (br s, 3H), 2.16–2.29 (m, 2H), 2.15 (s, 3H), 1.89–2.09 (m, 2H), 1.57 (d, J=6.9 Hz, 3H), 1.24–1.43 (m, 4H) and 0.87–0.96 (m, 3H); MS (FAB) *m/z* 584 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{38}N_5O_5S_3$ 584.2030, found 584.2032 (M+H)$^+$.

(7*S*)-7-Deoxy-7-[5-(2-thienyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (7)
Reaction of **2** (240 mg, 0.39 mmol) with 5-(thiophen-2-yl)-1,3,4-thiadiazole-2-thiol (100 mg, 0.50 mmol) gave **7** as a colorless solid in 17% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +90° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.96 (d, J=8.8 Hz, 1H), 7.48–7.54 (m, 2H), 7.11–7.16 (m, 1H), 5.34 (d, J=5.5 Hz, 1H), 5.31 (br s, 1H), 4.36–4.46 (m, 1H), 4.22–4.33 (m, 2H), 4.15 (dd, J=10.0, 5.5 Hz, 1H), 3.68–3.74 (m, 1H), 3.58 (dd, J=10.0, 3.4 Hz, 1H), 3.38 (dd, J=7.7, 5.5 Hz, 1H), 3.07 (dd, J=10.2, 4.9 Hz, 1H), 2.37 (s, 3H), 2.17 (s, 3H), 1.86–2.15 (m, 4H), 1.53 (d, J=6.9 Hz, 3H), 1.28–1.39 (m, 4H) and 0.86–0.96 (m, 3H); MS (FAB) *m/z* 589 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}N_4O_5S_4$ 589.1641, found 589.1646 (M+H)$^+$.

(7*S*)-7-Deoxy-7-[5-(2-furanyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (8)
Reaction of **2** (240 mg, 0.39 mmol) with 5-(furan-2-yl)-1,3,4-thiadiazole-2-thiol (100 mg, 0.54 mmol) gave **8** as a colorless solid in 38% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +88° (*c* 1.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, J=8.5 Hz, 1H), 7.58–7.62 (m, 1H), 7.13–7.17 (m, 1H), 6.57–6.63 (m, 1H), 5.34 (d, J=5.5 Hz, 1H), 5.26 (br s, 1H), 4.35–4.44 (m, 1H), 4.31 (qd, J=6.9, 3.3 Hz, 1H), 4.24 (d, J=10.2 Hz, 1H), 4.11–4.21 (m, 2H), 3.67–3.74 (m, 1H), 3.53–3.64 (m, 2H), 3.47 (s, 1H), 3.29–3.39 (m, 1H), 3.06 (dd, J=10.0, 4.5 Hz, 1H), 2.36 (s, 3H), 2.14 (s, 3H), 2.02–2.11 (m, 2H), 185–1.99 (m, 2H), 1.51 (d, J=6.9 Hz, 3H), 1.11–1.40 (m, 4H) and 0.82–0.97 (m, 3H); MS (FAB) *m/z* 573 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}N_4O_6S_3$ 573.1870, found 573.1870 (M+H)$^+$.

(7*S*)-7-Deoxy-7-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-ylthio] lincomycin (9)
Reaction of **2** (320 mg, 0.51 mmol) with 5-(2-nitrophenyl)-1,3,4-thiadiazole-2-thiol (160 mg, 0.67 mmol) gave **9** as a colorless solid in 54% yield by a similar

Table 4 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



No.	Test organism[b]	Characteristics	12	13	14	24	15
1	*Streptococcus pneumoniae* DP1 TypeI	susceptible	0.03	0.03	0.03	0.03	0.015
2	*S. pneumoniae* #2	susceptible	0.03	0.03	0.03	0.06	0.03
3	*S. pneumoniae* #3	susceptible	0.03	0.015	0.03	0.03	0.015
4	*S. pneumoniae* #4	*ermB methylase* (c)	32	2	8	4	8
5	*S. pneumoniae* #5	*ermB methylase* (c)	64	32	8	16	16
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	128	128	64	64	64
7	*S. pneumoniae* #7	*ermB methylase* (i)	16	16	4	ND	4
8	*S. pneumoniae* #8	*ermB methylase* (i)	32	8	4	8	8
9	*S. pneumoniae* #9	*mefE efflux*	0.03	0.03	0.03	0.06	≦0.008
10	*S. pneumoniae* #10	*mefE efflux*	0.06	0.03	0.03	0.06	0.015
11	*Streptococcus pyogenes* Cook	susceptible	0.03	0.03	0.03	0.06	0.015
12	*S. pyogenes* #2	*ermB methylase* (c)	8	8	4	8	4
13	*S. pyogenes* #3	*mefE efflux*	0.03	0.03	0.06	0.06	0.03
14	*Haemophilus influenzae* #1	susceptible	32	32	8	16	ND
15	*H. influenzae* #2	susceptible	16	16	4	16	4
16	*H. influenzae* #3	susceptible	64	64	32	64	32

Abbreviations: c, constitutive; i, inducible; MIC, minimum inhibitory concentration; ND, not determined.
[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.
[b]All strains except standard organisms were clinically isolated.

procedure to **3**. mp 235–240 °C (decomp.); $[\alpha]_D^{30}$ +91° (*c* 0.52, $CHCl_3$); IR (KBr) 3399, 2922, 1654 and 1533 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (d, *J* = 8.0 Hz, 1H), 7.68–7.79 (m, 1H), 7.68–7.79 (m, 3H), 5.36 (d, *J* = 5.5 Hz, 1H), 4.39–4.49 (m, 1H), 4.20–4.38 (m, 2H), 4.15 (dd, *J* = 9.9, 5.5 Hz, 1H), 3.71 (br s, 1H), 3.53–3.61 (m, 1H), 3.31–3.38 (m, 1H), 3.09 (dd, *J* = 10.3, 4.8 Hz, 1H), 2.40 (s, 3H), 2.19 (s, 3H), 2.03–2.16 (m, 4H), 1.57 (d, *J* = 7.1 Hz, 3H), 1.24–1.40 (m, 4H) and 0.86–0.96 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.1, 164.9, 163.5, 148.5, 132.9, 132.0, 131.6, 124.9, 123.8, 89.1, 71.8, 71.0, 69.2, 68.4, 68.2, 62.5, 53.0, 44.9, 41.7, 38.1, 37.9, 35.7, 21.5, 18.5, 14.8 and 14.2; MS (FAB) *m/z* 628 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{38}N_5O_7S_3$ 628.1928, found 628.1934 $(M+H)^+$.

(7*S*)-7-Deoxy-7-[5-(3-nitrophenyl)-1,3,4-thiadiazol-2-ylthio] lincomycin (10)

Reaction of **2** (320 mg, 0.51 mmol) with 5-(3-nitrophenyl)-1,3,4-thiadiazole-2-thiol (160 mg, 0.67 mmol) gave **10** as a colorless solid in 41% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +85° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (d, *J* = 9.1 Hz, 1H), 8.07–8.75 (m, 1H), 8.35–8.42 (m, 1H), 8.24–8.29 (m, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 5.36 (d, *J* = 5.2 Hz, 1H), 5.31 (br s, 1H), 4.34–4.52 (m, 2H), 4.23 (d, *J* = 10.2 Hz, 1H), 4.15 (dd, *J* = 10.0, 5.4 Hz, 1H), 3.69–3.75 (m, 1H), 3.57 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.36–3.44 (m, 1H), 3.11 (dd, *J* = 10.0, 4.8 Hz, 1H), 2.43 (s, 3H), 2.18 (s, 3H), 2.08–2.17 (m, 2H), 1.90–2.03 (m, 2H), 1.58 (d, *J* = 6.9 Hz, 3H), 1.24–1.44 (m, 4H) and 0.88–0.99 (m, 3H); MS (FAB) *m/z* 628 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{38}N_5O_7S_3$ 628.1928, found 628.1938 $(M+H)^+$.

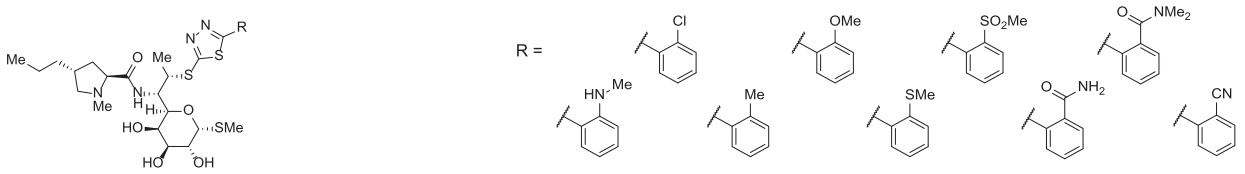
(7*S*)-7-Deoxy-7-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-ylthio] lincomycin (11)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(4-nitrophenyl)-1,3,4-thiadiazole-2-thiol (120 mg, 0.50 mmol) gave **11** as a colorless solid in 33% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +67° (*c* 0.81, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (d, *J* = 9.1 Hz, 1H), 8.35 (d, *J* = 8.5 Hz, 2H), 8.08 (d, *J* = 8.5 Hz, 2H), 5.34 (d, *J* = 5.5 Hz, 1H), 4.34–4.51 (m, 2H), 4.22 (d, *J* = 9.9 Hz, 1H), 4.16 (dd, *J* = 10.3, 5.6 Hz, 1H), 3.72 (d, *J* = 3.0 Hz, 1H), 3.58 (dd, *J* = 10.0, 3.3 Hz, 1H), 3.33–3.39 (m, 1H), 3.11 (dd, *J* = 10.0, 4.5 Hz, 1H), 2.41 (s, 3H), 2.14 (s, 3H), 2.06–2.12 (m, 2H), 1.86–2.04 (m, 2H), 1.57 (d, *J* = 6.9 Hz, 3H), 1.23–1.38 (m, 4H) and 0.85–0.94 (m, 3H); MS (FAB) *m/z* 628 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{38}N_5O_7S_3$ 628.1928, found 628.1927 $(M+H)^+$.

(7*S*)-7-Deoxy-7-[5-(1-methyl-5-nitro-1*H*-pyrazol-4-yl)-1,3,4-thiadiazol-2-ylthio]lincomycin (12)

Reaction of **2** (280 mg, 0.45 mmol) with 5-(1-methyl-5-nitro-1*H*-pyrazol-4-yl)-1,3,4-thiadiazole-2-thiol (120 mg, 0.49 mmol) gave **12** as a colorless solid in 35% yield by a similar procedure to **3**. $[\alpha]_D^{31}$ +51° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.89 (d, *J* = 8.8 Hz, 1H), 5.34 (d, *J* = 5.5 Hz, 1H), 5.27 (br s, 1H), 4.37–4.48 (m, 2H), 4.23 (d, *J* = 10.4 Hz, 1H), 4.14 (dd, *J* = 10.0, 5.5 Hz, 1H), 4.08 (s, 3H), 3.70 (br s, 1H), 3.53–3.62 (m, 1H), 3.32–3.40 (m, 1H), 3.09 (dd, *J* = 10.3, 4.8 Hz, 1H), 2.40 (s, 3H), 2.17 (s, 3H), 1.85–2.15 (m, 4H), 1.55 (d, *J* = 6.9 Hz, 1H), 1.25–1.39 (m, 4H) and 0.85–0.96 (m, 3H); MS (FAB) *m/z* 632 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}N_7O_7S_3$ 632.1989, found 632.1991 $(M+H)^+$.

Table 5 Antibacterial activities of novel lincomycin derivatives (MIC; μg ml^{-1})[a]



No.	Test organism[b]	characteristics	16	17	18	19	20	21	27	28	22
1	*Streptococcus pneumoniae* DP1 TypeI	susceptible	0.06	0.13	0.13	0.25	0.13	0.06	0.06	0.06	0.06
2	*S. pneumoniae* #2	susceptible	0.13	0.25	0.13	0.25	0.13	0.13	0.06	0.06	0.06
3	*S. pneumoniae* #3	susceptible	0.13	0.25	0.13	0.25	0.13	0.13	0.06	0.06	0.06
4	*S. pneumoniae* #4	*ermB methylase* (c)	ND	128	4	64	16	64	8	16	1
5	*S. pneumoniae* #5	*ermB methylase* (c)	8	128	8	16	16	16	8	16	4
6	*S. pneumoniae* #6	*ermB methylase* (c) + *mefE*	32	>128	128	128	128	128	32	64	32
7	*S. pneumoniae* #7	*ermB methylase* (i)	4	16	8	16	16	16	1	2	4
8	*S. pneumoniae* #8	*ermB methylase* (i)	4	32	N.D.	16	16	16	1	2	4
9	*S. pneumoniae* #9	*mefE efflux*	0.06	0.13	0.06	0.13	0.06	0.06	0.03	0.015	0.06
10	*S. pneumoniae* #10	*mefE efflux*	0.13	0.13	0.13	0.06	0.13	0.13	0.06	0.06	0.06
11	*Streptococcus pyogenes* Cook	susceptible	0.13	0.13	0.13	0.25	0.13	0.06	0.06	0.06	0.06
12	*S. pyogenes* #2	*ermB methylase* (c)	4	32	8	16	16	8	4	4	4
13	*S. pyogenes* #3	*mefE efflux*	0.13	0.25	0.13	0.13	0.25	0.13	0.06	0.06	0.13
14	*Haemophilus influenzae* #1	susceptible	32	128	32	64	128	16	16	16	16
15	*H. influenzae* #2	susceptible	16	128	16	32	32	16	16	16	16
16	*H. influenzae* #3	susceptible	32	>128	64	128	128	64	32	64	64

Abbreviations: c, constitutive; i, inducible; MIC, minimum inhibitory concentration; ND, not determined.
[a]All antibacterial evaluations were performed as hydrochloride. Gray shading strains are target strains.
[b]All strains except standard organisms were clinically isolated.

(7*S*)-7-Deoxy-7-[5-(1-methyl-4-nitro-1*H*-pyrazol-3-yl)-1,3,4-thiadiazol-2-ylthio]lincomycin (13)

Reaction of **2** (280 mg, 0.45 mmol) with 5-(1-methyl-4-nitro-1*H*-pyrazol-3-yl)-1,3,4-thiadiazole-2-thiol (120 mg, 0.49 mmol) gave **13** as a colorless solid in 18% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +79° (*c* 0.52, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (d, J=8.8 Hz, 1H), 8.33 (s, 1H), 5.35 (d, J=5.5 Hz, 1H), 5.30 (br s, 1H), 4.37–4.49 (m, 2H), 4.23 (d, J=10.4 Hz, 1H), 4.10–4.20 (m, 2H), 4.08 (s, 3H), 3.67–3.76 (m 2H), 3.52–3.63 (m, 2H), 3.49 (s, 1H), 3.29–3.44 (m, 2H), 3.09 (dd, J=10.3, 4.3 Hz, 1H), 2.83–2.95 (m, 1H), 2.69 (d, J=7.7 Hz, 1H), 2.41 (s, 3H), 2.14 (s, 3H), 2.04–2.13 (m, 2H), 1.79–2.00 (m, 2H), 1.56 (d, J=7.1 Hz, 3H), 1.27–1.38 (m, 4H) and 0.86–0.95 (m, 3H); MS (FAB) *m/z* 632 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}N_7O_7S_3$ 632.1989, found 632.1982 (M+H)$^+$.

(7*S*)-7-[5-(5-Amino-1-methyl-1*H*-pyrazol-4-yl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (14)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(5-amino-1-methyl-1*H*-pyrazol-4-yl)-1,3,4-thiadiazole-2-thiol (115 mg, 0.54 mmol) gave **14** as a colorless solid in 65% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +88° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (d, J=8.2 Hz, 1H), 7.46 (s, 1H), 5.38 (br s, 1H), 5.34 (d, J=5.5 Hz, 1H), 5.23 (br s, 1H), 4.33–4.44 (m, 1H), 4.21–4.32 (m, 2H), 4.14 (dd, J=10.0, 5.5 Hz, 1H), 3.70 (s, 3H), 3.55 (m, 1H), 3.26–3.33 (m, 1H), 3.07 (dd, J=10.0, 4.5 Hz, 1H), 2.35 (s, 3H), 2.15 (s, 3H), 2.03–2.14 (m, 2H), 1.85–2.01 (m, 2H), 1.49 (d, J=7.1 Hz, 3H), 1.25–1.38 (m, 4H) and 0.86–0.97 (m, 3H); MS (FAB) *m/z* 602 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{40}N_7O_5S_3$ 602.2248, found 602.2243 (M+H)$^+$.

(7*S*)-7-[5-(3-Aminopyrazin-2-yl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (15)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(3-aminopyrazin-2-yl)-1,3,4-thiadiazole-2-thiol (140 mg, 0.66 mmol) gave **15** as a colorless solid in 59% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +62° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (d, J=8.8 Hz, 1H), 8.11 (d, J=2.4 Hz, 1H), 7.94 (d, J=2.4 Hz, 1H), 5.34 (d, J=5.5 Hz, 1H), 5.20 (br s, 1H), 4.33–4.52 (m, 2H), 4.22 (d, J=10.2 Hz, 1H), 4.17 (dd, J=10.5, 5.5 Hz, 1H), 3.67–3.75 (m, 1H), 3.60 (dd, J=10.2, 3.3 Hz, 1H), 3.23–3.32 (m, 1H), 3.09 (dd, J=10.0, 4.5 Hz, 1H), 2.39 (s, 3H), 2.10 (s, 3H), 1.84 (m, 2H), 1.56 (d, J=6.9 Hz, 3H), 1.20–1.40 (m, 4H) and 0.85–0.96 (m, 3H); MS (FAB) *m/z* 600 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{24}H_{38}N_7O_5S_3$ 600.2091, found 600.2092 (M+H)$^+$.

(7*S*)-7-Deoxy-7-{5-[2-(methylamino)phenyl]-1,3,4-thiadiazol-2-ylthio}lincomycin (16)

Reaction of **2** (160 mg, 0.26 mmol) with 5-[2-(methylamino)phenyl]-1,3,4-thiadiazole-2-thiol (100 mg, 0.45 mmol) gave **16** as a colorless solid in 22% yield by a similar procedure to **3**. $[\alpha]_D^{31}$ +55° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (d, J=9.1 Hz, 1H), 8.09–8.21 (m, 1 H), 7.27–7.45 (m, 2H), 6.63–6.85 (m, 2H), 5.35 (d, J=5.5 Hz, 1H), 5.26 (br s, 1H), 4.38–4.51 (m, 1H), 4.22–4.36 (m, 2H), 4.17 (dd, J=9.9, 5.5 Hz, 1H), 3.68–3.77 (m, 1H), 3.60 (dd, J=10.0, 3.4 Hz, 1H), 3.24–3.36 (m, 1H), 3.04–3.13 (m, 1H), 3.00 (d, J=4.9 Hz, 3H), 2.38 (s, 3H), 2.14 (s, 3H), 1.88–2.12 (m, 4H), 1.54 (d, J=6.9 Hz, 3H), 1.25–1.40 (m, 4H) and 0.85–0.97 (m, 3H); MS (FAB) *m/z* 612 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{42}N_5O_5S_3$ 612.2343, found 612.2339 (M+H)$^+$.

(7*S*)-7-[5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (17)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(2-chlorophenyl)-1,3,4-thiadiazole-2-thiol (100 mg, 0.44 mmol) gave **17** as a colorless solid in 34% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +102° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (d, J=9.1 Hz, 1H), 8.25–8.33 (m, 1H), 7.37–7.60 (m, 3H), 5.35 (d, J=5.5 Hz, 1H), 5.30 (br s, 1H), 4.21–4.44 (m, 5H), 3.66–3.75 (m, 1H), 3.51–3.64 (m, 2H), 3.30–3.43 (m, 1H), 3.29–3.39 (m, 1H), 3.09 (dd, J=10.2, 4.7 Hz, 1H), 2.39 (s, 3H), 2.16 (s, 3H), 2.02–2.13 (m, 2H), 185–2.00 (m, 2H), 1.54 (d, J=6.9 Hz, 3H), 1.19–1.42 (m, 4H) and 0.85–0.98 (m, 3H); MS (FAB) *m/z* 617 (M+H)$^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{38}ClN_4O_5S_3$ 617.1687, found 617.1691 (M+H)$^+$.

(7*S*)-7-Deoxy 7-[5-(*o*-tolyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (18)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(*o*-tolyl)-1,3,4-thiadiazole-2-thiol (150 mg, 0.72 mmol) gave **18** as a colorless solid in 22% yield by a similar procedure to **3**. $[\alpha]_D^{31}$ +88° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 9.20 (d, *J*=9.1 Hz, 1H), 7.64 (d, *J*=7.9 Hz, 1H), 7.28–7.44 (m, 3H), 5.40 (br s, 1H), 5.36 (d, *J*=5.5 Hz, 1H), 4.41–4.47 (m, 1H), 4.32 (qd, *J*=7.1, 3.4 Hz, 1H), 4.27 (d, *J*=10.3 Hz, 1H), 4.13–4.18 (m, 1H), 3.70–3.75 (m, 1H), 3.59 (dd, *J*=10.1, 3.5 Hz, 1H), 3.39 (dd, *J*=7.9, 5.4 Hz, 1H), 3.11 (dd, *J*=10.5, 4.5 Hz, 1H), 2.61 (s, 3H), 2.41 (s, 1H), 2.19 (s, 3H), 2.06–2.17 (m, 3H), 1.86–2.03 (m, 2H), 1.57 (d, *J*=7.1 Hz, 3H), 1.29–1.37 (m, 4H) and 0.85–0.96 (m, 3H); MS (FAB) *m/z* 597 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{41}N_4O_5S_3$ 597.2234, found 597.2238 $(M+H)^+$.

(7*S*)-7-Deoxy-7-[5-(2-methoxyphenyl)-1,3,4-thiadiazol-2-ylthio] lincomycin (19)

Reaction of **2** (240 mg, 0.39 mmol) with 5-(2-methoxyphenyl)-1,3,4-thiadiazole-2-thiol (130 mg, 0.58 mmol) gave **19** as a colorless solid in 48% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +114° (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 8.31 (dd, *J*=8.0, 1.6 Hz, 1H), 7.55 (ddd, *J*=8.0, 7.0, 1.6 Hz, 1H), 7.24 (d, *J*=8.0 Hz, 1H), 7.10–7.18 (m, 1H), 5.27 (d, *J*=5.7 Hz, 1H), 4.57 (dd, *J*=9.7, 3.2 Hz, 1H), 4.43 (d, *J*=9.7 Hz, 1H), 4.34 (qd, *J*=7.0, 3.1 Hz, 1H), 4.06–4.16 (m, 1H), 4.04 (s, 3H), 3.80–3.83 (m, 1H), 3.58 (dd, *J*=10.3, 3.2 Hz, 1H), 3.26 (dd, *J*=8.6, 6.0 Hz, 1H), 2.99 (dd, *J*=10.4, 5.0 Hz, 1H), 2.35 (s, 3H), 2.15–2.26 (m, 1H), 2.02–2.14 (m, 2H), 2.01 (s, 3H), 1.96–2.00 (m, 1H), 1.71–1.91 (m, 1H), 1.54 (d, *J*=7.0 Hz, 3H), 1.27–1.41 (m, 4H) and 0.86–0.95 (m, 3H); MS (FAB) *m/z* 613 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{41}N_4O_6S_3$ 613.2183, found 613.2174 $(M+H)^+$.

(7*S*)-7-Deoxy-7-{5-[2-(methylthio)phenyl]-1,3,4-thiadiazol-2-ylthio} lincomycin (20)

Reaction of **2** (240 mg, 0.39 mmol) with 5-[2-(methylthio)phenyl]-1,3,4-thiadiazole-2-thiol (150 mg, 0.62 mmol) gave **20** as a colorless solid in 44% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +141° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 9.13 (d, *J*=8.8 Hz, 1H), 8.00–8.06 (m, 1H), 7.42–7.54 (m, 2H), 7.29–7.37 (m, 1H), 5.35 (d, *J*=5.5 Hz, 1H), 4.37–4.47 (m, 1H), 4.22–4.35 (m, 2H), 4.07–4.22 (m, 2H), 3.72 (t, *J*=3.3 Hz, 1H), 3.57 (td, *J*=10.0, 3.3 Hz, 1H), 3.41 (dd, *J*=7.9, 5.4 Hz, 1H), 3.09 (dd, *J*=10.6, 4.6 Hz, 1H), 2.50 (s, 3H), 2.18 (s, 3H), 2.04–2.16 (m, 2H), 1.89–2.01 (m, 3H), 1.55 (d, *J*=7.1 Hz, 1H), 1.25–1.39 (m, 4H) and 0.84–0.97 (m, 3H); MS (FAB) *m/z* 629 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{41}N_4O_5S_4$ 629.1954, found 629.1960 $(M+H)^+$.

(7*S*)-7-Deoxy-7-{5-[2-(methylsulfonyl)phenyl]-1,3,4-thiadiazol-2-ylthio}lincomycin (21)

Reaction of **2** (240 mg, 0.39 mmol) with 5-[2-(methylsulfonyl)phenyl]-1,3,4-thiadiazole-2-thiol (120 mg, 0.44 mmol) gave **21** as a colorless solid in 32% yield by a similar procedure to **3**. $[\alpha]_D^{30}$ +73° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 8.22–8.26 (m, 1H), 7.82–7.87 (m, 2H), 7.69–7.73 (m, 1H), 5.28 (d, *J*=5.6 Hz, 1H), 4.63 (dd, *J*=9.8, 3.1 Hz, 1H), 4.51 (qd, *J*=6.9, 2.9 Hz, 1H), 4.45 (d, *J*=9.8 Hz, 1H), 4.12 (dd, *J*=10.3, 5.6 Hz, 1H), 3.80–3.84 (m, 1H), 3.56–3.64 (m, 2H), 3.34–3.39 (m, 2H), 3.25 (dd, *J*=8.5, 6.2 Hz, 1H), 3.00 (dd, *J*=10.4, 5.1 Hz, 1H), 2.40 (s, 3H), 2.16–2.27 (m, 1H), 2.02–2.10 (m, 3H), 2.02 (s, 3H), 1.80–1.90 (m, 1H), 1.59 (d, *J*=6.9 Hz, 3H), 1.28–1.39 (m, 4H) and 0.89–0.95 (m, 3H); MS (FAB) *m/z* 661 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{41}N_4O_7S_4$ 661.1853, found 661.1843 $(M+H)^+$.

(7*S*)-7-[5-(2-Cyanophenyl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (22)

Reaction of **2** (240 mg, 0.39 mmol) with 2-(5-mercapto-1,3,4-thiadiazol-2-yl) benzonitrile (100 mg, 0.46 mmol) gave **22** as a colorless solid in 32% yield by a similar procedure to **3**. mp 223–229 °C (decomp.); $[\alpha]_D^{30}$ −64° (*c* 1.5, $CHCl_3$); IR (KBr) 3397, 2922, 2227, 1655 and 1510 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.05 (d, *J*=8.8 Hz, 1H), 8.04 (d, *J*=7.7 Hz, 1H), 7.75–7.82 (m, 1H), 7.68 (td, *J*=7.8, 1.4 Hz, 1H), 7.52–7.60 (m, 1H), 5.27 (d, *J*=5.8 Hz, 1H), 4.22–4.44 (m, 2H), 4.15 (d, *J*=9.6 Hz, 1H), 4.07 (dd, *J*=9.9, 5.5 Hz, 1H), 3.61–3.68 (m, 1H), 3.44–3.56 (m, 1H), 3.31–3.39 (m, 1H), 2.99–3.09 (m, 1H), 2.36–2.47 (m, 3H), 2.25–2.36 (m, 1H), 2.11 (s, 3H), 1.80–2.09 (m, 4H), 1.43–1.58 (m, 3H) and 1.16–1.31 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.8, 177.7, 153.9, 134.7, 133.3, 131.6, 131.1, 129.6, 117.1, 110.7, 90.9, 70.9, 70.3, 69.2, 68.5, 67.9, 63.0, 54.4, 51.4, 42.1, 37.7, 37.6, 35.8, 21.6, 15.8, 15.2 and 14.2; MS (FAB) *m/z* 608 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{38}N_5O_5S_3$ 608.2030, found 608.2033 $(M+H)^+$.

(7*S*)-7-[5-(2-Aminopyridin-3-yl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (24)

To a solution of **23**[10–12] (200 mg, 0.29 mmol) and K_2CO_3 (118 mg, 0.85 mmol) in *N,N*-dimethylformamide (DMF) (2.0 ml) was added 5-(2-aminopyridin-3-yl)-1,3,4-thiadiazole-2-thiol (120 mg, 0.57 mmol) and the mixture was stirred at 80 °C for 10 h. After cooled to room temperature, the mixture was diluted with ethyl acetate and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane-ethyl acetate) to give a colorless solid (54 mg). To a solution of the compound obtained above (54 mg) in MeOH (1 ml) was added 1 N HCl (1 ml) and the reaction mixture was stirred at room temperature for 10 min. The mixture was diluted with ethyl acetate and extracted with H_2O. The aqueous phase was neutralized with 10% aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting residue was purified by preparative thin-layer chromatography ($CHCl_3/CH_3OH$/28% aq NH_4OH=10/1/0.1) to afford **24** (16 mg, 9%) as colorless solid. $[\alpha]_D^{30}$ +84° (*c* 0.22, $CHCl_3$); ^1H NMR (300 MHz, CD_3OD) δ 8.10 (dd, *J*=4.9, 1.7 Hz, 1H), 7.87 (dd, *J*=7.8, 1.7 Hz, 1H), 6.75 (dd, *J*=7.8, 4.9 Hz, 1H), 5.27 (d, *J*=5.6 Hz, 1H), 4.58–4.63 (m, 2H), 4.12 (dd, *J*=10.2, 5.6 Hz, 1H), 3.81–3.83 (m, 1H), 3.55–3.60 (m, 1 H), 3.20–3.28 (m, 1H), 3.01 (dd, *J*=10.4, 5.0 Hz, 1H), 2.37 (s, 3H), 2.14–2.25 (m, 1H), 2.02–2.10 (m, 1H), 2.01 (s, 3H), 1.79–1.89 (m, 1H), 1.57 (d, *J*=6.8 Hz, 3H), 1.27–1.37 (m, 4H) and 0.86–0.94 (m, 3H); MS (FAB) *m/z* 599 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{25}H_{39}N_6O_5S_3$ 599.2139, found 599.2152 $(M+H)^+$.

(7*S*)-7-Deoxy-7-{5-[2-(methoxycarbonyl)phenyl]-1,3,4-thiadiazol-2-ylthio}lincomycin (26)

To a solution of **25**[11] (80 mg, 0.19 mmol) in DMF (0.5 ml) were added 1 M sodium hexamethyldisilazane tetrahydrofuran solution (0.38 ml, 0.38 mmol) and methyl 2-(5-chloro-1,3,4-thiadiazol-2-yl)benzoate (53 mg, 0.21 mmol) and the mixture was stirred at room temperature for 10 min. The mixture was diluted with ethyl acetate and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$-MeOH) to give a colorless solid (93 mg). ^1H NMR (400 MHz, $CDCl_3$) δ 9.00 (d, *J*=9.0 Hz, 1H), 7.90–7.96 (m, 1H), 7.55–7.68 (m, 3H), 5.36 (d, *J*=5.6 Hz, 1H), 5.31 (br s, 1H), 4.30–4.46 (m, 2H), 4.27 (d, *J*=10.2 Hz, 1H), 4.16 (dd, *J*=10.2, 5.5 Hz, 1H), 3.77–3.84 (m, 3H), 3.68–3.75 (m, 2 H), 3.59 (dd, *J*=10.0, 3.2 Hz, 1H), 3.48 (s, 1H), 3.35 (dd, *J*=7.9, 5.5 Hz, 1H), 3.09 (dd, *J*=10.6, 4.5 Hz, 1H), 2.37 (s, 3H), 2.15–2.22 (m, 3H), 1.83–2.13 (m, 5H), 1.55 (d, *J*=6.8 Hz, 3H), 1.29–1.36 (m, 3H) and 0.85-0.93 (m, 3H); MS (FAB) *m/z* 641 $(M+H)^+$.

(7*S*)-7-Deoxy-7-[5-(2-dimethylcarbamoylphenyl)-1,3,4-thiadiazol-2-ylthio]lincomycin (28)

To a solution of **26** (268 mg, 0.42 mmol) in MeOH (3.0 ml) were added 2 N NaOH (2.0 ml) and the mixture was stirred at room temperature for 30 min. The mixture was concentrated *in vacuo* and acidified with 1 N HCl and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a carboxylic acid (150 mg). To a solution of the compound obtained above (60 mg, 0.096 mmol) in DMF (0.30 ml) were added hydroxybenzotriazole (16 mg, 0.12 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (22 mg, 0.12 mmol) and 2 M dimethylamine MeOH solution (96 µl, 0.19 mmol) and stirred at room temperature for 2 h. The mixture was diluted with ethyl acetate and washed with 10% aqueous

$NaHCO_3$ to afford **28** (52 mg, 43%) as colorless solid. $[\alpha]_D^{30}$ +131° (*c* 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.92 (d, *J* = 9.1 Hz, 1H), 7.88–7.93 (m, 1H), 7.49–7.58 (m, 2H), 7.36–7.40 (m, 1H), 5.34 (d, *J* = 5.4 Hz, 1H), 5.30 (d, *J* = 3.4 Hz, 1H), 4.34–4.44 (m, 2H), 4.23 (d, *J* = 10.0 Hz, 1H), 4.10–4.16 (m, 1H), 3.69–3.72 (m, 1H), 3.53–3.60 (m, 1H), 3.34–3.39 (m, 1H), 3.12 (s, 3H), 3.07–3.11 (m, 1H), 2.83 (s, 3H), 2.70–2.77 (m, 1H), 2.41 (s, 3H), 2.15–2.20 (m, 3H), 2.06–2.14 (m, 2H), 1.87–2.01 (m, 2H), 1.53 (d, *J* = 6.8 Hz, 3H), 1.29–1.38 (m, 4H) and 0.86–0.93 (m, 3H); MS (FAB) *m/z* 654 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{29}H_{44}N_5O_6S_3$ 654.2448, found 654.2456 $(M+H)^+$.

(7*S*)-7-[5-(2-Carbamoylphenyl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (27)

Reaction of the carboxylic acid obtained in the first step of **28** (46 mg, 0.073 mmol) with 7 N NH_3 MeOH solution (0.020 ml, 0.14 mmol) gave **27** as a colorless solid in 44% yield by a similar procedure to **28**. mp 228–235 °C (decomp.); $[\alpha]_D^{30}$ +127° (*c* 1.1, $CHCl_3$); IR (KBr) 3397, 2924, 1664 and 1510 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.76–7.83 (m, 1H), 7.57–7.67 (m, 3H), 5.27 (d, *J* = 5.6 Hz, 1H), 4.60 (dd, *J* = 9.7, 3.2 Hz, 1H), 4.37–4.47 (m, 2H), 4.11 (dd, *J* = 10.2, 5.6 Hz, 1H), 3.81 (d, *J* = 2.2 Hz, 1H), 3.55–3.60 (m, 2H), 3.27 (dd, *J* = 8.6, 6.2 Hz, 1H), 3.53–3.63 (m, 1H), 2.34–2.42 (m, 3H), 2.01–2.08 (m, 2H), 2.00 (s, 3H), 1.79–1.90 (m, 1H), 1.56 (d, *J* = 6.8 Hz, 3H), 1.26–1.40 (m, 4H) and 0.86–0.97 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 170.2, 166.8, 164.4, 135.7, 130.9, 130.8, 130.7, 128.3, 127.3, 89.0, 71.6, 71.1, 69.3, 68.4, 68.3, 62.6, 53.1, 44.7, 41.8, 38.1, 38.0, 35.7, 21.6, 18.9, 14.7 and 14.3; MS (FAB) *m/z* 626 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{40}N_5O_6S_3$ 626.2135, found 626.2137 $(M+H)^+$.

(7*S*)-7-[5-(2-Aminophenyl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (29)

To a solution of compound **9** (390 mg, 0.63 mmol) in ethanol (12.0 ml) was added $SnCl_2 \cdot H_2O$ (560 mg, 2.5 mmol), $NaBH_4$ (16.0 mg, 0.42 mmol) and stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure. The resulting residue was dissolved by ethyl acetate, washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by preparative thin-layer chromatography ($CHCl_3/CH_3OH$/28% aq NH_4OH = 20/1/0.1) to obtain the title compound as a colorless solid (123 mg, 33%). $[\alpha]_D^{31}$ +62° (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.94 (d, *J* = 9.3 Hz, 1H), 7.37 (dd *J* = 7.9, 1.4 Hz, 1H), 7.21–7.26 (m, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.74 (t, *J* = 7.3 Hz, 1H), 6.10 (br s, 2H), 5.36 (d, *J* = 5.6 Hz, 1H), 5.28–5.35 (m, 1H), 4.23–4.33 (m, 2H), 4.12–4.18 (m, 1H), 3.68–3.75 (m, 1H), 3.53–3.13 (m, 1H), 3.31 (dd, *J* = 8.0, 5.7 Hz, 1H), 3.09 (dd, *J* = 10.5, 4.7 Hz, 1H), 2.72–2.81 (m, 1H), 2.39 (s, 3H), 2.18 (s, 3H), 2.04–2.16 (m, 2H), 1.87–2.02 (m, 3H), 1.55 (d, *J* = 7.1 Hz, 3H), 1.25–1.38 (m, 5H) and 0.87–0.96 (m, 3H); MS (FAB) *m/z* 598 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{40}N_5O_5S_3$ 598.2186, found 598.2185 $(M+H)^+$.

(7*S*)-7-[5-(3-Aminophenyl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (30)

Compound **30** was obtained from compound **10** (75 mg, 0.13 mmol) as a colorless solid in 24% yield by a similar procedure to **29**. $[\alpha]_D^{30}$ +140° (*c* 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.94 (d, *J* = 9.1 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.20–7.26 (m, 1H), 6.80 (d, *J* = 8.1 Hz, 1H), 6.73 (t, *J* = 7.6 Hz, 1H), 6.11 (br s, 2H), 5.35 (d, *J* = 5.5 Hz, 1H), 5.32 (br s, 1H), 4.37–4.49 (m, 1H), 4.23–4.35 (m, 2H), 4.15 (dd, *J* = 10.0, 5.3 Hz, 1H), 3.71 (d, *J* = 2.5 Hz, 1H), 3.58 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.31 (dd, *J* = 7.4, 5.3 Hz, 1H), 3.09 (dd, *J* = 10.2, 4.9 Hz, 1H), 2.39 (s, 3H), 2.18 (s, 3H), 2.05–2.16 (m, 2H), 1.86–2.02 (m, 3H), 1.55 (d, *J* = 6.9 Hz, 3H), 1.22–1.40 (m, 4H) and 0.87–0.97 (m, 3H); MS (FAB) *m/z* 598 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{40}N_5O_5S_3$ 598.2186, found 598.2192 $(M+H)^+$.

(7*S*)-7-[5-(4-Aminophenyl)-1,3,4-thiadiazol-2-ylthio]-7-deoxylincomycin (31)

Compound **31** was obtained from compound **11** (50 mg, 0.84 mmol) as a colorless solid by a similar procedure to **29**. $[\alpha]_D^{30}$ +67° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 7.59–7.65 (m, 2H), 6.70–6.75 (m, 2H), 5.26 (d, *J* = 5.6 Hz, 1H), 4.55 (dd, *J* = 9.8, 3.2 Hz, 1H), 4.42 (d, *J* = 10.4 Hz, 1H), 4.30 (qd, *J* = 6.9, 3.2 Hz, 1H), 4.07–4.15 (m, 2H), 3.79–3.82 (m, 1H), 3.58 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.25 (dd, *J* = 8.2, 6.2 Hz, 1H), 2.98 (dd, *J* = 10.5, 5.1 Hz, 1H), 2.34 (s, 3H), 2.13–2.25 (m, 1H), 2.03 (s, 3H), 1.97–2.02 (m, 1H), 1.78–1.91 (m, 1H), 1.53 (d, *J* = 7.0 Hz, 3H), 1.29–1.39 (m, 4H) and 0.89–0.95 (m, 3H); MS (FAB) *m/z* 598 $(M+H)^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{40}N_5O_5S_3$ 598.2186, found 598.2192 $(M+H)^+$.

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 (Nissui Pharmaceutical, Tokyo, Japan) 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 colony-forming units per ml was inoculated into sensitivity disk agar (Nissui Pharmaceutical) supplemented with 5% horse blood and incubated at 37 °C for 20 h. Then, minimum inhibitory concentration was defined as the lowest drug concentration that prevented visible growth.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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