Semisynthetic Modification of Antibiotic Lincomycin

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(Received for publication April 4, 1996)

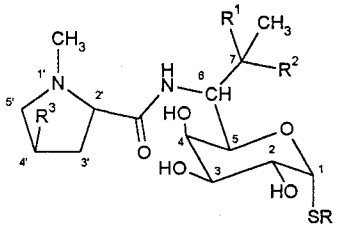
During the past three decades numerous analogues of lincomycin[1] (**1**, lincocin) and related antibiotics, such as celesticetin[2] (**2**) have been prepared[3] by synthetic and microbiological transformation, involving modification of the parent antibiotics both at the sugar moiety and the amino acid side-chain (Fig. 1). Of these new derivatives 7(*S*)-chloro-7-deoxylincomycin[4] (**3**, cleocin, clindamycin) is one of the most important, possessing more pronounced and wider spectrum of antibiotic activity, as well as enhanced absorption capability than lincomycin (**1**).

The present paper describes our results on the chemical modification of clindamycin (**3**). During our work a primary goal was to perform syntheses of the new antibiotic analogues which do not require temporary protection of the existing functions of the starting molecules.

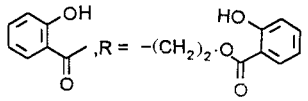
Nucleophilic substitution of the 7(*S*)-chlorine atom of **3** offers the synthesis of novel antibiotic analogues modified at position C-7 (Fig. 2). By employing sodium azide (**4a**) and various heteroaromatic mercapto compounds (**4b**~**4e**) and DMF as the solvent 7-azido-7-deoxylincomycin (**5a**) and the 7-thioether derivatives **5b**~**5e** were obtained probably with inversion of the configuration. In the latter cases fused potassium carbonate was used as the acid scavenger. In the above new compounds the steric position of the introduced R_2 substituent is equal with that of the C-7 hydroxyl group of **1**. However an opposite C-7 chirality can not be excluded on the basis on NMR-spectroscopic[6] evidences. Selected physico-chemical properties and spectroscopic data of **5a**~**5e** are listed in Table 1. All of the newly synthesized compounds gave satisfactory microanalytical and spectroscopic evidence. The fragmentations of the $(M+H)^+$ and $(M^{+\cdot})$ ions are in good agreement with the chemical structures of synthesized compounds. The most characteristic ^1H NMR data are shown in Table 1.

Table 2 shows the *in vitro* antibacterial activity of the 7-substituted lincomycins. The exchange of the C-7 hydroxyl group of **1** to an azido function with the same

Fig. 1. Structures of lincomycin (**1**), celesticetin (**2**) and clindamycin (**3**).

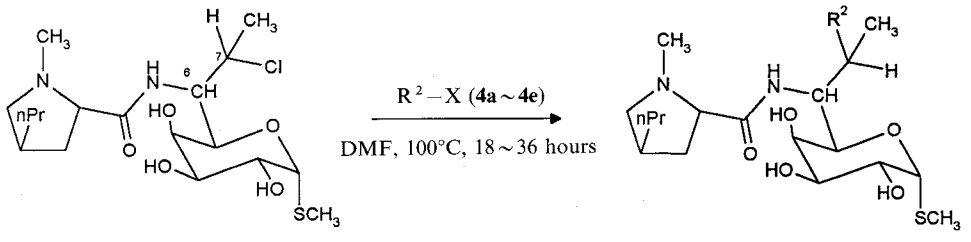


1 R^1=OH, R^2=H, R^3=*n*Pr, R=CH_3

2 R^1=OH, R^2=H, R^3=  ,R= $-(CH_2)_2-O$

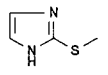
3 R^1=H, R^2=Cl, R^3=*n*Pr, R=CH_3

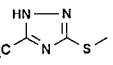
Fig. 2. Synthetic route to 7-substituted lincomycins.

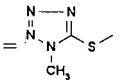


3 **5a**~**5e**

4a $R^2=N_3$; X=Na

4b $R^2=$  ; X=H

4c $R^2=$  ; X=H

4d $R^2=$  ; X=H

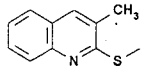
4e $R^2=$  ; X=H

Table 1. Physico-chemical properties and spectroscopic data of novel semisynthetic analogues of lincomycin.

Compound	Yield (%)	MP (°C)	$[\alpha]_D^{20}$	Ms (*m/z*)	IR (KBr) cm^{-1}	^1H NMR (200 MHz, $CDCl_3$) δ ppm
5a	68.3	71.5~73	+119.6° (*c* 0.5, H_2O)	432[a]	3400, 2180, 1654, 1532, 1380, 1335, 1082, 1050, 675	*5.36 (d, H1), 4.54 (dd, H6), 4.13 (dd, H2), 2.37 (s, NCH_3), 2.12 (s, SCH_3), 1.25 (d, 7-CH_3)
5b	11	84~85	+159.0 (*c* 0.5, $CHCl_3$)	489[a]	3390, 1652, 1456, 1380, 1328, 1090, 1054, 696	7.8~7.5 (1H, hetero Ar), 5.36 (d, H1), 5.26 (m, 1H, hetero Ar), 2.35 (s, NCH_3), 2.15 (s, 3H, SCH_3) 1.30 (d, 7-CH_3)
5c	15	107~110	+135.5 (*c* 0.2, MeOH)	503[b]	3400, 1654, 1418, 1424, 1382, 1312, 1088, 1054, 668	8.1 (br s, NH), 5.32 (d, H1), 2.45 (s, NCH_3), 2.15 (br s, 6H, SCH_3+CH_3), 1.48 (d, 7-CH_3)
5d	39.2	58~60	+156.0 (*c* 0.2, CH_2Cl_2)	504[b]	3416, 1660, 1454, 1390, 1280, 1086, 1050, 702	8.22 (d, NH), 5.35 (d, H1), 3.90 (s, NCH_3), 2.49 (s, NCH_3), 2.22 (s, SCH_3), 1.60 (d, 7-CH_3)
5e	18.7	88~92 (decomp)	+163.4 (*c* 0.5, $CHCl_3$)	563[b]	3380, 1654, 1622, 1600, 1558, 1490, 1514, 1390, 1332, 668	7.5~7.35 (m, 5H, Ar), 5.47 (d, H1), 4.63 (dt, H6), 2.35 (s, SCH_3), 1.56 (d, 7-CH_3)

[a] FAB $(M+H)^+$, [b] NH_4Cl-CI (M^+)[5], * in D_2O.

Table 2. *In vitro* antibacterial activity of **5a**~**5e** in comparison with lincomycin (**1**) and clindamycin (**3**).

No.	Test organism	Medium	MIC (μg/ml)						
			Compound						
			1	**5a**	**5b**	**5c**	**5d**	**5e**	**3**
1	*Staphylococcus aureus* KB 210 (ATCC 6538p)	MHA	0.20	0.20	0.20	3.13	3.13	1.56	0.05
2	*Staphylococcus aureus* KB 199 (MLs^r)	MHA	>100	>100	>100	>100	>100	>100	>100
3	*Staphylococcus aureus* KB 222 (MLs^r)	MHA	>100	>100	>100	>100	>100	>100	>100
4	*Bacillus subtilis* KB 211 (ATCC 6633)	MHA	25	12.5	50	>100	>100	100	1.56
5	*Bacillus cereus* KB 143 (IFO 3001)	MHA	12.5	6.25	6.25	100	50	25	0.78
6	*Micrococcus luteus* KB 212 (ATCC 9341)	MHA	0.20	0.20	<0.1	1.56	3.13	1.56	0.10
7	*Mycobacterium smegmatis* KB 42 (ATCC 607)	MHA	25	50	50	>100	>100	50	25
8	*Escherichia coli* KB 213 (NIHJ)	MHA	100	100	>100	>100	>100	100	50
9	*Escherichia coli* KB 176 (NIHJ JC-2)	MHA	>100	>100	>100	>100	>100	100	25
10	*Escherichia coli* KB 198 (MLs^s)	MHA	6.25	12.5	6.25	50	>100	12.5	0.78
11	*Klebsiella pneumoniae* KB 214 (ATCC I0031)	MHA	>100	>100	>100	>100	>100	100	100
12	*Proteus vulgaris* KB 127 (IFO 3167)	MHA	>100	>100	>100	>100	>100	>100	>100
13	*Pseudomonas aeruginosa* KB 115 (IFO 3080)	MHA	>100	>100	>100	>100	>100	100	>100
14	*Clostridium perfringens* KB 129 (ATCC 3624)	GAM	0.78	0.39	0.40	1.56	3.13	1.56	0.05
15	*Clostridium perfringens* KB 130	GAM	6.25	1.56	6.25	25	12.5	6.25	0.78
16	*Clostridium kainantoi* KB 133 (IFO 3353)	GAM	1.56	1.56	1.56	12.5	3.13	6.25	0.39
17	*Bacteroidis fragilis* KB 169 (ATCC 23745)	GAM	1.56	0.39	1.56	50	12.5	6.25	0.10
18	*Fusobacterium varium* KB 234 (ATCC 8501)	GAM	6.25	12.5	12.5	100	100	100	3.13

Method: Agar dilution method.
Solvents: Dist. water (**1**, **3**); 30% MeOH (**5b**); 50% MeOH (**5a**, **5c**, **5d**); DMSO-H_2O (7:3) (**5e**).
MHA: Mueller Hinton agar (Nissui) 37°C, 20 hours.
GAM: GAM Agar Nissui, 37°C, 23 hours. Gas pack method (BBL).

configuration retained the order of magnitude of the antimicrobial activity. The azido compound (**5a**) possesses higher activity towards the microorganisms in entries 4 and 5 than **1**, but these effects are still lower than those of **3**. Of the new antibiotic analogues having a heteroaryl-thio group at C-7 compound **5b** is almost as active as lincomycin itself, but it is less effective than clindamycin.

Experimental

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. ^1H NMR spectra were recorded at 200 MHz on a Bruker WP 200 SY spec-

trometer. Mass spectrometry was performed using VG-7035 GC-MS and VG-7070 HS FAB (matrix: glycerol, gas: Xe, 8 kV). IR spectra (KBr discs) were recorded on a Perkin-Elmer 16 PC FT-IR spectrophotometer. Specific optical rotations were measured at room temperature on a Perkin-Elmer 141 MC polarimeter. TLC and column chromatography was carried out on Silicagel 60 (0.063~0.2 Merck) with *A* $CHCl_3$-MeOH (1:1); *B* $CHCl_3$-CH_2Cl_2-MeOH-NH_4OH (5:5:1:0.2); *C* $CHCl_3$-MeOH-NH_4OH (8.5:1.5:0.2); *D* $CHCl_3$-MeOH-NH_4OH (9.5:0.5:0.2); *E* $CHCl_3$-MeOH (9.5:0.5). Evaporations were carried out under diminished pressure at $\leq$40°C.

7-Azido-7-deoxylincomycin (**5a**)

A mixture of **3** (0.25 mmol) and sodium azide (2.5 mmol) in abs. DMF (5 ml) was stirred at 100°C for 18 hours and the progress of reaction was monitored by TLC (*A*). When all of the starting **3** had reacted the reaction mixture was filtered, the filter-cake was washed with chloroform and the combined filtrate was concentrated and co-distilled with toluene under diminished pressure. The residue was purified with the aid of column chromatography (*A*).

7-Heteroaryl-thio-lincomycins (**5b~5e**)

A mixture of **3** (1 mmol), **4b~4f** (1.1 mmol) and freshly fused and well-powdered potassium carbonate (2.2 mmol) in abs. DMF (8 ml) was stirred at 100~105°C for 24~36 hours. After filtration the filtrate was concentrated and co-distilled with toluene under diminished pressure. The crude products (**5b~5e**) were then submitted to column chromatography (eluent to furnish pure): **5b** (*B*), **5c** (*C*), **5d** (*D*) and **5e** (*E*).

Acknowledgments

The authors are indebted to the Kitasato Institute (Tokyo, Japan) for financial support. We thank Mrs. ERZSÉBET RŐTH for her skillful technical assistance.

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