SYNTHESIS AND BIOLOGICAL EVALUATION OF NEOPYRROLOMYCIN ANALOGS

Sir:

Neopyrrolomycin (**1**) is an optically active phenylpyrrole antibiotic isolated from cultured broth of *Streptomyces* sp. with broad antibacterial and antifungal activities and the structurally unique atrope isomerism[1].

Very recently, (+)- and (−)-neopyrrolomycins (**1**) have been efficiently synthesized from 3,5-dichloroanisole in our laboratories as briefly described below[2].

The advantage of our route also makes it practical to synthesize neopyrrolomycin analogs that are required for studies on structure-activity relationship.

Herein we report the synthesis and biological evaluation of neopyrrolomycin analogs.

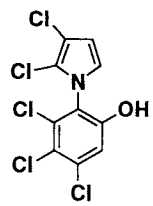
3,5-Dichloroanisole was converted into 2,3,4-trichloro-6-methoxyaniline (**2**) (mp 85°C) by regioselective chlorination, nitration and reduction[2]. Reaction of **2** with 2,5-dimethoxytetrahydrofuran generated the phenylpyrrole **3** (mp 117°C), which was also regioselectively brominated by NBS to give the 2-bromo compound **4** (mp 70°C). Lithiation of **4** with *n*-BuLi followed by treatment with CO_2 gas gave the carboxylic acid **5** (mp 246°C), which was chlorinated by trichloroisocyanuric acid (TCIA) to afford exclusively the 4,5-dichloropyrrole **6** (mp 233°C). Decarboxylation by heating in quinoline with Cu powder to give **7** (mp 113°C) followed by de-*O*-methylation with $AlCl_3$ led to (±)-neopyrrolomycin (**1**) (mp 91°C). The atrope isomers were readily resolved by acylation with *N*-(*p*-toluenesulfonyl)-L-phenylalanyl chloride[3] to yield the diastereomers **8a** ($[\alpha]_D$ −15° (*c* 1.0, MeOH)) and **8b** ($[\alpha]_D$ −33° (*c* 1.0, MeOH)). Deacylation of **8a** with KOH followed by acidification with HCl gave (+)-neopyrrolomycin (**1**) (oil, $[\alpha]_D$ +41° (*c* 0.07, $CHCl_3$)) identical with natural neopyrrolomycin[1,2], which was converted into potassium salt (mp 72 °C, $[\alpha]_D$ −46° (*c* 0.50, $CHCl_3$)). Similarly, (−)-neopyrrolomycin (**1**) (oil, $[\alpha]_D$ −41° (*c* 0.07, $CHCl_3$)) and its (+)-potassium salt (mp 72°C, $[\alpha]_D$ +45° (*c* 0.49, $CHCl_3$)) were obtained from **8b**.

A variety of analogs were prepared from the aforesaid key intermediates.

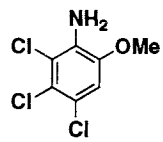
Iodination of **3** with NIS in DMF at room temperature for 15 hours gave the 2-iodopyrrole **9** (77%, mp 96°C), which was chlorinated by TCIA in DMF at room temperature for 2 hours to give a mixture of dichloropyrrole derivatives. The mixture was hydrogenated in the presence of Pd-C to give, after silica-gel column chromatography (PhH-hexane, 1:2), the aforesaid **7** (10%) and the 2,4-dichloropyrrole derivative **10** [58%; mp 67°C, ^{1}H NMR (90 MHz, $CDCl_3$): δ 3.83 (3H, s), 6.20 (1H, d, J=2 Hz), 6.53 (1H, d, J=2 Hz), 7.10 (1H, s)].

Direct chlorination of **3** with NCS in DMF at room temperature for 15 hours, followed by silica-gel column chromatography (PhH-hexane, 1:9) produced the monochloropyrrole derivatives **11** [55%; mp 72°C, ^{1}H NMR (90 MHz, $CDCl_3$): δ 3.84 (3H, s), 6.30 (2H, m), 6.58 (1H, m), 7.13 (1H, s)] and **12** [9%; mp 98°C, ^{1}H NMR (90 MHz, $CDCl_3$): δ 3.80 (3H, s), 6.28 (1H, m), 6.60 (2H, m), 7.08 (1H, s)]. 2-Chloropyrrole derivative **11** was further chlorinated by TCIA in DMF at 0→10 °C for 2 hours to give 2,5-dichloropyrrole derivative **13** [52%; mp 69°C, ^{1}H NMR (90 MHz, $CDCl_3$): δ 3.82 (3H, s), 6.20 (2H, s), 7.13 (1H, s)].

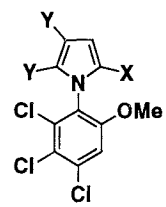
De-*O*-methylation of **3**, **4**, **10**, **11**, and **12** with $AlCl_3$ in benzene at room temperature for 14 hours gave the corresponding phenol derivatives **14** [91%; mp 84°C, ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.46 (2H,



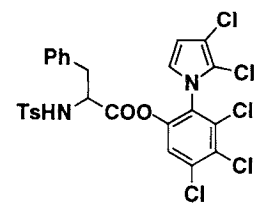
1 : Neopyrrolomycin



2



3 : X=Y=H
4 : X=Br,Y=H
5 : X=COOH,Y=H
6 : X=COOH,Y=Cl
7 : X=H,Y=Cl



8a, b

t, $J=2$ Hz), 6.58 (2H, t, $J=2$ Hz), 7.17 (1H, s)], **15** [87%; mp 78°C (dec.), ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.43 (2H, m), 6.72 (1H, m), 7.19 (1H, s)], **16** [89%; oil, ^{1}H NMR (90 MHz, $CDCl_3$): δ 5.87 (1H, br s), 6.30 (1H, d, $J=2$ Hz), 6.60 (1H, d, $J=2$ Hz), 7.20 (1H, s)], **17** [67%; mp 121°C, ^{1}H NMR (90 MHz $CDCl_3$): δ 5.20 (1H, s), 6.40 (2H, m), 6.62 (1H, m), 7.30 (1H, s)] and **18** [81%; oil, ^{1}H NMR (90 MHz, $CDCl_3$): δ 5.37 (1H, s), 6.40 (1H, m), 6.63 (2H, m), 7.17 (1H, s)], respectively.

To our surprise, de-*O*-methylation of **13** with $AlCl_3$ by the similar way gave exclusively the aforesaid 2,4-dichloropyrrole derivative **16** through the unexpected migration of the chlorine atom.

The antibacterial and antifungal activities of the synthesized neopyrrolomycin analogs (**1**, **3**~**7** and **10**~**18**) are shown in Table 1.

Both enantiomers of neopyrrolomycin (**1**) and the racemate showed almost the same activities, although the activity of the natural antibiotic (+)-**1** against Gram-negative bacteria was slightly weaker than the unnatural (−)-**1**.

The *O*-methyl analogs (**3**~**7** and **10**~**13**) showed no significant activities.

Remarkably, 2,4-dichloro (that is, 3,5-dichloro) and 3-chloro analogs (**16** and **18**) exhibited the same activities as neopyrrolomycin (**1**), suggesting that the chlorine atom at the C-3 position (β-position) of the

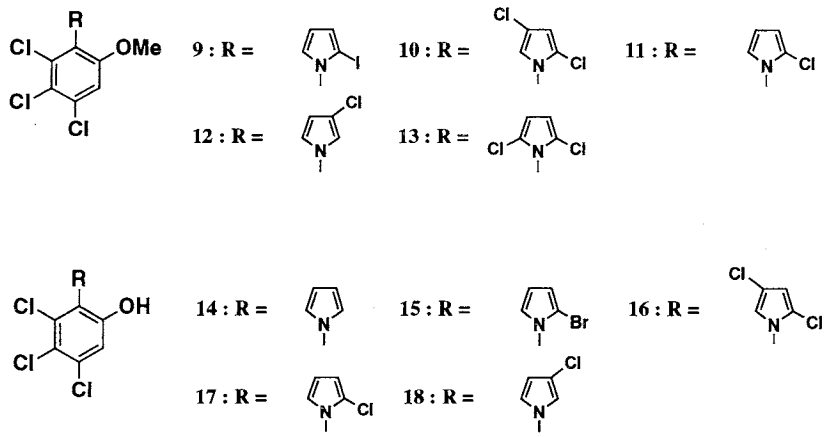


Table 1. Antibacterial and antifungal activities of neopyrrolomycin analogs.[a]

Test organism	MIC (μg/ml)					
	(+)-**1**[b]	(−)-**1**[b]	(±)-**1**[b]	**3**	**4**	**5**
Citrobacter freundii IFO 12681	50	50	50	>100	>100	>100
Enterobacter cloacae IFO 12935	25	6.25	12.5	>100	>100	>100
Escherichia coli NIHJ JC-2	50	6.25	12.5	>100	>100	>100
Klebsiella pneumoniae IFO 3317	12.5	1.56	3.13	>100	>100	>100
Proteus vulgaris GN 5298	25	1.56	3.13	>100	>100	>100
Pseudomonas aeruginosa IFO 3445	50	50	50	>100	>100	>100
Serratia marcescens 3759	50	50	50	>100	>100	>100
Streptococcus epidermidis IFO 13889	0.39	0.39	0.39	>100	>100	>100
Enterococcus faecalis IFO 12964	0.78	0.39	0.78	>100	>100	>100
E. faecium IFO 12367	0.20	0.20	0.39	>100	>100	>100
Staphylococcus aureus IFO 12732	0.20	0.20	0.39	>100	>100	>100
Methicillin-resistant *S. aureus* 4[c]	0.20	0.20	0.20	>100	>100	>100
Methicillin-resistant *S. aureus* 69[c]	0.20	0.20	0.39	>100	>100	>100
Candida albicans IFO 1269	3.13	3.13	3.13	>100	>100	>100
C. albicans IFM 40009	6.25	6.25	6.25	>100	>100	>100
Cryptococcus neoformans TIMM 0354	0.39	0.39	0.39	>100	>100	>100
C. neoformans TIMM 0362	≤0.20	≤0.20	≤0.20	>100	50	>100
Aspergillus fumigatus TIMM 0063[d]	6.25	6.25	6.25	>100	>100	>100
A. fumigatus IMF 4942[d]	6.25	6.25	6.25	>100	>100	>100

Table 1. (Continued)

Test organism	MIC (μg/ml)					
	6	**7**	**10**	**11**	**12**	**13**
Citrobacter freundii IFO 12681	>100	>100	>50	>100	>50	>50
Enterobacter cloacae IFO 12935	>100	>100	>50	>100	>50	>50
Escherichia coli NIHJ JC-2	>100	>100	>50	>100	>50	>50
Klebsiella pneumoniae IFO 3317	>100	>100	>50	>100	>50	>50
Proteus vulgaris GN 5298	>100	>100	>50	>100	>50	>50
Pseudomonas aeruginosa IFO 3445	>100	>100	>50	>100	>50	>50
Serratia marcescens 3759	>100	>100	>50	>100	>50	>50
Streptococcus epidermidis IFO 13889	25	>100	>50	>100	>50	>50
Enterococcus faecalis IFO 12964	>100	>100	>50	>100	>50	>50
E. faecium IFO 12367	>100	>100	>50	>100	>50	>50
Staphylococcus aureus IFO 12732	>100	>100	>50	>100	>50	50
Methicillin-resistant *S. aureus* 4[c]	>100	100	>50	>100	>50	>50
Methicillin-resistant *S. aureus* 69[c]	100	25	>50	100	>50	50
Candida albicans IFO 1269	>100	>100	>50	>100	>50	>50
C. albicans IFM 40009	>100	>100	>50	>100	>50	>50
Cryptococcus neoformans TIMM 0354	>100	>100	25	>100	50	>50
C. neoformans TIMM 0362	>100	6.25	6.25	6.25	3.13	>50
Aspergillus fumigatus TIMM 0063[d]	>100	>100	>50	>100	>50	>50
A. fumigatus IMF 4942[d]	>100	>100	>50	>100	>50	>50

Test organism	MIC (μg/ml)				
	14	**15**	**16**	**17**	**18**
Citrobacter freundii IFO 12681	>100	>100	50	>50	>25
Enterobacter cloacae IFO 12935	1.56	12.5	25	6.25	6.25
Escherichia coli NIHJ JC-2	1.56	12.5	25	12.5	6.25
Klebsiella pneumoniae IFO 3317	0.20	3.13	12.5	1.56	1.56
Proteus vulgaris GN 5298	1.56	6.25	12.5	3.13	1.56
Pseudomonas aeruginosa IFO 3445	>100	>100	50	>50	>25
Serratia marcescens 3759	100	>100	50	>50	25
Streptococcus epidermidis IFO 13889	3.13	3.13	0.39	1.56	0.39
Enterococcus faecalis IFO 12964	12.5	6.25	0.78	3.13	0.78
E. faecium IFO 12367	3.13	1.56	0.20	1.56	0.39
Staphylococcus aureus IFO 12732	0.78	1.56	0.20	1.56	0.39
Methicillin-resistant *S. aureus* 4[c]	0.78	1.56	0.10	1.56	0.20
Methicillin-resistant *S. aureus* 69[c]	1.56	1.56	0.20	1.56	0.39
Candida albicans IFO 1269	12.5	12.5	6.25	12.5	3.13
C. albicans IFM 40009	12.5	12.5	6.25	12.5	3.13
Cryptococcus neoformans TIMM 0354	1.56	0.78	≤0.20	0.78	≤0.20
C. neoformans TIMM 0362	1.56	0.78	≤0.20	0.78	0.39
Aspergillus fumigatus TIMM 0063[d]	6.25	6.25	6.25	6.25	3.13
A. fumigatus IMF 4942[d]	6.25	12.5	6.25	12.5	6.25

[a] MIC values were determined by an agar dilution method using Mueller-Hinton agar for antibacterial tests with incubation at 37°C for 18 hours and a Sabouraud Dextrose agar for antifungal tests with incubation at 30°C for 24 hours.

[b] K salt.

[c] Clinical isolate.

[d] Incubation: 48 hours.

pyrrole moiety is essential for the preservation of the potent biological activities.

Acknowledgment

We are grateful to Mochida Pharmaceutical Co., Ltd., Yamanouchi Pharmaceutical Co., Ltd. and Shikoku Chemicals Co. for the generous support of our program.

KUNIAKI TATSUTA*
MANABU ITOH

Graduate School of Science and Engineering,
Waseda University,
3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169, Japan

(Received October 12, 1993)

References

1) NOGAMI, T.; Y. SHIGIHARA, N. MATSUDA, Y. TAKAHASHI, H. NAGANAWA, H. NAKAMURA, M. HAMADA, Y. MURAOKA, T. TAKITA, Y. IITAKA & T. TAKEUCHI: Neopyrrolomycin, a new chlorinated phenylpyrrole antibiotic. J. Antibiotics 43: 1192~1194, 1990
2) TATSUTA, K. & M. ITOH: Total synthesis of chlorinated phenylpyrrole antibiotics, (+)- and (−)-neopyrrolomycins. Tetrahedron Lett. in press.
3) HASHIMOTO, S.; S. KASE, A. SUZUKI, Y. YANAGIYA & S. IKEGAMI: A practical access to optically pure (*S*)-1-octyn-3-ol. Synth. Commun. 21: 833~839, 1991