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Pyridonecarboxylic Acids as Antibacterial Agents. XII.[1] Synthesis and Antibacterial Activity of Enoxacin Analogues with a Variant at Position 1

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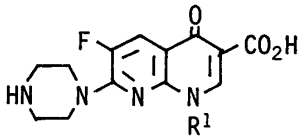
Synthesis and antibacterial activity of enoxacin analogues [1-substituted 6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acids] were studied. Alkyl, hydroxyalkyl, chloroalkyl, aralkyl and alkenyl groups were selected as substituents at position 1. Among the compounds prepared in this work, the 1-(2-chloroethyl) analogue is the most active.

Keywords——enoxacin; antibacterial agent; antibacterial activity; 1,8-naphthyridine; structure–activity relationship

In the previous paper[2] of this series, a synthesis of enoxacin (**1**), a potent and broad-spectrum antibacterial agent, was reported along with that of its 1-vinyl, 1-(2-hydroxyethyl) and 1-(2-fluoroethyl) analogues (**2**—**4**). With respect to analogues with other N-1 substituents, the cyclopropyl and phenyl derivatives (**5**[3] and **6**[4]) have been reported thus far. However, comparable activity data for those compounds have not been available. In this paper, we wish to report a synthesis of enoxacin analogues (**10a**—**o**, **12** and **14**) with an N-1 variant such as alkyl, hydroxyalkyl, chloroalkyl, aralkyl and alkenyl groups, and to describe their structure–activity relationships as compared with those of compounds **1**—**6**.

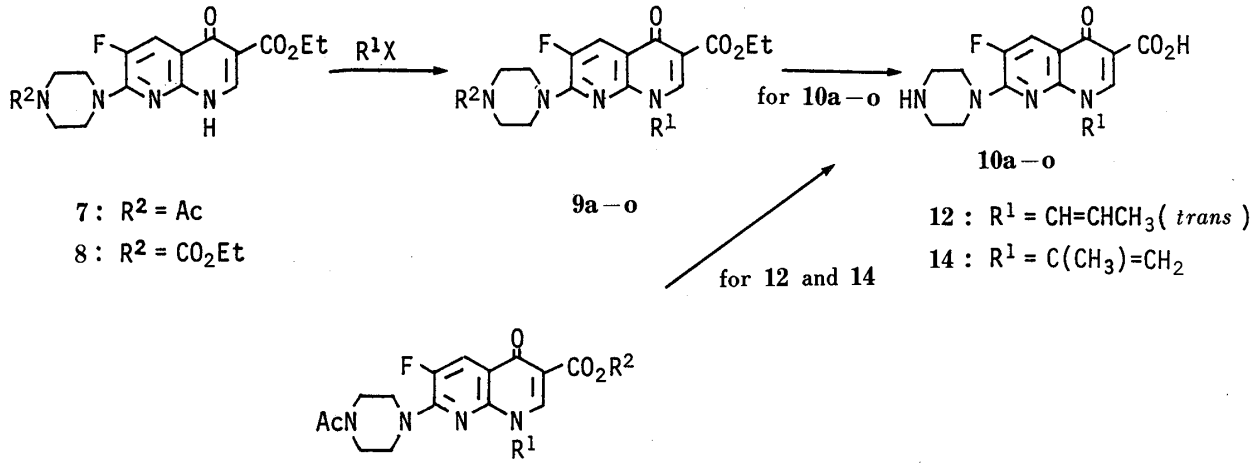


1: R^1 = Et (enoxacin) 4: R^1 = CH_2CH_2F
2: R^1 = $CH{=}CH_2$ 5: R^1 = *cyclo*-C_3H_5
3: R^1 = CH_2CH_2OH 6: R^1 = Ph

Chart 1

Chemistry

The desired 1-substituted 6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acids (**10a**—**o**) were synthesized by alkylation of ethyl 7-(4-acetyl- or 4-ethoxycarbonyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**7** or **8**) with alkyl halides followed by hydrolysis of **9**, according to the method[2] reported previously (Chart 2). Compound **12** was prepared *via* **11a**→**11b**→**11c**. Thus the 1-(2-hydroxypropyl) analogue **11a**, derived from alkylation of **7**, was chlorinated with thionyl chloride followed by treatment of the chloro compound **11b** with a base to give the 1-(1-propenyl) derivative **11c**. The structure of **11c** was assigned on the basis of the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the ethyl ester of **11c**, which showed signals due



11a : $R^1 = CH_2CH(OH)CH_3$, $R^2 = Et$
11b : $R^1 = CH_2CHClCH_3$, $R^2 = Et$
11c : $R^1 = CH{=}CHCH_3$ (*trans*), $R^2 = H$
13a : $R^1 = CH(CH_3)CN$, $R^2 = Et$
13b : $R^1 = CH(CH_3)CH_2NH_2$, $R^2 = Et$
13c : $R^1 = CH(CH_3)CH_2OAc$, $R^2 = Et$
13d : $R^1 = CH(CH_3)CH_2OH$, $R^2 = Et$
13e : $R^1 = CH(CH_3)CH_2Cl$, $R^2 = Et$
13f : $R^1 = C(CH_3){=}CH_2$, $R^2 = H$

Chart 2

TABLE I. 1,8-Naphthyridine Derivatives **9a**—**o**[a]

Compd.	R^2	R^1	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)				
						C	H	Cl	F	N
9a	Ac	Me	222—223 (EtOH)	72	$C_{18}H_{21}FN_4O_4$	57.44 (57.38	5.62 5.71		5.05 5.11	14.89 14.61)
9b	Ac	*n*-Pr	160—161 (AcOEt)	76	$C_{20}H_{25}FN_4O_4$	59.39 (59.37	6.23 6.21		4.70 4.57	13.86 13.76)
9c	Ac	iso-Pr	197—199 (AcOEt)	65	$C_{20}H_{25}FN_4O_4$	59.39 (59.25	6.23 6.34		4.70 4.70	13.86 13.96)
9d	Ac	*n*-Bu	148—150 (AcOEt)	76	$C_{21}H_{27}FN_4O_4$	60.27 (60.23	6.50 6.64		4.54 4.45	13.39 13.38)
9e	Ac	$(CH_2)_4CH_3$	154—156 (AcOEt)	61	$C_{22}H_{29}FN_4O_4$	61.09 (61.06	6.76 6.81		4.39 4.21	12.96 12.99)
9f[b]	Ac	$(CH_2)_3OH$								
9h	Ac	$(CH_2)_3Cl$	188—190 (MeCN)	33	$C_{20}H_{24}ClFN_4O_4$	54.73 (54.73	5.51 5.52	8.08 8.32	4.33 4.49	12.77 13.00)
9i	Ac	$(CH_2)_4Cl$	185—187 (EtOH)	50	$C_{21}H_{26}ClFN_4O_4$	55.69 (55.46	5.79 6.04	7.83 7.74	4.20 4.02	12.37 12.37)
9j	Ac	CH_2Ph	195—196 (EtOH)	78	$C_{24}H_{25}FN_4O_4$	63.71 (63.47	5.57 5.70		4.20 4.03	12.38 12.06)
9k	Ac	$(CH_2)_2Ph$	171—173 (AcOEt)	82	$C_{25}H_{27}FN_4O_4$	64.36 (64.62	5.83 5.67		4.07 3.94	12.01 12.13)
9l	Ac	$(CH_2)_3Ph$	161—162 (AcOEt)	29	$C_{26}H_{29}FN_4O_4$	64.98 (65.15	6.08 5.97		3.95 3.84	11.66 11.63)
9m	Ac	$(CH_2)_4Ph$	141—142 (AcOEt)	77	$C_{27}H_{31}FN_4O_4$	65.57 (65.80	6.32 6.33		3.84 3.68	11.33 11.41)
9n	Ac	$CH_2CH{=}CH_2$	154—156 (AcOEt)	79	$C_{20}H_{23}FN_4O_4$	59.69 (59.49	5.76 5.86		4.72 4.57	13.92 13.85)
9o	CO_2Et	$(CH_2)_2CH{=}CH_2$	126—127 (AcOEt)	93	$C_{22}H_{27}FN_4O_5$	59.18 (59.05	6.10 6.39		4.26 4.00	12.55 12.66)

a) Compound **9g** was previously reported.[2] *b*) Compound **9f** was not isolated.

TABLE II. 1,8-Naphthyridine Derivatives **10a—o**, **11a—c**, **12**, **13a—f** and **14**

Compd.[a]	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)				
				C	H	Cl	F	N
10a	280—283	72	$C_{14}H_{15}FN_4O_3$	54.90	4.94		6.20	18.29
				(54.96	4.94		6.04	18.29)
10b	190—192	40	$C_{16}H_{19}FN_4O_3$	57.47	5.73		5.68	16.76
				(57.33	5.53		5.72	16.79)
10c	257—258	36	$C_{16}H_{19}FN_4O_3$	57.47	5.73		5.68	16.76
				(57.18	5.83		5.59	16.86)
10d	184—187	21	$C_{17}H_{21}FN_4O_3$	58.61	6.08		5.45	16.08
				(58.78	5.95		5.37	15.97)
10e	168—170	23	$C_{18}H_{23}FN_4O_3$	59.65	6.40		5.24	15.46
				(59.52	6.10		4.98	15.37)
10f	211—214	41	$C_{16}H_{19}FN_4O_4$	54.85	5.47		5.42	15.99
				(54.75	5.39		5.66	16.06)
10g	240—245 (dec.)	49	$C_{15}H_{16}ClFN_4O_3$	50.78	4.55	9.99	5.36	15.79
				(50.81	4.81	9.89	5.28	15.89)
10h	275—290 (dec.)	62	$C_{16}H_{18}ClFN_4O_3$	52.11	4.92	9.61	5.15	15.19
				(51.93	4.74	9.48	5.05	15.09)
10i	261—263	27	$C_{17}H_{20}ClFN_4O_3$	53.33	5.27	9.26	4.96	14.64
				(53.57	5.20	9.38	5.05	14.56)
10j	249—251	30	$C_{20}H_{19}FN_4O_3$	62.82	5.01		4.97	14.65
				(62.96	4.80		4.99	14.71)
10k	262—264	63	$C_{21}H_{21}FN_4O_3$	63.62	5.34		4.79	14.13
				(63.86	5.12		4.80	14.13)
10l	183—185	35	$C_{22}H_{23}FN_4O_3$	64.38	5.65		4.63	13.65
				(64.61	5.68		4.54	13.63)
10m	194—195	23	$C_{23}H_{25}FN_4O_3$	65.08	5.94		4.48	13.20
				(65.37	5.91		4.32	13.33)
10n	187—188	36	$C_{16}H_{17}FN_4O_3$	57.82	5.16		5.72	16.86
				(57.54	5.07		5.51	16.73)
10o	191—193	15	$C_{17}H_{19}FN_4O_3$	58.95	5.53		5.49	16.18
				(59.14	5.80		5.56	16.32)
11a	152—154 (AcOEt)	90	$C_{20}H_{25}FN_4O_5$	57.14	5.99		4.52	13.33
				(57.11	6.04		4.27	13.38)
11b	149—150 (AcOEt)	57	$C_{20}H_{24}ClFN_4O_4$	54.73	5.51	8.08	4.33	12.77
				(54.68	5.32	7.84	4.47	12.54)
11c	258—260 (MeCN)	75	$C_{18}H_{19}FN_4O_4$	57.75	5.12		5.07	14.97
				(57.71	5.21		5.28	14.82)
12	197—198	75	$C_{16}H_{17}FN_4O_3$	57.83	5.16		5.72	16.86
				(57.60	5.06		5.92	16.81)
13a	244—245 (MeCN)	73	$C_{20}H_{22}FN_5O_4$	57.83	5.34		4.57	16.86
				(57.61	5.33		4.64	16.61)
13b	266—268 (MeCN)	79	$C_{20}H_{26}FN_5O_4 \cdot 3.5\,H_2O$	49.71	6.90		3.92	14.52
				(49.87	6.70		4.03	14.50)
13c	140—141 (AcOEt)	24	$C_{22}H_{27}FN_4O_6 \cdot 0.25\,H_2O$	56.60	5.94		4.50	12.00
				(56.32	5.88		4.29	12.03)
13d	209—210 (MeCN)	61	$C_{20}H_{25}FN_4O_5$	57.27	5.77		4.53	13.36
				(56.99	6.07		4.45	13.29)
13e	174—175 (AcOEt)	62	$C_{20}H_{24}ClFN_4O_4$	54.73	5.51	8.08	4.33	12.77
				(54.47	5.80	7.79	4.29	12.69)
13f	292—295 (MeCN)	65	$C_{18}H_{19}FN_4O_4$	57.75	5.12		5.07	14.97
				(57.62	5.38		4.96	14.92)
14	244—247	65	$C_{16}H_{17}FN_4O_3$	57.83	5.16		5.72	16.86
				(57.68	5.22		5.73	16.73)

a) Compounds **10a—o**, **12** and **14** were purified by reprecipitation, by treatment with the acid and subsequently with the base or *vice versa*.

TABLE III. *In Vitro* Antibacterial Activity[a)]

Compd.	R^1	Minimum inhibitory concentrations, μg/ml		
		S. aureus 209P JC-1	*E. coli* NIHJ JC-2	*P. aeruginosa* 12
1	Et	0.78	0.10	1.56
2	$CH=CH_2$	1.56	0.05	0.78
3	CH_2CH_2OH	6.25	1.56	50
4	CH_2CH_2F	0.39	0.20	0.78
5	*cyclo*-Pr	0.39	0.05	0.20
6	Ph	1.56	0.20	0.78
10a	Me	6.25	0.20	6.25
10b	*n*-Pr	0.78	0.20	3.13
10c	iso-Pr	3.13	0.20	6.25
10d	*n*-Bu	6.25	0.39	25
10e	$(CH_2)_4CH_3$	50	6.25	>100
10f	$(CH_2)_3OH$	25	1.56	50
10g	CH_2CH_2Cl	0.39	0.10	0.78
10h	$(CH_2)_3Cl$	6.25	0.78	6.25
10i	$(CH_2)_4Cl$	25	3.13	100
10j	CH_2Ph	3.13	0.39	1.56
10k	CH_2CH_2Ph	12.5	50	100
10l	$(CH_2)_3Ph$	1.56	12.5	>100
10m	$(CH_2)_4Ph$	>100	>100	>100
10n	$CH_2CH=CH_2$	3.13	0.39	3.13
10o	$(CH_2)_2CH=CH_2$	3.13	0.39	6.25
12	$CH=CHCH_3$	25	0.20	0.78
14	$C(CH_3)=CH_2$	1.56	0.20	0.78

a) See the experimental section.

to the methyl and vinyl protons [δ 1.90 (3H, dd, $J=8$, 1.5 Hz), δ 6.06 (1H, dd, $J=13$, 8 Hz), δ 7.30 (1H, dd, $J=13$, 1.5 Hz)]. Compound **14** was prepared as follows. Alkylation of **7** with 2-chloropropionitrile gave the 1-cyanoethyl derivative **13a**. Hydrogenation of **13a** with Raney Ni afforded the amino analogue **13b**, which was diazotized with isoamyl nitrite in acetic acid, followed by acetylation with acetic anhydride, giving the 1-(acetoxymethyl)ethyl derivative **13c**. Treatment of **13c** with 1 N potassium carbonate followed by chlorination of **13d** with thionyl chloride gave the corresponding chloro analogue **13e**. The chloride **13e** was treated with potassium hydroxide in ethanol to give the 1-isopropenyl derivative **13f**, which, on hydrolysis with 2 N potassium hydroxide, was converted to the desired compound **14**.

Physical data for compounds **9**—**14** are shown in Tables I and II.

Biological Results

The *in vitro* antibacterial activity of compounds **10a**—**o**, **12** and **14** against representatives of Gram-positive (*Staphylococcus aureus* 209P JC-1) and Gram-negative (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* 12) bacteria was tested and the results are summarized in Table III, which includes, for comparison, the antibacterial activity of compounds **1**—**6** reported previously.

Among the compounds prepared in the present work, the most active was the 2-chloroethyl derivative **10g**, which was twice as potent as enoxacin (**1**) as regards activity against both *S. aureus* and *P. aeruginosa*. The 1-propyl and 1-isopropenyl derivatives (**10b** and **14**) had fairly potent antibacterial activity against all the bacteria tested. However, no compound which was markedly superior to enoxacin was found. Based on the results for all

enoxacin analogues including **1**—**6**, the ethyl, vinyl, 2-fluoroethyl, cyclopropyl, *n*-propyl, 2-chloroethyl and isopropenyl groups as the N-1 substituent were found to be efficient for enhancing *in vitro* antibacterial activity. In particular, the cyclopropyl group, as in **5**, was the most effective for increasing the activity against all the bacteria tested. Thus, one can conclude that the steric bulk of the N-1 substituent is important, namely nearly the same bulk as the ethyl, 2-chloroethyl or cyclopropyl group is optimum for the antibacterial activity. Quantitative relationships between the antibacterial activity and the physical properties (including the steric factor) of this series remain to be studied.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus, and are uncorrected. The ^{1}H-NMR spectra were taken at 60 MHz with a Varian EM-360A. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard.

Ethyl 1-Substituted 7-(4-Acetyl-1-piperazinyl) and 7-(4-Ethoxycarbonyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (9a—o, 11a and 13a)——Method A: An appropriate alkyl halide (30 mmol) was added at 80 °C to a stirred mixture of ethyl 7-(4-acetyl or ethoxycarbonyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**7** or **8**)[5] (10 mmol) and anhydrous K_2CO_3 (20 mmol) in dimethylformamide (DMF) (50 ml). The mixture was heated at the same temperature for 2—5 h and then filtered. The filtrate was concentrated to dryness *in vacuo*. The residue was extracted with $CHCl_3$. The extract was washed with water and concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH and the main fraction was crystallized from the solvent given in Table I or Table II to give the corresponding products **9a**—**o**, **11a** and **13a** (Table I and Table II).

1-Substituted 6-Fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic Acid (10a—o, 12 and 14)——Method B: A mixture containing **9a**—**n** or **13f** (10 mmol) and 20% HCl (60 ml) was heated to reflux for 2—4 h and then concentrated to dryness *in vacuo*. The residue was crystallized with water. The crystals were dissolved in hot water, and the solution was neutralized with NH_4OH to give the corresponding products **10a**—**n** and **14** (Table II and Table III).

Method C: A mixture containing **9o** or **11c** (10 mmol) and 2 N NaOH (50 ml) was heated to reflux for 2 h and then neutralized with AcOH. The resulting precipitate was collected and dissolved in 10% AcOH. The mixture was filtered to remove insoluble materials. The filtrate was neutralized with NH_4OH to give **10o** and **12** respectively (Table II and Table III).

Ethyl 7-(4-Acetyl-1-piperazinyl)-1-(2-chloropropyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (11b)——A mixture containing **11a** (3.0 g, 7.1 mmol), $SOCl_2$ (2.5 g, 21 mmol) and $CHCl_3$ (100 ml) was heated to reflux for 2 h. After addition of water and 10% NaOH, the chloroform layer was separated and concentrated to dryness *in vacuo*. The residue was crystallized from AcOEt to give **11b** (1.8 g). Compound **13e** was similarly prepared from **13d** (Table II).

7-(4-Acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(1-propenyl)-1,8-naphthyridine-3-carboxylic Acid (11c)——Compound **11b** (1.15 g, 2.6 mmol) was added to a mixture of KOH (440 mg, 7.9 mmol) and EtOH (20 ml) at 70 °C. The mixture was heated to reflux for 2 h. After neutralization with AcOH, the resulting solid was collected and recrystallized from CH_3CN to give **11c** (630 mg). Compound **13f** was similarly prepared from **13e**. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 2.29 (3H, s), 3.80 (8H, s), 5.19—6.62 (2H, m), 8.13 (1H, d, J=13 Hz), 8.68 (1H, s) (Table II).

Ethyl 7-(4-Acetyl-1-piperazinyl)-1-[1-(aminomethyl)ethyl]-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (13b)——A mixture containing **13a** (8.5 g, 20.5 mmol), Raney Ni (3 g), 10% NH_4OH (10 ml) and EtOH (270 ml) was shaken under H_2 gas until the required volume of hydrogen was absorbed. The mixture was filtered to remove the catalyst, and the filtrate was concentrated to dryness *in vacuo*. The residue was recrystallized from CH_3CN to give **13b** (6.8 g) (Table II).

Ethyl 1-[1-(Acetoxymethyl)ethyl]-7-(4-acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (13c)——Isoamyl nitrite (1.5 g, 19 mmol) was added to a mixture of **13b** (5.3 g, 12.6 mmol), AcOH (30 ml) and $CHCl_3$ (170 ml) at room temperature and the mixture was heated at 70—80 °C for 1.5 h, then concentrated to dryness *in vacuo*. After addition of Ac_2O (20 ml) to the residue, the mixture was heated at 90—100 °C for 0.5 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was chromatographed on silica gel with $CHCl_3$–MeOH to give **13c** (1.38 g) (Table II).

Ethyl 7-(4-Acetyl-1-piperazinyl)-1,4-dihydro-1-[1-(hydroxymethyl)ethyl]-4-oxo-1,8-naphthyridine-3-carboxylate (13d)——A mixture containing **13c** (1.3 g, 2.8 mmol), 1 N K_2CO_3 (40 ml) and EtOH (10 ml) was stirred at room temperature for 5 h, and then allowed to cool on an ice-bath. The resulting solid was collected and crystallized from CH_3CN to give **13d** (720 mg) (Table II).

Ethyl 7-(4-Acetyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-1-(1-propenyl)-1,8-naphthyridine-3-carboxylate (15)

——A mixture containing **11c** (200 mg, 0.53 mmol), Et_3N (81 mg, 0.80 mmol) and $CHCl_3$ (20 ml) was stirred under ice-cooling, and ethyl chloroformate (87 mg, 0.80 mmol) was added. The reaction mixture was stirred for 1 h at the same temperature. After addition of EtOH (3 ml), the mixture was stirred for an additional 3 h. The resulting solution was concentrated to dryness *in vacuo* and the residue was taken up in a mixture of water and $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness *in vacuo* and the residue was crystallized from AcOEt to give **15** (160 mg, 74%). ^{1}H-NMR (60 MHz, DMSO-d_6) δ: 1.28 (3H, t, J=7 Hz), 1.90 (3H, dd, J=8, 1.5 Hz), 2.06 (3H, s), 3.71 (8H, br), 4.23 (2H, q, J=7 Hz), 6.06 (1H, dd, J=13, 8 Hz), 7.30 (1H, dd, J=13, 1.5 Hz), 7.78 (1H, d, J=13 Hz), 8.48 (1H, s).

Biological Screenings——The *in vitro* antibacterial activity was tested by the same method as reported in a previous paper.[2)]

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References

1) Part XI: Y. Nishimura, A. Minamida and J. Matsumoto, *J. Heterocycl. Chem.*, "in press."
2) J. Matsumoto, T. Miyamoto, A. Minamida, Y. Nishimura, H. Egawa, and H. Nishimura, *J. Med. Chem.*, **27**, 292 (1984).
3) T. Miyamoto, H. Egawa, K. Shibamori and J. Matsumoto, *J. Heterocycl. Chem.*, **24**, 1333 (1987).
4) D. T. W. Chu, P. B. Fernandes, A. K. Claiborne, E. H. Gracey and A. G. Pernet, *J. Med. Chem.*, **29**, 2363 (1986).
5) J. Matsumoto, T. Miyamoto, A. Minamida, Y. Nishimura, H. Egawa, and H. Nishimura, *J. Heterocycl. Chem.*, **21**, 673 (1984).