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Studies on the Antibacterial Activity of 1-Substituted 1,4-Dihydro-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine Derivatives[1]

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The antibacterial activities of 64 naphthyridine derivatives were tested. Ultimately, potassium 1,4-dihydro-1-methyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine-3-carboxylate (677K) was selected as the most active compound. Compound 677K exhibited excellent antibacterial activities against either gram-positive or gram-negative bacteria *in vitro*. Furthermore, 677K exhibited high activities against experimental infection of streptococci, diplococci, or staphylococci in mice when administered orally. The activity on diplococcal infection in mice was higher than that of aminobenzylpenicillin, tetracycline, chloramphenicol, sulfisomezole, and dihydroxymethylfuratrizine. The effect of 677K against experimental typhosis was small. Cross resistance was seen between 677K and DF, but not between 677K and NA.

Nalidixic acid having a naphthyridine ring has been known to show excellent antibacterial activity, especially against gram-negative bacteria. As a part of the investigations on new antibacterial agents, a series of 1,8-naphthyridine derivatives have been prepared.[3] On the basis of antibacterial activity *in vitro* and *in vivo*, potassium 1,4-dihydro-1-methyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine-3-carboxylate (677K) was found as the most promising compound as a novel antibacterial agent in this series. Structure-activity relationships are also discussed.

Material[4] and Method

I. In Vitro Studies——a) Antibacterial Tests: The antibacterial activities were determined against various gram-positive and gram-negative bacteria by the standard serial dilution method in the semi-synthetic medium of Gakken and Mueller-Hinton agar medium containing 1% glucose. Test compounds were dissolved in ethylene glycol and added to the medium. Table I shows the microorganisms and media used

1) This work was presented at the 88th Annual Meeting of the Pharmaceutical Society of Japan, April 1968.

2) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*

3) *a*) S. Nishigaki, F. Yoneda, K. Ogiwara, T. Naito, R. Dohmori, S. Kadoya, Y. Tanaka, and I. Takamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1827 (1969); *b*) R. Dohmori, S. Kadoya, Y. Tanaka, I. Takamura, R. Yoshimura, and T. Naito, *Chem. Pharm. Bull.* (Tokyo), **17**, 1832 (1969).

4) Abbreviation used are: DF for dihydroxymethylfuratrizine, NA for nalidixic acid, CP for chloramphenicol, SM for streptmycin, TC for tetracycline, AB-PC for aminobenzylpenicillin, KM for kanamycin, SIZ for sulfisomezole.

for the sensitivity determination. Further, 14 strains of other bacteria were also used which were isolated recently from clinical specimens and known to be multiple drug resistant strains. In the case of broth-dilution method, these organisms were inoculated into the medium containing the respective test compounds and incubated at 37° for 48 hr. The inoculum size was 0.05 ml of a 10^{-3} diluted overnight broth culture to 5 ml of medium. In the case of agar-dilution method, a loopful of 10^{-3} diluted overnight culture in broth was used as inoculum. The activity of these compounds was expressed as the minimum inhibitory concentration (MIC) in μg/ml.

TABLE I. Microorganisms and Medium used for Antibacterial Test

Microorganism	Medium	Microorganism	Medium
E. coli K-12	G	*Staph. aureus* R 4	G
Sh. dysenteriae Hanabusa	G	*Diplo. pneumoniae* DP-1	M.H.
Sh. flexineri 257	G	*Coryne. diphtheriae* P.W. 8	M.H.
Sal. typhosa H 901	G	*Str. agalactiae* 9925	M.H.
Ps. aeruginosa Tsuchijima	G	*Erys. rhusiopathiae* Chiran	M.H.
Staph. aureus Terajima	G		

Composition of Gakken Medium

$Na_2HPO_4 \cdot 12\ H_2O$	2.5 g	DL-Tryptophan	10 mg
KH_2PO_4	0.4 g	Thiamine hydrochloride	10 mg
NaCl	5.0 g	$MgSO_4 \cdot 7H_2O$	0.1 g
Casamino acid	2.0 g	Water	1000 ml
Glucose	1.0 g		pH 7.0

G: Gakken medium M.H.: Mueller-Hinton agar medium containing 1% glucose

b) Effect of Bovine Serum Albumin on the MIC: The effect of 0.5% bovine serum albumin on the activity of the test compounds against *Escherichia coli* K-12 were examined by the serial dilution method with Gakken medium.

c) Development of Resistance and Cross Resistance: For the resistance study, serial two-fold broth dilutions were made in 5 ml of Gakken medium and inoculated with 0.05 ml of 10^{-3} diluted overnight broth culture of *Shigella dysentriae* Hanabusa. After 48 hr of incubation at 37°, the MIC was recorded, and the contents of the tubes containing one-half or one-quarter of the MIC of 677K or 837K were used as inocula for the succeeding transfer series. Experiments for cross resistance were carried out with *E. coli* K-12 resistant to DF, NA, 677K, or 837K. These resistant strains were obtained by treatment with each of the drug *in vitro*.

d) *In Vitro* Antimycobacterial Activity: The MIC of test compounds for *Mycobacterium tuberculosis* $H_{37}Rv$ was determined by two-fold serial dilution in Kirchner medium, and was recorded after 3 weeks of incubation at 37°.

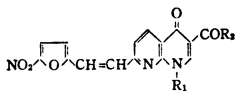
e) *In Vitro* Antifungal Activity: The antifungal activities *in vitro* were tested on the glucose-peptone-yeast extract agar by ten-fold serial dilution method. The activities were determined after incubation at 30° for 1 week.

II. In Vivo Studies——Chemotherapeutic activities of 677K and 837K were compared in experimental infections of *Streptococcus pyogenes*, *Diplococcus pneumoniae*, *Staphylococcus aureus*, and *Salmonella enteritidis* in mice. The infections were established by intraperitoneal injection of the organisms except *Staph. aureus* 39, which is a TC-resistant strain of human origin, and was injected intravenously for chronic kidney infection in mice. The inocula used for the infections were as follows:

Str. pyogenes G-36	1.2×10^6 cells/mouse
Diplo. pneumoniae DP-I	1.1×10^6 cells/mouse
Staph. aureus 39	6.8×10^6 cells/mouse
Sal. enteritidis 11	2.2×10^3 cells/mouse

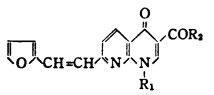
Aqueous suspension of the compound was administered orally to mice a few hours (2—4 hr) after infection of streptococci or diplococci. In staphlococcal infection, oral medication was made once daily for ten consecutive days, and once daily for five consecutive days in salmonella infection. Effect of test compounds in experimental infection of streptococci, diplococci, or salmonella was evaluated by survival rate of mice. In staphylococcal infection, the effect was evaluated not only by the survival rate of mice, but also the

TABLE II. Chemical Structures and Antibacterial Activities of 1-Substituted 7-[2-(5-Nitro-2-furyl)vinyl]-1,8-naphthyridine-3-carboxylic Acid Derivatives

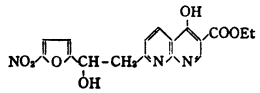


Compd. No.	Substituent R_1	Substituent R_2	MIC (μg/ml) *E. coli*	*Sh. dysen.*	*Sh. flex.*	*Sal. typh.*	*Ps. aeru.*	*Staph. aure.*	*Staph. R 4*	*Dip. pneu.*	*Cor. diph.*	*Str. agal.*	*Ery. rhus.*
675	H	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
677	CH_3	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
643	CH_2CH_3	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
705	$CH_2CH_2CH_3$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
706	$CH(CH_3)_2$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
730	CH_2COCH_3	OH	>100	>100	>100	>100	>100	100	50	<1.6	<1.6	<1.6	<1.6
800	CH_2CO-C$_6$H$_5$	OH	25	25	3.2	3.2	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
735	CH_2COOH	OH	<1.6	<1.6	<1.6	<1.6	12.5	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
797	$CH(CH_3)COOH$	OH	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
796	$CH(CH_3)COOEt$	OH	<1.6	3.2	<1.6	<1.6	12.5	<1.6	<1.6	3.2	<1.6	3.2	3.2
795	CH_2CONH_2	OH	>100	>100	>100	>100	>100	>100	>100	100	100	100	6.3
799	CH_2CN	OH	<1.6	<1.6	<1.6	<1.6	50	<1.6	<1.6	3.2	3.2	3.2	<1.6
714	CH_2CH-CH_2	OH	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
915	$CH_2CH=C(CH_3)_2$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
719	CH_2-C$_6$H$_5$	OH	>100	>100	>100	>100	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
753	CH_2-C$_6$H$_4$-NO_2	OH	>100	>100	>100	>100	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
734	CH_2CH_2OH	OH	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
758	$CH_2CH(OH)CH_3$	OH	<1.6	<1.6	<1.6	<1.6	3.2	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
752	$CH_2CH(OH)CH_2OH$	OH	6.3	6.3	3.2	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
757	$CH_2CH(OH)CH_2Cl$	OH	<1.6	<1.6	<1.6	<1.6	3.2	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
837	$CH_2CH_2N(Et)_2$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
832	$CH_2CH_2N(Me)_2\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
801	$CH_2CH_2CH_2N(Me)_2\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
857	$CH_2CH(CH_3)CH_2N(Et)_2\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	3.2	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
855	$CH_2CH_2NHC_2H_5\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
917	CH_2CH_2-N$\square\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	3.2
856	CH_2CH_2-N$\bigcirc\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
858	CH_2CH_2-N$\bigcirc$O$\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
916	CH_2CH_2-N$\bigcirc$S$\cdot HCl$	OH	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
641	H	OC_2H_5	<1.6	<1.6	<1.6	<1.6	100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
676	CH_3	OC_2H_5	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
642	C_2H_5	OC_2H_5	25	>100	50	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
704	$CH_2CH_2CH_3$	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	>100	<1.6	<1.6	<1.6
707	$CH(CH_3)_2$	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	100	<1.6	<1.6	<1.6
829	H	OCH_3	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	3.2	<1.6	>100	<1.6
1040	CH_3	OCH_3	<1.6	<1.6	<1.6	<1.6	3.2	<1.6	<1.6	3.2	<1.6	<1.6	<1.6
830	H	OC_4H_9	<1.6	3.2	<1.6	6.3	>100	<1.6	<1.6	>100	50	>100	<1.6
708	C_2H_5	OC_4H_9	100	>100	25	6.3	>100	<1.6	<1.6	>100	<1.6	<1.6	<1.6
711	C_2H_5	$OC_{10}H_{21}$	25	25	6.3	12.5	>100	<1.6	<1.6	6.3	<1.6	6.3	<1.6
715	$CH_2CH=CH_2$	OC_2H_5	>100	>100	>100	3.2	>100	<1.6	<1.6	>100	<1.6	3.2	<1.6
718	CH_2-C$_6$H$_5$	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	>100	>100	>100	>100
751	CH_2-C$_6$H$_4$-NO_2	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	>100	>100	>100	>100
729	CH_2COCH_3	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	>100	>100	>100	<1.6
710	CH_2COOH	OC_2H_5	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	100	<1.6	>100	<1.6
716	CH_2COOEt	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	>100	<1.6	>100	<1.6
790	$CH(CH_3)COOEt$	OC_2H_5	>100	>100	>100	>100	>100	<1.6	<1.6	>100	>100	>100	>100

Compd. No.	Substituent R_1	Substituent R_2	MIC (μg/ml) *E. coli*	*Sh. dysen.*	*Sh. flex.*	*Sal. typh.*	*Ps. aeru.*	*Stap. aure.*	*Stap.* R 4	*Dip. pneu.*	*Cor. diph.*	*Str. agal.*	*Ery. rhus.*
892	$CH_3CH_2N(Me)_2 \cdot CH_3SO_3H$	OC_2H_5	3.2	6.3	3,2	1.6	25	1.6	1.6	3.2	1.6	6.3	1.6
791	$CH_3CH_2N(Et)_2 \cdot HCl$	OC_2H_5	12.5	25	12.5	6.3	>100	<1.6	<1.6	6.3	6.3	12.5	3.2
712	CH_2COOEt	OCH_2COOEt	>100	>100	>100	>100	>100	<1.6	<1.6	>100	<1.6	>100	<1.6
798	$CHCOOEt$ CH_3	$OCHCOOEt$ CH_3	>100	>100	>100	>100	>100	<1.6	<1.6	50	6.3	12.5	6.3
794	C_2H_5	OCH_2CH_2OH	<1.6	6.3	<1.6	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
793	C_2H_5	OCH_2CHCH_2 (OH, OH)	6.3	12.5	3.2	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
792	C_2H_5	OCH_2CH_2Cl	<1.6	6.3	<1.6	<1.6	50	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
732	C_2H_5	$OCH_2CH_2N(Et)_2$	<1.6	3.2	<1.6	<1.6	25	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
831	H	$OCH_2CH_2N(Et)_2$	<1.6	<1.6	<1.6	<1.6	12.5	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
713	C_2H_5	NH_2	<1.6	<1.6	<1.6	<1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
789	C_2H_5	NHC_2H_5	<1.6	<1.6	1.6	1.6	>100	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
756	C_2H_5	NH-⟨⟩	3.2	6.3	3.2	3.2	25	<1.6	<1.6	6.3	<1.6	3.2	<1.6
755	C_2H_5	NH-⟨H⟩	50	50	12.5	12.5	>100	3.2	<1.6	6.3	3.2	12.5	<1.6
754	C_2H_5	N-⟨HO⟩	<1.6	6.3	<1.6	<1.6	25	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6
731	C_2H_5	$N(CH_2CH_2OH)_2$	6.3	6.3	12.5	6.3	50	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6



Compd. No.	Substituent R_1	Substituent R_2	MIC (μg/ml) *E. coli*	*Sh. dysen.*	*Sh. flex.*	*Sal. typh.*	*Ps. aeru.*	*Stap. aure.*	*Stap.* R 4	*Dip. pneu.*	*Cor. diph.*	*Str. agal.*	*Ery. rhus.*
788	C_2H_5	OC_2H_5	>100	>100	>100	>100	>100	25	50	>100	>100	>100	>100
717	C_2H_5	OH	>100	>100	>100	>100	>100	<1.6	<1.6	>100	>100	>100	25
1041	$CH_2CH_2N(Et)_2 \cdot HCl$	OC_2H_5	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100



Compd. No.	MIC (μg/ml) *E. coli*	*Sh. dysen.*	*Sh. flex.*	*Sal. typh.*	*Ps. aeru.*	*Stap. aure.*	*Stap.* R 4	*Dip. pneu.*	*Cor. diph.*	*Str. agal.*	*Ery. rhus.*
1039	6.3	12.5	12.5	6.3	>100	<1.6	3.2	50	>100	>100	6.3

E. coli=*E. coli* O 111, *Sh. dysen.*=*Sh. dysenteriae* Hanabusa, *Sh. flex.*=*Sh. flexneri* 258, *Sal. typh.*=*Sal. typhosa* H 901, *Ps. aeru.*=*Ps. aeruginosa* Tsuchijima, *Stap. aure.*=*Staph. aureus* Terajima, *Stap.* R 4=*Staph. aureus* Resistance, *Dip. pneu.*=*Diplo. pneumoniae* DP-1, *Cor. diph.*=*Coryne. diphtheriae* P.W. 8, *Str. agal.*=*Str. agalactiae* 9995, *Ery. rhus.*=*Erys. rhusiopathiae* Chiran

severity of abscess formation and viable counts of staphylococci in the kidneys. Namely after evaluation of the degrees of abscess formation, the kidneys were excised and the homogenized in 10 ml of sterilized physiological saline solution, and homogenate were serially diluted ten times with the same solution. Aliquots of these homogenates were added to nutrient agar medium containing 5% mannitol and 0.2% bromthymol blue. Staphylococci in the homogenates were counted after incubation at 37° for 48 hr.

Result

I. *In Vitro* Studies

1) Antibacterial Activity of Naphthyridine Derivatives——The *in vitro* activities of 64 compounds against laboratory strains of bacteria are shown in Table II. Naphthyridine 3-carboxylic acid derivatives were more active than the corresponding esters or amides. Further, the compounds substituted with lower alkyl (methyl, ethyl, propyl) or aminoalkyl group at 1-position of naphthyridine ring were more active than other compounds. Com-

pound 641 having a vinyl group exhibited a high activity, and 1039 lacking vinyl group was less active than 641. Compounds 717, 788, and 1041 lacking nitro group in furan ring exhibited no activity against most strains except staphylococci. In view of these activities, the compounds were divided into three groups; (1) compounds active against both gram-positive and negative bacteria, (2) compounds which were more active against gram-povitive than gram-negative bacteria, and (3) compounds which were inactive against gram-negative bacteria but active against gram-positive bacteria.

From these results, 13 compounds (643, 675, 677, 705, 706, 801, 832, 837, 855, 856, 858, 916, and 917) were selected preliminarily whose MIC were less than 1.6 μg/ml against all strains.

2) Antibacterial Activities of 13 Compounds——More detailed studies were made in order to obtain more precise information of antibacterial activities of these compounds.

i) Antibacterial Activities against Four Strains of Common Pathogenic Bacteria: Seven compounds (643, 675, 677, 705, 801, 832, and 837) were chosen on the basis of activities against *Staph. aureus* Terajima, *Sh. dysenteriae* Hanabusa, *Ps. aeruginosa* Tsuchijima, and *E. coli* K-12 (Table III). The most active compound was 1,4-dihydro-1-methyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine-3-carboxylic acid (677).

Table III. Antibacterial Activities of 13 Compounds

		643	675	677	705	706	801	832	837	855	856	858	916	917
MIC (μg/ml)	*Staph. aure.*	0.0125	0.05	0.0125	0.0125	0.2	0.1	0.05	0.025	0.8	0.05	0.05	0.0125	0.1
	Sh. dysen.	0.4	0.2	0.05	0.2	1.6	0.4	0.2	0.2	1.6	0.8	0.8	0.8	0.4
	Ps. aeru.	0.8	1.6	0.1	0.2	1.6	0.8	0.4	0.2	1.6	1.6	1.6	0.8	0.8
	E. coli	0.2	0.1	0.05	0.4	1.6	0.4	0.1	0.2	1.6	0.8	0.8	0.8	0.8
	Mycobac.	1.6	100	1.6	1.6		25	12.5	6.3					
	E. coli DF-R[a]	6.3	6.3	0.8	6.3	6.3	6.3	6.3	6.3	50	12.5	50	100	12.5
	E. coli NA-R[a]	0.4	0.4	0.1	0.2	12.5	0.8	0.4	0.8	3.2	1.6	1.6	1.6	0.8
Effect of 0.5% Albumin(MIC for *E. coli*)		1.6	1.6	0.2	0.8	100	0.8	0.8	0.8	3.2	3.2	1.6	1.6	1.6
Development of resistance in Shigella (6th passage)		250-fold		16-fold					4-fold					

Staph. aure.=*Staph. aureus* Terajima; *Sh. dysen.*=*Sh. dysenteriae* Hanabusa; *Ps. aeru.*=*Ps. aeruginosa* Tsuchijima; *Mycobac.*=*Mycobac. tuberculosis* $H_{37}Rv$; DF=dihydroxymethyl-furatrizine;NA=nalidixic acid

a) These strains are resistant to DF or NA with MIC volues higher than 100 μg/ml respectively.

ii) Antibacterial Activities against *E. coli* Resistant Strains: The antibacterial activities of 13 compounds were higher against NA resistant strain than DF resistant strain (Table III). The compounds, 677, 801, 832, and 837 were more active than the other nine compounds. Among these four compounds, 677 showed the highest antibacterial activity.

iii) Effect of Bovine Serum Albumin on the MIC: In the middle column of Table III is shown a comparison of the effect of 0.5% bovine serum albumin on the MIC of these 13 compounds. The decrease of activities of the compounds against *E. coli* K-12 was found in the presence of 0.5% bovine serum albumin in the medium, and a marked decrease was observed in the case of 706. The most active compound was 677K.

iv) Antimycobacterial Activities: The seven compounds (643, 675, 677, 705, 801, 832, and 837), which showed high activities in the previous experiments, were tested to determine antimycobacterial activity *in vitro*. As shown in Table III, *M. tuberculosis* $H_{37}Rv$ exhibited high sensitivity to 643, 677, 705 (MIC: 1.6 μg/ml), and 837 (MIC: 6.3 μg/ml).

v) Development of Resistance: The development of resistance of *Sh. desenteriae* Hanabusa to 643, 677, and 837 was determined by the stepwise dose-increasing method. Degree of resistance of the organism to 643, 677, and 837 increased respectively to 250, 16, and 4-times of the primary MIC in six serial transfers (Table III).

3) Comparison of Antibacterial Activities of 677 and 837——From the results described above, 677 and 837 were selected from the seven compounds according to the MIC, and the antibacterial activities of 677 and 837 were compared with those of DF and NA, both *in vitro* and *in vivo*. Potassium salts of 677 and 837 (677K and 837K) were used for further experiments because they were more soluble in water than the corresponding free acids.

i) Activities against Common Pathogenic Bacteria, Tubercle Bacilli, and Fungi: The activities of 677K and 837K against 18 strains of gram-negative bacteria were compared with

Table IV. Minimal Inhibitory Concentration (μg/ml) against Gram-negative Bacteria

Microorganism	677K	837K	DF	NA
E. coli 0111	0.025	0.2	0.1	1.6
E. coli K-12	0.05	0.4	0.2	6.3
E. coli B	0.05	0.4	0.4	1.6
Kleb. pneumoniae Type I	0.025	0.1	0.1	1.6
Kleb. pneumoniae Type II	0.1	0.8	0.8	0.8
Sh. dysenteriae Hanabusa	0.05	0.2	0.2	1.6
Sh. flexineri Komagome	0.05	0.1	0.1	3.2
Sh. flexineri 253	0.025	0.1	0.1	1.6
Sal. typhosa H 901	<0.0125	0.05	0.05	0.4
Sal. enteritidis 11	0.05	0.4	0.4	0.1
Sal. paratyphi B 8006	0.05	0.1	0.1	0.1
Ps. aeruginosa Tsuchijima	0.4	0.4	12.5	>100
Pro. vulgaris 3045	0.8	6.3	6.3	6.3
Pro. vulgaris 3167	0.2	1.6	1.6	3.2
Aero. liquefaciens T-ET	<0.0125	0.2	0.025	
Aero. liquefaciens Y-62	<0.0125	0.2	0.05	
Aero. salmonicida	<0.0125	0.1	0.025	
Vibrio piscium	<0.0125	0.0125	<0.0125	0.2

medium: Gakken medium
DF: dihydroxymethylfuratrizine, NA: nalidixic acid

Table V. Minimal Inhibitory Concentration (μg/ml) against Gram-positive Bacteria, My. tuberculosis, and Pathogenic Fungi

Medium	Microorganism	677K	837K	DF	NA
1	*Staph. aureus* Terajima	0.0063	0.025	0.2	25
	Staph. aureus R4	0.0063	0.025	0.2	100
	Staph. aureus 39	0.0063	0.025	0.2	50
	B. subtilis PCI 219	0.05	0.1	0.1	0.4
	Diplo. pneumoniae DP-1	0.05	0.8	1.6	>100
	Diplo. pneumoniae DP-2	0.0125	0.05	0.1	>100
	Str. pyogenes G-36	0.05	0.1	0.1	>100
	Str. pyogenes S-8	0.05	0.4	0.1	>100
	Str. agalactiae 9925	0.2	0.4	0.8	>100
	Str. dysgalactiae 9926	0.0125	0.1	0.2	>100
	Coryne. pyogenes Daini-Nojo	0.1	0.4	1.6	>100
	Coryne. pyogenes Tojo-Hai	0.1	0.1	0.2	>100
2	*My. tuberculosis* $H_{37}Rv$	<1.6	6.3	3.2	
3	*Tri. mentagrophytes*	1	>100	10	
	Tri. interdigitale	10	>100	10	
	Mic. gypseum	1	>100	10	
	Asp. fumigatus	10	>100	10	
	Can. albicans	1	>100	100	
	Cry. neoformans	1	>100	10	

medium: 1=Mueller-Hinton medium, 2=Kirchner medium, 3=glucose-yeast extract-peptone agar

those of DF and NA *in vitro*. As shown in Table IV, the activities of 677K and 837K were higher than those of control agents, DF and NA, and especially marked difference of MIC was found in the case of *Pseudomonas*.

MIC of 677K against gram-negative bacteria was 0.0125 to 0.8 μg/ml, and this compound was about 2 to 10 times as effective as 837K. Table V presents the MIC of 677K and 837K against gram-positive bacteria. Similar result was obtained as in the case of gram-negative bacteria.

Antimycobacterial and antifungal activities of 677K, 837K, and DF are shown in Table V. The activity of 677K against *M. tuberculosis* $H_{37}Rv$ was higher than that of 837K and DF.

The *in vitro* activities of 677K, 837K, and DF against several pathogenic fungi were examined. The antifungal activities of 677K were proved to be the best among these three compounds. The inhibition of growth of the fungi was not observed even in the presence of 100 μg/ml of 837K.

ii) Development of Resistance and Cross Resistance: Examination on development of resistance was carried out by serial transfer in Gakken medium containing 677K or 837K. As seen in Table VI, the development of resistance to 677K or 837K in *Sh. dysenteriae* Hanabusa was slow.

Table VII shows *in vitro* antibacterial activities of 677K and 837K against DF- or NA-resistant strain derived from *E. coli* K-12. It is interesting that cross resistance was seen between the test compounds (677K and 837K) and DF but not between these test compounds and NA.

TABLE VI. *In Vitro* Development of Resistance to 677K and 837K in *Sh. dysenyeriae* Hanabusa

Compound	MIC (μg/ml)									
	1[a]	2	3	4	5	6	7	8	9	10
677K	0.2	0.2	0.2	0.2	0.4	0.8	1.6	1.6	1.6	1.6
837K	0.4	0.8	0.8	1.6	1.6	1.6	1.6	1.6	1.6	1.6

[a] transfer No.

TABLE VII. Cross Resistance Patterns of 677K, 837K, DF, and NA

E. coli K-12	MIC (μg/ml)			
	677K	837K	DF	NA
Susceptible parent culture	0.4	3.2	1.6	6.3
DF-resistance	6.3	25	100	100
NA-resistant	0.4	1.6	1.6	100
677K-resistant	25	50	12.5	50
837-K-resistant	50	100	12.5	50

inoculum size: 4.6×10^6 cells/ml

iii) Antibacterial Activities against Multiple Drug Resistant Strains: The antibacterial activity of 677K and 837K against multiple drug resistant strains isolated from clinical specimen was tested by agar streak method on heart infusion medium. The inoculum given was 10^3 times as large in size as that used in the MIC test. Although the organisms were resistant to CP, SM, TC, AB-PC and KM as shown in Table VIII, 677K and 837K showed high activities against these organisms, and 677K was superior to 837K in activity.

II. *In Vivo* Studies

1) Effect against Experimental Streptococcal Infection in Mice——The chemotherapeutic effect of 677K, 837K, and DF against experimental streptococcal infection in mice is shown in Fig. 1. The mice treated with 677K or 837K showed a higher survival rate than those treated with DF during 7 days of experiment, while all the control mice died within 2 days. ED_{50} values were calculated from the survival rate on the 4th day after infection according to the method of Litchfield-Wilcoxon. ED_{50} values of 677K and 837K were 10.0 and 17.8 mg/kg, respectively.

TABLE VIII. Sensitivity of Clinical Isolates to 677K and 837K

Microorganism	MIC (μg/ml)						
	CP	SM	TC	AB-PC	KM	677K	837K
Staph. aureus 19	50	>100	>100	1.6	25	0.0125	0.05
Staph. aureus 20	12.5	>100	100	3.2	1.6	0.0125	0.0125
Staph. aureus 45	100	100	>100	3.2	1.6	0.0125	0.025
Staph. aureus 76	50	>100	100	3.2	100	0.0125	0.025
Ps. aeruginosa 5	>100	25	>100	>100	100	6.3	12.5
Ps. aeruginosa 6	>100	25	>100	>100	100	1.6	1.6
Ps. aeruginosa 7	>100	>100	>100	>100	100	3.2	3.2
Ps. aeruginosa 8	>100	>100	>100	>100	100	1.6	3.2
E. coli 78746 (0146)	100	>100	>100	>100	3.2	0.8	1.6
E. coli 78741 (0146)	>100	>100	>100	>100	3.2	0.8	3.2
E. coli 74468 (0127)	6.3	0.8	50	>100	3.2	0.8	1.6
E. coli 84408 (0136)	0.8	1.6	3.2	50	6.3	0.4	0.4
Sh. sonnei 60238	>100	>100	>100	>100	1.6	0.4	0.8
Sh. sonnei 60237	>100	>100	>100	>100	3.2	0.4	0.8
Sh. sonnei 60263	>100	>100	>100	100	3.2	0.4	0.8
Sh. sonnei 70024	>100	>100	>100	100	3.2	0.4	0.8

medium: heart infusion agar

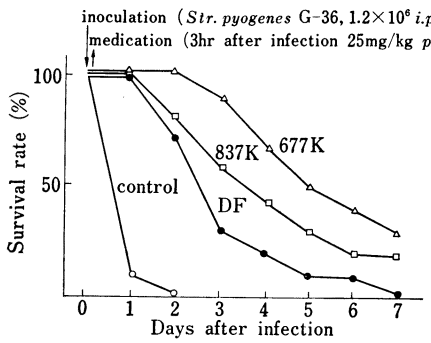


Fig. 1. Chemotherapeutic Activity of Naphthyridine Derivatives against Experimental Streptococci Infection in Mice

animal: JCL-ICR male mice, weighting 19—21 g and 25—28 days of age were used. ED_{50} on the 4th day after infection (Litchfield-Wilcoxon's method)
677 k: 10.0 (2.4—41.1) mg/kg 837 k: 17.8 (5.4—58.2) mg/kg DF: 48.4 (30.0—79.1) mg/kg

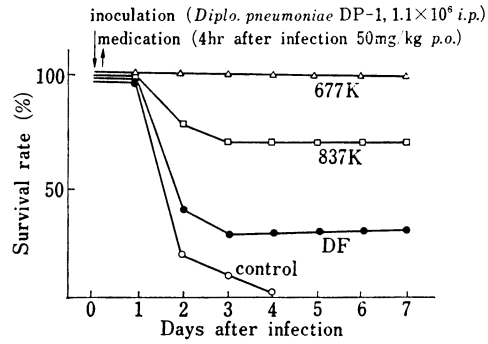


Fig. 2. Chemotherapeutic Activity of Naphthyridine Derivatives against Experimental Pneumococcal Infection in Mice

animal: JCL-ICR male mice, weighting 17—20 g and 25—28 days of age were used.
ED_{50} on the 4th day after infection (Litchfield-Wilcoxon's method) 677k: 100 mg 12.5 mg/kg 837 k: 31.9 (19.0—53.6) mg/kg DP>100 mg/kg

2) Effect against Experimental Pneumococcal Infection in Mice——As shown in Fig. 2,

the survival rate of mice medicated with 677K on the 7th day after infection was 100%, and was higher than that of 837K (70%) and DF (30%), while all the control mice died within 4 days after infection. ED_{50} values of 677K on the 4th day after infection was less than 12.5 mg/kg, and those of 837K and DF were 31.9 and more than 100 mg/kg, respectively.

3) Effect against Experimental Staphylococcal Infection in Mice——The effect of 677K, 837K, and DF in doses of 25, 50, or 100 mg/kg against experimental staphylococcal infection in mice was evaluated by the survival rate of mice, the grade of abscess formed, and viable count of bacteria in the kidneys. Fig. 3 shows the degree of abscess formation and the viable count of bacteria in the kidneys. Judging from the remarkable multiplication of bacteria in dead mice, septicemia might be the cause of death in these mice. It was assumed that bacterial count of dead mice was more numerous than that of survived mice. As maximum bacterial count of a survived mice was 10^9 cells per homogenated 1 ml, accordingly, the bacterial count was assumed to be more than 10^9 in every dead mouse.

As a result, 677K was proved to be the best in all respects, and 837K and DF were arranged in the order of decreasing potency. In these respects the effectiveness of these three compounds was agreement with that evaluated ED_{50} ranking.

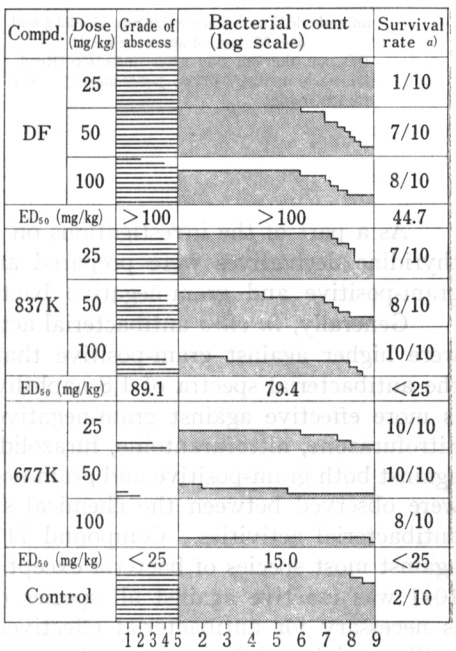


Fig. 3. Chemotherapeutic Activity of Naphthyridine Derivatives against Experimental Staphylococcal Infection in Mice

a) No. mice alive/No. used

4) Effect against Experimental Typhosis in Mice——Several experiments have been carried out to determine the chemotherapeutic effect of the test compounds against experimental typhoid infection in mice. In these experiments three or four different doses have been administered. Fig. 4 illustrates the survival rate which was obtained in mice treated with a dose of 200 mg/kg. In this experiment, *Sal. enteritidis* 11 was injected intraperitoneally and medications were carried out orally once daily for five days. The medication started about 2 hr after injection. The effects were determined by the survival rate of mice. As shown in Fig. 4, all of the control mice died within 8 days. The mice medicated with 677K or 837K were survived on the 7th day after infection at the rate of 10% and 70%, respectively. ED_{50} values calculated by the method of Reed-Muench were more than 200 mg/kg for 677K and 129 mg/kg for 837K. Compound 837K was better than 677K in this experiment, contrary to the results obtained from infection experiments with gram-positive bacteria.

5) Comparison of Chemotherapeutic Effect of 677K with that of Commercial Antibacterial Drugs——Since compound 677K was active both *in vitro* and *in vivo*, chemotherapeutic effect of 677K against experimental peumococcal infection in mice was compared with that of other commerical antibacterial drugs (AB–PC, TC, SIZ, DF, and CP). The results obtained are summarized in Fig. 5. In view of such effects of these drugs after 7 days of infection, the test compounds might be divided into two groups, a higher active group of 677K and AB–PC, and a lower active group of TC, DF, CP, and SIZ. Compound 677K was more active than the others.

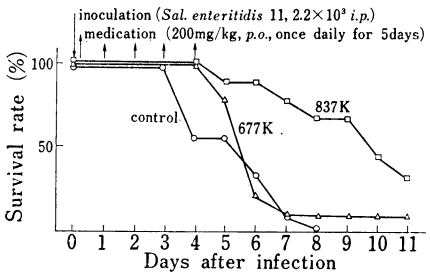


Fig. 4. Chemotherapeutic Activity of Naphthyridine Derivatives against Experimental Typhosis in Mice

animal: ddN male mice weighting 17—22 g and 35—37 days of age were used.
ED_{50} on the 7th day after infection (Reed-Muench's method) 677k: >200 mg/kg 837 K: 129 mg/kg

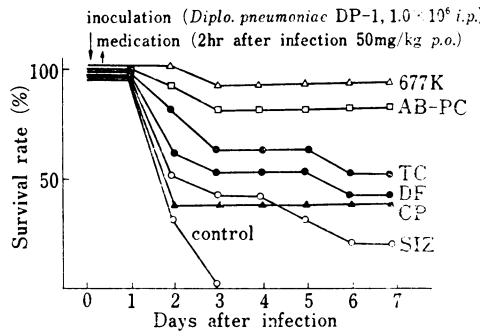


Fig. 5. Comparison of Chemotherapeutic Activity of 677K with that of other Commercial Antibacterial Drugs in Experimental Pneumococcal Infection in Mice

animal: JCL-ICR male mice, weighting 19—20 g and 24—27 days of age were used.
AB-PC: aminobenzylpenicillin TC: tetracycline
SIZ: sulfisomezole DF: dihydroxymethylfuratrizine
CP: chloramphenicol

Discussion

As a part of the investigations on new antibacterial agents, 64 compounds of 1,8-naphthyridine derivatives were prepared and their antibacterial activities were tested against gram-positive and gram-negative bacteria in comparison with NA and DF.

Generally, *in vitro* antibacterial activities of 64 compounds of naphthyridine derivatives were higher against gram-positive than gram-negative bacteria. This fact indicates that the antibacterial spectra of 1,8-naphthyridine derivatives is different from that NA[5] which is more effective against gram-negative than gram-positive bacteria. Nitrofurans such as nitrofurazone, nitrofurantoine, furazolidone, and furaltadone are known to be equally active against both gram-positive and gram-negative bacteria *in vitro*.[6] The following relationships were observed between the chemical structures of 1,8-naphthyridine derivatives and their antibacterial activities. Compound 717 and 788 lacking nitro group were almost inactive against most species of bacteria except *Staph. aureus* and *Ery. rhusiopathiae*, and compound 1041 was inactive against all species of bacteria used. This indicates that a nitro group is necessary for antibacterial effectiveness, as reported by many workers.[7] Further, the antibacterial activities of 3-carboxynaphthyridine were higher than those of esterified or amidated compounds. In connection with this respect, Lesher[8] reported that esterified or amidated NA was active as NA *in vivo*, although the former was less active than the latter *in vitro*. He suggested that esterified or amidated NA might be hydrolysed *in vivo*. Similar phenomenon was reported by Kaminsky[9] in the case of oxolinic acid. Accordingly, esterified or amidated 3-carboxynaphthyridine derivative might be active *in vivo* as the corresponding

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3-carboxylic acid derivative which has been proved to be active *in vivo*.

Compound 677K and 837K were selected as highly active among 64 kinds of 1,8-naphthyridine derivatives. The antibacterial activities of these two compounds were superior to those of DF and NA *in vitro*. Chemotherapeutic activities of 677K were compared with those of 837K in experimental infections in mice. The results showed that 677K was more active than 837K. Despite the high effectiveness against all the bacteria used *in vitro*, 677K was active against only gram-positive bacteria *in vivo*.

Further, the studies of blood, organ, and urinary levels of 677K are in progress in order to clarify the reason for its ineffectiveness against experimental typhoid infection.

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