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Prominent Chemotherapeutic Properties of the N-Oxide Derivatives of (5-Nitro-2-furyl)vinyl Heterocycles

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On the way of the study to improve the *in vivo* antibacterial activity of nitrofuran compounds, it was found that the some N-oxide derivatives of (5-nitro-2-furyl)vinyl heterocycles had the superior *in vivo* activity to the corresponding original compounds. To clarify whether such tendency was observed in general, thirteen (5-nitro-2-furyl)vinyl heterocycles and their N-oxide derivatives were co mpared on their oral protective effects in mice and additionally on the *in vitro* antibacterial activities and the plasma levels in rats. It was generally observed that the N-oxide derivatives were superior to the corresponding original compounds in the protective effects. Such superiority seemed to be partially attributed to the increase of the *in vitro* antibacterial activities and the plasma levels of the N-oxide derivatives.

It has been reported that (5-nitro-2-furyl)vinyl heterocycles generally show outstanding antibacterial activity *in vitro*.[2] However, their *in vivo* activity is not always sufficient as compared with the *in vitro* one. Such is the case especially when the compounds are administered by an oral route.

Various chemical modifications were tried to improve the *in vivo* activity. It was found in the many compounds of this type that the conversion of the nitrogen of heterocycles into N-oxide markedly improved oral protective effects on the experimental infections in mice.

This paper is concerned with the influence of the conversion on oral protective effects, and additionally on *in vitro* antibacterial activities and plasma levels. The possible reasons for the improvement were also discussed.

Experimental

a) **Compounds**——All nitrofuran compounds tested were synthesized in Research Laboratry, Dainippon Pharmaceutical Co., Ltd.[3]

b) ***In vitro* Tests**——The serial tube dilution method was employed, details of which were described elsewhere.[2]

c) ***In vivo* Tests**——Infection with *Staphylococcus aureus*; Each group of ten male mice (*dd* strain) weighing 18—20 g was intraperitoneally infected with 100—1000 LD_{50} of the bacterium with 3% mucin (Wako). Immediately, the infected mice were orally treated once with 100 mg/kg of the compounds suspended in 3% Gum Arabic solution. The mice were daily observed for one week, in which survival rate was determined. Infection with *Salmonella typhimurium*; Without added mucin, the infection procedure was the same as above. The infected mice were orally treated twice a day for four days from the day of the infection with graded doeses of the compounds suspended in 3% Gum Arabic solution. The mice were daily observed for two weeks. ED_{50} was calculated from Behrenes–Kaerber's formula[4] at the terminal day.

1) Location: *Ebie-kami-2-chome, Fukushima-ku, Osaka.*
2) K. Fujimoto, *Chemotherapy*, **15**, 288 (1967).
3) A. Fujita, T. Yamamoto, J. Matsumoto, S. Minami and H. Takamatsu, *Yakugaku Zasshi*, **85**, 565 (1965); A. Fujita, M. Nakata, S. Minami and H. Takamatsu, *ibid.*, **86**, 1014 (1966); A. Fujita, J. Aritomi, S. Minami and H. Takamatsu, *ibid.*, **86**, 427 (1966).
4) G. Kaerber, *Arch. Exptl. Pathol. Pharmakol.*, **162**, 480 (1931).

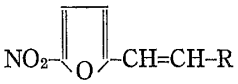
d) **Plasma Levels**——A compound suspended in 3% Gum Arabic solution was orally administered to three male rats (*Donryu* strain) weighing 250—300 g in a dose of 200 mg/kg. Blood was drawn by cardiac puncture with a heparinized syringe under ether anesthesia one, two and four hours postadiministration. The collected blood was pooled together at each time and centrifuged to separate plasma, which was then assayed by the cup-plate method using *Bacillus subtilis* as an indicator strain. Plasma levels were expressed as the concentrations of the compounds administered.

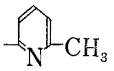
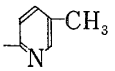
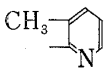
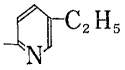
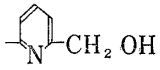
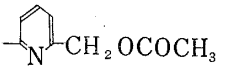
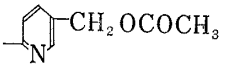
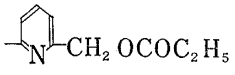
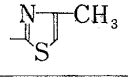
Results

(a) *In Vitro* Tests

In vitro antibacterial activity of (5-nitro-2-furyl)vinyl heterocycles (non-N-oxides) are shown in Table I. Most compounds were considerably active against various gram positive and negative bacteria, while some compounds were inactive against *Streptococcus hemolyticus* and *Proteus vulgaris*.

Table I. *In Vitro* Antibacterial Activity of (5-Nitro-2-furyl)vinyl Heterocycles

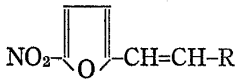


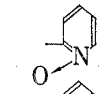
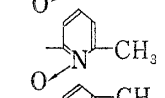
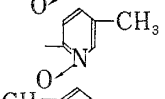
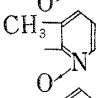
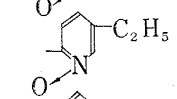
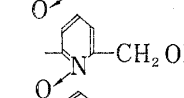
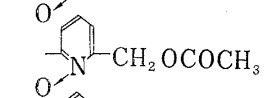
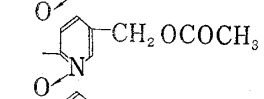
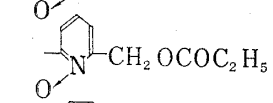
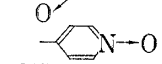
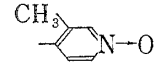
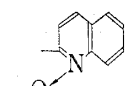
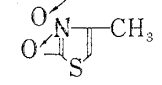
No.	R	A	B	C	D	E	F	G	H	I	J
		(MIC: μg/ml)									
1		10	1	>100	1	0.1	0.1	1	10	0.3	3
2		3	0.3	100	1	0.01	0.1	0.1	10	0.3	1
3		1	0.1	>100	0.3	0.03	0.3	0.3	30	0.3	1
4		100	1	>100	1	0.1	0.1	1	100	3	3
5		3	0.3	>100	1	0.03	1	1	>100	1	3
6		1	0.3	10	1	0.01	0.3	0.3	10	0.3	1
7		10	1	>100	10	0.03	0.3	0.1	>100	1	10
8		3	0.3	30	3	0.03	0.1	0.3	30	1	3
9		3	0.3	>100	10	0.03	1	0.3	>100	3	10
10		3	0.3	30	3	0.03	0.1	0.3	3	0.3	0.3
11		3	0.3	30	1	0.03	0.3	0.3	10	1	0.3
12		1	0.1	0.3	0.3	0.03	1	1	100	3	1
13		10	1	10	3	0.1	0.3	0.3	100	1	1

abbreviation
MIC: minimum inhibitory concentration
A: *Staphylococcus aureus* Terajima, B: *Staphylococcus albus*,
C: *Streptococcus hemolyticus*, D: *Diplococcus pneumoniae* I,
E: *Bacillus subtilis* PCI 219, F: *Klebsiella pneumoniae*,
G: *Escherichia coli* NIHJ JC-1, H: *Proteus vulgaris* OX_{19},
I: *Salmonella typhimurium*, J: *Shigella flexneri* 2a EW 10

In vitro antibacterial activity of their N-oxide derivatives (N-oxides) are presented in Table II. The all derivatives were also active against all of the bacteria tested, although the strength of the activities varied. It can be found by the comparison of Table I and Table II that N-oxides are usually more active than non-N-oxides.

Table II. *In Vitro* Antibacterial Activity of the N-oxide Derivatives of (5Nitro-2-furyl)vinyl Hetrocycles



No.	R	A	B	C	D	E	F	G	H	I	J
		(MIC: μg/ml)									
1′		1	0.3	3	1	0.03	0.3	0.1	3	0.1	0.3
2′		1	0.3	30	3	0.01	0.1	0.1	10	0.1	1
3′		1	0.3	30	1	0.01	0.1	0.1	3	0.1	0.3
4′		3	1	100	3	0.1	0.3	1	30	1	3
5′		1	1	100	1	0.03	0.3	1	10	0.3	1
6′		0.3	0.1	1	1	0.01	0.1	0.1	3	0.1	0.3
7′		1	0.3	100	3	0.01	0.1	0.3	100	0.3	3
8′		0.3	0.3	3	1	0.1	0.1	0.3	10	0.3	3
9′		0.3	1	100	3	0.1	0.3	0.3	100	1	10
10′		1	0.3	3	0.3	0.01	0.3	0.3	3	0.3	1
11′		0.3	0.1	10	0.3	0.03	0.3	1	30	3	1
12′		1	0.3	1	1	0.01	0.1	0.1	1	0.1	1
13′		1	0.3	3	1	0.03	0.3	0.3	3	0.1	0.3

(b) *In Vivo* Tests

Table III shows oral protective effects on the experimental infection with *Staphylococcus aureus*. None of non-N-oxides was singnificantly effective, whereas nine out of thirteen N-oxides were effective showing more than 30% of survival rate. It is clear that N-oxides are always superior to the corresponding non-N-oxides.

Almost similar tendency was obtained in the infection with *Salmonella typhimurium* as shown in Table IV. Four out of thirteen non-N-oxides were effective to some extent, although nine remainders were ineffective even at 100 mg/kg/dose. On the other hand all N-oxides showed effectiveness, especially some of them having ED_{50} less than 10 mg/kg/dose. Thus, N-oxides are more effective than non-N-oxides in any pair tested.

Table III. Oral Protective Effects on the Infection with *Staphylococcus aureus*

No.	Survival rate[a)]	No.	Survival rate[a)]
1	10	1′	100
2	0	2′	40
3	0	3′	80
4	0	4′	0
5	20	5′	60
6	0	6′	50
7	0	7′	0
8	0	8′	30
9	0	9′	70
10	10	10′	40
11	0	11′	0
12	0	12′	0
13	10	13′	50

a) %

Table IV. Oral Protective Effects on the Infection with *Salmonella typhimurium*

No.	ED_{50}[a)]	No.	ED_{50}[a)]
1	>100	1′	18
2	>100	2′	7
3	>100	3′	8
4	>100	4′	20
5	70	5′	12
6	50	6′	8
7	100	7′	20
8	40	8′	16
9	>100	9′	10
10	>100	10′	13
11	>100	11′	50
12	>100	12′	70
13	>100	13′	11

a) mg/kg/dose

(c) Plasma Levels

In Fig. 1 plasma levels are compared in the several pairs of non-N-oxides and N-oxides. The plasma levels of the former were hardly detectable or very low even when detected. It is noteworthy that the compounds showing detectable plasma levels are such ones as observed effectiveness on the infection with *Salmonella typhimurium*. On the other hand, all of the latter exhibited relatively high plasma levels except one compound (7′), in which the plasma level was slightly over the assay limit and the protective effects were not so good as the rest of this type. Aside from such an exception, N-oxides always attained to higher plasma levels than the corresponding non-N-oxides.

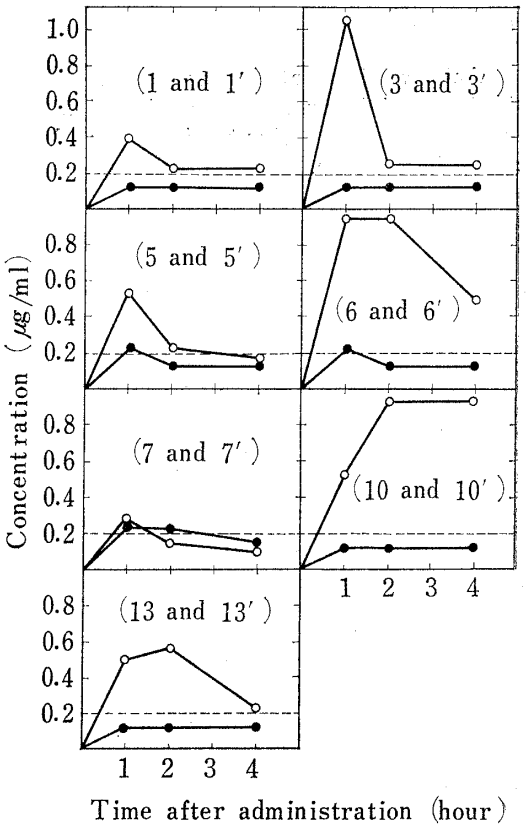


Fig. 1. Plasma Levels of Several (5-Nitro-2-furyl)vinyl Heterocycles and Their N-Oxide Derivatives

—○—: N-oxides —●—: non-N-oxides
--------: assay limit

Discussion

As shown in Results, it is obvious that the conversion of the nitrogen of heterocycles such as pyridine, quinoline and thiazole into N-oxide markedly improved oral protective effects on the experimental infections in mice. Also the *in vitro* antibacterial activity of N-oxides was usually superior to the corresponding non-N-oxides. For instance, eleven N-oxides (1′, 2′, 4′, 5′, 6′, 7′, 8′, 9′, 10′, 11′ and 13′) were superior and two (3′ and 12′) equal to the corresponding non-N-oxides against *Staphylococcus aureus*. Similarly, eleven N-oxides (1′, 2′, 3′, 4′, 5′, 6′, 7′, 8′, 9′, 12′ and 13′) were superior, one (10′) being equal and one (11′) inferior against *Salmonella typhimurium*. It is conceivable from these results that the improvement of N-

oxides in the oral protective effects can be partially ascribed to the increase of the *in vitro* antibacterial activities. However, a few N-oxides showing the equal or inferior *in vitro* activities to the corresponding non-N-oxides were also more effective on the infections. The plasma levels of some such derivatives (3′ and 10′) were higher than the corresponding non-N-oxides. To the contrary, one (7′) of N-oxides exceptionally showed a low plasma level, which was ineffective on the infection with *Staphylococcus aureus* and rather less effective on that with *Salmonella typhimurium*, although it was still more effective than the corresponding compound (7). Three non-N-oxides showing low but detectable plasma levels were effective to some extent on the infection with *Salmonella typhimurium*. These results suggest that the plasma levels are closely related to the protective effects on the systemic infections employed here. As N-oxides generally showed high plasma levels compared with the corresponding non-N-oxides, it also seems to be some of the reasons for the improvement.

All non-N-oxides used here are very slightly soluble in water. Such solubility may affect disadvantageously the rate of absorption and the amount absorbed from intestinal tracts. As the solubility of N-oxides in water is usually a little higher than that of non-N-oxides,[5] the former may be advantageous to distribute over the surface of intestinal tracts. Thus, it is likely that the absorption of N-oxides might be superior to non-N-oxides. However, to elucidate more clearly the high plasma levels of N-oxides, it is nescessary to clarify the other drug-host interactions such as excretion and metabolic degradation, which remain to be studied in future.

Acknowledgement The authors wish to thank Dr. Ose, Director of Research and Development Division, Dainippon Pharmaceutical Co., Ltd., and H. Takamatsu, Director of this laboratory, for their encouragement throughout this work and also express their gratitude to Mr. I. Nakanishi for his skillful technical assistance.

5) unpublished data.