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Anticancer Activity of 2-(2-(5-Nitro-2-furyl)vinyl)-8-(β-(N,N-Diethylamino)ethoxy)quinoline[1]

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2-(2-(5-Nitro-2-furyl)vinyl)quinoline (NQ) and its related nitrofurans have been reported to have antibacterial and anticancer effects,[3] to preferentially inhibit deoxyribonucleic acid (DNA) synthesis in susceptive bacteria,[4] and to have phage inducing activity in lysogenic bacteria.[5]

Many efforts have been made to obtain more potent anticancerous compounds, for example, by means of introduction of appropriate substituent(s) into quinoline ring of NQ. As an attempt in this line of works, eight nitrofurans were newly prepared and their anticancer effectiveness was examined *in vivo*. In this paper, the data with some discussion were presented.

Experimental

Preparation of Chemicals

Representative preparations were described below.

2-(2-(5-Nitro-2-furyl)vinyl)-8-(β-(N,N-diethylamino)ethoxy)quinoline (III)——A mixture of 5.0 g of 8-hydroxyquinaldine, 6.0 g of diethylaminoethyl chloride hydrochloride, 10.0 g of K_2CO_3, 1.0 g of NaI and 40 ml of acetone was refluxed for 8 hr. After cooling, addition of large amount of water produced deposition of oily materials, which were extracted with ether. After treating with dil. NaOH and water, ether evaporation gave viscous oil. Condensation of 4.0 g of the oil and 3.0 g of 5-nitro-2-furaldehyde in the presence of 8 ml of acetic anhydride gave yellowish needles. mp 119—120° (from dil. EtOH).

8-(β-(N,N-Diethylamino)ethylamino)quinaldine——A mixture of 3.16 g of 8-aminoquinaldine and 3.4 g of diethylaminoethyl chloride was heated at 130° for 20 hr. After cooling, reaction mixture was dissolved in hot water, insoluble materials were discarded by filtration. The filtrate was made alkaline with 10% NaOH. By steam distillation unreacted 8-aminoquinaldine was distilled out. Extraction of residues with ether and ether evaporation gave 3.7 g of oily product. Picrate, mp 167—169° (from EtOH). *Anal.* Calcd. for $C_{16}H_{23}N_3 \cdot C_{12}H_6O_4N_6$: C, 47.00; H, 4.50; N, 17.60. Found: C, 47.04; H, 4.24; N, 17.58.

2-(2-(5-Nitro-2-furyl)vinyl)-8-(β-(N,N-diethylamino)ethylamino)quinoline (VII)——A mixture of 2.0 g of crude 8-diethylaminoquinaldine and 2.0 g of 5-nitro-2-furaldehyde in 6.0 ml of acetic anhydride was heated at 90° for 1 hr. After cooling and evaporating excess acetic anhydride with reduced pressure, residues were dissolved in 1N HCl. Treatment of the filtrate with 5% ammonia gave yellowish precipitate which was crystallized from dil. EtOH. mp 69—72° (from EtOH). By desication of the specimen anhydrated material was obtained. mp 103—105°.

Other Materials

AF-5 (Panfuran, 3-amino-6-(2-(5-nitro-2-furyl)vinyl)-1,2,4-triazine hydrochloride) and 5-nitro-2-fur aldehyde diacetal were kindly supplied from Toyama Chemical Industries Co., Ltd. Mitomycin C (MMC) was purchased from Sankyo Co., Ltd., and 6-mercaptopurine (6MP) from Kohjin Co., Ltd.

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1) The data were partly presented to the Annual Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, October, 1968.
2) Location: *Takara-machi, 13-1, Kanazawa.*
3) *a*) K. Miura, T. Oohashi, S. Matsuda, and Y. Igarashi, *Yakugaku Zasshi*, **83**, 771 (1963); *b*) K. Miura, M. Ikeda, T. Oohashi, I. Okada, and Y. Igarashi, *ibid.*, **84**, 341 (1964); *c*) *Idem, ibid.*, **84**, 537 (1964); *d*) T. Ujiie, *Chem. Pharm. Bull.* (Tokyo), **14**, 461 (1966); *e*) H. Katae, H. Iwana, Y. Takase, and M. Shimizu, *Arzneim. Forsch.* (*Drug Res.*), **17**, 1030 (1967).
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Anticancer Experiments

Experimental Animal——Male albino mice of dd strain, weighing 20—22 g, were used for primary anticancer experiments with Ehrlich carcinoma, leukemia SN 36, and Sarcoma 180. Employed for further testing were the following: Mouse leukemia L1210, female BDF_1 mice, weighing 20—22 g; Yoshida sarcoma and ascites hepatomas AH 13 and AH 66, male Donryu rats weighing 90—110 g. Six to ten animals were used in each experiment.

Acute Toxicity——Median lethal dose (LD_{50}) of the compounds was estimated following the method described in previous paper.[3d]

Implantation of Cancer Cells——Cancer cells were harvested from animals bearing appropriate time lapsed ascites tumors. The peritoneal fluids were collected, and centrifuged at 1000 rpm for 5 min, followed two times washing with cold saline. The sedimented cells were resuspended in cold saline to obtain an appropriate cell density. To each test animal, the cells were implanted intraperitoneally or subcutaneously in the groin (Inoculum sizes were footnoted in the tables.).

Treatment——Administration of chemicals was started 24 hr after implantation of cancer cells. A daily dose of the compound to be tested was given intraperitoneally into each animal for five consecutive days. MMC was dissolved in saline, and nitrofurans (I—VIII) were also dissolved in saline by the aid of small amount of dil. HCl. NQ and 6MP were suspended in 0.5% carboxymethyl cellulose (CMC). The survivors were sacrificed and autopsied for detecting the tumor invasion. In the case of experiments on solid tumors, the tumors were excised at the termination of the experiment (14 day) and weighed individually.

Evaluation of Anticancer Effect——The anticancer activity of the compounds is estimated from T/C (treated/control) values. In ascites tumors the T/C value is the ratio of the mean survival time of the treated animals to the mean survival time of the control animals. In solid tumors the value is the ratio of the mean tumor weight of the treated group to the mean tumor weight of the control group.

Measurement of Difference Spectra——The chemicals to be examined were dissolved in 10 mM Tris buffer containing appropriate EtOH or dimethylformamide. Sample solution is composed of the chemicals and deoxyribonucleic acid or ribonucleic acid, while reference is of only the chemical at the same concentration. Difference spectra were obtained by measuring absorbances ranging from 320 nm to 500 nm in Hitachi spectrophotometer, model EPU, using 1 cm light path.

Results and Discussion

Preparation of Nitrofurans

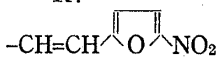
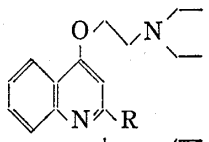
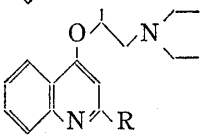
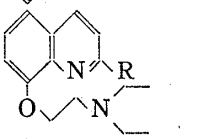
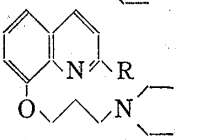
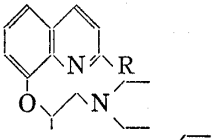
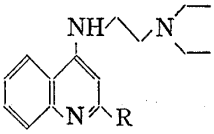
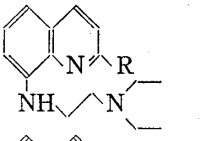
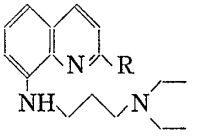
Condensation of 5-nitro-2-furaldehyde with 4-(β-(N,N-diethylamino)ethoxy)quinaldine and with 4-(β-(N,N-diethylamino)isopropoxy)quinaldine in the presence of acetic anhydride afforded 2-(2-(5-nitro-2-furyl)vinyl)-4-(β-(N,N-diethylamino)ethoxy)quinoline (I) and 2-(2-(5-nitro-2-furyl)vinyl)-4-(β-(N,N-diethylamino)isopropoxy)quinoline (II), respectively. Similarly, reaction of the various kinds of substituted quinaldine with 5-nitro-2-furaldehyde gave the compounds (III—VIII), as indicated in Table I.

Anticancer Experiments

The *in vivo* anticancer effect of these compounds was evaluated by employing Ehrlich carcinoma (ascites and solid form), Sarcoma 180 (solid form), leukemias SN 36 and L1210, Yoshida sarcoma, and ascites hepatomas AH 13 and AH 66 as experimental tumor species. As positive control, MMC and 6MP were run in parallel. Treatment of mice bearing Ehrlich ascites carcinoma or leukemia SN 36 with I, III, and VII, as indicated in Table II, did give the results similar to those obtained with MMC treatment to cause prominent prolongation of life-span of the mice. Table III indicated the results of anticancer experiments with compounds (I, III, and VII) against solid form of Ehrlich carcinoma. The compound (III) was moderately effective in inhibiting tumor growth to the same extent as with MMC, while I and VII were very slightly effective. Moreover, effects of several nitrofurans against tumor growth of solid form of Sarcoma 180 in mice were tested. As shown in Table IV, treatment with III also exhibited tumor growth retardation indicating that III had approximately equal effectiveness to MMC, but was inferior to 6MP. In other side, another compounds, (I, II, IV, VII, and VIII) were all ineffective in this assay system.

Then, III was submitted to the second step in a program to evaluate the anticancer activity from the results described above. Comparative experiments carried out with III and NQ showed that III was found to be effective against ascites hepatoma AH 13, but almost

Table I. Nitrofurans containing Diethylaminoalkyl Moiety

No.	Compound R: 	mp (°C)[a]	Recryst. solvent[b]	Formula	Analysis (%) Calcd. (Found) C	H	N
I		181—182	B	$C_{21}H_{23}O_4N_3$	66.13 (66.04)	6.08 (5.78)	11.02 (11.14)
II		138—141	B	$C_{22}H_{25}O_4N_3$	66.82 (66.41)	6.37 (6.32)	10.63 (10.78)
III		119—120	A	$C_{21}H_{23}O_4N_3$	66.12 (65.88)	6.08 (6.07)	11.02 (11.02)
IV		105—106	C	$C_{22}H_{25}O_4N_3$	66.82 (66.89)	6.37 (6.42)	10.63 (10.33)
V		104—105	C	$C_{22}H_{25}O_4N_3$	66.82 (66.58)	6.37 (6.36)	10.63 (10.29)
VI		205 (decomp.)[c]	D	$C_{24}H_{35}O_4N_4Cl_3$	52.41 (52.14)	6.37 (6.14)	10.19 (9.92)
VII		103—105	C	$C_{21}H_{24}O_3N_4$	66.30 (66.28)	6.36 (6.43)	14.73 (14.89)
VIII		65— 67[d]	C	$C_{22}H_{28}O_4N_4$	64.06 (63.61)	6.84 (6.45)	13.58 (13.17)

a) uncorrected b) A, dil. EtOH; B, EtOH; C, pet. benzine; D, isopropanol c) 3HCl. isopropanol d) hydrate

not effective against AH 66 and Yoshida sarcoma, while NQ did retain anticancer effect against AH 13 and AH 66, but not against Yoshida sarcoma (Fig. 1). In any case, it is obvious that III shows inhibitory effect on the growth of Ehrlich carcinoma (ascites and solid form), leukemia SN 36, Sarcoma 180 (solid form), and ascites hepatoma AH 13. Additionally, both III and NQ did not give a life prolongation of mice bearing leukemia L1210 and of mice bearing intravenous implants of Ehrlich ascites carcinoma.

It may be noted here that in a standpoint of structure activity relationship, substitution of a diethylamino-alkoxy and -alkylamino group for a hydrogen atom at 4 or 8 position on quinoline ring of NQ resulted in a marked increase in toxicity, and that diethylamino-alkoxy derivatives are more toxic than -alkylamino derivatives so far tested.

Meanwhile, it has been reported that the fate and tissue distribution of the antimalarial drugs, chloroquine and atabline, brought to a focus in selective accumulation in certain parenchymatous organs. With the cell, these drugs localize and accumulate in the nucleus. This has been thought to be due to the fact that these drugs have a selective affinity for nucleic

Table II. Effect on Ehrlich Ascites Carcinoma and Leukemia SN 36 in Mice

Compound	Toxicity: LD_{50} in mice (mg/kg, *i.p.*)	Dose (mg/kg/day, *i.p.*) × 5	Ehrlich ascites carcinoma[a]		Leukemia SN 36[a]	
			Survival days (mean)[b]	*T/C*	Survival days (mean)[b]	*T/C*
I	12.5	1.0	35.3	2.06	50.0	3.18
		2.0	38.4	2.25	43.0	2.74
		4.0	40.9	2.39	—	—
III	22.5	1.0	37.5	2.19	50.0	3.18
		2.0	44.0	2.57	43.3	2.76
		4.0	48.7	2.85	50.0	3.18
VII	160.0	4.0	16.0	0.94	36.0	2.29
		8.0	23.2	1.36	33.7	2.15
		16.0	26.5	1.55	50.0	3.18
MMC	5.2	0.25	24.6	1.44	36.7	2.34
		0.50	38.0	2.22	44.3	2.82
		1.00	41.8	2.44	50.0	3.18
Saline			17.1	—	15.7	—

a) Tumor cell implantation: Ehrlich ascites carcinoma, 6×10^6 cells/head, *i.p.*; leukemia SN 36, 3×10^6 cells/head, *i.p.*
b) Survivors were sacrificed on the 50th day of implantation and autopsied.

Table III. Effect on Solid Form of Ehrlich Carcinoma in Mice[a]

Compound	Dose (mg/kg/day, *i.p.*) × 5	*T/C*[b] (mean)
I	1.0	0.71
	2.0	0.84
III	2.0	0.64
	4.0	0.43
VII	4.0	0.86
	8.0	0.81
MMC	1.0	0.50

a) Tumor cells (3.4×10^6) were implanted subcutaneously into the left groin of mice.
b) The mean tumor weights of control mice in separate experiments were ranging from 0.75 g to 0.90 g.

Table IV. Effect on Solid Form of Sarcoma 180 in Mice[a]

Compound	Toxicity: LD_{50} in mice (mg/kg, *i.p.*)	Dose (mg/kg/day, *i.p.*) × 5	*T/C*[b] (mean)
I	12.5	1.0	0.98
		2.0	0.85
II	34.0	3.5	0.94
		7.0	1.34
III	22.5	2.0	0.79
		4.0	0.48
IV	26.0	2.5	0.92
		5.0	1.44
V	34.0	3.5	1.23
		7.0	1.28
VI	120.0	15.0	1.35
		30.0	1.17
VII	160.0	15.0	1.44
		30.0	0.91
VIII	160.0	15.0	0.86
		30.0	1.18
MMC	5.2	0.5	0.55
		1.0	0.51
6MP	250.0	50.0	0.13

a) Tumor cells (5×10^6) were implanted subcutaneously into the left groin of mice.
b) The mean tumor weights of control mice in separate experiments were ranging from 0.62 g to 1.22 g.

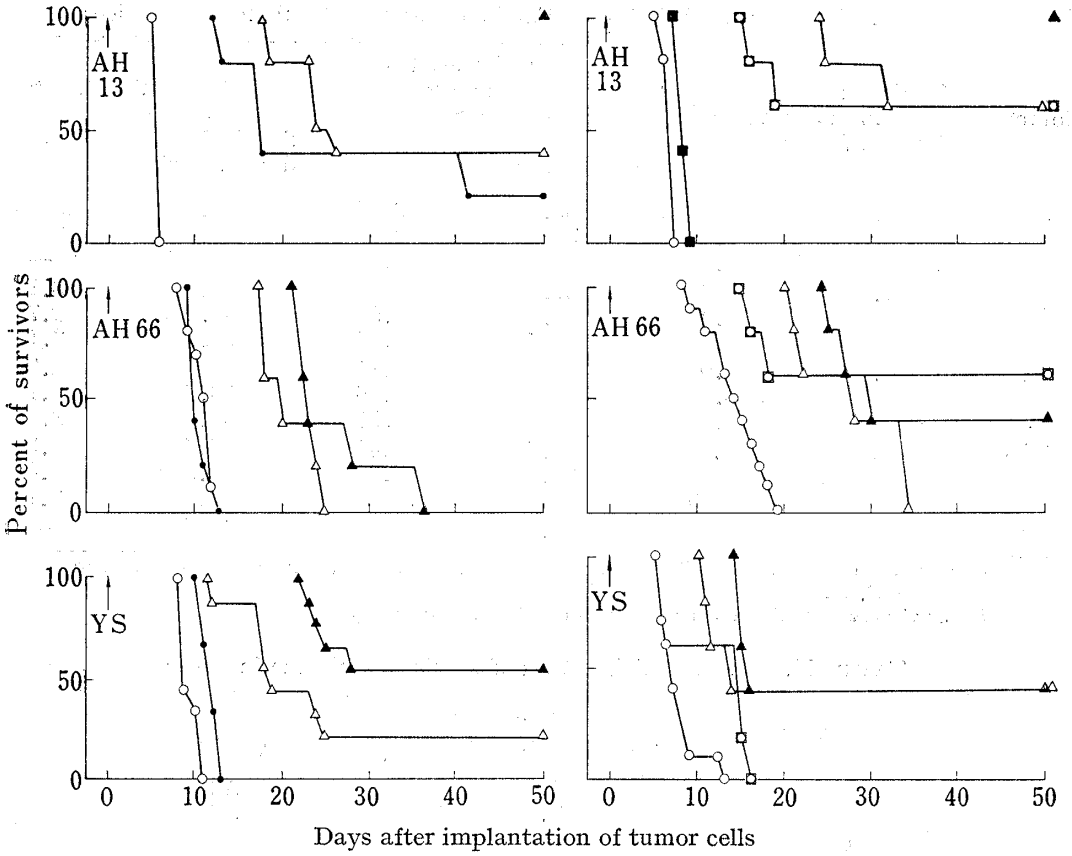


Fig. 1. Effect of III and NQ on Ascites Hepatomas AH 13 and AH 66 and Yoshida Sarcoma[a)]

a) tumor cell implantation: ascites hepatoma AH 13, 3×10^6 cells/rat, *i.p.*; ascites hepatoma AH 66, 3×10^6, *i.p.*; Yoshida sarcoma (YS), 2×10^6 cells/rat, *i.p.*
—○—: control; —●—: III, 2.0 mg/kg, *i.p.*; —□—: NQ, 10 mg/kg, *i.p.*; —■—: NQ, 20 mg/kg, *i.p.*; —△—: MMC, 0.3 mg/kg, *i.p.*; —▲—: MMC, 0.6 mg/kg, *i.p.*

acids and particularly for deoxyribonucleic acid.[6)] Chloroquine and atabline possess in these molecular structures a side chain, diethylaminoalkyl group, which is thought to be attributable to strengthen the interaction of these compounds with nucleic acids. Besides, some papers have been appeared that involved in anticancer properties of these antimalarial drugs.[7)] Moreover, Miracil D (1-diethylaminoethylamino-4-methyl-10-thiaxanthenone) being an effective agent in the treatment of schistosomiasis has been established by Hirschberg, *et al.*[8)] to exhibit anticancer effect in rodents bearing a variety of transplantable neoplasms. Thus, it was proposed that the presence of diethylaminoethylamino side chain is profoundly affected to the biological activity of Miracil D.[8b,9)]

By difference spectrum measurements, some interaction of nitrofurans having diethylaminoalkyl side chain but no one of those not having the side chain (NQ, AF-5, and 2-(2-(5-nitro-2-furyl)vinyl)-8-aminoquinoline) with nucleic acid was observed (Fig. 2).

Possibility of interaction of III with nucleic acid was further examined, using gel filtration method. Taketo, *et al.*[5a)] have reported that no reaction between DNA and 2-(2-(5-nitro-2-

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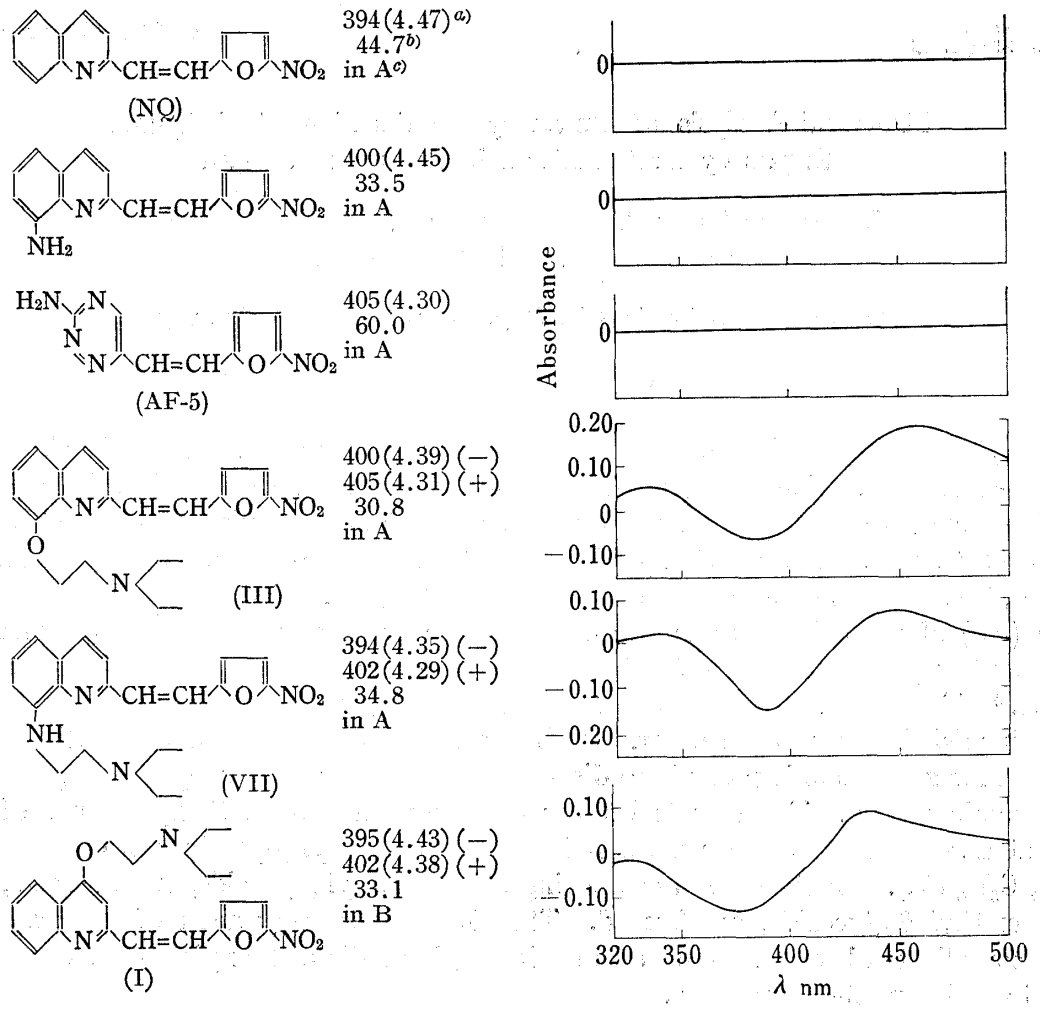

Fig. 2. Difference Spectra of Nitrofurans with DNA

a) λ_{max} nm (log ε)
b) concentration (μM)
c) Nitrofurans were dissolved in 10mM Tris (pH 7.3 containing 5% EtOH (A) or 15% dimethylformamide (B) in the presence (+) or absence (−) of calf thymus DNA (300 μg/ml).

furyl)vinyl)-4-aminoquinoline appears to occur, since after mixing the products goes through a Sephadex G-25 column in separate fractions. In contrast, III and DNA were filtrated in the same fraction. Moreover, III raised the Tm of calf thymus DNA (Sigma) by 10° in heat denaturation test.

The next paper will be concerned with the mode of action of III and of NQ in comparative studies.

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