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Substituent effect of a fluorine atom on the mutagenicity of nitroquinolines

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Abstract

Some 16 nitroquinolines (NQs) and their fluorinated derivatives were tested for mutagenicity in *Salmonella typhimurium* TA100 without S9 mix to investigate the effect of fluorine-substitution on the mutagenicity. These NQs consist of 5-NQs, 5-nitroquinoline *N*-oxides (5-NQOs), *N*-methyl-5-nitroquinolinium methanesulfonates (*N*-Me-5-NQs) and 8-NQs, including three *ortho*-F-NQs, one *meta*-F-NQ, four *para*-F-NQs and four 3-F-NQs. For this purpose, eight F-NQs were newly synthesized. The data indicated that the ratio of the mutagenic activities (revertants/plate/nmol) of fluorinated NQs to those of the corresponding parent non-fluorinated compounds ranged from 0.6- to 119-fold. The fluorine atom located *para* to the nitro group markedly enhanced the mutagenicity (24-fold and more), while three *ortho*-fluorinated derivatives showed no significant increase in mutagenicity (enhancement ratio were 0.6, 0.8 and 1.7). With respect to 8-NQs, its meta-fluorinated derivative also had an enhanced mutagenicity over the parent compound (53-fold). In addition, although *N*-Me-5-NQ was less mutagenic than 5-NQ and 5-NQO, the mutagenicity of *N*-Me-5-NQ was most significantly enhanced by fluorine-substitution. These results suggest that introduction of a fluorine atom to the molecule in question may be a useful tool to modify their mutagenic potency and to better understand the mechanism of mutation.

Keywords: Nitroquinoline; Fluoroquinoline; Mutagenicity; Ames test

1. Introduction

We have investigated fluorine-substituted heteroaromatic compounds with special attention to their biological effects. Replacement of a hydrogen atom with a fluorine results not only in changes in electron-density distribution within the molecule but also in electric repulsive/attractive interactions with intra/intermolecular environments. These changes may significantly affect such interactions as those between an enzyme and its substrate or between a receptor and its ligand. In addition, the metabolism of xenobiotics, including drugs, food-additives and

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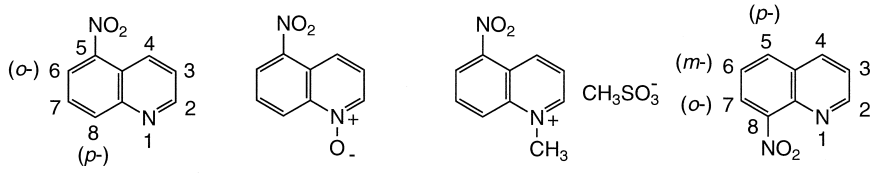
other environmental chemicals, is sometimes crucially altered by fluorine-substitution, rendering these substances generally resistant to enzymic oxidation at the site of substitution [1–5]. This may result in either a desirable or undesirable modification of the biological activity. As we previously reported [6–11], one example of this is the abolishment of genotoxicity in carcinogenic [12–14] and mutagenic [15] quinoline by fluorine-substitution at position-3, probably because of an inhibition of the metabolic transformation of quinoline to its active metabolite responsible for the production of mutagenic DNA lesions. Introduction of fluorine atom(s) to a molecule in question may also allow us to better understand the mechanism of induction of the mutation by these mutagens. In fact, we have proposed the enamine epoxide hypothesis of quinoline mutagenicity, i.e., the ultimate mutagenic structure of the 2,3-epoxide of 1,4-hydrated quinoline, through our studies with various fluorine-substituted quinoline derivatives [6–9].

Nitroquinolines (NQs), nitro derivatives of quinoline, form a group of the most simple structural nitroazaarenes. Nitroazaarenes are poorly understood except a limited number of compounds such as 4-nitroquinoline *N*-oxide and azabenzo[*a*]pyrenes, although they are distributed ubiquitously as potent environmental mutagens/carcinogens [16–19]. The present study was undertaken to investigate structure–mutagenicity relationships by fluorine-substitution on the molecules of NQs, aiming at investigating the fluorine-substitution effects on the mutagenicity, and at better understanding the mechanism of induction of the mutation. In this study, we used 12 fluorinated derivatives of 5- and 8-NQs for the above purpose, and the Ames test was conducted for measurement of the mutagenicity.

2. Materials and methods

2.1. Materials

5-NQ, 8-NQ, *m*-fluoroaniline and 2,4-difluoroaniline were purchased from Tokyo Kasei Kogyo (Tokyo); 4-fluoro-2-nitroaniline from Aldrich Chemical (Milwaukee, USA). 3-Fluoroquinoline, 6-fluoroquinoline, 7-fluoroquinoline, 8-fluoroquinoline, 5-nitroquinoline *N*-oxide (5-NQO), 6-fluoroquinoline *N*-oxide, 8-fluoroquinoline *N*-oxide and 6-fluoro-8-nitroquinoline (Registry Nos. 396-31-6, 396-30-5, 396-32-7, 394-68-3, 7613-19-6, 2338-74-1, 2795-43-9 and 343-26-0, respectively) were synthesized according to the reported methods [20–23]. 3-Fluoro-5-nitroquinoline, 5-fluoro-8-nitroquinoline and 8-fluoro-5-nitroquinoline (Registry Nos. 191861-20-8, 152167-85-6 and 94832-39-0, respectively) were synthesized in our laboratory [4]. Melting points were determined with a YANACO MP-500D micro melting point apparatus without correction. Mass spectra were measured by a JEOL AX 505HA spec-



5-nitroquinoline (5-NQ) (**1**)
3-F-5-NQ (**2**)
6-F-5-NQ (**3**)
8-F-5-NQ (**4**)

5-nitroquinoline *N*-oxide (5-NQO) (**5**)
3-F-5-NQO (**6**)
6-F-5-NQO (**7**)
8-F-5-NQO (**8**)

N-methyl-5-nitroquinoline methanesulfonate (*N*-Me-5-NQ) (**9**)
3-F-*N*-Me-5-NQ (**10**)
8-F-*N*-Me-5-NQ (**11**)

8-nitroquinoline (8-NQ) (**12**)
3-F-8-NQ (**13**)
5-F-8-NQ (**14**)
6-F-8-NQ (**15**)
7-F-8-NQ (**16**)

Scheme 1. List of 5-nitroquinolines and 8-nitroquinolines examined.

trometer. UV spectra were recorded on a SHIMADZU UV-2100 spectrophotometer. ^{1}H NMR spectra were measured by a JEOL JMN-EX 270 or JMN-GSX 400 spectrometer in $CDCl_3$ or Me_2SO-d_6 using tetramethylsilane as an internal standard.

The following compounds were newly synthesized in this study (Scheme 1).

*2.1.1. 6-Fluoro-5-nitroquinoline (6-F-5-NQ) (**3**)*

6-Fluoroquinoline (309 mg) was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 12 h. Purification of the reaction mixture by column chromatography (aluminium oxide, benzene) yielded **3** in 38% yield: mp 93–96°C; ^{1}H NMR (Me_2SO-d_6) δ 9.02 (dd, 1H, $J_{2-3}=4.3$ Hz, $J_{2-4}=1.2$ Hz, H-2), 8.37 (d, 1H, $J_{3-4}=8.5$ Hz, H-4), 8.35 (dd, 1H, $J_{7-8}=10.4$ Hz, $J_{6F-8}=4.9$ Hz, H-8), 7.64 (dd, 1H, H-3), 7.63 (t, 1H, $J_{6F-7}=9.8$ Hz, H-7); HR-MS m/z: 192.033, Calcd. for $C_9H_5FN_2O_2$: 192.032.

*2.1.2. 3-Fluoro-5-nitroquinoline N-oxide (3-F-5-NQO) (**6**)*

3-Fluoro-5-nitroquinoline (**4**) (300 mg) was allowed to react with *m*-chloroperbenzoic acid (70%, 576 mg, 1.5 eq) in 10 ml of $CHCl_3$ at room temperature for 45 h. The reaction mixture was washed with 5% Na_2CO_3 (20 ml × 2), dried with MgSO4 anhydrous, filtered and evaporated in vacuo. The residue was purified by column chromatography (silica gel, $CHCl_3$). Recrystallization from hexane yielded **6** as pale yellow needles in 80% yield: mp 182–183°C; MS m/z: 208 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 9.12 (dd, 1H, $J_{2-3F}=4.9$ Hz, $J_{2-4}=2.4$ Hz, H-2), 8.90 (dd, 1H, $J_{6-7}=8.6$ Hz, H-6), 8.61 (d, 1H, $J_{7-8}=7.9$ Hz, H-8), 8.18 (dd, 1H, $J_{3F-4}=9.8$ Hz, H-4), 7.95 (t, 1H, H-7); Anal. Calcd. for $C_9H_5FN_2O_3$: C, 51.93; H, 2.42; N, 13.46. Found: C, 51.65; H, 2.51; N, 13.22.

*2.1.3. 6-Fluoro-5-nitroquinoline N-oxide (6-F-5-NQO) (**7**)*

6-Fluoroquinoline *N*-oxide (264 mg) was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 4 days. The reaction mixture was poured into ice water (300 ml), neutralized with Na_2CO_3, and extracted with $CHCl_3$ (200 ml × 2). Recrystallization from benzene yielded **7** as brown needles in 30% yield: mp 170–173°C; MS m/z: 208 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 8.89 (dd, 1H, $J_{6F-8}=5.1$ Hz, $J_{7-8}=9.5$ Hz, H-8), 8.73 (d, 1H, $J_{2-3}=5.9$ Hz, H-2), 8.04 (t, 1H, $J_{6F-7}=9.9$ Hz, H-7), 7.89 (d, 1H, $J_{3-4}=9.2$ Hz, H-4), 7.71 (dd, 1H, H-3); Anal. Calcd. for $C_9H_5FN_2O_3$: C, 51.93; H, 2.42; N, 13.46. Found: C, 52.13; H, 2.47; N, 13.45.

*2.1.4. 8-Fluoro-5-nitroquinoline N-oxide (8-F-5-NQO) (**8**)*

8-Fluoroquinoline *N*-oxide (200 mg) was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 3 days. The reaction mixture was poured into ice water (400 ml), neutralized with K_2CO_3, and extracted with $CHCl_3$ (200 ml × 2). Purification of the extract by column chromatography (silica gel, benzene:MeOH = 2:1) and recrystallization from benzene–hexane yielded **8** as yellow powder in 53% yield: mp 178–180°C; MS m/z: 208 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 8.63 (d, 1H, $J_{2-3}=6.4$ Hz, $J_{2-4}=1.5$ Hz, H-2), 8.49 (dd, 1H, $J_{3-4}=9.3$ Hz, H-4), 8.24 (dd, 1H, $J_{6-7}=8.8$ Hz, $J_{6-8F}=3.9$ Hz, H-6), 7.69 (dd, 1H, H-3), 7.70 (dd, 1H, $J_{7-8F}=12.2$ Hz, H-7); Anal. Calcd. for $C_9H_5FN_2O_3$: C, 51.93; H, 2.42; N, 13.46. Found: C, 51.82; H, 2.64; N, 13.54.

*2.1.5. N-methyl-5-nitroquinolinium methanesulfonate (N-Me-5-NQ) (**9**)*

5-NQ (352 mg) was allowed to react with methylmethanesulfonic acid (1.71 ml, 10 eq) at 80°C for 1 day. After addition of a small amount of hexane, the reaction mixture was left standing in a freezer. Recrystallization of the crude **9**, thus obtained, from ethanol yielded **9** as pale yellow solid in 64% yield: mp 127–131°C; ^{1}H NMR (Me_2SO-d_6) δ 9.68 (d, 1H, $J_{2-3}=5.6$ Hz, H-2), 9.53 (d, 1H, $J_{3-4}=8.9$ Hz, H-4), 8.92 (d, 1H, $J_{6-7}=9.2$ Hz, H-6), 8.78 (d, 1H, $J_{7-8}=7.6$ Hz, H-8), 8.43 (dd, 1H, H-7), 8.37 (dd, 1H, H-3), 4.72 (s, 3H, *N*-CH_3), 2.30 (s, 3H, $CH_3SO_3^-$); Anal. Calcd. for $C_{11}H_{12}N_2O_5S$: C, 46.47; H, 4.25; N, 9.85. Found: C, 46.22; H, 4.22; N, 9.96.

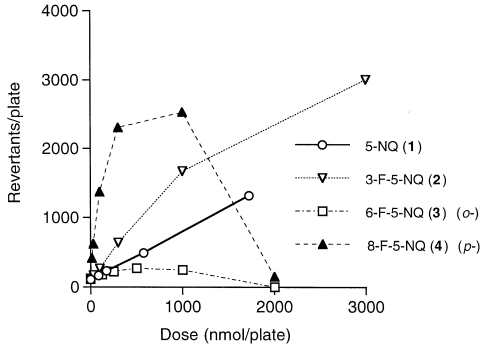


Fig. 1. Mutagenicity of 5-NQs in *S. typhimurium* TA100 without S9 mix. The symbols shown indicate the means of at least three independent experiments.

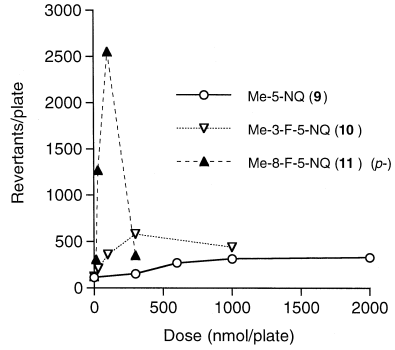


Fig. 3. Mutagenicity of *N*-Me-5-NQs in *S. typhimurium* TA100 without S9 mix. The symbols shown indicate the means of at least three independent experiments.

*2.1.6. 3-Fluoro-N-methyl-5-nitroquinolinium methanesulfonate (3-F-N-Me-5-NQ) (**10**)*

3-Fluoro-5-nitroquinoline (**2**) (300 mg) was allowed to react with methylmethanesulfonic acid (1.32 ml, 10 eq) in 1 ml dimethylformamide at 80°C for 1 day. The reaction mixture was left standing in a freezer. The precipitate was washed with hexane and ether. **10** was obtained as brown solid in 90% yield: mp 162–163°C; ^{1}H NMR (Me_2SO-d_6) δ 10.12 (t, 1H, $J_{2-3F} = 3.1$ Hz, H-2), 9.45 (dd, 1H, $J_{3F-4} = 9.2$ Hz, H-4), 8.95 (d, 1H, $J_{6-7} = 8.8$ Hz, H-6), 8.83 (d, 1H, $J_{7-8} = 7.9$ Hz, H-8), 8.40 (t, 1H, H-7), 4.76 (s, 3H, *N*-CH_3), 2.30 (s, 3H, $CH_3SO_3^-$); Anal. Calcd. for $C_{11}H_{11}FN_2O_5S \cdot H_2O$: C, 41.25; H, 4.09; N, 8.75. Found: C, 41.12; H, 4.24; N, 8.99.

*2.1.7. 8-Fluoro-N-methyl-5-nitroquinolinium methanesulfonate (8-F-N-Me-5-NQ) (**11**)*

8-Fluoro-5-nitroquinoline (**9**) (100 mg) was allowed to react with methylmethanesulfonic acid (0.44 ml, 10 eq) in 1 ml of dimethylformamide at 85°C for 18 h. The reaction mixture was left standing in a freezer. The precipitate was washed with ether. **11** was obtained as brown solid in 74% yield: mp 122–123°C; ^{1}H NMR (Me_2SO-d_6) δ 9.67 (d, 1H, $J_{2-3} = 5.5$ Hz, H-2), 9.54 (d, 1H, $J_{3-4} = 9.2$ Hz, H-4), 8.80 (d, 1H, $J_{6-7} = 8.8$ Hz, $J_{6-8}F = 4.0$ Hz, H-6), 8.41 (dd, 1H, H-3), 8.31 (dd, 1H, $J_{7-8F} = 13.6$ Hz H-7), 4.81 (d, 3H, *N*-CH_3), 2.30 (s, 3H, $CH_3SO_3^-$); Anal. Calcd. for $C_{11}H_{11}FN_2O_5S \cdot 2H_2O$: C, 39.05; H, 4.47; N, 8.28. Found: C, 38.74; H, 4.74; N, 8.71.

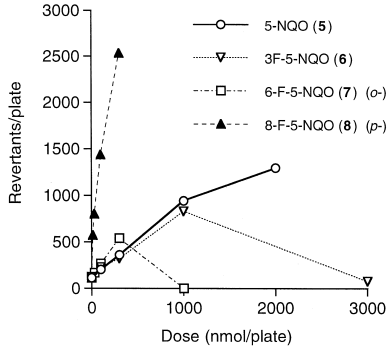


Fig. 2. Mutagenicity of 5-NQOs in *S. typhimurium* TA100 without S9 mix. The symbols shown indicate the means of at least three independent experiments.

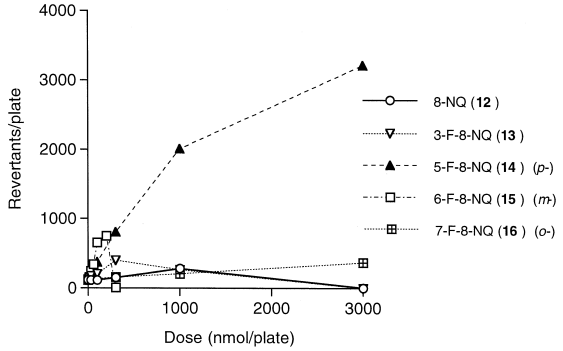


Fig. 4. Mutagenicity of 8-NQs in *S. typhimurium* TA100 without S9 mix. The symbols shown indicate the means of at least three independent experiments.

*2.1.8. 3-Fluoro-8-nitroquinoline (3-F-8-NQ) (**13**)*

3-Fluoroquinoline (10.3 g) was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 4.5 h. Purification of the reaction mixture by column chromatography (aluminium oxide, benzene:hexane = 1:1) yielded **13** in 24% yield (3-F-5-NQ was also obtained in 38% yield): mp 131–133°C; ^{1}H NMR ($CDCl_3$) δ 8.97 (d, 1H, $J_{2-3F} = \sim 0$ Hz, $J_{2-4} = 3.0$ Hz, H-2), 8.01–8.05 (m, 2H, H-5 and H-7), 7.90 (dd, 1H, $J_{3F-4} = 8.3$ Hz, H-4), 7.68 (t, 1H, $J_{5-6} = J_{6-7} = 7.9$ Hz, H-6); HR-MS m/z: 192.033, Calcd. for $C_9H_5FN_2O_2$: 192.030.

*2.1.9. 7-Fluoro-8-nitroquinoline (7-F-8-NQ) (**16**)*

7-Fluoroquinoline (251 mg) was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 6 days. Purification of the reaction mixture by column chromatography (silica gel, $CHCl_3$) and recrystallization from benzene–hexane yielded **16** as white needles in 81% yield: mp 149–150°C; ^{1}H NMR ($CDCl_3$) δ 9.05 (dd, 1H, $J_{2-3} = 4.0$ Hz, $J_{2-4} = 1.3$ Hz, H-2), 8.26 (dd, 1H, $J_{3-4} = 8.4$ Hz, H-4), 8.00 (dd, 1H, $J_{5-6} = 9.2$ Hz, $J_{5-7F} = 5.5$ Hz, H-5), 7.56 (dd, 1H, H-3), 7.50 (t, 1H, $J_{6-7F} = 8.8$ Hz, H-6); Anal. Calcd. for $C_9H_5FN_2O_2$: C, 56.26; H, 2.62; N, 14.58. Found: C, 56.25; H, 2.87; N, 14.65.

2.2. Mutation assay

Chemicals were tested for mutagenicity using *Salmonella typhimurium* TA100 without S9 mix according to the procedure of the Ames test with a

Table 1
Mutagenicity of NQs in *S. typhimurium* TA100 without S9 mix

Substituent	Revertants per plate per nmol[a]	Maximum revertants per plate[b]	*F*-substitution effect on mutagenicity[c]
5-NQs			
none (**1**)	0.71	1321	(1.0)
3-F (**2**)	1.43	3011	2.0
6-F (**3**) (*o*-)	0.45	268	0.6
8-F (**4**) (*p*-)	17.0	2528	24
5-NQOs			
none (**5**)	0.83	1295	(1.0)
3-F (**6**)	0.70	833	0.8
6-F (**7**) (*o*-)	1.41	539	1.7
8-F (**8**) (*p*-)	22.6	2533	27
N-Me-5-NQs			
none (**9**)	0.32	270	(1.0)
3-F (**10**)	2.31	578	7.2
8-F (**11**) (*p*-)	38.1	2551	119
8-NQs			
none (**12**)	0.10	281	(1.0)
3-F (**13**)	0.22	364	2.2
5-F (**14**) (*p*-)	2.61	3221	26
6-F (**15**) (*m*-)	5.29	747	53
7-F (**16**) (*o*-)	0.08	436	0.8

[a] Calculated from the slope of the linear portion of each curve near the origin. The numbers indicate the means of at least three independent experiments.
[b] Obtained from the assay up to 5 μmol/plate. The numbers indicate the means of at least three independent experiments.
[c] The ratio of the mutagenic activity (revertants/plate/nmol) of the fluorinated quinoline to that of the corresponding parent non-fluorinated compound.

modification of pre-incubation at 37°C for 20 min as previously reported [24–26]. Briefly, The chemical to be tested was dissolved in 0.1 ml of dimethyl sulfoxide and added to a mixture of 0.5 ml of 100 mM sodium phosphate buffer (pH 7.4) and 0.1 ml of the overnight cell culture. After being incubated at 37°C for 20 min under gentle shaking, 2.0 ml of 0.05 mM L-histidine–0.05 mM biotin molten top agar was added. The mixture was layered on minimal glucose–agar plates. The number of histidine prototroph revertants was counted after incubation at 37°C for 2 days. In this study, each compound was assayed in triplicate or more replicate at each dose level. The mean number of revertants obtained at each dose is illustrated in Figs. 1–4.

3. Results

The NQs listed in Chart 1 were tested for mutagenicity in *S. typhimurium* TA100 in the absence of S9 mix according to the procedure of the Ames test [24–26]. The results are summarized in Table 1, where the number of revertants per plate per nmol was calculated from the slope of the initial linear portion of each dose–response curve shown in Figs. 1–4, and the ratio of the number of revertants per plate per nmol for fluorinated NQs to that for non-fluorinated parent NQs is termed as the *F*-substitution effect on mutagenicity. In addition, the maximal number of revertants per plate obtained from the assay up to 5 μmol/plate were included in Table 1.

3.1. Mutagenicity of 5-NQs

As shown in Figs. 1–3 and Table 1, mutagenic potencies (revertants/plate/nmol) of the isomers examined in the Ames assay system decrease in the following order:

5-NQs

8-F-5-NQ (*para*-position)

≫ 3-F-5-NQ

> 5-NQ

> 6-F-5-NQ (*ortho*-position)

5-NQOs

8-F-5-NQO (*para*-position)

≫ 6-F-5-NQO (*ortho*-position)

> 5-NQO ≈ 3-F-5-NQ ≈ (5-NQ)

N-Me-5-NQs

8-F-*N*-Me-5-NQ (*para*-position)

≫ 3-F-*N*-Me-5-NQ

> (5-NQ) > *N*-Me-5-NQ.

In all the three 5-nitro groups, the fluorine atom located para to the nitro group greatly enhanced the mutagenicity. 3-Fluorinated derivatives, in which the fluorine atom was located on the pyridine ring, showed mutagenicities slightly more potent than, or almost the same as, the corresponding non-fluorinated derivatives. *Ortho*-fluorine-substitution on the 5-NQ molecule decreased the mutagenicity, while the same substitution on the 5-NQO molecule slightly enhanced the mutagenicity, compared to their respective parent molecules. Although *N*-Me-5-NQ was less mutagenic than 5-NQ and 5-NQO, its mutagenicity was most markedly enhanced by fluorine-substitution among the above three parent compounds.

3.2. Mutagenicity of 8-NQs

As shown in Fig. 4 and Table 1, the mutagenic potencies (revertants/plate/nmol) of the 8-NQs in the Ames assay system decrease in the following order:

6-F-8-NQ (*meta*-position)

> 5-F-8-NQ (*para*-position)

≫ 3-F-8-NQ

> 8-NQ ≈ 7-F-8-NQ (*ortho*-position).

The fluorine atom located at the *meta*- or *para*-position of the nitro group markedly enhanced the mutagenicity. Fluorine-substitution in the pyridine moiety (i.e., 3-F-8-NQ) slightly potentiated the mutagenicity of 8-NQ. *Ortho*-fluorine-substitution showed no remarkable effect on the mutagenicity of 8-NQ.

4. Discussion

We measured the mutagenicity of 16 5- and 8-NQs and their fluorinated derivatives in the absence of S9 mix (without metabolic activation systems) using the Ames test with a modification of pre-incubation at 37°C for 20 min to investigate the structure-mutagenicity relationship after fluorine-substitution.

Many nitroarenes are transformed to their proximate active forms, *O*-acetylarylhydroxylamines, by bacterial nitroreductase and *O*-acetyltransferase without help of S9 mix to exert their mutagenicity [27–30]. 5- and 8-NQs and their derivatives tested in this study were all mutagenic in *S. typhimurium* TA100 without S9 mix. The ratio of the mutagenic activities (revertants/plate/nmol) of the fluorinated NQs to those of the corresponding parent non-fluorinated NQs ranged from 0.6- to 119-fold (Table 1). Greatest enhancing effect (24- to 119-fold) on the mutagenicity was obtained by *para*-fluorine-substitution to the nitro group (compounds (**4**), (**8**), (**11**) and (**14**)). With regard to 8-NQs, the mutagenicity was also enhanced by *meta*-substitution (**15**).

The structure of NQs may remind us of three possible hypotheses of the mechanism of mutagenicity; (1) a mechanism with nitro aromatic compounds, (2) a mechanism based on an aromatic nucleophilic substitution, and (3) the mechanism involving metabolically activated 1,2-epoxide-1,4-hydrated quinoline (enamine epoxide) in the case of quinoline mutagenicity. Firstly, the involvement of enamine epoxides can be denied in NQs mutagenicity by the fact that the 3-fluorinated derivatives of NQs are mutagenic in the absence of S9 mix as well as in the presence of S9 mix (data not shown). Because the formation of arene oxides requires a metabolic activation by S9 mix and the introduction of a fluorine atom at position-3 of NQs probably inhibits the formation of the enamine epoxides responsible for production of mutagenic DNA lesions. The second mechanism described above is a hypothesis based on DNA-adduct formation analogous to dinitrophenylation of amino acids with dinitrofluorobenzene, which is often used in identification of the N-terminal of proteins. In fact, we observed that similar rapid substitution reactions proceeded between the *ortho*- and *para*-fluorine-substituted NQs and *N*-acetyl-L-cysteine at 37°C in phosphate buffer solution, and *meta*- and 3-fluorinated NQs did not cause this substitution under the same conditions (unpublished result). As shown in Table 1, the *ortho*-fluoro-NQs are about one order less mutagenic than the corresponding *para*-fluoro-NQs. In addition, the *meta*- and 3-fluorinated NQs are almost as mutagenic as, or more mutagenic than, the corresponding *ortho*-fluoro-NQs. These findings may suggest that the mutagenicity of F-NQs is not mainly due to the mechanism based on the nucleophilic substitution reaction of the F-NQs with the target DNA molecule. Furthermore, these aromatic nucleophilic substitutions will selectively occur with highly nucleophilic substances such as proteins or amino acids containing sulfur atom. Such compounds will be so efficiently quenched by cellular proteins or amino acids that they will not reach the target DNA. Although the possibility of mutation by this mechanism cannot completely be excluded, we believe that the mutagenicity of NQs was induced by the mechanism widely accepted with nitro aromatic compounds.

The enhancing effect of *para*-fluorine substitution on the mutagenicity of 5- and 8-NQs might be explained by their potent electron-donating R-effect, which leads to the formation of a more stable nitrenium cation, an ultimate active form of NQs generated by ionic cleavage of the N–O bond of *O*-acetylhydroxylaminoquinolines. Although the *ortho*-fluorines of 6-F-5- and 7-F-8-NQs may exert the enhancing R-effects similar to those of the *para*-fluorines, the mutagenicities of *ortho*-fluorinated 5- and 8-NQs were ca. one order lower than those of the corresponding *para*-fluorinated compounds, as described before. One possible reason for this is that the *ortho*-fluorines to the nitro group hinder access of the enzymes involved in mutagenic activation, which include bacterial nitroreductase and

O-acetyltransferase. In any way, further studies are needed for better understanding the mechanism of induction of the NQ mutagenicity.

In conclusion, the present study with a series of fluorine-substituted NQs provides further basic data for possible mutagenic modification by fluorine-substitution and may offer a tool for mechanistic studies.

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