Dual Stimulatory and Inhibitory Effects of Fluorine-Substitution on Mutagenicity: An Extension of the Enamine Epoxide Theory for Activation of the Quinoline Nucleus

Ken-ichi SAEKI,*[,a] Hiroshi KAWAI,[a] Yutaka KAWAZOE,[a] and Atsushi HAKURA*[,b]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] Tanabedori, Mizuho-ku, Nagoya 467, Japan and Department of Drug Safety Research, Eisai Co., Ltd.,[b] 5-chome, Tokodai, Tsukuba 300–26, Japan.
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Nineteen mono- and di-fluorinated derivatives of quinoline, 1,7-phenanthroline, 1,10-phenanthroline, benzo[*h*]quinoline, and benzo[*f*]quinoline were subjected to analysis of their structure–mutagenicity relationships. For this purpose, six new fluorinated derivatives were synthesized. The results support that the enamine epoxide structure of the pyridine moiety, as well as the bay-region epoxide structure, is responsible for mutagenicity. Formation of K-region epoxides might involve a detoxification process rather than mutagenic activation.

Key words quinoline; fluoroquinoline; mutagenicity; metabolism; benzoquinoline; phenanthroline

It is well known that aromatic xenobiotics are enzymatically oxidized to arene oxides (aromatic epoxides), which are highly toxic in general due to their potent electrophilicity, leading to modification of biological macromolecules such as nucleic acid, proteins, *etc.* However, the epoxides are readily detoxified by their conversion to dihydro-diols and/or phenolic structures by epoxide hydrase. Alternatively, glutathione-*S*-transferase transforms them to their non-toxic glutathione conjugates. However, these quenching mechanisms do not necessarily work well, especially toward sterically hindered arene oxides, which could then result in a higher chance of producing genotoxic lesions in the cell. This concept has been widely accepted, based on the finding that the genotoxicity of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene is attributed mainly to their metabolic transformation to the bay-region epoxides resistant to enzymatic detoxification (Chart 1).[1] In addition, our previous findings revealed that the genotoxicity of quinoline, an aza-analog of naphthalene with no bay-region in its molecule, might be attributable to the formation of an enamine epoxide resistant to enzymatic transformation (Chart 1).[2] It is also known that K-region epoxides (Chart 1), readily produced enzymatically as products of a major detoxification mechanism for polycyclic aromatic hydrocarbons,[3] undergo rapid enzymatic hydration; hence, the contribution of K-region epoxides to genotoxicity might not be significant in general. Thus, in the metabolic processes of polycyclic aromatic hydrocarbons, aromatic nuclei probably face competition among the transformation pathways for bay-region epoxides, K-region epoxides, and, if a pyridine moiety exists in the molecule, enamine epoxides.

It is worth noting here that when the aromatic nucleus is substituted with a fluorine (F) atom, enzymatic oxidation is generally inhibited at the site of F-substitution.[4]

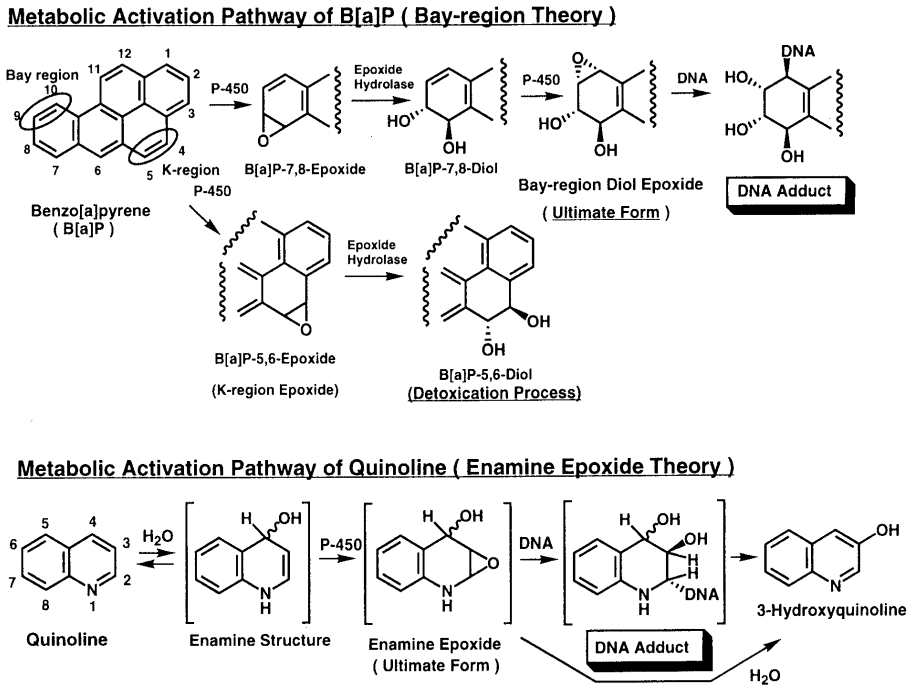

Chart 1. Proposed Metabolic Activation Pathways of B[*a*]P and Quinoline

* To whom correspondence should be addressed.

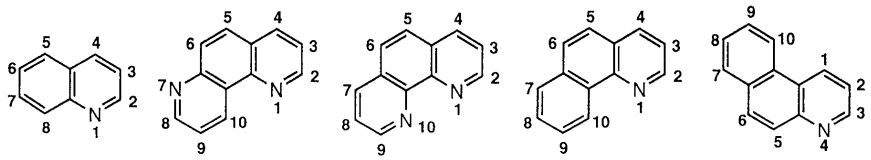

Chart 2. Structure of *N*-Containing Aromatic Hydrocarbons

Table 1. Mutagenicity toward *Salmonella typhimurium* TA100 with S9 Mix

Substituent	Location of fluorine				Revertants per nmol[a]	Maximum revertants[a]
	β to N	β to N′	K-Region	Bay-region		
Quinolines (Q)						
None	H	/	/	/	0.6	703
3-F	3F	/	/	/	Negative	136 (BG)[b]
5-F	H	/	/	/	1.3	1888
3,5-diF	3F	/	/	/	Negative	126 (BG)
5,8-diF	H	/	/	/	1.9	1154
1,7-Phenanthrolines (Ph_{1-7})						
None	H	H	H	/	1.4	398
6-F	H	H	6F	/	6.7	323
9-F	9F	H	H	/	1.3	334
3,9-diF	3F	9F	H	/	Negative	157 (BG)
1,10-Phenanthrolines (Ph_{1-10})						
None	H	H	H	/	Negative	151 (BG)
5-F	H	H	5F	/	1.4	405
Benzo[*h*]quinolines (B[*h*]Q)						
None	H	/	H	(H)[c]	Negative	240 (<BG × 2)
3-F	3F	/	H	(H)	Negative	238 (<BG × 2)
5-F	H	/	5F	(H)	3.0	317
6-F	H	/	6F	(H)	3.7	441
7-F	H	/	H	(7F)	2.4	834
9-F	H	/	H	(9F)	0.9	366
7,10-diF	H	/	H	(7F,10F)	0.7	278
3,6-diF	3F	/	6F	(H)	Negative	184 (<BG × 2)
Benzo[*f*]quinolines (B[*f*]Q)						
None	H	/	H	H	1.3	279
2-F	2F	/	H	H	5.0	1146
7-F	H	/	H	7F	4.1	420
10-F	H	/	H	10F	3.5	730
7,10-diF	H	/	H	7F,10F	8.0	692

a) Figure indicates the means of at least 3 independent experiments. *b*) BG indicates the number of revertants at the background level. *c*) Figures in the parentheses indicate that these positions may not be regarded as a bay-region because of the presence of an N atom in this location.

Therefore, F-substitution may affect the mutagenicity of aromatic compounds as follows: a decrease in mutagenicity by F-substitution at the activation site and an increase in mutagenicity by substitution at the detoxification site.[5] In order to confirm whether the enamine epoxide mechanism participates in the activation process for *N*-containing polycyclic aromatic hydrocarbons in general, the present study was undertaken to investigate structure–mutagenicity relationships among fluorinated and non-fluorinated *N*-containing tricyclic aromatic compounds; fluorinated compounds included monofluorinated (F-) and difluorinated (diF-) derivatives of 1,7-phenanthroline (Ph_{1-7}), 1,10-phenanthroline (Ph_{1-10}), benzo[*h*]quinoline (B[*h*]Q), and benzo[*f*]quinoline (B[*f*]Q), in addition to several fluorinated quinolines (Q). Nineteen fluorinated derivatives, newly or previously[6] prepared in our laboratory, were used as the model compounds for the present purpose (Chart 2 and Table 1).

RESULTS

Mutagenicities were tested in *Salmonella typhimurium* TA100 in the presence of an S9 mix. Chemicals inducing more than twice the number of revertants on the background were considered mutagenic. The results are summarized in Table 1, where the number of revertants per nmol, a measure of mutagenic potency, was calculated from the linear portion of the dose–response curve. The maximum number of revertants shown in the dose–response curve is related to both mutagenic potency and cytotoxicity, and hence should not be used for evaluation of the mutagenic potency of the test compound. Dose–response curves of quinolines are shown as examples in Fig. 1.

Mutagenicity of Fluorinated Quinolines Fluorine-substitution at position-5 potentiated mutagenicity of the quinoline nucleus,[5] probably because the F atom at

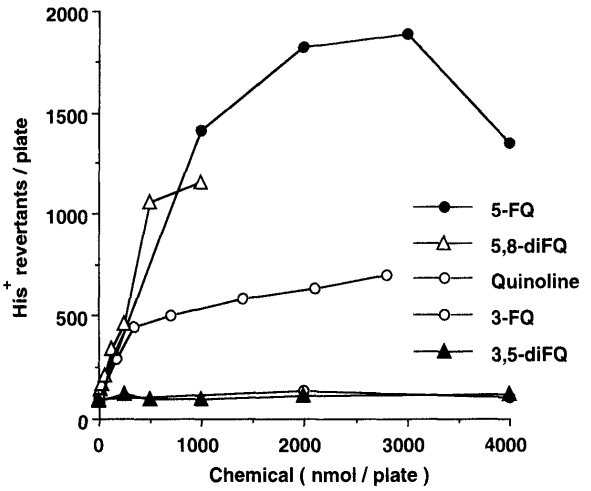

Fig. 1. Mutagenicity of Fluorinated Quinolines in *S. typhimurium* TA100 in the Presence of an S9 Mix

The symbols shown indicate the means of at least 3 independent experiments.

position-5 stabilizes the 1,4-hydrated structure, the key intermediate leading to the ultimate enamine epoxide, as previously reported.[7] The second F-substitution at position-3 of 5-F-Q deprived this molecule of mutagenicity completely, just as quinoline was deprived of mutagenicity by 3-F-substitution.

Mutagenicity of Fluorinated $Ph_{1\text{-}7}$ $Ph_{1\text{-}7}$ is regarded as having three sites of metabolism for mutagenic activation, *i.e.*, the two α,β,γ-free pyridine moieties and the K-region. Greater mutagenicity was induced by 6-F-substitution (in the K-region), probably because of the greater chance for mutagenic metabolism at the pyridine sites. On the other hand, F-substitution on both pyridine moieties (in 3,9-diF-$Ph_{1\text{-}7}$) completely deprived the molecule of mutagenicity. It seems that F-substitution at only one of the pyridine moieties (in 9-F-$Ph_{1\text{-}7}$) was not enough to abolish mutagenicity, probably because the other pyridine moiety site might become more susceptible to metabolic activation.

Mutagenicity of $Ph_{1\text{-}10}$ Although $Ph_{1\text{-}10}$ itself did not induce any mutation, 5-F-$Ph_{1\text{-}10}$ was appreciably mutagenic, probably because the F atom at the K-region blocked a less mutagenic pathway to allow more chances for more mutagenic activation at the pyridine sites.

Mutagenicity of Fluorinated B[*h*]Q It is assumed that the N atom at position-1 might interfere with epoxidation at closely located double bonds such as the 9,10-double bond of B[*h*]Q's and the 7,8-double bond of quinolines.[2,5,8] This aromatic ring system might, therefore, be regarded as having no bay-region in the molecule. B[*h*]Q itself did not induce mutation. However, appreciable mutagenicity was demonstrated by 5-F-B[*h*]Q and 6-F-B[*h*]Q, detoxification of which might have been blocked by F-substitution at position-5 or -6 (the K-region). The mutagenicity of 6-F-B[*h*]Q was lost by the second F-substitution at position-3 (in 3,6-diF-B[*h*]Q).

Mutagenicity of Fluorinated B[*f*]Q Only this class of compounds has a typical bay-region, as well as a pyridine moiety and a K-region in the molecule, among the quinoline analogs examined in the present study. It is worth noting that F-substitution at position-2 (the mutagenic metabolic site of pyridine moiety) potentiated the mutagenicity of B[*f*]Q, suggesting that the bay-region epoxidation might play a dominant role in its mutagenicity. On the other hand, 7-F-B[*f*]Q and 10-F-B[*f*]Q were still mutagenic, although an F atom at either position-7 or -10 might block the oxidative metabolism leading to formation of the bay-region epoxide. These results suggest that the enamine epoxide formation in the pyridine moiety might be partly responsible for their mutagenicity, together with the bay-region epoxide formation. It is worth mentioning here that Kumar *et al.*[7] stated that there may be another site, in addition to the bay region epoxidation, for the mutagenic activation of B[*f*]Q.

DISCUSSION

Alteration of the mutagenic property by F-substitution was investigated using fluorinated derivatives of four kinds of *N*-containing tricyclic aromatic ring systems, in addition to several fluorinated quinolines, in *S. typhimurium* TA100. It has now been proven on these ring systems that the enamine epoxide structure of the pyridine moiety, as well as the bay-region epoxide structure, is responsible for mutagenicity, and that the K-region epoxide is important for detoxification rather than for mutagenic activation. The molecules which comprise α,β,γ-free-pyridine moieties, a bay-region, and a K-region in the molecule might competitively undergo metabolic oxidations in these regions. Mutagenicity might be altered depending on which region is blocked by F-substitution. In other words, blocking at all mutagenic sites, *i.e.*, at the α,β,γ-free-pyridine moieties and the bay-region, would result in loss of mutagenicity, whereas blocking at the K-region would result in an increase in mutagenicity. The expected results in the present study provided further support for the mechanism previously proposed for mutagenic activation of the quinoline nucleus (Chart 1) and, at the same time, would prompt us to intentionally modify the mutagenic potency of heteroaromatic prodrug candidates by F-substitution. Recently, our medium term-assay for carcinogenicity indicated that the mutagenic capacities of 5-F-Q, Q, and 3-F-Q were in the same order as found for their relative carcinogenic potencies.[9] Further studies are being pursued to examine whether the manipulation of mutagenicity by F-substitution discussed so far might be practically applied to modify the carcinogenicity of these classes of aromatics.

MATERIALS AND METHODS

Materials 2,5-Difluoroaniline was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). 3-Fluoroquinoline was synthesized from 3-aminoquinoline by the Schiemann reaction as described in the literature.[10] Fluorinated B[*h*]Q's and B[*f*]Q's were synthesized in our laboratory as previously reported.[6] Sodium nitromalonaldehyde monohydrate was prepared as described in the literature.[11] Melting points were determined with a Yamato MP-500D micro melting point apparatus without correction. Mass spectra were measured with a JEOL AX 505HA spectrometer. ^{1}H-NMR spectra were recorded with a

JEOL JNM-EX 270 or a JNM-GSX 400 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard.

5-Fluoroquinoline (5-F-Q[5]) 5-F-Q was synthesized from 5-aminoquinoline by the Schiemann reaction using the same method as described in the synthesis of 3,5-difluoroquinoline (3,5-diF-Q). Colorless oil. bp 110 °C (18 mmHg). *Anal.* Calcd for C_9H_6FN: C, 73.46; H, 4.11; N, 9.52. Found: C, 73.66; H, 4.17; N, 9.51.

8-Fluoroquinoline (8-F-Q[5]) 8-F-Q was synthesized from 2-fluoroaniline by the Skraup reaction using the same method as described in the synthesis of 5,8-difluoroquinoline (5,8-diF-Q). Colorless oil. bp 125 °C (20 mmHg). *Anal.* Calcd for C_9H_6FN: C, 73.46; H, 4.11; N, 9.52. Found: C, 73.27; H, 4.17; N, 9.36.

3,5-diF-Q 3-Fluoroquinoline was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 5 h. Purification of the reaction mixture by column chromatography (aluminum oxide, benzene : hexane = 1 : 1) yielded 3-fluoro-5-nitroquinoline (3-F-5-NO_2-Q) in 38% yield. mp 92—93 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.80 (t, H-7), 8.45—8.53 (m, H-6 and H-8), 8.78 (ddd, H-4), 8.95 (d, H-2); $J_{2\text{-}3F}$ = —0, $J_{2\text{-}4}$ = 2.6, $J_{3F\text{-}4}$ = 10.2, $J_{6\text{-}7}$ = 8.3, $J_{7\text{-}8}$ = 8.3 Hz. HR-MS *m/z*: 192.032, Calcd for $C_9H_5FN_2O_2$: 192.033. 3-F-5-NO_2-Q thus prepared (2.51 g) was hydrogenated with 5% Pd–C in benzene. Without any purification, the product was dissolved in EtOH (150 ml) and mixed at 0 °C with 42% HBF_4 (10.5 ml, 5 eq) and isoamyl nitrite (5.6 ml, 3 eq). After 3 h, the precipitates produced by the addition of ether were washed with ether and hexane, then suspended in xylene (100 ml) and refluxed for 2 h. The filtrate of the reaction mixture was purified by column chromatography (aluminum oxide, benzene). 3,5-diF-Q was obtained as pale yellow prisms in 25% yield from 3-F-5-NO_2-Q. mp 52—55 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.28 (m, H-6), 7.62 (dt, H-7), 7.94 (d, H-8), 8.02 (dd, H-4), 8.85 (d, H-2); $J_{2\text{-}3F}$ = —0, $J_{2\text{-}4}$ = 2.6, $J_{3F\text{-}4}$ = 8.5, $J_{5F\text{-}6}$ = 9.8, $J_{5F\text{-}7}$ = 6.1, $J_{6\text{-}7}$ = 8.6, $J_{7\text{-}8}$ = 8.6 Hz. HR-MS *m/z*: 165.039, Calcd for $C_9H_5F_2N$: 165.039.

5,8-diF-Q 2,5-Difluoroaniline (2.0 g), glycerol (3.4 ml, 3 eq) and sodium *m*-nitrobenzenesulfonate (10.1 g, 3 eq) were dissolved in 80% H_2SO_4 (20 ml), and the mixture was stirred at 140 °C for 4 h. The reaction mixture was poured into ice water (300 ml). The filtrate was neutralized with aqueous NH_3 and extracted with $CHCl_3$. Purification of the extract by column chromatography (aluminum oxide, benzene) yielded 5,8-diF-Q in 81% yield. mp 68—69 °C. MS *m/z*: 165 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.16 (ddd, H-7), 7.35 (ddd, H-6), 7.57 (dd, H-3), 8.44 (ddd, H-4), 9.03 (dd, H-2); $J_{2\text{-}3}$ = 4.2, $J_{2\text{-}4}$ = 1.6, $J_{3\text{-}4}$ = 8.6, $J_{4\text{-}8F}$ = 1.6, $J_{5F\text{-}6}$ = 8.9, $J_{5F\text{-}7}$ = 4.6, $J_{6\text{-}7}$ = 8.6, $J_{6\text{-}8F}$ = 3.7, $J_{7\text{-}8F}$ = 9.9 Hz. *Anal.* Calcd for $C_9H_5F_2N$: C, 65.46; H, 3.05; N, 8.48. Found: C, 65.37; H, 3.17; N, 8.68.

6-Fluoro-1,7-phenanthroline (6-F-Ph$_{1\text{-}7}$) 8-F-Q was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 1 h. Purification of the reaction mixture by column chromatography (silica gel, $CHCl_3$) yielded 8-fluoro-5-nitroquinoline (8-F-5-NO_2-Q) in 60% yield. mp 135—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.51 (dd, H-7), 7.75 (dd, H-3), 8.48 (dd, H-6), 9.11 (dd, H-2), 9.15 (ddd, H-4); $J_{2\text{-}3}$ = 4.3, $J_{2\text{-}4}$ = 1.3, $J_{3\text{-}4}$ = 8.9, $J_{4\text{-}8F}$ = 1.0, $J_{6\text{-}7}$ = 8.6, $J_{6\text{-}8F}$ = 4.6, $J_{7\text{-}8F}$ = 8.9 Hz. HR-MS *m/z*: 192.032, Calcd for $C_9H_5FN_2O_2$: 192.033. 8-F-5-NO_2-Q thus prepared (2.51 g) was hydrogenated with 5% Pd–C in benzene. Purification of the reaction mixture by column chromatography (aluminum oxide, benzene) yielded 5-amino-8-fluoroquinoline (5-NH_2-8-F-Q) in 65% yield. mp 115—117 °C. MS *m/z*: 162 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 4.06 (br, 2H, D_2O-exchangeable NH_2), 6.71 (dd, H-6), 7.22 (dd, H-7), 7.42 (dd, H-3), 8.20 (ddd, H-4), 8.94 (dd, H-2); $J_{2\text{-}3}$ = 4.0, $J_{2\text{-}4}$ = 1.5, $J_{3\text{-}4}$ = 8.4, $J_{4\text{-}8F}$ = 1.8, $J_{6\text{-}7}$ = 8.4, $J_{6\text{-}8F}$ = 4.0, $J_{7\text{-}8F}$ = 10.6 Hz. *Anal.* Calcd for $C_9H_7FN_2 \cdot 1/4H_2O$: C, 64.86; H, 4.54; N, 16.81. Found: C, 64.62; H, 4.66; N, 16.77. 5-NH_2-8-F-Q thus prepared (300 mg), together with glycerol (0.4 ml, 3 eq) and sodium *m*-nitrobenzenesulfonate (0.42 g, 1 eq), was dissolved in 80% H_2SO_4 (20 ml) and the mixture was stirred at 140 °C for 1 d. The reaction mixture was poured into ice water (100 ml). The filtrate was neutralized with aqueous NH_3 and extracted with $CHCl_3$. Purification of the extract by column chromatography (aluminum oxide, benzene) and recrystallization from hexane yielded 6-fluoro-1,7-phenanthroline as colorless needles in 43% yield. mp 129—131 °C. MS *m/z*: 198 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.62 (d, H-5), 7.75 (m, 2H, H-3 and H-9), 8.20 (dd, H-4), 8.99 (dd, H-2 or H-8), 9.13 (dd, H-2 or H-8), 9.58 (dt, H-10); $J_{2\text{-}3}$ = 4.3, $J_{2\text{-}4}$ = 1.7, $J_{3\text{-}4}$ = 8.3, $J_{5\text{-}6F}$ = 10.6, $J_{6F\text{-}10}$ = 1.7, $J_{8\text{-}9}$ = 4.3, $J_{8\text{-}10}$ = 1.7, $J_{9\text{-}10}$ = 8.6 Hz. *Anal.* Calcd for $C_{12}H_7FN_2$: C, 72.72; H, 3.56; N, 14.13. Found: C, 72.57; H, 3.75; N, 13.98.

9-Fluoro-1,7-phenanthroline (9-F-Ph$_{1\text{-}7}$) 3-F-5-NO_2-Q (2.25 g) was hydrogenated with 5% Pd–C in benzene. Without any purification, the product, together with glycerol (2.6 ml, 3 eq) and sodium *m*-nitrobenzenesulfonate (2.6 g, 1 eq), were dissolved in 80% H_2SO_4 (20 ml) and the mixture was stirred at 140 °C for 2 d. The reaction mixture was poured into ice water (100 ml). The filtrate was neutralized with aqueous NH_3 and extracted with $CHCl_3$. Purification of the extract by column chromatography (aluminum oxide, benzene) and recrystallization from hexane yielded 9-F-Ph$_{1\text{-}7}$ as colorless needles in 59% yield. mp 111—113 °C. MS *m/z*: 198 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.62 (d, H-3), 7.91 (d, H-5), 8.08 (d, H-6), 8.26 (dd, H-4), 8.93 (dd, H-8), 9.02 (dd, H-2), 9.19 (dd, H-10); $J_{2\text{-}3}$ = 4.4, $J_{2\text{-}4}$ = 1.7, $J_{3\text{-}4}$ = 8.1, $J_{5\text{-}6}$ = 9.2, $J_{8\text{-}9F}$ = —0, $J_{8\text{-}10}$ = 2.9, $J_{9F\text{-}10}$ = 9.3 Hz. *Anal.* Calcd for $C_{12}H_7FN_2$: C, 72.72; H, 3.56; N, 14.13. Found: C, 72.98; H, 3.62; N, 14.47.

3,9-Difluoro-1,7-phenanthroline (3,9-diF-Ph$_{1\text{-}7}$) 3-F-5-NO_2-Q (0.64 g) was hydrogenated with 5% Pd–C in benzene. Without any purification, the product was treated with sodium nitromalonaldehyde monohydrate (0.6 g, 1 eq) in 2% aqueous HCl (100 ml) at 50 °C for 30 min to give *N*-(2-formyl-2-nitroethylidene)-5-amino-3-fluoroquinoline, which was then treated with $ZnCl_2$ in dimethylacetamide at 180 °C for 2 d. Purification by column chromatography (silica gel, $CHCl_3$) gave 9-fluoro-3-nitro-1,7-phenanthroline in 13% yield from 3-F-5-NO_2-Q. mp 220—224 °C. MS *m/z*: 243 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 8.06 (d, H-5), 8.25 (d, H-6), 9.03 (d, H-8), 9.10 (d, H-4), 9.21 (dd, H-10), 9.77 (d, H-2); $J_{2\text{-}4}$ = 2.6, $J_{5\text{-}6}$ = 9.2, $J_{6\text{-}10}$ = 0.7, $J_{8\text{-}9F}$ = —0, $J_{8\text{-}10}$ = 2.9, $J_{9F\text{-}10}$ = 9.2 Hz. 9-Fluoro-3-nitro-1,7-phenanthroline (110 mg) was catalyti-

cally hydrogenated quantitatively with 5% Pd–C in benzene to 3-amino-9-F-1,7-phenanthroline. mp 197—202 °C. MS *m/z*: 213 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 4.08 (br s, 2H, D_2O-exchangeable, $-NH_2$), 7.35 (d, H-8), 7.71 (d, H-5), 7.96 (d, H-6), 8.55 (d, H-4), 8.79 (d, H-2), 8.99 (d, H-10); $J_{2\text{-}4}=2.9$, $J_{5\text{-}6}=9.2$, $J_{8\text{-}9F}=-0$, $J_{8\text{-}10}=2.6$, $J_{9F\text{-}10}=9.6$ Hz. 3-Amino-9-F-1,7-phenanthroline (64 mg) in MeOH (5 ml) was mixed at 0 °C with 42% HBF_4 (0.23 ml, 5 eq) and isoamyl nitrite (0.12 ml, 3 eq). The precipitates produced by addition of ether were suspended in xylene (20 ml) and refluxed for 1 h. The filtrate of the reaction mixture was evaporated *in vacuo*. Purification by column chromatography (silica gel, $CHCl_3$) gave 3,9-difluoro-1,7-phenanthroline in 71% yield. mp 117—120 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.87 (d, H-5), 7.90 (dd, H-4), 8.12 (d, H-6), 8.90 (d, H-2 or H-8), 8.92 (d, H-2 or H-8), 9.11 (ddd, H-10); $J_{2\text{-}3F}=-0$, $J_{2\text{-}4}=2.9$, $J_{3F\text{-}4}=8.8$, $J_{5\text{-}6F}=9.2$, $J_{6\text{-}10}=0.7$, $J_{8\text{-}9F}=-0$, $J_{8\text{-}10}=2.9$, $J_{9F\text{-}10}=9.2$ Hz. HR-MS *m/z*: 216.049, Calcd for $C_9H_5F_2N_2$: 216.050.

5-Fluoro-1,10-phenanthroline (5-F-Ph$_{1\text{-}10}$) 5-F-Q was nitrated with 61% nitric acid and conc. sulfuric acid at room temperature for 1 d. Recrystallization of the reaction mixture from EtOH yielded 5-fluoro-8-nitroquinoline as yellow needles in 59% yield. mp 122—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.30 (dd, H-6), 7.65 (dd, H-7), 8.13 (dd, H-3), 8.53 (dd, H-4), 9.16 (dd, H-2); $J_{2\text{-}3}=4.3$, $J_{2\text{-}4}=1.7$, $J_{3\text{-}4}=8.6$, $J_{5F\text{-}6}=8.6$, $J_{5F\text{-}7}=4.3$, $J_{6\text{-}7}=9.8$ Hz. HR-MS *m/z*: 192.034, Calcd for $C_9H_5FN_2O_2$: 192.033. 5-F-8-NO_2-Q thus prepared (189 mg) was hydrogenated with 5% Pd–C in benzene. Without any purification, the product, together with glycerol (1.0 ml, 15 eq) and sodium *m*-nitrobenzenesulfonate (0.2 g, 1 eq), was dissolved in 80% H_2SO_4 (20 ml) and the mixture was stirred at 160 °C for 9 h. The reaction mixture was poured into ice water (100 ml). The filtrate was neutralized with aqueous NH_3 and extracted with $CHCl_3$. Purification of the extract by column chromatography (aluminum oxide, benzene : MeOH = 19 : 1) yielded 5-F-Ph$_{1\text{-}10}$ as a pale yellow solid in 40% yield. mp 147—149 °C. MS *m/z*: 198 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.44 (d, H-6), 7.64 (dd, H-3 or H-8), 7.73 (dd, H-3 or H-8), 8.21 (dd, H-4 or H-7), 8.54 (dd, H-4 or H-7), 9.15 (dd, H-2 or H-9), 9.26 (dd, H-2 or H-9); $J_{2\text{-}3}=4.3$, $J_{2\text{-}4}=1.7$, $J_{3\text{-}4}=8.3$, $J_{5F\text{-}6}=10.2$, $J_{7\text{-}8}=4.3$, $J_{7\text{-}9}=1.7$, $J_{8\text{-}9}=8.3$ Hz. *Anal.* Calcd for $C_{12}H_7FN_2$: C, 72.72; H, 3.56; N, 14.13. Found: C, 72.51; H, 3.75; N, 13.55.

Mutation Assay Chemicals were tested for mutagenicity as previously reported,[12,13] using *Salmonella typhimurium* TA100 in the presence of an S9 mix consisting of the S9 fraction (50 μl) obtained from rat liver (induced by phenobarbital and 5,6-benzoflavone, Oriental Yeast Co., Tokyo) and co-factors (Oriental Yeast Co., Tokyo). Assays were carried out after preincubation of the test chemical in the S9 mix at 37 °C for 20 min.

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