2-Aryl-1,4-naphthoquinone-1-oxime Methyl Ethers: Their Cytotoxic Activity

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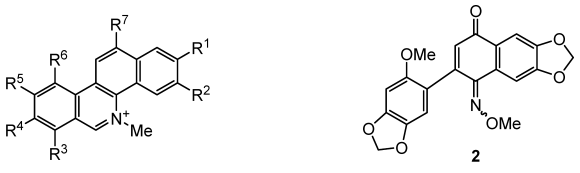
Preliminary examination for the structure–activity relationship of quinone monooxime derivatives on cytotoxicity against HeLa S3 cell and further trials using eight different cell lines suggested that 2-aryl-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ethers, carrying 2-methoxy-4,5-methylenedioxyphenyl, 7-methoxy-2-methylbenzofuran-4-yl, and 2-methoxycarbonyl-3,4-dimethoxyphenyl as the 2-aryl substituent, were potential candidates for anti-cancer drugs.

Key words 2-aryl-1,4-naphthoquinone-1-oxime methyl ether; benzophenanthridine; cytotoxicity; structure–activity relationship

Fully-aromatized benzo[*c*]phenanthridine alkaloids **1** with an isoquinoline core skeleton are structurally classified into O_4-type (R^1, R^2, R^3, R^4 or R^1, R^2, R^4, R^5=OR), O_5-type (R^1, R^2, R^3, R^4, R^6=OR), and O_6-type (R^1, R^2, R^3, R^4, R^6, R^7=OR) bases dependent upon the number of oxygen functions in their molecules.[1)] They have been attracted much attention due to their valuable pharmacological activities,[1—3)] especially the anti-tumor activity of O_4-type alkaloids such as nitidine[4)] (**1a**), fagaronine[4)] (**1b**), sanguinarine[5)] (**1c**), and NK109[6)] (**1d**). For their structure–activity relationship (SAR) on anti-tumor activity, we have established the practical synthetic methods through 2-aryl-1-tetralones as key intermediates under the modified Robinson's route[7)] for the O_4- and O_5-type benzo[*c*]phenanthridine alkaloids. On the other hand, macarpine (**1e**), a O_6-type alkaloid, has been prepared by an alternative route through 2-(2-methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ether (**2**) as a key synthetic precursor[8)] (Fig. 1). In screening for the discovery of potential anti-tumor active seed compounds from natural and unnatural sources, we found that the 2-aryl-1,4-naphthoquinone-1-oxime methyl ether **2** shows strong cytotoxic activity. In this paper we report preliminary results on the SAR of the naphthoquinone monooximes and the related compounds for cytotoxic activity against HeLa S3 cell and more precise examination on the potent growth inhibitory effects of the selected 2-aryl-1,4-naphthoquinone-1-oxime methyl ethers **2** and **7** using eight cancer cell lines, in which effective inhibition against HeLa and MCF-7 cells was observed.



2

nitidine (**1a**) : R^1+R^2=OCH_2O, R^3=R^6=R^7=H, R^4=R^5 =OMe
fagaronine (**1b**) : R^1=OH, R^2=R^4=R^5=OMe, R^3=R^6=R^7=H
sanguinarine (**1c**) : R^1+R^2=R^3+R^4=OCH_2O, R^5 = R^6 = R^7 = H
NK109 (**1d**) : R^1+R^2=OCH_2O, R^3=OH, R^4=OMe, R^5=R^6 =R^7=H
macarpine (**1e**) : R^1+R^2=R^3+R^4=OCH_2O, R^6=R^7=OMe, R^5=H

Fig. 1. Structures of Benzo[*c*]phenanthridine Alkaloids **1a**—**e** and Naphthoquinone Monooxime Ether **2**

Results and Discussion

The cytotoxic activity of 2-(2-methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ether (**2**) was evaluated using HeLa S3 cell by methylene blue staining method and the IC_{50} was estimated to be less than 0.1 μg/ml using Litchfield and Wilcoxon method. Thus, the 2-aryl-1,4-naphthoquinone-1-oxime methyl ether **2** was decided to be used as a positive control for further SAR examination of the naphthoquinone monooximes and the related compounds and, firstly, substituent variation of skeletal alkoxy functions of **2** was focused on. We had reported the preparation methods of 1,4-naphthoquinone-1-oxime methyl ethers by either basic nitrosation [isoamyl nitrite in dimethylformamide (DMF) in the presence of potassium carbonate (K_2CO_3)] of phenol substrate followed by methylation with dimethyl sulfate[9)] or heating 2-aryl-1-tetralone oxime methyl ethers with 5,6-dichloro-2,3-dicycano-1,4-benzoquinone (DDQ) in acetic acid.[10)] Three known 2-aryl-1,4-naphthoquinone-1-oxime methyl ethers **3**—**5** with different alkoxy functions in the naphthoquinone skeleton and/or in the 2-aryl substituent, which had been prepared by application of the latter DDQ oxidation method, were subjected to evaluation of cytotoxic activity using the same protocol for a positive control **2** (Table 1). Displacement of the methylenedioxy moieties either in the naphthoquinone skeleton (see **3**) or in the 2-aryl substituent (see **4**) with dimethoxy ones led to the reduction of activity (entries 2 and 3 in Table 1). No activity was observed when both methylenedioxy moieties in **2** were converted to methoxy groups like **5** (entry 4 in Table 1). 2-(3,4-Dimethoxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ether (**6**), carrying the same substitution pattern that an anti-tumor active benzo[*c*]phenanthridine alkaloid, nitidine[4)] (**1a**), was newly prepared according to the

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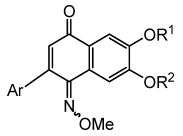
Table 1. Cytotoxic Activity of 2-Aryl-1,4-naphthoquinone-1-oxime Methyl Ethers **2**—**8**

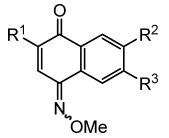


Entry	Compd.	Ar	R^1	R^2	Results[a)]
1	**2**[8)]	2-Methoxy-4,5-methylenedioxyphenyl	CH_2		(+)
2	**3**[10)]	2-Methoxy-4,5-methylenedioxyphenyl	Me	Me	(±)
3	**4**[10)]	2,4,5-Trimethoxyphenyl	CH_2		(±)
4	**5**[10)]	2,4,5-Trimethoxyphenyl	Me	Me	(−)
5	**6**	3,4-Dimethoxyphenyl	CH_2		(±)
6	**7**[9)]	7-Methoxy-2-methylbenzofuran-4-yl	CH_2		(+)
7	**8**[11)]	2-Methoxycarbonyl-3,4-dimethoxyphenyl	CH_2		(+)

a) Evaluated by methylene blue staining method using HeLa S3 cell. IC_{50} (μg/ml) was estimated using Litchfield and Wilcoxon method and the following symbols are used as criteria: (+)=<0.1 μg/ml; (±)=0.1—1.0 μg/ml; (−)=>1.0 μg/ml.

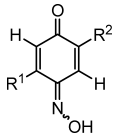
Table 2. Cytotoxic Activity of Other 1,4-Quinone Oximes

I. 1,4-Naphthoquinone-1-oxime Methyl Ethers **9** and **10**



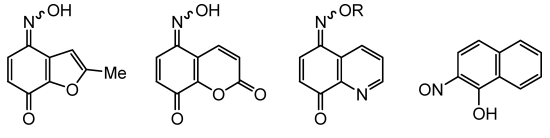
Entry	Compd.	R^1	R^2	R^3	Results[a)]
I-1	**9**[9)]	7-Methoxy-2-methylbenzofuran-4-yl	OCH_2O		(−)
I-2	**10**[9)]	H	H	H	(±)

II. 1,4-Benzoquinone-1-oximes **11**—**15**



Entry	Compd.	R^1	R^2	Results[a)]
II-1	**11**[9)]	Me	CO_2Me	(±)
II-2	**12**[9)]	H	CO_2Me	(−)
II-3	**13**[9)]	H	CHO	(±)
II-4	**14**[9)]	OMe	CHO	(−)
II-5	**15**[9)]	OMe	H	(−)

III. Heterocyclic 1,4-Quinone Oximes **16**—**19** and 2-Nitroso-1-naphthol **20**



16 **17** **18**: R=H **19**: R=Me **20**

Entry	Compd.	Results[a)]
III-1	2-Methylbenzofuran-4,7-quinone-4-oxime[9)] **16**	(±)
III-2	Coumarin-5,8-quinone-5-oxime[9)] **17**	(−)
III-3	Quinoline-5,8-quinone-5-oxime[9)] **18**	(±)
III-4	Quinoline-5,8-quinone-5-oxime methyl ether[9)] **19**	(±)
III-5	2-Nitroso-1-naphthol[9)] **20**	(±)

a) See footnote *a*) in Table 1.

DDQ oxidation method; however, its activity was estimated to be moderate (entry 5 in Table 1). On the other hand, alternative 1,4-naphthoquinone-1-oxime methyl ethers with 7-methoxy-2-methylbenzofuran-4-yl[9] **7** or 2-methoxycarbonyl-3,4-dimethoxyphenyl[11] **8** as the 2-aryl pendant, which had been prepared by the former basic nitrosation-methylation method, were interestingly proven to maintain strong cytotoxic activity (entries 6 and 7 in Table 1).

A variety of quinone monooxime derivatives had also been prepared from the corresponding phenolic compounds by application of the basic nitrosation method.[9] Thus, selected samples in our hands, including 3-(7-methoxy-2-methylbenzofuran-4-yl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ether (**9**), a positional isomer of aryl substituent in the cytotoxic 2-(7-methoxy-2-methylbenzofuran-4-yl) derivative **7**, were subjected to evaluation in order to discuss on the skeletal requirement of 2-aryl-1,4-naphthoquinone-1-oxime methyl ether core to cytotoxic activity. The results of 1,4-naphthoquinone-1-oxime ethers, 1,4-benzoquinone-1-oximes, and heterocyclic 1,4-quinone oximes are shown in I, II, and III in Table 2, respectively, and all quinone monooxime derivatives examined were found to be negative.

These experimental facts obtained in Tables 1 and 2 suggest that the presence of 6,7-methylenedioxy function in the 2-aryl-1,4-naphthoquinone-1-oxime skeleton is crucial for the positive cytotoxic activity, in spite of substituent tolerance in the 2-aryl group. It should be noted here that no distinct change in conformation of the 2-aryl-1,4-naphthoquinone-1-oxime skeleton was observed in crystal structures between an active **2** and an inactive pentamethoxy-substituted derivatives **5**.[12]

Next, our attention turned to the structural modification of the oxime moiety in the 2-aryl-1,4-naphthoquinone-1-oxime methyl ether for the examination of the contribution of the ether unit in oxime methyl ether function to positive cytotoxicity. In general, 2-aryl-1,4-naphthoquinone-1-oxime methyl ether derivatives are hard to dissolve in the test solution for evaluation. Thus, introduction of polar functional group to a positive control **2** was targeted for the increase of solubility, and the basic nitrosation method was applied to the preparation of oxime-modified derivatives from 3-(2-methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1-naphthol **21** (Chart 1). Naphthoquinone oxime derivatives **22** and **23** with acetic acid residue were prepared by alkylation with bromoacetate and by alkaline hydrolysis of the resulting oxime acetate **22**, respectively, after the basic nitrosation of **21**. The aminoethyl-inserted ether **24** was given by alkylation using dimethylaminoethyl chloride in place of bromoacetate in **22**. The known iminoquinone[8] **25**, in addition to the three derivatives **22**—**24** prepared above, were subjected to the evaluation of cytotoxic activity under the same condition that on a positive control **2** (Table 3). However, all of the four *N*-modified analogues[13] **22**—**25** showed no activity, indicating that the oxime methyl ether function in the 1,4-naphthoquinone-1-oxime skeleton plays an important role for the positive activity.

Finally, based on the above results, cell prolification of a positive control **2** and an alternative potential **7** on a concentration-dependent manner was further examined using eight cancer cell lines, in which expected activity was observed except for H28 cell (Table 4). In particular, these naphthoquinone oximes effectively attacked HeLa (IC_{50} 0.10 μM on **2** and 0.23 μM on **7**, respectively) and MCF-7 cells (IC_{50} 0.20 μM on **2** and 0.51 μM on **7**, respectively).

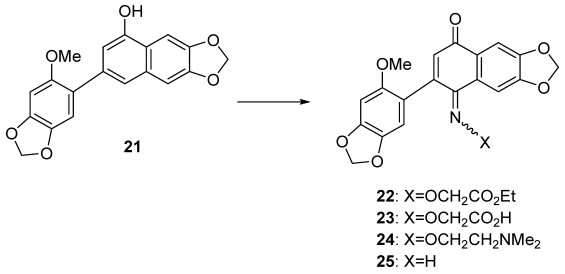


Chart 1. Preparation of *N*-Modified 2-(2-Methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime Ether Derivatives **22**—**25**

Table 3. Cytotoxic Activity of 2-(2-Methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime Methyl Ether (**2**) and Its *N*-Modified Derivatives **22**—**25**

Entry	Compd.	Results[a]
1	**2**[8]	(+)
2	**22**	(−)
3	**23**	(−)
4	**24**	(−)
5	**25**[8]	(−)

a) See footnote *a*) in Table 1.

Table 4. IC_{50} Values of Two Naphthoquinone Monooximes **2** and **7** in Different Cancer Cell Lines[a]

Cell	Derivation	IC_{50} (μM)±S.D.	
		2	**7**
HeLa	Ovarian cancer	0.10±0.02	0.23±0.04
Caki-1	Renal cancer	0.60±0.14	0.91±0.06
786-O	Renal cancer	0.28±0.07	0.71±0.09
A549	Lung cancer	0.43±0.04	1.06±0.07
MCF-7	Breast cancer	0.20±0.07	0.51±0.09
H28	Mesothelioma	5.78±10.50	6.52±3.39
H2052	Mesothelioma	0.54±0.25	0.47±0.15
MSTO-211H	Mesothelioma	0.38±0.25	1.05±0.79

a) Cells were treated with **2** and **7** for 48 h. Data are from 3 independent experiments.

In conclusion, it was found that 2-aryl-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ethers showed cytotoxic activity in a wide range of cancer cell lines, especially HeLa and MCF-7 cells. These facts suggest that the quinone monooxime core could serve as a potential scafford in screening for the discovery of bioactive small molecules because of known antiviral activity.[9] Mechanistic approaches to the cytotoxicity of these 2-aryl-1,4-naphthoquinone-1-oxime methyl ethers, in addition to further modification of the substituent in the 2-aryl moiety focusing on increasing hydrophilicity for extension to *in vivo* trials due to the hard solubility of the 1,4-naphthoquinone-1-oxime ethers to the test solution, are under investigation in our laboratory.

Experimental

General IR spectra were recorded on a JASCO IR-300E spectrophotometer (ATR). High resolution (HR)-FAB-MS and HR-electrospray ionization (ESI)-MS spectra were measured by JEOL JMS-HX 110A and JMS-

T100LP, and Thermo Scientific Exactive Bentitop Orbitrap spectrometers, respectively. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on JEOL JNM ECP 400 in $CDCl_3$ unless otherwise stated. For TLC was used SiO_2 60 F_{254}, 0.25 mm (Merck) and for column chromatography SiO_2 60 (63—210 mm) (Kanto-Cica) or FL100D SiO_2 (Fuji Silysia Chemical Ltd., Japan).

2-(3,4-Dimethoxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime Methyl Ether (6) According to the reported method for the DDQ oxidation,[10] a mixture of 2-(3,4-dimethoxyphenyl)-6,7-methylenedioxy-1-tetralone oxime methyl ether (0.11 g, 3.1×10^{-4} mol), easily derived from the corresponding 1-tetralone[14] by conventional treatment with hydroxylamine methyl ether, and DDQ (0.30 g, 1.3×10^{-3} mol) in benzene (3 ml) containing acetic acid (0.2 ml) was heated at 80 °C for 22 h. After worked up, purification of a crude product by SiO_2 column chromatography (ethyl acetate : hexane=1 : 3) provided the naphthoquinone-1-oxime ether **6** (0.009 g, 8%) as yellow prisms, mp 248—250 °C. IR cm^{-1}: 1641. ^{1}H-NMR δ: 3.92 (3H, s, OMe), 3.94 (3H, s, OMe), 4.13 (3H, s, OMe), 6.13 (2H, s, OCH_2O), 6.63 (1H, s, C3-H), 6.91 (1H, d, J=8.4 Hz, C5′-H), 7.06 (1H, d, J=1.6 Hz, C2′-H), 7.10 (1H, dd, J=8.4 Hz, 1.6 Hz, C6′-H), 7.69 (1H, s, C5-H), 8.36 (1H, s, C8-H). ^{13}C-NMR δ: 55.9, 56.0, 64.5, 102.2, 106.0, 110.2, 110.4, 113.3, 122.8, 124.6, 127.5, 128.7, 129.7, 145.6, 148.1, 149.3, 149.8, 151.2, 151.7, 183.4. HR-FAB-MS m/z: 368.1145 (Calcd for $C_{20}H_{18}NO_6$: 368.1134).

2-(2-Methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime Ethoxycarbonylmethyl Ether (22) According to the reported method for basic nitrosation,[8,9] isoamyl nitrite (0.02 ml, d=0.87, 14×10^{-4} mol) was added to a mixture of the naphthol **21** (0.030 g, 8.0×10^{-5} mol) and K_2CO_3 (0.060 g, 4.0×10^{-4} mol) in DMF (0.3 ml) at rt and then the whole was stirred at rt for 1 h. After addition of ethyl bromoacetate (0.012 ml, d=1.51, 11×10^{-4} mol) under ice-cooling, the resulting mixture was stirred at rt for 1 h, quenched with water, and extracted with ethyl acetate. The organic solution was washed with water and brine, dried (K_2CO_3), and evaporated. Purification of the residual red brown oil by preparative TLC (ethyl acetate : hexane=1 : 2, Rf=0.30—0.38) afforded orange prisms (0.030 g, 76%), mp 125—129 °C, which were triturated with hexane. IR cm^{-1}: 1768, 1639. ^{1}H-NMR δ: 1.28 (3H, t, J=7.1 Hz, $CH_2\underline{CH_3}$), 3.70 (3H, s, OMe), 4.22 (2H, q, J=7.1 Hz, $O\underline{CH_2}CH_3$), 4.74 (2H, s, OCH_2CO), 5.97 (2H, s, OCH_2O), 6.11 (2H, s, OCH_2O), 6.54 (2H, s, C5′-, C3-H), 6.72 (1H, s, C2-H), 7.67 (1H, s, C5-H), 8.46 (1H, s, C8-H). ^{13}C-NMR δ: 14.1, 56.6, 61.2, 72.7, 94.8, 101.4, 102.1, 106.0, 110.2, 110.6, 118.6, 124.5, 128.7, 129.7, 140.8, 147.0, 148.7, 149.4, 149.6, 151.3, 152.5, 168.6, 183.6. HR-ESI-MS m/z: 476.0927 (Calcd for $C_{23}H_{19}NNaO_9$: 476.0958).

2-(2-Methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime Hydroxycarbonylmethyl Ether (23) A mixture of the naphthoquinone ester **22** (0.025 g, 5.5×10^{-5} mol) and 2 mol/l aq NaOH (0.5 ml) in EtOH (1 ml) was heated at 50 °C for 10 min, quenched with water, and extracted with ethyl acetate. The aqueous solution was acidified with 5% HCl solution and extracted with ethyl acetate. The organic solution was dried ($MgSO_4$) and evaporated to give red prisms (0.023 g, quant.), mp 202—205 °C, which were triturated with hexane. IR cm^{-1}: 1758, 1622. ^{1}H-NMR δ: 3.70 (3H, s, OMe), 4.79 (2H, s, OCH_2CO), 5.98 (2H, s, OCH_2O), 6.13 (2H, s, OCH_2O), 6.55 (1H, s, C5′- or C3-H), 6.56 (1H, s, C5′- or C3-H), 6.72 (1H, s, C2-H), 7.68 (1H, s, C5-H), 8.36 (1H, s, C8-H). ^{13}C-NMR (CD_3OD) δ: 57.0, 73.1, 95.8, 102.4, 103.6, 105.9, 110.6, 111.1, 119.6, 125.3, 129.4, 129.9, 141.7, 147.4, 149.8, 150.6, 151.0, 152.3, 153.7, 170.0, 183.3. HR-ESI-MS m/z: 448.0663 (Calcd for $C_{21}H_{15}NNaO_9$: 448.0645).

2-(2-Methoxy-4,5-methylenedioxyphenyl)-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime 2-Dimethylaminoethyl Ether (24) According to the reported method for basic nitrosation,[8,9] isoamyl nitrite (0.04 ml, d=0.87, 3.0×10^{-4} mol) was added to a mixture of the naphthol **21** (0.030 g, 8.9×10^{-5} mol) and K_2CO_3 (0.10 g, 7.2×10^{-4} mol) in DMF (1.6 ml) under ice-cooling and then the whole was stirred at rt for 2 h. After addition of 2-dimethylaminoethyl chloride hydrochloride (0.017 g, 1.2×10^{-4} mol) under ice-cooling, the resulting mixture was stirred at rt for 15 h, quenched with ice-water, and extracted with ethyl acetate. The organic solution was washed with water and brine, dried (K_2CO_3), and evaporated. Purification of the residual dark brown gummy product by SiO_2 column chromatography (ethyl acetate : hexane : methanol=10 : 10 : 1) afforded yellow prisms (0.023 g, 59%), mp 109—112 °C, which were triturated with hexane. IR cm^{-1}: 1639. ^{1}H-NMR δ: 2.28 (6H, s, NMe_2), 2.65 (2H, t, J=5.9 Hz, $CH_2\underline{CH_2}N$), 3.71 (3H, s, OMe), 4.33 (2H, t, J=5.9 Hz, $O\underline{CH_2}CH_2$), 5.98 (2H, s, OCH_2O), 6.11 (2H, s, OCH_2O), 6.52 (1H, s, C3-H), 6.56 (1H, s, C5′-H), 6.72 (1H, s, C2′-H), 7.68 (1H, s, C5-H), 8.40 (1H, s, C8-H). ^{13}C-NMR δ: 45.8, 56.8, 58.0, 74.9, 95.0, 101.6, 102.3, 106.2, 110.3, 119.4, 124.8, 128.8, 129.4, 141.0, 145.7, 148.7, 149.4, 150.4, 151.4, 152.5, 183.8. HR-ESI-MS m/z: 439.1526 (Calcd for $C_{23}H_{23}N_2O_7$: 439.1505).

Preliminary Screening Using HeLa S3 The sample was dissolved in dimethyl sulfoxide (DMSO) and then diluted with saline at the final concentrations of 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 μg/ml. HeLa S3 cell containing 1×10^4 cells/ml were inoculated into 96-well microplates with 0.2 ml/well and incubated at 37 °C for 24 h under 5% CO_2 atmosphere. After addition of the test solution (0.1 μl) cells were cultured for 72 h. Following cells were fixed with methanol and cells were stained with 0.05% methyleneblue in 10 mmol/l Tris–HCl (pH 8.5) (0.1 ml/well) at rt for 30 min. After removal of the staining solution using aspirator the residue was washed with water three times and the stained dye was extracted with 3% HCl (0.2 ml/well). The inhibition ratio was estimated by measurement of absorbance of each well using a wave length of 660 nm and IC_{50} (μg/ml) was calculated by Litchfield and Wilcoxon method.

Cell Cytotoxicity Assay All cell lines were provided by Tomohiro Yano (Graduate School of Life Sciences, Toyo University, Japan), unless otherwise indicated. Caki-1 (human kidney cancer cell line from ATCC) was grown in McCoy's 5A (Wako). HeLa (human ovarian cancer cell line from RIKEN), 786-O (human kidney cancer cell line), and A549 (human lung cancer cell line) were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Wako). MCF-7 (human breast cancer cell line), H2052 (human malignant mesothelioma cell line), and MSTO-211H (human malignant mesothelioma cell line) were grown in RPMI 1640 (Wako). H28 (human malignant mesothelioma cell line) was grown in RPMI 1640 supplemented with 0.01 M *N*-(2-hydroxyethyl)piperazine-*N*′-2-(ethanesulfonic acid) (HEPES) buffer solution (GIBCO BRL, Grand Island, NY, U.S.A.), 1 mM sodium pyruvate (GIBCO BRL), and 9.0 g/l glucose. All cells were cultured with 10% fetal bovine serum (FBS) (EQUITECH-BIO, Kerrville, TX, U.S.A.), 1.0 units/ml penicillin (GIBCO BRL), and 2.0 mg/ml streptomycin (GIBCO BRL) in atmosphere at 37 °C with 5% CO_2.

A total of 3.0×10^3 cells/well was seeded in a 96-well plate. After 24 h incubation, an optimum concentration gradient of the 2-aryl-1,4-naphthoquinone-1-oxime methyl ethers **2** or **7** was added to each well, followed by culturing for 48 h. Finally, to assess the sensitivity of the cells to these compounds, cell viability was assessed using the Proliferation Reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (DOJINDO, Kumamoto, Japan) according to the manufacturer's instructions. Control cells were treated with 0.1% DMSO, which is the vehicle for **2** and **7**. The inhibition ratio was estimated by measurement of absorbance of each well using a wave length of 570 nm and IC_{50} (μg/ml) was calculated by GraphPad PRISM 5J (Graphpad Software, Inc., San Diego, CA, U.S.A.).

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