Synthesis of Dibenzoylmethane Derivatives and Inhibition of Mutagenicity in *Salmonella typhimurium*

Tominari CHOSHI,[a] Shigenori HORIMOTO,[a] Ching Y. WANG,*[,b] Hisamitsu NAGASE,[b] Masataka ICHIKAWA,*[,c] Eiichi SUGINO,[d] and Satoshi HIBINO*[,d]

Department of Pharmacy, Hiroshima General Hospital,[a] 1-3-3 Jigozen, Hatsukaichi, Hiroshima 738, Japan, Department of Chemical Carcinogenesis, Michigan Cancer Foundation,[b] 110 E. Warren Avenue, Detroit, Michigan 48201, U.S.A., Department of Hospital Pharmacy, Nagasaki University School of Medicine,[c] 7-1 Sakamoto-Machi, Nagasaki 852, Japan, and Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University,[d] 985 Higashimura, Fukuyama, Hiroshima 729-02, Japan. *Received September 12, 1991*

Twenty dibenzoylmethanes with methyl, methoxy, bromo, chloro, or fluoro substitution on either one or both benzene rings were synthesized and assayed for inhibition of the mutagenicity of 2-nitrofluorene in *S. typhimurium* TA98. 2,2-Dimethoxy, 3,3-dimethoxy and 3,3,4,4-tetramethoxydibenzoylmethane was as active as dibenzoylmethane. None of the halogen-substituted dibenzoylmethanes were active. These results demonstrate that dibenzoylmethanes can inhibit the mutagenicity of 2-nitrofluorene, and that modifications made on the benzene rings of dibenzoylmethane cannot enhance the antimutagencity of this parent compound.

Keywords dibenzoylmethane derivative; 1,3-diphenyl-1,3-propanedione derivative; synthesis; antimutagenicity; mutation test; *Salmonella typhimurium* TA98

Introduction

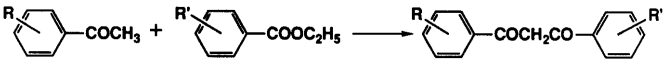
Chemical mutagenes are electrophilic *per se* or they require metabolic activation to form electrophilic agents to react with deoxyribonucleic acid (DNA). As a consequence of DNA modification, base-substitution or frame-shift mutations can occur during the subsequent DNA replication. Most chemical carcinogens also undergo a similar reaction with DNA to initiate tumor induction. Thus, nucleophilic agents which can react with electrophilic mutagens are potential antimutagenic/anticarcinogenic agents. Several classes of nucleophilic chemicals have been shown to have these activities.[1,2] Most active are the polyhydric phenol, ellagic acid, and the sulfur-containing compound, phenethyl isothiocyanate. The inhibitory activity of these compounds may also be due to their effects on the enzymes that metabolize mutagens/carcinogens.

Diacylmethanes possess an active methylene function which forms carbanions or enolic ions in neutral solutions. These ions can react with electrophilic mutagens and carcinogens and, thus, may inhibit mutagenesis and carcinogenesis. Indeed, we have previously observed that several diacylmethanes inhibit the binding of activated *N*-hydroxy-2-acetylaminofluorene,[3] benzo[*a*]pyrene and aflatoxin B_1[4] to transfer ribonucleic acid (tRNA). These diacylmethanes also inhibit the mutagenicity of 2-nitrofluorene, 2-naphthohydroxamic acid, benzo[*a*]pyrene, aflatoxin B_1 and methylnitrosourea in *S. typhimurium*.[3,4] Among the diacylmethanes investigated, dibenzoylmethane is the most active; 0.5 μmol/plate of this compound inhibits by 50% the mutagenicity of these mutagenes.[3,4] Its potency is less than that of phenethyl isothiocyanate, ellagic acid and epigallocatechin-3-gallate. The amount required for 50% inhibition of the mutagenicity of benzo[*a*]pyrene is approximately 0.1 μmol/plate for phenethyl isothiocyanate[5] and approximately 0.05 μmol/plate for epiglocatechin-3-gallate.[6] Ellagic acid, 1 nmol/plate, inhibits by 50% the mutagenicity of the dihydrodiol epoxide metabolite of benzo[*a*]pyrene[7]; 0.5 μmol/plate of this compound inhibits by 50% the mutagenicity of methylnitrosourea.[8] Since the inhibitory activity of diacylmethanes may be related to the structure of the acyl group, several diacylmethanes that differ in acyl structures were synthesized and assayed for their activities against the mutagenicity of 2-nitrofluorene in *S. typhimurium* TA98.

Results and Discussion

Chemistry The ethyl benzoate derivatives (**2d—m**) were prepared in the usual manner. The acetophenone derivative (**1e**) was prepared by reaction of 4-hydroxyacetophenone with benzylbromide in dimethylformamide (DMF) using potassium carbonate as a catalyst.

Twenty dibenzoylmethane derivatives (**3**) were prepared according to the method of Swamer *et al.*[9] Briefly, acetophenone (**1**), ethyl benzoate (**2**) and sodium hydride were refluxed in benzene (Chart 1 and Table I). The yields



1a—o 2a—n 3a—t

Chart 1

TABLE I. Reaction of the Acetophenone (**1**) with the Ethyl Benzoate (**2**)

Substrates		React. time (h)	Dibenzoylmethanes (**3**)	Yields (%)
Acetophenones (**1**)	Ethyl benzoates (**2**)			
1a : 4-OCH_3	**2a** : H[a]	3	**3a** : 4-OCH_3	42
1b : 2,4-$(OCH_3)_2$	**2a** : H	3	**3b** : 2,4-$(OCH_3)_2$	60
1c : 3,4-$(OCH_3)_2$	**2a** : H	2	**3c** : 3,4-$(OCH_3)_2$	59
1d : 2,5-$(OCH_3)_2$	**2a** : H	3	**3d** : 2,5-$(OCH_3)_2$	48
1e : 4-OCH_2Ph	**2a** : H	4	**3e** : 4-OCH_2Ph	55
1a : 4-OCH_3	**2b** : 4-OCH_3	5	**3f** : 4,4-$(OCH_3)_2$	56
1f : 2,4,6-$(CH_3)_3$	**2a** : H	8	**3g** : 2,4,6-$(CH_3)_3$	13
1f : 2,4,6-$(CH_3)_3$	**2b** : 4-OCH_3	10	**3h** : 2,4,6-$(CH_3)_3$-4-OCH_3	12
1g : 4-CH_3	**2c** : 4-CH_3	10	**3i** : 4,4-$(CH_3)_2$	66
1h : 2-OCH_3	**2d** : 2-OCH_3	14	**3j** : 2,2-$(OCH_3)_2$	45
1i : 3-OCH_3	**2e** : 3-OCH_3	7	**3k** : 3,3-$(OCH_3)_2$	51
1j : 3,4-$(CH_3)_2$	**2f** : 3,4-$(CH_3)_2$	6	**3l** : 3,3,4,4-$(CH_3)_4$	62
1b : 2,4-$(OCH_3)_2$	**2g** : 2,4-$(OCH_3)_2$	2	**3m**: 2,2,4,4-$(OCH_3)_4$	53
1c : 3,4-$(OCH_3)_2$	**2h** : 3,4-$(OCH_3)_2$	2	**3n** : 3,3,4,4-$(OCH_3)_4$	53
1d : 2,5-$(OCH_3)_2$	**2i** : 2,5-$(OCH_3)_2$	2	**3o** : 2,2,5,5-$(OCH_3)_4$	45
1k : 3,4,5-$(OCH_3)_3$	**2j** : 3,4,5-$(OCH_3)_3$	4	**3p** : 3,3,4,4,5,5-$(OCH_3)_6$	38
1l : 4-Br	**2k** : 4-Br	1	**3q** : 4,4-Br_2	54
1m: 4-Cl	**2l** : 4-Cl	3	**3r** : 4,4-Cl_2	55
1n : 3,4-Cl_2	**2m**: 3,4-Cl_2	3	**3s** : 3,3,4,4-Cl_4	95
1o : 2,4-F_2	**2n** : 2,4-F_2	3	**3t** : 2,2,4,4-F_4	35

a) Non-substituted ethyl benzoate.

for the products were moderate. However, the yields were very poor for those that were derived from the acetophenone (**1f**), which possessed two methyl groups at both *o*-positions, probably due to steric hindrance. It has been reported that this type of reactants does not provide a good yield.[10]

The signals of the olefinic protons of 17 compounds (**3**) were observed clearly at near δ_H 5.5—7.0 and the hydroxyl protons of 14 compounds were also observed at δ_H 16.5—17.3 in the proton nuclear magnetic resonance (^{1}H-NMR) spectra. The olefinic protons of **3l**, **3n** and **3t** were not observed because of overlapping with other signals. It has been suggested that these dibenzoylmethane derivatives (**3**) may exist in a chelated enols form and the *Z*-configuration of *β*-hydroxychalcone as reported by Tsuda and co-workers.[11]

Antimutagenicity The number of spontaneous revertants obtained for *S. typhimurium* TA98 was between 24 to 34 per plate. 2-Nitrofluorene, 10 nmol, produced 456± colonies. The antimutagenicities of the tested diketones (**3a**—**t**) were graded as −(without inhibitory activity at 5 μmol/plate), +(the amount to inhibit by 50% the mutagenicity of 2-nitrofluorene was greater than 1 μmol/plate), and ++(the amount was less than 1 μmol/plate).

TABLE II. Physical Data and Antimutagenicity of Dibenzoylmethane Derivatives (**3**)

Compd. No.	mp (°C) (lit.,) Recryst. solv.[a]	Molecular formula	Analysis (%) Found (Calcd) C	H	Antimutagenicity
3a	126—127 (130—131)[15] b	$C_{16}H_{14}O_3$	75.78 (75.57)	5.68 5.55)	+
3b	52—54 (57)[16] e/h	$C_{17}H_{16}O_4$	71.90 (71.82)	5.77 5.67)	+
3c	63—64 (67)[17] b	$C_{17}H_{16}O_4$	71.92 (71.82)	5.65 5.67)	+
3d	63.5—65.5 et	$C_{17}H_{16}O_4$	71.89 (71.82)	5.70 5.67)	+
3e	83—84 e/h	$C_{22}H_{18}O_3$	80.19 (79.98)	5.54 5.49)	+
3f	114—116 (114)[18] e/h	$C_{17}H_{16}O_4$	72.03 (71.82)	5.88 5.67)	+[b]
3g	74—75 et	$C_{18}H_{18}O_2$	81.27 (81.17)	6.79 6.81)	+
3h	105—107 et	$C_{19}H_{20}O_3$	77.31 (77.00)	6.77 6.80)	+
3i	123.5—125 et	$C_{17}H_{16}O_2$	76.25 (76.10)	6.18 6.18)	+
3j	63—65 e/h	$C_{17}H_{16}O_4$	71.79 (71.82)	5.88 5.67)	++
3k	68.5—69 e/h	$C_{17}H_{16}O_4$	71.94 (71.82)	5.91 5.67)	++
3l	139.5—140 et	$C_{19}H_{20}O_2$	81.35 (81.39)	7.20 7.19)	−
3m	129—130 et	$C_{19}H_{20}O_6$	66.37 (66.27)	5.90 5.85)	−
3n	100.5—101 et	$C_{19}H_{20}O_6$	66.49 (66.27)	5.92 5.85)	++
3o	130—132 et	$C_{19}H_{20}O_6$	66.55 (66.27)	5.79 5.85)	−[b]
3p	154.5—155.5 et	$C_{21}H_{24}O_8$	62.67 (62.37)	6.25 5.98)	−
3q	195—196 et	$C_{15}H_{10}Br_2O_2$	47.35 (47.16)	2.78 2.63)	−
3r	157.5—159.5 et	$C_{15}H_{10}Cl_2O_2$	61.77 (61.46)	3.55 3.44)	−
3s	180—184 et	$C_{15}H_8Cl_4O_2$	49.98 (49.77)	2.49 2.23)	—[b]
3t	125.5—128 e	$C_{15}H_8F_4O_2$	60.87 (60.83)	2.90 2.72)	—[b]

a) Recrystallization solvent: b=benzene, e=ether, h=hexane, et=ethanol. *b*) Due to poor solubility, these compounds precipitated on the plates.

The positive control, dibenzoylmethane, inhibited the mutagenicity of 2-nitrofluorene by 50% at 0.5 μmol/plate.

As shown in Table II, 2,2-dimethoxy (**3j**), 3,3-dimethoxy (**3k**) and 3,3,4,4-tetramethoxydibenzoylmethane (**3n**) were as active as dibenzoylmethane. None of the halogen-substituted dibenzoylmethanes were active. The present study demonstrates that dibenzoylmethanes can inhibit the mutagenicity of 2-nitrofluorene, and that modifications made on the benzene rings cannot enhance the inhibitory activity of the parent compound. The inhibitory activity of dibenzoylmethanes may be due to protection of the bacterial DNA from electrophilic attack by this chemical carcinogen.

Experimental

Melting points were determined by a Yanagimoto micro melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR 408 spectrometer. ^{1}H-NMR spectra were measured on a JEOL PMX 60Si spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard and chemical shifts were given in δ unless otherwise stated. Mass spectra (MS) were taken by a Shimadzu GC-MS 9020DF instrument at 70 eV chamber voltage on a direct inlet system. Silica gel (60—100 mesh, Merck art 7734) was used for column chromatography. Pre-coated plates (Silica gel 60 Kieselguhr F_{254}, Merck art 5737) were used for thin layer chromatography. All reactions were performed under N_2 stream. Ethyl benzoate (**2a**), ethyl 4-methoxybenzoate (**2b**), ethyl 4-methylbenzoate (**2c**), and acetophenone derivatives (**1**), except 4-benzyloxyacetophenone (**1e**), were purchased from commercial sources.

4-Benzyloxyacetophenone (1e) A stirred mixture of 4-hydroxyacetophenone (13.6 g, 0.1 mol), benzylbromide (10 g, 0.11 mol) and K_2CO_3 (15.2 g, 0.11 mol) in DMF (150 ml) was heated at 100 °C for 14 h. After being cooled to room temperature, the reaction mixture was quenched with water and extracted with benzene. The organic layer was separated, washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was recrystallized from EtOH to give **1e** (19.7 g, 87%), mp 93 °C (lit.,[12] mp 93 °C).

General Procedure for the Synthesis of Ethyl Benzoate Derivatives (2d—n) A solution of the corresponding benzoic acid (60 mmol) in absolute EtOH (40—50 ml) in the presence of conc. H_2SO_4 (2 ml) was refluxed for 2—3 h. After being cooled to room temperature, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with 0.1 N NaOH and brine, dried over $MgSO_4$ and concentrated. The residue was distilled. Yields and physical data of all compounds (**2d—n**) were obtained as follows.

Ethyl 2-Methoxybenzoate (**2d**): (87%), bp 121—122 °C (7 Torr) (lit.,[13] bp 104.4—104.5 °C (2 Torr)).

Ethyl 3-Methoxybenzoate (**2e**): (68%), bp 126—127.5 °C (10 Torr) (lit.,[13] bp 97.6—97.8 °C (1 Torr)).

Ethyl 3,4-Dimethylbenzoate (**2f**): (91%), bp 115—116 °C (9 Torr) (lit.,[14] bp 120 °C (12 Torr)).

Ethyl 2,4-Dimethoxybenzoate (**2g**): (78%), bp 168—172 °C (9 Torr). ^{1}H-NMR: 1.43 (3H, t, J=6.6 Hz, $OCH_2\underline{CH_3}$), 3.80 (3H, s, $O\underline{CH_3}$), 3.84 (3H, s, $O\underline{CH_3}$), 4.28 (2H, q, J=6.6 Hz, $O\underline{CH_2}CH_3$), 6.03—6.48 (2H, m, aromatic protons). 7.71 (1H, d, J=9.4 Hz, aromatic proton). MS: *m/z* 210 (M^+). *Anal.* Calcd for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.80; H, 6.75.

Ethyl 3,4-Dimethoxybenzoate (**2h**): (88%), bp 152—153 °C (6 Torr) (lit.,[15] bp 160 °C (10 Torr)).

Ethyl 2,5-Dimethoxybenzoate (**2i**): (81%), bp 158—160 °C (9 Torr). ^{1}H-NMR: 1.35 (3H, t, J=7.4 Hz, $OCH_2\underline{CH_3}$), 3.71 (3H, s, $O\underline{CH_3}$), 3.77 (3H, s, $O\underline{CH_3}$), 4.54 (2H, q, J=7.4 Hz, $O\underline{CH_2}CH_3$), 6.89 (3H, br s, aromatic protons), 7.28 (1H, d, J=2.4 Hz, aromatic proton). MS: *m/z* 210 (M^+). *Anal.* Calcd for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.90; H, 6.70.

Ethyl 3,4,5-Trimethoxybenzoate (**2j**): (98%), bp 166—168 °C (6 Torr) (lit.,[16] bp 117 °C (1 Torr)).

Ethyl 4-Bromobenzoate (**2k**): (97%), bp 107—109 °C (7 Torr) (lit.,[17] bp 131 °C (13 Torr)).

Ethyl 4-Chlorobenzoate (**2l**): (87%), bp 101—101.5 °C (6 Torr) (lit.,[18] bp 110—111 °C (11 Torr)).

Ethyl 3,4-Dichlorobenzoate (**2m**): (90%), bp 130—132 °C (10 Torr), mp 32.5—33.5 °C (lit.,[19] bp 262—263 °C (760 Torr) and lit.,[14] mp 37 °C).

Ethyl 2,4-Difluorobenzoate (**2n**): (85%), bp 86.5—87.5 °C (8 Torr). MS: *m/z* 172 (M^+). *Anal.* Calcd for $C_8H_6F_2O_2$: C, 55.86; H, 3.52. Found: C,

55.99; H, 3.68.

General Procedure for the Synthesis of Dibenzoylmethane Derivatives (3a—t) The acetophenone (**1**) (20 mmol) in dry benzene (20 ml) and the ethyl benzoate (**2**) (22 mmol) in dry benzene (20 ml) were added dropwise to a suspension of 60% NaH (washed with pet. ether, 22.5 mmol). The mixture was refluxed for 1—14 h and monitored with thin layer chromatography using 30% EtOAc/hexane as the development solvent. Then the reaction mixture was cooled to room temperature and quenched with 10% HCl solution. The mixture was extracted with EtOAc, washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from an appropriate solvent or purified by silica gel column chromatography using 30% EtOAc/hexane. mp and elemental analysis (C, H), of all compounds (**3**) are listed in Table I and II, respectively.

1-(4-Methoxyphenyl)-3-phenyl-1,3-propanedione (**3a**): mp 126—127 °C (lit.,[20] mp 130—131 °C). IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.78 (3H, s, OCH_3), 6.65 (1H, s, =CH–), 6.83 (2H, d, J=9 Hz, aromatic protons), 7.12—7.91 (7H, m, aromatic protons), 16.78 (1H, br s, OH). MS: m/z 254 (M^+).

1-(2,4-Dimethoxyphenyl)-3-phenyl-1,3-propanedione (**3b**): mp 52—54 °C (lit.,[21] mp 57 °C). IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.81 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.51 (1H, s, =CH–), 6.64—8.05 (8H, m, aromatic protons). MS: m/z 284 (M^+).

1-(3,4-Dimethoxyphenyl)-3-phenyl-1,3-propanedione (**3c**): mp 63—64 °C (lit.,[22] mp 67 °C). IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.91 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.74 (1H, s, =CH–), 6.83—8.00 (8H, m, aromatic protons), 16.99 (1H, br s, OH). MS: m/z 284 (M^+).

1-(2,5-Dimethoxyphenyl)-3-phenyl-1,3-propanedione (**3d**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.71 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 6.83 (1H, s, =CH–), 6.86—7.93 (8H, m, aromatic protons), 16.77 (1H, br s, OH). MS: m/z 284 (M^+).

1-(4-Benzyloxyphenyl)-3-phenyl-1,3-propanedione (**3e**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 5.12 (2H, s, OCH_2Ph), 6.69 (1H, s, =CH–), 6.95 (2H, d, J=9 Hz, aromatic protons), 7.15—7.44 (10H, m, aromatic protons), 7.87 (2H, d, J=9 Hz, aromatic protons), 16.86 (1H, br s, OH). MS: m/z 330 (M^+).

1,3-Di-(4-methoxyphenyl)-1,3-propanedione (**3f**): mp 114—116 °C (lit.,[23] mp 114 °C). IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.84 (6H, s, $OCH_3 \times 2$), 6.61 (1H, s, =CH–), 6.86 (4H, d, J=9 Hz, aromatic protons), 7.86 (4H, d, J=9 Hz, aromatic protons), 16.99 (1H, br s, OH). MS: m/z 284 (M^+).

1-Phenyl-3-(2,4,6-trimethylphenyl)-1,3-propanedione (**3g**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 2.29 (9H, s, $CH_3 \times 3$), 6.24 (1H, s, =CH–), 6.83 (2H, s, aromatic protons), 7.79—8.06 (5H, m, aromatic protons). MS: m/z 266 (M^+).

1-(4-Methoxyphenyl)-3-(2,4,6-trimethyl)-1,3-propanedione (**3h**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 2.33 (9H, s, $CH_3 \times 3$), 3.82 (3H, s, OCH_3), 6.16 (1H, s, =CH–), 6.82 (2H, s, aromatic protons), 6.86 (2H, d, J=9 Hz, aromatic protons), 7.82 (2H, d, J=9 Hz, aromatic protons). MS: m/z 296 (M^+).

1,3-Di-(4-methylphenyl)-1,3-propanedione (**3i**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 2.39 (6H, s, $CH_3 \times 2$), 6.76 (1H, s, =CH–), 7.23 (4H, d, J=8 Hz, aromatic protons), 7.85 (4H, d, J=8 Hz, aromatic protons), 16.80 (1H, br s, OH). MS: m/z 268 (M^+).

1,3-Di-(2-methoxyphenyl)-1,3-propanedione (**3j**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.93 (6H, s, $OCH_3 \times 2$), 6.78—7.84 (7H, m, =CH– and aromatic protons), 7.83 (2H, dd, J=4 and 1 Hz, aromatic protons), 16.69 (1H, br s, OH). MS: m/z 284 (M^+).

1,3-Di-(3-methoxyphenyl)-1,3-propanedione (**3k**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.80 (6H, s, $OCH_3 \times 2$), 6.69 (1H, s, =CH–), 6.86—7.51 (8H, m, aromatic protons), 16.69 (1H, br s, OH). MS: m/z 284 (M^+).

1,3-Di-(3,4-dimethylphenyl)-1,3-propanedione (**3l**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 2.32 (12H, s, $CH_3 \times 4$), 6.72 (1H, s, =CH–), 7.17 (2H, d, J=2 Hz, aromatic protons), 7.53—7.67 (4H, m, aromatic protons). MS: m/z 280 (M^+).

1,3-Di-(2,4-dimethoxyphenyl)-1,3-propanedione (**3m**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.83 (6H, s, $OCH_3 \times 2$), 3.90 (6H, s, $OCH_3 \times 2$), 6.47 (1H, s, =CH–), 6.63 (2H, d, J=2 Hz, aromatic protons), 7.29 (2H, d, J=8 Hz, aromatic protons), 7.93 (2H, d, J=8 Hz, aromatic protons), 17.12 (1H, br s, OH). MS: m/z 344 (M^+).

1,3-Di-(3,4-dimethoxyphenyl)-1,3-propanedione (**3n**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.80 (6H, s, $OCH_3 \times 2$), 3.87 (6H, s, $OCH_3 \times 2$), 6.97 (3H, br s, aromatic protons and =CH–), 7.42 (4H, br s, aromatic protons), 16.76 (1H, br s, OH). MS: m/z 344 (M^+).

1,3-Di-(2,5-dimethoxyphenyl)-1,3-propanedione (**3o**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.95 (12H, s, $OCH_3 \times 4$), 6.68 (1H, s, =CH–), 6.87 (2H, d, J=9 Hz, aromatic protons), 7.49—7.63 (4H, m, aromatic protons), 17.30 (1H, br s, OH). MS: m/z 344 (M^+).

1,3-Di-(3,4,5-trimethoxyphenyl)-1,3-propanedione (**3p**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 3.91 (18H, s, $OCH_3 \times 6$), 6.59 (1H, s, =CH–), 7.15 (4H, s, aromatic protons), 16.92 (1H, br s, OH). MS: m/z 404 (M^+).

1,3-Di-(4-bromophenyl)-1,3-propanedione (**3q**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 6.66 (1H, s, =CH–), 7.50 (4H, d, J=9 Hz, aromatic protons), 7.77 (4H, d, J=9 Hz, aromatic protons), 16.75 (1H, br s, OH).

1,3-Di-(4-chlorophenyl)-1,3-propanedione (**3r**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 6.72 (1H, s, =CH–), 7.42 (4H, d, J=8 Hz, aromatic protons), 7.79 (4H, d, J=8 Hz, aromatic protons), 16.61 (1H, br s, OH).

1,3-Di-(3,4-dichlorophenyl)-1,3-propanedione (**3s**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 6.60 (1H, s, =CH–), 7.15—7.51 (2H, m, aromatic protons), 7.70 (2H, dd, J=8 and 2 Hz, aromatic protons), 7.93 (2H, d, J=2 Hz, aromatic protons).

1,3-Di-(2,4-difluorophenyl)-1,3-propanedione (**3t**): IR (KBr): 1600 cm^{-1} (C=O). ^{1}H-NMR: 6.62—7.23 (5H, m, =CH–, aromatic protons), 7.81—8.22 (2H, m, aromatic protons), 16.56 (1H, br s, OH).

Procedure for Mutation Test The mutation test was done according to the protocol described previously.[4] The reaction mixtures in 0.5 ml quantities contained overnight 0.1 ml cultures of *S. typhimurium* TA98, 10 nmol 2-nitrofluorene, 0.1 to 5 μmol of test compounds, 20 μl dimethylsulfoxide (DMSO) and 25 μmol Na phosphate, pH 7.0. The mixtures were kept at 24 °C for 30 min and then plated. After incubation at 37 °C in the dark for 2 d, the revertant colonies were counted with an Artek System model 880 colony counter (Artek System Corp., Farmingdale, NY). All experiments were performed in triplicate.

Acknowledgment This work was supported in part by United States Public Health Service grant CA23800.

References

1) P. E. Hartman and D. M. Shankel, *Environ. Mol. Mutagen.*, **15**, 145 (1990).
2) S. De Flora and C. Ramel, *Mutation Res.*, **202**, 285 (1988).
3) C. Y. Wang, M.-S. Lee, H. Nagase, and K. Zukowski, *J. Natl. Cancer. Inst.*, **81**, 1743 (1989).
4) C. Y. Wang, M.-S. Lee, and K. Zukowski, *Mutation Res.*, **262**, 189 (1991).
5) L. M. Calle and P. D. Sullivan, *Mutation Res.*, **101**, 99 (1982).
6) Z. Y. Wang, S. J. Cheng, Z. C. Zhou, M. Athar, W. A. Khan, D. R. Bickers, and H. Mukhtar, *Mutation Res.*, **223**, 273 (1989).
7) A. W. Wood, M.-T. Huang, R. L. Chang, H. L. Newmark, R. E. Lehr, H. Yagi, J. M. Sayer, D. M. Jerina, and A. H. Conney, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 5513 (1982).
8) R. Dixit and B. Gold, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8039 (1986).
9) F. W. Swamer and C. R. Hauser, *J. Am. Chem. Soc.*, **72**, 1352 (1950).
10) C. R. Hauser, F. W. Swamer, and J. T. Adams, "Organic Reactions," Vol. VIII, ed. by R. Adams, A. H. Blatt, V. Boeckelheide, A. C. Cope, D. Y. Curtin, F. C. McGrew, and C. Niemann, John Wiley & Sons, Inc., New York, 1954, pp. 59—196.
11) F. Kikuchi, X. Chen, and Y. Tsuda, *Chem. Pharm. Bull.*, **38**, 1862 (1990).
12) H. M. Priestly and E. Moness, *J. Org. Chem.*, **5**, 355 (1940).
13) D. D. Thompson, *J. Am. Chem. Soc.*, **59**, 816 (1937).
14) B. Jons and J. Robinson, *J. Chem. Soc.*, **1955**, 3845.
15) M. M. Byrne and N. H. P. Smith, *J. Chem. Soc. B*, **1968**, 809.
16) N. L. Dlake and W. B. Tuemmler, *J. Chem. Soc.*, **77**, 1204 (1955).
17) H. McCombie and H. A. Scarborough, *J. Chem. Soc.*, **107**, 160 (1915).
18) S. Tanaka, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.*, **24**, 2825 (1976).
19) F. Beilstein and A. Kuhrberg, *Justus Liebigs Ann. Chem.*, **152**, 228 (1869).
20) H. Ishihara and Y. Hirabayashi, *Chem. Lett.*, **1978**, 1007.
21) R. Robinson and M. R. Turner, *J. Chem. Soc.*, **113**, 874 (1918).
22) J. M. Gulland and R. Robinson, *J. Chem. Soc.*, **127**, 1493 (1925).
23) T. Tasaki, *Acta Phytochim. Jpn.*, **3**, 259 (1927).